Tumor necrosis and >20 mitoses per 50 high-power fields can distinguish 'very high-risk' and 'highest-risk' within 'high-risk' gastric gastrointestinal stromal tumor

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Aim: We aimed to investigate the optimal criteria for classifying higher risk forms of gastric gastrointestinal stromal tumor (gGIST). **Materials & methods:** A total of 246 high-risk gGIST patients were enrolled. Univariate and multivariate analyses were conducted to determine the association between clinicopathological features and overall survival. Appropriate cut-off values were calculated to identify those at higher risk of gGIST. **Results:** Multivariate and univariate analyses revealed that tumor necrosis and mitotic counts are independent risk factors for overall survival. The optimal cut-off value of mitotic counts was 20. Patients with both necrosis and >20 mitoses/50 high-power fields were worse than those with either one. **Conclusion:** Tumor necrosis and >20 mitoses/50 high-power fields are independent risk factors for high-risk gGIST. Patients with both risk factors indicate worse prognosis.

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The gastrointestinal stromal tumor (GIST) is one of the most common sarcomas, with an incidence of 10–13 per million cases per year [1]. Although the origin and pathogenesis have been clarified, the risk factors for recurrence, metastasis and death still confuse most clinical doctors. More than half of all GISTs are located in the stomach [2], and are termed ‘gastric GISTs (gGISTs)’. At present, several scales are used to assess the recurrent and metastatic risk of GISTs, such as the modified NIH criterion [3] and the Armed Forces Institute of Pathology classification [4]; these scales are used to decide the adjuvant therapy after surgery. However, these criteria have limitations. They cannot predict the exact recurrence probability, especially for patients with high-risk GISTs. Some patients with high-risk GISTs experience recurrence in a short time after a 3-year adjuvant therapy, while others experience disease-free survival for a long period. Therefore, some researchers have proposed the new concept of ‘very high-risk GISTs’ to indicate those patients with a higher risk of recurrence [5]. To date, few studies have attempted to investigate the optimal criteria for defining cases at very high risk. Therefore, the criteria remain controversial.

Given this situation, the present study aimed to determine the optimal criteria for defining patients at very high risk of developing gGISTs.

Patients & methods

From August 2000 to November 2015, a total of 246 high-risk gGIST patients from four hospitals, including Guangdong General Hospital, Sun Yat-sen University Cancer Hospital, Nanfang Hospital, Southern Medical University and Fujian Medical University Union Hospital were enrolled in this study. According to the Scandinavian Sarcoma Group (SSG) XVIII (NCT00116935) [6], a high-risk gGIST is defined as both a tumor with > five mitoses per 50 high-power fields (HPFs) and >5 cm in diameter. The inclusion criteria were as follows: high-risk gGIST; with tumor R0 resection; without tumor rupture or metastasis; used imatinib after surgery; received follow-up and having complete clinicopathological data and follow-up data. The patients received follow-up according to the guidelines recommended [7]. High-risk gGIST patients underwent a follow-up with an abdominal CT scan every 3–6 months for 3 years during adjuvant therapy; then every 3 months for 2 years, every 6 months until 5 years from stopping adjuvant therapy and annually for an additional 5 years. This study was approved by the Ethics Committee of Guangdong General Hospital, and written operation informed consent was obtained from all patients prior to surgery.

We collected important clinicopathological data, including age, gender, tumor location, tumor size, mitotic counts, tumor necrosis, types of resections and operative approach. Tumor necrosis was determined to be present or absent. The follow-up data included whether recurrence, metastasis or death occurred and the exact time. Based on the follow-up data, we calculated overall survival (OS).

SPSS 22.0 software (IBM Corporation, NY, USA) was used to prove that mitotic count is an independent prognostic factor of OS by univariate and multivariable analysis. And then we used the X-tile software (Yale University, CT, USA) to calculate the cut-off values of tumor size and mitotic counts for the prognosis of gGISTs. At last, we performed the survival analysis to verify the cut-off value. Significant risk factors were identified by univariate and multivariate analyses using the Cox regression model. OS was analyzed using the Kaplan–Meier method. The *p*-values <0.05 were considered statistically significant.

Results

A total of 246 high-risk gGIST patients, including 141 males (57.3%) and 105 females (42.7%) were enrolled in this study. The clinicopathological characteristics of these patients were presented in Table 1. The mean age of the patients was 59.7 (range: 26–86 years). The mean size of the tumors was 9.4 cm (range: 5.1–29.0 cm). The mean mitotic counts were 15.8 (range: 6–126). The mean follow-up period was 34.5 months (range: 1–137 months), and the 1-, 3- and 5-year OS rates for these high-risk patients were 99.1, 88.9 and 78.5%, respectively.

We carried out univariate and multivariate analyses to identify the risk of obtaining a prognosis of high-risk gGISTs in these patients. As shown in Table 2, univariate analysis demonstrated that tumor necrosis and mitotic counts were significant prognostic factors for OS, whereas age, gender, tumor location, type of resection, tumor size and operative approach were not. To avoid issues associated with univariate analysis regarding the potential

Table 1. Clinicopathological characteristics of high-risk gastric gastrointestinal stromal tumor patients.

