RESEARCH ARTICLE

Does Immunohistochemistry Provide Additional Prognostic Data in Gastrointestinal Stromal Tumors?

Lutfiye Demir[1]*, Nese Ekinci[2], Cigdem Erten[1], Yuksel Kucukzeybek[1], Ahmet Alacacioglu[1], Isil Somali[3], Alper Can[1], Ahmet Dirican[1], Vedat Bayoglu[1], Murat Akyol[1], Fulya Cakalagaoglu[2], Mustafa Oktay Tarhan[1]

Abstract

Background: To investigate the predictive and prognostic effects of clinicopathologic and immunohistochemical (IHC) features in patients with gastrointestinal stromal tumours (GISTs). Materials and Methods: Fifty-six patients who were diagnosed with GIST between 2002 and 2012 were retrospectively evaluated. Relationships between clinicopathologic/immunohistochemical factors and prognosis were investigated. Results: Median overall survival (OS) of the whole study group was 74.9 months (42.8-107.1 months), while it was 95.2 months in resectable and 44.7 months in metastatic patients respectively (p=0.007). Epitheliolid tumor morphology was significantly associated with shortened OS as compared to other histologies (p=0.001). SMA(+) tumours were significantly correlated with low (<10/50HPF) mitotic activity (p=0.034). Moreover, SMA(+) patients tended to survive longer and had significantly longer disease-free survival (DFS) times than SMA (-) patients (37.7 months vs 15.9 months; p=0.002). High Ki-67 level (≥30%) was significantly associated with shorter OS (34 vs 95.2 months; 95%CI; p=0.001). CD34 (-) tumours were significantly associated with low proliferative tumours (Ki-67<%10) (p=0.026). Median PFS (progression-free survival) of the patients who received imatinib was 36 months (27.7-44.2 months). CD34 (-) patients had significantly longer PFS times than that of negative tumours; (50.8 vs 29.8 months; p=0.045). S100 and desmin expression did not play any role in predicting the prognosis of GISTs. Multivariate analysis demonstrated that ≥10/50HPF mitotic activity/HPF was the only independent factor for risk of death in GIST patients. Conclusions: Despite the negative prognostic and predictive effect of high Ki-67 and CD34 expression, mitotic activity remains the strongest prognostic factor in GIST patients. SMA positivity seems to affect GIST prognosis positively. However, large-scale, multicenter studies are required to provide supportive data for these findings.

Keywords: Gastrointestinal stromal tumor - immunohistochemistry - predictive potential - prognosis

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Introduction

Gastrointestinal stromal tumor (GIST) is a recently diagnosed tumor and known as the most common type of mesenchymal tumors of the GI tract (Rubin et al., 2007). GISTs include a wide spectrum of tumors with different clinical behaviour and can occur in the entire gastrointestinal (GI) tract and may also arise from the omentum, mesenteries, and retroperitoneum. Gain-of-function mutations of c-kit proto-oncogene and PDGFRA (platelet-derived growth factor receptor-α) protein occur in around 90% and <5% of GISTs respectively (Hirota et al., 1998; Tornillo and Terracciano, 2006). The definite diagnosis of GIST depends on histological and immunohistochemical evaluation. Expression of the receptor tyrosine kinase, KIT which is demonstrated by an IHC marker, CD117, is seen in almost all GISTs (95%), regardless of the site of origin, histologic morphology, or biologic behavior, and thus, is regarded as one of the key diagnostic markers (Kindblorn et al., 1998; Hornick and Fletcher, 2002). Smooth muscle actin (SMA), S-100, CD34, desmin are other markers that can be observed in various rates. About 60-70% of GISTs are CD34 positive, 30-40% are positive for smooth muscle actin, 5% for S-100 protein and 1-2% are positive for desmin or keratin (Miettinen et al., 1999; Miettinen et al., 2006). The resection of the tumor with a negative surgical margin is the mainstay of therapy for primary GIST. However about 40-90% of resected GIST patients experience disease recurrence and 28-35% five-year survival rates have been reported for these patients (DeMatteo et al., 2000). The assesment of relapse risk for GIST patients is based on mitotic rate, tumor size, tumor site, surgical margins and the status of tumor rupture (Joensuu, 2008). Currently,

