The Types of Tumoral Vessels Associated to GISTs Are Conditioning the Effectiveness of Anti - Vascular Therapy with Tyrosine Kinase Receptor Inhibitors

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Gastrointestinal stromal tumors (GIST'sare the most common mesenchymal neoplasms of the alimentary tract. Angiogenesis is an essential condition in the growth and development of tumors, especially in the case of GISTs due to their particular behavior. The aggressive behavior depends on the size and site of the tumor in close relationship with the intratumoral vascular development. The purpose of this study is to investigate, using immunohistochemical stainings, the types of intratumoral vessels in GIST's, knowing the interrelation between the tumor angiogenesis and the response to the targeted antivascular therapy, influencing directly the prognosis. In our research, in all 37 cases the most numerous vessels were the immature and intermediate ones, signaling an increased angiogenic activity. The perivascular cell free vessels are the most sensitive at antivascular therapy with tyrosine kinase receptor inhibitors, providing a good response and prognosis to the treatment. Immature vessels evaluation especially, could be a an important sign in assessment of the effectiveness of antivascular therapy in GIST.

Keywords: types of vessels, GIST, anti-vascular therapy, tyrosine kinase receptor inhibitor

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the alimentary tract. This tumors count less than 1% of digestive neoplasms and around 10% of soft tissue tumors of this site [1]. The global incidence is 15cases/million/year [2]. For a long period of time this tumors were considered leiomyomas or leiomyosarcomas, schwannomas and remained unrecognized as a separate type. Initially, GISTs were regarded to be extremely rare, but as immunohistochemical studies have improved, much more cases were reported also allowing to discover and understand the origin of the tumors, the molecular mechanism of carcinogenesis, the behavior according to the malignant potential and last but not the least the oncologic treatment including surgery and the therapy with biologic agents. All this findings have determined the distinction of GIST [3].

GIST is originating from the interstitial cell of Cajal (ICC) [4]. The cell was named interstitial because of the interposition between the intramural neurons and smooth muscle cells of the digestive tract. ICC is serving as pacemaker, mediating propagation of intrinsec slow electrical wave of gut peristaltics [5,6]. Both GIST cell and ICC are presenting the similar expression of CD34 and CD117 [7]. The immunohistochemical staining for CD117 is the main criterion in diagnosis of GIST, having a sensitivity of 95% and a high specificity, compared to the expression for CD34 positive in 60-70% of tumors [8]. CD34 and CD117 are part of the cluster of differentiation protocol used in the investigation of cell surface molecules helping cells immunophenotyping [9,10]. CD34 encodes a cell surface glycoprotein and functions as a cell-cell adhesion factor. It is normally found in mesenchymal stem cells, endothelial cells of blood vessel and in tumors like giant cell fibroblastoma, Kaposis sarcoma, dermatofibrosarcoma, granulocytic sarcoma, liposarcoma, papillary thyroid carcinoma, neurofibromas, malignant fibrous histiocytoma, meningiomas, gastrointestinal stromal tumors [11-13]. CD117 encodes a protein which is the tyrosine kinase receptor type III, known as KIT or stem cell factor receptor(SCFR) [14-16]. In normal physiology KIT is activated by binding the extracellular ligand called stem cell factor(SCF) [17-19]. The result is the appearance of intracellular signaling pathways leading to increase cell proliferation. In GIST carcinogenesis is involved a gain of excess function at the stem cell factor receptor, in the absence of ligand SCF, due to a mutation of proto-oncogene c-Kit located on chromosome arm 4q12 [20]. Between 5-15% of GISTs are considered Wild Type GIST not presenting KIT mutation, but having a gain of function activity [21]. The mutation in this cases is showing up in platelet-derived growth factor receptor A (PDGFRA).

GISTs are more frequent after the age of 50, being uncommon under the age of 40 and extremely rare at children [6,22]. The most common site is the stomach 50-60%, followed by the small intestine 20-25%, colon and rectum 5%, esophagus, appendix 2% [23-25]. Very rare locations are the liver, pancreas, gallbladder, vaginal septum, portal vein, genitor-urinary tract, pleura [26-33]. Extra-gastrointestinal stromal tumors (EGIST) are tumors developed outside the digestive tract. Most common sites are the omentum, mesentery, mesocolon, followed by the retroperitoneal and retrogastric location [34].

