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Original article

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

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Abstract

Background. **Malignant gastrointestinal stromal tumors (GISTs), previously termed leiomyosarcomas or epithelioid leiomyosarcomas, are known to show wide variability in their malignancy. We evaluated the clinicopathological features of a large number of primary malignant gastric GISTs to clarify which features were independent prognostic factors.**

Methods. **Clinicopathologic features (age, sex, tumor location, mode of growth and size, surgical method, ulceration, cell type, nuclear atypia, cellularity, mitotic index, growth pattern, necrosis, hemorrhage, direct tumor invasion, peripheral lymphoid cuffing, expression of α-smooth muscle actin [α-SMA], desmin, caldesmon, vimentin, CD34, c-kit protein and S-100 protein, and MIB-1 index) were evaluated by multivariate analysis in 140 patients with resected primary malignant gastric GISTs to identify independent prognostic factors.**

Results. **Univariate analysis showed that each of the following factors had a significant deleterious influence on prognosis: male sex, tumor size 10 cm or more, presence of ulceration, an epithelioid cell component, severe nuclear atypia, high cellularity, a mitotic index of more than 10, an exogastric or invasive growth pattern, necrosis, hemorrhage, direct tumor invasion of surrounding tissue, negative caldesmon immunoreactivity, positive S-100 protein immunoreactivity, and a MIB-1 antigen labeling index of more than 10%. Multivariate analysis showed that male sex, tumor size 10 cm or more, presence of an epithelioid cell component, and a mitotic index of more than 10 were statistically significant indicators of a poor prognosis (P = 0.013, 0.001, 0.014, and <0.001, respectively). Multivariate analysis using the MIB-1 index instead of a mitotic count showed that male sex, tumor size 10 cm or more, presence of necrosis, and a MIB-1 antigen labeling index of more than 10% were independent predictors of a poor prognosis (P = 0.009, 0.001, 0.043, and <0.001, respectively).**

Conclusion. **Male sex, tumor size 10 cm or more, and cell proliferation as estimated by the mitotic index or MIB-1 index are independent indicators of a poor prognosis in primary malignant gastric GIST.**

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Key words Malignant gastric GIST · Clinicopathologic study · Prognostic factor · Multivariate analysis · Immunohistochemistry

Introduction

The term "gastrointestinal stromal tumor (GIST)" is now used to describe a specific group of tumors which includes the majority of gastrointestinal mesenchymal tumors and encompasses most of the gastric and intestinal mesenchymal tumors previously known as leiomyomas, cellular leiomyomas, leiomyoblastomas, and leiomyosarcomas. Most of the tumors historically designated as leiomyosarcomas are now classified as GISTs; hence, the older literature on gastric and intestinal leiomyosarcomas largely pertains to malignant GISTs [1].

The advent of electron microscopy and immunohistochemistry has allowed the cellular origins of GISTs to be investigated, and the interstitial cell of Cajal, an intestinal pacemaker cell, or the mesenchymal stem cells of the gut have recently been proposed as candidates [2,3]. On ultrastructural examination, the Cajal cell has characteristics indicating both smooth muscular and neural differentiation; thus, neoplastic Cajal cells could preferentially express one, both, or neither of these features, and this would account for the variants of GIST [4,5]. In addition, CD34 and c-kit protein have been shown to be markers for the interstitial cell of Cajal [2,3,6]. The majority of GISTs are positive for CD34, a hematopoietic progenitor cell antigen, and are frequently marked by the presence of the c-kit proto-oncogene product, a transmembrane tyrosine kinase receptor protein [2,3,6,7].

Malignant GISTs (previously termed "leiomyosarcomas" or "epithelioid leiomyosarcomas") are known to show wide variability in their malignancy. Several

factors, such as a large tumor size, a high mitotic rate, and the presence of severe nuclear atypia, high cellularity, and tumor necrosis, have been correlated with a poor prognosis [8–17]. However, there have been no studies in which clinicopathological findings, including cell markers such as CD34 and c-kit, have been evaluated by multivariate analysis to identify further indicators of a poor prognosis. In the current study, of a large number of primary malignant gastric GISTs, we used multivariate analyses to evaluate their clinicopathological features, including the expression of Cajal cell markers, to clarify whether they were independent prognostic factors.

Patients and methods

Patients

Between 1962 and December 1999, 144 patients with a diagnosis of primary malignant gastric GIST (original diagnoses included 123 leiomyosarcomas, 7 leiomyoblastomas, and 14 epithelioid leiomyosarcomas, and excluded benign tumors such as leiomyomas, schwannomas, and neurofibromas) received their initial treatment at the National Cancer Center Hospital, Tokyo, Japan. The patients underwent surgery without prior radio- or chemotherapy. The present study included 140 patients (119 with leiomyosarcomas, 7 with leiomyoblastomas, and 14 with epithelioid leiomyosarcomas) whose records and histological specimens were available for review. In our series, malignant gastric GIST was defined as gastric GIST with at least one mitotic figure in the tumor cells.

Clinical findings

Age, sex, tumor location (upper [U], middle [M], or lower [L] third of the stomach; anterior or posterior wall [AW and PW, respectively]; lesser or greater curvature [LC and GC, respectively]); and surgical method (total, proximal, or distal gastrectomy, wedge resection, or enucleation) were checked in the surgical records. The occurrence of metastasis or local recurrence was determined from the clinical records. Mortality statistics for all patients were obtained from the follow-up records and the city registry office.

