RESEARCH ARTICLE

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Clinicopathologic Characteristics of Gastrointestinal Stromal Tumors and Prognostic Importance of Ki-67 Labeling Index: May be a New Prognostic Marker

ABSTRACT

Objective: The biological behavior of gastrointestinal stromal tumors (GISTs) varies widely and it is diffucult to predict their malignant potential with the current risk classification criterias. Therefore, we aimed to analyse the prognostic importance of Ki-67 LI for estimating survival outcomes in patients with GISTs.

Methods: For the last 11 years, between 2006 and 2017, who had been treated after surgery were included. A single pathologist re-defined the histologic examples of all cases retrospectively.

Results: Totally, 104 patients were included in the study. The median follow-up time was 73 months (range; 6 to 148 months). Seven of the 104 patients (7%) had local recurrence, 26 of the 104 patients (25%) had distant metastases and 11 of the 104 patients (11%) died during the follow-up period. The mean OS was 133 (range; 124 to 141) and the mean DFS was 117 (range; 107 to 127) months for patients. The disease progression or recurrence during follow up and increasing age were the significant prognostic factors for OS. Mitotic count, growth pattern, tumor location and Ki-67 LI were the significant prognostic factors for DFS. According to multivariate analyses, the Ki-67 LI was the only prognostic factor for estimating disease progression or recurrence (p=0.04).

Conclusions: The most important prognostic factors that affect OS were the age and disease progression or recurrence of disease. Ki-67 LI was the only prognostic factor for estimating disease progression or recurrence. As the follow-up period increases, we think that Ki-67 LI also will affect overall survival.

Keywords: Gastrointestinal Stromal Tumors, Ki-67, Prognostic Factors, Survival.

Gastrointestinal Stromal Tümörlerin Klinikopatolojik Özellikleri ve Ki-67 proliferasyon İndeksinin Prognostik Önemi: Yeni Bir Prognostik Belirteç Olabilir

ÖZET

Amaç: Gastrointestinal stromal tümörler (GIST'ler), gastrointestinal sistemin en sık görülen mezenkimal neoplazmlarıdır. GIST' lerin biyolojik davranışı çok değişkendir ve mevcut risk sınıflandırma kriterleri ile malignite potansiyellerini tahmin etmek oldukça güçtür. Bu nedenle, çalışmamızda GIST' li hastalarda sağ kalım sonuçlarını tahmin etmek için Ki-67'nin prognostik önemini analiz etmeyi amaçladık.

Gereç ve Yöntem: 2006 ve 2017 yılları arasında cerrahi sonrası tedavi altına alınan hastalar çalışmaya dahil edildi., Tüm vakaların histolojik örnekleri geriye dönük olarak tek patolog tarafından yeniden değerlendirildi.

Bulgular: 104 hasta çalışmaya dahil edildi. Ortalama takip süresi 73 aydı (6- 148 ay). Takip süresince 104 hastanın 7' sinde (% 7) lokal nüks, 26' sında (% 25) uzak metastaz mevcut olup 11' i (% 11) ex idi. Tüm hastalar için ortalama genel sağ kalım (OS) 133 (124-140) aydı. Ortalama hastalıksız sağ kalım (DFS) 117 (107-127) aydı. Takip süresince hastalığın progresyonu, rekürrensi ve yaş artışı OS için önemli prognostik faktörlerdi. Mitoz sayısı, büyüme paterni, tümör yerleşimi ve Ki-67 indeksi DFS için önemli prognostik faktörlerdi. Çok değişkenli analizlere göre, Ki-67 hastalığın ilerlemesi veya nüksünü tahmin etmede tek prognostik faktördü (p = 0.04).

Sonuç: OS' yi etkileyen en önemli prognostik faktörler; yaş, hastalık progresyonu veya nüx idi. Ki-67, hastalık progresyonu veya nüksünü tahmin etmede tek prognostik faktördü. Bu sonuçlar ışığında takip süresi uzadıkça Ki-67'nin genel sağ kalımı da etkileyeceğini düşünüyoruz.