The oncologic treatment is including surgery and therapy with biologic agents. Obviously, different surgical

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techniques lead to different results [35-41]. The size of organ resection and the surgical approach, preceded or followed by the treatment with tyrosine kinase inhibitors is influenced by the malignant potential of GIST, according with the size of the tumor and mitotic count.

Angiogenesis is an essential condition in the growth and development of tumors, especially in the case of GISTs due to their particular behavior. The aggressive behavior depends on the size and site of the tumor in close relationship with the intratumoral vascular development. Tumor expansion over 1 mm depends on neo-vascularization, because the oxygen can diffuse from the capillaries only to 1.5-2 mm, the cells starting to degenerate when this distance is longer [42]. The study of angiogenesis in GIST tumors also it is important in evaluation of the intratumoral vascular microdensity, tumoral progresion, resulting in the reduction of recurrence –free survival. The therapy with biologic agents, is effective according with the type of intratumoral vessels. The tirozin kinase inhibitors like imatinib ($C_{29}H_{31}N_7O$), sunitinib ($C_{22}H_{27}FN_4O_2$), sorafenib ($C_{21}H_{16}ClF_3N_4O_3$), nilotinib ($C_{28}H_{22}F_3N_7O$), dasatinib ($C_{22}H_{26}ClN_7O_2S$) used in the treatment of GISTs as adjuvant biologic therapy, neoadjuvant therapy followed by surgery or in the treatmeant of metastatic disease are inhibiting CD117, PDGFRA and vascular endothelial growth factor receptors (VEGFRs), preventing tumorigenesis, due to the antiangiogenesis effect on the immature tumoral vessels [43-,46].

Experimental part

The purpose of this study is to investigate the types of intratumoral vessels in GISTs ,knowing the interrelation between the tumor angiogenesis and the response to the targeted antivascular therapy, influencing directly the prognosis. The study included 37 patients of County Emergency Clinical Hospital Arad, Department of Surgery, diagnosed with GISTs having different locations, who had undergone surgery. The material used for the research, consisted in tumor specimens obtained by surgical resection or excision. The tumoral fragments with 1 cm^3 maximum size, were included in paraffin following the standardized automated technique. Multiple sections having 3 um in thickness were made from the paraffin blocks, for the immunohistochemical studies. For the confirmation of the diagnosis were used immuno-histochemical stainings for CD117 (7mL CD117 (EP10) BOND RTU Primary ,Product code PAOO7,Leica Biosystems) and CD34 (7mL CD34 BOND RTU, Product code PAO212, Leica Biosystems). In evaluation of tumor vessels were used double immunostainings methods CD34-SMA and CD 31-SMA ; SMA representing the marker for smooth muscle actin (7mL SMA BOND RTU, Product code PA0943, Leica Biosystems) and CD31 representing the membrane staining of endothelial cells (7mL CD31 BOND RTU, Product code PA0250, Leica Biosystems).The tissue sampling for the immunohistochemical studies was accomplished using TMA Grand Master , automatic system. The examination and image acquisition were performed using Axiocam 506 color, Zeiss, Jena, Germany, microscope. The microvascular density was calculated using Weidner method.

Results and discussions

The blood vessels are essential in malignant tumor growth, metastasizing or invasion. The tumor cells are sending signals to stimulate the development of new tumor vessels from the preexisting one, process well known as angiogenesis.

Angiogenesis is very important in malignant tumors, even the process can be encountered also in other human diseases or lesions. In the absence of the vascular development the tumor cannot grow over 3mm in size. Also the inhibition or destruction of new vessels is stopping the tumor proliferation. The tumor angiogenesis concept refers to tumor cell capacity to increase angiogenic activity, stimulating the proliferation of endothelial cells, followed by the assembling of neoformation vessels. The antivascular therapy is worldwide accepted and applied in a large variety of tumors, even the results are not very satisfying. The growth factors and specific receptors were studied intensely in angiogenesis, being used in multiple antivascular therapy strategies. The informations are more less about the profile of tumor vessels and the relation with the antiangiogenic treatment. The tumor vessels are different from the normal, healthy, tissue vessels. Was established that the vessels of the tumor stroma are irregularly arranged, are unequal and are not forming ordered networks.

The goal of the study was to evaluate the vessels associate to GISTs, according with the morphology, vascular density and the structure of the wall of neoformation vessels. Using the stainings for CD117 and CD34, firstly, the diagnosis of GISTs was confirmed in all the 37 cases, the reactions being positive. The reaction for CD117 was positive and intense in all the cases in the tumor cells (Fig1).

