Combination of Microsatellite Instability and Lymphocytic Infiltrate as a Prognostic Indicator in Colon Cancer

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Background: Microsatellite instability (MSI) is a genetic aberration associated with less aggressive tumor biology. Some tumors with MSI also have lymphocytic infiltrate (LI), which suggests a heightened immune response against the tumor.

Objective: To evaluate the combined prognostic significance of MSI and LI in a colon cancer population.

Design: Colon cancers were prospectively evaluated for MSI by assessing 11 satellite markers and were classified as MSI+/LI− if 2 or more satellite markers displayed instability. Tumors were classified as LI+/H11001 if at least 5 lymphocytes were observed per 10 high-power fields.

Setting: Community hospital system.

Patients: Individuals undergoing definitive surgery for colon cancer.

Main Outcome Measures: Overall and disease-free survival were compared according to combined MSI and LI status.

Results: In 150 patients, tumors were classified as follows: 95 were MSI−/LI−, 9 were MSI−/LI+, 30 were MSI+/LI−, and 16 were MSI+/LI+. Median follow-up was 40.6 months. Five-year disease-free survival was 56.7% for patients with MSI−/LI− tumors and 88.9% for those with MSI+/LI+ tumors (P=.01). Patients with MSI+/LI− and MSI−/LI+ tumors had 5-year survival of 75.4% and 75.0%, respectively.

Conclusions: Patients with colon cancer and MSI−/LI+ tumors have worse disease-free survival rate regardless of stage at diagnosis. Patients exhibiting both MSI+ and LI− tumors have more favorable disease-free survival rates. Both MSI and LI show promise as a combined prognostic marker and with further study may prove to be particularly useful in selecting patients with stage II disease for adjunctive therapy.

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THE COLON IS THE THIRD leading cancer tumor site, with an estimated 112,340 cases diagnosed per year in the United States.1 Significant progress has been made in the realm of treatment. New chemotherapeutic regimens are improving response rates. However, we have yet to develop prognostic markers to select patients who would benefit from more aggressive adjunctive therapy. To date, physicians still base that decision on nodal involvement.

Microsatellite instability (MSI) is a phenomenon in colorectal cancer that has attracted attention owing to the prognostic implications associated with it. Almost all cases of colon cancer with hereditary nonpolyposis colorectal cancer demonstrate MSI. Several studies have demonstrated improved survival rates in patients with colorectal cancer whose tumors demonstrate MSI. The mechanism by which these tumors lead to a more favorable prognosis has been unclear.

A large proportion of tumors with MSI, in sporadic cases and in hereditary nonpolyposis colorectal cancer carriers, are associated with immune host response features. One such feature is a Crohn disease-like peritumoral reaction that consists of lymphoid follicles with germinal centers.6,7 Intratumoral lymphocytic infiltrate (LI), which is often seen in association with a peritumoral stromal lymphocytic inflammatory infiltrate, is another feature often identified.6 CD3 lymphocyte counts in excess of 40 per 1000 epithelial cells occur in 70% of tumors with high-frequency MSI, 33% with low-frequency MSI, and 17.5% of microsatellite-stable tumors.6 CD3 counts in excess of 100 per 1000 epithelial cells are highly sensitive for high-frequency MSI tu-
The presence of LI (referred to as LI
survival after surgery were performed between the MSI
rized into 4 groups according to MSI and LI status. Time-to-
generated using the Fisher exact test. Patients were catego-
cal data, such as sex, tumor grade, pathological stage of dis-
formed under a protocol approved by the institutional review
and lymph node involvement. All the procedures were per-
tumor epithelium. Patients were prospectively followed for
greater than 5 lymphocytes per 10 high-power (×40) fields
microsatellite stability and low-frequency MSI are referred to
frequency MSI are subsequently referred to as MSI
Tumors were examined for intratumoral LI using light
microscopy and staining with hematoxylin-eosin (Figure 1).

All patients undergoing definitive resection of colorectal can-
cancers in a community hospital system were prospectively
January 1, 2001, and December 31, 2003. No restrictions were imposed on patient age, tumor loca-
history of colorectal cancer. Tumors of con-
clinical Molecular Diagnostic Laboratories, City of Hope
1997 National Cancer Institute guidelines (Clinical Molecular Diagnostic Laboratories, City of Hope
Tumors in which none of the loci demonstrated instability were classified as micro-
satellite stable. Those with a single unstable locus were classified as low-frequency MSI. Tumors with 2 or more unstable
loci were classified as high-frequency MSI. Patients with high-
frequency MSI are subsequently referred to as MSI+; those with microsatellite stability and low-frequency MSI are referred to as MSI−. Tumors were examined for intratumoral LI using light
microscopy and staining with hematoxylin-eosin (Figure 1).