Anahtar Kelimeler: Gastrointestinal Stromal Tümör, Ki-67, Prognostik Faktörler, Sağ Kalım.

INTRODUCTION

Gastrointestinal stromal tumors (GISTs), are the most common mesenchymal neoplasms of the gastrointestinal tract and arise from interstitial cells of cajal (1). These tumors are most frequently occuring in the stomach (60%), small intestine, ileum and jejunum (30%), duodenum (5%), rectum and colon (3–5%), respectively (2). The signs and symptoms of GISTs are varied, depends on tumor size and location. Abdominal distension, gastrointestinal bleeding, and vague pain are the most common clinical symptoms of disease (3).

The mainstay treatment modality for localized tumors is surgery with clear resection margins (4). The characteristics of pathologic specimens are crucial for directing adjuvan treatment and predicting the survival (5). The most significant pathological parameters for estimating prognosis are tumor size and mitosis. Fletcher et al. determined the "National Institutes of Health consensus" which defined aggressive tumors according to the mitotic count (>5mitoses/ 50HPF) and tumor size (> 5cm) accepted as high-risk characteristics (6). Gold et al. from Memorial Sloan Kettering Cancer Center (MSKCC) and Miettinen et al. from the Armed Forces Institute of Pathology (AFIP) identified two additional risk stratification systems included in tumor location as a 3th pathologic parameter related with enhanced risk of recurrence (7).

In spite of consistent data on clinicopathological features of GISTs, almost all risk stratification systems for predicting the prognostic subgroups of GISTs have some constraints in estimating survival. In this context, there is a necessity to define new prognostic markers on the purpose of predicting tumor behavior and prognosis. Immunohistochemical determination of Ki-67 is the method most widely utilized in clinical practice to evaluate the proliferative activity of cancer cells. Except for the resting phase of cell cycle (G0-phase), Ki-67 is determined in all proliferative phases (G1-, S-, G2- and M-phase) (8). Some studies showed that the percentage of Ki-67 positive cells, also named as ‘labeling index (LI)’, can be utilized for the risk stratification of GISTs (9,10). But, the prognostic value of Ki-67 index in GISTs patients is still uncertain.

In the current research, we therefore aimed to analyse the prognostic importance of Ki-67 LI in GISTs patients who were treated with definitive approachs.

MATERIAL AND METHODS

Patient Characteristics: Patients with GISTs who had been operated between 2006 and 2017 were included in this study. The excluding criterias were: follow-up time <6 months, age <18 years, Karnofsky Performance Status (KPS) < 70, a history of other type of cancer within the last 5 years and documented metastasis at the time of diagnosis. The patients who received preoperative chemo or radiotherapy were excluded from the study. Finally, the data of 104 patients with GISTs were evaluated.

This research was confirmed by the board of our university and complied with the Declaration of Helsinki.

Treatment and Follow-up: After radical surgery, if the disease was localized primary GISTs, no adjuvant therapy was recommended. Adjuvant imatinib was recommended, if the disease was locally advanced or patients with intermediate-to-high risk disease. Patients were examined for tumor status in three month intervals for 2 years and in six month interval for 3 to 5 years, and annually thereafter. Follow-up information was collected by review of electronic inpatient records.

Histopathological Evaluation: A single pathologist (F.S.) re-defined the histologic examples of all cases retrospectively based on the guideline recommendation of the Collage of American Pathologists (CAP). Hemotoxylen-Eosine stained, formaline fixed and paraffin wax-embedded tumor slides were re-evaluated to verify tumour morphology. All the microscopic and gross characteristics of surgical specimens were recorded including primary tumor location, tumor size, number of mitosis, tumor morphology, and prognostic group. The diagnosis was comfirmed by immunohistochemistry with one of the CD-117 or DOG1. Immunohistochemical stained sections were used for the assesment of Ki-67. Only nuclear staining of Ki-67 was considered positive when scoring Ki-67. Proliferation index is identified as the percentage of positive staining cells among the total number of tumor cells in the area scored. The slides were analyzed with x4 and x10 object lenses to define the region of most intense staining. The expression level of Ki-67 index was identified by numbering at least 500 tumor cells in the high-power (x40 objective) areas. The Ki-67 LI was determined to be below or above 10%. The mitotic index was identified by numbering the count of mitotic cells per 50 HPFs.

