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Gastrointestinal Stromal Tumors, Identification of C-Kit Mutation and Differential Diagnosis

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Abstract
Background: Prior to the recognition of C-Kit mutations, GIST were most commonly classified as leiomyoma, leiomyosarcoma, leiomyoblastoma or Autonomic nervous system gastrointestinal tumor. This is why, for the accurate histologic diagnosis of these tumors, it is necessary to record the C-Kit mutation and to identify some other molecular markers that help in differential diagnosis. C-Kit mutation detection is a criterion for morphologic diagnosis and also it serves as a predictive factor for determining the therapy with Imatinib (Glivec) if it is present in these tumors**.Objectives**: Evaluation of 49 cases of C-Kit mutations and differential diagnosis assessment by IHC examinations.**Material and Methods:** The study is a retrospective cohort type. 49 patients was evaluated the C-Kit mutation and further IHC examinations for differential diagnosis with other similar lesions. For this purpose the immunoreactivity for CD117, CD34, Actin, Desmin, S100 was evaluated. In these cases, the proliferative index with Ki67 was also evaluated as a prognostic factor together with the tumor diameter and presence or not of necrosis.**Results**: In the cases studied the presence of c-kit mutation (CD117 positive) was detected in 97% of the examined cases, CD34 was found positive in 77% of the examined cases, SMA was found positive in 34% of the examined cases, Desmin was found positive in 17% of the examined cases, S100 was found positive in 7% of the examined cases. Ki67 resulted to be on average 17.6% in cases with mitotic index> 5/50 HPF and on average 5.7% in cases with mitotic index <5/50% HPF **Conclusions:** Most of the mesenchymal tumors in the gastrointestinal system are Gastrointestinal Stromal type. This tumor is diagnosed in most cases if C-Kit mutation (positive immunoreactivity for CD117) is identified. Other immunostains like CD34, SMA, Desmin, S100, help in the diagnose by differentiating this tumor from other histologically similar lesions. Proliferative index determination by Ki67 serves for the differentiation of these tumors into two prognostic categories.
Keywords: GIST, C-KIT mutations, differential diagnosis, malignant behavior

Introduction

Gastrointestinal Stromal Tumors (GIST) became a clear histological diagnostic entity in 1998, when it was found that nearly all GIST-s present mutations in the KIT gene. Prior to recognizing KIT mutations, GIST were most commonly classified as leiomyoma, leiomyosarcoma, leiomyoblastoma and as autonomic nervous system gastrointestinal tumors. Other mesenchymal tumors that constitute the differential diagnosis of GIST are precisely these tumors, as well as fibromatosis, angiomatous, neuronal, neuroendocrine tumors, and undifferentiated sarcoma, or carcinoma and melanoma metastases. That's why, for the accurate histologic diagnosis of this tumor, which is thought to derive from the progenitor cells of the "Interstitial Cells of Cajal", it is necessary to record the C-Kit Mutation and to identify some other molecular markers that help in differential diagnosis such as CD34, SMA, Desmin and S100. Tyrosine-kinase Inhibitor receptors such as imatinib mesylate and others of the younger generations such as sunitinib are effective in treating GIST. Thus, proper diagnosis of these tumors is important to provide adequate and effective therapy. For this reason, the positive immunereactivity of the c-kit has become an essential tool in evaluating neoplastic mesenchymal lesions of GI tract and for establishing GIST diagnosis (1). However, 4% to 15% of GIST does not stain with c-kit, and the morphology of these tumors is often similar to other mesenchymal tumors which constitute the differential diagnosis. Thus, Immunochemistry is an essential diagnostic examination in evaluating the neoplastic mesenchymal lesions of GI tract (1-4). On the other hand, the determination of proliferative index with Ki67, apart from the determination of maximal diameter tumor and the presence or absence of necrosis, divides these tumors into different prognostic categories.

Material and Methods

The tissue samples taken during the surgical intervents were fixed 10% buffered formalin for about 48 hours. From the examiner pathologist, measurements of the tumor dimensions were made and the cutting surface was inspected for possible macroscopic changes and evidence of the lesion on the organ wall. Representative tissue samples were taken for histopathologic examination, including continuity from the central area to the peripheral area. Selected tissue samples for histopathological examination were placed on tissue blocs and then placed on Leica 1020 automatic processor. Histopathologic examination has been performed by diagnosis and classification based on the histological type and the histological degree determined by WHO criteria described in

the "Blue Book" (5). In the respective microscopic magnification is evaluated the morphology of cells; Cellularity; Atypism, mitotic activity who is estimated by the presence of mitosis and is quantified for 50HPF; The level of invasion in the organ wall and the presence of necrosis. After evaluating the macroscopic and microscopic features described above, the histopathologic diagnosis and classification was made according to WHO classification in the "Blue Book" (5- WHO of GI).