*[1]Department of Medical Oncology, [2]Department of Pathology, Ataturk Training and Research Hospital, Izmir Katip Celebi University, [3]Department of Medical Oncology, Institute of Oncology, Dokuz Eylul University, Turkey *For correspondence: demir.lutfiye77@gmail.com*

small thyrosine kinase inhibitors (TKI) such as, imatinib mesylate (van Oosterom et al., 2001) sunitinib (Demetri et al., 2006), and sorafenib (Heinrich et al., 2012) inhibit the function of PDGFRA and c-kit receptor and have been used for the treatment of metastatic GIST. Over 80% of metastatic GIST patients benefit from TKI therapy, and in the past few years, the efficacy of adjuvant treatment with imatinib mesylate specifically in high risk patients has been proven (Li et al., 2011). However, primary or secondary resistance to TKI therapy is the most important factor that decrease the chance for cure of these patients (Nilsson et al., 2005; Heinrich et al., 2006; Desaj et al., 2007). Genetic mutation analysis of metastatic GIST patients revealed that, KIT gene exon 11 mutations had better median progression-free survival times than the patients whose GIST encoded exon 9 mutations or those with wild-type kit independent from the effect of KIT (CD117) expression intensity (Hornick and Fletcher, 2007). The expression level of Ki-67 labeling index and its correlation with tumor mitotic activity is also examined in many studies. Higher Ki-67 levels were associated with poor prognosis in these studies, however, a cut-off level of for Ki-67 labeling index is still unclear (Ohdaira et al., 2005; Liang et al., 2008; Aoyagi et al., 2009). Moreover, it is unknown whether the presence of other immunohistochemical markers (CD34, SMA, desmin, S100 protein) predicts patient outcome and response to imatinib therapy. In this report, we tried to investigate both prognostic and predictive effects of these immunohistochemical parameters in a GIST cohort, including both resectable and metastatic patients. We also investigated their relationship with each other.

Materials and Methods

Patients

Fifty-six GIST patients diagnosed at a single center, Izmir Katip Celebi University, Ataturk Training and Research Hospital, between July 2003 and February 2012 including both early (localized resectable diease) and advanced (unresectable or metastatic) stages were retrospectively assessed. The clinical data included age, gender, primary tumor site, tumor size, the initiation and completion date of TKIs, progression date (or relapse date for resected patients) and last visit (or death) date. Follow-up time was defined as the time from the diagnosis to the last visit. Disease-free survival (DFS) was used for completely resected (early stage) patients and defined as the time from the surgery to the relapse date or last visit which came first. Progression-free survival (PFS) was used for the patients who received TKI treatment for advanced disease and defined as the time from the iniatiation of TKI until the disease progression or last visit which came first. Overall survival (OS) was defined as the time from the diagnosis to the last visit or death which came first. The evaluation of response to TKIs in metastatic patients was performed by using RECIST criteria (Eisenhauer et al., 2009).

Histopathological and immunohistochemical analysis

Pathologic and morphologic features (such as tumor morphology, mitotic activity, tumor size, ulceration, necrosis) were obtained retrospectively from the medical records of these patients which were archieved in the out-patient clinic of Department of Medical Oncology. Diagnosis of GIST was based on the criteria published by Miettinen et al. (2001). Tumor size was accepted as the largest diameter in any dimension. The following three histologic types were defined according to the criteria: pure spindle, epithelioid and mixed (both spindle and epithelioid). The hematoxylin and eosin (HE) sections were used to assess the number of mitotic figures from 50 consecutive high power fields (HPF) (×40 magnification) (Figure 1). Risk stratification was carried out using National Institutes of Health (NIH) consensus classification system and completely resected (early stage) GISTs were classified as very low risk, low risk, intermediate risk, high risk (Dematteo et al., 2008). The expression of the markers such as CD117,CD34, SMA, S100, desmin, Ki-67 proliferation (labeling index) which were previously evaluated by immunohistochemical (IHC) staining analysis and the pathologic morphology (histologic type, the status of ulceration and necrosis) were obtained from the pathology reports. The staining intensity of KIT positive cells was subjectively evaluated by the same pathologist according to following degrees: + (weak), ++ (intermediate) and strong (+++) positive. The immunoreactivity for CD34, desmin, SMA, and S100 protein was defined as positive or negative (The apperance of positive expression for these markers are demonstared in Figure 2).