Statistical Evaluation of Data: All statistical analyses were carried out using Statistical Package for Social Sciences software version 22.0 (SPSS, Chicago, IL, USA). Patient, treatment and disease characteristics were evaluated using descriptive statistics. The overall survival (OS) was defined as the time from surgery to the date of the death or last follow-up. The disease-free survival (DFS) was defined as the time from surgery to the date of documented recurrence/progression or to the date of death from cancer or last follow-up. Kaplan and Meier test was performed for survival analyses and two-sided log rank test was fulfilled to make comparisons between subgroups. The estimation of hazard ratios and 95% confidence intervals (CIs) were evaluated using Cox regression analysis. The parameters which had statistical significance in univariate analysis ($p < 0.05$) were added in

multivariate analysis as covariates. A p value less than 0.05 was accepted statistically significant.

RESULTS

Patients, Tumor and Treatment Characteristics: Overall, 104 patients with GISTs were included in this study. The median follow up time was 73 months (range; 6 to 148 months). The median age was 60 years (range: 29 to 88 years; median 60 years). All cases were categorised into different risk group as regards to modified NIH and AFIP risk classification systems. The detailed patients, treatment and histopathologic features of GISTs are presented in Table 1.

Table 1. Patient, tumor and treatment characteristics

Variables	No. of patients (Total:104)	%
Age (years)		
Median	60	
Range	29-88	
Sex		
Male	51	49
Female	53	51
Karnofsky Performance Status		
90-100	95	91
70- 89	9	9
Tumor Site		
Gastric	61	59
Non-gastric	43	41
Tumor size (cm)		
<2	15	14
2-5	24	23
5-10	40	39
>10	25	24
Mitotik rate		
<10	81	78
≥10	23	22
Cell Type		
Spindle	62	60
Epithelioid	12	11
Mixt	30	29
Growth Pattern		
Expansile	82	79
İnfiltrative	22	21
Atypia		
Slight	76	73
Modarate	6	21
Significant	22	6
Cellularity		
Slight	90	87
Significant	14	13
Ki-67		
<10%	67	64
≥10%	37	36
Surgical margin status		
R0	102	98
R1/R2	2	2
Postoperative Imatinib		
Yes	50	48
No	54	52

Survival Analysis: The median follow-up time was 73 months (range; 6 to 148 months). Seven of the 104 patients (7%) had local recurrence, 26 of the 104 patients (25%) had distant metastases and 11 of the 104 (11%) patients died during the follow-up period.

The mean OS was 133 (range; 124 to 141) months for all the patients. 2-, 5- and 10- year OS rates were 93%, 89% and 89%, respectively. According to univariate analysis, only the disease progression or recurrence during follow up was significant prognostic factor for OS (Fig1, p=0.001). In multivariate Cox regression analysis, the patient age and progression or recurrence of disease were independent prognostic factors for OS. Patients with increasing age had a shorter OS (p=0.03) and the older age was associated with 1.09- fold higher risk of death (p=0.03; HR: 10.9 [1.06- 1.18]). Patients who had progression or recurrence during the follow-up time had a shorter OS (p=0.002) and was associated with 27.64- fold higher risk of death (p=0.002; HR: 27.64 [3.55-215.02]).

