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# Predictive values of clinical and pathological parameters for malignancy of gastrointestinal stromal tumors

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**Summary.** Gastrointestinal stromal tumors (GISTs) possess a wide spectrum of biological properties, from indolent to highly aggressive. In this study, we evaluated a set of clinical and pathological parameters for their predicative values for malignancy of GISTs by retrospective reviews of tumor specimens and their relevant medical records from 840 patients. All GIST cases were first assigned as malignant if they met any of the following criteria: gross spreads, including liver metastassis and/or peritoneal dissemination, microscopic spreads, including lymph node metastasis, infiltrations to vascular, fat, nerve and muscularis mucosal tissues, or relapse. The remaining cases were recorded as biological behavior uncertain. This initial assignment revealed a set of five morphological features to be associated with malignancy. They were: mitotic counts greater than 10 per 50HPFs (P<0.0001), muscularis propria infiltration (P<0.0001), coagulative necrosis (P<0.0001), perivascular growth pattern (P=0.005), and severe nuclear atypia (P=0.014). Therefore, a new classification system, including criteria of 2 gross spreads, 5 microscopic spreads, and 5 histopathological parameters was developed. All the GIST cases were re-classified into a group of 485 malignant tumors, and a group of 355 nonmalignant tumors. Patient follow-up data revealed 5-year disease-free and overall survival rates as high as 99.3% and 100% for the nonmalignant group, but low rates of 43.9% and 59.7% for the malignant group. These results demonstrated a correlation of the new classification with clinical outcomes. Therefore, this set of 12 parameters has predictive values for malignancy of GISTs, and is potentially useful in the grading of the tumors.

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**Key words:** Gastrointestinal stromal tumor (GIST); Malignancy

## Introduction

Although gastrointestinal stromal tumors (GISTs) constitute only 1-3% of all gastrointestinal malignancies, they are the most common mesenchymal tumors of the gastrointestinal tract (Miettinen et al., 1998). They display a wide range of clinical and pathological features reflecting various biological behaviors. In general, bigger tumor size and elevated mitotic activities are associated with poor prognosis, and are also the basis of the consensus and most accepted criteria for risk stratification adopted by National Institutes of Health (NIH) (Fletcher et al., 2002). The NIH criteria categorize these tumors into four groups: very low, low, intermediate, and high risk groups.

Although high risk GISTs are generally interpreted as malignant, since neither a small tumor size nor a low mitotic activity can rule out malignancy, determination of the malignant potential of GISTs is still a clinical challenge (Shirin et al., 2007). This necessitates the development of a new classification system to identify less unambiguous parameters associated with aggressive behaviors. Many clinical and pathological parameters have been indicated to be useful in the prediction of aggressive behaviors in GISTs (Cooper et al., 1992; Haque and Dean, 1992; Ueyama et al., 1992; Tworek et al., 1999a,b; Nilsson et al., 2005; Takahashi et al., 2007), such as invasion, coagulative necrosis, liver metastasis and peritoneal dissemination. In this study, we re-evaluated the usefulness of these traditional clinical and pathological parameters in the prediction of the malignant potentials in GISTs based on their correlations with clinical outcomes, which identified a new set of parameters different from those used by the NIH criteria.

## Materials and methods

### *Tumor specimens*

Medical records and tissue specimens for 1155 primary mesenchymal tumors of the gastrointestinal (GI) tract were retrieved within certain clinical ranges from 12 hospitals in Shanghai. The hospitals and clinical ranges were Zhongshan Hospital, Fudan University, 1993-2006; Cancer Hospital, 1985-2002; Huashan Hospital, 1980-1999; Huadong Hospital, 1981-2000; Changning Centre Hospital, 1992-2002; Zhabei Centre Hospital, 1996-2003; Yangpu Center Hospital, 1990-2004; Chongming Centre Hospital, 1980-2001; Qinpu Centre hospital, 1971-2000; Putuo Centre hospital, 1983-2000; The Tenth People's Hospital, 1998-2003; and Gongli Hospital, 1996-2003. The 1155 cases were primary mesenchymal tumors previously characterized as leiomyoma, leiomyosarcoma, leiomyoblastoma, schwannoma, stromal or smooth muscle tumors originating from gastrointestinal (GI) tract.

All tumor specimens were reviewed by at least two experienced pathologists. Of these, 771 cases from surgeries were identified as GISTs based on their c-KIT positivity by immunohistochemistry, or their histopathologic features. GISTs identified by both methods similarly recognized spindle cells and epithelioid variants of tumors. Among the rest of the non-GIST cases, there were 247 smooth muscle tumors, 31 schwannomas, 4 neurilemomas, 17 fibromatoses, 16 inflammatory fibroid polyps, 28 lipomas, 1 liposarcoma, 18 miscellaneous tumors, and 22 unclassified tumors that were phenotypically indistinguishable and morphologically undifferentiated, as well as negative for c-KIT, CD34, markers of muscle cells and nerve cells. Another 69 tumor specimens were directly collected by surgical excision from patients in the clinical consultation files of Zhongshan Hospital from January 2005 to March 2007. The collection and use of the human tumor tissues were pre-approved by the institutional review boards of involved hospitals.

### *Parameters for evaluation*

Tumor specimens associated with patient demographics and clinical data were retrieved from their medical records. Parameters selected for analyses were the following: patient age (<50 years or >50 years) and gender, complaints and main symptoms, tumor size (<5 cm, >5 cm to <10 cm, and ≥ 10 cm), tumor site (stomach, duodenum, small intestine, rectum, and others including esophagus, colon, extra-GI tract, and unspecified), predominant growth pattern (sub-mucosa, intramural, outgrowth, and others including extra-GI as well as unspecified), as well as presence of ulceration, adhesion, tumor rupture, pedicle, liver metastasis, peritoneal dissemination, and type of surgical procedure, curative or palliative.

### *Histological evaluation*

A total of 1-27 hematoxylin and eosin (H&E) stained slides (with a median of 4 slides) were reviewed for each case, and were recorded for the following features: predominant cell type, pleomorphism, nuclear atypia, necrosis, perivascular growth pattern, mitotic count, and invasion.

A predominant cell type referred to the cell type constituting greater than 70% of total cells in the tumor specimen, including spindle cells, epithelioid cells or mixed cell type (Trupiano et al., 2002). The severity of pleomorphism of the tumor cells was defined by the degree of nuclear atypia that was classified by the criteria previously described (Yan et al., 2003) with the following specifications: no or mild pleomorphism indicated tumor cell nuclei showing little or no difference in size of nuclei from those of normal cells, having a diameter transversely equal to that of a lymphocyte; moderate pleomorphism indicated modest variation in the size of the nuclei, showing some nuclear enlargement to a size of approximately that of two lymphocytes; severe pleomorphism indicated extensive variation in the nuclear size, or significantly enlarged nuclei having a size transversely three times of a lymphocyte in diameter. The severity of nuclear atypia was further referred to as a focal or diffused pattern (Miettinen et al., 2005, 2006). A few cases of scattered focal severe atypia were combined into a group of moderate atypia for statistical analysis, and diffused severe nuclear atypia was classified in the severe group (Fig. 1). Necrosis was classified as coagulative necrosis identified by the existence of apoptotic or dead tumor cells (Yan et al., 2003) (Fig. 2). Perivascular growth pattern was seen where tumor cells packed and grew around vessels as perivascular collars (Miettinen et al., 2000b, 2005, 2006) (Fig. 3). Mitotic count is the total number of mitotically active cells identified from 50 consecutive high-power fields (HPF) at a magnification of 400x using an Olympus B x 41 microscope with 40x objective and 10x ocular lens (0.159 $mm^2$). All slides were first screened for the most proliferative areas prior to counting the mitoses. Then mitotic counts in 50 HPFs were recorded as <5/50 HPFs, or ≥ 5 to <10/50 HPFs, and ≥ 10/50 HPFs.

