Summary of the Phd Thesis:
THE HISTOLOGICAL AND HISTOPATHOLOGICAL STUDY OF GASTRO-INTESTINAL AND RETROPERITONEAL MESENCHYMAL TUMORS
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This PhD thesis consists in two major parts, a general and a personal contributions one.

The general part has three subchapters. In the first subchapter I briefly presented the general histological structure of the digestive tract, along with its regional particularities. The second subchapter focuses exclusively on the Cajal interstitial cells, which are considered to be the precursors of gastro-intestinal stromal tumors (GISTs), the most frequent mesenchymal tumors found in the digestive tract. Within the third subchapter I included a brief description of the new terminology used in gastro-intestinal mesenchymal tumors, of the 2002 WHO classification of these tumors and of the six types of mesenchymal tumors that can develop in the digestive tract and retroperitoneally.

At the beginning of the personal part I underline the premise of the study: since gastro-intestinal mesenchymal tumors do not have a highly characteristic histopathological aspect, most spindle cell sarcomas of the digestive tract were classified as leiomyomas or leiomyosarcomas (due to the resemblance with smooth muscle) and occasionally as neural tumors before the advent of immunohistochemistry. At a later time, the main histological, histopathological and immunohistochemical features of gastro-intestinal mesenchymal tumors were established by means of immunohistochemical and molecular studies. Most of these studies showed that a large part of the gastro-intestinal mesenchymal tumors originate in a certain group of cells, the interstitial Cajal cells and are, as such, GISTs.

Thus, the purpose of this thesis is a complex evaluation of the gastro-intestinal mesenchymal tumors included in the study, through classic, histological methods, and modern, immunohistochemical ones. I also aimed to establish the importance of a correct and exact histopathological diagnosis from a macroscopic, microscopic and immunohistochemical point of view, because this diagnosis represents the basis of subsequent assessment of the patient and establishment of an adequate therapeutic approach.

The main objectives were to assess the importance of immunohistochemical examination in the diagnosis of gastro-intestinal mesenchymal tumors, as GISTs have a specific targeted therapy, and to establish certain positive, differential and malignancy diagnostic criteria based upon a morphologic, histologic and immunohistochemical study.

I carried out a retrospective study based, on one hand, upon the content of the cases’ pathological reports (previous macroscopic, microscopic and immunohistochemical examinations) and, on the other hand, upon a microscopic and immunohistochemical re-evaluation of the cases included in my study. The material of the study was selected from the registry comprising cases examined at the Pathology Department from Tîrgu Mureș, between 2002 and 2008. Observing the definitions, classification criteria and WHO coding of gastro-intestinal mesenchymal studies, as presented in the general part, I selected a total of 213 cases, of which 193
cases matched the inclusion criteria. The selected cases were divided into six initial study groups: GISTs – 51 cases, real smooth muscle tumors – 51 cases, nervous tumors – 16 cases, fibrous tumors – 11 cases, adipose tissue tumors – 50 cases and vascular tumors – 12 cases. Each group of tumors was assigned a study chart based upon microscopic and immunohistochemical criteria specific to each class of gastrointestinal mesenchymal tumors.

Following microscopic re-evaluation, it was apparent that in most cases of mesenchymal tumors microscopic features are very similar, especially in the first four groups, and they have frequently caused confusions in establishing the final pathologic diagnosis. As such, cases pertaining to these groups (GISTS, tumors of muscular, nervous and fibrous origins), that pose the most positive and differential diagnosis problems, as well as classification problems, were subjected to an immunohistochemical study. Adipose tissue and vascular tumors were excluded from this study because their microscopic features are specific and positive and differential diagnoses do not raise any particular problems. The immunohistochemical study comprised four types of primary antibodies with specificity for a certain type of tumor: CD117 (DAKO) and CD34 (DAKO) for GISTs, SMA (Lab Vision) for muscle tissue tumors and S100 (DAKO) for nervous tumors. Primary antibodies were not used only for positive diagnosis; they also served in differential diagnosis. Negative control was obtained through processing a tumor section by the standard protocol except the primary antibody, which was replaced with a control solution. Immunohistochemical staining according to each antibody’s antigen localization was considered a positive reaction, whereas lack of staining was considered negative.

Data obtained were included within an individual study chart, coded and statistically analyzed.

The results were grouped as follows:
- In the GISTs group:
  - CD117+, CD34±, SMA± and S100± tumors: 47 cases; the diagnosis of GIST was proposed
  - CD117-, CD34-, SMA+ and S100- tumors: 3 cases; the diagnosis of muscle tissue tumor was proposed
  - CD117-, CD34-, SMA- and S100+ tumors: 1 case; the diagnosis of nervous tumor was proposed
- In the muscle tissue tumors group:
  - CD117-, CD34-, SMA+ and S100- tumors: 25 cases; the diagnosis of muscle tissue tumor was proposed
  - CD117+, CD34±, SMA± and S100± tumors: 26 cases; the diagnosis of GIST was proposed
- In the nervous tumors group:
  - CD117-, CD34-, SMA- and S100+ tumors: 10 cases; the diagnosis of nervous tumor was proposed
  - CD117+, CD34±, SMA± and S100± tumors: 6 cases; the diagnosis of GIST was proposed
- In the fibrous tumors group:
  - CD117-, CD34-, SMA± and S100± tumors: 11 cases, the diagnosis of fibrous tumor was proposed

The final study groups were established according to these results. Thus, the number of GIST cases increased to 79 cases, especially on the expense of muscle tissue tumors, which decreased to 28 cases, and less so with regards to nervous
tumors, which decreased to 11 cases. The number of fibrous tumors remained the same as the initial one, 11 cases.