The mean DFS was 117 months (range; 107 to 127 months) for all the patients. 2-,5- and 10- year DFS rates were 94%, 88% and 67%, respectively. According to Kaplan Meier analysis, mitotic count, growth pattern, disease location, and Ki-67 index were the significant prognostic factors for DFS. Patients with >5mitoses/ 50HPF had a poorer DFS than ≤5mitoses/ 50HPF. Kaplan Meier analysis of the mean DFS was 126 months for the patients with ≤5mitoses/ 50HPF vs 105 months for the patients with >5mitoses/ 50HPF (p=0.03; Fig 2a). Patients with infitrative growth pattern had a shorter DFS than expansile growth pattern. Kaplan Meier analysis of the mean DFS was 122 months for the patients with expansile growth pattern vs 98 months for the patients with infiltrating growth pattern (p=0.013; Fig 2b). In addition to these factors, the DFS was affected by primary tumor site. Patients with primary gastric GISTs had longer DFS than non-gastric GISTs. Kaplan Meier analysis of the mean DFS was 128 months for the patients with gastric GISTs vs 99 months for the patients with non-gastric GISTs (p=0.004; Fig 2c). Patients with a Ki-67 LI value <10% had a longer DFS than patients with a Ki-67 LI value ≥10%. Kaplan Meier analysis of the mean DFS was 131 months for the patients with a Ki-67 LI value <10% vs 103 months for the patients with a Ki-67 LI value ≥10% (p= 0.003; Fig2d). In multivariate Cox regression analysis, Ki-67 index was the only significant prognostic factor for predicting disease progression or recurrence (p=0.04). The patients who had the level of Ki-67 ≥10% had a poorer DFS and these group of patients had 3.78- fold higher risk of progression or recurrence (p=0.04; HR: 3.78 [1.05-13.61]).

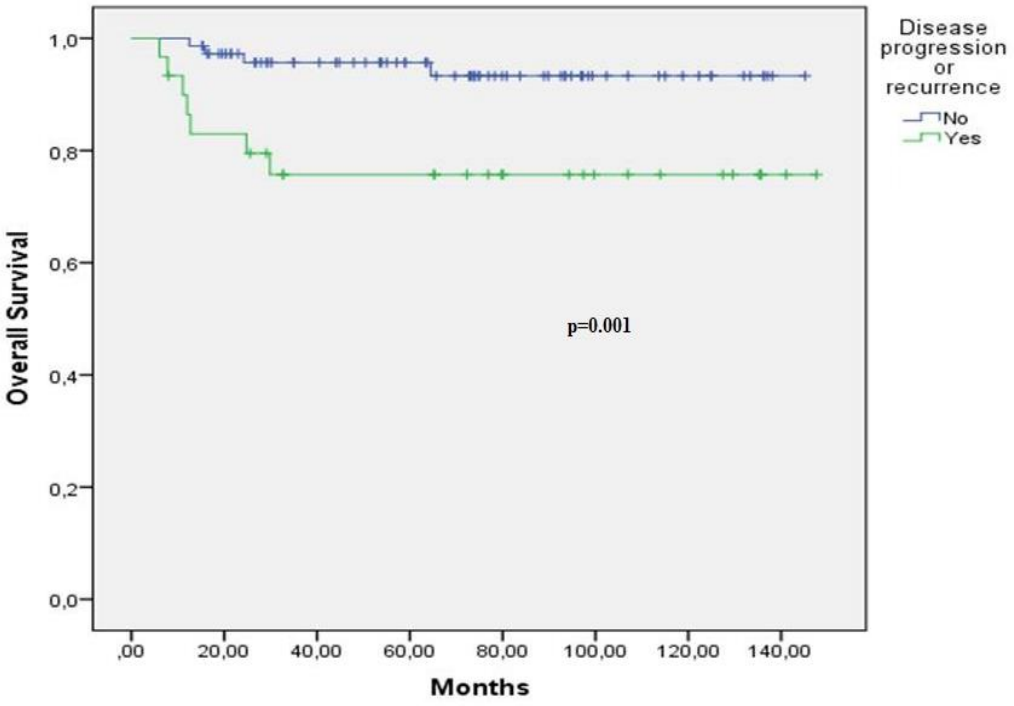

Figure 1. Overall survival according to the disease progression and recurrence.