Tumor growth pattern inside the invaded tissues was examined under a light microscope and recorded by the method reported previously (Shiu et al., 1982; Koga et al., 1995; Trupiano et al., 2002; Fujimoto et al., 2003; Yan et al., 2003) with modifications. Muscular propria infiltration or "muscle infiltration" indicated the presence of tumor cells between smooth muscle fibers as tongue-like, nest-like or sheet structures, and the fibers were splayed or dissected by tumor cells (Tworek et al., 1999a,b) (Fig. 4). Mucosal infiltration was registered when tumor cells infiltrated inside the normal epithelial layers (Miettinen et al., 2000b, 2005, 2006). Invasion into fat (Miettinen et al., 2000a, 2006), nerve, or

vascular tissues, and tumor emboli (Cooper et al., 1992), and lymph node metastasis were all recorded. Pathological changes of the tumor stromal structures were recorded as cystic or hemorrhagic. Slides were independently reviewed by two experienced pathologists who did not have access to the patients' medical records and were unaware of the disease outcomes.

### *Immunohistochemical evaluations*

Immunohistochemical staining was performed based on a previously reported method (Hou et al., 2006). One representative formalin-fixed paraffin-embedded block from each case was sectioned and subjected to IHC staining with a panel of antibodies against CD117 (rabbit polyclonal anti-human c-KIT, diluted 1:150, Dako, Denmark), CD34 (mouse monoclonal antibody, clone QBEnd 10, diluted 1:200, Dako), SMA (mouse monoclonal antibody, clone 1A4, diluted 1:200, Dako), desmin (mouse monoclonal antibody, clone D33, diluted 1:200, Dako) and S-100 protein (polyclonal, diluted 1:300, Dako). The slides were first treated for antigen retrieval by the microwave method with 0.01 M citrate buffer, PH 6.0, and incubated overnight at 4°C. IHC detection was performed with EnVision–avidin-biotin based polymer system with a commercial kit (Dako). Diaminobenzidine was used as the chromogen, and all sides were counterstained with hematoxylin.

### *Patient follow-up information*

The follow-up information for patients after the surgery and treatment was provided by the referring pathologists and clinicians, or obtained from patients and their family members through direct contact by mailing and telephone calls.

### *Tumor classification and statistical analysis*

GISTs were first temporarily assigned as malignant when they displayed any of the following invasive behaviors: gross spreads, including liver metastasis and peritoneal dissemination, microscopic spreads to sites, including lymph nodes, vascular, fat and nerve tissues, as well as muscularis mucosal infiltration or tumor relapse. All other cases were regarded as biological behavior uncertain. Correlation of malignant status with clinicopathological parameters was analyzed by Chi square test. Those statistically significant variables identified by the univariate analysis were entered into a multivariate analysis with the binary logistic analysis.

Kaplan-Meier method was used to calculate disease-free survival (DFS) and overall survival (OS) rates for patients with GISTs in different groups. DFS measured

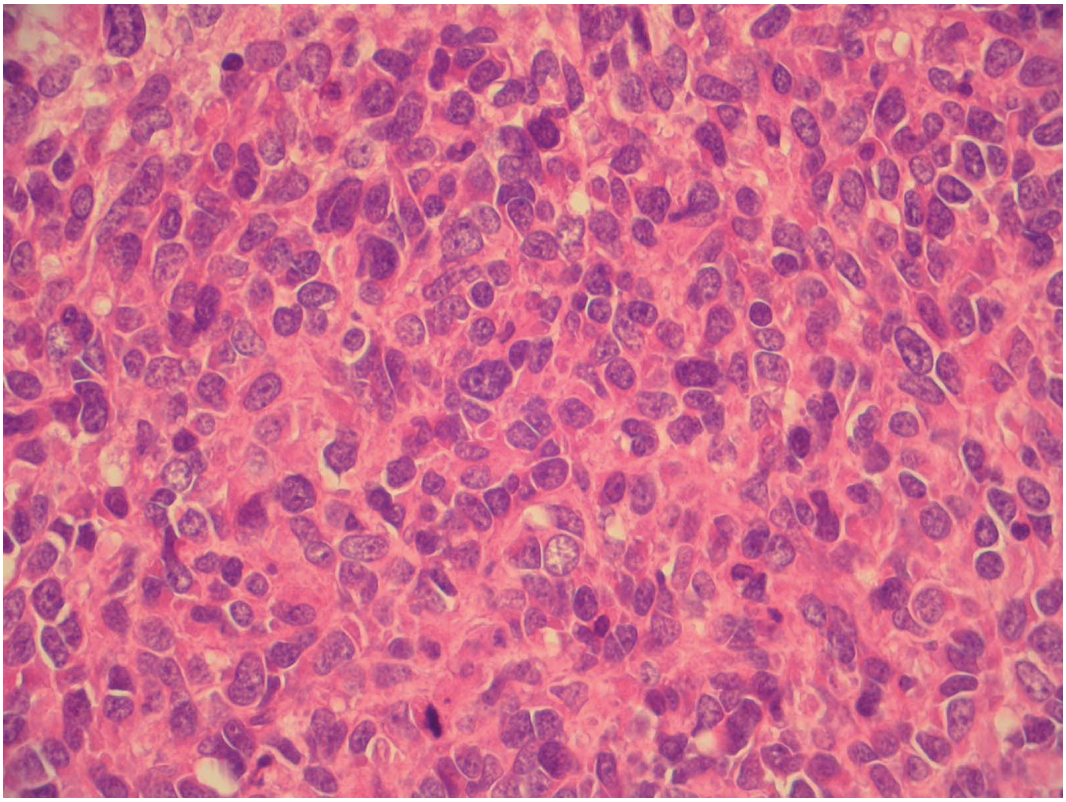

**Fig. 1.** Image of H&E stained GIST specimen showing severe nuclear atypia under a light microscope. x 400

the time from surgery to the time of first tumor recurrence or the most recent follow-up visit; OS measured the time from surgery to the time of patient death related to GIST. Among those, follow-ups for 181 patients with curative resection and 5 patients with palliative resection were completely lost due to unknown changes of residential addresses and telephone numbers; 24 patients who had been followed up for less than 1 year were not included in the survival analysis; of the 33 patients with palliative resections, 7 died of other diseases or accidents within 1 year and were not included in the DFS data. Therefore, a total of 590 patients were evaluated for DFS. Of those, patients with palliative resection were included, but patients with imatinib mesylate therapy were excluded from OS. In the end, 562 patients were evaluated for OS. Long-rank test of survival analysis was used to compare DFS and OS curves as the function of variables, and to identify significant difference.

