

Clinical implications of microsatellite instability in sporadic colon cancers

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Purpose of review

To review data demonstrating the prognostic and predictive impact of microsatellite instability (MSI) in human colon carcinomas.

Recent findings

MSI is a molecular marker of defective DNA mismatch repair that is detected in approximately 15% of sporadic colon cancers. Most, but not all retrospective studies, have shown that colon cancers with MSI have better stage-adjusted survival rates compared with non-MSI tumors. Furthermore, analyses of colon cancers from participants in randomized adjuvant therapy trials have suggested that MSI tumors do not benefit from treatment with 5-fluorouracil. Recent studies, including a pooled analysis, validate prior data demonstrating the prognostic and predictive impact of MSI status in colon cancer.

Summary

MSI is a molecular marker that can provide valuable prognostic and predictive information in colon cancer patients. In the appropriate clinical setting, MSI data can be used in clinical decision-making. Specifically, the favorable outcome of stage II colon cancers with MSI indicates that such patients should not receive adjuvant chemotherapy. Although data for stage III colon cancers with MSI suggest a lack of benefit from 5-fluorouracil alone, the benefit of the current standard treatment, 5-fluorouracil, leucovorin, and oxaliplatin, in this subgroup remains unknown and awaits further study.

Keywords

colon cancer, microsatellite instability, prediction, prognosis

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Introduction

Although surgical tumor staging remains the key determinant of colorectal cancer (CRC) prognosis and treatment, there is considerable stage-independent variability in clinical outcome underscoring the need for robust prognostic and predictive markers to guide the use of adjuvant chemotherapy. Approximately 15% of sporadic colon cancers develop via an alternative pathway of tumorigenesis characterized by microsatellite instability (MSI), a molecular marker of defective function of the DNA mismatch repair (MMR) system [1•,2]. Multiple retrospective studies [3–6], as well as a population-based study [7] and meta-analysis [8], have shown that patients whose colon cancers exhibit MSI, that is, MSI-high-frequency (MSI-H) tumors [9], have a more favorable stage-adjusted prognosis compared with patients with non-MSI-H tumors. Evidence also suggests that patients with MSI-H colon cancers do not benefit from 5-fluorouracil (5-FU)-based adjuvant therapy in contrast to those with non-MSI-H tumors [8,10–13]. Despite these results, some studies have failed to show an impact of MSI status upon patient outcome [14•] or suggest a better outcome for non-MSI tumors

[15,16]. Recently, a retrospective analysis was reported that has validated the prognostic and predictive impact of MSI-H in colon cancers from participants in randomized adjuvant therapy trials [17••]. It is our objective to highlight the importance of recognizing the MSI-H phenotype in clinical practice and to discuss its implications for clinical decision-making, including the use of adjuvant chemotherapy.

Sporadic microsatellite instability-high-frequency colon cancers

MSI-H can be detected in nearly all cases of Lynch syndrome and in about 15% of sporadic colon cancers [1•]. Whereas Lynch syndrome is due to germline mutations in MMR genes, sporadic colon cancers develop MSI-H due to inactivation of the *hMLH1* gene by DNA methylation, an epigenetic and nonheritable event [1•,18,19••]. Of approximately 108 000 incident colon cancers that were diagnosed in the United States in 2008 [20], 16 000 are expected to be MSI-H tumors. Sporadic MSI-H colon cancers and those from patients

with Lynch syndrome share distinct clinical and pathological features that include proximal colon predominance, poor differentiation with or without mucinous histology, a marked peritumoral lymphocytic infiltration, wild-type *p53* status, and diploid DNA content [3–5,11,21,22]. In contrast to Lynch syndrome cases, sporadic MSI-H cancers are distinct epidemiologically in that they are associated with older age at diagnosis, female sex, and with cigarette smoking [19^{••},22,23]. MSI-H status has been shown to be very rare in rectal carcinomas [24,25].