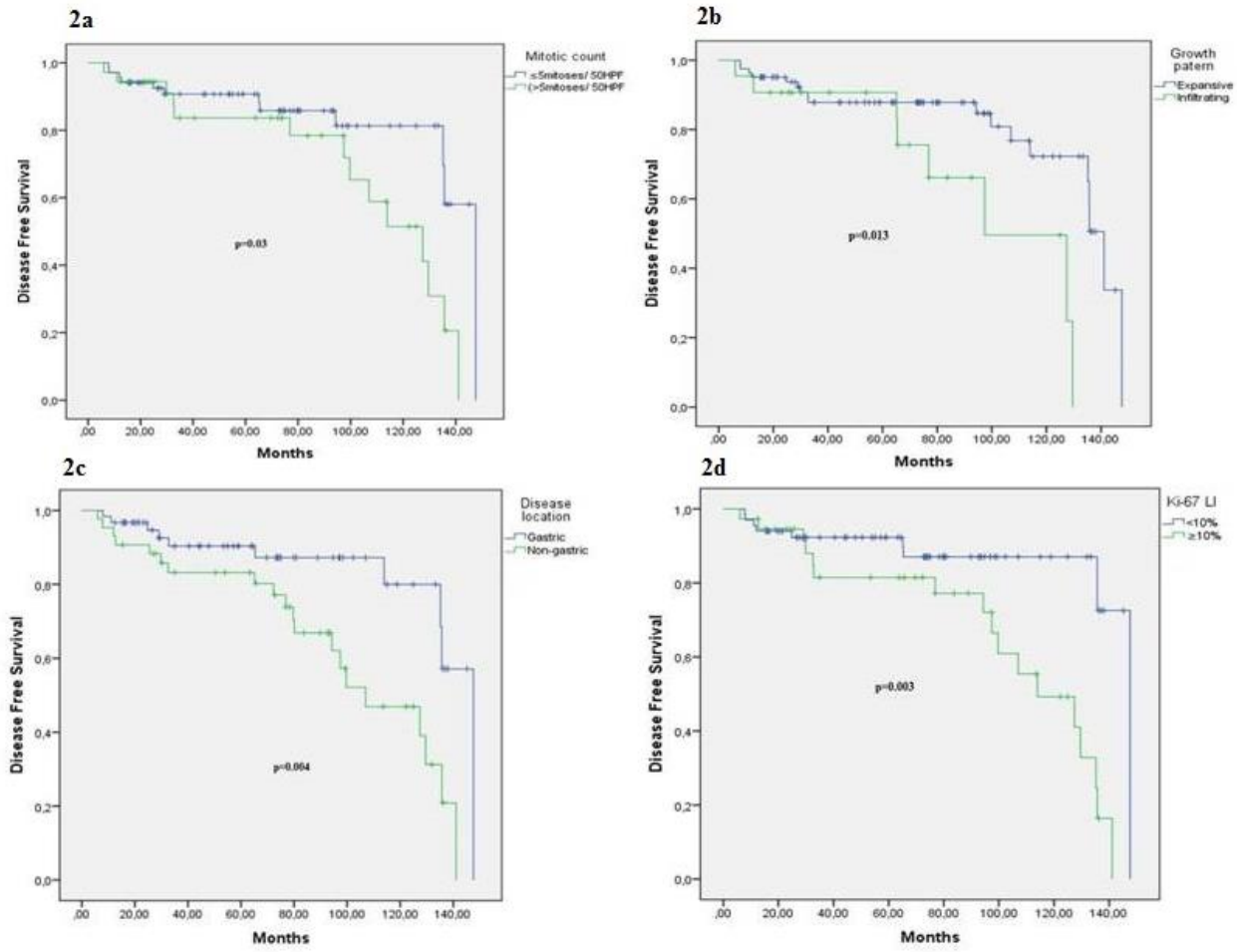

Figure 2. Disease free survival according to mitotic count (2a), growth pattern (2b), disease location (2c), and Ki-67 LI (2d).

DISCUSSION

The biological behavior of GISTs varies widely and it is diffucult to estimate their aggressiveness using the current risk classification criterias. There is a large heterogeneity exists among patients with GISTs, even those with the same risk classification. So that, the researchers continue to investigate the additional prognostic factors to improve the current risk classification criterias.

