Serosal penetration is an important prognostic factor for gastrointestinal stromal tumors

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Abstract. Predicting the malignant potential of gastrointestinal stromal tumors (GISTs) remains difficult. We assessed the value of serosal penetration, an established prognostic factor in solid tumors, to determine the clinical outcome in patients with GISTs. From 1996-2002, 25 consecutive patients with GIST underwent surgical resection at our Department. The histopathological presence of serosal penetration was assessed to predict clinical outcome. In addition, the established histopathological classification system by Franquemont (modified by using the Ki-67 proliferation index), was applied to each study patient. A Ki-67 index ≥5% ($p<0.001$) and a mitotic rate ≥5/50 high-power fields ($p<0.047$) significantly correlated with a shorter survival, whereas a tumor size >5 cm ($p=0.07$) tended towards a worse prognosis. The survival of patient groups defined by Franquemont ($p=0.03$) were of prognostic relevance. The presence of serosal penetration significantly correlated ($p<0.01$) with a shorter survival. Our data suggest that the presence of serosal penetration is a negative prognostic factor for GISTs. Serosal penetration may become a useful additional parameter for the classification of the malignant potential of GISTs. Since our data are merely hypothesis-generating, serosal penetration should be evaluated in large prospective databases.

Introduction

Predicting the malignant potential of GISTs remains difficult. Three key prognostic factors have been characterized: tumor size, mitotic index and tumor site. Multiple studies have shown that tumors with a small size, low mitotic index and localization in the stomach have a significantly better prognosis compared with tumors that have a large size, high mitotic index and localization in the small bowel or rectum (1-4). Nonetheless, Tornoczky *et al* revealed a frequent occurrence of low-grade cases among patients with metastatic GISTs, indicating that better parameters are still required to accurately predict the clinical course of this tumor (5).

The depth of tumor infiltration, including the presence of serosal penetration, is known to be an important prognostic factor in solid gastrointestinal tumors such as esophageal, gastric and colorectal cancers (6-8). However, this known prognostic factor has yet to be reported in patients with GISTs. Yan *et al* demonstrated in 69 patients with GISTs that the presence of tumor invasion in adjacent tissue/organs is of prognostic relevance, suggesting the depth of tumor infiltration to be a marker of interest in the evaluation of clinical outcome in GIST patients (9).

The targeted inhibitor of tyrosine kinase activity imatinib (Gleevec®) has demonstrated marked efficacy in the majority of patients with advanced GISTs (10). While unresectable, recurrent and metastatic GISTs are treated with imatinib, evidence suggests that this therapy is also effective in the adjuvant setting after complete surgical resection. DeMatteo *et al* recently demonstrated in a phase III trial that adjuvant therapy with imatinib increases recurrence-free survival in patients with completely resected localized primary GISTs (11). Therefore, considerable interest exists in the identification of prognostic markers that may be used to select patients for adjuvant imatinib treatment after R0 resection.

The existing data regarding prognostic factors in GISTs emphasize the necessity to develop additional reliable parameters for this tumor entity to better predict clinical behaviour. In this retrospective study, we assessed the value of serosal penetration, an established prognostic factor in solid tumors, to determine the clinical outcome in patients with GISTs.

Patients and methods

From 1996 to 2002, 264 patients with mesenchymal tumors underwent surgical therapy at the Department of Visceral and Vascular Surgery, University of Cologne, Germany. Patients had a minimum follow-up of 5 years. The histopathological

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Table I. Parameters of the original and modified Franquemont classification.

Risk	Tumor size	Mitotic index	PCNA index (%)	Ki-67 index (%)
Low risk	<5 cm	<5 mitoses/10 HPF	<10	<5
High risk	≥5 cm	≥5 mitoses/10 HPF	≥10	≥5

HPF, high-power fields and PCNA, proliferating cell nuclear antigen.