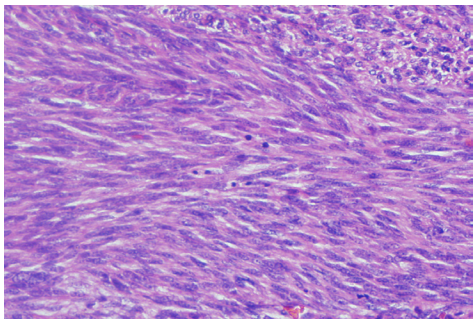


Figure 1. The Apperance of Atypia and Mitotic Activity in GIST (×100 Magnification)

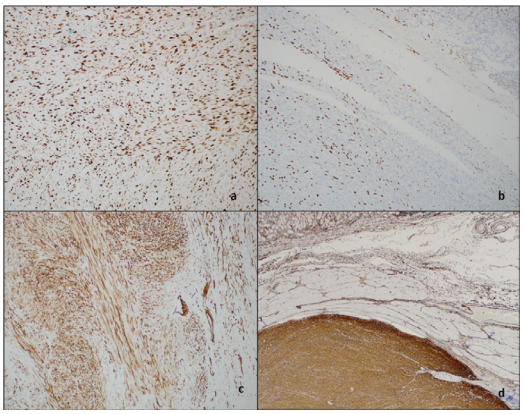
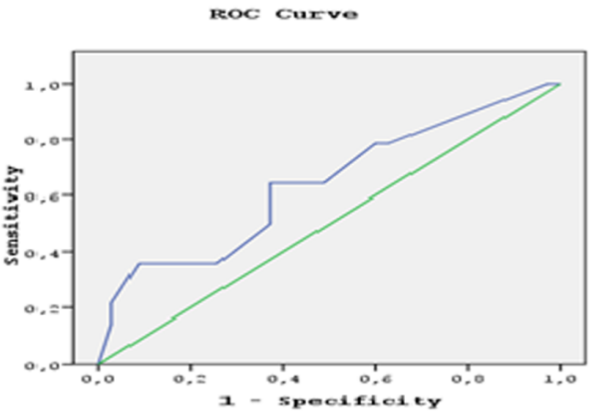


Figure 2. The Appearence of Immunoreactivity of GIST Cells for A) Ki-67 (×100 Magnification) B) S-100 Protein (×40 Magnification) C) SMA (×40 Magnification) and D) CD34 (×40 Magnification)



Diagonal segments are produced by ties.

Area Under the Curve

Test Result Variable(s): Ki-67

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.641	.091	.127	.463	.818

The test result variable(s): KY67SAYI has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 3. The ROC curve and the statistical significance of cut-off value for Ki-67 Labeling index

Statistical analysis

Statistical analysis was performed using SPSS 16 (Statistical Package for the Social Sciences, version 16) statistical programme. Clinical features was defined by descriptive analysis; median and mean values were calculated. The difference between two variables was sought by x^2 (Chi-square) test. Nonparametric test (Kruskal Wallis) was used to compare the effect of a parameter between two multi-sorted variable. Survival analysis was carried out by Kaplan-Meier analysis; all of the ranges were stated with a 95% confidence interval (CI). The survival comparison between two different parameters was fulfilled by using Log-rank test. Due to the lack of any accepted cut-off level for Ki-67 labeling index, the prognostic and predictive effect of Ki-67 was sought by using median levels. Additionally ROC curve analysis was performed to determine the cut-off level. However, area under curve was 0.64 and thus, this analysis could not be used appropriately (Figure 3). The most common prognostic levels for Ki-67 labeling index from the previous analyses are 10%, 30%, 40%. Therefore, we also analysed the prognostic and predictive effects according to the these levels besides the median value. The correlation between two variables was performed by using Pearson correlation test. Multivariate analysis was performed to search for the independent factors on OS by using the backward stepwise method of the Cox proportional hazard regression model with a 95% confidence interval (CI). Statistical significance limit was accepted as p values under 0.05 for all tests.

Results

Patient characteristics

Median age was 56 years SD±12 (25-84 years) and 73.2% (n=41) of the patients were over fifty. The rate of male and female patients were 53.6% (n=30) and 46.4% (n=26) respectively. The most common localization of GISTs were stomach followed by omentum, small intestine, colorectal and eosaphagus respectively. Twenty seven patients had undergone surgical procedure and total removal of the tumor without capsule rupture or residual tumoral tissue could be achieved in 85.1% (n=23) of these patients. Among these operable patients, three patients received adjuvant imatinib treatment. Five patients (8.9%) were initially irresectable of whom two of them could be operated following 9 months of neodjuvant imatinib treatment. Twenty-four patients (42.9%) presented with metastatic disease at initial diagnosis, and the leading site of metastases was liver followed by intraabdominal lymph nodes and peritoneal cavity. Imatinib mesylate, 400 mg, was the initial treatment in these 29 patients. Twelve patients were treated by second-line sunitinib after imatinib failure. Two patients who were progressed after imatinib imatinib and sunitinib received sorafenib.