After histological examination, was performed the selection of the most representative paraffin block for immunohistochemistry analysis. In all the 49 cases, full sections of the selected paraffin blocks were examined. Immunohistochemical staining was done automatically with Ventana Benc Mak XT system where antibodies were used according to the specifications presented in Table 1.

Antibodies	Clones	Manufacturer	Dilution
CD117	A4502	DAKO	1:250
CD34	QBEnd10	DAKO	1:50
SMA	1A4	DAKO	1:100
Ki-67	MIB-5	DAKO	1:100
DESMIN	D33	DAKO	1:100
S100	ZO311	DAKO	1:100

Table 1. Antibodies, clones, manufacturers and dilutions.

Presentation of the data recorded is based on tables and diagrams. The processing of data is based on the SPSS 20 statistical program.

Results:

In 44 cases diagnosed as GIST after IHC examinations, the male-female ratio was 1.44: 1. Below this report is presented graphically. The age interval in our study was 32-81 years. The average age turned out to be 61.5 years and the average age by gender was 63.2 for males and 59.5 for females.

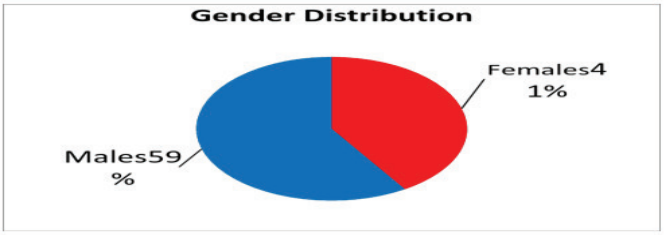

Figure 1: Gender distribution of cases diagnosed with GIST.

Age	Total	Female	Male
Average	61.5	59.5	63.2

Table 2: Age as per gender.

The most frequent localization resulted to be stomach with 59% of cases, followed by intestine with 32% of cases.

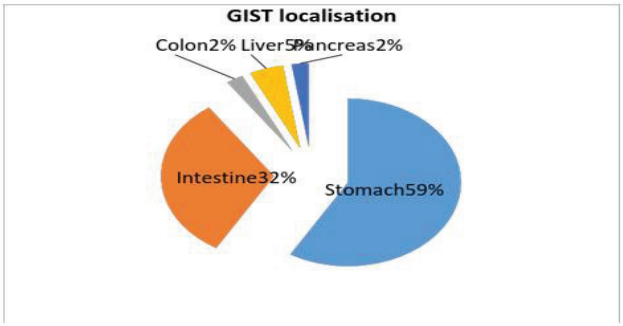

Figure 2: Distribution of cases by location

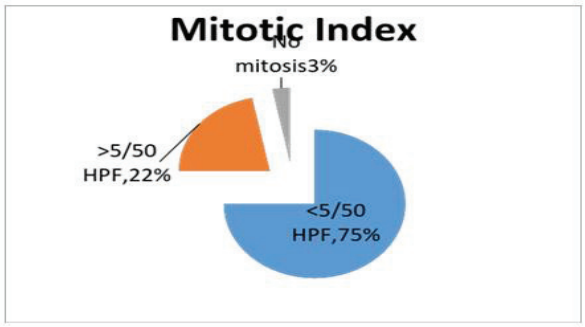

Figure 3: Case distribution according to mitotic index

The following graph was processed by analyzing the mitotic indexes. 75% of the cases resulted with low mitotic index, 22% with high mitotic index and in 3% of cases there were no mitosis. The average tumor diameter resulted to be 6.9 cm.

CD117 was applied in 82% of cases and resulted positive in 97% .CD34 was applied in 80% of cases and resulted positive in 77% of them. Actin was reviewed in 73% of cases and resulted positive in 34% of them. S100 was applied in 64% of cases and resulted positive in 7% of them. Desmin was applied in 68% of cases and resulted positive in 17% of them.

