

# CLINICAL CASE REPORT

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## A Primary Primitive Neuroectodermal Tumor of the Central Nervous System in a 51-year-old Woman: a Case Report and Literature Review

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**Key words:** primitive neuroectodermal tumor; central nervous system; magnetic resonance imaging; immunohistochemistry; CDKN2A gene deletion.

**Summary.** Primitive neuroectodermal tumors are a group of rare, aggressive, and highly malignant embryonal tumors of unknown etiology of the central and peripheral nervous systems. It is a term for a group of small round cell tumors thought to be derived from fetal neuroectodermal precursor cells. Primitive neuroectodermal tumor is usually described as a tumor of children younger than 15 years and is very rare in adults.

The article presents a short literature review and a rare case of a primary primitive neuroectodermal tumor of the central nervous system diagnosed in a 51-year-old woman.

### Introduction

In 1973, Hart and Earle described the term of primitive neuroectodermal tumor (PNET) (1). PNET is a broad term that includes a wide array of lesions with varying differentiating potential affecting both the central and peripheral nervous systems. PNETs predominantly show a proliferation of undifferentiated or poorly differentiated neuroepithelial cells and, thus, are histologically similar to the much more common infratentorial medulloblastomas (2). These are small round cell tumors of neuroectodermal origin with high malignant potential (3). PNETs of the central nervous system (CNS) are uncommon malignant neoplasms of the cerebral hemispheres and suprasellar region and include supratentorial, infratentorial, brain stem, and spinal cord tumors (4). PNET usually occurs in children and only sporadically in adults (older than 20 years) (1) accounting for approximately 2.5% of brain tumors in children and 0.46% in adults (2). In the pediatric population, prognosis is worse than for infratentorial medulloblastoma. Older age appears to be prognostically favorable (5).

This article describes a rare case of a 51-year-old woman with a disseminated primary primitive neuroectodermal tumor of the CNS.

### Case Report

A 51-year-old Lithuanian woman applied to a family doctor with progressive vision loss, vertigo,

headache, and weakness in the legs lasting for two months. During clinical examinations, an ophthalmologist suspected a progressive edema in the ocular fundus, and a CT scan showed a tumor of the mesencephalic-pinealis region with internal occlusive hydrocephalus and brain edema. After such findings, the patient was referred to the Clinic of Neurosurgery, Hospital of the Lithuanian University of Health Sciences. Magnetic resonance imaging (MRI) revealed an irregularly shaped heterogeneous solid mass showing strong contrast enhancement and measuring 5 cm in maximal diameter in the quadrigeminal cistern. The tumor infiltrated the surrounding brain structures including the posteromedial temporal lobes and the posterior thalami, the quadrigeminal plate, and particularly the superior cerebellar vermis. The mass extended superiorly, but did not extensively infiltrate the pineal gland. The tumor showed protrusion into the posterior third and superior fourth cerebral ventricles causing mild occlusive hydrocephalus with mild periventricular edema (Fig. 1).

**Pathological Findings.** Histopathology revealed a highly cellular neoplasm. Tumor cells were small, poorly differentiated having hyperchromatic nuclei and little cytoplasm (Figs. 2A and 2B). There were occasional Homer Wright rosettes and tumor cell palisades. The mitotic rate was high. The Ki-67 proliferation index was about 25% (Fig. 3A). Tumor cells were immunohistochemically positive

