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	JIF = 1.500	SJIF (Morocco) = 7.184	OAJI (USA) = 0.350

SOI: 1.1/TAS DOI: 10.15863/TAS

International Scientific Journal

Theoretical & Applied Science

p-ISSN: 2308-4944 (print) **e-ISSN:** 2409-0085 (online)

Year: 2022 **Issue:** 07 **Volume:** 111

Published: 13.07.2022 **http://T-Science.org**

Issue Article

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MODERN OPPORTUNITIES OF DIAGNOSTICS AND PROGNOSIS OF PERIPHERAL PRIMITIVE NEUROECTODERMAL BONE TUMORS (PNET)

Abstract: *In this research are present modern opportunities for diagnosing and predicting peripheral primitive neuroectodermal bone tumors based on the analysis of literature data and results of our observations.*

Key words: *modern opportunities, diagnosing, predicting, peripheral primitive neuroectodermal bone tumors.*

Language: *English*

Citation: Muradov, H. K., et al. (2022). Modern opportunities of diagnostics and prognosis of peripheral primitive neuroectodermal bone tumors (PNET). *ISJ Theoretical & Applied Science, 07 (111),* 24-29.

Soi: http://s-o-i.org/1.1/TAS-07-111-5 ***Doi***: https://dx.doi.org/10.15863/TAS.2022.07.111.5

Scopus ASCC: *2700.*

Introduction

Oncoginecology and patohystomorphology

Recently, based on the data of classical morphology and application of new research methods (tissue culture, cytogenetics, molecular genetics, and immunophenotypic analysis), a group of poorly differentiated small round cell tumors designated by the term "peripheral primitive neuroectodermal tumor", which are related to bone marrow tumors and

observed in children, adolescents, and adults, has been identified (PNET) (3, 5).

The mentioned tumor form needs further study since it has certain features of the clinical course and requires correct histological diagnosis to select an adequate specific treatment (1, 10).

Many of the solid malignant tumors of childhood have a similar histological structure, which is a reflection of their dysembryogenetic and primitive nature (4, 7, 10). Some of the members of this group, Ewing's sarcoma and primitive neuroectodermal tumors (hereinafter referred to as PNETs) are localized mainly in bones and soft tissues (5). These tumors were reported long before the introduction of electron microscopy and immunohistochemistry (8, 9, 11).

The term "primitive neuroectodermal tumor" (PNET) was first used in 1973 by Hart and Earle to refer to a group of tumors, which are derivatives of fetal neuroectodermal cells and have immunomorphological features of a tumor of small hyperchromic cells with variable signs of neural, glial and ependymal differentiation (1, 2). Despite the ongoing discussion regarding this term, the designation "PNET" (primitive neuroectodermal tumor) has found wide use in the classification of tumors of the CNS recommended by WHO (6,12).

Purpose of the research. To present modern opportunities for diagnosing and predicting peripheral primitive neuroectodermal bone tumors based on the analysis of literature data and results of our observations.