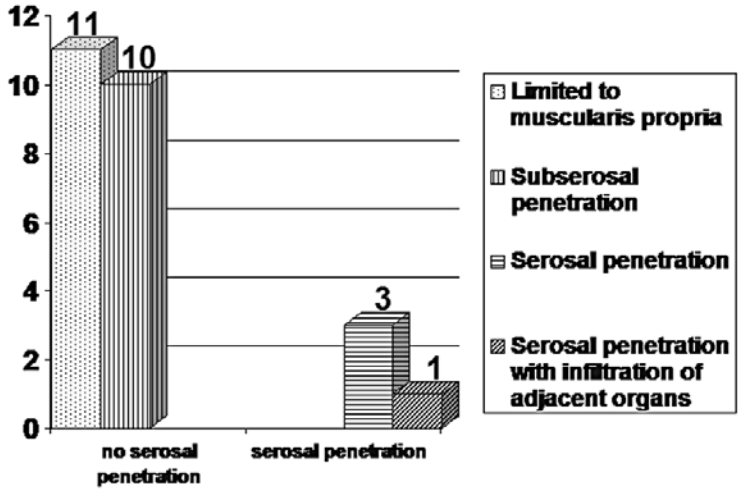

Figure 1. Frequency of serosal penetration in study patients.

reports of these patients were reviewed to identify patients with GIST. In addition, the visceral sarcomas were re-reviewed by two staff pathologists (S.E.B and U.D) for the identification or confirmation of a GIST including c-kit immunohistochemistry. Only those GISTs primarily diagnosed and treated at our Department were included in this retrospective study.

Documented patient data included age, gender, tumor localisation, date and type of operation, tumor size, presence of serosal penetration, mitotic rate, immunohistochemical variables, adjuvant therapy, recurrence, distant metastases, date of succumbing or last date of follow-up.

In addition, all tumors were re-classified according to the histopathological classification system by Franquemont (modified), as described below.

Imatinib therapy in the adjuvant setting. The European Commission approved imatinib for use in the treatment of patients with unresectable or metastatic GISTs in February 2002. Only one patient of our study group with recurrent disease received adjuvant therapy with imatinib, so that its prognostic influence was not evaluated in this study.

Histopathology and immunohistochemistry. To determine the mitotic rate (haematoxylin and eosin-stained sections) and Ki-67-positive tumor cells, we evaluated 50 high-power fields (HPF, magnification x400) of the tumor tissue of each patient. The frequencies were recorded as the number of positive cells (n)/50 HPF.

Immunohistochemical staining was performed by applying monoclonal antibodies directed against Ki-67 (MIB-1; 1:150; DakoCytomation, Hamburg, Germany), c-kit (CD117; 1:100; DakoCytomation) and CD34 (Qbend10; 1:100; DakoCytomation). Microwave pre-treatment was performed (2x7 min in citrate buffer, pH 6.0) previous to Ki-67 and CD34 staining. c-kit immunoreactivity was enhanced by autoclave pre-treatment.

Paraffin-embedded tumor tissues from pre-therapeutic biopsies and resected specimens were cut into 2-μm sections and mounted onto Superfrost Plus slides (Menzel-Glaeser, Braunschweig, Germany).

Tissue sections were deparaffinized according to routine histopathological procedures. Immunostaining was performed using a DakoCytomation Tech-Mate 500 plus immunostainer according to the manufacturer's instructions by applying the reagents: ChemMate buffer kit (K5006), peroxidase/AEC detection kit (K5003), ChemMate peroxidase blocking solution (S2023) and ChemMate Hematoxylin (S2020), all from DakoCytomation.

The slides were analyzed by two staff pathologists (S.E.B. and U.D.) who were blinded to the clinical data.

Original and modified classification system according to Franquemont. The original/modified classification according to Franquemont (2) is shown in Table I. In the original classification system the proliferation marker PCNA was used. However, studies have shown that the use of PCNA as a proliferation marker is not practical. In addition, Wong *et al* demonstrated in 108 patients with GIST that a Ki-67 proliferation index ≥5% is significantly associated with a worse prognosis (14). Therefore, in this study the Ki-67

Table II. Franquemont classification of GISTs.