Statistic analysis was carried out for all the data with SPSS 15.0 software (SPCC, Inc. Chicago, IL, USA). A value of $P<0.05$ was considered statistically significant.

## Results

### *Clinical and pathological features*

Table 1 lists the clinical and pathological features of GISTs from 840 patients reviewed in the study. The ages of the patients ranged from 17 to 89 years with a median of 58 and a mean of 57.4 years of age. There were 486 males and 354 females. At diagnosis, abdominal pains were presented in 146 patients, abdominal discomfort in 148 patients, palpable mass in 117 patients, and GI bleeding in 276 patients. At the time of operation, 17 patients developed liver metastases, 37 had peritoneal dissemination, and 10 patients had both. The tumor sizes ranged from 0.2 to 35 cm in diameter with a median of 6.0 cm and a mean of 7.17 cm. There were 447 gastric tumors, 51 duodenal tumors, 202 small intestinal tumors, 81 rectal tumors and 59 tumors at other primary sites (11 esophagus, 8 colon, 19 extra-GI tract, 19 at unspecified sites with local dissemination, and 2 at multiple sites). Among them, 569 tumors were predominantly intramural, 22 were chiefly submucosal, 206 were

**Table 1.** Univariate analysis of the clinical and pathological parameters used for the assumed classification of GIST malignancy.

| Parameters | Classification | Total (840) | Assumed malignancy | | *P* value |
|---|---|---|---|---|---|
| | | | no=269 | Rate=32.0% | |
| Sex | Male:female | 486:354 | 187:82 | 38.5: 23.2 | <0.0001 |
| Age (years) | <50: ≥ 50 | 230:610 | 100:169 | 43.5: 27.7 | <0.0001 |
| GI bleeding | Yes:no | 276:564 | 98:171 | 35.5: 30.3 | 0.130 |
| Abdominal discomfort | Yes:no | 148:692 | 45:224 | 30.4:32.4 | 0.642 |
| Abdominal pain | Yes:no | 146:694 | 63:206 | 43.2:29.7 | 0.002 |
| Palpable mass | Yes:no | 117:723 | 56:213 | 47.9:29.5 | <0.0001 |
| Ulceration | Yes:no | 245:595 | 106:163 | 43.3:27.4 | <0.0001 |
| Adhesion | Yes:no | 251:589 | 164:105 | 65.3:17.8 | <0.0001 |
| Tumor rupture | Yes:no | 40:800 | 31:238 | 77.5:29.8 | <0.0001 |
| Pedicle | Yes:no | 44:796 | 5:264 | 11.4:33.2 | 0.003 |
| Tumor size (cm) | <5: ≥ 5 to <10: ≥ 10 | 332:278:230 | 50:96:123 | 15.1:34.5:53.5 | <0.0001 |
| Tumor site | Stomach (yes:no) | 447:393 | 89:180 | 19.9:45.8 | <0.0001 |
| | Duodenum (yes:no) | 51:789 | 16:253 | 31.4:32.1 | 0.918 |
| | Small bowel (yes:no) | 202:638 | 102:167 | 50.5:26.2 | <0.0001 |
| | Anorectum (yes:no) | 81:759 | 33:236 | 40.7:31.1 | 0.077 |
| | Others (yes:no) | 59:781 | 29:240 | 49.2:30.7 | 0.003 |
| Growth pattern | Submucosa (yes:no) | 22:818 | 3:266 | 13.6:32.5 | 0.061 |
| | Transmural (yes:no) | 569:271 | 173:96 | 30.4:35.4 | 0.145 |
| | Subserosa (yes:no) | 206:634 | 66:203 | 32.0:32.0 | 0.996 |
| | Others (yes:no) | 43:797 | 27:242 | 62.8:30.4 | <0.0001 |
| Cell type | Spindle:Epithelioid:Mixed | 675:76:89 | 141:22:50 | 23.0:30.6:58.8 | <0.0001 |
| | Spindle (yes:no) | 675:165 | 192:77 | 28.4:46.7 | <0.0001 |
| | Epithelioid (yes:no) | 76:764 | 25:244 | 32.9:31.9 | 0.865 |
| | Mixed (yes:no) | 89:751 | 52:217 | 58.4:28.9 | <0.0001 |
| Nuclear atypia | Mild:Moderate:Severe | 159:482:199 | 2:152:115 | 1.3:31.5:57.8 | <0.0001 |
| Mitotic count (50HPFs) | <5: ≥ 5 to <10: ≥ 10 | 469:78:293 | 54:20:195 | 11.5:25.6:66.6 | <0.0001 |
| Muscle infiltration | Yes:no | 288:552 | 186:83 | 64.6:15.0 | <0.0001 |
| Coagulative necrosis | Yes:no | 229:611 | 157:112 | 68.6:18.3 | <0.0001 |
| Perivascular pattern | Yes:no | 170:670 | 117:152 | 68.8:22.7 | <0.0001 |
| Hemorrhagic change | Yes:no | 398:442 | 147:122 | 36.9:27.6 | 0.0001 |
| Cystic change | Yes:no | 420:420 | 151:118 | 36.0:28.1 | 0.015 |

Bold figures indicate correlation with malignancy; Italicised figures indicate correlation with nonmalignancy.

mainly outgrowth or loose attachment to the outer coats of the GI tract, and 43 had other patterns (18 extra-GI and 25 unspecified patterns). Adhesion to neighboring organs was recorded in 251 patients and tumor ruptures were observed in 40 patients.

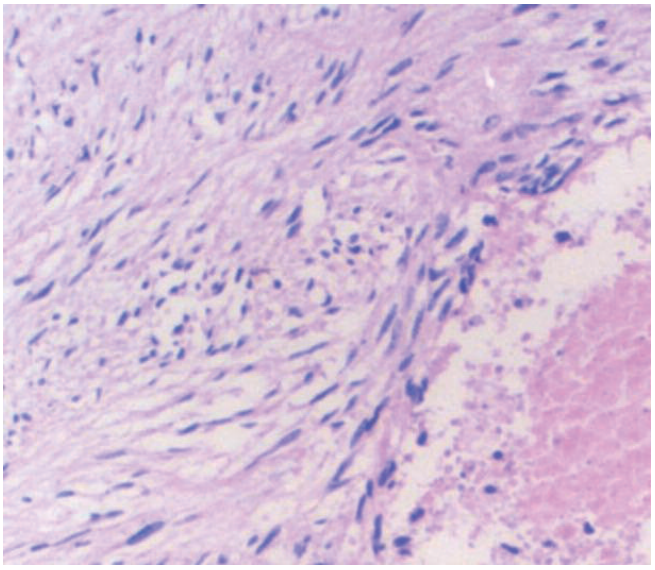

**Fig. 2. I**mage of H&E stained GIST specimen with coagulative necrosis showing ghost of dead tumor cells under a light microscope. x 200

The cell types in these tumors were spindle in 675, epithelioid in 76, and mixed in 89 cases. Mitotic activities were noted in 543 of 840 tumors, and the frequency of mitotic figures ranged from 0 to 410 per 50 HPFs with a median of 3 per 50 HPFs, and a mean of 17.8 per 50 HPFs. The frequencies of other morphological characters were summarized in Table 1.