Macroscopic and microscopic findings

The mode of tumor growth (endogastric or exogastric), tumor size, and ulceration were checked using the resected specimens, all of which had been fixed in 10% formalin and embedded in paraffin. Tumor size was defined as the largest diameter in any dimension of the primary tumor and was stratified as less than 5 cm, 5 to 10 cm, or 10 cm or more.

Nine types of microscopic features were evaluated on hematoxylin-and-eosin-stained tissue sections containing the maximum tumor diameter. (1) Cell type: spindle, blastoma, or epithelioid (Fig. 1). The spindle-cell type is composed of elongated fusiform cells with

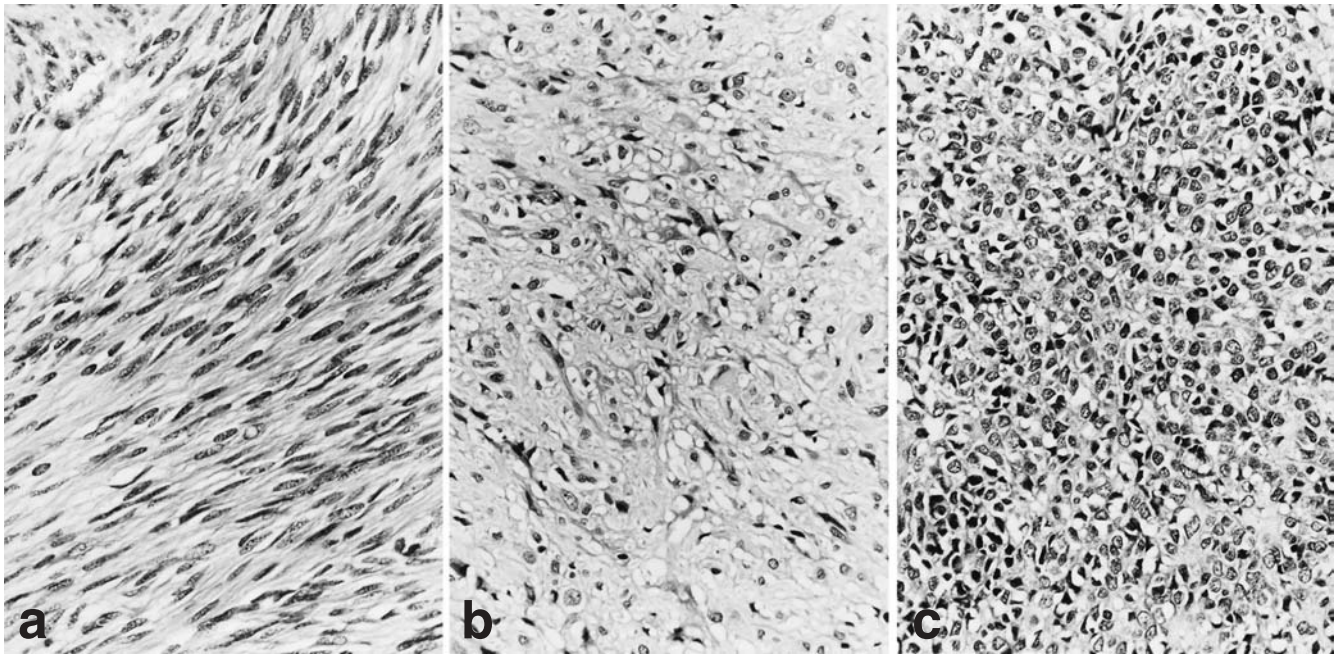

Fig. 1a–c. Histological features of the various cell types of gastric gastrointestinal stromal tumors (GISTs). **a** Spindle; **b** blastoma; **c** Epithelioid. All, H&E, ×100

