Nuclear morphometry and proliferative activity evaluation in the gastrointestinal stromal tumors

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Abstract
Twenty-two cases with gastrointestinal stromal tumors (GISTs) have been studied, sized from 2 cm to invasive gigantic tumors and also from low to high degree of malignancy. The altering of the form and the size of the nucleus is a reference point of malignancy, being used in the histological grading of many types of tumors and also as an appreciating parameter of the tumoral prognosis, with a high degree of accuracy in the colorectal, uterine, prostatic or ovarian cancers, as it was pointed in the previous researches. The aim of this study is to evaluate the dimensional characteristic of the nuclei and the mitosis in GIST with a cholic and gastric localization, attempting a quantitative differentiation of the two tumors, by studying the following aspects: nuclear dimensions, mitotic activity index and the mitotic density. The results of the proliferative activity quantification (mitotic activity index and mitotic density) have shown that this can be a decisive criterion for the precocious appreciation of the evolution. The most important morphological criterion with a predictive role is the mitotic activity index, but is recommended to be applied correlated with the size and the localization of the tumor. Although various nuclear morphometry studies in different types of malignant tumors have been performed, the data in gastrointestinal stromal tumors is scarce and only few similar studies have been reported in the specialty literature; from this point of view, the present study is new and original and is also trying to point out that even with GIST, such analysis and prognosis is as valuable as in any other malignant diseases.

Keywords: stromal tumors, aggressivity, nuclear morphometry, mitosis.

Introduction
The gastrointestinal stromal tumors (GISTs) are defined as mesenchymal stromal or epithelioid tumors of the gastrointestinal tract, emerging/developing due to the affinity of the receptor/receiving factor of the formant stem cells for tyrosine kinase, also known as KIT (CD117). GISTs represent the great majority of the formant stem cells for tyrosine kinase, also known as KIT (CD117). GISTs represent the great majority of the mesenchymal tumors of the digestive tract and their clinico-pathological profile is not completely clarified [1]. In order of frequency, it affects the stomach, the small intestine and the colon, rectum, or esophagus [2], but occasionally originates outside the intestinal tract.

The altering of the form and of the size of the nucleus is a pointing mark of malignancy, being used in histological grading, with a high degree of accuracy in colorectal, uterine, prostatic or ovarian cancers as it was shown in the previous researches [3, 4].

Different methods of diagnosis and prognosis are studied due to the similar morphological aspect, which cannot be differentiated by conventional methods. This way, the assisted analysis systems on PC, brings important data. The pathologist may obtain quantitative measurement on histological preparations. Nuclear measurements (area, roundness, perimeter) alone or combined, are useful instruments for potential estimation of prognosis on different types of cancer. Using the nuclear morphometry in GISTs is mentioned in the specialty literature [2–5]. Generally, in GIST, atypical nuclei are not obvious and their meaning is unclear [6].

The aim of this study is to evaluate the dimensional characteristics of nuclei and of mitosis in GIST, with gastric and cholic localization, attempting a quantitative differentiation of the two tumors.

Materials and Methods
Twenty-two cases with GISTs were studied, with sizes from 2 cm to invasive gigantic tumors and also from a low degree of malignancy to a high degree of malignancy. Paraffin blocks have been provided by 1st Surgery Clinic of Galați, Romania (two cases) and by the Laboratory of Microanatomy, “Grigore T. Popa” University of Medicine and Pharmacy, Iassy, Romania (Prof. Lucia Doina Frâncu) – 20 cases. The GISTs have been clinically diagnosed as malignant, following the next criteria: peripheral invasive growth, ganglionic metastasis as well as metastasis in other distant organs, tumoral recurrence. The following characteristics have been taken into evaluation with all cases: primary localization of the tumor, the size of the tumor, the presence of necrosis as well as the number of mitoses in 10 high-power fields (HPF). As for the gender distribution, there were 12 men and 10 women. Out of the 22 tumors, 14 were gastric and eight were cholic, one ordered ascendingly, three crosswise and five descendingly and sigmoid. Out of the 22 cases, six were tumors with sizes between 2 to 5 cm and the rest were bigger than 5 cm; the biggest ones were found at the level of the stomach and the right colon, the smallest ones were found in the left colon (2 cm). Sixteen cases presented with ganglionic invasion and the mitotic index was directly proportional to the size of the tumor.
and the ganglionic metastasis. Five cases presented with tissue necrosis associated with metastasis in other distant organs (i.e., liver).

Tumor aggressiveness appreciated correlating clinical symptoms and intraoperative and laboratory aspects.

For immunohistochemical analysis, sections were prepared from formalin-fixed paraffin-embedded tissue samples after deparaffinization and were stained with Hematoxylin and Eosin (HE). HE stained slides were reviewed for each case.