Immunohistochemistry revealed that CD117, CD34, α-SMA, S-100 and desmin were positive in 95.2%, 80.0%, 28.9%, 18.0% and 0.4% of the 460 cases, respectively. All other 380 cases unambiguously identified by morphological features without immunohistochemical staining showed typical clinical and pathological features.

### *Analysis of clinical outcomes*

Among the 590 patients being followed up for disease free survival (DFS) and 562 for overall survival (OS), the DFS ranged from 0.22 to 18.63 years with a median value of 14.4 years and a mean value of 11.7 years of age. Values for OS ranged from 0.23 to 31.48 years, with a mean of 21.1 years, and a median was not available. Among all patients with follow-up information, there were 154 cases of tumor-related recurrence and 93 deaths. The 5-year DFS and OS rates

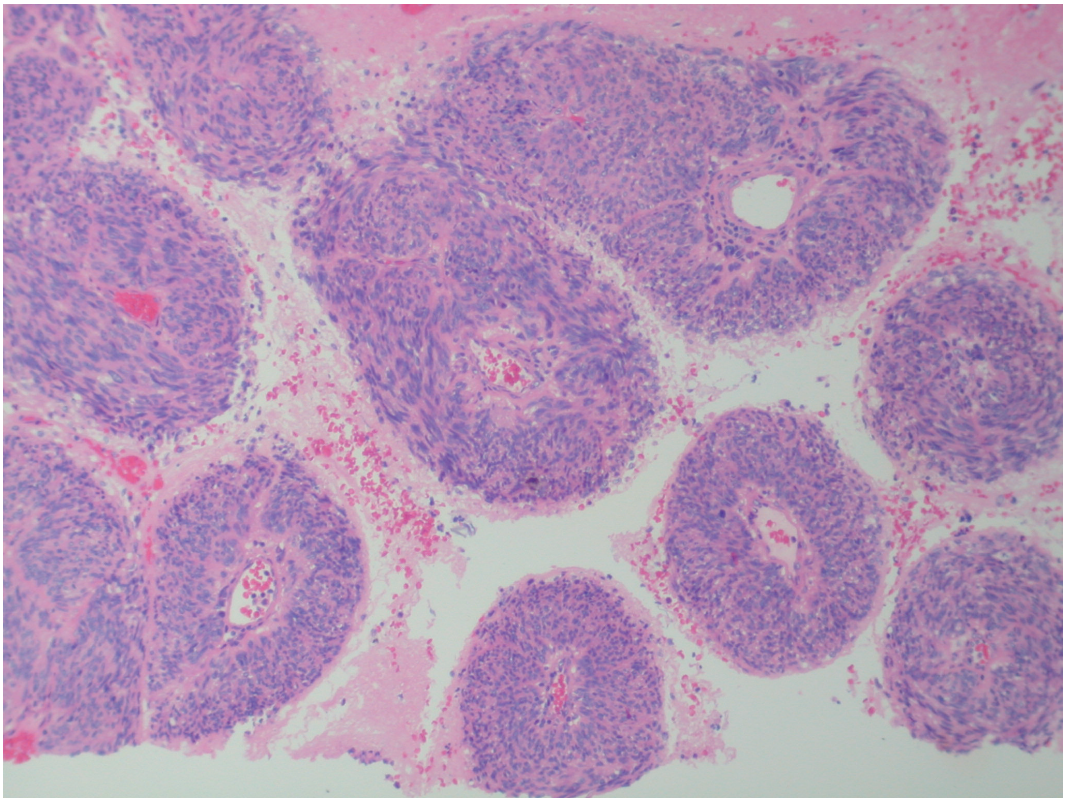

**Fig. 3.** Image of H&E stained GIST cells grown around vessels - recorded as perivascular growth pattern under a light microscope. x 200

were 71.2% and 80.9, respectively.

### *Correlations of clinical and pathological parameters with malignancy of GISTs*

First, based on the main features of tumor invasion and metastasis as shown by the two gross spreads, five microscopic spreads and tumor relapse, of the 840 GIST cases, 269 tumors were classified as malignant and 571 as biological behavior uncertain (Table 1). From the presumably malignant GISTs, a set of clinical and pathological parameters were identified, their frequencies were recorded and further analyzed by Univariate analysis in order to assess their predictive values for tumor malignancy. A set of 19 parameters were identified to be associated with malignancy (Table 1): male (P<0.0001), younger age (P<0.0001), abdominal pain (P=0.002), palpable mass (P<0.0001), ulceration (P<0.0001), adhesion (P<0.0001), tumor rupture (P=0.0001), tumor size ≥ 10cm (P<0.0001), smaller bowel (P<0.0001), metastasis to any site outside the primary lesion (P=0.003), other growth pattern (P<0.0001), tumor with mixed cell types (P<0.0001), severe nuclear atypia (P<0.0001), high mitotic count of ≥ 10 in 50 HPFs (P<0.0001), muscle infiltration (P<0.0001), coagulative necrosis (P<0.0001), perivascular growth pattern (P<0.0001), hemorrhagic change (P=0.0001) and cystic change (P=0.015). On the other hand, tumors with pedicle (P=0.003), gastric (P<0.0001) and spindle cell types (P<0.0001) were associated with nonmalignancy.

For the 19 parameters, multivariate analyses revealed the correlations of 6 clinical and 5 pathological parameters with malignancy (Table 2). Features of the 5 clinical parameters were being male (P=0.027), a younger age (P<0.0001), tumor adhesion (P<0.0001), tumor rupture (P=0.021), a small bowel (P=0.009), and another growth pattern (P=0.005); the pathological features were severe nuclear atypia (P=0.014), a high mitotic rate of ≥ 10 in 50 HPFs (P<0.0001), muscle infiltration (P<0.0001), coagulative necrosis (P<0.0001) and perivascular growth pattern (P=0.005). Since a tumor size ≥ 10 cm only presented a marginal correlation with malignancy (P=0.056), only the remaining 5 pathological features were deduced as morphological parameters in situ predictive of malignancy for further assessment.

### *Application of morphological parameters in situ predictive of malignancy*

Parameters used for our primary assumption and in situ morphological evaluation were combined into a new assessment system that employed a set of 12 criteria: 2 clinical spreads, 5 microscopic spreads, and 5 morphological parameters in situ, alone or in