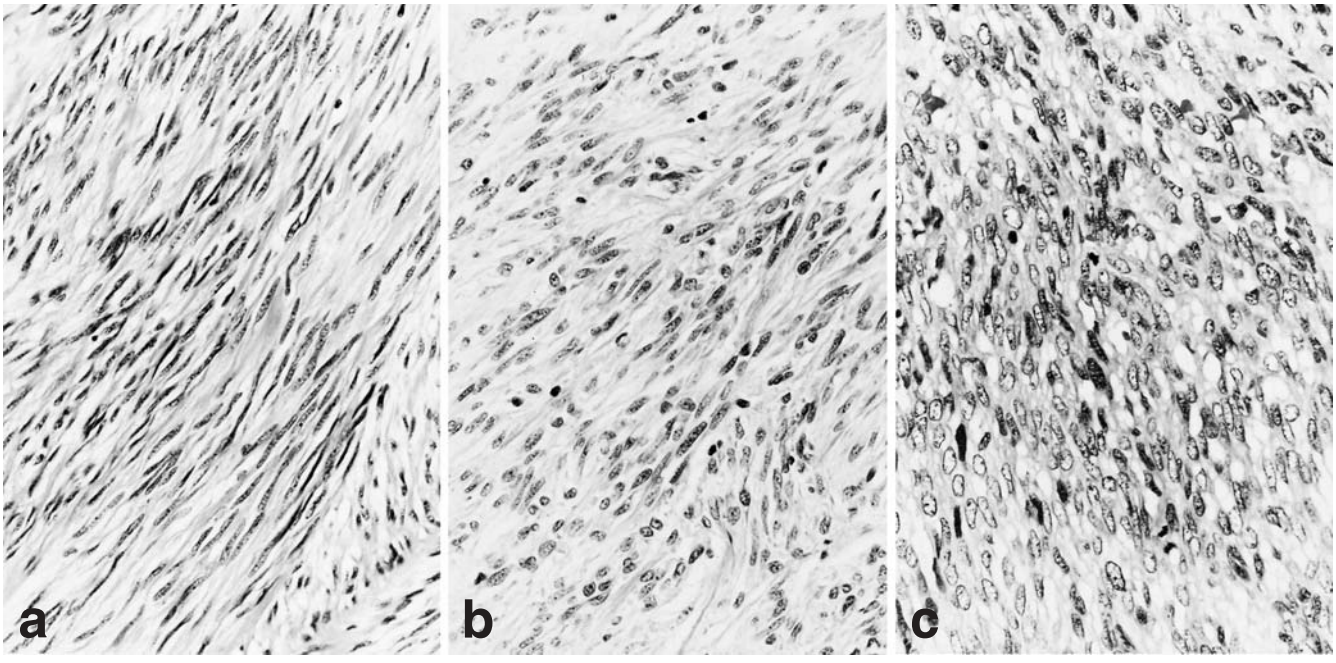

Fig. 2a–c. Degrees of nuclear atypia. **a** Mild; **b** moderate; **c** severe. All, H&E, ×100

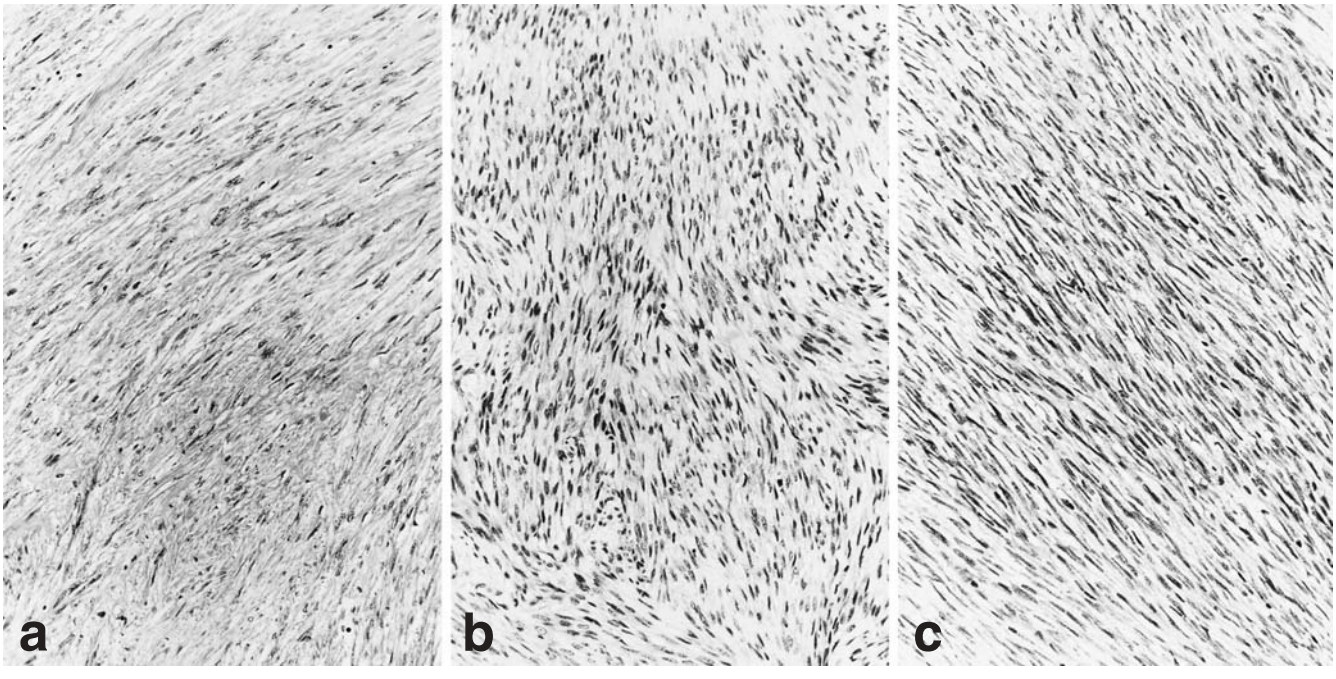

Fig. 3a–c. Degrees of cellularity. **a** Low; **b** moderate; **c** high. All, H&E, ×50

eosinophilic cytoplasm. The blastoma-cell type is composed of round or polygonal cells with only vague organization, and has either homogeneously clear, vacuolated, or eosinophilic cytoplasm. The epithelioid-cell type is composed of clusters and sheets of polygonal cells. (2) The degree of nuclear atypia: mild, moderate or severe (Fig. 2). Mild nuclear atypia shows elongated spindle nuclei with tapered ends. Moderate nuclear atypia shows round spindle nuclei. Severe nuclear atypia shows pleomorphic and hyperchromatic nuclei. (3) The degree of cellularity: low, moderate, or high (Fig. 3). The degree of cellularity is estimated by visual impression without counting the actual number of tumor cells. (4) Mitotic index: the average number of

mitotic cells in ten high-power fields (HPF; ×20 objective and ×10 ocular lenses) covering the most active areas, assessed using an Olympus BH-2 microscope (Olympus Optical, Tokyo, Japan). (5) Growth pattern: expansive or invasive. (6) The presence or absence of necrosis. (7) The presence or absence of hemorrhage on the cut surface. (8) The presence or absence of direct tumor invasion into the surrounding organs. (9) The presence or absence of lymphocytic infiltration around the tumor tissue (peripheral lymphoid cuffing).