The association of histologic and immunohistochemical markers with clinical and prognostic features

The association of morphologic features (such as tumor morphology, the status of ulceration or necrosis) and immunohistochemical markers (CD34, SMA, S100, desmin and Ki-67 labeling index) with mitotic activity, tumor size and clinical factors (age, localization, tumor resectability, peritoneal or hepatic involvement) were individually investigated in whole study group (both for patients with completely resected and advanced disease). Spindle cell morphology was the predominant histologic type. Tumor morphology did not play any role in tumor origin, resectability or mitotic activity; however, the epithelioid morphology was significantly associated with larger tumors and peritoneal metastasis when compared to the tumors with other morphologies (Kruskal-Wallis test, p=0.035 and p=0.02 respectively). No relationship was observed between the tumor ulceration and the following variables: age (p=0.57), tumor diameter (p=0.51), mitotic activity (p=0.66), the origin of tumor (p=0.16), peritoneal/hepatic metastases (p=0.44/p=0.78). Tumor necrosis was much more observed in larger tumors when compared to smaller tumors (73.3% for ≥10 cm tumors vs 26.7% for <10 cm tumors; p=0.018). Additionally high mitotic activity was more common in the tumors with necrosis (51.7% for tumors with necrosis vs 19% for tumors without necrosis; p=0.019). The patients older than fifty had a tendency to present more frequently with CD34 (+) tumors than the younger patients (79.1% vs 20.9%) and CD34 (+) tumors found to be more frequently seen in tumors larger than 10 cm (61.9% vs 38.1%). However these two results were not supported by statistically significance level (p=0.09 and p=0.32 respectively). Conversely, the majority of CD34 positive tumors had significantly lower mitotic activity rates than CD34 (-) tumors (31% vs 63.6%; p=0.047). SMA (+) tumors were significantly associated with smaller tumors (<10 cm) (SMA positivity in smaller tumors was 86.7% vs 55.6 % in larger tumors; p=0.034). Both S100 and desmin did not show any relationship with the above parameters. Median Ki-67 labeling index value for the whole study group was 6% (SD:14.02; ranges:1-50).

The prognostic effect of Ki-67 Labeling index and it's relationship with other factors Ki-67 proliferation index was evaluated also according to three risk levels: Ki-67 <10%, Ki-67 10-29% and Ki-67 ≥30%. The tumors with low Ki-67 level tended to have low mitotic activity, however a statistically significant relationship between tumor mitotic activity and Ki-67 labeling index could not be demonstrated (p=0.16). Additionally no correlation was observed between the Ki-67 labeling index and mitotic activity (Pearson correlation test: r=0.011, p=0.942). Moreover, no relationship was noted between Ki-67 and age (p=0.64), tumor localization (p=0.83), tumor size (p=0.42), metastasis (p=0.73) or resectability (p=0.73) respectively. Similarly Ki-67 proliferation index did not significantly differ according to SMA, S100, or desmin immunoexpression. However, a meaningful relationship between CD34 immunoexpression and Ki-67 labeling index was observed. CD34 (-) tumors had significantly lower Ki-67 labeling index levels than CD34 (+) tumors [90% vs 51.3% for CD34 (-) and CD34 (+)tumors respectively; p=0.026].

Relationship with GIST risk groups among resectable patients

The same parameters were reevaluated among only resectable patients to seek the relationship with risk groups which were stratified according to NIH classification system. Histologic morphology, ulceration and Ki-67 status, and the expression of markers such as CD34, SMA, Desmin, S100 did not significantly vary according to different GIST risk groups. However, the tumors with necrosis significantly tended to belong to high-risk tumors (p=0.04) (see Table 1).

