Ki67 and Tumor Size as Prognostic Factors of Gastrointestinal Stromal Tumors

JMAJ 48(12): 586–592, 2005

Hironori Ohdaira,*1 Shigekazu Ohyama,*2 Toshiharu Yamaguchi,*2 Akio Yanagisawa,*3 Yo Kato,*3 Mitsuyoshi Urashima*4

Abstract

Background The aim of the study is to determine useful prognostic factors of gastrointestinal stromal tumors (GIST).

Methods 135 patients with GIST in the stomach resected at the Cancer Institution Hospital were retrospectively reviewed.

Results 84% were positive for KIT and/or CD34. All events of death due to GIST, metastasis to lymph nodes, liver and other sites occurred in patients with KIT and CD34 positive GIST. Univariate Cox regression analyses revealed that ulceration, tumor size and Ki67 index were significant predictors of death due to GIST with KIT and/or CD34 positive stain. On the other hand, multivariate Cox regression analysis showed tumor size $\geq$50mm [hazard ratio: 6.9 (95% confidence interval: 1.6–30.1)] and Ki67 index $\geq$40/mm$^2$ [hazard ratio: 8.0 (95% confidence interval: 2.6–25.1)] were the only significant poor prognostic factors. Prognosis of patients with KIT and/or CD34 positive GIST satisfying both conditions of larger than 50mm in tumor size and Ki67 index $\geq$40/mm$^2$ was significantly poorer than in others (log-rank test: $P<0.0001$): 5 year-survival rate: 36% vs. 95%.

Conclusion These results suggest that patients with KIT and CD34 positive GIST larger than 50mm in tumor size and greater than 40 in Ki67 index may have poor prognosis.

Key words Gastrointestinal stromal tumor, Stomach, Clinicopathology, Prognostic factor

Introduction

Until recently, most submucosal tumors of the stomach had been considered to be myogenic or neurogenic and were diagnosed as leiomyoma, leiomyosarcoma, and schwannoma. However, immunohistochemical staining and electron microscopic studies revealed that most of these tumors did not differentiate into smooth muscle or Schwann cells. Mazur termed these tumors gastrointestinal stromal tumors (GIST) in 1983.1 In 1996, Rosai defined GIST as a general term for mesenchymal tumors of the gastrointestinal tract and classified these tumors into 1) smooth muscle type, 2) neural type, 3) combined type and 4) uncommitted type according to differentiation to smooth-muscle cells or Schwann cells.2 In 1998, Hirota demonstrated mutations of the KIT proto-oncogene in GIST.3 Recently, the term GIST tends to be referred to mesenchymal tumors that are positive for the KIT receptor (CD117, stem cell factor receptor) and/or CD34, and do not differentiate into smooth-muscle cells or Schwann cells. Since both GIST and interstitial cells of Cajal, intestinal pacemaker cells, are positive for KIT protein and/or CD34, the cellular origin of GIST is considered to be mesenchymal

*1 Department of Surgery, Jikei University School of Medicine, Tokyo
*2 Department of Surgery, Cancer Institute Hospital, Tokyo
*3 Department of Pathology, Cancer Institute Hospital, Tokyo
*4 Division of Clinical Research & Development, Jikei University School of Medicine, Tokyo
Corresponding to: Hironori Ohdaira MD, Department of Surgery, Jikei University School of Medicine, 3-25-8 Nishishinbashishi, Minato-ku, Tokyo 105-8461, Japan. Tel: 81-3-3433-1111, Fax: 81-3-5472-4140, E-mail: nori-o@fj8.so-net.ne.jp
stem cells or interstitial cells of Cajal. A gain-of-function mutation of the KIT proto-oncogene may play a part in development of GIST. Thus, at present, two definitions of GIST are used depending on the pathologists. In a broad sense, GIST is a general term for mesenchymal tumors based on the definition by Rosai. In a narrow sense, GIST means neutral tumors negative for smooth muscle markers, as well as nerve markers, based on the latest WHO classification and the definition by Miettinen.