Immunohistochemical findings

Representative tissue sections from each lesion were subjected to immunohistochemical staining using the avidin-biotinyl-peroxidase complex (ABC) method [18]. The target differentiation antigens visualized by the monoclonal and polyclonal antibodies were α-smooth muscle actin (α-SMA; clone 1A4, 1:100 dilution; Dako, Glostrup, Denmark), desmin (D33; 1:100; Dako), caldesmon (a heavy molecular weight marker for the development and maturation of smooth muscle cells [19]; h-CD; 1:100; Dako), vimentin (V9; 1:200; Dako), CD34 (My10; 1:100; Becton Dickinson, Mountain View, CA, USA), c-kit protein (polyclonal antiserum, 1:80; Immuno-Biochemical Laboratories, Fujioka, Japan), and S-100 protein (polyclonal antiserum; 1:2000; Dako). The presence of proliferative activity was investigated using MIB-1 antigen antibody (MIB-1; 1:100; Immunotech, Marseille Cedex, France). The selected sections were autoclaved (autoclaved for 10 min with heat-induced epitope retrieval in 10 mmol/l citrate buffer, pH 6.0) prior to reaction with the diluted caldesmon, CD34, c-kit protein, and MIB-1 antigen antibodies.

Positive reactions were classified using the following criteria: (−) negative, (+) 10% or more to less than 40%, (++) 40% or more to less than 70%, and (+++) 70% or more. Tumors were designated positive when more than 10% of the tumor cells showed a positive reaction. The internal positive controls used for the various immunoreactions were: smooth muscle cells for α-SMA, desmin, and caldesmon; schwann cells for S-100 protein; mesenchymal tissue cells (such as vessel wall or connective tissue) for vimentin; endothelial or perimuscular fibroblastic cells for CD34; and tissue mast cells or Cajal cells for c-kit protein. In addition, great care was taken to exclude nonneoplastic smooth muscle bundles, which were frequently entrapped within the tumor tissue and strongly expressed α-SMA and desmin, but not vimentin. Negative controls were run by substituting control mouse IgG1 at the same dilution as the active antibody. The GISTs were divided into four groups (smooth muscular type, neural type, combined smooth muscular-neural type, and uncommitted type), according to their immunoreactivity [4,20]. The tumor was defined as a smooth muscular type when it showed a positive reaction for the smooth muscle markers α-SMA and/or desmin, as a neural type when it showed a positive reaction for the neural marker S-100 protein, as a combined smooth muscular-neural type when it showed a positive reaction for both α-SMA and/or desmin and S-100 protein, and as an uncommitted type when no positive reaction for any of these markers was obtained.

Tumor cell nuclei that stained brown to dark brown were considered positive for MIB-1 antigen. Sections from a follicular hyperplasia of the tonsil were used as positive controls for MIB-1 antigen. To calculate the MIB-1 antigen labeling index, the number of positive nuclei among at least 500 tumor cells in the most active areas was counted and expressed as a percentage.

Statistical analysis

Patient survival rates were calculated using the Kaplan-Meier method, and statistically significant differences in survival were identified using the Log-Rank test. Patients who died of diseases other than GIST were treated as censored cases. Prognostic factors were analyzed using Cox's proportional hazards model. The level of significance was set at $P < 0.05$.

Results

The clinicopathological features of the patients and GISTs are summarized in Table 1.

Clinical findings

The patients' ages ranged from 28 to 78 years (mean, 59.8 years). There were 76 male and 64 female patients, and the male-to-female ratio was 1.2:1. The tumors tended to be located in the upper portion of the stomach, with 90 (64%), 35 (25%), and 15 (11%) tumors in the upper (U), middle (M), and lower (L) thirds, respectively. Thirty (21%) tumors presented in the anterior wall (AW), 40 (29%) in the posterior wall (PW), 43 (31%) in the lesser curvature (LC) and 27 (19%) in the greater curvature (GC) of the stomach, respectively. Surgical methods included 96 partial resections (95 wedge resections and 1 enucleation) and 44 gastrectomies (5 total, 18 proximal, and 21 distal). Partial resection was performed in 20 (48%) of the 42 patients who underwent surgery during the first 23 years of the study period (1962–1984), whereas this technique was used in 76 (78%) of the 98 patients treated during the last 15 years (1985–1999). Sixty-two patients (44%) underwent systematic or sampling lymph node dissection and no

Table 1. Clinicopathological features of the patients and gastrointestinal stromal tumors (GISTs)