Whereas Amsterdam and Bethesda criteria were developed to identify patients with Lynch syndrome [26,27], established criteria to identify sporadic colon cancers with MSI-H are lacking and the clinician must rely upon clinical and pathological features. MSI testing can be performed in paraffin-embedded tumor tissues using a polymerase chain reaction (PCR)-based assay for detection of instability at selected microsatellite loci [2,9]. CRCs are categorized according to the extent of MSI exhibited as high frequency (MSI-H), low frequency (MSI-L) or microsatellite stable (MSS) [9]. MSS tumors show no instability at any loci that is typical of most sporadic CRCs that show chromosomal instability [1[•]]. Nearly all studies have shown that MSI-L and MSS colon cancers have similar clinicopathological features and clinical outcome; therefore, they are generally grouped together [4,5,9,14[•],22,28]. Analysis of tumor tissue for MMR protein expression by immunohistochemistry (IHC) is a complementary or alternative test whose results are highly concordant with MSI testing [6,29]. IHC will reveal loss of the protein product of MMR genes of which only hMLH1 loss has been described in sporadic MSI-H colon cancers [18,19^{••},22]. Therefore, this test can suggest Lynch syndrome if loss of any protein besides hMLH1 is found. Importantly, neither IHC nor MSI indicates the mechanism of the MMR defect, namely germline mutation versus epigenetic inactivation. Accordingly, these data must be interpreted within the relevant clinical context, which dictates whether any additional testing and/or genetic evaluation/counseling is warranted.

Prognostic impact of microsatellite instability phenotype

When MSI was discovered in CRCs, it was noted that patients with MSI-H tumors had better survival rates compared with those with non-MSI-H (i.e., MSS, MSI-L) tumors [2]. MSI-H was also found to be associated with lower tumor stage at diagnosis [30] and is rare in metastatic CRCs [31,32]. Subsequently, several retrospective case studies as well as a population-based study [7] found that MSI-H colon cancers have a more favorable survival compared with non-MSI-H tumors that was independent

of tumor stage [4–6,11,30]. Similar results were found among participants in phase III adjuvant trials, in which patients with MSI-H stage II and III colon cancers not treated with chemotherapy showed significantly better overall survival rates compared with untreated patients with non-MSI-H tumors [3,10]. Not all studies [14[•],33], however, have shown a prognostic impact for MSI-H, including a cooperative group trial whereby no survival differences were found for patients with MSI-H versus those with non-MSI-H colon cancers [14[•]]. Several factors may account for these differences, including an insufficient sample size as MSI-H tumors represent a relatively small subset, and that the magnitude of the MSI-H prognostic effect is modest. Furthermore, selection bias is a potential confounder in retrospective case series including adjuvant clinical trials, in which tissue availability has generally been limited to a patient subset. In addition, the microsatellite markers used to detect MSI-H cases have not been uniform, which can result in false positive results that can dilute the prognostic effect [9]. Despite these limitations, the prognostic impact of MSI status is strongly supported by a meta-analysis of 32 studies including 1277 MSI-H CRCs that demonstrated a 35% reduction in the risk of death for patients with MSI-H colon cancers compared with those with non-MSI-H tumors [8]. This analysis included untreated patients as well as patients treated in phase III randomized, 5-FU-based adjuvant therapy trials. The observed survival benefit of MSI-H was maintained when restricting the analysis to clinical trial participants.

In a recent study aimed at validating the prognostic and predictive impact of MSI status, stage II and III colon cancers from participants in North American and European adjuvant therapy trials were analyzed for MSI or MMR protein expression [17^{••}]. MMR expression, determined by IHC, has been shown to be highly concordant with MSI test results [29] and confers similar prognostic information [4,6]. After pooling of studies and considering only patients not receiving chemotherapy ($n = 515$), tumors with MSI-H or loss of an MMR protein showed a 49% reduction in disease-free survival compared with non-MSI-H cases or those with intact protein expression [17^{••}]. These results from an independent dataset confirm prior studies demonstrating the prognostic impact of MSI-H. To date, the exact mechanism underlying the better outcome of MSI-H colon cancers remains unknown and awaits further study. However, the pronounced lymphocytic infiltration seen in these cancers may contribute to their better prognosis [34[•],35,36].

