

Comparative study of the different degrees of risk of gastrointestinal stromal tumor

Estudo comparativo dos diferentes graus de risco no tumor estromal gastrointestinal

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A B S T R A C T

Objective: To evaluate the applicability of the main categories of risk and morphological factors in the prognosis of gastrointestinal stromal tumors. **Methods:** we retrospectively studied fifty-four cases of GIST, assessing the main prognostic factors of this neoplasia: risk levels, topography, size, mitotic index, necrosis, histological subtype and immunophenotype. We also verified their association and the reduction of overall survival. **Results:** Univariate analysis showed that tumors with mitoses number greater than 5 per 50CGA (high-power fields), the presence of necrosis and a high risk for both the systems proposed by Fletcher and Miettinen had a significant association with reduced survival ($p = 0.00001$, 0.0056 , 0.03 and 0.009 , respectively). The remaining analyzed factors (size, histological subtype, topography and immunophenotype) had no such association. Multivariate analysis (Jacard index) showed that the Miettinen degree of risk was the one that best correlated with prognosis. **Conclusion:** the risk criteria of Fletcher and Miettinen are important in assessing the prognosis of patients with gastrointestinal stromal tumors, especially the latter, which adds to the mitotic index and the presence of tumor necrosis.

Key words: Gastrointestinal Stromal Tumors. Risk Factors. Digestive System Neoplasms. Prognosis. Mitotic Index.

INTRODUCTION

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal neoplasms of the gastrointestinal tract¹, their incidence being estimated at 14 to 20 cases per million population²; they are more frequent in male patients older than 50 years old³. The pathogenesis is related to mutational changes in two tyrosine kinase receptors: KIT and PDGFR-alpha (platelet-derived growth factor receptor alpha) on the surface of the interstitial cells of Cajal, the former being the most common (85% of cases)^{4,5}. Gastrointestinal stromal tumors can develop in any topography, from the esophagus to the rectum. However, they are more common in the stomach (50% to 60%), followed by small intestine (20% to 30%), colon (10%), rectum or esophagus (5%)⁶. Macroscopically, the tumor lesions often have a nodular form, transmural involvement and submucosal growth, with ulceration of the mucosa or not. In light microscopy, histology reveals three types: the most common spindle (70%), epithelioid (20%) and the mixed type (10%),

when there is combination of epithelioid and spindle ones⁷.

The diagnosis of stromal neoplasms is based on immunohistochemical study with CD117 marker, expressed in most such neoplasias¹. Noteworthy are also other markers: DOG 1, nestin, theta protein kinase C and carbonic anhydrase II⁸. The differential diagnosis includes: desmoid tumor, inflammatory myofibroblastomas, leiomyoma, inflammatory fibroid polyp, neuroma, neurosarcoma, sarcomatoid mesothelioma and metastatic melanoma^{7,9,10}

GIST prognosis is still matter of discussion. Currently there are different classifications^{7,11-15} aimed to stratify tumors into groups, linking them to a higher or lower risk of tumor recurrence and/or distant metastasis^{8,12}. Of all the classifications mentioned above, the two most commonly used are the one of Fletcher *et al.*⁷ and Miettinen *et al.*¹¹. The first classification established two factors as prognostic parameters, one macroscopic and the other microscopic. This combination resulted in a system that ranked the stromal tumors in different degrees of risk. The second,

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based on a larger number of cases, whose diagnosis was supported both by immunohistochemical and genetic studies, and especially with a prolonged follow-up. In addition to the criteria used in Fletcher *et al.*⁷ classification, a third was added to Miettinen *et al.*¹¹ classification, the location of the tumor, allowing greater stratification of risk groups and the establishment of a percentage related to the chances of development of recurrence and / or metastasis.

The aim of this study was to evaluate the applicability of the main risk categories (Fletcher and Miettinen) and morphological factors in the prognosis of gastrointestinal stromal tumor.