Factors		$n = 140$
Age (years)	<60:≥60	59:81
Sex	Male:Female	76:64
Tumor location	U:M:L	90:35:15
	AW:PW:LC:GC	30:40:43:27
Surgical method	T:P:D:W:E	5:18:21:95:1
Mode of growth	Endogastric:exogastric	71:69
Tumor size (cm)	<5:≥5 to <10:≥10	88:30:22
Ulceration	(−):(+)	96:44
Cell type	Spindle:blastoma:epithelioid	119:7:14
	Spindle component, (−):(+)	12:128
	Blastoma component, (−):(+)	123:17
	Epithelioid component, (−):(+)	116:24
Nuclear atypia	Mild:moderate:severe	8:99:33
Cellularity	Low:moderate:high	12:60:68
Mitotic index	<10:≥10	118:22
Growth pattern	Expansive:invasive	107:33
Necrosis	(−):(+)	104:36
Hemorrhage	(−):(+)	53:87
Direct tumor invasion	(−):(+)	136:4
Lymphoid cuffing	(−):(+)	140:0

U, M, and L, Upper, middle, and lower third of the stomach, respectively; AW and PW, anterior and posterior walls, respectively; LC and GC, lesser and greater curvatures, respectively; T, P, and D, total, proximal, and distal gastrectomy, respectively; W, wedge resection; E, enucleation

lymph node metastasis was observed. None of the patients died during their hospital stay.

Macroscopic findings

Endogastric growth was observed in 71 (51%) patients, while an exogastric pattern was seen in 69 (49%). The tumor diameters ranged from 8 to 380mm (mean, 57.1mm). After stratification, 88 (63%) tumors measured less than 5cm, 30 (21%) measured 5 to 10cm, and 22 (16%) measured 10cm or more. Ulceration was present in 44 (31%) patients.

Microscopic findings

Cell type. Of the 140 tumors, 119 were classified as leiomyosarcomas, 7 as leiomyoblastomas, and 14 as epithelioid leiomyosarcomas, based on their predominant histologic features. Among the tumors, 128 (92%) had a spindle-cell component of more than 10%, while 17 (12%) contained more than 10% blastoma cells, and 24 (17%) contained more than 10% epithelioid cells.

Degree of nuclear atypia. The degree of nuclear atypia was mild in 8 (6%) patients, moderate in 99 (71%), and severe in 33 (23%).

Degree of cellularity. The degree of cellularity was low in 12 (9%) patients, moderate in 60 (43%), and high in 68 (48%).

Mitotic index. The mitotic index ranged from 1 to 22 (mean, 3.9). The tumors were divided into two groups (one with fewer than ten, and the other with ten or more mitotic cells) to isolate groups with a poor prognosis, because 17 (77.3%) of the 22 patients whose mitotic index was 10 or more died of GIST.

Growth pattern. Expansive growth was found in 107 (76%) patients and invasive growth in 33 (24%).

Presence or absence of necrosis. Necrosis was present in 36 (26%) tumors.

Presence or absence of hemorrhage. Hemorrhage was present in 87 (62%) tumors.

Presence or absence of direct tumor invasion into the surrounding organs. Direct invasion was observed in four (3%) patients (two, diaphragm; one, spleen; one, pancreas).

Presence or absence of lymphocytic infiltration around the tumor tissue. None of the patients exhibited lymphocytic infiltration around the tumor tissue. Peripheral lymphoid cuffing is a distinctive histologic feature of benign neurogenic tumors with extensive S-100 protein expression (data not shown).

Immunohistochemical findings

The immunoreactivity of the 140 GISTs for each marker is summarized in Table 2. A positive reaction for α-SMA was obtained in 26 (19%) patients, desmin in 9 (7%), caldesmon in 111 (82%), vimentin in 122 (88%), CD34 in 138 (99%), c-kit protein in 138 (99%), and S-100 protein in 9 (6%). Based on these immunohistochemical findings, 23 of the GISTs were classified as muscular type (16%), 4 as neural type (3%), 5 as combined type (4%), and 108 as uncommitted type (77%).

The distribution of MIB-1 antigen labeling indices among the 130 GISTs tested is summarized in Table 3. There were 24 (18.4%) of the 130 patients whose MIB-1 antigen labeling index was 10% or more. The cutoff value for the MIB-1 antigen labeling index was set at 10% to isolate groups with a poor prognosis, because 13 (54.2%) of the 24 patients whose MIB-1 antigen labeling index was 10% or more died of GIST.

Metastasis and recurrence

Follow-up information was available for all 140 patients. Of these, 135 (96%) underwent curative resection, while 5 (4%) received palliative resection because of the presence of liver metastases (4 patients) or peritoneal dissemination (1 patient). Metastasis or recurrence occurred in 20 (15%) of the 135 patients who underwent resection with curative intent. Fourteen (70%) of these 20 patients showed no evidence of metastasis at surgery. The remaining 6 (30%) had liver metastases (4 patients) or peritoneal dissemination (2 patients) at surgery; however, the metastastic foci were curatively resected.