Survival analysis

Overall survival (OS): Median overall survival (OS) of the whole study group was 74.9 months (range 42.8-107.1 months, 95%CI). Median OS was 95.2 months in resectable patients while it was 44.7 months in patients with advanced disease (p=0.007). Survival time did not differ according to age groups (p=0.52). Epithelioid morphology was significantly associated with shorter survival when compared with spindle cell and/or mixt histology (29.2 months vs 96.1 months for spindle cell, 61 months for mixt histology; 95%CI; p=0.001) (Figure 4a). Tumor ulceration consituted a tendency of shortened survival, however no significant difference could be demonstrated (68 months vs 101 months; 95%CI; p=0.065). Both tumor size and mitotic activity had an important negative effect on survival; the patients with tumors larger than 10 cm survived significantly shorter than the patients with smaller tumors (61 months vs 93 months; 95%CI; p=0.013) (Figure 4b). Similarly, tumors with high mitotic activity had significantly shortened survival as compared to the tumors with low mitotic activity (61 months vs 98 months; 95%CI; p=0.032) (Figure 4c). Tumor localization had an impact on survival of GIST patients as expected; 95.2 months for gastric tumors vs 68.6 months for nongastric tumors), however it did not reach a statistical significance level (95%CI; p=0.46). CD34 (-) patients tended to survive longer than positive patients, however no significant difference was demonstrated (95 vs 68 months, 95%CI; p=0.49). Conversely SMA positivity comprised a tendency to have longer OS; however similar with CD34 results no significant difference could be demonsrated statistically (99 months vs 53.4 months, 95%CI; p=0.06). Immunoreactivity for desmin (95% CI; p=0.12) and S-100 (95% CI; p=0.87) did not perform a valuable difference in survival rates of GIST patients as well. The patients with

Table 1. The Relationship between Clinicopathologic Factors and GIST Risk Groups

Risk groups Variable	Very risk		Low risk		Intermediate risk		High risk		p value
	n	%	n	%	n	%	n	%	
Histologic morphology									
Spindle cell	2	100	0	0	5	71.4	9	52.9	
Epithelioid	0	0	0	0	0	0	1	5.9	0.33
Mixt	0	0	1	100	2	28.6	7	41.2	
Ulceration									
Yes	1	50	0	0	3	42.9	7	43.8	
No	1	50	1	100	4	57.1	9	56.2	0.85
Necrosis									
Yes	0	0	0	0	1	16.7	12	75	
No	1	100	1	100	5	83.3	4	25	0.04
CD34									
Positive	1	50	1	100	7	100	14	82.4	
Negative	1	50	0	0	0	0	3	17.6	0.34
SMA									
Positive	1	50	0	0	3	42.9	5	31.2	
Negative	1	50	1	100	4	57.1	11	68.8	0.80
Desmin									
Positive	0	0	0	0	1	14.3	1	6.2	
Negative	2	100	1	100	6	85.7	15	93.8	0.87
S100									
Positive	1	50	0	0	1	14.3	7	41.2	
Negative	1	50	1	100	6	85.7	10	58.8	0.51
Kİ-67 proliferation index									
>6	1	50	0	0	2	28.6	9	60	
≤6	1	50	1	100	5	71.4	6	40	0.43
Age									
>50 y	1	50	0	0	7	100	9	52.9	
≤50 y	1	50	1	100	0	0	8	47.1	0.09
Relapse									
Yes	0	0	0	0	3	42.9	10	71.4	
No	1	100	1	100	4	57.1	4	28.6	0.07
Exitus									
Yes	0	0	0	0	1	14.3	4	23.5	
No	2	100	1	100	6	85.7	13	76.5	0.79

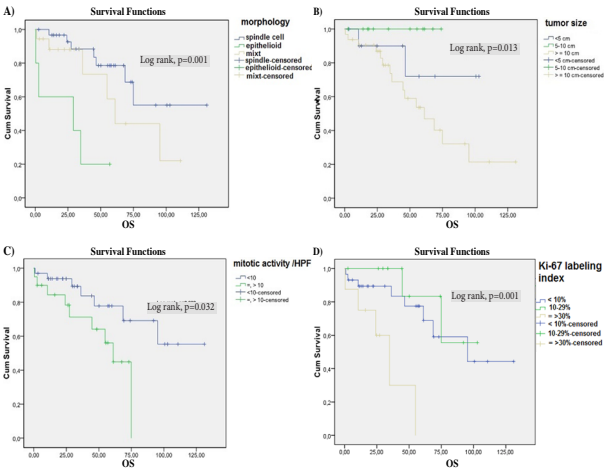


Figure 4. Kaplan-Meier Curve for Overall Survival Analysis According to A) Histologic Type B) Tumor Size C) Tumor Mitotic Activity D) Ki-67 Labeling Index

a Ki-67 labeling index ≤6% (under median) tended to have slightly better survival than the patients with higher Ki-67 level (>6%) without any statistically significance. However, Ki-67 proliferation index greater than 30% was found as an important negative prognostic factor for GIST patients. The patients with high Ki-67 level (≥30%) had significantly shorter OS than the patients with Ki-67 10-29% or Ki-67<10% (34.7 vs 95.2 and 85.4 months respectively, 95%CI; p=0.001) (Figure 4d).