METHODS

We retrospectively studied a cohort of 54 cases of gastrointestinal stromal tumors with positive immunohistochemistry for the expression of anti-CD117 antibody, completely excised with no evidence of disseminated disease and not associated with other malignancies. These came from the files of the Pathology Services of the Hospital Univeristário Gaffrée e Guinle (HUGG/UNIRIO) and Hospital Univeristário Clementino Fraga Filho (HUCFF/UFRJ). We obtained data on age, gender and clinical outcomes of patients from medical records and requests from pathological examinations. The study evaluated the data of patients alive and without recurrence, of patients who died from the disease, and of live patients

with recurrent disease. The time with disease evolution was counted from the date of surgery until the last contact of the patient, recorded in the medical record. The project was approved by the Ethics in Research Committee of HUCFF under number 079/05.

Morphological data and risk degrees

The topography and tumor size were obtained from requests from pathological examinations. The mitotic index was assessed in 50CGA (high-power fields), using an Olympus BX40 microscope with a 40X objective and 10x eyepiece⁷. The mitotic count was performed by two pathologists. The histological subtype was determined by the predominant presentation form at microscopy (spindle, epithelioid, mixed); the presence of necrosis was evaluated in areas distant from those corresponding to the ulceration of the overlying mucosa, when present⁷. When assessing degrees of risk, we employed the ones proposed by Fletcher *et al.*⁷ (Table 1) and Miettinen *et al.*¹¹ (Table 2).

Immunohistochemistry

The following antibodies were used: anti-CD117 (Dako, Carpinteria, California / USA) diluted 1:100; anti-protein S-100 (Dako, Carpinteria, California / USA) diluted 1:1,000; and anti-smooth muscle actin (Dako, Carpinteria, California / USA) diluted 1: 250. Subsequently, sections were incubated with Universal LSAB™2 kit / HRP Rabbit / Mouse - K0675 (Dako, Carpinteria, California / USA). The positivity for anti-protein S-100 antibody and muscle-specific anti-actin defined the immunophenotype of neoplasms,

Table 1 - Fletcher risk degrees.

Risk degree	Macroscopic size (cm)	Mitotic index (50 CGA)
Very low	<2	<5
Low	2-5	<5
Intermediary	<5 5-10	6-10 <5
High	> 5> 10any size	> 5any index> 10

Fletcher *et al.*⁷

Table 2 - Miettinen Risk Degrees.

Tumor parameters		Risk of disease progression				
Index	Mitotic	Size	Stomach	Duodenum	Ileum/ jejunum	Rectum
< 5 per50CGA		< 2 cm	No(0)	None (0)	No(0)	No(0)
		2 > to < 5 cm	Very low (1.9 percent)	Low(4.3%) and	Low(8.3 percent)	Low(8.5 percent)
		5 > to < 10 cm	Low(3.6 percent)	Moderate(24)	Datainsufficient	DataInsufficient
> 5 per50 CGA		> 10 cm	Moderate(10% off)	High(52% off)	High(34% off)	High(57)
		< 2 cm	None (0)	High(61)	Datainsufficient	High(54% off)
		2 > to < 5 cm	Moderate(16% off)	High(73)	High(50% off)	High(52% off)
		5 > to < 10 cm	High(55% off)	High(85 percent)	Datainsufficient	DataInsufficient
		> 10 cm	High(86)	High(90 percent)	High(86)	High(71)

Miettinen *et al.*¹¹



Figure 1 - Gastrointestinal stromal tumor of the jejunum, with serosal bulging.

classifying them respectively as muscular, neural, double or null (no expression)⁷.

Statistical analysis

All factors were submitted to analysis of the direct and univariate frequencies with the chi-square test. The statistical significance was set at $p < 0.05$. The criteria that showed statistical significance in the univariate analysis were submitted to the Jaccard similarity index.

RESULTS

The study group consisted of 54 patients, 30 (59.5%) of them women and 24, men (40.5%) with a mean age of 57.34 ± 13.71 , ranging from 24 to 83. Regarding the topography of neoplasms, tumors were located: one (1.8%) in the esophagus, 27 (50%) in the stomach, 20 (37.1%) in the small intestine (Figure 1) and six (11.1%) in the large intestine.

