Immunohistochemical diagnosis of gastrointestinal stromal tumor (GIST) and nurse knowledge

Diagnóstico imuno-histoquímico do tumor estromal gastrointestinal (GIST) e conhecimento da enfermeira

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Mariana de Souza Barbosa
Enfermeira pela Unifesp
Instituição de atuação atual: Hospital São Paulo/Universidade Federal de São Paulo
Endereço: Rua Napoleão de Barros, 715 - Vila Clementino, São Paulo - SP, cep: 04024-002
E-mail: mari.marianasbarbosa@gmail.com

Débora Vieira da Silva
Formação acadêmica mais alta: Enfermeira pela Unifesp
Instituição de atuação atual: Complexo Hospitalar dos Estivadores / Santa Casa de Misericórdia de Santos
Endereço completo (pode ser institucional ou pessoal, como preferir): Rua Dom Pedro II, 674 - Vila Nova, Cubatão – SP, cep: 11520040
E-mail: deboravieiras96@gmail.com

Andreza Scapinello Fortini
Mestranda
Instituição de atuação atual: Universidade Federal de São Paulo
Endereço: Rua Pedro de Toledo, 781, 5º andar, Vila Clementino São Paulo – SP, cep: 04039-032
E-mail: andrezasf@gmail.com

Celina Tizuko Fujiyama Oshima
Doutora em Ciências
Instituição de atuação atual: Universidade Federal de São Paulo
Endereço: Rua Pedro de Toledo, 781, 5º andar, Vila Clementino São Paulo – SP, cep: 04039-032
E-mail: celina.oshima@gmail.com

Daniel Araki Ribeiro
Livre docente
Instituição de atuação atual: Universidade Federal de São Paulo
Endereço: Rua Silva Jardim136, sala 332, Vila Mathias, Santos – SP, cep: 11050-020
E-mail: daribeiro@unifesp.br
ABSTRACT
Gastrointestinal Stromal Tumor is a rare neoplasm which affects cajal interstitial cells due to dysfunction in the KIT or PDGFRA genes. It is considered a rare but common mesenchymal tumor in the digestive tract. The objective was to analyze the incidence, epidemiology and immunolocation of KIT protein and the nurse's performance in relation to patients with GIST. The inclusion criteria were pathological reports issued by the Pathological Anatomy service of UNIFESP / EPM, as of 2010, and diagnosed as GIST.
Twenty-one samples were selected for KIT immunohistochemical analysis. Among women, 54.6% were over 60 years old and among men 90%. The most common location among women was the stomach with 63.7%, while in men, the stomach had the same percentage as the intestine (40%). Immunohistochemistry showed C-KIT positivity in 81.8% of women and 90% in men. The average size of the lesion was 5.58 cm and 3.8 cm in women and men, respectively. The presence of necrosis was verified in 27.3% of women and in 30% of men. The most prevalent morphology was the spindle cell with approximately 70% in both women and men. Of the 21 cases, three patients died, two had relapse and, in five cases, adjuvant treatment with imatinib mesylate was performed. For the performance of the nurse in front of the patient with GIST, it was necessary to confirm the expression of the KIT protein, which allows a differential diagnosis. The analysis of the incidence and epidemiology of this population allowed nurses to add more knowledge about this tumor.

Key words: Gastrointestinal neoplasia, Gastrointestinal Stromal Tumor (GIST), Immunohistochemistry, Epidemiology.