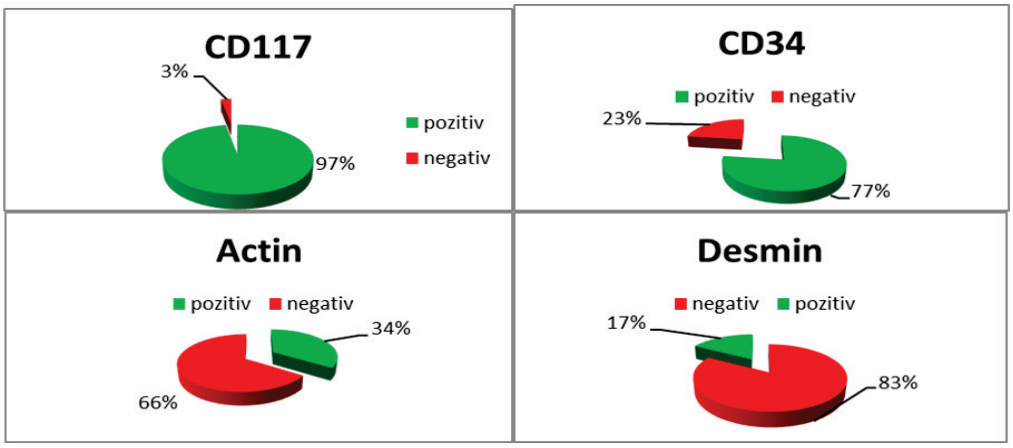

Figure 4: Distribution of immune-markers in examined cases

The average of proliferative index Ki 67 was 12%. The correlation between the proliferative index Ki67 and the mitotic index was analyzed. Ki67 resulted to be on average 17.6 in cases with mitotic index> 5/50 HPF. Ki67 resulted to be on average of 5.7 in cases with mitotic index <5/50 HPF. At the end 89.8% of the morphologically suspected diagnoses for GIST in Hematoxylin-Eosin stain resulted Gist in the final diagnosis after IHC examinations. The most common histopathologic diagnoses encountered were GIST with low malignancy in 50% of cases and GIST with intermediate malignity in 31% of cases. 75% of cases resulted with low mitotic index, 22% with high mitotic index and in 3% of cases no mitosis were observed.

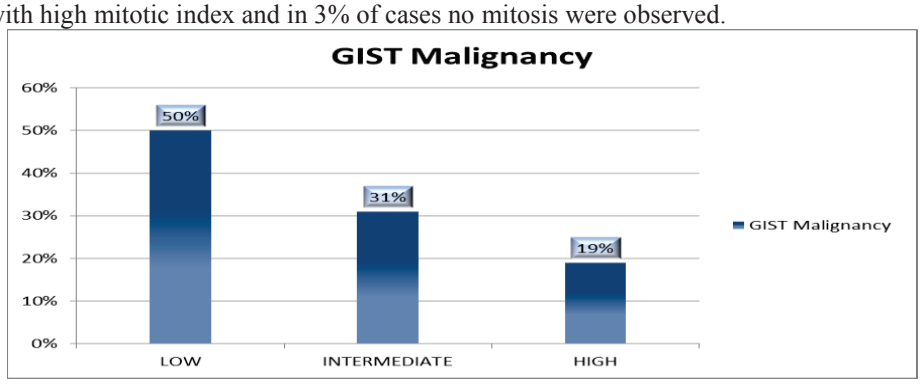

Figure 5. Distribution of Gist according to malignant potential.

Discussion

GIST-s, gastrointestinal leiomyoma or leiomyosarcoma, schwannoma, local extension of a retroperitoneal primary undifferentiated liposarcoma, benign and malignant vascular tumors, intra-abdominal fibromatosis (desmoid tumor), carcinoid tumor with a fusiform morphology, and melanoma metastasis with fusiform morphology, or carcinoma are the predominant tumors that need to be considered in the differential diagnosis.
Most of these tumors can be accurately evaluated based on accurate clinical data and careful microscopy examinations fulfilled by proper immunohistochemical stains, and sometimes even ultrastructural data and molecular biology examinations.

The differential diagnosis of GIST from primary tumors of smooth muscle may sometimes be difficult. Although the positive immunoreactivity for CD117 in GIST should greatly facilitate Gist-s distinction from primary smooth muscule tumors. In 5% of cases CD117 (c-kit) may result negative in immunohistochemistry(1-5), in GISTs with extreme myoid differentiation, thus making very difficult the distinction between the two entities with the current techniques. In this case the diagnosis remains largely based on the results of CD34, SMA and Desmin and may often not be totally definitive with current techniques.

Another potential problem are GISTs with epithelioid morphology, with negative immunoreactivity for CD117 and CD34, which may be encumbered by a metastasis of melanoma with a primary non-target focus. In this case, S100 can be found that are commonly found positive in melanoma and more rarely in GIST (4). Proliferative index Ki67 correlates in our study with the mitotic index in these tumors and thus serves for the differentiation of these tumors into two categories, which may be also prognostic categories.

Conclusions

Gastrointestinal stromal tumors (GIST) are the most common primary mesenchymal neoplasms of the GI tract. These tumors are found mostly in stomach and small intestine. This tumor is mostly diagnosed if C-Kit

mutation (positive immunoreactivity for CD117) is identified but positive immunoreactivity for Cd34 can help the diagnosis especially in cases with negative CD117. Other Immunostains as SMA, Desmin, S100, help in the differential diagnosis with other lesions with similar histologic patterns. Proliferative index Ki67 correlates with the mitotic index and helps identifying prognostic groups.

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