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

Eugene Y. Chang, MD; Paul B. Dorsey, MS; Joseph Frankhouse, MD; Randall G. Lee, MD; Deb Walts, MSN; William Johnson, MD; George Anadiotis, DO; Nathalie Johnson, MD

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+/H11001 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+/H11001, 30 were MSI+/H11001/LI−, and 16 were MSI+/H11001/LI+/H11001. 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+/H11001 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+/H11001 and LI+/H11001 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 presence of LI (referred to as LI+) was categorized into 4 groups according to MSI and LI status. Time-to-event analyses of disease-free survival (DFS) and overall survival after surgery were performed between the MSI+/LI+ and MSI−/LI− groups. Kaplan-Meier survival estimates were plotted and stratified according to stage of disease, nodal involvement, and tumor grade. When comparing the survival estimates, the log-rank test was used. Multivariate analysis of survival according to MSI status, LI status, T stage, and N stage was performed using a Cox regression analysis. Statistical calculations were performed using a software program (SPSS 13; SPSS Inc, Chicago, Illinois).

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 patients 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 involvement, 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 differences 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.
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 an insufficient number of patients to detect the association 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 intra-tumoral 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.
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.

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Correspondence: Nathalie Johnson, MD, Legacy Health System, Surgical Associates, 1330 NW 22nd St, Ste 500, Portland, OR 97210 (njohnson@lhs.org).

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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>MSI+/LI+</th>
<th>MSI+/LI−</th>
<th>MSI−/LI+</th>
<th>MSI−/LI−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well differentiated</td>
<td>NA</td>
<td>83.0 (9.0)</td>
<td>83.3 (15.2)</td>
<td>59.3 (6.0)</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>100</td>
<td>63.6 (14.5)</td>
<td>50.0 (35.4)</td>
<td>44.4 (16.6)</td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>66.7 (27.2)</td>
<td>75.4 (8.1)</td>
<td>75.0 (15.3)</td>
<td>56.7 (5.4)</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 (0.882-0.433)</td>
<td>.13</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; LI, lymphocytic infiltrate; MSI, microsatellite instability.
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REFERENCES