Disease-free survival (DFS): Median DFS was 34.7 months (24.6-44.8 months, 95%CI) in resectable patients, it did not differ according to age groups (median 30 months in patients over fifty vs 27 months in younger patients; 95%CI; p=0.74). Similarly, both the tumor origin and size did not affect DFS (28 months for gastric, 24 months for nongastric; p=0.91, and 28 months for ≥10 cm tumors and 30 months for <10 cm tumors; 95%CI; p=0.84). However, the tumors with high mitotic activity significantly tended to relapse earlier (27 months vs 37 months; p=0.01). Coherent with OS results, tumors with epithelioid morphology tended to relapse earlier, but statistical significance could not be demonstrated (15.9 months for epithelioid vs 24 months-for mixed vs 34.7 months for spindle cell types; 95%CI; p=0.11). Tumor ulceration or necrosis were not effective markers on relapse times (95%CI; p=0.31, p=0.44 respectively). No significant association with immunoexpression of CD34, S100, desmin and relapse times was found as well (95%CI; p=0.51, p=0.15, p=0.73 respectively). However, SMA (+) patients relapsed significantly later than the SMA (-) patients (37.7 months vs 15.9 months; 95%CI; p=0.002) (Figure 5). Consistent with the OS results, the relapse was developed earlier in patients with high Ki-67 level (Ki-67 ≥30%) than the patients with lower levels (15.9 months vs 34.7 and 37.7 months; 95%CI; p=0.01).

Progression-free survival (PFS): Median PFS of the advanced -staged patients (who received imatinib) was 36 months (range: 27.7-44.2 months, 95%CI). PFS analysis was evaluated according to age, primary location of the tumor (gastric-extragastric) histologic morphology, mitotic activity, SMA, S100, histologic morphology or Ki-67. No significant difference was observed in PFS according to these variables. However, CD34 (+) patients had significantly shorter PFS than CD34 (-) patients (29.8 vs 50.8 months; 95%CI; p=0.045) (Figure 6).

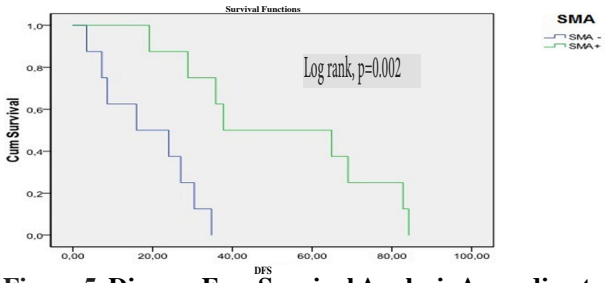


Figure 5. Disease-Free Survival Analysis According to Tumor SMA Expression

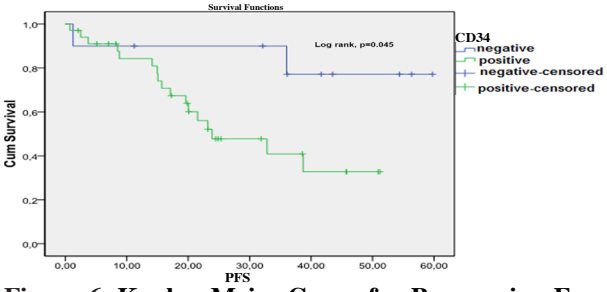


Figure 6. Kaplan-Meier Curve for Progression-Free Survival Analysis According to CD34 Expression

Table 2. Cox Multivariate Regression Analysis for Risk of Death

Variable		Exp(B)	95% CI	p
Epithelioid morphology	Yes	2.58	0.42-15.72	0.3
	No	1		
SMA negativity	Yes	1.26	0.27-5.88	0.76
	No	1		
CD34 positivity	Yes	0.56	0.08-3.7	0.54
	No	1		
Ki-67 proliferation ≥30%	Yes	1.28	0.29-5.65	0.73
	No	1		
Tumor diameter ≥10 cm	Yes	2.67	0.56-12.6	0.21
	No	1		
Mitotic activity ≥10/HPF	Yes	5.46	1.58-18.8	0.007
	No	1		