The presence of LI (referred to as LI+ herein) was defined as greater than 5 lymphocytes per 10 high-power (×40) fields
of the tumor epithelium. Patients were prospectively followed for the development of recurrence and death. Data were collected
regarding age, sex, pathological stage of disease, tumor grade, and lymph node involvement. All the procedures were performed
under a protocol approved by the institutional review board of the Legacy Health System.

Comparisons were made between patients with LI+ and LI−tumors. The unpaired t test was used to compare age. Categori-
cal data, such as sex, tumor grade, pathological stage of dis-
case, and nodal involvement, were compared and P values were
generated using the Fisher exact test. Patients were catego-
rized into 4 groups according to MSI and LI status. Time-to-
event analyses of disease-free survival (DFS) and overall sur-
vival after surgery were performed between the MSI+/LI+ and

RESULTS

One hundred sixty-seven patients were enrolled in the study. Testing for MSI was inconclusive in 6 patients. In
11 patients, there was insufficient tumor mass for analysis. The remaining 150 patients had MSI and LI results and were included for analysis. In 25 patients (16.7%), the tumors demonstrated LI. Of these 25 patients, 9 (36.0%) had MSI+ tumors. Of the 125 patients without LI, 30 (24.0%) had MSI+ tumors. The mean age of pa-
tients was 72.5 years for MSI+/LI+ tumors, 71.3 years for MSI+/LI−, 61.3 years for MSI−/LI+, and 69.2 years for MSI−/LI−.

Forty-three patients had stage I disease; 56, stage II; 34, stage III; and 16, stage IV. One patient underwent resection for a local recurrence and was not included in the stage groups. Stage distribution, lymph node involve-
ment, and tumor grade were similar between LI+ and LI− patients.

During follow-up (median, 40.6 months), 49 patients
developed recurrence or died (Table 1). Patients with LI+ tumors had 5-year DFS of 84.9% compared with
61.0% in those with LI− tumors (P = .03). The differ-
eses were greatest in patients with stage II disease, where 5-year DFS was 100% for patients with LI+ tumors and 61.1% for those with LI− tumors (P = .09).

When patients were grouped according to MSI and LI
status, those with MSI−/LI− tumors had 5-year DFS of
56.7% compared with 88.9% for those with MSI+/LI+ (P = .01). Five-year DFS for patients with MSI−/LI+ and
MSI+/LI− tumors was 75.0% and 75.4%, respectively (Figure 2).

Similar patterns of survival were seen when patients
were stratified by tumor grade (Table 2). Of patients with moderately differentiated tumors, those with MSI−/LI− tumors had 5-year DFS of 59.3% compared with 100% for those with MSI+/LI+ tumors (P = .03). Of patients with poorly differentiated tumors, those with MSI−/LI−
tumors had 5-year DFS of 44.4% compared with 66.7% for those with MSI+/LI+ tumors (P = .20).

Of patients with node-negative disease, those with
MSI−/LI− tumors had 5-year DFS of 65.3% compared with 100% for those with MSI+/LI+ (P = .04). In patients with nodal involvement, those with MSI−/LI− tumors had 5-year DFS of 42.9% compared with 66.7% for those with
MSI+/LI+ (P = .18).

Multivariate analyses of MSI, LI, T stage, and N stage were performed using Cox regression (Table 3). High-
frequency MSI was associated with a hazard ratio of 0.433 (P = .04). The presence of LI was associated with a hazard ratio of 0.425 (P = .16). T3 (or greater) and N1 (or greater) staging were associated with hazard ratios of 3.171 (P = .006) and 1.567 (P = .13), respectively.
Table 1. Patients Who Developed Disease Recurrence or Died, According to Microsatellite Instability (MSI) and Lymphocytic Infiltrate (LI) Status

<table>
<thead>
<tr>
<th>Disease Stage</th>
<th>MSI+/LI+</th>
<th>MSI+/LI−</th>
<th>MSI−/LI+</th>
<th>MSI−/LI−</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0/6</td>
<td>7/16 (43.8)</td>
<td>0/1</td>
<td>7/26 (26.9)</td>
<td>7/43 (16.3)</td>
</tr>
<tr>
<td>II</td>
<td>0/5</td>
<td>7/16 (43.8)</td>
<td>0/2</td>
<td>11/33 (33.3)</td>
<td>18/56 (32.1)</td>
</tr>
<tr>
<td>III</td>
<td>1/5 (20.0)</td>
<td>0/6</td>
<td>1/2 (50.0)</td>
<td>7/21 (33.3)</td>
<td>9/34 (26.5)</td>
</tr>
<tr>
<td>IV</td>
<td>0/0</td>
<td>0/0</td>
<td>1/1 (100.0)</td>
<td>14/15 (93.3)</td>
<td>15/16 (93.8)</td>
</tr>
<tr>
<td>Overall</td>
<td>1/16 (6.3)</td>
<td>7/29 (24.1)</td>
<td>2/9 (22.2)</td>
<td>39/95 (41.1)</td>
<td>49/149 (32.9)</td>
</tr>
</tbody>
</table>

Abbreviations: LI+, LI present; LI−, LI absent; MSI+, high-frequency MSI; MSI−, microsatellite stable or low-frequency MSI.