Predictive role of microsatellite instability

Human CRC cell lines with MSI-H display resistance to compounds that induce DNA damage, including 5-FU

[37]. Importantly, resistance to 5-FU was overcome by restoring normal DNA MMR function in CRC cells [38]. As predicted by the preclinical data, retrospective studies have suggested that patients with sporadic MSI-H colon cancers did not derive benefit from 5-FU-based chemotherapy in contrast to those with non-MSI tumors [8,10–13]. Among these reports is a study of 256 MSI-H colon cancer patients from three institutions in Italy, whereby 5-FU-based chemotherapy failed to improve survival in these patients [11]. Further support derives from adjuvant therapy trials, whereby patients with MSI-H tumors (stage II and III) did not benefit from 5-FU-based chemotherapy, whereas patients with non-MSI cancers demonstrated a significant survival advantage in favor of treatment [10]. In a study in which stage II and III CRC patients received adjuvant chemotherapy and were followed prospectively, the survival benefit of 5-FU treatment was again limited to non-MSI tumors or those with intact MMR proteins [12]. Extended follow-up of this cohort has confirmed these earlier findings [39**]. Despite these data, the predictive utility of MSI has generated much controversy, given that some retrospective studies have failed to demonstrate a predictive impact of MSI [14*,33] and other reports [15,16,40] have shown that patients with MSI colon cancers receive a greater benefit from 5-FU-based adjuvant treatment than do non-MSI patients. In one of these reports, patients receiving 5-FU were on average 13 years younger than those who did not [16]. In addition to potential selection bias, another issue is the need to compare treated with untreated MSI cases given the prognostic impact of MSI. Another report evaluating the predictive value of MSI-H in colon cancers from adjuvant therapy trials failed to confirm that MSI testing can predict response to 5-FU-based chemotherapy [14*]. As previously stated, potential explanations include retrospective study design, limited statistical power to detect survival differences, and technical issues related to defining MSI-H status.

Importantly, a meta-analysis of retrospective data from patients with tumor node metastasis (TNM) stage II and III colon cancers enrolled in randomized clinical trials found that MSI-H tumors do not derive benefit from adjuvant 5-FU compared with non-MSI-H cases [8]. A study was recently reported at the 2008 American Society of Clinical Oncology (ASCO) meeting, in which the effect of 5-FU in relation to MSI status was determined in pooled data from stage II and III colon cancer patients treated in randomized adjuvant studies from North America and Europe. This study confirmed that patients whose tumors showed MSI-H or loss of MMR protein expression did not benefit from 5-FU compared with those patients randomized to untreated control arms [17**].

Implications of microsatellite instability for adjuvant therapy

Since publication of the ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer [41], further evidence has accumulated confirming the prognostic utility of MSI status in colon cancer patients and more recently, validating its predictive impact for 5-FU-based adjuvant therapy [3,6,12,17**]. Although nearly all of the data are from retrospective analyses, results from more recent, large studies are concordant and statistically robust. These studies demonstrate that MSI-H is a favorable prognostic marker and that MSI-H tumors do not benefit from adjuvant 5-FU. A prospective phase III study in stage II colon cancer patients is being conducted by the Eastern Cooperative Oncology Group (ECOG 5202), whereby MSI status and chromosome 18q allelic imbalance (an adverse prognostic marker in some colon cancer studies [42,43]) are determined in tumor specimens. Patients are categorized into low-risk (MSI-H or 18q intact) or high-risk (non-MSI-H or 18q allelic imbalance) groups. In this trial, low-risk patients receive observation, whereas high-risk patients are treated with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) with or without bevacizumab. Although this study aims to provide definitive information regarding the influence of these molecular markers on clinical outcome, the trial will not provide needed prospective data as to responsiveness of MSI-H tumors to FOLFOX therapy, as no MSI cases are being treated. This clinical trial is currently accruing patients and will require several years for the data to mature.