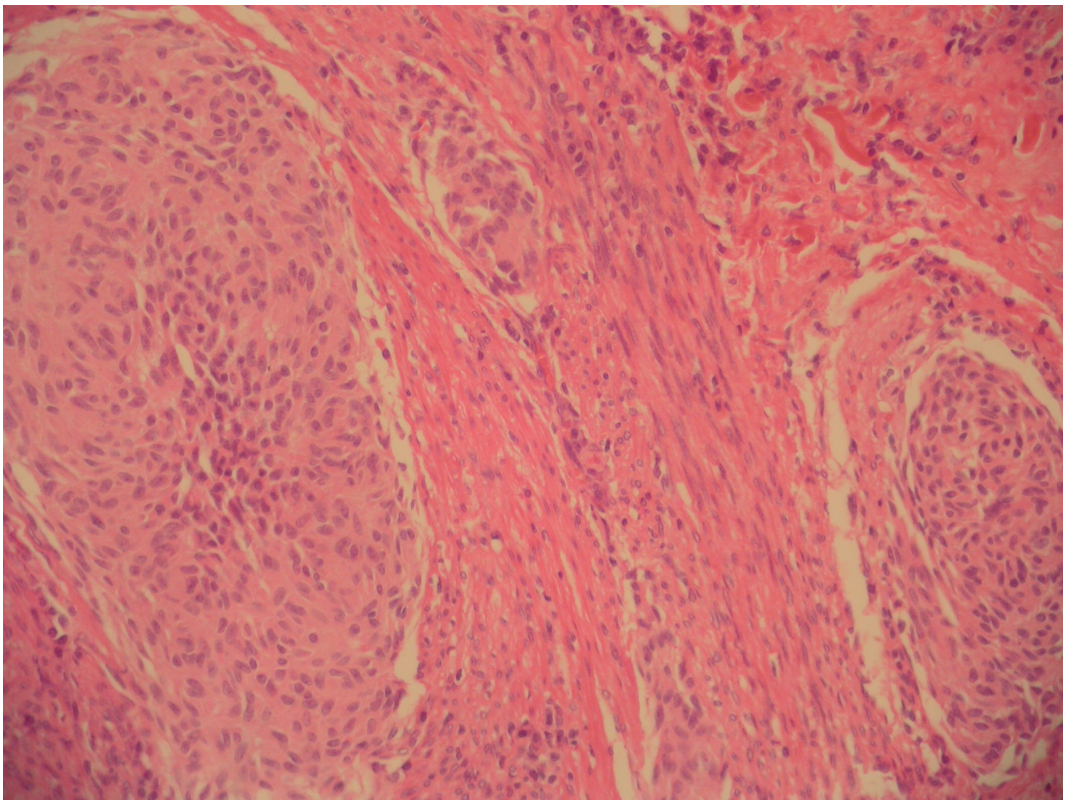

**Fig. 4.** Image of H&E stained GIST cells with invasion into normal muscularis propria displaying a nest-like structure under a light microscope. x 200

combination. To test this new system, the same set of GIST cases were re-categorized into one of two groups: a malignant group of 485 cases showing features of any of these 12 parameters, and a nonmalignant group of the remaining 355 cases. In the nonmalignant group, three patients had local tumor recurrence but survived disease free after a subsequent surgical removal of the secondary tumor without any additional treatment; therefore, these cases were not regarded as malignant. Based on these 12 parameters, patients in the nonmalignant group had higher rates of average 5-year DFS and OS, 99.3% and 100%, respectively; while those in the malignant group had DFS and OS rates of 43.9% and 59.7%, respectively (Table 3).

**Table 2.** Multivariate analysis of the clinical and pathological parameters used for the assumed classification of GIST malignancy.

| | | | Exp (B) and 95% CI | | | |
|---|---|---|---|---|---|---|
| | | Wald | Exp(B) | Lower | Upper | P value |
| Sex | Male:female | 4.9 | 0.6 | 0.4 | 0.9 | 0.027 |
| Age (years) | <50: ≥ 50 | 13.2 | 0.4 | 0.3 | 0.7 | <.0001 |
| Abdominal pain | Yes:no | 0.1 | 0.9 | 0.5 | 1.6 | 0.705 |
| Palpable mass | Yes:no | 0.4 | 1.2 | 0.6 | 2.3 | 0.544 |
| Ulceration | Yes:no | 0.6 | 0.8 | 0.5 | 1.4 | 0.448 |
| Adhesion | Yes:no | 22.6 | 0.3 | 0.2 | 0.5 | <.0001 |
| Tumor rupture | Yes:no | 5.3 | 0.3 | 0.1 | 0.8 | 0.021 |
| Tumor size (cm) | <5 | 1 | | | | |
| | ≥ 5 to <10 | 3.5 | 0.6 | 0.3 | 1.0 | 0.063 |
| | ≥ 10 | 3.6 | 0.5 | 0.2 | 1.0 | 0.056 |
| Tumor site | Small bowel (yes:no) | 6.7 | 0.5 | 0.3 | 0.8 | 0.009 |
| | Others (yes:no) | 0.8 | 1.8 | 0.5 | 6.0 | 0.361 |
| Growth pattern | Others(Yes:no) | 7.9 | 0.1 | 0.03 | 0.5 | 0.005 |
| Cell type | Mixed(Yes:no) | 0.1 | 0.9 | 0.4 | 1.7 | 0.714 |
| Nuclear atypia | Mild | 1 | | | | |
| | Moderate | 5.7 | 6.2 | 1.4 | 27.7 | 0.017 |
| | Severe | 6.0 | 7.0 | 1.5 | 33.4 | 0.014 |
| Mitotic count | <5 | 1 | | | | |
| (50HPFs) | ≥ 5 to <10 | 0.5 | 1.3 | 0.6 | 2.8 | 0.479 |
| | ≥ 10 | 26.3 | 3.8 | 2.3 | 6.4 | <.0001 |
| Muscle infiltration | Yes:no | 48.1 | 0.2 | 0.1 | 0.3 | <.0001 |
| Coagulative necrosis | Yes:no | 14.0 | 0.4 | 0.2 | 0.6 | <.0001 |
| Perivascular pattern | Yes:no | 7.9 | 0.5 | 0.3 | 0.8 | 0.005 |
| Hemorrhagic change | Yes:no | 2.0 | 1.6 | 0.9 | 3.0 | 0.158 |
| Cystic change | Yes:no | 0.6 | 1.3 | 0.7 | 2.4 | 0.458 |

Bold figures indicate close correlation with malignancy.

**Table 3.** Re-classification of the 840 GIST cases based on the 12 parameters: 2 gross spreads, 5 microscopic spreads, and 5 morphological parameters in situ.

| Reclassification | Frequency | Percentage (%) | Cases entering | Cumulative cases | | | | Remaining cases | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | no | Percentage (%) | 5-year DFS (%) | 5-year OS (%) | no | 5-year DFS (%) | 5-year OS (%) |
| Liver metastases | 27 | 3.2 | 27 | 27 | 3.2 | 25.0 | 9.7 | 813 | 71.6 | 83.2 |
| Peritoneal dissemination | 47 | 7.6 | 37 | 64 | 7.6 | 6.3 | 8.2 | 776 | 73.2 | 86.0 |
| Lymph node metastasis | 6 | 0.7 | 2 | 66 | 7.9 | 5.9 | 11.2 | 774 | 73.3 | 86.0 |
| Vascular infiltration | 46 | 5.5 | 41 | 107 | 12.7 | 14.4 | 28.5 | 733 | 76.9 | 86.6 |
| Fatty infiltration | 18 | 2.1 | 9 | 116 | 13.8 | 16.5 | 30.7 | 724 | 77.4 | 86.8 |
| Nerve infiltration | 23 | 2.7 | 11 | 127 | 15.1 | 18.1 | 33.8 | 713 | 78.0 | 87.1 |
| Mucosal infiltration | 86 | 10.2 | 61 | 188 | 22.4 | 28.5 | 46.1 | 652 | 80.8 | 88.2 |
| Mitoses ≥ 10/50HPF | 293 | 34.9 | 159 | 347 | 41.3 | 30.6 | 46.4 | 493 | 94.4 | 97.7 |
| Muscle infiltration | 288 | 34.3 | 66 | 413 | 49.2 | 38.6 | 55.7 | 427 | 96.0 | 97.4 |
| Coagulative necrosis | 229 | 27.3 | 30 | 443 | 52.7 | 39.4 | 55.7 | 397 | 98.4 | 99.5 |
| Perivascular pattern | 170 | 20.2 | 10 | 453 | 53.9 | 39.8 | 56.5 | 387 | 99.0 | 99.4 |
| Severe nuclear atypia | 199 | 23.7 | 32 | 485 | 57.7 | 43.9 | 59.7 | 355 | 99.3 | 100.0 |