RESUMO
O tumor estromal gastrointestinal é uma neoplasia rara que afecta as células intersticiais cajal devido a disfunções nos genes KIT ou PDGFRA. É considerado um tumor mesenquimal raro mas comum no tracto digestivo. O objectivo era analisar a incidência, epidemiologia e imunolocalização da proteína do KIT e o desempenho da enfermeira em relação a pacientes com GIST. Os critérios de inclusão foram os relatórios patológicos emitidos pelo serviço de Anatomia Patológica da UNIFESP / EPM, a partir de 2010, e diagnosticados como GIST.
Vinte e uma amostras foram selecionadas para análise imuno-histoquímica do KIT. Entre as mulheres, 54,6% tinham mais de 60 anos de idade e entre os homens 90%. O local mais comum entre as mulheres era o estômago com 63,7%, enquanto nos homens, o estômago tinha a mesma percentagem que o intestino (40%). A imuno-histoquímica mostrou a positividade do C-KIT em 81,8% das mulheres e 90% nos homens. O tamanho médio da lesão era de 5,58 cm e 3,8 cm nas...
mulheres e nos homens, respectivamente. A presença de necrose foi verificada em 27,3% das mulheres e em 30% dos homens. A morfologia mais prevalente foi a célula fusiforme, com aproximadamente 70% tanto nas mulheres como nos homens. Dos 21 casos, três pacientes morreram, dois tiveram recaídas e, em cinco casos, foi realizado tratamento adjuvante com mesilato de imatinibe. Para o desempenho da enfermeira na frente do paciente com GIST, foi necessário confirmar a expressão da proteína KIT, o que permite um diagnóstico diferencial. A análise da incidência e epidemiologia desta população permitiu aos enfermeiros acrescentar mais conhecimentos sobre este tumor.

**Palavras-chave:** Neoplasia gastrintestinal, Tumor Estromal Gastrointestinal (GIST), Imuno-histoquímica, Epidemiologia.

### 1 INTRODUCTION

Gastrointestinal Stromal Tumor (GIST) is a neoplasm that affects cells of gastrointestinal tract (GI) called interstitial cells of Cajal (ICCs)\(^8\). These cells are known as “pacemakers” of the gastrointestinal tract with mesenchymal origin and make the connection between smooth muscle and innervation of the intestinal wall. In addition, they are pluripotent, have smooth muscle neural characteristics and are responsible for gastrointestinal motility\(^18\).

This neoplasm occurs due to mutations that lead to changes in two tyrosine kinase receptors (C-KIT and PDGRF-\(\alpha\)), both located on the cell membrane. The C-KIT gene is responsible for some cellular functions, such as: proliferation, apoptosis and differentiation. PDGRF-\(\alpha\) is a platelet-derived growth factor receptor. Alteration in the KIT receptor is the most common in 85% of cases and results in the stimulation of continuous cell proliferation\(^16\).

Chronologically, GIST studies began in the 1940s, when mesenchymal tumors were classified as smooth muscle sarcomas, receiving names like leiomyomablastomas\(^19\). From 1948 on, mesenchymal neoplasms came to be called stromal tumors. In 1983, with the use of electron microscopy and the emergence of immunohistochemistry, the name became known as today by Mazur and Clark and was confirmed in 1998 by Hirota through the evidence of the CD34 (hematopoietic mesenchymal precursor cell marker) and CD117 antigens (protein C-KIT)\(^18\).

GIST is rare and corresponds to 0.1-3% of all neoplasms of the gastrointestinal tract, however it is the most common mesenchymal neoplasm of this system\(^1\). It mainly affects the stomach, ranging from 50% to 60% of cases, followed by the small intestine, colon, rectum and esophagus\(^8\). It can also develop in places outside the gastrointestinal tract, as rarely in the peritoneum and mesentery. It represents 2.2% of gastric neoplasms, 13.9% of the small intestine and 0.1% of colon neoplasms\(^2\).

According to a Sweden's study the incidence of GIST was 13 cases per million inhabitants.
and the highest incidence was found in Hong Kong 15 to 20 cases per million inhabitants. In the United States, according to Surveillance, Epidemiology, and End Results (SEER) Programs of the National Cancer Institute (NCI), the annual incidence was 6.8 per million and in Europe, this neoplasm was found in 10 to 14 people per million inhabitants.

GIST affects adults in the sixth decade of life and is associated with neurofibromatosis (specifically Neurofibromatosis-1) or Carney’s triad (gastric stromal tumor, extra-adrenal paraganglioma and pulmonary chordoma). In young adults and children, it is rarer. According to the Ministry of Health of Brazil, the average age at diagnosis is between 58 and 63 years and there are no risk factors for this neoplasm.

Determining the symptoms of this neoplasm is still very difficult, as it depends on the location and size of the tumor. In addition, the data available in the literature indicate non-specific symptoms such as melena, hematemesis, bleeding, abdominal pain, dyspepsia, nausea, anorexia and a recent case report in which the patient was asymptomatic.