In the current study, the age and the disease progression or recurrence during follow up were significant prognostic factors for OS. Patients who had progression or recurrence during the follow-up period and older age had a shorter OS. The mitotic count, growing pattern, tumor location, and Ki-67 index were the significant prognostic parameters for DFS. Among them, only the Ki-67 index was independent prognostic factor for DFS according to multivariate analysis. It may be a signal that, as the follow-up period increases, the Ki-67 LI will affect the OS. The prognostic affect of Ki-67 LI in patients with GISTs was examined by previous researchs. But the results of these studies conflicted with each others results (9,10,19-21). Zhao et al. investigated the prognostic importance of Ki-67 LI and identified the cut-off point of Ki-67 LI as ≤5, 6-8 and >8%. They revealed that Ki-67 LI is a significant prognostic factor for recurrence free survivals and concluded that Ki-67 LI >8% can influentially subdivide high risk patients with different outcomes in the same group according to NIH criteria (19). Liu et al. divided the patients into two groups according to Ki-67 index as ≤6 or >6% and found that patients with Ki-67 index >6% had considerably shorter OS than patients with ≤6 (20). In contrast to these results, Sozutek et al. revealed that Ki-67 LI was associated with mitotic index but there was not any association between Ki-67 index and survival outcomes (21).

Besides the prognostic affect of Ki-67 LI on survival outcomes, we also investigated the other clinicopathologic factors. The prognostic importance of age was investigated in several researchs but the results of studies conflict with each others. In Chinese population, it was reported that patients over 60 years of age had a longer survival time (11) on the other hand Kramer et al. found that patients with ≥50 years displayed significantly shorter DFS compared to patients with <50 years (12). More recently, Yang et al. (2) revealed their study results which investigating the clinicopathologic characteristics and prognostic factors of GISTs and they did not find any association between the age and survival outcomes. According to our results, the patients with increasing age had a poorer OS and was associated with 1.09 fold higher risk of death.

Location of disease is the other clinicopathologic factor for GISTs. We know that, GISTs are most commonly occuring in the stomach. Some of the previous studies demonstrated that longer OS for primary gastric GISTs and non-gastric tumor location accepted to be associated with poor prognosis and tumor recurrence (2,5,7,11-13). According to our results, when comparing with the other tumor locations, patients with primary gastric GISTs had longer DFS in accordance with the literature.

The other independent parameters for determining risk groups in GISTs are tumor diameter and mitosis in reference to NIH and AFIP risk classification systems (6,7). According to our results, patients with increasing tumor diameter tended to have a shorter survival outcomes but the results did not reach significance (p=0.07). Additionally, mitotic count was determined as a significant prognostic factor for DFS. Patients with >5mitoses/ 50HPF had a poorer DFS than ≤5mitoses/ 50HPF. Invasive growth pattern of tumor was associated with poor prognosis (16-18). Miettinen and Losata developed a method to predict the risk of metastasis and recurrence which included the high cellularity, invasion and tumor rupture in addition with tumor size, disease location, and mitotic rate (7). We didn't find any association between the high cellularity and prognosis but growth pattern of tumor was the significant prognostic factors for DFS. Patients with infitrative growth pattern had a poorer DFS than expansile growth pattern.

Microscopically, three main histologic subtypes were defined: spindle cell type (most common), epithelioid type and mixed type (6,7). Some authors found that epithelioid morphology is associated with poor prognosis (14,15) and the others argued that survival rates have increased in tumors of epithelioid cell type (11). But, there was not any association between the cell type and prognosis according to our results.

We are aware of that there are some limitations of the study; including limited sample size and its retrospective nature. But, this single institution study is particulary important because all the histologic examples of cases re-defined by a single pathologist who is unaware of the survival of patients.

CONCLUSION

In conclusion; the current investigation demonstrated that the patient age and disease progression or recurrence of disease were the most important prognostic factors for OS. Ki-67 LI was the only independent prognostic factor for estimating disease progression or recurrence according to multivariate analysis. As the follow-up period increases, we think that Ki-67 LI also will affect OS. The Ki-67 LI may be a new prognostic factor for GISTs and it may be use as an effective component of current risk classification criterias. Further prospective randomized controlled studies are needed to support our study.

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