**Table 4.** Classification of GIST cases from patients in the 12 hospitals and clinical consultation according to the reverse order of the 12 parameters.

| Classification process | Frequency | Cases entering | The Cumulative cases | | | | The left cases | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | no | Rate | 5-year DFS (%) | 5-year OS (%) | no | 5-year DFS (%) | 5-year OS (%) |
| Severe nuclear atypia | 199 | 199 | 199 | 41.0 | 35.5 | 49.2 | 641 | 80.1 | 88.0 |
| Perivascular pattern | 170 | 90 | 289 | 59.6 | 37.4 | 51.8 | 551 | 85.8 | 92.8 |
| Coagulative necrosis | 229 | 67 | 356 | 73.4 | 37.5 | 51.5 | 484 | 91.3 | 96.9 |
| Muscle infiltration | 288 | 87 | 443 | 91.3 | 41.7 | 57.7 | 397 | 98.0 | 99.6 |
| Mitotic counts ≥ 10/50HPFs | 293 | 36 | 479 | 98.8 | 43.2 | 59.3 | 361 | 99.4 | 100.0 |
| 5 microscopic spreads | 179 | 6 | 485 | 100.0 | / | / | 355 | / | / |
| 2 gross spread | 74 | 0 | 485 | 100.0 | / | / | 355 | / | / |

**Table 5.** Distribution and disease outcomes of 840 GIST cases based on our classification and the NIH consensus stratification systems.

| NIH criteria | Nonmalignant* | Malignant* | Disease free survival | | | Overall survival | | |
|---|---|---|---|---|---|---|---|---|
| | | | No1 | No2 | Rate (%) | No1 | No2 | Rate (%) |
| Very low | 84 | 4 | 81 | 5 | 6.2 | 79 | 0 | 0 |
| Low | 169 | 48 | 179 | 10 | 5.6 | 177 | 3 | 1.7 |
| Intermediate | 71 | 87 | 97 | 14 | 14.4 | 93 | 5 | 5.4 |
| High | 31 | 346 | 233 | 125 | 53.3 | 213 | 85 | 39.9 |
| Total | 355 | 485 | 590 | 154 | 26.1 | 562 | 93 | 16.5 |

No1: number of patients with follow-up information; No2: number of patients with recurrence or death; *: these are classifications based on the 12 parameters described in the paper.

The classification process revealed two important findings. First, application of the 2 gross spreads and 5 microscopic spreads alone resulted in a malignant group of only 188 (22.4%) cases. Despite the significant differences in the rates of DFS and OS between the malignant and the remaining groups (P<0.0001), there were still 81 recurrences and 40 deaths in the remaining group. However, after the 5 morphologic parameters in situ were applied, there were overall 479 (98.8%) of malignant cases identified and only 6 cases were left, and DFS and OS rates were 43.2% and 59.3%, respectively, in the malignant group, and 99.4% and 100%, respectively, in the remaining group (Table 4). Therefore, only after the addition of 5 morphological parameters in situ, did the outcomes as indicated by DFS and OS in the re-classified malignant group significantly diverge from the nonmalignant group.

### *Correlations of disease outcome with malignancy or risk with different classification systems*

When the same pool of GIST cases was classified into different risk groups according to the NIH consensus risk-group stratification system, 88 cases were classified into a very-low-risk group, 217 in a low-risk group, 158 in an intermediate-risk group, and 377 in a high-risk group. The distributions of all GIST cases by the NIH system and with our malignant and nonmalignant classification are shown in Table 5. Analysis of disease outcomes revealed 125 recurrences and 85 deaths in high-risk groups, 5 relapses (4 local recurrences and 1 liver metastases) in the very-low-risk group, 10 relapses (5 local recurrences, 1 peritoneal dissemination and 4 liver metastases) and 3 deaths in the low-risk group, 14 relapses (peritoneal dissemination and/or liver metastases) and 5 deaths in the intermediate-risk group. These results indicated that the low risk groups by NIH standard did not show uniformly favorable outcomes; instead, they included a certain number of malignant cases. Furthermore, both the nonmalignant and malignant GISTs classified by our criteria were distributed in all of the risk levels by the NIH criteria (Table 5).

## Discussion

Since 2002 there have been two dichotomous approaches to determine the malignancy of GISTs. The first one is to stratify all GISTs under the generic risk levels without differentiating tumor natures. This approach, although popular, often categorises true benign and borderline GISTs into different risk levels. The second approach differentiates GISTs into benign or non-benign ones (Trupiano et al., 2002; Takahashi et al., 2007), which was the approach adopted in this study.

We approached this problem based on the hallmarks of malignant tumors proposed by Hanahan and Weinberg (Hanahan and Weinberg, 2000): immortality, abnormal growth regulation, self-sufficient growth, escapes from apoptosis, sustained angiogenesis, invasion and

metastasis. We focused on invasion and metastasis as the primary classification criteria to define malignancy (Eccles and Welch, 2007) since they can be objectively examined under a light microscope and by a gross observation. Parameters indicative of tumor invasion and metastasis include gross spreading to liver and peritoneal and microscopic spreading to five other sites. Among them, two gross spreads, liver metastasis and peritoneal dissemination, are highly objective, and so are the most reliable (Miettinen, 1988; Cooper et al., 1992; Ueyama et al., 1992). Based on this, GISTs originating in the muscularis propria but extending outside their origin at the time of diagnosis was also regarded as malignant. The five microscopic spreads are considered less objective. Another five histological features in situ identified by multivariate analysis associated with malignancy were deduced based on the gross and microscopic spreads. It is interesting that the deduced parameters, or the histological features in situ, were more commonly present in malignant tumors, thus should be more useful than objective parameters.

Objective parameters, such as gross and microscopic evidence of tumor spreading, are easier to recognized and be agreed upon among observers. However, gross and microscopic spreads only exist in one third of patients with malignant tumors (Table 3). Since the majority of malignant GISTs were identified by the histopathological features in situ in our analysis (Table 4), it is important to pay more attention and properly use them in routine diagnosis.