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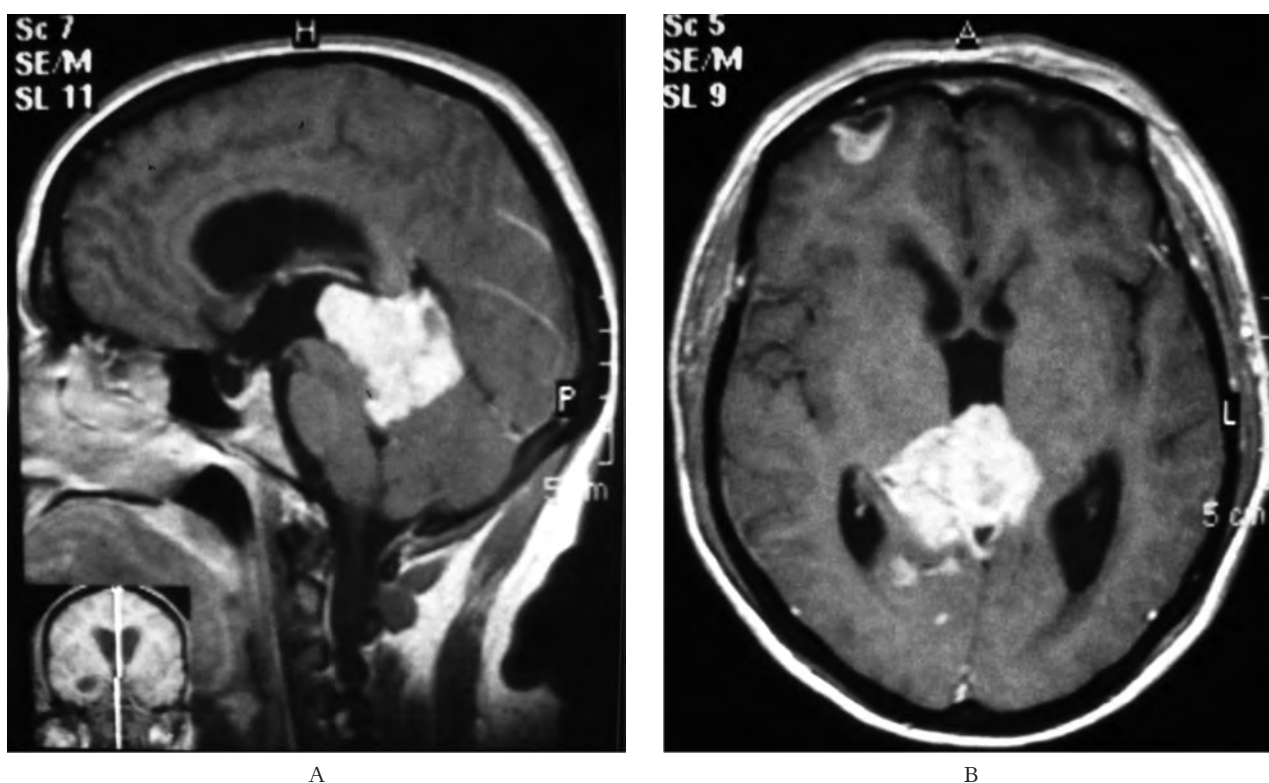


Fig. 1. Magnetic resonance imaging showing a disseminated heterogeneous tumor in the thalamus region and in the rear of the third ventricle (A) and in the right frontal lobe (B)

for synaptophysin (Fig. 3B), chromogranin A (Fig. 3C), CD56, and neuron-specific enolase (Fig. 3D). Neurofilaments showed weak positivity. Some tumor cells expressed S-100 protein and glial fibrillary acidic protein. Tumor cells were negative for vimentin, CD99, and cytokeratin MNF116.

**Molecular Genetics. Detection of Homozygous Deletion of *CDKN2A*.** The status of cyclin-dependent kinase inhibitor 2A gene, *CDKN2A*, which regulates 2 cell cycle regulatory pathways, the p53 and RB (retinoblastoma) pathways, was also checked. The *CDKN2A* tumor suppressor locus on chromosome band 9p21 encodes p16INK4A protein, a negative regulator of cyclin-dependent kinases, and p14ARF protein, an activator of TP53, in an alternative reading frame. *CDKN2A* locus (*p16INK4* and *p14ARF*) is inactivated in many human cancers by point mutation, promoter hypermethylation, and often deletion. Homozygous deletions are prevalent at this locus in very different human cancers.

**Differential Polymerase Chain Reaction.** Genomic DNA from frozen tumor tissue was extracted using a NucleoSpin Tissue<sup>®</sup> kit (Machery-Nagel, Germany). DNA from peripheral blood was extracted using the standard phenol-chloroform extraction method. Control blood DNA from peripheral blood of a healthy individual was used as a negative control, and DNA from human promyelocytic cell line NB4 was used as a positive control in each