Subgroup	n	%
Low risk	15	60
High risk	10	40

proliferation index with a cut-off value of ≥5% was used to classify patients instead of using PCNA as a proliferation marker. Therefore, we refer to this as the modified classification of Franquemont.

Statistical analysis. Kaplan-Meier plots were used to describe the survival distribution by the classification system of Franquemont (modified) or other clinicopathological variables. The log-rank test was used to evaluate the prognostic significance. The level of significance was set at $p<0.05$. The p-values were given for two-sided testing. All statistical tests were performed using the software package SPSS for Windows, version 11.0 (Chicago, IL).

Results

Histopathological factors. The median tumor size was 4.7 cm (range: 0.3-21). The median mitotic rate was 3 mitoses/50 HPF (range: 0-175). The tumors were completely resected (R0 resection). Twenty-one (84%) tumors did not penetrate the serosa, while in 4 cases a serosal penetration was described (Fig. 1). No lymph-node metastases were detected in the 12 patients, in which a lymph node dissection was performed.

Immunohistochemical factors. The 25 tumors were c-kit-positive and 24 of the tumors were CD34-positive. The median Ki-67 proliferation index was 2% (range 1-20). In 19 (76%) patients the Ki-67 proliferation index was ≥5% and in 6 (24%) patients <5%.

Classification system by Franquemont (modified). Table II shows the classification of the 25 tumors according to the modified Franquemont classification, as described above.

Survival analysis based on Ki-67 proliferation index, tumor size, mitotic index and serosal penetration. The log-rank test was used to evaluate the association between the Ki-67 proliferation index, tumor size, mitotic index and survival. Nineteen (76%) patients had a Ki-67 proliferation index ≥5% and in 6 (24%) patients the index was <5%. The median survival of patients with a low proliferation index was not achieved, while it was 22.93 months in patients with a high index (p=0.001; log-rank test).

Using a tumor size cut-off value of 5 cm, 12 (48%) patients had a tumor size ≥5 cm and in 13 (52%) patients the tumor was <5 cm. The median survival in the two groups was not achieved. As shown in Fig. 4B, patients with a small tumor tended to have a longer survival compared with patients that had a large tumor (p=0.07; log-rank test).

The cut-off value for the mitotic rate was 5 mitoses/50 HPF. Fourteen (56%) patients had <5 mitoses/50 HPF and 11 (44%) patients ≥5 mitoses/50 HPF. The median survival in the two groups was not achieved. Patients with a low mitotic rate had a significantly longer survival compared with patients that had a high mitotic rate (p=0.047; log-rank test).

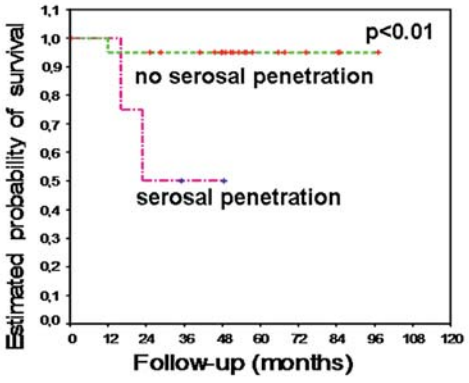

Figure 2. Plot of the probability of survival for the study patients in relation to the presence of serosal penetration.

The median survival of the 21 patients with no serosal penetration was not achieved, while it was 22.93 months in the 4 patients with serosal penetration. Patients with a serosal penetration had a significantly poorer prognosis compared with patients that had no serosal penetration (p<0.01; log-rank test; Fig. 2; Table III).

Prognostic evaluation by the Franquemont classification system. The median survival of the 2 patient groups classified according to the modified Franquemont classification (low- and high-risk) was not achieved. No tumor-related death occurred in the low-risk group while 3 of the 10 patients in the high-risk succumbed because of the tumor. There was a significantly longer survival in the low-risk compared with the high-risk group (p=0.03; log-rank test).