Materials and methods. In our study, PNET was observed in 28 cases. In the patients with PNET examined by us (13 male (46.42%) and 15 female (53.58%) patients), the tumor was most often localized in the ribs - 19 cases (67.85%), in the femur - 4 cases (14.28%), in the tibia - 1 patient (3.57%), in the bones of the spine - 1 patient (3.57%), in the scapula - 1 patient (3.57%), in the clavicle - 1 patient (3.57%) and in the skull - 1 patient (3.57%). Morphological examination of buffered formalin-fixed surgical material and tumor tissue fragments obtained by fine-needle biopsy included microscopic examination of serial sections stained by routine methods (hematoxylin-eosin) using decalcification conventional in cases of bone tumors. An immunohistochemical study using the immunoperoxidase method was carried out on sections from paraffin blocks 3–4 μm thick. After deparaffinization and dehydration, in order to block endogenous peroxidase, the sections were treated with 0.3% H_2O_2 for 20 min, washed in distilled water. To unmask antigenic determinants, they were subjected to temperature treatment using buffers with pH = 6.0 (Target Retrieval solution, DAKO) in the microwave for 20 minutes or in a "water bath" for 30 minutes at 98°C. For a number of antibodies, an EDTA buffer with pH = 9.0 was used; in this case, thermal treatment was carried out for 30–35 min. After washing in TBS three times for 5 min, primary antibodies of mouse or rabbit were applied. Incubation with primary antibodies was carried out in a humid chamber for 30-60 min (depending on markers - cytoplasmic, membrane, or nuclear) at room temperature.After incubation with primary antibodies, sections were washed in TBS three times for 5 min, then peroxidase-conjugated avidin-biotin complex was applied for 30 min at room temperature using LSAB+, Dako (two-step method), or EnVision+ detection system (Dako) (one-step method) for 30 min at room temperature. Peroxidase activity was detected using DAB+ (Dako). The nuclei were stained with hematoxylin. When using the EnVision+ detection system, the procedure was reduced by one “step”, since, after the primary antibodies, a polymer conjugated with secondary antibodies to mouse and rabbit immunoglobulins and an enzyme was applied. Primary antibodies to CD99 (“Dako”), S-100 (“Dako”), HBA-710 (“Dako”), EMA (“Dako”), Aktin (“Dako”), Vimentin (“Dako”), Desmin (“Dako”), Myogenin (“Dako”), HBA-71 (“Dako”), NSE (“Dako”), NF(“Dako”), OC(“Dako”) were used. Biotinylated antibodies to mouse and rabbit immunoglobulins were used as secondary antibodies (EnVision, “Dako”).

Results of the research

Clinical research methods of PNET. In most patients, clinical signs of the disease emerged within the first 4–5 months after the injury or immediately after the injury, or shortly after it. Some patients (9 cases 32.1%) denied the role of trauma in their anamnesis. In the majority of the patients studied by us (20 cases, 71.4%), the symptoms of this pathology were noted for no apparent reason or after a certain period following the injury. We believe the frequent definite coincidence in time and localization of the injury with the subsequent development of the tumor suggests that the damage in some cases is the impetus for the acceleration of the growth of a malignant tumor, which until then proceeded without symptoms.

As in Ewing sarcoma, the characteristic triads of symptoms repeated: pain in the affected area of the skeleton, swelling, violation of the functions of the limbs.

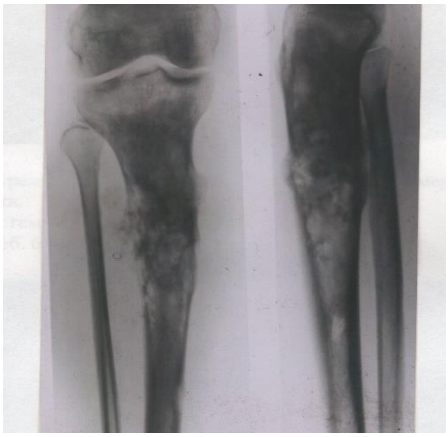
In most cases, the pain manifested itself earlier than changes in the bones visible on the radiogram. There is wide variability in the intensity of pain - from dull to sharp. In our studies, in 7 patients (25.0%) the disease developed slowly, gradually; mild, transient pains in the affected part of the bone (ribs) with significant light intervals between attacks were manifested.

The tumor was determined in 21 (75.0%) of 28 patients (100%) and as the first sign of the disease in 9 (32.1%) patients. The sizes of the tumors varied

from a small localized swelling to extensive deformity of the affected bone. The tumors were dense, immobile, without clear boundaries, painful, located together.

