Hypothesis: Microsatellite instability (MSI) correlates with clinicopathologic characteristics and long-term prognosis in patients having gastric carcinoma.

Design: Analysis of prospectively collected data and biologic material.

Setting: Tertiary University Hospital, Policlinico “Le Scotte,” Siena, Italy.

Patients: Two hundred fifty patients with gastric carcinoma.

Main Outcome Measures: Five mononucleotide repeats (BAT-26, BAT-25, NR-24, NR-21, and NR-27) were analyzed in these patients.

Results: An MSI phenotype was identified in 63 patients (25.2%) and correlated with specific clinicopathologic characteristics. Favorable prognosis was confirmed for patients with an MSI phenotype in univariate (P < .001) and multivariate (P = .05) analyses. Significant differences in clinicopathologic characteristics and long-term prognoses were observed among patients with microsatellite-stable tumors, tumors having instability at 2 to 4 markers, and tumors having instability at all 5 markers (MSI/5). The MSI/5 phenotype was associated with older age (P < .001), female sex (P = .001), antral tumor location (P = .04), intestinal histotype (P = .003), and less infiltration of the serosa (P = .006); lymph node involvement was rare (P < .001) and was limited to few (median, 3) metastatic lymph nodes (P = .001). Long-term survival of patients with the MSI/5 phenotype is favorable and was confirmed in multivariate analysis (relative risk vs patients with stable tumors, 0.32; 95% confidence interval, 0.16-0.63; P = .002).

Conclusions: Compared with stable tumors, MSI tumors have distinct clinicopathologic features and are associated with a better prognosis. Patients with the MSI/5 phenotype have a very good prognosis.

Arch Surg. 2009;144(8):722-727
tal, Policlinico “Le Scotte,” University of Siena, Siena, Italy, between January 1, 1990, and December 31, 2004. All patients underwent resection, including 183 patients with potentially curative (R0) tumors and 35 patients with microscopic (R1) and 32 patients with macroscopic (R2) residual tumors; 20 patients (17 with R1 and R2 residual tumors) had metastatic disease at the time of operation. Clinico-pathologic and follow-up data were prospectively collected for the whole cohort. The median follow-up period for surviving patients was 92.1 months (range, 36.3-260.9 months).

Informed consent was obtained from all patients. The study was approved by the appropriate local ethics committees.

SURGERY

The objective of surgery was complete resection of the tumor, although gastrectomy was performed even in patients with untreatable disease when required for symptom palliation. Gastric resection was performed based on the extent of tumor in the stomach. Distal subtotal gastrectomy was preferred for tumors located in the lower and middle thirds of the stomach provided that the proximal resection margin remained at least 5 cm from the tumor edge; otherwise, total gastrectomy was performed. Extended (D2) and superextended (D3) lymphadenectomy (in 157 patients [62.8%]) was performed in most R0 resections and has been routinely used since 1995, except for patients deemed unfit for this procedure. None of the patients received preoperative chemotherapy or chemoradiotherapy.

HISTOPATHOLOGIC CLASSIFICATION AND STAGING

Tumor size was calculated by measuring the largest diameter of the neoplasm. The most recent version of the TNM classification by the American Joint Committee on Cancer and the International Union Against Cancer was used for histopathologic staging. Level and number of metastatic lymph nodes were also recorded for each patient. Histologic classification followed the criteria by Lauren; mixed-type tumors were considered together with diffuse (nonintestinal) type. Lymphatic and vascular invasion was assessed for 176 and 184 patients, respectively.

SAMPLES AND DNA EXTRACTION

Frozen samples of normal mucosa and tumor tissue were available for each patient in our biologic material bank. Tumoral mucosa and corresponding normal mucosa of the stomach were flash-frozen in liquid nitrogen. Tumoral and constitutional DNA were extracted after histopathologic confirmation using a commercial DNA purification kit (Puregene; Gentra Systems, Inc, Minneapolis, Minnesota) and following the manufacturer’s instructions.

PENTAPLEX POLYMERASE CHAIN REACTION AND MICROSATELLITE ANALYSIS

Microsatellite analysis was evaluated using 5 quasimonomorphic mononucleotide repeats, namely, BAT-26, BAT-25, NR-24, NR-21, and NR-27. The 5’ antisense primer was labeled with a fluorescent dye using FAM for BAT-26 and NR-21, NED for BAT-25 and NR-27, and VIC for NR-24 (ABI PRISM Primer Pairs; Applied Biosystems, Foster City, California).