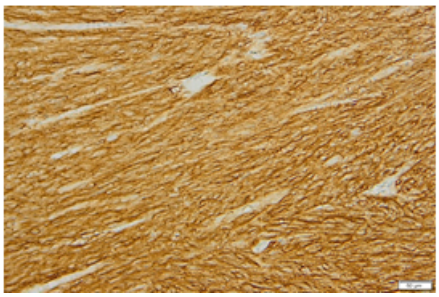

Fig.1. Immunohistochemical staining for CD117(viewed in brown), positive reaction in tumoral cells of GISTs

In the situation of CD34 immunohistochemical staining, all the cases were positive , but the distribution of the reaction result was different. The intensity of the reaction was not the same because in some cases CD34 antibodies bound only endothelial cells of tumor vessels and a few tumor cells (Fig.2). In majority of cases the staining for CD34 was positive ,intensely in the tumor cells of GISTs (Fig.3). A particular morphology of tumor vessels can be revealed in the cases with CD34 staining positive only in the endothelial cells (Fig.2). The vessels had anarchic disposition, with increased density, narrow lumen very

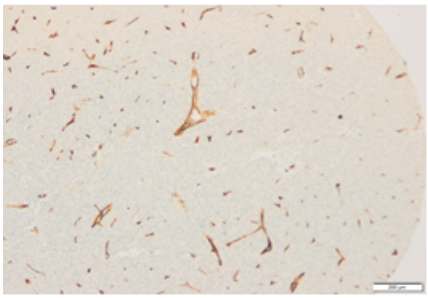

Fig.2. Immunohistochemical staining for CD34 (viewed in brown) positive in endothelial cells of tumoral vessels.

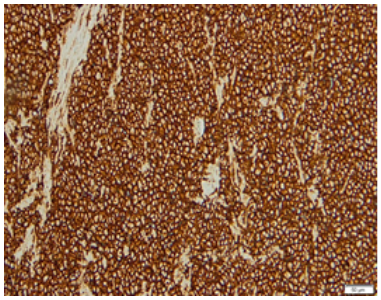

Fig.3. Immunohistochemical staining for CD34 (viewed in brown) positive intensely in tumoral cells of GIST masking the neoformation vessels

difficult to be identified on histological sections. Compared to peritumoral vessels, the intratumoral vessels had small sizes, narrow lumen, many ramifications and the perivascular cells could not be quantified.

For the evaluation of intratumoral vessels were used two double immunohistochemical stainings. The double staining CD34 –SMA, is mixing a marker of endothelial cells, but also of the GIST's cells and a marker of perivascular cells , smooth muscle actin. This combination is useful especially in cases with negative reaction for CD34 staining in tumor cells, allowing the classification of vessels and appreciation of vascular density (Fig.4). The intense positive staining for CD34 in tumor cells in majority of cases is masking the tumor vessels (Fig.5).

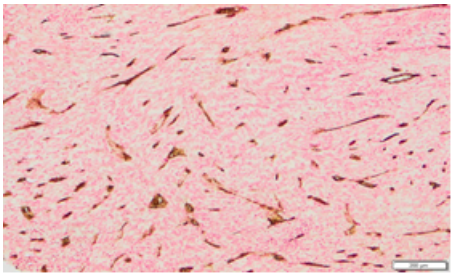

Fig.4. Double imunohistochemical staining CD34-SMA (CD34 viewed in brown, SMA in red). CD34 positive in endothelial cells, SMA marker of perivascular cells allowing the evaluation of types of intratumoral vessels. The immature and intermediate vessels are the most numerous.

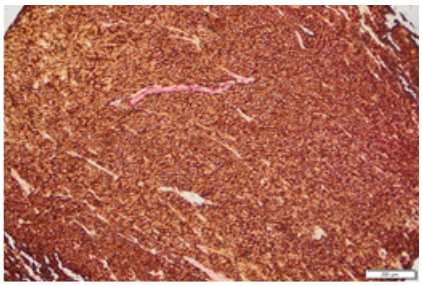

Fig.5. Double imunohistochemical staining CD34-SMA (CD34 viewed in brown, SMA in red). CD34 positive intensely in tumoral cells of GIST masking the vessels, SMA marker of perivascular cells .The immature and intermediate vessels are the most numerous.