Imatinib mesylate, a selective tyrosine kinase inhibitor, has been shown in preclinical models and preliminary clinical studies to have activity against such tumors. However, no patient had a complete response to the treatment and the median duration of response had not been reached after a median follow-up of 24 weeks after the onset of response. Moreover, early resistance to imatinib was noted in 14 percent of treated patients. Thus, still delineating prognostic factors for patients with GIST may be important even in post imatinib era.

In this study, we aimed to evaluate significant prognostic factors and determine the subgroup with poor prognosis, who may need intensive treatments.

Patients and Methods

Patients
The study was conducted with the approval of the Institutional Review Board of Jikei University School of Medicine and Cancer Institute Hospital, and patient confidentiality was preserved without going back to obtain individual consent.

From 1946 to 2000, 274 patients diagnosed as having submucosal tumors of the stomach underwent resection at the Cancer Institute Hospital. Among these patients, we examined 135 patients whose postoperative course could be followed and immunohistochemical staining was performed on their tumors. The original diagnoses of these tumors included leiomyoma, leiomyosarcoma and Schwannoma. According to the broad definition of GIST by Rosai, these tumors were considered to be GIST. Submucosal tumors other than spindle cell tumors, such as carcinoid, accessory pancreas and malignant lymphoma, were not included in this study. In addition, minute tumors of gastric cancer incidentally found in the resected stomach were also excluded.

Immunohistochemical staining
In addition to hematoxylin and eosin-staining, immunohistochemical staining was performed with KIT (CD117; c-kit proto-oncogene product), CD34 (stem cell factor receptor), SMA (α smooth-muscle actin; smooth-muscle marker), and S100 (nerve marker). Tumors were classified according to the result of immunohistochemical staining. Patients’ sex, age, location of the tumor, tumor size, macroscopic growth type, presence or absence of ulceration and metastases were examined by category. Macroscopic growth types of tumors were categorized into the following types according to the classification by Skandalakis: 1) endoluminal type, 2) exoluminal type, 3) intramural type and 4) mixed type. The location of the tumor was classified into 1) upper, 2) middle and 3) lower stomach. To demonstrate the proliferative activity of tumor cells, the number of Ki67 positive cells per mm² (defined as Ki67 index in this study) was examined.

The presence of metastasis was identified in image findings such as CT and US.

Statistics
Chi-squared test or Student’s t-test was used for statistical comparisons of baseline characteristics. Logistic regression model was applied to examine factors that associate with metastasis. The Cox’s proportional hazard model was used in univariable and multivariable analyses. Survival rates were calculated by the Kaplan-Meier method, and statistical significance was determined by the log-rank test. A value of P<0.05 was considered statistically significant. All statistical analyses were performed using STATA 8.0 (STATA Corporation, College Station, TX).

Results

Patients’ characteristics: Comparison between KIT and/or CD34 positive GIST and others
Patients’ characteristics including immunohistochemical staining stratified KIT and/or CD34 positive GIST (N = 114) and others (N = 21) are summarized in Table 1. There were 114 (84%) KIT and/or CD34-positive tumors, 15 (11%) smooth-muscle marker-positive tumors, 7 (5%) neuron marker-positive tumors. Others included
cases with both SMA-positive and -negative lesions. Thus, the tumors were classified into the following three categories: 1) smooth-muscle marker positive, 2) nerve marker positive, and 3) KIT and/or CD34 positive.

Patients with KIT and/or CD34 positive GIST were significantly older in age and more male dominant than others. There were no significant differences in macroscopic growth types, presence of ulcer, location of tumor, size of tumor, score of Ki67 between KIT and/or CD34 positive GIST and others.

The sites of relapse or recurrence in the 23 patients were as follows: Liver: 13.3% (18/135); lung: 0.7% (1/135); bone: 1.4% (2/135); peritoneal: 3.0% (4/135); lymph node: 4.4% (6/135). All events of death due to GIST, metastasis to lymph nodes, liver and metastases at any sites occurred in patients with KIT and CD34 positive GIST (N = 107). There were 23 cases of metastases and 22 of tumor death, of which 21 overlapped.