In terms of overall survival, patients with LI+ tumors had 5-year overall survival of 83.6% compared with 59.0% in those with LI− tumors (P = .12). Patients with MSI+ tumors had 5-year overall survival of 74.4% compared with 57.3% in those with MSI− tumors (P = .08). Patients with MSI+/LI+ tumors had 5-year overall survival of 88.9% compared with 55.3% in those with MSI−/LI− tumors (P = .06).

The results of this study confirm that tumors with MSI and LI have a more favorable prognosis. When patients are grouped by MSI and LI, the group with MSI+ and LI+ tumors demonstrated the most favorable survival rate, whereas the survival rates of those with neither MSI+ nor LI+ tumors were the least favorable. This pattern of DFS was seen regardless of tumor grade (Table 2).

Multivariate analysis of MSI and LI status in conjunction with known prognostic indicators confirmed that increased depth of tumor invasion and nodal involvement are associated with worse DFS, as expected. Moreover, this analysis suggests that MSI and LI status are independently associated with improved DFS. The analysis of LI status did not reach statistical significance, but this may be due to an insufficient number of patients to detect the association because nodal status (an accepted staging criterion) also did not reach statistical significance in this analysis. However, we submit that combining MSI and LI status to the currently accepted tumor depth of invasion and nodal status gives a more accurate prediction of outcome.

In stage II disease, LI+ tumors are associated with improved DFS even in patients with MSI− tumors. Similarly, patients with MSI+/LI− tumors also had an intermediate survival rate, which suggests that MSI status and LI status play a role in determining the behavior of the tumor, perhaps through separate mechanisms. Elucidation of these mechanisms may yield strategies toward the development of new approaches to disease treatment.

The mechanism by which MSI is associated with longer survival is not well understood. Some researchers have attributed the less aggressive biological mechanism of MSI+ tumors to a lower prevalence of mutations in the K-ras gene or a loss of heterozygosity in the DCC or p53 genes, each of which have been associated with a worse prognosis. Another postulated mechanism is that MSI is often associated with LI, which suggests a heightened immune response to MSI+ tumors. The presence of intratumoral lymphocytes may represent part of a more complex host immune response to the tumor.

One theorized mechanism is that MSI may be associated with the production of immunogenic peptides. High-frequency MSI tumors have been found to have a significantly higher percentage of cells undergoing apoptosis. Dolcetti et al showed that most CD8+ lymphocytes infiltrating MSI+ tumors are cytotoxic effectors characterized by a high degree of activation, with polarization of cytotoxic granules in close proximity to apoptotic bodies or cells with DNA fragmentation. This suggests that these lymphocytes may be involved in targeted cell-mediated killing of neoplastic cells.

The finding that LI alone correlated with 5-year DFS suggests a heightened immune response to these tumors, but the exact mechanism remains unknown. One possibility is that LI represents an inflammatory reaction to tumors that are particularly immunogenic. Further studies will hopefully clarify what kinds of genes or protein expressions enhance an immune response. Another possible mechanism is that the immune system of certain individuals enables them to mount a heightened immune response to a tumor. If the latter proves to be true, it may suggest a potential role for immunomodulatory drugs in the treatment of LI− tumors.
are not sensitive markers for MSI. Thus, this approach would miss approximately 65% of MSI−/LI− tumors with worse outcome in stage II disease. This subgroup of patients may stand to gain the most benefit from adjuvant chemotherapy. When patients were stratified by stage, we found that the more favorable DFS associated with LI was seen predominantly in patients with stage II disease. Clinically, this is an exciting finding. Currently, patients with stage II disease do not usually receive adjunctive therapy. Larger studies should be performed to bear out the association of MSI−/LI− tumors with worse outcome in stage II disease. This subgroup of patients may stand to gain the most benefit from adjuvant chemotherapy. This study was performed on hematoxylin-eosin–stained samples using a specific cutoff value for identifying tumors with LI+. performed by an expert pathologist. Although this technique is subject to interobserver variation, it seems to be effective in identifying the population of patients with improved prognosis at Legacy Health System. This technique is, therefore, a feasible alternative to formal quantitation of intratumoral T-cell infiltrates, performed with anti-CD3 pan–T-cell antibody. Although the latter technique may provide more objective results, it requires additional expense and processing time.