The second double staining CD31-SMA is more useful because is mixing a marker of the endothelial cells with a marker of the perivascular cells. CD31 has a greater specificity for the endothelial cell than CD 34 and is not coloring the tumor cells (Fig.6). The actin is very helpful in specifying the type of vessels, according with the classification of tumor vessels proposed by Gee et al. in 2003 [47]. According with the findings at the two double stainings the tumor vessels are classified in immature, intermediate and mature. This aspect is very important in the effectiveness of antivascular therapy, because only the

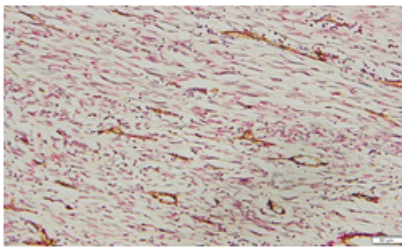

Fig.6. Double imunohistochemical staining CD31-SMA (CD31 viewed in brown, SMA in red) positive reaction. CD31 marker of endothelial cells, SMA marker of perivascular cells. Many immature vessels, cords of endothelial cells, without lumen, vascular sprouts and intermediate vessels in the GISTs

immature and intermediate vessels are sensitive at the targeted biological agents. The stainings revealed very rare mature tumor vessels, showing permeable lumen, with or without content, perivascular cells, having coexpression of CD34-SMA or CD31-SMA. Also intermediate vessels were found having irregular paths, thin wall, obvious lumen. This kind of vessels are very instable morphologically because are not presenting perivascular cells. Immunohistochemically are positive for CD31,CD34 and negative for actin. The immature vessels were the most numerous in our group of patients and are also known as vascular sprouts. Are formed by cords of endothelial cells, without lumen and perivascular cells, with a great proliferation capacity. The vascular sprouts are evolving into small caliber vessels, without perivascular cells forming intermediate vessels. The perivascular cell coverage comes later, the vessels achieve a larger caliber forming the mature vessels.

In the research literature was already proved that the instable, immature vessels are the most sensitive to the antivascular therapy. In a large variety of tumors the decrease of vessels density under therapy is due to loss of perivascular - negative vessels. The presence of perivascular cells it seems to provide protection to the endothelial cell against the antivascular drugs.

The malignant potential of GISTs is variable according with the size of tumor and the mitotic count. GISTs with size over 5 cm and mitotic count over 5/50HPF are presenting high risk of aggressive behavior. The malignant behavior consists in great capacity of spreading locally and hematogenously away especially in liver and peritoneum, this two being the most common sites of metastasis. The malignant potential is influencing the oncological treatment, dictating the size of surgical resection, the type of tirozin kinase receptor inhibitors used, duration of therapy, doses of drugs and also the sequence between drug and surgical treatment. To get to a high potential of recurrence the GIST must reach a specific size or mitotic count depending only by the development of intratumoral vascularization. That is the reason why the evaluation of vessels it is very important in GIST. The discovery of carcinogenesis in GIST helped the introduction of targeted therapy with tyrosine kinase inhibitors in the treatment with good results increasing the 2 year survival rates over 70%. Imatinib and the second line drug sunitinib, used in cases refractory to imatinib are multi targeted small molecule tyrosine kinase inhibitors being active on the mutant tyrosine kinase receptors and inhibiting vascular endothelial growth factor receptor, VGEF receptor. Also sorafenib, nilotinib, dasatinib kinase inhibitors were used inhibiting cell proliferation and angiogenesis. The most sensitive targets of the drug therapy are the immature and intermediate vessels, also described as perivascular free vessels. The tyrosine kinase inhibitors inhibiting

angiogenesis are causing several changes in the tumor like necrosis, cystic or myxoid degeneration, extended or patched hypocellularity and focal points of residual tumor.

Conclusions

The study of angiogenesis in GISTs it is very important because of the features of this pathology. The behavior of tumor is in close relation with the intratumoral vascular development. In our research, using immunohistochemical techniques, in all 37 cases the most numerous vessels were the immature and intermediate ones, signaling an increased angiogenic activity. The perivascular cell free vessels are the most sensitive at antivascular therapy with tyrosine kinase receptor inhibitors, providing a good response and prognosis to the treatment. Immature vessels evaluation especially, could be a an important sign in assessment of the effectiveness of antivascular therapy in GIST [48,49]. Anticoagulant therapy proved to be very important to prevent vascular risks [50-53].

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