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## CLINICAL AND STATISTICAL ANALYSIS AND IMMUNOHISTOCHEMICAL ASPECTS OF ANGIOGENESIS IN EWING SARCOMA

**Abstract:** One of the main types of cells that form blood vessels are endothelial cells, which line the inner surface of capillaries, arteries and veins. By immunohistochemical study of Ewing sarcoma, it was found that CD31 is produced by endotheliocytes, as well as perivascular elements. CD31 detected in the cell wall and cytoplasm of these cells. The production of CD31 increases in cases of high intensity of vascularization. The obtained data once again confirm the prognostic the significance of CD31 in the study of Ewing sarcoma.

**Key words:** Ewings sarcoma, CD31, angiogenesis, bone tumour.

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### Introduction

The hypothesis that the growth of solid tumors and the occurrence of metastases depend on the formation of new blood vessels was proposed by J.Folkman more than 25 year ago, and now largely

confirmed by recent discoveries in the field of molecular and cellular processes, concerning to the regulation of proliferation, migration and interaction cells that form vessels [9, 10, 12].

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One of the main types of cells that form blood vessels are endothelial cells, which line the inner surface of capillaries, arteries and veins [11]. With the formation of capillary tubes the walls of which consist of endothelial cells, the formation of the blood system begins at embryonic development. Further, around the capillaries gather other mesodermal cells, such as the pericytes, which are replaced by fully differentiated smooth muscle cells, which form the walls of large vessels as the vessels become larger.

Because of this, the study of the processes, regulating proliferation, migration and interaction of these types of cells is primarily necessary to understand the mechanisms of angiogenesis.

According to the WHO International Histological Classification of Bone Marrow tumours (Ewing sarcoma and bone malignant lymphoma) make up 30-35% of all primary malignant bone tumors [2, 5]. Consideration in the present study of Ewing sarcoma needs further study, as it has certain features of the clinical course [3, 4] and requires correct histological diagnosis [6] to choose adequate specific treatment [1, 7, 8].

**Purpose of the study:** Analysis of clinical and statistical signs and immunohistochemical study of angiogenesis in Ewing sarcoma.

### Material and Methods:

This work in accordance with the tasks set, includes data from 68 patients with Ewing sarcoma who were treated at the A.T. Abbasov City Oncological Dispensary (Baku), the Research Institute of Traumatology, City Oncological Dispensary (Baku) and the AMU Oncology Clinic from 2001 to 2009.

To solve the tasks set in the work the following research methods were used; clinical, radiological, cytological, histological, histochemical, immunohistochemical and statistical.

For the immunohistochemical study, a material frozen in liquid nitrogen was used, from which cryostatic sections were made followed by fixation in acetone, as well as a material of paraffin blocks fixed in 10% formalin. The studies were carried out by the method using enzyme immune complexes.

We used DAKO reagents in these studies (CD31, Endothelial Cell 0,2\1 ml).

All digital data obtained during the experiments were subjected to statistical processing taking into account modern requirements. The obtained data were subjected to statistical processing by nonparametric methods according to Wilcoxon –Mann –Whitney methods.

### Results and Discussions.

In our study Ewing sarcoma was: 68 cases males were affected by this pathology more often (43 men-62,24% and 25 women -36,76%). In the patients with Ewing sarcoma studied by us the tumor was most

often localized in the femur-24 observations (35,29%), on the tibia-8 observation (11,76%), in the fibula-7 observations (10,29%), in the pelvic bones-10 observations (14,7%), in the humerus-9 observations (13,24%), in the radius-3 observations (4,41%) in the ribs – 3 observation (4,25%), in the scapula -2 observation (2,94%), in the ulna – (observation 1,47%) also the tumor was localized in the clavicle – 1 observation (1,47%), in the jaw- (observation 1,0%).

In our studies, Ewing sarcoma was most often observed at the age of 1 to 20 years. Thus from 104 cases (5,89%), from 11 to 20-49 cases, from 21 to 30 -12 cases (17,65%), from 31 to 40 – 3 cases (4,43%)

According to the anamnesis of patients with Ewing sarcoma, the onset of the disease is characterized by a violation of the general condition, an increase in body temperature to 38-39 C, The appearance of pain in the affected part of the bone. Then there is swelling and local signs of the inflammatory process. After one to two weeks, the swelling disappears, the inflammatory processes subside, the temperature normalizes, the pain decreases or completely subsides. Remissions and relapses may alternate several times, then an outbreak occurs with a sharp further progression of the process.

However, only in a small group of patients the clinical course corresponds to the classical description of Ewing. In our observations, only 20 patients had acute onset of the disease. In most cases the disease begins either in full health, for no apparent reason, or after some period of time after the injury. According to our data, the injury preceded the appearance of the tumor in 40 % of cases. Average time of tumor occurrence after injury is 5 months. The shortest period of time is a few days the longest is 12 months. A history of trauma was noted in 25 of our patients. Clinical manifestation of the diseases occurred at various intervals from a few days to 2 years.

In most patients, clinical signs of the diseases occurred during the first 3 months after the damage, or immediately after injury, or shortly after it.