**Factors affect on metastasis**

Using logistic regression model, we examined factors that may associate with liver metastasis (Table 2) and metastases at any sites (Table 3) focusing only on patients with KIT and/or CD34 positive GIST (N = 114). Univariate logistic regression analyses revealed that in men, the presence of ulcerative lesions in the tumor, tumor size ≥50 mm in longer axis and Ki67 index ≥40/mm² independently increased frequency of liver metastases. On the other hand, multivariate logistic regression analysis showed that only tumor size ≥50 mm and Ki67 index ≥40/mm² significantly increased frequency of liver metastases.
Similarly, univariate logistic regression analyses revealed that for men with tumor size ≥50 mm in longer axis and Ki67 index ≥40 there was a positive association with the frequency of metastasis at any sites. Multivariate logistic regression analysis showed that only tumor size ≥50 mm and Ki67 index ≥40/mm² independently and positively associated with frequency of metastases at any sites.

**Survival analyses**

Cox hazard regression analyses were applied to determine factors associating with death due to KIT and/or CD34 positive GIST using univariate and multivariate manner (Table 4). Univariate Cox regression analyses revealed that ulceration, tumor size and Ki67 index were significant predictors of death due to GIST with KIT and/or CD34 positive GIST. On the other hand, multivariate Cox regression analysis showed tumor size ≥50 mm [hazard ratio: 6.9 (95% confidence interval: 1.6–30.1)] and Ki67 index ≥40/mm² [hazard ratio: 8.0 (95% confidence interval: 2.6–25.1)] were the only significant poor prognostic factors. The likelihood of a five-year survival of patients with KIT and/or CD34 positive GIST was 81.5%, of which patients satisfying both conditions of larger than 50 mm in tumor size and Ki67 index ≥40/mm² was significantly poorer than in others (log-rank test: $P<0.0001$):

| Table 2 Univariate and multivariate logistic regression analyses of the factors associating with liver metastasis (N=18) focusing on patients with KIT and/or CD34 positive GIST (N=114) |
|---|---|---|---|
| Univariate analysis | OR (95%CI) | P value | Multivariate analysis |
| Male | 4.50 (1.38–14.68) | 0.013 | 3.47 (0.72–16.71) | NS |
| Presence of ulcer | 3.36 (1.19–9.51) | 0.022 | 4.22 (0.84–21.23) | NS |
| Size ≥50 mm | 20.56 (2.63–160.79) | 0.004 | 22.7. (2.30–213.54) | 0.007 |
| Ki67 ≥40 | 29.20 (6.19–137.71) | <0.001 | 32.12 (5.67–182.03) | <0.001 |

| Table 3 Univariate and multivariate logistic regression analyses of the factor associating with metastasis at any sites (N=23) focusing on patients with KIT and/or CD34 positive GIST (N=114) |
|---|---|---|---|
| Univariate analysis | OR (95%CI) | P value | Multivariate analysis |
| Male | 3.78 (1.36–10.47) | 0.011 | 3.06 (0.79–11.83) | NS |
| Size ≥50 mm | 13.73 (3.04–62.10) | 0.001 | 16.27 (2.93–90.37) | 0.001 |
| Ki67 ≥40 | 19.84 (5.97–65.94) | <0.001 | 24.24 (6.08–96.66) | <0.001 |

| Table 4 Univariate and multivariate Cox regression analyses of the death from KIT and/or CD34 positive GIST (N=113*) |
|---|---|---|---|
| Univariable analysis | HR (95%CI) | P value | Multivariable analysis |
| Male | 3.82 (1.41–10.38) | 0.009 | 2.01 (0.71–5.72) | NS |
| Ulcer | 2.51 (1.09–5.78) | 0.031 | 1.70 (0.72–4.01) | NS |
| Size ≥50 mm | 9.76 (2.28–41.77) | 0.002 | 6.90 (1.58–30.07) | 0.010 |
| Ki67 ≥40 | 12.12 (4.08–36.03) | <0.001 | 8.02 (2.56–25.09) | <0.001 |

*1: One patient with KIT positive GIST was loss to follow-up.
Discussion

In recent reports, GIST has been narrowly interpreted and defined as tumors positive for KIT and CD34. However, tumors negative for a smooth-muscle marker and nerve marker may include tumors 1) positive for both KIT and CD34, 2) positive for only KIT, 3) positive for only CD34 and 4) negative for both KIT and CD34. In this study, KIT and/or CD34-positive tumors accounted for 84% of all spindle cell tumors. This means that 84% of submucosal tumors diagnosed as leiomyoma, leiomyosarcoma or Schwannoma in the past were positive for KIT and/or CD34.