experiment. Screening for the homozygous deletion of *p16INK4A* (exon-1 $\alpha$ ) and *p14ARF* (exon-1 $\beta$ ) was performed using the differential PCR analysis.  $\beta$ -Globin gene as an internal control was coamplified with either of the genes. The primer sequences were as follows: for detecting *p16INK4A* exon-1 $\alpha$ , 5'-ACCGGAGGAAGAAAGAGGAG-3' (sense) and 5'-AGAATCGAAGCGCTACCTGA-3' (antisense) (product size, 356 bp); *p14ARF* exon-1 $\beta$ , 5'-TCCCAGTCTGCAGTTAAGG-3' (sense) and 5'-GTCTAAGTCGTTGTAACCCG-3' (antisense) (product size, 447 bp); and  $\beta$ -globin, 5'-GAAGAGCCAAGGACAGGTAC-3' (sense) and 5'-CAACTTCATCCACGTTCCACC-3' (antisense) (product size, 268 bp). The PCR reaction in a total volume of 25  $\mu$ L for *p16INK4A* gene and 20  $\mu$ L for *p14ARF* gene was carried out using 12.5  $\mu$ L and 10  $\mu$ L of PCR Master Mix (2X) (Thermo Fisher Scientific), respectively, 0.2  $\mu$ M for each of *p16INK4A* primers, 0.25  $\mu$ M for each of *p14ARF* primers, and 0.125  $\mu$ M for each of  $\beta$ -globin primers. Besides, 2  $\mu$ L of target DNA was added to the PCR reaction. In addition, 2.5% of DMSO was added to the PCR reaction of exon-1 $\alpha$  of *p16* gene. PCR conditions for the amplification of *p16* gene consisted of 5-min denaturation at 95°C, 35 cycles of 30 s at 95°C, 1 min at 59.2°C, and 1 min at 72°C. PCR conditions for *p14* gene consisted of 3-min denaturation at 95°C, 30 cycles of 1 min at 95°C, 1 min at 55°C,

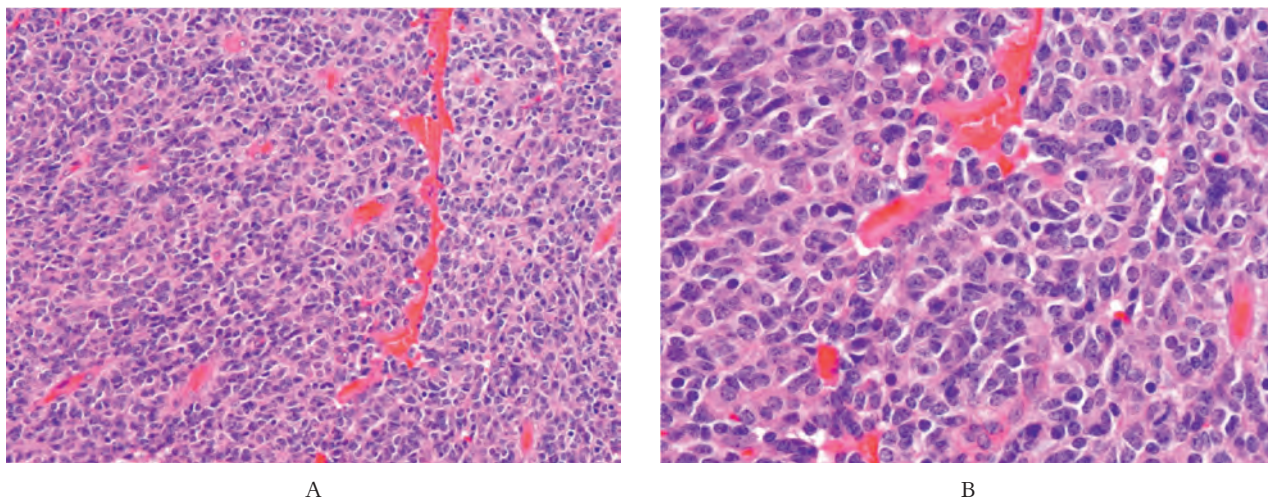


Fig. 2. Photographs showing a cellular poorly differentiated tumor composed of small cells with hyperchromatic nuclei and little cytoplasm (hematoxylin-eosin)

A, magnification  $\times 200$ ; B, magnification  $\times 400$ .