Although prospective studies remain the ‘gold standard’ for validating prognostic and predictive biomarkers, circumstances exist in which the preponderance of evidence is consistent and in which the results have been validated in an independent dataset [17**]. We regard this situation to be the case for the use of MSI status to inform patient prognosis and to influence treatment decisions in stage II colon cancer patients. Similarly, the predictive utility of *K-ras* mutational status for responsiveness to antibodies against the epidermal growth factor receptor (EGFR) has been consistently shown in retrospective analyses and these data have led to changes in clinical practice [44]. We believe that MSI testing can provide useful prognostic information in CRC patients and inform decision-making with regard to adjuvant therapy. The use of adjuvant chemotherapy in patients with stage II colon cancer remains controversial [45*,46,47] and represents a dilemma for medical oncologists with regard to the potential benefits versus risks of treatment. It is estimated that one-third of all stage II patients receive adjuvant therapy in the United States. We believe that the strength of the available evidence is sufficient at the present time to recommend that MSI-H stage II colon cancer patients not receive adjuvant chemotherapy given

that such tumors have a favorable prognosis and exhibit resistance to 5-FU. This recommendation is evidence-based and can spare such patients unnecessary treatment-related toxicities and reduced quality of life during chemotherapy. We emphasize that non-MSI-H status by itself does not indicate a high-risk stage II tumor nor does it provide a rationale for adjuvant chemotherapy. The majority (~85%) of stage II CRCs are non-MSI-H tumors and decisions regarding adjuvant therapy in these patients should be individualized. In stage III disease, however, the issue is more complex. Standard adjuvant therapy for stage III colon cancer is the FOLFOX regimen [48] and the predictive impact of MSI status upon the chemosensitivity to FOLFOX is unknown. At present, the available data do not justify the exclusion of MSI-H stage III colon cancer patients from receiving FOLFOX chemotherapy despite the fact that MSI-H tumors demonstrate resistance to 5-FU. We regard the impact of MSI status upon responsiveness to FOLFOX as a critical research question that awaits further evaluation. Clearly, MSI status needs to be taken into account in future adjuvant therapy trials in CRC.

As previously stated, sporadic MSI-H colon cancers are associated with proximal tumor site, poor differentiation, older age at diagnosis, and with female sex [3,4,19^{••},21,22]. We and others [49] are developing a model to predict the likelihood of MSI-H colon cancers using clinically available data. To identify MSI-H CRC patients in clinical practice and to utilize such information for clinical decision-making at this time, we recommend that MSI or IHC testing for defective DNA MMR testing be performed on all resected, node-negative nonmetastatic cancers of the proximal colon, wherein adjuvant therapy is a consideration. An advantage of MSI testing is that it does not identify the suspected mutated gene and is therefore not a genetic test but is a clinical test that can be utilized to influence patient management. An argument can be made that the cost of MMR testing may be offset by more efficient use of adjuvant chemotherapy.

Conclusion

Accumulated evidence demonstrates the prognostic and predictive utility of MSI status, including a recent study validating the clinical utility of defective MMR (MSI-H or loss of MMR protein expression) in colon cancers from patients treated in adjuvant therapy trials [17^{••}]. We believe that the current evidence is sufficient to utilize MSI results for prognosis and to influence clinical decision-making regarding the use of adjuvant chemotherapy in stage II colon cancer patients. At present, we restrict our recommendations to stage II patients. While patients with stage III MSI-H tumors have a better prognosis and may not benefit from 5-FU, data regarding the benefit of FOLFOX in such patients are lacking.

Therefore, all stage III patients should continue to receive standard adjuvant chemotherapy as of this writing. An analysis of MMR status in colon cancers from completed adjuvant studies that evaluated FOLFOX, for example, the Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) [45[•]], as well as ongoing adjuvant studies (NSABP C-08, NCCTG N0147) could shed further light on this issue. Going forward, prospective clinical trials are needed to evaluate non-5-FU-based regimens in patients with MSI-H stage III colon cancers. In summary, MSI is an important molecular marker of tumor biology that can provide clinically useful information and represents one further step toward individualized cancer therapy.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 391).

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