The size of the tumors ranged from 2.0 to 33 cm (median 8) with 12 measuring up to 5 cm (included) along the longest axis (22.3%) and 42 were greater than 5 cm (77.7%). As for the mitotic index, 36 cases had less than five mitosis per 50 CGA (66.8%) and the others (18), more than five (33.2%). The histological subtype of 32 tumors (59.3%) were spindle, of nine (16.6%), epithelioid and of 13, (24.1%) mixed. Necrosis was absent in 33 cases (61.2%) and present in 21 (38.8%). The immunophenotype was as follows: 26 (48.1%) tumors showed muscle differentiation, 11 (20.4%) neural, seven (13.3%), double differentiation (nerve and muscle) and 10 (18.2%), none.

Regarding the degree of risk, according to Fletcher *et al.*⁷ tumors were thus classified: Ten (18.6%) of low-risk, 17 (31.4%) of intermediate risk, 27 (50%) of high-risk and none of very low risk; and according to Miettinen *et al.*¹¹, three (5.4%) had no risk, four (7.4%) of very low risk, ten (18.6%) of low-risk, 14 (26%) of intermediate risk and 23 (42.6%) of high risk.

As for the status of the patients, 33 (61.2%) were alive without disease (good prognosis) and 21 (38.8%), alive with disease and / or died due to the neoplasia, and the follow-up period ranged from one to 248 months, with a median of 53 months. Among the parameters studied and tested in the univariate analysis, those who showed an adverse effect on overall survival was the mitoses number greater than five mitosis per 50 CGA ($p = 0.00001$). In multivariate analysis, employing the Jaccard Index, we found that the Miettinen degree of risk showed better association with reduced overall survival.

DISCUSSION

The characterization of the biological behavior of GIST is essential for signaling of patients who are outpatients, those who have the indication of treatment with imatinibmesylate. In this series, the gender distribution was different from the ones in the literature, there was a predominance of females (59.5%), while literature indicates a homogeneous distribution or a slight predominance of males^{11,16-18} and, by contrast, the average age of patients (57 years) was similar to that found in other series^{2,17}.

In this sample, the preferred location in the stomach, followed by small intestine, large intestine and esophagus was also observed by other authors^{2,11}. Although we found no statistically significant difference between the different organs involved ($p=0.08$), some studies^{11,19,20} have shown that the GIST location directly influences the prognosis and those located in the stomach had more favorable course than those found in other topographies. Statistically, we found that the colonic topography directly influenced prognosis, since five out of the six tumors had unfavorable follow-up (metastasis/death), which was also found in another study³.

The importance of tumor size in GIST prognosis has undergone major changes since the publication of Miettinen *et al.*¹¹, and came to corroborate evidence that linked the neoplastic diameter with tumor topography, ie, gastric tumors showed worse prognosis when their size was greater than 10cm, while cancer of the small intestine showed poor prognosis when greater than 5cm^{19,20}. However, in general, gastrointestinal stromal tumors with more than five centimeters are related to a worse prognosis. In this series there was no demonstration of prognosis associated with tumor size, a fact possibly influenced by the number of cases. Independently of this demonstration, some data needs to be emphasized, because the average size of the tumors of patients with worse prognosis was higher than in patients with good prognosis. Also, of the 21 tumors with recurrence, 17 were larger than 5cm, from which it can be inferred that size is an important prognostic criteria.

The mitotic index (MI) of more than 5 per 50 CGA was also a variable associated with prognosis, as observed

by other authors^{7,11,15,17,18,20}. The evaluation of this variable is present in the two main risk levels^{7,11} used to characterize GIST prognosis. The risk degree of Fletcher *et al.*⁷, has three MI subdivisions (< 5, 6-10 and > 5), while for Miettinen *et al.*¹¹, there is a binary division (< 5 and > 5). These forms of division diminish the discriminatory power of MI, a fact described after analysis of 929 tumors and observation of differences in patient survival when the stratification was done in four segments (< 5, 5-10, 10-30 and > 30)²¹. In addition, the correct interpretation of true mitosis is another problem since the material fixation time may impair its identification¹⁸.

The histological subtype in our study was not related to patients' prognosis. However, it is noteworthy that among the 12 epithelioid and mixed neoplasms, ten had unfavorable follow-up. The poor survival of patients with tumors of the mixed or epithelioid patterns has also been reported, but this finding only held for the mixed pattern in multivariate analysis^{22,23}. Another study suggests that the spindle histological pattern is associated with longer patients survival²⁰. In view of these conflicting results, we believe that this point still needs further study.