Firstly, it is known that mitotic index is the most critical parameter to determine malignancy in the prognosis of GISTs (Miettinen, 1988), but its threshold values vary from case to case. High mitotic activity is an indication of active growth and tumor malignancy. The threshold for mitotic count has been set at one or more mitotic figures per 10 HPFs (Shiu et al., 1982; Evans, 1985; McGrath et al., 1987; Carrillo et al., 1997; Fujimoto et al., 2003), or over five mitoses per 50 HPFs (Ueyama et al., 1992), or per 10 HPFs (Miettinen et al., 1995). In rare instances, the threshold values are set at > 2/50 HPFs (Morgan et al., 1990; Goldblum and Appelman, 1995). Very recently, Huang et al. (2007) found only high mitotic activity (>10/50 HPFs) significantly correlated with survival. In our study, mitoses greater than 10/50 HPFs were fairly secure in predicting malignancy, but its converse conclusion may not hold. Cases with relatively few or no mitoses sometimes also manifested as typical malignant tumors; therefore, there should not be a rigid limit of mitotic counts for malignancy. Cases with lower levels of mitotic counts but showing evidence of other malignant features should still be confidently diagnosed as malignant GISTs.

Secondly, challenges and discrepancies exist in distinguishing muscularis propria infiltration and interdigitation. GISTs are not encapsulated; their cells and fibers often integrate with non-tumor cells at the periphery, or even entrap normal cells in the center of the tumor. The entrapped smooth muscle cells often grow in parallel with the tumor cells or occult that can be detected by α-SMA or desmin immunostaining. Infiltration should be noted only when tumor cells protrude destructively as a tongue-like sheet or nest into smooth muscle tissue, most of which present vertically with normal tissue and dissected muscularis propria fibers. Tworek and colleagues found that an infiltrative growth pattern in the muscularis propria correlated significantly with both metastasis and death from tumor (Tworek et al., 1999a,b). Based on their observations, muscle infiltration could be identified and applied in diagnosis.

Thirdly, coagulative necrosis has attracted much attention in recent years. Its many similar forms in tumors have not been described in detail. For example, superficial necrosis eroded by mucosal ulceration is not coagulative necrosis. Ulcers commonly leave deep necrotic zones within the tumor, and inflammatory necrosis exists in fistulous tract or in abscess formation. In these cases, many neutrophil debris and bacterial colonies can be found in the lesion, thus helping to differentiate them from coagulative necrosis. Infarction can be observed by the appearance of an inflammatory reaction band and foamy histocytes at the peripheral of necrosis, and sometimes thrombus can also be found in neighboring blood vessels. Liquefactive necrosis is very common and should not be mistaken as coagulative necrosis. It is formed by foci of cystic changes that can gather to form small or large cysts.

Fourthly, it is worth noticing the special structure of tumor cells grown around thin vessels. Miettinen and colleagues also observed this structure in GISTs (Miettinen et al., 2000b, 2002, 2006), and described it as "gastrointestinal stromal tumors with extensive degenerative changes with preserved tumor cells as perivascular collars" in esophageal GISTs (Miettinen et al., 2000b), and as "massive coagulative necrosis with preserved tumor cells as perivascular collars" in small intestinal GISTs (Miettinen et al., 2006). For the first time, this study identified its association with malignancy. Although further exploration is needed to obtain a mechanistic view, the correlation of such tumor growth pattern with malignancy may be due to the following features: (1) It is often accompanied by coagulative necrosis (Miettinen et al., 2002), a traditionally accepted parameter predictive of malignancy or adverse prognosis (Miettinen et al., 2002); (2) The structure may reflect the needs of tumor cells for nutrients and oxgen. It forms gradually with coagulative necrosis, and degeneration occurs in tumor cells far from the central vessel, possibly due the lack of nutrients; (3) It is also found in other malignant tumors, especially highly malignant ones such as hepatic cell carcinoma, melanoma, and Ewing sarcoma.

Fifthly, severe nuclear atypia or high pleomorphism has often been mentioned in earlier literature as a prognostic factor. A good amount of normal lymphocytes co-existing in the tumor tissue can serve as

the internal control to determine the size of the cells or nuclei and to minimize the possible discrepancies among pathologists.

Presently, only tumor size and mitotic count are adopted in the NIH consensus criteria (Fletcher et al., 2002). Royster found that tumor size became a less effective prognostic indicator when many more parameters had been integrated into multivariate analysis system (Royster, 2007). A similar result was obtained by our multivariate analysis. When more clinical and pathological parameters were entered into the analytic system, tumor size only presented marginal correlation with malignancy; therefore, it is less important than the histopathological parameters.

Based on the NIH consensus criteria, high risk GISTs are explained as malignant GISTs because they are associated with high rates of tumor-related deaths. However, patients in the very-low-risk, low-risk, and intermediate-risk groups also display significant incidences of tumor-related death (Hasegawa et al., 2002; Nakamura et al., 2005; Nilsson et al., 2005; Takahashi et al., 2007). For example, in the series reported by Hasegawa et al., some patients with small tumors and a few mitoses still showed recurrences/metastases after surgical removal (Hasegawa et al., 2002). In the report of Nakamura et al. (2005), in addition to seven high-grade GISTs, one low-grade (liver metastasis) and three intermediate-grade GISTs also showed gross spreads. We found that both nonmalignant and malignant GISTs were present in all risk groups from very low to high risk levels; it indicates that the NIH criteria based on tumor size and mitotic activity alone may be insufficient to delineate malignant from nonmalignant GISTs. Nonmalignant tumors of large size can be incorrectly classified into the high risk group, especially when the tumor develops part or complete cystic change, since cystic changes are not uncommon in GISTs; at the same time, malignant tumors with small and/or indolent mitotic activities but which present other parameters predictive of malignancy can be underestimated for malignancy.

In summary, we identified new clinical and pathological criteria predictive of malignancy for GISTs. They are a set of 12 parameters including 2 gross spreads, 5 microscopic spreads and 5 histological parameters in situ. Based on the new system, our evaluation on a large panel of GIST cases made three important findings: (1) Morphological parameters in situ are common in malignant cases and more closely reflect the tumor's biological nature, therefore, they may offer higher diagnostic values in differentiating tumor natures. (2) Perivascular growth pattern is associated with malignancy of GISTs. (3) Determination of GIST malignancy based solely on tumor size and mitosis are not sufficient. Our observations support the classification of GISTs into malignant and nonmalignant groups (Trupiano et al., 2002). Patients with nonmalignant GISTs can be mostly cured by surgical removal alone without additional therapy; on the other hand, those with malignant GISTs often show a high frequency of relapses, and usually require additional medical care and post-surgical treatment.

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## References

Carrillo R., Candia A., Rodriguez-Peralto J.L. and Caz V. (1997). Prognostic significance of DNA ploidy and proliferative index (mib-1 index) in gastrointestinal stromal tumors. Hum. Pathol. 28, 160-165.

Cooper P.N., Quirke P., Hardy G.J. and Dixon M.F. (1992). A flow cytometric, clinical, and histological study of stromal neoplasms of the gastrointestinal tract. Am. J. Surg. Pathol. 16, 163-170.

Eccles S.A. and Welch D.R. (2007). Recent discoveries and novel treatment strategies. Lancet 369, 1742-1757.

Evans H.L. (1985). Smooth muscle tumors of the gastrointestinal tract. A study of 56 cases followed for a minimum of 10 years. Cancer 56, 2242-2250.