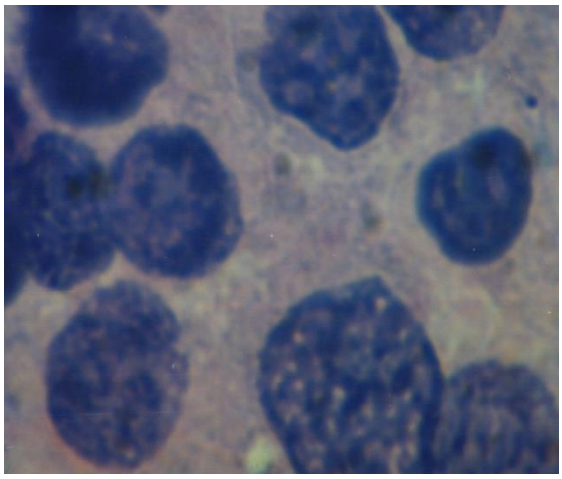
The general reaction of the body to the development of the tumor was manifested in the form of elevated body temperature, changes in the blood – leukocytosis, and acceleration of erythrocyte sedimentation rate (ESR), sometimes a decrease in body weight, and exhaustion was noted in the terminal stages. An increase in body temperature during various periods of the disease was noted in 11 (39.2%) of the patients we examined. A characteristic feature of PNET was an extremely aggressive clinical course of the tumor process. Lifespan in 18 (72.0%) patients averaged 8 months, in the remaining 10 (28.0%), an average of 12 months after diagnosis.

Radiological methods of the research. Radiographic symptoms of PNET are associated with two related processes: bone destruction, endosteal and periosteal osteogenesis. Bone destruction can manifest itself in the formation of small foci of a round or oval shape, giving the affected area a “porous” appearance – small focal destruction. In some cases, lamellar destruction is observed with the presence of a large, rounded, clearly delimited area with a sclerosis zone around and thin or tough partitions inside. Endosteal and periosteal growths are in the form of linear and layered periostitis, in the form of fringed or needle-like growths directed perpendicularly or at an angle to the long axis of the affected bone. The frequency of occurrence of certain radiological signs in PNET is not constant and their combinations in each case are extremely diverse.



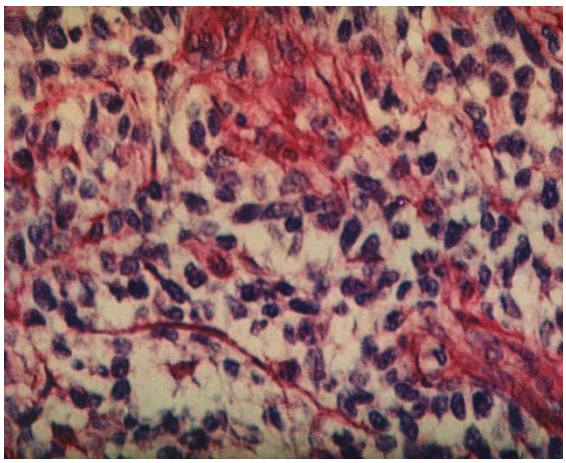
Pic.1.

Cytological methods in the research of PNET. The cytological picture is characterized by the presence of cells of the same type in shape and size. Tumor cells have a round, oval, and polymorphic shape. The cytoplasm is stained with intense basophilic tones, the boundaries are clear, there are vacuoles. The nuclei slightly vary in size, they are almost the same type: rounded, with proper contours of the nucleolemma. "Notches" and invaginates of the nuclear envelope are found only in the cells of the perinecrotic zones. The nucleoli are small, 1-2 each, the chromatin is uniform: it is uniformly reticulated in imprint smears, and fine-grained in histological preparations. The number of mitoses is 14-18 per 10 fields x 40. Pathological figures of mitosis are rare.



Pic.2.

Microscopic description. Tumor tissue in PNET is characterized by diffuse growths of rounded, oval, and polymorphic cells. Cells are usually closely adjacent to each other. With a good blood supply, especially with a developed capillary network, the formation of pseudo-rosettes (of the Homer Wright type) and pseudo-alveoli, which are accumulations of tumor cells around the capillaries, is noted. At high magnification, in the center of such a pseudorosette or pseudoalveoli, a lumen formed by an endotheliocyte can always be found. In the presence of vessels of a larger caliber (venules, veins, arterioles) in the tumor, with simultaneous underdevelopment of the capillary network, confluent fields of hemorrhagic necrosis are formed. In this case, the cells are preserved only in the form of sleeves around wide-walled vessels, forming the so-called pericytic structures. It should be noted that, although it is not an exclusive feature of Ewing's sarcoma and PNET, this type of necrosis is still uncharacteristic of bone lymphosarcomas and neuroblastoma metastases, where it is mainly mosaic. Regarding the issue of pseudorosettes, it should be noted that they are still rare in Ewing's sarcoma and, in a total study of a tumor section, make up less than 10% of its area. Besides, in PNET, almost the only specific morphological sign at the light level is pronounced rosette formation (more precisely, pseudorosette formation), which makes up the majority of the tumor area (about 70%).