Multivariate analysis

The factors which were found to have an influence on survival or considered to be important were reanalysed by Cox multivariate regression analysis. A cox regression model to identify the independent factors for risk of death was set by using the following variables: having tumor with epithelioid morphology or not, ≥10/HPF mitotic activity or not, ≥10 cm in diameter or not, Ki-67 proliferation level ≥30% or not, SMA negativity or not, CD34 positivity or not. This analysis demonstrated that among these parameters, high mitotic activity [Exp(B): 3.58 (1.03-12.4, 95%CI), p=0.045] was the strongest independent predictor for risk of death (Table 2).

Discussion

After the recognition of the GIST as a separate tumor from gastrointestinal sarcomas, several predictive and prognostic factors have been defined in the past two decades. Tumor size, origin and mitotic activity are the leading features that have been widely approved as being predictive of clinical outcome for resected GIST patients (Dematteo et al., 2008). The discovery of the particular molecular abnormalities and mutational analyses provided to define certain GIST subtypes that demonstrate divergent responses to TKI therapy and had different clinical behavior (Mazurenko et al., 2011). Previous studies demonstrated that KIT exon 11 mutations show the greatest benefit from imatinib treatment, while the patients with exon 9 mutations require a higher imatinib dosage to reach a higher response and are associated with a worse course than the patients with exon 11 mutations (Singer et al., 2002; Debiec-Rychter et al., 2006). The tumors without any mutations (PDGFRA or KIT) are defined as 'wild type' and these tumors usually demonstrate shorter survival times than the tumors with KIT or PDGFRA mutations.

The expression of immunohistochemical markers such as CD34, SMA, S100, desmin, Ki-67 varies in different sarcomas including GIST (Fletcher et al., 2002). However, we do not know whether these markers have a predictive or prognostic role in GISTs yet. In this study, we aimed to clarify the prognostic and predictive factors in GISTs, besides to answer this question in a GIST cohort, including both resectable and unresectable patients.

The spindle cell morphology is the most common histologic type in GISTs. However, the prognostic effect of tumor morphology is not clear from previous studies. Epithelioid cell morphology is associated with poor prognosis in the majority of published GIST series (Fujimoto et al., 2003; Kim et al., 2005; Koay et al., 2005; Miettinen et al., 2006). In a report, the five-year survival rates were significantly higher in patients with spindle cell type when compared to epithelioid or mixed morphology (Singer et al., 2002). However, some studies revealed no relationship between tumor morphology and survival (Reith et al., 2000; Martin et al., 2005; Sciot et al., 2008). In our study, also spindle cell morphology was the predominant histology and the overall survival of the patients with spindle cell tumors were significantly longer than the other two morphologies; and epithelioid cell type was significantly associated with poor outcome.

Tumor ulceration or necrosis are also other prognostic factors that were previously proposed that they have a negative impact on patient outcome (Fujimoto et al., 2003). Consistent with the literature, tumor ulceration and necrosis comprised a tendency to have shorter OS; however no statistical significance can be demonstrated; perhaps this may be due to the smaller number of resectable patients in the study population.

The tumor site and size are widely known prognostic factors, as both of them constitute the basis of GIST risk classification systems. After the adjuvant imatinib mesylate trials, it was shown that the most benefit was observed in high risk groups (Casali et al., 2012). However, there is not any data supporting the impact of tumor mitotic activity and origin on imatinib response in metastatic patients. In this study, we also explored this question and could not find any association with PFS times. In this study, we demonstrated the negative impact of high mitotic activity and larger tumors on the survival of our GIST patients. However, we could not establish any association between the treatment response and above important pathologic factors in patients with advanced stage receiving imatinib mesylate. Time to tumor progression times did not differ betwen gastric and extragastric tumors and between the tumors with high or low mitotic activity.

The prognostic significance of Ki-67 in GISTs have been explored in several studies (Wang, 2002; Nagasako, 2003; Liang et al., 2008; Aoyagi et al., 2009). Similar results were obtained from these studies and the prognostic role of Ki-67 and the relationship with mitotic activity were demonstrated. However there is not any accepted cut-off value for Ki-67 labeling index for use in clinical practice to predict prognosis (Wang, 2002; Liang et al., 2008; Ogino et al., 2013). In this study, the patients with a Ki-67 labeling index greater than 30% had significantly shorter overall survival and disease free survival times than the patients with Ki-67<10%, and also this provides an additional data for the prognostic effect of Ki-67 in GISTs.