Fletcher C.D., Berman J.J., Corless C., Gorstein F., Lasota J., Longley B.J., Miettinen M., O'Leary T.J., Remotti H., Rubin B.P., Shmookler B., Sobin L.H. and Weiss S.W. (2002). Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum. Pathol. 33, 459-465.

Fujimoto Y., Nakanishi Y., Yoshimura K. and Shimoda T. (2003). Clinicopathologic study of primary malignant gastrointestinal stromal tumor of the stomach, with special reference to prognostic factors: Analysis of results in 140 surgically resected patients. Gastric Cancer 6, 39-48.

Goldblum J.R. and Appelman H.D. (1995). Stromal tumors of the duodenum. A histologic and immunohistochemical study of 20 cases. Am. J. Surg. Pathol. 19, 71-80.

Hanahan D. and Weinberg R.A. (2000). The hallmarks of cancer. Cell 100, 57-70.

Haque S. and Dean P.J. (1992). Stromal neoplasms of the rectum and anal canal. Hum. Pathol. 23, 762-767.

Hasegawa T., Matsuno Y., Shimoda T. and Hirohashi S. (2002). Gastrointestinal stromal tumor: Consistent cd117 immunostaining for diagnosis, and prognostic classification based on tumor size and mib-1 grade. Hum. Pathol. 33, 669-676.

Hou Y.Y., Tan Y.S., Xu J.F., Wang X.N., Lu S.H., Ji Y., Wang J. and Zhu X.Z. (2006). Schwannoma of the gastrointestinal tract: A clinicopathological, immunohistochemical and ultrastructural study of 33 cases. Histopathology 48, 536-545.

Huang H.Y., Li C.F., Huang W.W., Hu T.H., Lin C.N., Uen Y.H., Hsiung C.Y. and Lu D. (2007). A modification of nih consensus criteria to better distinguish the highly lethal subset of primary localized gastrointestinal stromal tumors: A subdivision of the original high-risk group on the basis of outcome. Surgery 141, 748-756.

Koga H., Ochiai A., Nakanishi Y., Sasako M., Mizuno S., Kinoshita T.,

Maruyama K. and Hirohashi S. (1995). Reevaluation of prognostic factors in gastric leiomyosarcoma. Am. J. Gastroenterol. 90, 1307-1312.

McGrath P.C., Neifeld J.P., Lawrence W. Jr, Kay S., Horsley J.S. III and Parker G.A. (1987). Gastrointestinal sarcomas. Analysis of prognostic factors. Ann. Surg. 206, 706-710.

Miettinen M. (1988). Gastrointestinal stromal tumors. An immunohistochemical study of cellular differentiation. Am. J. Clin. Pathol. 89, 601-610.

Miettinen M., El Rifai W., Sobin H.L. and Lasota J. (2002). Evaluation of malignancy and prognosis of gastrointestinal stromal tumors: A review. Hum. Pathol. 33, 478-483.

Miettinen M., Makhlouf H., Sobin L.H. and Lasota J. (2006). Gastrointestinal stromal tumors of the jejunum and ileum: A clinicopathologic, immunohistochemical, and molecular genetic study of 906 cases before imatinib with long-term follow-up. Am. J. Surg. Pathol. 30, 477-489.

Miettinen M., Sarlomo-Rikala M. and Lasota J. (1998). Gastrointestinal stromal tumours. Ann. Chir Gynaecol. 87, 278-281.

Miettinen M., Sarlomo-Rikala M., Sobin L.H. and Lasota J. (2000a). Gastrointestinal stromal tumors and leiomyosarcomas in the colon: A clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases. Am. J. Surg. Pathol. 24, 1339-1352.

Miettinen M., Sarlomo-Rikala M., Sobin L.H. and Lasota J. (2000b). Esophageal stromal tumors: A clinicopathologic, immunohistochemical, and molecular genetic study of 17 cases and comparison with esophageal leiomyomas and leiomyosarcomas. Am. J. Surg. Pathol. 24, 211-222.

Miettinen M., Sobin L.H. and Lasota J. (2005). Gastrointestinal stromal tumors of the stomach: A clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. Am. J. Surg. Pathol. 29, 52-68.

Miettinen M., Virolainen M. and Maarit S.R. (1995). Gastrointestinal stromal tumors--value of cd34 antigen in their identification and separation from true leiomyomas and schwannomas. Am. J. Surg. Pathol. 19, 207-216.

Morgan B.K., Compton C., Talbert M., Gallagher W.J. and Wood W.C. (1990). Benign smooth muscle tumors of the gastrointestinal tract. A 24-year experience. Ann. Surg. 211, 63-66.

Nakamura N., Yamamoto H., Yao T., Oda Y., Nishiyama K., Imamura M., Yamada T., Nawata H. and Tsuneyoshi M. (2005). Prognostic significance of expressions of cell-cycle regulatory proteins in gastrointestinal stromal tumor and the relevance of the risk grade. Hum. Pathol. 36, 828-837.

Nilsson B., Bumming P., Meis-Kindblom J.M., Oden A., Dortok A., Gustavsson B., Sablinska K. and Kindblom L.G. (2005). Gastrointestinal stromal tumors: The incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era--a population-based study in western sweden. Cancer 103, 821-829.

Royster J.D. (2007). Agai technical review statements regarding gist are troubling. Gastroenterology 132, 822-823.

Shirin H., Kravtsov V., Shahmurov M., Sehayek S.V., Krinshpon I., Alin A., Avinoach I. and Avni Y. (2007). The cyclin-dependent kinase inhibitor, p27, has no correlation with the malignant potential of gist. Digestion 75, 4-9.

Shiu M.H., Farr G.H., Papachristou D.N. and Hajdu S.I. (1982). Myosarcomas of the stomach: Natural history, prognostic factors and management. Cancer 49, 177-187.

Takahashi T., Nakajima K., Nishitani A., Souma Y., Hirota S., Sawa Y. and Nishida T. (2007). An enhanced risk-group stratification system for more practical prognostication of clinically malignant gastrointestinal stromal tumors. Int. J. Clin. Oncol. 12, 369-374.

Trupiano J.K., Stewart R.E., Misick C., Appelman H.D. and Goldblum J.R. (2002). Gastric stromal tumors: A clinicopathologic study of 77 cases with correlation of features with nonaggressive and aggressive clinical behaviors. Am. J. Surg. Pathol. 26, 705-714.

Tworek J.A., Goldblum J.R., Weiss S.W., Greenson J.K. and Appelman H.D. (1999a). Stromal tumors of the abdominal colon: A clinicopathologic study of 20 cases. Am. J. Surg. Pathol. 23, 937-945.

Tworek J.A., Goldblum J.R., Weiss S.W., Greenson J.K. and Appelman H.D. (1999b). Stromal tumors of the anorectum: A clinicopathologic study of 22 cases. Am. J. Surg. Pathol. 23, 946-954.

Ueyama T., Guo K.J., Hashimoto H., Daimaru Y. and Enjoji M. (1992). A clinicopathologic and immunohistochemical study of gastrointestinal stromal tumors. Cancer 69, 947-955.

Yan H., Marchettini P., Acherman Y.I., Gething S.A., Brun E. and Sugarbaker P.H. (2003). Prognostic assessment of gastrointestinal stromal tumor. Am. J. Clin. Oncol. 26, 221-228.

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