UNIVERSITY OF MEDICINE AND PHARMACY TÎRGU MUREȘ

SCHOOL OF DOCTORAL STUDIES

IMMUNOHISTOCHEMICAL AND MOLECULAR PROGNOSTIC FACTORS IN GASTROINTESTINAL STROMAL TUMORS

PhD Candidate: **Attila Kövecsi**
Scientific Supervisors: **Prof. Dr. Simona Gurzu, Prof. Dr. Ioan Jung**

ABSTRACT

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal neoplasms of the gastrointestinal tract. The correct identification of these tumors is decisive, once biological targeted therapy with tyrosine kinase inhibitors has become available. In recent years have been their biological behavior, clinical and pathological characteristics, molecular aspects and therapeutic possibilities extensively investigated. GISTs have a broad spectrum, varying in size, benign/malignant behavior, clinical symptomatology, location, histological and prognostic aspects. It may affect any segment of the gastrointestinal tract, sometimes extragastrointestinal structures. An important feature of these tumors is the varied prognosis, 4 prognostic groups being accepted, based on the risk of recurrence. Morphological parameters based on which the risk of recurrence is estimated are mitotic rate, tumor size, and location.

The personal contribution part consists five observational studies.

1. **The prognostic value of clinicopathological parameters in gastrointestinal stromal tumors**

The aim of our study was to analyze the clinicpathological factors and their prognostic value in patients diagnosed with GIST. After the analysis of the 80 cases, a maximum incidence of GISTs was observed in the 61-70 age group, and a slight female predominance. The mean age of the patients was 61.58 ± 11.84 years (range 19-80 years). In most of the cases, the GIST was located in the stomach (n=35), followed by the small intestine (n=25), extragastrointestinal area (n=14), and colorectal segments (n = 6) respectively. The mean tumor size was 6.47 ± 4.67 cm (range 0.4 - 21 cm). The vast majority of the cases were included in the spindle cell type (n=64), followed by the mixed type (n=14), and only 2 cases were epithelioid cell tumors. The mean mitotic rate was 8.43 ± 14.02 (range 0-89). The mitotic rate of gastric tumors was lower than the extragastric (intestinal and E-GIST) (p=0.002). In case of gastric tumors the risk was very low/low and intermediate, while the risk in intestinal and extragastrointestinal tumors were higher (p=0.001). The presence of intratumoral necrosis was correlated significantly with the mitotic rate (p=0.0008), tumors with mitotic rate over 5/50 HPF showing more frequent necrosis. The presence of intratumoral necrosis was also associated with the risk of recurrence (p<0.0001), tumors at high risk of recurrence had more frequent necrosis. E-GISTs showed more frequent necrosis than those in the digestive tract (p=0.029). Tumors with a mitotic rate above 5/50 HPF, showed more frequent metastasis than those with less than 5/50HPF (p=0.01). The presence of local invasion was associated with higher mitotic rate (p<0.0001). In our study the age, sex of the patients, the histological type and the tumor location did not influence the overall survival. Patients with smaller tumors have a higher overall survival rate than those with large

tumors, but did not show a strong statistical difference (p=0.053). Overall survival was better patients with low mitotic rate (p=0.039), very low/low risk and intermediate risk groups (p=0.003), but was not influenced by the presence of intratumoral necrosis (p=0.18), metastasis (p=0.94) and local invasion (p=0.14). In 7 cases were synchronous tumors identified, most common digestive tract carcinomas.

The conclusions are that more than half of GISTs are diagnosed in patients over 60 years of age, with a male female ratio 0.77. The most frequent locations in decreasing order were: stomach, small intestine, extradigestive area and colorectal segments. GISTs located in the stomach are associated with smaller dimensions and with lower mitotic rate compared to extragastric tumors. The most common histological type is spindle cell type, followed by the mixed type and epithelioid type. Most GISTs (62.5%) have a low mitotic rate (below 5 mitoses/50 HPF). GISTs with intratumoral necrosis are associated with higher mitotic rate and increased risk of recurrence. 13.75% of the cases have metastases. The most common risk of recurrence group is the high-risk, followed by the low, intermediate and very low risk groups. Overall survival of patients is influenced significantly by the mitotic rate and the risk of recurrence. The most common synchronous tumors are digestive tract carcinomas, these tumors are more common with GISTs with gastric localization.

2. The role of immunohistochemical markers in the diagnosis of gastrointestinal stromal tumors

In this study we evaluated the diagnostic value of several immunohistochemical markers in the diagnosis of gastrointestinal stromal tumors.

The positivity rate of c-KIT and DOG-1 in GISTs was 92.50% (74/80) and 76.25% (61/80) respectively. PKCθ was positive in 90% of cases (72/80). The value of the Ki67 index was correlated with c-KIT expression, cases with c-KIT positivity showed predominantly a lower Ki67 index (<5%) (p=0.03). 25% of the cases (n=20) showed a higher Ki67 proliferation index. 70% of the cases expressed all of the three markers: c-KIT, DOG-1, PKCθ. All of the 61 DOG-1 positive GISTs and 13 of the 19 DOG-1 negative cases (68.42%) showed c-KIT positivity (p=0.0001). The expression of c-KIT and PKCθ did not show significant correlations with clinicopathological parameters. DOG-1 expression was significantly correlated with tumor location, a greater rate of positivity was observed in GISTs located in the small intestine or retroperitoneal (p=0.02). Examining the diagnostic markers, only the PKCθ expression influenced the overall survival rate (p=0.03). The higher Ki67 proliferation index was associated with lower overall survival rate (p=0.02).