Table 2. Immunohistochemical features of the GISTs

Antibody/Reactivity	−	+	++	+++	Total
α-SMA	114	18	5	3	140
Desmin	129	5	2	2	138
Caldesmon	25	13	27	71	136
Vimentin	16	12	45	65	138
CD34	2	2	11	125	140
c-kit protein	2	1	3	134	140
S-100 protein	131	7	1	1	140

−, Negative; +, ≥10% to <40%; ++, ≥40% to <70%; +++, ≥70%; SMA, Smooth muscle actin

At the time of writing this manuscript, 97 (69%) of the 140 patients were alive and 43 (31%) were dead. Ninety-six (99%) of the 97 patients still alive were well, with no evidence of recurrence. In 1 patient, liver metastases which had initially been successfully treated by hepatectomy occurred again 4 years after the operation, and the patient is now undergoing radiotherapy for bone metastases which occurred 5 years after the hepatectomy. Twenty-three (53%) of the 43 dead patients died of GIST; the remaining 20 (47%), including 1 patient with bone metastasis, died of other diseases. Liver metastasis occurred in 19 (82%) of the 23 patients who died of GIST. Among these 19 patients, liver metastasis alone occurred in 9 (48%), liver metastasis and peritoneal dissemination in 6 (32%), liver metastasis and bone metastasis in 2 (10%), and liver metastasis plus both peritoneal dissemination and local recurrence in 2 (10%). Peritoneal dissemination alone occurred in 2 (9%) of the 23 patients who died of GIST. Local recurrence without any metastasis was also observed in 2 (9%) of the 23 patients who died of subsequent peritoneal dissemination (Table 4).

The overall survival rates for the 140 patients were 86% at 5 years and 81% at 10 years. The 5- and 10-year overall survival rates for the 129 (92%) patients who underwent curative resection for nonmetastatic primary disease were 93% and 88%, respectively.

Analysis of prognostic factors

The relationships between the clinicopathological findings in the 140 patients and their prognoses, as evaluated by univariate analysis, are shown in Table 5. Univariate analysis showed that each of the following clinicopathological factors had a significant deleterious influence on prognosis: male sex, exogastric growth, invasive growth, tumor size 10cm or more, presence of ulceration, an epithelioid cell component, severe

Table 4. Sites of metastasis in patients who died of GIST

Site	*n*
Liver only	9
+ Peritoneal dissemination	6
+ Peritoneal dissemination + local	2
+ Bone	2
Peritoneal dissemination only	2
Local only	2

Table 3. The distribution of MIB-1 antigen labeling indices for GIST

	<10%	≥10% to <20%	≥20% to <30%	≥30% to <40%	≥40%	Total
MIB-1 index	106	6	7	4	7	130

Table 5. Univariate analysis of factors that influence survival

Factor		5-Year survival (% of patients)	*P* value
Age (years)	<60:≥60	87:86	NS (0.3433)
Sex	Male:female	81:92	0.0088
Tumor location	U:M:L	83:91:93	NS (0.3977)
	AW:PW:LC:GC	92:77:92:84	NS (0.1228)
Mode of growth	Endogastric:exogastric	95:77	0.0105
Tumor size (cm)	<5:≥5 to <10:≥10	98:83:39	<0.0001
Ulceration	(−):(+)	95:69	<0.0001
Cell type	Spindle:blastoma:epithelioid	85:100:85	NS (0.2048)
	Spindle component (−):(+)	100:85	NS (0.8315)
	Blastoma component (−):(+)	84:92	NS (0.1942)
	Epithelioid component (−):(+)	90:70	<0.0001
Nuclear atypia	Mild:moderate:severe	85:90:75	0.0059
Cellularity	Low:moderate:high	100:98:74	<0.0001
Mitotic index	<10:≥10	97:29	<0.0001
Growth pattern	Expansive:invasive	88:80	0.0247
Necrosis	(−):(+)	92:69	0.0001
Hemorrhage	(−):(+)	100:78	0.0245
Direct tumor invasion	(−):(+)	87:50	0.0014
α-SMA	(−):(+,++,+++)	87:83	NS (0.3813)
Desmin	(−):(+,++,+++)	87:85	NS (0.5584)
Caldesmon	(−):(+,++,+++)	74:90	0.0468
Vimentin	(−):(+,++,+++)	92:86	NS (0.7276)
CD34	(−):(+,++,+++)	100:86	NS (0.5986)
c-kit protein	(−):(+,++,+++)	100:86	NS (0.5986)
S-100 protein	(−):(+,++,+++)	91:22	<0.0001
MIB-1 index	<10%:≥10%	100:44	<0.0001

NS, Not significant

Table 6. Correlation between the mitotic index and the MIB-1 antigen labeling index

		MIB-1 antigen labeling index		
		<10	≥10	Total
Mitotic index	<10	106	7	113
	≥10	0	17	17
Total		106	24	130

$P < 0.0001$ (Fisher's exact test)

nuclear atypia, high cellularity, a mitotic index of 10 or more, necrosis, hemorrhage, direct tumor invasion of the surrounding tissues, negative caldesmon immunoreactivity, positive S-100 protein immunoreactivity, and an MIB-1 antigen labeling index of 10% or more (P = 0.0088, 0.0105, <0.0001, <0.0001, <0.0001, <0.0001, 0.0059, <0.0001, 0.0247, 0.0001, 0.0245, 0.0014, 0.0468, <0.0001, and <0.0001, respectively). When the GISTs were divided into four groups based on these immunohistochemical findings, the neural and combined types were significantly associated with a poor prognosis.