Variables	Number of patients	Percentage (%)
Age:		
– <60 years	120	48.8
– ≥60 years	126	51.2
Gender:		
– Male	141	57.3
– Female	105	42.7
Tumor location:		
– Gastric cardia	15	6.1
– Gastric fundus	86	35
– Gastric body	127	51.6
– Gastric antrum	18	7.3
Tumor necrosis:		
– Yes	76	30.9
– No	170	69.1
Type of resection:		
– Wedge	154	62.6
– Distal	18	7.3
– Proximal	49	19.9
– Total	21	8.5
– Others	4	1.6
Operative approach:		
– Laparoscopy	51	20.7
– Laparotomy	195	79.3

Table 2. Univariate and multivariate analyses of high-risk gastric gastrointestinal stromal tumor patients for overall survival.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	1.017 (0.991–991.043)	0.213		
Gender	1.167 (0.604–602.256)	0.645		
Tumor location	0.739 (0.447–441.222)	0.239		
Tumor necrosis	0.426 (0.220–220.823)	0.011	0.479 (0.246–240.935)	0.031
Type of resection	1.194 (0.929–921.534)	0.166		
Operative approach	1.111 (0.641–641.924)	0.708		
Tumor size	1.050 (0.991–991.112)	0.098		
Mitotic count	1.025 (1.014–011.037)	0.000	1.024 (1.012–011.035)	0.000

HR: Hazard ratio.

prognostic variables, we also examined these factors using a multivariate Cox analysis and found that tumor necrosis (HR: 0.426; 95% CI: 0.220–0.823; $p = 0.011$) and mitotic counts (HR: 1.025; 95% CI: 1.014–011.037; $p < 0.001$) were independent prognostic factors for OS.

The patients were divided into two groups according to tumor necrosis (a tumor necrosis group and a without tumor necrosis group). We performed a survival analysis using the Kaplan–Meier method. As shown in [Figure 1](#), patients without tumor necrosis exhibited a significant survival benefit compared with patients with tumor necrosis. The 5-year survival rates were 60.6 and 85.5% for patients with necrosis and without necrosis, respectively ($p = 0.009$).

We used the X-tile software (Yale University) to calculate cut-off values of mitotic counts for the prognosis of gGISTs. As shown in [Figure 2](#), the cut-off value of mitotic counts for the prognosis of gGISTs was 20 in the high-risk gGIST patients. Patients with high-risk gGISTs were divided into two groups according to the cut-off value of the

Figure 1. Survival analysis according to tumor necrosis.
OS: Overall survival.

mitotic count. Survival analyses of these two groups were performed using the Kaplan–Meier method. The OS of high-risk gGIST patients based on the mitotic counts are shown in Figure 3. Patients with six to 20 mitoses per 50 HPFs exhibited a significant survival benefit compared with that of patients with more than 20 mitoses per 50 HPFs. And the 5-year survival rates were 82.8 and 0.0% for these two groups, respectively ($p < 0.001$).

On the basis of the results, tumor necrosis and tumors with more than 20 mitoses per 50 HPFs can be regarded as risk factors for high-risk gGISTs. We divided these patients into three groups according to how many risk factors the patients were exposed to ('no risk factor', 'one risk factor' and 'two risk factors'). There was a significant difference in the OS among these three groups. The 5-year survival rates of these three groups were 89.7, 57.9 and 0%, respectively ($p < 0.001$; Figure 4).

Discussion

The stomach is the most common tumor site of GISTs, and more than half of these tumors are located in this organ [8]. For patients with high-risk gGISTs after R0 resection, adjuvant imatinib therapy is necessary. Based on the results of the SSGXVIII/AIO trial (NCT00116935) [6], the National Comprehensive Cancer Network (NCCN) guidelines [7] recommend at least 3 years of adjuvant imatinib therapy after surgery rather than 1 year of therapy in patients with high-risk GISTs. However, some patients with high-risk gGIST experience recurrence soon after adjuvant imatinib therapy. Preliminary results of the PERSIST-5 study show that patients with GISTs can benefit from 5 years of adjuvant imatinib therapy [9]. Lin JX *et al.* [10] also suggest that patients with high-risk GISTs

Figure 2. Calculation of the cut-off value of mitotic counts by X-tile software.

Figure 3. Survival analysis according to mitotic counts.
OS: Overall survival.

should receive adjuvant imatinib therapy for at least 5 years. Nevertheless, adjuvant imatinib therapy should not be prolonged blindly; instead, patients who can benefit from prolonged therapy should be identified before adjuvant imatinib therapy is prolonged. We term this tumor type ‘very high-risk gGIST’. Some studies have attempted to identify these patients [11]; however, few data have been published.

