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## Estimation of Diagnostic Value of Chosen Markers of Neural Differentiation in Neuroblastoma Group of Tumors and pPNETs

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**Neuroblastoma and peripheral PNETs both are typical examples of wide group of small round blue cell tumors of childhood. Matured neuroblastoma show typical clinical presentation and easy to interpret microscopic picture. Unfortunately in everyday practice much commonly appeared less differentiated neuroblastomas with difficult to predict clinical behavior and impossible to diagnose in routine stain histologic view. Peripheral PNETs are found as morphologically similar entities to some neuroblastoma subtypes but they are treated as separate CD99 positive group of tumors with different biology and clinical behavior. The aim of our study was to estimate the usefulness of neural markers expression (Neuroblastoma Marker, neurospecyfic enolase and neurofilaments) in routine separation between neuroblastoma tumors and pPNETs and between neuroblastoma subtypes according to currently used classification of those entities. We investigated 63 tumor tissue samples and found differences in expression of investigated markers between both neuroblastoma subgroups and neuroblastoma group of tumors and pPNETs.**

### Introduction

In recent decades, dramatic improvements in survival have been achieved for cancer patients. Guidelines for treatment arise from clinical trials and experience of group of specialist. Treatment protocols are available for most type of cancer that occurs in children and adolescents and they are designed to achieve better results of performed therapy and quality of life. Current oncology is based on pathologic examination of tumor tissue samples and histological examination became a standard procedure with influence on

the way of treatment and found as a new prognostic factor. In the case of less differentiated tumors routine diagnostic procedures which is microscopic examination must be supported by immunohistochemistry and/or molecular genetic. Despite visible progress in those diagnostic methods some group of tumors still appear as an important diagnostic problem for pathologists. Very good examples of this type of difficulties are small round blue cell tumors of childhood [24, 26, 27]. From this wide group of malignancies we choose for our study neural tumors – neuroblastoma histologic subtypes and peripheral PNETs. Neuroblastoma originates in the renal medulla or the paraspinal sites where sympathetic nervous tissue is present. Histologic examination involves evaluation of tumor tissue sample for the amount of stromal development, the degree of neuroblastic maturation, and the mitosis-karyorrhexis index of the neuroblastic cells. Neuroblastoma containing many differentiating cells is named ganglioneuroblastoma, less matured tumors are composed of poorly differentiated cells and they have typical microscopic look of small round cell tumors of childhood [2, 9, 14, 15, 28, 31]. Peripheral primitive neuroectodermal tumors (pPNETs) were previously found as only one of the morphological subgroup of neural tumors. They consist of small round cells also and they have characteristic primitive chromatin. Current immunohistochemistry allow to distinguish pPNETs from other less differentiated neural tumor and the examination of typical for pPNETs CD95 (MIC2) antigen is mandatory for diagnosis. Despite common similarity to neuroblastoma group of tumors in routine (hematoksylin and eosin) stain and immunohistochemical evaluation of typically used neural marker (neurospecyfic enolase), in clinical course pPNETS are separate entities with special biological behavior and proper diagnosis is a necessity for the best treatment of patients [5, 21, 22, 23].

## Materials and Methods

We selected for our study 63 formalin-fixed and paraffin-embedded tumor tissue sections from the files of the Department of Pathology of the Age of Development and Department of Pathology Konopnicka Memorial Hospital Medical University of Lodz and Department of Pathology Mother and Child Institute in Warsaw (57 neuroblastoma cases and 6 pPNETs). For the purpose of our study all of the previously diagnosed neuroblastoma became reclassified according current criteria for this group. From all these tissues samples paraffin blocks about the thickness 3-4 of micrometers were prepared and stained with hematoxylin and eosin (HE) and they were used for immunohistochemical research with use of Neuroblastoma Marker (NB84), neurospecific enolase (NSE) and neurofilaments (NF) and with ABC detection system produced by Novocastra Company. The estimation of the expression of investigated markers were examined with computer image analysis system (Multi Scan Base v. 8.08 - Computer Scanning System, Ltd.). All examined microscopic pictures (Nikon Microphot FXA) were transferred to the computer by camera (CC20P). Expression of neural markers we estimated qualitatively – their presence or absence in neoplastic cells. For the analysis we used the statistical pack SYSTAT for Windows (Version 5.03, SYSTAT, Inc, Evaston, Illinois, USA, the license No: DA021594). For all tests  $p < 0.05$  was accepted.