The 5 mononucleotide repeats were coamplified in one multiplex (pentaplex) polymerase chain reaction (PCR) using the protocol for amplification of microsatellite loci (Multiplex PCR; Qiagen, Studio City, California). The pentaplex PCR was performed on tumor and matched constitutional DNA of the same patient with GC. The allelic profiles of these 5 mononucleotides were detected on an automated DNA sequencer (ABI PRISM 3100 Genetic Analyzer, Applied Biosystems) according to the manufacturer’s protocol (Figure 1).

SCORING OF MSI

According to the definition of the National Cancer Institute workshop on MSI for cancer detection and familial predisposition, we considered a tumor as having MSI whenever 2 or more markers showed instability on 5 loci. The clinicopathologic characteristics and survival rates associated with tumors having low instability (one locus involved) are similar to those of stable tumors and were considered together (microsatellite-stable [MSS] phenotype). DNA samples showing an abnormal allelic shift were confirmed by a second PCR and gene scan.

STATISTICAL ANALYSIS

Analyses were performed using commercially available statistical software (SPSS 14.0 for Windows; SPSS Inc, Chicago, Illinois). Statistical associations between clinicopathologic characteristics and MSI status were assessed by χ² test for categorical variables and by t test or analysis of variance for continuous variables. Survival curves were estimated using the Kaplan-Meier method and were compared using log-rank test. Multivariate analysis was performed using a Cox proportional hazards regression model by considering the following risk factors: sex, age (older vs the median or younger), tumor location (other vs antrum), Lauren histotype (nonintestinal vs intestinal), depth of tumor invasion (pT2, pT3, or pT4 vs pT1), lymph node involvement (pN1, pN2, or pN3 vs pN0), presence of systemic metastasis (M1 vs M0), and R category (R1 or R2 vs R0). Postoperative mortality was assessed, with deaths unrelated to tumor recurrence considered censored observations at the time of death. Because differences in clinicopathologic features and survival rates were observed within the MSI phenotype for tumors with instability at 2 to 4 markers (MSI/4) vs tumors with instability at all 5 markers (MSI/5), an analysis was performed...
Microsatellite instability is defined as the presence of replication errors resulting in insertions or deletions of bases within nucleotide repeats, known as microsatellite regions. Although a consensus panel for determination of MSI phenotype has been proposed and accepted for colorectal cancer, several markers with differing results have been used for determination of MSI in GC.\textsuperscript{14,19} Based on evaluation using several markers, the MSI phenotype generally characterizes 15% to 25% of patients with GC tumors, more frequently in older women.\textsuperscript{3,18} Most MSI tumors are of intestinal histotype located in the distal part of the stomach, with limited lymph node involvement. Our data regarding MSI phenotype are consistent with other findings reported to date.\textsuperscript{3,18}

Several observations of unexpectedly good prognoses in patients with advanced tumors and instability at all 5 markers led us to look at this subset of patients. The case of a 31-year-old woman who had undergone palliative sub-

\textbf{MSI AT ALL 5 MARKERS}

\textbf{Table 1} shows differences in clinicopathologic characteristics associated with the types of tumors. These include MSS, MSI/$<5$, and MSI/5 tumors.

The MSI/5 phenotype correlated with older age ($P < .001$), female sex ($P = .001$), antral tumor location ($P = .04$), and intestinal histotype ($P = .003$). Invasion of the serosa ($P = .006$) and lymph node metastasis ($P < .001$) were rarely observed. Among 22 patients having MSI/5 tumors with lymph node involvement, deposits were observed in few (median, 3; range, 1-8) metastatic lymph nodes ($P = .001$).

Lymphovascular MSI invasion was rare compared with that in MSS tumors ($P < .001$). Vascular invasion was observed in 33.3% of MSI/$<5$ tumors and in 14.8% of MSI/5 tumors ($P = .24$). Lymphatic invasion was equally frequent (33.3%) in MSI/$<5$ and MSI/5 tumors.

\textbf{Figure 2} shows Kaplan-Meier curves for patients with MSS, MSI/$<5$, and MSI/5 tumors ($P < .001$). Survival rates for patients with M0 disease who underwent potentially curative (R0) resections are shown in \textbf{Figure 4} ($P = .004$). Limiting the analysis to patients who underwent noncurative (R1 or R2) resections, the median (95% confidence interval) survival was 9.0 months (5.6-12.4 months) for 55 patients with MSS tumors, 5.2 months (2.1-8.3 months) for 7 patients with MSI/$<5$ tumors, and 58.4 months (0.0-132.5 months) for 5 patients with MSI/5 tumors ($P < .009$).