Multivariate analysis. A multivariate analysis was not performed because of the small study group and the low number of events.

Discussion

This retrospective study evaluated the prognostic potential of serosal penetration for the clinical outcome of patients with GISTs. We demonstrated that the presence of serosal penetration was a negative prognostic factor in this tumor entity.

Molecular abnormalities of GISTs were detected, which provided new options for the therapy of these particular mesemchymal tumors. Since the introduction of imatinib (Gleevec), a targeted inhibitor of tyrosine kinase activity in the therapy of patients with GISTs, this drug was included in the standard management of advanced disease, i.e. patients with unresectable, recurrent or metastatic disease (10). Moreover, recent data advocate the use of imatinib even in the adjuvant setting after a complete surgical resection of localized primary GISTs (11). As this drug has side-effects and is very expensive, prognostic factors identifying patients that will most likely benefit from adjuvant therapy are of particular importance.

Table III. Characteristics of patients with and without serosal penetration.

	Tumors without serosal penetration (n=21)	Tumors with serosal penetration (n=4)
Gender		
Male	13	2
Female	8	2
Age		
Median (range), years	63 (29-81)	63.5 (55-69)
Tumor location		
Esophagus	1	0
Stomach	12	3
Duodenum	1	0
Small bowel	6	0
Rectum	1	1
Tumor size		
Median (range), cm	3.8 (0.3-23)	14 (7-21)
Mitotic index		
<5 mitoses/10 HPF	14	0
≥5 mitoses/10 HPF	7	4
Ki-67 index		
<5%	18	1
≥5%	3	3
Type of surgery		
Esophagectomy	1	0
Local gastric resection	9	3
(Sub-) total gastrectomy	3	
Partial small bowel resection	7	
Anterior rectal resection	1	1

HPF, high-power fields.

Our understanding of the molecular events in the pathology of GISTs has increased. Consequently, recent studies have described sensitive predictive/prognostic factors for the therapy of patients with GISTs, such as oncogenic mutations of the c-kit or platelet-derived growth factor α (PDGFRA) kinases (12,13).

Mitotic index and tumor size are the two major clinico-pathological factors for risk stratification of GIST patients (1). Tumors with a low mitotic index and small size have a significantly better clinical outcome compared with tumors that have a high mitotic index and large tumor size. In addition, Franquemont *et al* demonstrated a prognostic value of the proliferating cell nuclear antigen (PCNA) in patients with GISTs, while Wong *et al* showed that the Ki-67-proliferation index is the more practical immunohistochemical factor for the prediction of clinical outcome in patients with GISTs (2,14). We re-confirmed the prognostic value of the mitotic index, showing that patients with tumors with a low mitotic rate had a significantly longer survival compared with a high mitotic rate. Patients with tumors <5 cm tended to have a longer survival compared with patients that had tumors ≥5 cm but this did not reach statistical significance probably due to the small number of study patients. Furthermore, the Ki-67 proliferation index was the factor with the strongest prognostic value in our study, showing a high Ki-67 proliferation index to be a negative prognostic factor in patients with GISTs. Finally, we showed that the modified Franquemont classification (using the Ki-67 proliferation index instead of PCNA) is of significant prognostic importance as patients classified in the low-risk group had a significantly longer survival compared with patients classified in the high-risk group.

Our data suggest that serosal penetration is a prognostic factor in patients with GISTs. Tumors with a serosal penetration had a significantly poorer prognosis compared with tumors without serosal penetration. To the best of our knowledge, this is the first report showing that the presence of serosal penetration in GISTs appears to be of significant prognostic impact. These findings are consistent with studies in other gastrointestinal tumors, such as esophageal, gastric and colorectal cancers, indicating that the depth of tumor invasion is significantly correlated with the clinical outcome (6-8).

In conclusion, our data imply that serosal penetration appears to be a prognostic factor for patients with GISTs. Due to the retrospective nature of our study and the small sample size our data are hypothesis-generating and should therefore be validated in larger clinical studies.

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