In this study, we performed univariate and multivariate analysis to identify the independent risk factors for patients with high-risk gGIST. The results showed that tumor necrosis and mitotic count are independent risk factors of high-risk gGISTs. Tumor necrosis may occur if tumors grow so rapidly that the blood supply cannot keep up, and necrosis has been shown to indicate a worse prognosis for many solid tumors [12–14]. Based on univariate and multivariate analyses, we found that tumor necrosis was an independent risk factor for high-risk gGIST. We divided the patients into two groups based on whether the tumor was necrotic or not, and then performed the survival analysis, retrospectively. From the survival curve, we can see that patients without tumor necrosis had a significantly longer OS than patients with tumor necrosis. Hence, tumor necrosis indicates a worse prognosis for patients with high-risk gGIST.

We also find that mitotic count is another independent risk factor for patients with high-risk gGIST. Mitotic count is always regarded as an important prognostic factor [15]; therefore, it has been included in various prognostic criteria for GISTs, including the NIH classification, the modified NIH classification [8] and the Armed Forces Institute of Pathology classification [4]. Based on the results of our univariate and multivariate analyses, we find that mitotic count is also an independent prognostic factor for high-risk gGISTs. According to the NCCN guidelines [7], gGISTs with more than five mitotic counts and a diameter of more than 5 cm are identified as high-risk gGISTs.

Figure 4. Survival analysis according to the number of risk factors patients are exposed to. OS: Overall survival.

However, we wondered whether gGISTs with more than five mitotic counts have the same prognosis, regardless of the number. The answer is, obviously not. As is known, the greater the number of mitotic counts, the worse the prognosis obtained for the same tumor size [8,16]. X-tile is a tool for biomarker assessment and outcome-based cut-point optimization [17]. Some studies have also used this program to determine the optimal cut-point for clinical problems [18]. In this study, we used X-tile software to determine the optimal cut-off value for mitotic count to separate two groups. As a result, we found that the OS rate for patient groups with more than 20 and those with six to 20 mitoses per 50 HPFs were separated most clearly ($p < 0.001$). Thus, 20 mitoses per 50 HPFs can be regarded as the optimal cut-off value to separate high-risk gGIST cases into two groups. The survival analysis also showed that patients with more than 20 mitoses per 50 HPFs had a worse prognosis for high-risk gGISTs.

Based on the above findings, we regard tumor necrosis and more than 20 mitoses per 50 HPFs as risk factors for patients with high-risk gGISTs. Therefore, we conducted separate survival analyses for patients with two, one and no risk factors. The survival curves showed that patients with two risk factors have the worst prognosis, and patients with one risk factor have the second worst prognosis. This result indicates that these two risk factors are synergistic. Patients with more risk factors show a worse OS. Therefore, we think that gGIST cases with tumor necrosis with >5 mitoses per 50 HPFs and >5 cm in diameter, and gGIST cases without tumor necrosis with >20 mitoses per 50 HPFs and >5 cm in diameter can be regarded as 'very high-risk' gGISTs. Furthermore, gGISTs with tumor necrosis, >20 mitoses per 50 HPFs and >5 cm in diameter can be regarded as 'highest-risk' gGISTs. The SSGXVIII study [6] noted that 3 years of adjuvant imatinib therapy are not sufficient for high-risk patients; therefore, we strongly recommend that adjuvant imatinib therapy should be appropriately prolonged for patients

with ‘very high-risk’ gGISTs, and that patients with ‘highest-risk’ gGISTs should be given longer adjuvant therapy than patients at ‘very high risk’.

A limitation of this study is that the clinicopathological characteristics did not include immunohistochemical and gene detection data. This is a retrospective study and the inclusion of patients began in 2000, meaning the collection of immunohistochemical and gene detection data was not yet universal at that time so we were unable to obtain all the data that are currently required.

Conclusion

In conclusion, tumor necrosis and >20 mitoses per 50 HPFs are independent risk factors for high-risk gGISTs. Patients with both risk factors have a worse prognosis than patients with either risk factor alone. These factors represent optimal criteria for distinguishing ‘very high-risk’ and ‘highest-risk’ gGISTs.

Summary points

- A total of 246 patients with high-risk gastric gastrointestinal stromal tumor (GIST) undergoing treatment at four hospitals in China were enrolled in this study.
- Multivariate and univariate analyses revealed that tumor necrosis and mitotic counts are independent risk factors of overall survival in high-risk gastric GIST patients.
- Next, we used X-tile software to determine an optimal cut-off value for mitotic counts. The optimal cut-off value for mitotic counts for patients with high-risk gastric GIST was 20.
- We found that tumor necrosis and >20 mitoses per 50 high-power fields are risk factors for high-risk gastric GIST.
- The prognosis of patients with both risk factors was worse than that of patients with either necrosis or >20 mitoses per 50 high-power fields alone.
- Thus, these criteria can be considered optimal for the ‘very high-risk’ and ‘highest-risk’ categories of gastric GIST.

Financial & competing interests disclosure

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Ethical conduct of research

Approved by the Ethics Committee of Guangdong General Hospital, and written informed consent was obtained from all patients prior to surgery. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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