Its diagnosis can be made by biopsy of the lesion to identify the KIT receptor by immunohistochemistry, in addition to the presence of mutations in the Kit or PDGFRA gene. This biopsy can be done through endoscopy, colonoscopy or echoendoscopy, depending on your location. Computed tomography of the abdomen can assist in biopsy and scale the tumor. The differential diagnosis of GIST includes immunohistochemical analysis of C-KIT (CD-117), which is positive in approximately 90% of cases, but which can be detected in other tumors, such as melanoma, large cell lymphoma, among others. To aid the diagnosis, the CD-34 marker, positive in about 80% of cases, can be used, in addition to the proteins S100, Desmina and Vimentina. Positivity for CD-117 is responsible for confirming the diagnosis of GIST.

GIST can be classified into three histological subtypes: fusiform (70%), epithelioid (10%) and mixed (20%). Currently, parameters such as mitotic index, tumor size, mucosal invasion, and presence of tumor necrosis are used, whose objective is to determine the prognosis.

The most appropriate treatment for GIST is total resection of the injured tissue, but it is not always possible. Imatinib mesylate is an adjunctive treatment for this type of neoplasia and has the effect of selective inhibition of tyrosine kinase that blocks the activity of KIT protein in tumor cells. Imatinib can be used to reduce the pre-surgical tumor and for palliative treatment. This antineoplastic agent has edema, diarrhea and fatigue as side effects. For patients with intolerance or resistance to this antineoplastic agent or who cannot undergo surgery, radiotherapy or use of Sunitinibe is indicated. Imatinib mesylate is an adjuvant treatment for this type of neoplasia and has the effect of selective tyrosine kinase inhibition that blocks KIT protein activity in tumor cells. Imatinib can be used to reduce the pre-surgical tumor and also for palliative treatment.
treatment. This antineoplastic agent has edema, diarrhea and fatigue as side effects. For patients who have intolerance or resistance to this antineoplastic agent or are not able to undergo surgery, radiotherapy or use of Sunitinib is indicated 3.

The Unified Health System (Sistema Único de Saúde-SUS) in Brazil provides imatinib mesylate to GIST-Tumor of Gastrointestinal Stroma Tumor in adults (Ordinance SAS / MS 494/2014) through state health departments. Until 2016, the value of this antineoplastic in SUS was R $ 16.63 / 100mg and R $ 66.54 / 400mg. 13. Based on the information above, a very important factor is the fundamental role of the nursing team, as these professionals are able to understand the previous symptoms, during and after treatment, thus being able to act both in the assessment and clinical intervention of the patient and in the systematization of Nursing care. The analysis of the incidence and epidemiology of this population will allow nurses to add more knowledge about this tumor.

2 METHODS

Retrospective study identified only with the anatomopathological archives of Department of Pathology of UNIFESP / EPM (2010 - 2014) and diagnosed as GIST. This study was approved by the Research Ethics Committee of UNIFESP/EPM (CAAE 95996418.6.0000.5505).

After the survey and selection of cases, analyzes of the variables of incidence, epidemiology, clinical and immunohistochemistry were performed. The immunohistochemical reaction was performed at the Department of Pathology of UNIFESP / EPM for diagnostic purposes (17,21).

3 IMMUNOHISTOCHEMICAL ASSESSMENT

Slides with histological GIST sections were kept in an oven at 56-60°C for one night and subjected to dewaxing in xylene for 30 minutes (3 baths for 10 minutes), absolute alcohol (2 baths for 5 minutes) and rehydrated in running water at room temperature.

Antigenic recovery was performed with citrate buffer (pH = 6.0) for 40 minutes in an electric pressure cooker for 3 minutes. After this period, the slides were immersed in the phosphate buffer (pH = 7.4) for 20 minutes, followed by washing in distilled water (pH = 7). The endogenous peroxidase blockade was performed with the application of 3% hydrogen peroxide in distilled water (4 baths of 5 minutes), with subsequent washing in phosphate buffer (PBS) pH 7.4 for 30 min. Incubation with the Polyclonal Rabbit Anti-Human CD117 antibody, c-kit (code A4502), dilution 1: 400, was according to the manufacturer's instructions (DAKO, Glostrup, Denmark). Subsequently, the slides were incubated with secondary antibody for 20 min, from the Novo Link.
kit (New Castra®, New Castle Upon Tyne, United Kingdom), followed by the polymer from the same kit for 20 min.