The assessment of the proliferation activity was performed on 50 consecutive fields in the area of the tumor with the most intensive activity, using a 10× ocular, a 40× lens with 0.75 numerical aperture and a circular field with 450 μm diameter and 0.159043 mm² area. The results were: mitotic activity index (MAI), which represents the total number of mitotic figures counted on 10 HPF; mitotic density, which represents the number of mitoses/mm².

The evaluation of nuclear dimensions was done in the HE-stained sections, using the interactive software PRODIT 5.2. After the computerized acquisition of the images, the examiner marks successively the outline of 30 nuclei for each case, the selected morphometric images, the examiner marks successively the outline of PRODIT 5.2. After the computerized acquisition of the HE-stained sections, using the interactive software Miettinen was selected.

The descriptive statistic posts the nuclear parameters’ outline and roundness) being automatically calculated.

In GIST with colic localization, the average of quantified nuclear parameters was the following: nuclei area is 48.35 μm² averagely. The median line is 48.04 μm² and the module is 47.50 μm², so the value distribution presents with a positive asymmetry, the average being characteristic to the small values or nuclear areas. Nuclei perimeter is averagely of 25.14 μm. Maximum perimeter is 26.61 μm and minimum of 23.40 μm. Median line is 25.10 μm and the module is 24.45 μm, with an existing positive asymmetry of individual results. Nuclei diameter is averagely 7.83 μm, median line is 7.81 μm and the module is 7.79 μm, values that show the existence of a discreet positive asymmetry in individual results, and which testifies a very high degree of representativeness of the average value. The maximum diameter is 8.33 μm and the minimum is 7.40 μm. The long axis of nuclei is averagely 8.62 μm, the median line is 8.49 μm and the module is 8.44 μm, with a discreet positive asymmetry of individual results, which highlights a very high degree of representativeness of the average value. The maximum value is 9.73 μm and the minimum value is 8.06 μm.

In GIST with gastric localization, the average of quantified nuclear parameters was the following: nuclei area is 35.48±3.8 μm² (minimum 46.37 and maximum 64.39), median line of 55.84 μm² and the module of 53.17 μm², so the distribution of individual values presents with a positive asymmetry, low values of the characteristic being predominant. Average perimeter of nuclei is 26.90±0.94 μm (minimum 24.91 μm and maximum 29.17 μm). The median line is 26.94 μm and the module is 26.00 μm, with a positive asymmetry of the distribution of individual values. Average diameter of nuclei is 8.40±0.29 μm (maximum 9.06 and minimum 7.68), median line of 8.43 μm and module of 8.47 μm, with a slight negative asymmetry of individual values, which are small on a reduced area (varies 0.08), highlighting a very high degree of representativeness on average value. The long axis average of nuclei is 9.48±0.57 μm (maximum 10.66 and minimum 8.49), the median line and the module having values slightly equal (9.50 μm and respectively 9.52 μm), with a symmetry of individual result, which highlights the very high degree of representativeness of average values (Table 2).
The quantification of the mitosis by the two parameters, the mitotic activity index (MAI) and the mitotic density (MD = mitoses/mm²) have revealed differences in the tumors of the two studied localizations (Table 3).

| Table 2 – Synoptic table with the quantified nuclear parameters in the two localizations of GIST |
|--------------------------------------------------|----------------|----------------|
| Parameter                                      | Gastric GIST | Cholic GIST |
| Area [μm²]                                     | Average ±SD | Max. | Min. | Average ±SD | Max. | Min. |
| 48.29 ±2.67                                    | 55.48 ±3.80 | 64.49 | 46.38 |
| Perimeter [μm]                                 | 25.14 ±0.72 | 26.90 ±0.94 | 29.17 | 24.92 |
| Diameter [μm]                                  | 7.83 ±0.22 | 8.40 ±0.29 | 9.06 | 7.68 |
| Long axis [μm]                                 | 8.63 ±0.41 | 9.48 ±0.57 | 10.66 | 8.49 |

Both MAI and MD have higher averages in the colic location than the gastric. In addition, MAI has higher absolute values, being easy to interpret.

### Discussion

GISTS arise most commonly in the stomach followed by the and then small intestine, rectum, or esophagus. Occasionally, GISTs originate outside the intestinal tract [8, 9]. According to recent data, the most useful clinicopathological prognostic parameters in GISTs are tumor stage, size, histological type, degree of necrosis, mitosis activity and nuclear pleomorphism [2, 10, 11].

The most consistent histopathological features to predict aggressiveness are tumor size and number of mitoses. A high Ki-67 index and high expression of p53, PCNA, Bcl-2, and vascular endothelial growth factor are frequently associated with poor prognosis [12–14].

Computer-assisted quantitative image analysis is a method important in diagnostic procedures. The study of nuclear shape (nuclear morphometry) quantitatively, either alone or in combination with mitosis quantification, is a potentially useful tool for predicting the prognosis of GISTs. Nuclear morphometry analysis is a useful tool for estimating prognosis in various types of cancer [3, 15, 16].