\textbf{Table 2} summarizes results of the multivariate analyses using Cox proportional hazards regression models controlling for other risk factors. The MSI/5 phenotype was confirmed to be an independent predictor of long-term survival (relative risk vs patients with the MSS phenotype, 0.32; 95% confidence interval, 0.16-0.63; $P = .002$).
total gastrectomy with macroscopic residual (R2) tumor 63 months previously for a nonintestinal pT4N2 tumor is still alive. The favorable survival in patients with the MSI/5 phenotype was also evident among patients who underwent palliative resection (median survival, 38.4 months; 95% confidence interval, 0.0-132.5 months).

### Table 1. Clinicopathologic Characteristics of the Study Cohort According to Microsatellite Instability (MSI) Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MSS (n=187)</th>
<th>MSI/≤5 (n=22)</th>
<th>MSI/5 (n=41)</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>66.7 (10.6)</td>
<td>69.3 (9.0)</td>
<td>74.7 (11.0)</td>
<td>&lt;.001 [.06]</td>
</tr>
<tr>
<td>Sex, No. (%):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>117 (62.6)</td>
<td>15 (68.2)</td>
<td>13 (31.7)</td>
<td>.001 [.008]</td>
</tr>
<tr>
<td>Female</td>
<td>70 (37.4)</td>
<td>7 (31.8)</td>
<td>28 (68.3)</td>
<td></td>
</tr>
<tr>
<td>Tumor location, No. (%):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>89 (47.6)</td>
<td>9 (40.9)</td>
<td>28 (68.3)</td>
<td>.04 [.06]</td>
</tr>
<tr>
<td>Other</td>
<td>98 (52.4)</td>
<td>13 (59.1)</td>
<td>13 (31.7)</td>
<td></td>
</tr>
<tr>
<td>Lauren12 histotype, No. (%):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>112 (59.9)</td>
<td>15 (68.2)</td>
<td>36 (87.8)</td>
<td>.003 [.09]</td>
</tr>
<tr>
<td>Nonintestinal</td>
<td>75 (40.1)</td>
<td>7 (31.8)</td>
<td>5 (12.2)</td>
<td></td>
</tr>
<tr>
<td>Tumor size, mean (SD), mm</td>
<td>57.8 (3.2)</td>
<td>60.8 (3.2)</td>
<td>57.9 (2.8)</td>
<td>.92 [.72]</td>
</tr>
<tr>
<td>Depth of tumor invasion, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1</td>
<td>14 (7.5)</td>
<td>1 (4.5)</td>
<td>2 (4.9)</td>
<td></td>
</tr>
<tr>
<td>pT2</td>
<td>60 (32.1)</td>
<td>10 (45.5)</td>
<td>26 (63.4)</td>
<td>.006 [.36]</td>
</tr>
<tr>
<td>pT3 or pT4</td>
<td>113 (60.4)</td>
<td>11 (50.0)</td>
<td>13 (31.7)</td>
<td></td>
</tr>
<tr>
<td>Lymph node involvement, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN0</td>
<td>45 (24.1)</td>
<td>8 (36.4)</td>
<td>19 (46.3)</td>
<td></td>
</tr>
<tr>
<td>pN1</td>
<td>60 (32.1)</td>
<td>6 (27.3)</td>
<td>21 (51.2)</td>
<td>&lt;.001 [.003]</td>
</tr>
<tr>
<td>pN2</td>
<td>48 (25.7)</td>
<td>6 (27.3)</td>
<td>1 (2.4)</td>
<td></td>
</tr>
<tr>
<td>pN3</td>
<td>34 (18.2)</td>
<td>2 (9.1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No., mean (SD)</td>
<td>9.4 (12.1)</td>
<td>6.9 (10.9)</td>
<td>1.7 (2.1)</td>
<td>.001 [.005]</td>
</tr>
<tr>
<td>Presence of metastasis, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>168 (89.8)</td>
<td>21 (95.5)</td>
<td>41 (100.0)</td>
<td>.08 [.35]</td>
</tr>
<tr>
<td>M1</td>
<td>19 (10.2)</td>
<td>1 (4.5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>R category, No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R0</td>
<td>132 (70.6)</td>
<td>15 (68.2)</td>
<td>36 (87.8)</td>
<td>.07 [.09]</td>
</tr>
<tr>
<td>R1 or R2</td>
<td>55 (29.4)</td>
<td>7 (31.8)</td>
<td>5 (12.2)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MSS, microsatellite-stable tumors; MSI/≤5, MSI tumors having instability at 2 to 4 markers; MSI/5, MSI tumors having instability at all 5 markers.

a Brackets indicate P value differences between MSI/≤5 and MSI/5 tumors.