The presence of necrosis was associated with poor prognosis, which was also found in other studies^{18,20}. It is believed that necrosis is directly related to severe proliferative activity of the tumor, ie, the most aggressive would present necrosis areas.

As for the studied immunohistochemical markers, there was no correlation of the neoplasia immunophenotype with patients' prognosis, as evidenced in another series². Nonetheless, neoplasms with muscle differentiation showed greater disease-free intervals¹⁷. However, the authors point out that these results need to be evaluated carefully, since the tumors with muscle differentiation had a less aggressive biological course.

Regarding the two GIST prognostic characterization systems, there was evidence of association with prognosis in both Fletcher *et al.*⁷ and Miettinen *et al.*¹¹ classification, which was also found by other authors^{14,18,24,25}.

However, in this series we found a better risk statistical correlation with the Miettinen *et al.*¹¹ classification. This can be explained by the introduction of a third criterion (location), the study of more than 2,000 cases, with long follow-up of patients and mainly the greater stratification of the risk categories, allowing a reclassification of the neoplasias²⁵. This can be observed in our study, with the appearance of three tumors with no risk, four tumors with very low risk, and also reduction in both neoplasias at intermediate risk (from 17 to 14) and high risk (from 27 to 23).

Although the relationship between prognosis and the two classifications, there are still tumors that do not follow this natural history, ie there are neoplasms classified as low risk that progress to metastasis / death, and other categorized as high risk, whose patients present favorable follow-up (alive without recurrence)⁷. The foregoing can be explained due to various conflicting situations in the two proposed systems. Fletcher *et al.*⁷ fails to point out the mitosis counting method, the size measuring mode, the definition of what is a high-power field, and also does not define the risk degree to neoplasias with exactly five mitosis per 50 CGA²⁵. In Miettinen *et al.*¹¹, certain subgroups have few documented neoplasias, such as duodenal tumors less than 2cm and lesions with mitotic index greater than five per 50 CGA, which prevents their categorization⁸. In addition, there is no classification for GIST in the esophagus or colon, whose biological behavior can be misinterpreted²⁵.

We conclude that both the systems proposed by Fletcher *et al.*⁷ and Miettinen *et al.*¹¹ in cases of GIST showed correlation with prognosis, although in this series the latter has proved to be superior. However, we understand that such systems need to be reviewed, either through a new form of the current division criteria (number of mitosis per CGA and tumor size) or by including other morphological variables, such as necrosis and less frequent location sites. However, we understand that we still need more studies involving more cases, especially in those places where GISTs are less common.

R E S U M O

Objetivo: avaliar a aplicabilidade das principais categorias de risco e de fatores morfológicos no prognóstico tumor estromal gastrointestinal. **Métodos:** cinquenta e quatro casos de GIST foram estudados retrospectivamente considerando-se os principais fatores prognósticos da neoplasia: graus de risco, topografia, tamanho, índice mitótico, necrose, subtipo histológico e imunofenótipo. Foi também verificada a sua associação e a redução da sobrevida global dos pacientes. **Resultados:** a análise univariada mostrou que os tumores com número de mitoses maior que 5/50CGA (campos de grande aumento), a presença de necrose, de alto risco tanto para os sistemas propostos por Fletcher, quanto para Miettinen tiveram associação significativa com redução da sobrevida ($p=0,00001$, $0,0056$, $0,03$ e $0,009$, respectivamente). Enquanto que os demais fatores analisados (tamanho, subtipo histológico, topografia e imunofenótipo) não tiveram tal associação. A análise multivariada (índice de Jacard) demonstrou que o grau de risco de Miettinen foi aquele que melhor se relacionou com o prognóstico. **Conclusão:** os critérios de risco de Fletcher e de Miettinen são importantes na avaliação do prognóstico de pacientes com tumor estromal gastrointestinal, principalmente este último, que se soma ao índice mitótico e a necrose tumoral.

Descritores: Tumores do Estroma Gastrointestinal; Fatores de Risco; Neoplasias do Sistema Digestório. Prognóstico. Índice Mitótico.

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