General, histologic and histopathologic features were observed based upon the final study groups.

1. In the GISTs group, the mean age of the patients was 59.94 years and the gender distribution was almost equal. The most frequent localization was the stomach (40%). Macroscopically, the tumors are nodular (75%) and have a pale white color (81%). *Areas of necrosis (p=0.0217)* and *ulceration of the mucosa (p=0.0358)* are specific to tumors over 5 cm. Most GISTs present microscopically as *spindle cell* tumors (67%); less frequently they have epithelioid (13%), mixed (9%), signet ring cell (2%) or myxoid (9%) features. GANT variant, with specific features like *intestinal localization (p<0.0001)* and the presence of *skeinoid fibers (p=0.0485)* was encountered in 13 cases. The number of mitoses does not correlate with the size of the tumor (p=0.2462760). The age and gender of the patients and the localization of the primary tumor do not correlate with the degree of malignancy (p=0.4585, p>0.05, p=0.2647), while *invasion of the mucosa and tumor necrosis* are statistically significant when compared to the degree of malignancy (*p=0.0303 and p=0.0386*). Immunohistochemically, the antibody yielding the greatest specificity was CD117 (97%), followed by CD34 (63%) and, with greatly reduced values, SMA (24%) and S100 (13%).

2. In the muscle tissue tumors group, the mean age of the patients was 56.79 years and the gender distribution was almost equal. Benign tumors were localized mostly in the digestive tract, whereas the malignant ones were predominantly localized retroperitoneally. Macroscopically, these tumors are nodular (78%) and have a pale white color (52%). Microscopically, they are spindle cell tumors; high number of mitoses, tumor necrosis and cytonuclear pleomorphism significantly correlate with the degree of malignancy (*p=0.0011*). The immunohistochemical profile is specific for this group of tumors, as they are positive to SMA (100%) and negative to the other three antibodies – CD117, CD34 and S100.

3. In the nervous tumors group the mean age of the patients was 49.81 years and the gender distribution showed a feminine predominance (10/1). With regards to their localization, these tumors appear in the same proportion in the digestive tract and in the retroperitoneum. Macroscopically, these tumors are nodular (73%) and have a pale white color (81%). Microscopically, most of them are spindle cell tumors; atypical mitoses, tumor necrosis and cytonuclear pleomorphism are not characteristic. The immunohistochemical profile is specific for this group of tumors, as they are positive to S100 (100%) and negative to the other three antibodies – CD117, CD34 and SMA.

4. In the fibrous tumors group, the mean age of the patients was 55.27 years and the gender distribution was almost equal. Malignant fibrous tumors are localized in the retroperitoneum exclusively (100%). Macroscopically, these tumors are nodular (81%) and have a pale white color (63%). Microscopically, all fibrous tumors presented spindle cell features; atypical mitoses, tumor necrosis, cytonuclear pleomorphism and large dimensions are specific to malignant fibrous tumors. The immunohistochemical profile is specific for this group of tumors, as they negative to CD117 și CD34 and focally positive for SMA or S100.

**Conclusions:**

- Based upon immunohistochemical results, the number of cases from the 4 groups analyzed changed in favour of the GISTs.
- GISTs represent 41% of all mesenchymal tumors; other gastro-intestinal mesenchymal tumors share a smaller proportion - 26% adipose tissue tumors, 15% muscle tissue tumors, 12% vascular tumors, 11% nervous and fibrous tumors.
- The onset age in mesenchymal tumors is approximately 60 years.
- Gender distribution is in most cases uniform.
- With regards to localization, GISTs, benign muscle cell tumors and nervous tumors are located in different segments of the digestive tract whereas malignant muscular and fibrous tumors are found retroperitoneally.
- Macroscopically, most gastro-intestinal stromal tumors are nodular and have a pale white color. Areas of necrosis and mucosal ulceration are specific for larger tumors.
- Microscopic localization within the gastro-intestinal wall comprises its whole thickness (in tumors over 5 cm) and, more seldom, tumors are located within the submucosa and the muscularis propria.
- Cytomorphologically, most gastro-intestinal mesenchymal tumors have spindle cell features; other variants are present in smaller numbers.
- Metastases are extremely rare – the most frequent are lymph node metastases, followed by hepatic ones.
- In GISTs the number of mitoses and tumor size are correlated with the degree of malignancy and can be used as main prognostic factors.
- GIST size is not correlated with the number of mitoses
- Cytonuclear pleomorphism is not characteristic in GISTs
- Tumor necrosis and mucosal invasion are secondary prognostic factors in GISTs
- The age and gender of patients and primary tumor localization are not correlated with the degree of malignancy.
- A great number of atypical mitoses, marked cytonuclear pleomorphism and tumor necrosis are specific for sarcomas.
- The most suitable antibody for diagnosis in GISTs is CD117. Reactivity to CD34 is lower, whereas positivity to SMA and S100 is rarely observed.
- Muscle tissue tumors are 100% positive to SMA
- Nervous tumors are 100% positive to S100
- Fibrous tumors are sometimes focally and non-specifically positive to SMA or S100. The latter three types of tumors are negative to CD117 and CD34.
- A correct diagnosis concerning the different groups of mesenchymal tumors must include immunohistochemical examinations using a panel of antibodies specific to these tumors, in order to establish future patient management.