Figure 3. Kaplan-Meier survival estimates according to microsatellite instability (MSI) in 250 patients who underwent gastrectomy. The median (95% confidence interval) survival was 26.7 (20.8-32.5) months for patients with microsatellite-stable (MSS) tumors and 18.5 (0.0-50.1) months for patients with MSI tumors having instability at 2 to 4 markers (MSI/≤5), and the median was beyond the observation period for patients with MSI tumors having instability at all 5 markers (MSI/5) (P=.001).

Figure 4. Kaplan-Meier survival estimates according to the number of microsatellite instability (MSI) markers in 180 patients with M0 disease who underwent potentially curative (R0) gastrectomy. The median (95% confidence interval) survival was 48.9 (31.0-86.8) months for patients with microsatellite-stable (MSS) tumors, and the median was beyond the observation period for patients with MSI tumors having instability at 2 to 4 markers (MSI/≤5) or with MSI tumors having instability at all 5 markers (MSI/5) (P=.004).
of patients with the MSI/5 phenotype was significantly better than that with MSI/5 tumors (Figures 3 and 4). Survival of patients class of tumors, one could argue for an aggressive sur-

lymph nodes. Considering the locoregional growth of this

with a similar growth pattern but with less propensity

were reported in MSI tumors, but they seem to

tics have been reported in MSI tumors, but they seem to

Older age, female sex, antral tumor location, intesti-

nodal histotype, less invasion of the serosa, and involve-

ment of fewer lymph nodes significantly correlated with

MSI/5 phenotype. The number of involved lymph nodes

was particularly low in patients having tumors with in-

stability at all 5 markers. Among 22 patients with MSI/5 pN+ disease, 21 had fewer than 6 positive lymph nodes, while the 31-year-old patient already mentioned had 8 positive lymph nodes.

Significantly lower rates of lymphovascular invasion were

consistently observed for MSI/5 and MSI/5 tumors, and this difference

had a median of 1.06 (0.74-1.52) vs 1.56 (0.69-38.79).

Risk

P Value

Age, y

> vs ≤ Median

Sex

Male vs female

Tumor location

Antrum vs other

Lauren11021 histotype

Intestinal vs nonintestinal

Depth of tumor invasion

pT2 vs pT1

pT3 or pT4 vs pT1

Lymph node involvement

pN1 vs pN0

pN2 vs pN0

pN3 vs pN0

Presence of metastasis

M1 vs M0

R category

R1 or R2 vs R0

MSI status

MSI/<5 vs MSS

MSI/5 vs MSS

Abbreviations: MSS, microsatellite-stable tumors; MSI/<5, MSI tumors having instability at 2 to 4 markers; MSI/5, MSI tumors having instability at all 5 markers.

A Relative risks and P values are derived from Cox proportional hazards regression analyses controlling for all other variables.

In conclusion, our data confirm previous evidence that
tumors with MSI demonstrate distinct clinicopathologic fea-
tures and better prognosis compared with patients having
stable tumors. A subset of tumors was observed with in-
stability at 5 quasimonomorphic mononucleotide repeats
(BAT-26, BAT-25, NR-24, NR-21, and NR-27). These tu-
mors have a low predilection to spread systemically or to
involve lymph nodes, and they are associated with favora-

able long-term prognosis even in patients with advanced
disease. Further studies among more patients are neces-
sary to confirm our results and to render them reproduc-
able and useful in clinical practice.

Accepted for Publication: May 28, 2008.

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Author Contributions: All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Corso, Pedrazzani, Pinto, and Roviello.

Acquisition of data: Marrelli. Analysis and interpretation of data: Corso, Pedrazzani, Marrelli, and Pascale.

Drafting of the manuscript: Corso, Pedrazzani, Marrelli, and Pascale.

Critical revision of the manuscript for important intellectual content: Pedrazzani, Pinto, and Roviello. Statistical analysis: Pedrazzani and Marrelli. Obtained funding: Pinto and Roviello. Administrative, technical, and material support: Corso, Marrelli, and Pascale. Study supervision: Roviello.

Financial Disclosure: None reported.

Funding/Support: This study was supported by grant 20050604011004PRIN (Progetti di Ricerca di Interesse Nazionale) 2005, “Fattori genetici ed eredofamiliari nel carcinoma gastrico,” from Ministero Università e Ricerca.

Additional Contributions: Lorenzo Garosi provided valuable help in data collection, material storage, and technical support.