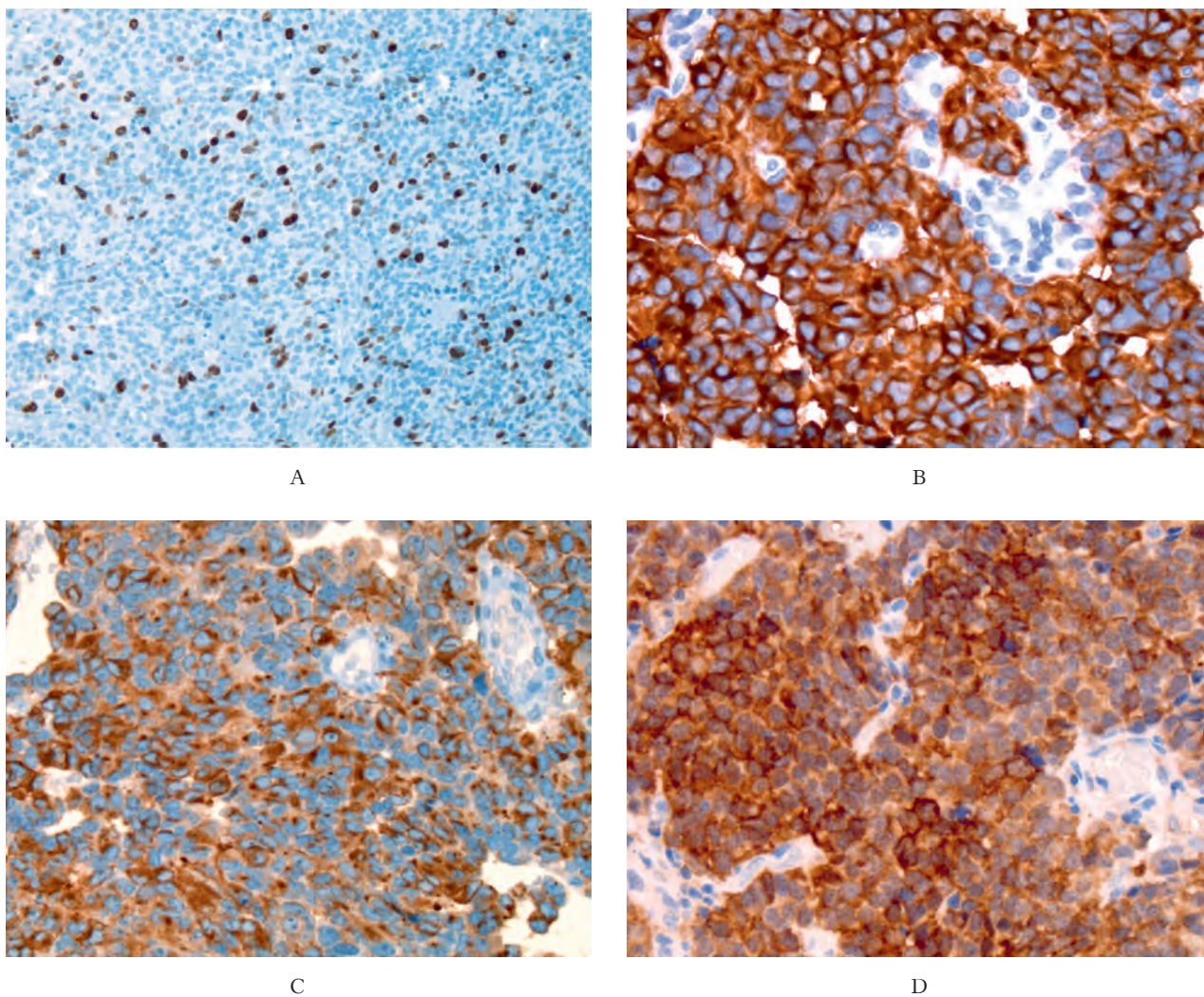


Fig. 3. Photographs of the tumors

A, Ki-67 staining (magnification  $\times 200$ ); B, immunohistochemical staining positive for synaptophysin (magnification  $\times 400$ ); C, immunohistochemical staining positive for chromogranin A (magnification  $\times 400$ ); and D, immunohistochemical staining positive for neuron-specific enolase (magnification  $\times 400$ ).

and 1 min at 72°C. PCR products were run on a 1.5% agarose gel and visualized under UV light after staining with ethidium bromide.

### Results

Exon-1 $\alpha$  of *p16INK4A* gene and exon-1 $\beta$  of *p14ARF* gene were amplified separately for homozygous deletions. No homozygous deletions were observed either for *p16INK4A* or *p14ARF* genes in the analyzed PNET (Fig. 4).

The bypass surgery with biopsy and partial tumor excision were performed. Postoperative period was comfortable and without complications. The patient received radiotherapy at a total dose of 58 Gy to the brain and 30 Gy to the spinal cord. The patient refused to undergo chemotherapy. The patient was followed-up by specialists. Magnetic resonance imaging performed just 3 years after the surgery revealed tumor regrowth. The patient died 3 years and 8 months after the first surgery.