The conclusions are that the sensitivity of the c-KIT, DOG-1 and PKCθ markers in GISTs are 92.5%, 76.25% and 90%, respectively. In the duble (c-KIT and DOG-1) negative GISTs PKCθ expression should be verified for a complex differential diagnosis. The PKCθ expression in GISTs may be a possible prognostic indicator. The Ki67 proliferation index above 5% may be an additional risk factor.

3. Epithelial-mesenchymal and mesenchymal-epithelial transition in gastrointestinal stromal tumors

In this study we analyzed the possible role of several immunohistochemical markers, which are generally involved in the epithelial-mesenchymal transition (EMT) and in mesenchymal epithelial (MET), respectively, in the plasticity of tumor stem cells and histogenesis of gastrointestinal stromal tumors. The reason of this study is based on recent literature reports emphasizing the role of EMT/MET-related markers with a negative prognostic role in malignant tumors, including GISTs.

We found no positivity for E-Cadherin in the examined cases. Most of the cases (88.75%, n=71) showed positivity to SLUG, respectively VSIG1 83.75% (n=67). CD44 and N-cadherin were positive in 36.25% (n=29) and 10% (n=8) respectively. None of the four positive markers (SLUG,

CD44, N-Cadherin, VSIG1) were correlated significantly with clinicopathological parameters. All six c-KIT negative cases expressed SLUG, were negative for N-Cadherin and positive or negative for CD44 and VSIG1. The SLUG expression was not correlated with N-Cadherine expression (p=0.58). A reverse correlation between the PKCθ and N-Cadherin expression was showed (p=0.029), and between N-cadherin and VSIG1 expression (p=0.021) was observed, respectively. The expression of VSIG1 was correlated with PKCθ expression (p=0.012). The VSIG1 expression has positively influenced the overall survival (p=0.01), but the SLUG, CD44, N-Cadherin expression did not influence the survival.

The conclusions are that the positivity rate of the used IHC markers are: 88.75% SLUG, 83.75% VSIG1, 36.25% CD44, 10% N-Cadherine, 0% E-Cadherine. The positive expression of VSIG1 in GISTs correlates with higher overall survival rate. The SLUG, N-Cadherine and CD44 expression were not correlated with the clinicopathological parameters and survival rate.

4. Angiogenesis in gastrointestinal stromal tumors

The aim of these study was to evaluate the prognostic value of immunohistochemical markers with role in the angiogenesis of gastrointestinal stromal tumors.

The mean intratumoral microvascular density (MVD) for CD31 was 3.56 ± 2.36 (1.15-13.09), for CD105 3.29 ± 2.11 (0.94-12.15). Cases with MVD≤4.10 (CD31) or MVD≤3.85 (CD105) were considered as low MVD tumors. Cases with high MVD were considered tumors with marked angiogenesis. 32.5% of the cases expressed VEGF (n=26). GISTs located in the small bowel showed a higher MVD (CD31) (p=0.01). The high MVD (CD31) was also associated with high Ki67 index (p=0.01). In the case of high MVD (CD105), we showed significant correlations with increased tumor size (p=0.02), higher mitotic rate (p=0.001), high-risk group of recurrence (p=0.00003), higher Ki67 proliferation index (p=0.01), local invasion (p=0.002) and intratumoral necrosis (p=0.0004). The expression of CD105 in tumor cells was observed in 20% of the cases, this expression was correlated with extragastric location (p=0.02). The VEGF expression were significantly correlated with high mitotic rate (p=0.0001), high-risk of recurrence (p=0.001), with intratumoral necrosis (p=0.001) and with the presence of distant metastases (p=0.03). A higher MVD (CD31) was associated with shorter overall survival rate (p=0.04), but a higher MVD (CD105) and VEGF expression did not influence the overall survival.

The conclusions are that intratumoral microvascular density determined with CD31 correlates with tumor localization in the small intestine, with high Ki67 index (>5%) and a shorter overall survival rate. Intravascular microvascular density determined with CD105 correlates with tumor size (>5 cm), high mitotic rate (>5/50 HPF), high risk of recurrence, higher Ki67 index (>5%), intratumoral necrosis and distant metastasis. VEGF expression in GISTs were correlated with increased mitotic rate, higher risk of recurrence, intratumoral necrosis and distant metastasis.

5. *KIT* and *PDGFRA* mutations in gastrointestinal stromal tumors

The aim of this study was to determine the mutational profile in gastrointestinal stromal tumors in order to highlight some correlations between the type of mutation and some morphological and immunohistochemical patricularities of these tumors.

c-KIT or *PDGFRA* mutations were identified in 14 tumors of the cases subjected to molecular analysis (n=28), the rest of the cases did not show detectable mutations of *KIT/PDGFRA* genes (*KIT/PDGFRA-WT*). Various mutations were detected on the *KIT* exon 11 gene (n=10) as follows: in 5 cases deletions, in 3 cases insertions, and in 2 cases point mutations. The two cases with *KIT* mutations on exon 9 showed point mutations. The two *PDGFRA* mutant cases were affected in exon 18 (D842V substitutions). No PDGFRA mutations on exon 12 were identified. No significant correlations were found between the presence of *KIT/PDGFRA* mutations and clinicopathological

parameters. Patients with *KIT/PDGFRA-WT* tumors showed higher overall survival rates, but the results were not significant (p=0.18).

The cocnclusions are that the presence of *KIT/PDGFRA* mutations did not prove to be prognostic factors, they have only predictive role. No correlations between *KIT/PDGFRA* mutations and classical prognostic parameters were observed: tumor size, mitotic rate or risk of recurrence.

Key words: gastrointestinal stromal tumor, c-KIT, DOG-1, PKCθ, epithelial-mesenchymal transition, angiogenesis, prognostic factors.