A multivariate analysis was then performed, using the clinicopathological factors which exhibited a significant deleterious influence on prognosis in the univariate analysis, except for the MIB-1 antigen labeling index, because this correlated strongly with the outcome predicted by the mitotic index (Table 6). This multivariate analysis (analysis A) showed that male sex, tumor size 10 cm or more, and the presence of an epithelioid cell component and a mitotic index of 10 or more were statistically significant indicators of a poor prognosis (P = 0.013, 0.001, 0.014, and <0.001, respectively) (Table 7). It is well known that assessments of the mitotic count lack reproducibility owing to the quality of the slides and differences in interpretation between pathologists. Furthermore, the microscopic identifiability of mitoses becomes more difficult as the length of time since fixation increases [21,22]. Therefore, we reanalyzed the same data using the MIB-1 antigen labeling index instead of the mitotic count (multivariate analysis B). This showed that male sex, tumor size of 10 cm or more, the presence of necrosis, and an MIB-1 antigen labeling index of 10% or more were independent factors predictive of a poor prognosis (P = 0.009, 0.001, 0.043, and <0.001, respectively) (Table 7). The various cell differentiation markers were not statistically significant indicators of a poor prognosis in the multivariate analyses.

Discussion

Several studies of GISTs have shown that various histological features of these tumors, such as mitotic counts [7,10,20,23], or a combination of mitotic counts and

Table 7. Multivariate analyses of factors that influence survival

Factors		Multivariate analysis A			Multivariate analysis B		
		HR	(95%CI)	*P* value	HR	(95%CI)	*P* value
Sex	M:F	0.469	(0.257–0.854)	0.013	0.358	(0.166–0.773)	0.009
Tumor size (cm)	<5	1			1		
	≥5 to <10	4.365	(0.707–26.954)	0.113	3.547	(0.610–20.632)	0.159
	≥10	20.989	(3.560–125.673)	0.001	21.273	(3.298–137.216)	0.001
Epithelioid component	(−):(+)	5.315	(1.402–20.149)	0.014			
Nuclear atypia	Mild	1					
	Moderate	0.521	(0.052–5.204)	0.579			
	Severe	0.086	(0.006–1.202)	0.068			
Mitotic index	<10:≥10	45.951	(8.811–239.657)	<0.001		Not analyzed	
Necrosis	(−):(+)				0.512	(0.268–0.978)	0.043
Hemorrhage	(−):(+)	0.410	(0.159–1.060)	0.066			
MIB-1 index	<10%:≥10%		Not analyzed		31.622	(6.130–163.109)	<0.001

Analysis B, Material from the same patients as for analysis A was analyzed using the MIB-1 antigen labeling index instead of the mitotic index
HR, Hazard ratio; CI, confidence interval

nuclear atypia [4,8,9,12], cellularity [9,12] and/or tumor necrosis [9], can be used to categorize their degree of malignancy. Mitotic activity has been regarded as indicating malignant potential by many previous investigators, who considered GISTs to be malignant when they showed one or more mitotic figures per 10 HPFs (×400) [9,10,12], or over five mitoses per 50 HPFs (×400) [8,23], or per 10 HPFs (×400) [7,20]. Evans [10] reported that fatal low-grade leiomyosarcomas could have maximum mitotic rates as low as one mitosis per 10 HPFs. In addition, Akwari et al. [24] reported that only a few mitotic figures could be found in some definitely metastatic leiomyosarcomas. Therefore, in our series, GISTs which showed at least one mitotic figure in the tumor cells were regarded as malignant.

Several studies using univariate-type analyses have reported that the mitotic rate [8,10,11,15], tumor cellularity [8,15,16] and size [8,11–15,17], and the presence of necrosis [8,15], hemorrhage [8], ulceration [11], nuclear atypia [13], and direct tumor invasion [11] are statistically significant indicators of a poor prognosis in patients with GISTs. In our series, these factors were also significantly predictive of a poor prognosis in the univariate analysis. However, additional histopathologic factors (male sex, an exogastric growth pattern, the presence of an epithelioid component, invasive growth, negative caldesmon immunoreactivity, positive S-100 protein immunoreactivity, and an MIB-1 antigen labeling index of 10% or more) were also found to be significantly associated with a poor prognosis in the univariate analysis. In previous studies, factors such as the mode of growth, the presence of an epithelioid component, and invasive growth into the surrounding tissues have not been examined.

In the only previous study in which a multivariate analysis has been used, DeMatteo et al. [14] found that only tumor size predicted disease-specific survival in patients with GISTs who underwent complete gross resection. In our study, multivariate analysis A showed that male sex, tumor size 10 cm or more, the presence of an epithelioid cell component, and a mitotic index of 10 or more were statistically significant indicators of a poor prognosis, while multivariate analysis B, which used the MIB-1 index instead of the mitotic index, showed that male sex, tumor size 10 cm or more, the presence of necrosis, and an MIB-1 antigen labeling index of 10% or more were independent factors predictive of a poor prognosis. Because both multivariate analyses showed that male sex, tumor size (more than 10 cm), and cell proliferation (as estimated by either the mitotic index or the MIB-1 index) were independent factors indicative of a poor prognosis, these three factors can be considered important prognostic factors for malignant gastric GIST. These findings were gathered from a series of 140 malignant gastric GISTs treated at a single institution, the largest clinicopathologic study of malignant gastric GISTs which has ever been reported. Based on our results, we recommend that more careful preoperative and more frequent postoperative follow-up examinations should be performed for male patients with large tumors whose cells show high proliferative activity.