GIST is clinically and pathologically different from myogenic and neurogenic tumors, and their behaviors differ. Thus, myogenic and neurogenic tumors were not included in this study, as recommended by NIH consensus conference. Because myogenic tumors and neurogenic tumors are relatively scarce, we have to accumulate more cases of such tumors, for future research and discussion.

Cases of tumor death almost exactly overlapped with cases of metastases. Thus, tumor size ≥50 mm and Ki67 index ≥40/μm² were common independent significant poor prognostic factors in both metastases and tumor death in multiple regression analyses. Prognosis of patients with KIT and/or CD34 positive GIST satisfying both conditions of larger than 50 mm in tumor size and Ki67 index ≥40/μm² was significantly poor than in others (log-rank test: P<0.0001): 5 year survival rate: 36% vs. 95%. Recent studies demonstrated that both tumor size and mitotic activity were significant prognostic factors, which were consistent with our results.

Tumor size could be a simple and reliable index for operative indication and postoperative follow-up. DeMatteo reported that only tumor size predicted survival in patients with primary lesions who underwent complete gross resection, based on multivariate analysis. Kwon stated that tumor size ≥50 mm was a poor prognostic factor, although this was based on univariate analysis only.

The Ki67 index is a cell proliferation marker and could be a good indicator of the risk of metastases and prognosis. In the past, the mitotic index was often used for diagnosis of leiomyoma and leiomyosarcoma. However, the mitotic
index has the following problems: 1) Authors use different criteria for determination of malignancy, 2) assessment of mitosis using HE-stained samples tends to be subjective and 3) there is a possibility that broken nuclei could be mistaken for mitoses. The advantages of the Ki67 index include: 1) Mitosis is clearly observed as brown, 2) concentrated nuclei and karyolysis are negative and 3) information on mitosis can be obtained only by observing the nuclear configuration in Ki67-positive cells. Carrillo concluded that MIB-1 (a monoclonal antibody to Ki67 antigen) index was the most powerful predictor of clinical behavior of GIST and should be used to support histological grading, based on multivariate analysis. Sedial stated that the expression of Ki67 in the nuclei of the tumor cells was the most important prognostic factor. Fujimoto reported the correlation with Ki67 and mitotic index, and each of them became prognostic factors in GISTs of the stomach. This was not examined in our study, but we were able to pick up mitosis certainly by calculating Ki67 positive cells.

From the Table 2 and 3 which analyzed factors related to metastasis, we can ascertain that the tumor size was small and Ki67 were low in cases without metastasis.

It is clear that the tumor size and Ki67 index are reliable prognostic factors. In future research it would be useful to determine whether preoperative diagnosis and estimation of prognosis are possible. GIST is usually diagnosed by immunohistochemical staining and histopathological examination of the resected specimen. To diagnose GIST before surgery is generally difficult. In our study, variation of Ki67 index was frequently detected depending on the location in a single tumor. There are reports on biopsy after artificial ulceration and EUS-guided fine needle aspiration. However, because sufficient tissue for pathological examination cannot be obtained in many cases, these methods have not become widespread. Preoperative percutaneous biopsy theoretically has the risk of peritoneal seeding or tumor rupture.

In conclusion, 1) 84% of GIST was positive for KIT and/or CD34, 2) all cases who developed to metastases and all cases who died of tumor were observed in patients with GIST positive for both KIT and CD34, 3) combination of tumor size and Ki67 index were further prognostic factors in GIST positive for both KIT and CD34.

References