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netic inactivation of MLH1 and outcome of patients with endometrial carcino-


tion of recurrence after radical surgery for gastric cancer: a scoring system ob-


Table 2. Relative Risks of Death From Gastric Carcinoma as a Function of Microsatellite Instability (MSI)4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Risk (95% Confidence Interval)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>&lt; Median vs ≤ Median</td>
<td>1.06 (0.74-1.52)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male vs female</td>
<td>1.22 (0.83-1.80)</td>
</tr>
<tr>
<td>Tumor location</td>
<td>Antrum vs other</td>
<td>0.85 (0.61-1.20)</td>
</tr>
<tr>
<td>Lauren11021 histotype</td>
<td>Intestinal vs nonintestinal</td>
<td>0.66 (0.45-0.98)</td>
</tr>
<tr>
<td>Depth of tumor invasion</td>
<td>pT2 vs pT1</td>
<td>5.16 (0.69-38.79)</td>
</tr>
<tr>
<td></td>
<td>pT3 or pT4 vs pT1</td>
<td>12.14 (1.61-91.23)</td>
</tr>
<tr>
<td>Lymph node involvement</td>
<td>pN1 vs pN0</td>
<td>2.40 (1.36-4.24)</td>
</tr>
<tr>
<td></td>
<td>pN2 vs pN0</td>
<td>3.68 (1.98-6.82)</td>
</tr>
<tr>
<td></td>
<td>pN3 vs pN0</td>
<td>3.67 (1.89-7.10)</td>
</tr>
<tr>
<td>Presence of metastasis</td>
<td>M1 vs M0</td>
<td>2.13 (1.22-3.72)</td>
</tr>
<tr>
<td>R category</td>
<td>R1 or R2 vs R0</td>
<td>2.37 (1.61-3.47)</td>
</tr>
<tr>
<td>MSI status</td>
<td>MSI/&lt;5 vs MSS</td>
<td>1.43 (0.79-2.59)</td>
</tr>
<tr>
<td></td>
<td>MSI/5 vs MSS</td>
<td>0.32 (0.16-0.63)</td>
</tr>
</tbody>
</table>


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The future in staging of cancer is molecular. Molecular markers will ultimately replace pathologic findings in our staging of gastrointestinal cancer. Corso et al from Italy show that genomic MSI in gastric adenocarcinoma is useful in determining the prognosis of patients. DNA from tumor and from adjacent normal tissue was amplified by pentaplex PCR, and DNA fragments were sequenced, which determined the presence of microsatellites.

DNA microsatellites are small (1-3 bp) repetitive DNA sequences, and instability results from the presence of these, which are absent from normal parental DNA. They are the result of mismatch repair gene mutations and have been implicated in hereditary nonpolyposis colorectal cancer; the present study confirms the utility of MSIs in gastric cancer as well. The authors showed that patients having tumors with all 5 MSI markers did better than those who did not.

Articles describing the use of molecular markers are being published frequently, and the names, functions, and utility of the markers are confusing. The first genetic investigations focused on mutations or deletions in host genomic DNA. A classic study by Shibata et al3 in 1996 demonstrated that patients with stage II colon cancer who harbored the deleted in colorectal carcinoma (DCC) gene mutation had prognoses similar to those of patients with stage III DCC-positive disease. In the Archives, I recently commented about the utility of cyclooxygenase 2 gene expression as a molecular predictor of the response of colon cancer to chemoradiotherapy.4

Newer molecular markers focus not on DNA mutations but on molecular changes in genetic expression. Epigenetics includes the study of DNA methylaton and of micro RNA inhibition of genetic expression.5 The former represents inhibition of tumor suppressor gene expression by changes in DNA methylation or enzymatic modification of histone proteins that lead to inhibited DNA repair, replication, and gene expression. The latter comprises inhibition of messenger RNA expression of tumor suppressor genes by small 22-bp endogenous anti-sense micro RNAs that destroy the message and inhibit expression of tumor suppressor genes. Each molecular marker is complicated to understand and requires extensive study to determine its utility.

Corso et al studied MSI in genomic DNA of gastric cancers and found that patients having tumors with 5 MSI markers have better survival than patients having tumors without MSI markers. This is potentially an important observation because it was true for lymph node–positive gastric cancers with MSI. It is critical to determine whether this is clinically important. One simple test would have been to determine whether survival by stage changes is based on MSI, as assessed by Shibata et al, but no TNM staging was presented in the current article. Similarly, Corso et al could have built on their finding that MSI was more frequent in older women with antral lesions. For example, it could have been determined whether younger men with MSI have survival similar to that of older women without MSI.

Molecular markers of cancer are the wave of the future. We need to determine which marker will be best for each type of cancer, and studies like this are important steps.