## Results

According current histologic criteria we diagnosed the following neuroblastoma subgroups characterized by different degree of morphological ‘maturation’:

- conventional neuroblastoma (NB subgroup) – 32 cases,
- neuroblastoma with differentiation to ganglioneuroblastoma (NB-GNB subgroup) - 12 cases,
- ganglioneuroblastoma (GNB subgroup) – 8 cases,
- ganglioneuroblastoma with differentiation to ganglioneuroma (GNB-GN subgroup) – 5 cases.

All the peripheral primitive neuroectodermal tumor (pPNETs) – 6 cases were confirmed by CD99 (MIC2) positive immunohistochemical stains.

### *Results of NB84 investigation*

We observed NB84 expression in 55/57 neuroblastoma tissue samples and none of pPNET tumors. The expression appeared as fine-grained cytoplasmic reaction, situated on one pole of the cell. In NB subgroup reaction

was found in 30/32 of cases, while in NB-GNB, GNB and GNB-GN in all tumor tissue samples (12, 8 and 5 cases, properly). Peripheral PNETs did not show the expression of NB84 (0/6).

### *Results of NSE investigation*

NSE expression was found in all examined cases both neuroblastomas and pPNETs. Immunohistochemical reaction was always of cytoplasmic type but its location was different in different types of cells. We found perinuclear round type reaction in neuroblasts and equally dispersed in ganglion cells.

### *Results of NF investigation*

NF positive reaction of cytoplasmic perinuclear round type was found in 41 neuroblastoma tissue samples and none of pPNET. In morphological neuroblastoma subgroups the number of cases was observed as follows: NB – 17/32, NB-GNB – 11/12, GNB – 8/8 and GNB-GN 5/5. Details of neural markers expression show Fig. 1 and Table 1.

In further analysis we did not found any statistically significant correlations between expression of investigated markers and type of the tumor or histologic subtypes of neuroblastoma.

## Discussion

Proper diagnosis of neural small round cell tumors still appears as a very important problem in routine histologic examination. Neuroblastoma is the most common extracranial solid tumor of childhood and should be differentiated from other small blue round cell tumors e.g: Wilms’ tumor, Ewing’s sarcoma, pPNETs, poorly differentiated soft tissues sarcomas and some variants of osteosarcoma, chondrosarcoma and non-Hodgkin lymphomas. Because of morphological resemblance of these tumors the use of additional diagnostic tools is mandatory. In the differential diagnosis immunohistochemic stains are routinely used and in the most difficult cases molecular genetic is necessary [1, 3, 8, 18, 22, 23, 25, 33].

Classification of neural tumors is still under discussion. In proposed by T. J. Triche, modernized classification of small round cell tumors of childhood the term pPNET refers to poorly differentiated neuroectodermal tumors (including Ewing’s sarcoma and Askin’s tumor). According to Hirose, Parham, Varienda and Sorensen esthesioneuroblastoma and olfactory neuroblastoma should be included