Testing for MSI, which is a relatively complex DNA assay, is fairly expensive and can be difficult for most laboratories to perform. It has been proposed that the presence of LI could be used as a screening tool to identify tumors that should undergo MSI testing. In this study, however, 25 of 30 patients without LI had MSI+ tumors. Thus, this approach would miss approximately 65% of MSI+ cases. Other histopathologic features, such as a medullary-type pattern, LI, and poor differentiation, also are not sensitive markers for MSI+ tumors.

Molecular profiling of tumors is currently the standard for several malignancies (including breast cancer, gastrointestinal stromal tumors, and lymphoma). To be successful, markers should be easily reproducible and widely available. Microsatellite instability and lymphocytic infiltration bear the potential to be included in the standard molecular profile of colorectal cancer. Although microRNA profiling has recently been described, it is far more difficult to perform and would not be readily available to most community laboratories. Recently, immunohistochemical staining for mismatch repair proteins has been used to complement MSI testing for the detection of mismatch repair mutations. Immunohistochemical analysis may be performed at a lower cost than formal MSI testing. Its accuracy depends on the quality of nuclear staining and the availability of an experienced pathologist. Immunohistochemical analysis will accurately detect the absence of mismatch repair proteins in mutations that truncate the protein or reduce its expression and will miss approximately 6% of mutations that involve more subtle alterations in the protein, such as those resulting from nonsense mutations. However, it can be readily added to the armamentarium of most pathology departments.

Given that the LI and MSI status of a tumor seems to affect cancer recurrence, these tests should, at a minimum, be more widely used and reported. The presence of lymphocytic infiltration should be routinely included in pathologic reports of colorectal cancer. These variables should be considered in the design of clinical studies evaluating the effect of chemotherapy in patients with colorectal malignancies. This may be of particular importance when studying adjuvant therapy in the early-stage population.

Table 2. Five-Year Disease-Free Survival of Patients According to Microsatellite Instability (MSI) Status and the Presence of Lymphocytic Infiltrate (LI), Stratified by Tumor Grade and Stage of Disease

<table>
<thead>
<tr>
<th>Tumor grade</th>
<th>5-y Disease-Free Survival, Mean (SE), %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSI+/LI+</td>
</tr>
<tr>
<td>Well differentiated</td>
<td>NA</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>66.7 (27.2)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>100</td>
</tr>
</tbody>
</table>

Stage of disease:

<table>
<thead>
<tr>
<th>Stage</th>
<th>T stage 3 or 4</th>
<th>T stage 1 or 2</th>
<th>Presence of LI</th>
<th>High-frequency MSI</th>
<th>N stage 1 or 2</th>
<th>N stage 1 or 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>100</td>
<td>83.0 (12.7)</td>
<td>66.7 (27.2)</td>
<td>0.425 (0.128-1.414)</td>
<td>0.433 (0.198-0.946)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>83.3 (15.2)</td>
<td>66.7 (27.2)</td>
<td>0.425 (0.128-1.414)</td>
<td>0.433 (0.198-0.946)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>100</td>
<td>50.0 (35.4)</td>
<td>66.7 (27.2)</td>
<td>0.425 (0.128-1.414)</td>
<td>0.433 (0.198-0.946)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>100</td>
<td>63.4 (9.5)</td>
<td>66.7 (27.2)</td>
<td>0.425 (0.128-1.414)</td>
<td>0.433 (0.198-0.946)</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>88.9 (10.5)</td>
<td>75.4 (8.1)</td>
<td>66.7 (27.2)</td>
<td>0.425 (0.128-1.414)</td>
<td>0.433 (0.198-0.946)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Hazard Ratios for Disease Recurrence or Death According to Multivariate Analysis by Cox Regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-frequency MSI</td>
<td>0.433 (0.198-0.946)</td>
<td>.04</td>
</tr>
<tr>
<td>Presence of LI</td>
<td>0.425 (0.128-1.414)</td>
<td>.16</td>
</tr>
<tr>
<td>T stage 3 or 4</td>
<td>3.171 (1.401-7.175)</td>
<td>.006</td>
</tr>
<tr>
<td>N stage 1 or 2</td>
<td>1.567 (0.882-0.943)</td>
<td>.13</td>
</tr>
</tbody>
</table>

Abbreviations: LI+, LI present; LI−, LI absent; MSI+, high-frequency MSI; MSI−, microsatellite stable or low-frequency MSI; NA, not applicable.

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REFERENCES