Although the mitotic rate has been regarded as an important prognostic factor by many previous investigators [4,7–12,20,23], assessments of the mitotic count are well known to lack reproducibility owing to the quality of the slides and differences in interpretation [21]. Donhuijsen et al. [22] have also shown that the microscopic identifiability of mitoses becomes more difficult with increasing time. Therefore, we reanalyzed our study material using the MIB-1 antigen labeling index instead of the mitotic index, because these two indices showed significant correlation. In both multivariate

analyses, the mitotic index and the MIB-1 antigen labeling index were confirmed to be independent predictors of a poor prognosis.

Five-year survival rates for patients with GISTs have been reported to be 35% (in 200 patients, of whom 78 had tumors in the stomach) [14], 28% (in 191 patients, 72 with stomach tumors) [13], 40% (in 51 patients, 26 with stomach tumors) [12], and 56% (in 41 patients, all with gastric GISTs) [9]. Five-year survival rates for patients with nonmetastatic GISTs who underwent curative resection have been reported to be 54% (in 80 patients, 43 with stomach tumors) [14], 48% (in 99 patients, in whom tumor location was not described) [13], and 63% (in 30 patients, in whom tumor location was not described) [12]. In our study, the 5-year survival rate for all 140 patients with malignant gastric GISTs was 86%, while that for the 129 (92%) patients who underwent curative resection for malignant but nonmetastatic gastric GISTs was 93%. Thus, our survival rates were higher than those in previous studies. The discrepancy between our results and those in other studies, which included intestinal GISTs, can be explained by the fact that gastric GIST has a more favorable prognosis than intestinal GIST [8,15,17]; in addition, these previous studies included a greater number of large tumors than ours, with 38% to 60% measuring more than 10 cm [12–14].

Positive reactions for SMA have been reported in 27%–74% of 46–109 GISTs [4,7,15,16], while desmin reactivity has been observed in 3%–53% of 45–109 GISTs [4,7,8,15,16,25], with caldesmon reactivity being observed in 50% of 58 GISTs [15]. Positive vimentin reactivity has been reported in 92%–100% of 46–96 GISTs [8,16,25], CD34 reactivity in 56%–82% of 49–109 GISTs [2,3,7,15], c-kit protein reactivity in 72%–100% of 49–85 GISTs [2,3,6,15], and S-100 protein reactivity in 1%–28% of 46–170 GISTs [4,7,8,15,16,25,26]. Coexpression of c-kit protein and CD34 has been found in 66%–78% of 49–78 GISTs [2,3,15]. With regard to GISTs located in the stomach, 59%–67% of 18–41 GISTs were reported to be positive for SMA [15,16], while 10%–50% of 18–57 GISTs showed desmin reactivity [8,15,16,25] and 59% of 41 GISTs were caldesmon-positive [15]. Furthermore, 88%–100% of 18–57 GISTs were positive for vimentin [8,16,25], 80% of 41 GISTs were positive for CD34 [15], 73% of 41 GISTs were positive for c-kit protein [15], and 0%–22% of 18–91 GISTs were positive for S-100 protein [8,15,16,25,26]. As for malignant GISTs, positive reaction rates for CD34 and c-kit protein have been reported to be 35%–52% of 23–31 GISTs [6,7] and 71% of 31 GISTs [6]. In the current study, a positive reaction for α-SMA was demonstrated in 19% (26/140) of the tumors, while 7% (9/138) were desmin-positive, 82% (111/136) were caldesmon-positive, 88% (122/138) were vimentin-positive, 99% (138/140) were CD34-positive, 99% (138/140) were c-kit protein-positive, and 6% (9/140) were S-100 protein-positive. The incidences of immunopositivity for the various differentiation markers in our present study differ somewhat from those in previous studies, which included not only malignant but also benign GISTs, in addition to both intestinal and gastric GISTs. Therefore, the differences in the immunopositivity rates for differentiation markers might reflect differences in the nature of the tumors themselves. Because almost all of our GISTs showed diffuse CD34 and c-kit protein reactivity, the vast majority of malignant gastric GISTs can be considered to show differentiation towards a Cajal cell phenotype. Negative caldesmon immunoreactivity and positive S-100 protein immunoreactivity were also statistically significant indicators of a poor prognosis in the univariate analysis; therefore, some GISTs that fail to abort smooth muscular differentiation or that show neural differentiation may be linked with a poor prognosis. However, cell differentiation markers were not statistically significant predictors of a poor prognosis in the multivariate analyses. There have been no other studies in which cell differentiation markers have been evaluated by multivariate analysis and found to indicate a poor prognosis.

Finally, because the current results showed that the type of surgery had no impact on the patient's prognosis, and none of the patients developed lymph node metastasis, it can be concluded that wedge resection without systematic lymph node dissection appears to be an adequate surgical procedure for malignant gastric GIST, especially because the functions of both the cardia and pylorus of the stomach are preserved when this technique is employed.

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