Finally, the slides were washed 3 times in PBS for 5 minutes each, followed by the development of the 3,3'-diaminobenzidine chromogenic agent (DAB Liquid - DAKO, Glostrup, Denmark) at room temperature for 3 minutes and washed in distilled water. Harris' hematoxylin was used for counterstaining and the slides mounted with Entellan resin. The slides were analyzed by the pathologist and the researcher.

4 STATISTICAL ANALYSES

The collected data were placed in an Excel spreadsheet for analysis of means, standard deviation and medians. Student's t-test, chi-square and Mann-Whitney tests. GraphPad Prism version 6.0 was used.

5 RESULTS

The data survey indicated that 52% of GIST diagnoses occurred in females and 48% in males. Regarding age, patients were divided into two groups: over and under 60 years old. The group over 60 years old had the highest number of patients with 72.3% of cases and only two patients were under 50 years old. In the female gender, in relation to the tumor location, we found 63.7% of GIST of the stomach, 27.2% of the intestine and 9.1%, with no location being indicated. In men, the neoplasm was found in 40% in the stomach, 40% in the intestine and 10% in the esophagus and rectum. The size of the lesion was greater than 5 cm in 45.4% of females, with an average size of 5.58 cm. The male gender presented equality in the size proportion, with an average size of 3.8 cm. Necrosis was present in 27.3% of female and 30% of male cases. As for female morphology, 72.7% were spindle cells, 9.1% were epithelioid and 18.2% were mixed cells. In males, 70% were spindle cells, 10% epithelioid and mixed cells and 10% did not present this information (Table 1).

Regarding immunohistochemistry, female and male showed positive C-KIT without distinction. In Figure 1, the microphotographs of the GIST Hematoxylin and Eosin staining (A) and the positivity for C-KIT / CD-117 are shown, both in spindle cells and in most neoplastic cells (B).

We considered the follow-up of patients undergoing treatment for more than 18 months (mean time of relapse). Of the 21 patients, 14 continued to be followed up by the outpatient service, three died and four were lost to follow-up.
6 DISCUSSION

The distribution of GIST types by gender revealed a slight dominance of the female gender in relation to the male. Even so, our data do not present statistical significance and as in the international and national literature, there is no gender distinction in relation to this neoplasia\(^4\)\(^5\).

The age distribution showed 54.6% of patients over 60 years of age female and 90% male. GIST is a rare neoplasm that affects people around the sixth decade of life\(^12\), as found in our research: the most prevalent age group was between 61 and 70 years old and had a median age of 60 years for females and 70 years for the male sex.

In the present survey, we found a percentage of approximately 52% of tumors in the stomach and only one case in the rectum and esophagus. According to the literature, in 50 - 60% of the cases, the most common site is the stomach, and are rarely found in extra-intestinal regions\(^7\)\(^8\).

In 2017, Florou et al. showed that the GIST recurrence around 18 months and had an average survival of 58 months\(^6\). In the present study, it was not possible to visualize the mean survival, as many patients lost follow-up treatment, what unviabled the statistics analyses due to lack of data. Even so, we found an average follow-up of 53.5 months and only three patients had confirmed death. All patients who were followed up are alive and without pathology.

Regarding the immunostaining of the C-Kit, in this study, the positivity was observed in 85.9% of the cases and according to Judson et al (2017)\(^10\) the positive C-KIT marking occurred, on average, in 95% of the cases, proving that this marker is essential for the diagnosis of GIST.

Ninomiya et al (2019)\(^14\) reports that some tumor variables must be analyzed in order to know their malignancy, among them are necrosis, tumor size and morphology. A total of 21 cases analyzed, we found six (28.6%) presented necrosis. In 47.7% of the patients, the lesion was larger than 5 cm. In addition, the average tumor size was 5.58 cm for females and 3.8 cm for males. Although the relationship between the size of the tumor and the presence of necrosis was not statistically significant, it was noted that there is a propensity for the lesion to present necrosis in tumors from 5 cm upwards.