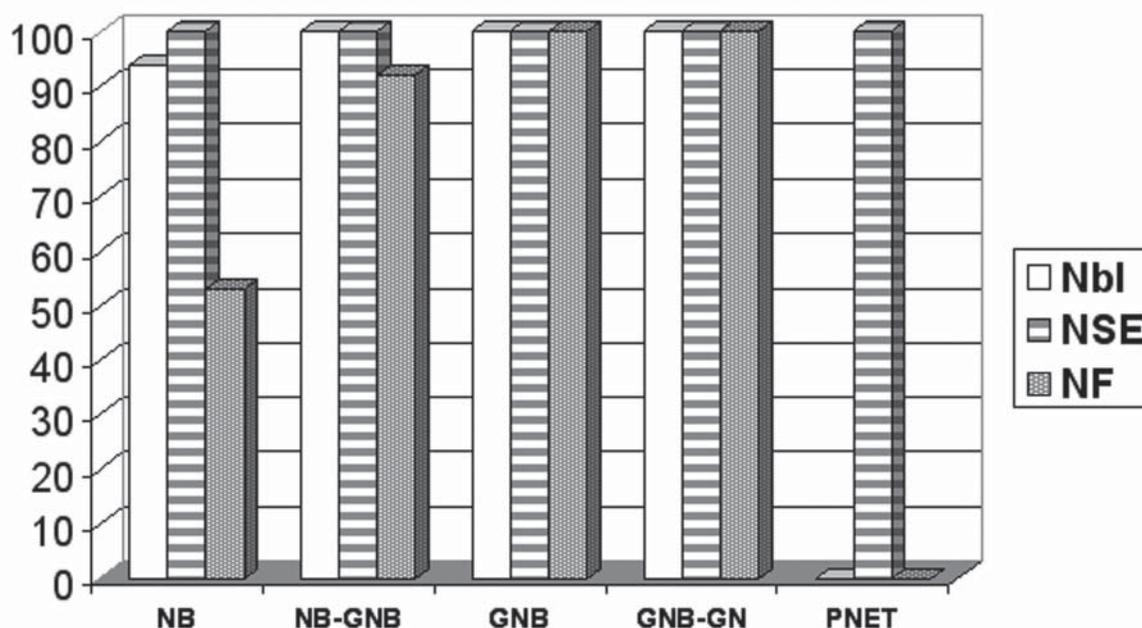


Fig. 1. Percentage (the y axis) of positive results of neural markers expression in neuroblastoma subtypes and pPNET. NB – conventional neuroblastoma, NB-GNB - neuroblastoma with differentiation to ganglioneuroblastoma, GNB – ganglioneuroblastoma, GNB-GN – ganglioneuroblastoma with differentiation to ganglioneuroma, PNET – peripheral primitive neuroectodermal tumor

**TABLE 1**  
Results of neural markers expression

Histologic type	NB 84		NF	
	N° of cases	%	N° of cases	%
NB	30	93,75	17	53,1
NB-GNB	12	100,0	11	91,7
GNB	8	100,0	8	100,0
GNB-GN	5	100,0	5	100,0
pPNET	0	0,0	0	0,0

to this group also [29]. It is proved that in spite the morphological resemblance and probably the common origin pPNET and less differentiated neuroblastoma (the conventional type) show cytogenetic and molecular heterogeneity and different clinical course and prognosis as well and should be separated each other [19, 20, 30, 32, 35]. Special attention deserve immunohistochemical stains, first of

all evaluation of expression of CD99 antigen (MIC2). The product of MIC2 gene is found in over 95% of pPNET/Ewing's sarcoma tumors and nowadays is the only widely accepted diagnostic marker for this group [7].

Described difficulties in diagnosing and classification of evaluated tumors point the necessity of further investigation of diagnostic value of currently available and some

new neural markers to separate pPNET and neuroblastoma.

In our research we found the expression of the most common neural marker which NSE is in all the investigated tumor tissue samples, both neuroblastomas and pPNETs, what is peaceable with other authors results [6, 10, 11, 12, 17, 34]. The expression of neurofilaments was observed 65% of investigated neuroblastoma subtypes and none of pPNETs. Research of Brinkhuis, Fletcher, Hachitanda, Hirose, Kelly and Parham showed the expression NF in the most of tumors from neuroblastoma group and in 38% of pPNETs [4, 11, 13]. This difference can probably be explained or by low number of pPNET cases in our research or by different MIC2 status (possible influence of MIC-2 status on the expression of neural markers). Kelly and Izbicki and their coworkers underline also the presence of NF expression in more differentiated and its lack in the more primitive tumors.

Neuroblastoma Marker (NB84) was observed in 87, 3% of investigated tumors and was strictly connected with neuroblastoma group, opposite to pPNETs where NB84 expression was not found. Kelly and his coworkers qualified NB84 as specific for neuroblastoma group of tumors, however its expression pPNET was also described in the literature. Our research did not lead to conclusion because of lack of the statistically important correlation despite to clear results of immunohistochemical stains.

Results of our research among neuroblastoma subtypes needs in our opinion further estimation. Wide spectrum of morphologic views of neuroblastoma group of tumors and the phenomenon of spontaneous or caused by chemotherapy maturation of tumor cells are known and they are evaluated in current histologic examination and influence on prognosis also [16]. Despite observed differences in results of immunohistochemical stains in evaluated subgroups we did not found statistically important correlations between neuroblastoma subtypes and expression of examined neural markers. We did not observe differences of NSE and/or NB84 expression among histological subgroups. NF expression was found in the half of conventional neuroblastoma and did not found in all of the tumors with morphological features of maturation. In summary only pPNETs tumors differ from other investigated subgroup because of the lack of NB84 and NF expression in all the examined cases.

We conclude that the evaluation of expression of Neuroblastoma Marker (NB84) as a helpful tool in proper diagnosis of neuroblastoma group of tumors. Neurospecyfic enolase expression observed in other than neuroblastoma neuronal tumors is in our opinion less specific but also useful in differential diagnosis of small round blue tumors of childhood. Evaluation of the neurofilaments expression in

neural tumors in our research had supplementary meaning only.

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