The growth of the nuclei has been recorded frequently in malignant tumors. Therefore, the size of the nuclei is an important indicator of prognosis in the oral carcinoma with squamous cells, in mammary carcinoma, renal carcinoma and prostate carcinoma [3, 15–18]. Shape modifications of the nuclei represent a distinctive factor in malignant tumors as well as in the evaluation of potentially metastasis of colorectal adenocarcinoma [19, 20]. It had been found that in ovarian carcinoma, shape modifications of the nuclei are more frequent than in borderline ovarian tumors [21].

Previously presented data shows the importance of clinical use of the nuclear morphometric parameters as prognosis factors, in comparison with the conventional systems of classification for malignancy in different organs. Such method also allows the exact measurement of the cells, the shape and their organization, quantification that cannot be realized by other methods. The computer-assisted analysis of the results is precise and with an over 99% sensitiveness [22].

Although various nuclear morphometry studies in different types of malignant tumors have been performed, the data in gastrointestinal stromal tumors is scarce and only few similar studies have been reported in the specialty literature; from this point of view, the present study is new and original and is also trying to point out that even with GIST, such analysis and prognosis is as valuable as in any other malignant diseases.

Comparing the data obtained for the two studied localizations, there are observed superior values in the colon localization, maybe due to the rapid evolution and a higher aggressivity percentage. Nuclear morphometric parameters obtained on personal cases have the same values as to the ones in other studies [2] done on GIST with different localizations, without making separate evaluations (Table 2).

GIST with gastric localization has a worse prognosis than the tumor of the same size but with intestinal localization [23]. Most studies have evaluated mitosis on 50 HPF. In specialty literature, recent studies have been done with a similar technique to ours in appreciating the number of mitosis and different values have been found, function of the size of the tumor: 5.63±7.39 in benign tumors between 0 and 27 cm, 2.6±5 in benign tumors between 0 and 16 cm and 7.7±8.2 in malignant tumors between 1 and 27 cm [24]. The quantified values in personal casuistry are within the limits set out above, proliferative activity was lower than in other forms of cancer.

Gastric and jejunum-ileal GIST with the size lower than 5 cm and five mitoses or more on 50 HPF has a high malignancy degree. In colon GIST, the ratio between benign and malignant forms is 1:2 [24].

The most important morphological criteria with a predictive role is the mitotic ratio [23, 25, 26], but it is recommended to be applied correlated with the size of the tumor. However, the localization of the tumor must be taken in consideration, because it prints the degree of aggressivity, gastric tumors being less aggressive than the intestinal ones [27].

Most researches believe that mitosis quantification represents the evaluation method with the highest degree of accuracy, if done on the areas with the most prolife-rative activity. The limit of five mitoses on 50 HPF is considered to be the limit to which a tumor is expected to have a benign behavior and also being the mark between benign and malignant [7, 23]. However, a small number of benign tumors with a low mitotic index may metastases ulcerior, especially in intestinal localizations [28], thus demonstrating the evolving individuality of each case.

In other types of tumors, the mitotic index is sure of malignancy with higher values, between 20–50 on 50 HPF [26]. On the other hand, it was shown that some tumors with a low mitosis index may metastases [1].

The number of mitosis represents the variable that

### Table 3 – Synoptic table with the quantified proliferative activity values in the two localizations of GIST

<table>
<thead>
<tr>
<th>Localization</th>
<th>Reduced aggressivity</th>
<th>High aggressivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric GIST</td>
<td>1.6</td>
<td>6</td>
</tr>
<tr>
<td>Cholic GIST</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

The number of mitoses in the gastric localization is higher than in the colic localization.
can evaluate with most accuracy the GIST malignancy and out results confirm it. We, thus, support the classification system of GIST suggested by Fletcher et al. (2002) [29], which correlated the size of the tumor to the number of mitosis. The morphologic features that appear to be most predictive of outcome and biological behavior are nuclear shape and the mitotic rate [30].

Variables’ measurements by digital programs may be a complementary method in tumor evaluation without being influenced by potential subjective interpretation on the part of the investigator. The malignant risk of GIST must be calculated by the pathologist based on traditional and morphometric criteria, in order to help the clinician to offer to the patient the most adequate therapy.

Conclusions

Dimensional quantification of the tumoral nucleus shows superior values at the level of the colon localization in comparison with the gastric one. The same sense of differences is found in the mitosis quantification. The results of the proliferative activity quantification (mitotic activity index and mitotic density) have shown that these can be decisive criteria for the precocious appreciation of the evolution. The localization of the tumor imprits the degree of aggressivity, gastric tumors being less aggressive than the intestinal ones. The most important morphologic criterion with a predictive role is mitotic activity index but it is recommended to be applied in correlation with the size and localization of tumor. We consider that studies performed on bigger batches of patients, with morphometric analysis on histological subtypes, correlated with immunohistochemical data, may represent an important prognosis factor in GIST with influences on the therapeutic modes.

References


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