In most of the cases studied by us the disease started slowly, gradually: light transient pains appeared in the empty part of the skin with significant light intervals between attacks. Over time, the pain attacks became more frequent, their duration increased, and the pain became constant. The described course of disease was observed in 52 out of 68 (100%) of our patients. Our own observations and literature data have convinced us that according to clinical manifestations, two groups of lesions with Ewing tumor can be distinguished; the first, numerically predominant, is more favorable in clinical course. The tumor remains within one bone for a relatively long time and gives late metastases to the lymph nodes and lungs. Metastases in other bones can be detected only in the terminal phase (43

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observation). The second group is when Ewing sarcoma, causing rapid and extensive destruction of the bone, rapidly metastasizes to other parts of the skeleton. From a solitary lesion during the first months the disease turns into a generalized one.

Duration of clinical symptoms from the moment of their appearance symptoms from the moment of their appearance to according to our data the patients first visit to the doctor ranges from several days to 3 months. As with other malignant neoplasms of the skeleton the characteristic triad of symptoms is repeated with the greatest constancy in Ewing sarcoma: pain in the affected part of the skeleton, tumor, violation function.

Pain is the dominant symptom of disease and in most cases pain appeared earlier than bone changes visible on the X-ray. There was a wide variability in the intensity of pain from sharp, shooting or insignificant, dull, occurring during physical exertion and passing at rest. As with most bone sarcomas, the intensity of pain increased at night. At the same time, according to our research, the pain with Ewing sarcoma never reached to acute "skeletal" pain, characteristic of osteosarcoma.

Another constant symptoms of the disease was a tumor. In our observations, the tumor was detected in 55 out of 68 patients, as the first sign of the disease in 28. The size of the tumor was different from a small localized swelling to extensive deformity to the affected limb.

The general reaction of the body to the disease manifests itself in the form of a rise in body temperature, changes in the blood, sometimes there is weight loss, exhaustion in the terminal stages.

In our observations, an increase in body temperature during various periods of the disease was noted in 36 patients, however, we did not note a direct relationship between the degree of malignancy of the tumor and temperature reaction.

The clinical manifestations of Ewing sarcoma are very diverse and to a certain extent depend on the localization of the tumor, they depend on the localization of the tumor, the extent of the lesion, the relationship between the symptoms of the disease and the localization of the tumor.

Immunohistochemical research angiogenesis.

By immunohistochemical study of Ewing sarcoma, it was found that CD31 is produced by endothelial cells, as well as perivascular elements. CD31 detected in the cell wall and cytoplasm of these cells.

Among endothelial cells, there is pronounced reactivity of CD31 positivity was identified in 57.1±10.8% of cells, a positive reaction in 33.3±10.3% of cells, constant negativity in the group approach is in 9.5±6.4% of cells.

Immunohistochemical study of angiogenesis in Ewing sarcoma depending on lymphoid infiltration of the tumor. Immunohistochemical examination of angiogenesis in Ewing sarcoma revealed a low degree of lymphoid infiltration of the tumor in 16 (31.5±6.3%) cases. In this pathology in 41 (64.7±11.6%) cases high secretion of CD31 was detected, only in 1 (5.9±5.7%) cases the constant negativity of CD31 was revealed.

Thus, an inverse correlation was found between the lymphoid infiltration of the pathological focus and the secretion of CD31 positive cells ( $r=0.400$ ,  $p<0.001$ ).

Immunohistochemical study of angiogenesis in sarcoma Ewing depending on spontaneous necrotization of the tumor. By histological examination revealed a low degree of spontaneous necrotization of the tumor process in 15 out of 54 cases (27.8±6.1%).

Among the studied cases, immunohistochemically, in 2/3 of cases (66.7±12.2%) a high degree of secretion by endothelial elements of CD31 was detected. In the group approach, only in 2 (13±8.8%) cases, the secretion of CD31 was absent.

Thus, a significant inverse correlation ( $r=0.312$ ) was established between spontaneous tissue necrotization and secretion of stromal elements of CD31 ( $x=17.7$ ,  $P<0.01$ ).

Analyzing the studied data, it can be argued that the activity of secretion by endothelial elements of CD31 directly correlates with the intensity of tumor vascularization ( $r=0.322$ ;  $p=0.01$ ). In other words, the production of CD31 increases in cases of high intensity of vascularization.

The obtained data once again confirm the prognostic significance of CD31 in the study of Ewing sarcoma.

Summarizing the above we can state.

1. The activity of secretion by endothelial elements of CD31 inversely and statistically significantly correlates with the degree of malignancy of the tumor process in Ewing sarcoma ( $r=0.438$ ;  $p<0.001$ )

2. Indicators of lymphoid infiltration of the pathological focus are inversely correlated with the secretion of CD31 positive cells ( $r=0.400$ ;  $p<0.001$ )

3. Between the secretion of CD31 by endothelial cells and spontaneous necrotization of tissue in Ewing sarcoma. It was revealed statistically significant, inversely proportional relationship ( $r=0.31$ ;  $p=0.01$ )

4. A direct reliable correlative relationship between angiogenesis of tumor tissue and the amount of CD31 ( $r=0.322$ ,  $p<0.01$ ).

**Conclusion:** Thus these immunohistochemical features of angiogenesis in Ewing sarcoma should be taken into account in their differential diagnosis, treatment and forecasting.

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