The predictive and prognostic effects of immunohistochemical markers were investigated only in a few number of studies previously. There are divergent results from these studies. The majority of these studies revealed no relationship between the expression of CD34, SMA, S-100, desmin protein and prognosis (Chirieac et al., 2006; Sanchez et al., 2007; Sciot et al., 2008). CD34 positive and larger tumors were significantly associated with aggressive behavior in a study (Liang et al., 2008). The predictive role of CD34 immunoreactivity was examined only in one study. This was a retrospective analysis of BFR-14 study which was performed by Bertucci et al. (2011) and established that CD34 (+) tumors were associated with longer PFS for imatinib. In our study, the majority of CD34 positive tumors were greater than 10cm in size and the patients with CD34 (+) patients tended to have shorter OS than CD34 (-) patients. Moreover, high mitotic activity (>10/HPF) was more frequently observed in CD34 (+) tumors. Thus, we concluded that CD34 immunoreactivity was a negative prognostic factor in this study population. Conversely with the results of Bertucci's analysis, CD34 (+) patients had significantly shorter progression-free intervals for imatinib. This finding was compatible with the result of another study which showed that the tumors which present with a cystic degeneration after imatinib therapy were mostly CD34 (-) tumors (Koh et al., 2012). Therefore, CD34 immunoreactivity seems to be both negative predictive and prognostic factor. Positive immunoexpression for smooth muscle actin (SMA) was associated with a poor (Bertin et al., 2007) clinical outcome in a study and 39% vs 100% 5-year survival rates were given for SMA positive and negative tumors respectively. Conversely, another study in which only small intestinal stromal tumors were evaluated, SMA positivity was associated with a benign behavior (Chiu et al., 2005). In our study, SMA positive tumors were frequently consisted of <10 cm tumors. Moreover, completely resected SMA (+) patients relapsed significantly later than SMA negative patients. Consistently, SMA (+) patients had a tendency to survive longer. However, no relationship between imatinib response and SMA expression was shown in patients with advanced disease. Similar with other markers, no association was reported between S-100 immunoexpression and GIST prognosis in several retrospective studies. However, only two studies proposed that S-100 expression had a negative prognostic effect on GISTs (Fujimoto et al., 2003; Perez et al., 2007). In our study group, both S-100 and desmin were not associated with GIST prognosis relevant with the results of the great majority of the studies.

In summary, this study points out the following important findings: *i*) Epithelioid morphology was significantly associated with poor clinical outcome (negative prognostic marker). *ii*) SMA immunoreactivity was associated with smaller tumors and with a better disease-free survival in completely resected GISTs (positive prognostic marker). *iii*) CD34 positive

immunoexpression was more commonly associated with larger tumors and high mitotic activity. The duration of imatinib response in CD34 (+) tumors were significantly shorter (negative prognostic and predictive marker). *iv*) S-100 and desmin expression status did not have an impact on prognosis and treatment responses. *v*) The primary site of the tumor origin was not prognostic or predictive in this GIS cohort. *vi*) Both tumor size and mitotic activity were prognostic factors in this study group. Tumors larger than 10 cm or tumors with a mitotic activity >10/50 HPF were significantly associated with poorer survival rates (negative prognostic marker) . However the size of the primary tumor and the mitotic activity were not directly associated with imatinib response in advanced stages. *vii*) No significant correlation was observed between Ki-67 proliferation (labeling) index and tumor mitotic activity. *viii*) Tumors with a Ki-67 labeling index of 30% and greater was related with shorter overall and disease-free survival times (negative prognostic marker). *ix*) Multivariate analysis demonstrated that the mitotic activity greater than 10/50HPF was found as the most effective and independent prognostic factor in GIST patients,

In conclusion, we demonstrated that besides the traditional risk factors, CD34 positivity, SMA negativity and high Ki-67 labeling index (≥30%) have additional negative influence on prognosis of GIST patients. Shorter PFS times in CD34 (+) patients, considers CD34 immunoreactivity as a candidate for being a predictive marker for imatinib treatment. However, further multi-center or prospective studies are required to support these findings and to use in daily clinical practice.

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