However, in the morphology, we observed in this study that 71.35% of the cases were composed of spindle cells and 9.55% epithelioids. According to Gönüllü et al (2018)\(^8\) the relationship between these cell types helps to identify malignancy. Our findings corroborate with the literature regarding the prevalence of spindle cells over the epithelioid cell type. Large volume tumors are composed of spindle cells, and they are more common in the stomach and so respond better to treatment compared to those composed of epithelioid cells. When relating the results of survival and morphology, it was possible to observe a confirmation of what was found in the
literature, since of the patients who maintained their follow-up at the service, 85.7% had fusiform cells (the most found) and, as seen previously, has a better response to treatment\textsuperscript{8,23}.

In 2005, the Ministry of Health implemented the National Oncological Care Plan (PNAO) which aims to implement everything from health promotion to palliative care\textsuperscript{15}. In a study carried out in Campo Grande (MS), most primary care nurses were unaware of this plan, which greatly implies supporting the population's health\textsuperscript{20}. So, in Brazil (2011), the Federal Court of Auditors\textsuperscript{22} presented an audit report and found that the care network was unable to provide equal access to the services present in PNAC. Greater knowledge of the network and its paths can enable a better quality of life, personalized treatment in case of illness and better rehabilitation.

We believe that this study is important for the entire health area, as it allowed us to add knowledge about the GIST. Nursing has an important role in the recognition of the pathology clinic, as one of its principles is health education. Through the knowledge of the entire health care network, personalized care is possible and has a fundamental role in the health / disease process.

Although KIT is expressed in other neoplasms, the evaluation of C-KIT expression in GIST cases confirmed to be a sensitive and specific marker for the differential diagnosis. We also believe that it is necessary to analyze other variables and more complete data sources for the GIST panorama in Brazil to be better known.
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CONFLICT OF INTEREST
The authors declare that they have no conflict of interests.
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Table 1: GIST epidemiological variables.

| Variables       | (%)   | (%)   |
|-----------------|-------|-------|
| Gender          | Female| Male  |
|                 | 11 (52)| 10 (48) |
| Age (years)     |       |       |
| Over 60 years   | 6 (54,6)| 9 (90) |
| Below 60 years  | 5 (45,4)| 1 (10) |
| Median          | 60 y  | 70 y  |
| Tumor Site      |       |       |
| Stomach         | 7 (63,7)| 4 (40) |
| Intestine       | 3 (27,2)| 4 (40) |
| Esophagus       | 0     | 1 (10) |
| Rectum          | 0     | 1 (10) |
| No information  | 1 (9,1)| 0     |
| Tumor Size      |       |       |
| ≥ 5 cm          | 5 (45,4)| 5 (50) |
| < 5 cm          | 6 (54,6)| 5 (50) |
| average size    | 5.58  | 3.8   |
| Necrosis        |       |       |
| Present         | 3 (27,3)| 3 (30) |
| Absent          | 5 (45,4)| 6 (60) |
| No information  | 3 (27,3)| 1 (10) |
| Morphology      |       |       |
| Fusiform        | 8 (72,7)| 7 (70) |
| Epithelioid     | 1 (9,1)| 1 (10) |
| Mixed           | 2 (18,2)| 1 (10) |
| No information  | 0     | 1 (10) |
| Immunohistochemistry |     |       |
| Positive C-kit  | 9 (81,8)| 9 (90) |
| Negative C-kit  | 1 (9,1)| 0     |
| Unperformed     | 1 (9,1)| 1 (10) |
Figure:

Figure 1: Microphotographs of Hematoxylin Eosin (HE) (A) and Immunohistochemistry staining (B, C).

Legend: Photomicrograph of a case of GIST. A) Hematoxylin & Eosin (HE) staining: presents a tripolar mitosis (shown in the arrow), which indicates an uncontrolled cell division. In addition, it presents an architectural pattern with interlaced beams (400x). B) Indicates positivity for the immunoexpression of C-KIT / CD-117 with fusiform cells presenting paranuclear intracytoplasmic vacuoles (400x). C) It shows immunolocation for C-KIT / CD-117 in most neoplastic cells with a greater 100-fold (100x) increase.