Pic.3.

In this pathology, staining with picrofuchsin reveals an extremely poor collagen stroma. Rarely, collagen in the form of small bundles can be found, which can simulate osteoid and will require differential diagnosis with small cell osteogenic sarcoma. In the stroma, silver impregnation reveals a poor argyrophilic carcass. This sign was used for differential diagnosis of malignant lymphomas (according to old ideas - reticulosarcoma) of the bone. Unlike Ewing's sarcoma in PNET (especially in the soft tissue variant), lobulation is determined, which is formed by tough fibrous septa.

Thus, the basic light-optical signs for the diagnosis of PNET are a diffuse pattern of growth, a pronounced polymorphism of cells and nuclei (among the porous units considered in the work, this feature is the most characteristic of Ewing's sarcoma and PNET), the presence of a perivascular nature of necrosis with the formation of pericyte sleeves. Taking into account the histogenetic similarity of Ewing's sarcoma and PNET, proved on the basis of molecular-genetic and cultural studies, as well as the sameness of therapeutic approaches for these tumors, the need for differential diagnosis between them is still insufficiently justified. The only significant morphological feature that distinguishes PNET from Ewing's sarcoma is distinct rosette formation.

Flow cytometry methods for studying PNET. We studied 18 cases of PNET (10 male and 8 female patients) in the first and second decades of life by Flow cytometry. We used 1 fresh material, and 17 materials of paraffin blocks (Table 1).

Table 1. Flow Cytometric Studies in PNET

Total material	$C_0/1$	S-phase	G_2+M	>4c
Quantity				
18	78.7	8.1	10.3	2.9

Based on the study of the total DNA content in cells, the hypodiploid DNA content was 11.6±1.6, diploid - 40.3±1.2, aneuploid tetraploid - 20.6±1.1, pentaploid 9.5±1.1, hexaploid - 3.9±0.6, octaploid - 9.1±1.2, and nonaploid - 4.0±0.8.

Given the nature of the ploidy and clonality of the tumor, it was found that in PNET, the aneuploid variant was in 10 cases (55.5%), diploid tumors were in 8 cases (45.5%). Aneuploid tetraploid (36.2%±1.2) DNA content predominated in aneuploid tumors - 10 cases (55.5%±1.5). In 8 detected cases of diploid tumors (45.5%±1.0), the predominant class with diploid (62.3%±2.6) DNA content was clearly expressed.

Immunohistochemical methods for studying PNET.

Immunophenotyping of PNET cells revealed a high degree of positivity only (+++) with CD99. A moderate degree of positivity was found with vimentin, neuron-specific enolase, HBA-71, neurofilaments, and protein S-100. Rare positive cells were detected with EMA. The reaction with actin, desmin, myogenin, and osteocalcin was negative (Table 2).

Table 2.

Immunohistochemical panel	
Quality	19
VIM	++
SD-99	+++
S-100	++
NSE	++
HBA-71	++
ACT	-
DES	-
EMA	+
NF	++
MIO	-
DES	-
EMA	+
NF	++
MIO	-
OC	-

Note: +++ high degree of positivity,
++ moderate degree of positivity,
+ rare positive cells,
- non-positive cells.

Thus, the above material indicates that regardless of the nature of the process, histogenetic sources of development, and type of differentiation, PNET differed from other malignant bone marrow tumors in clinical, radiological, and morphological parameters. Tumor cells can implement a variety of differentiation methods, and thus, acquire certain histochemical and immunohistochemical properties. Specified features should be taken into account when clarifying the histogenetic sources of the studied tumors and conducting their differential diagnosis and prognosis.

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