### Discussion

Primitive neuroectodermal tumors (PNETs) include a heterogeneous group of tumors thought to originate from primitive or undifferentiated neuroepithelial cells that typically occur in pediatric patients and are rare in adult patients. The prototype of these tumors is a cerebellar medulloblastoma, which constitutes 13%–25% of all pediatric brain tumors (6) and, in contrast, only 1% of adult brain tumors. Hart and Earle described the term of primitive neuroectodermal tumor – PNET – in 1973 (1). Originally, PNET was considered as a cerebral high-grade undifferentiated neuroepithelial tumor of childhood, rarely demonstrating focal differen-

tiation along glial and neuronal lines. However, the term was soon used for undifferentiated embryonal tumors of all CNS sites and all ages and was promoted by Rorke in 1983 (7, 8). According to literature, PNETs may occur in almost any location within or outside the central nervous system (9–11). PNETs recognized outside the CNS are diagnosed as peripheral PNETs (pPNET). CNS PNET and pPNET are different entities with different immunohistochemical profiles and genetic backgrounds. Clinically, they are both aggressive tumors, but exhibit different characteristics in their local manifestation and metastatic spread (CNS PNET metastasize rarely, <5%). However, the survival rates are quite similar (12). In the classification of tumors of the central nervous system provided by the WHO in 2007, the Working Group recommended to use a more general term PNET for similar tumors located in the brain stem and spinal cord and to add the prefix CNS to these entities in order to avoid any confusions concerning the localizations of extracerebral tumors (WHO grade IV) (13). CNS PNETs can be divided into 2 large groups: infratentorial tumors (medulloblastoma or iPNETs) and supratentorial tumors (sPNETs). All these tumor types are very rare in adult age. In the present case, the PNET tumor mass was disseminated and involved the supratentorial and infratentorial brain parts. Ohba et al. have recently reviewed 57 published cases of supratentorial PNETs (independently of tumor location). The mean age of these patients at diagnosis was 35.2 years with the peak between 20 and 30 years. Moreover, they also found a difference in the sex ratio (male:female, 32:23) (14). Primary intraspinal PNETs are an exceedingly rare entity.

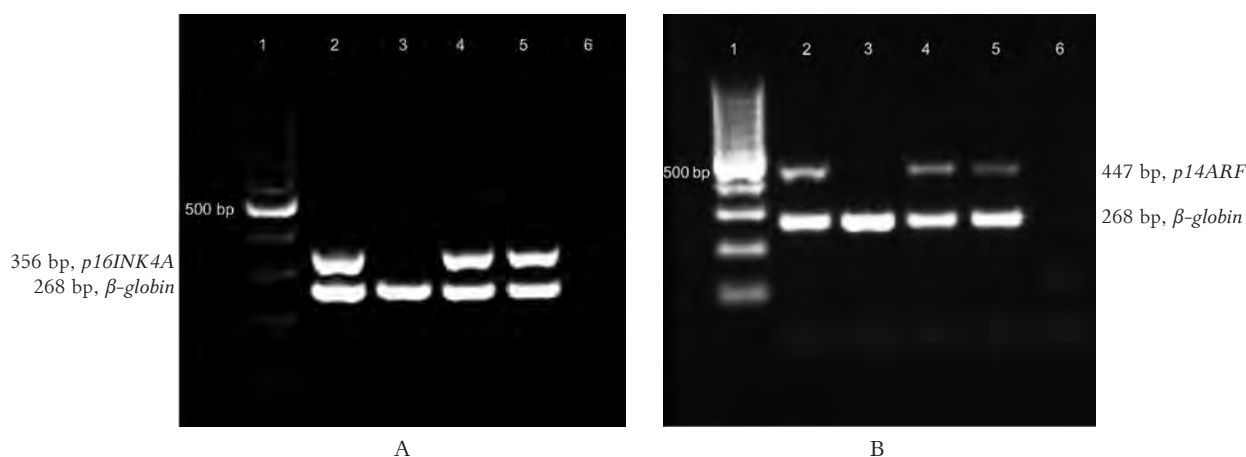


Fig. 4. *CDKN2A* locus (*p16INK4A* and *p14ARF*) study in the patient with primitive neuroectodermal tumor PNET, control blood sample, and NB4 cell line

$\beta$ -Globin gene (268 bp) was used as an internal control. Homozygous deletions of *p16INK4A* (A) and *p14ARF* (B). A – *p16INK4A* exon-1 $\alpha$  (amplicon length, 356 bp): lane 1, DNA 100-bp ladder; lane 2, control blood DNA; lane 3, NB4 cell line with *p16INK4A* deletion; lanes 4 and 5, tumor DNA of patient with PNET; lane 6, water control. B – *p14ARF* gene exon-1 $\beta$  (amplicon length, 447 bp): lane 1, DNA 100-bp ladder; lane 2, control blood DNA; lane 3, NB4 cell-line with *p14ARF* deletion; lanes 4 and 5, tumor DNA of patient with PNET; lane 6, water control.

These neoplasms can be found extradurally, extramedullary, intraextramedullary, and purely intramedullary (15). A recent literature research has revealed 28 cases reported (16). Only 10 cases of purely intraspinal PNETs have been reported with the mean age of 12.9 years, no sex differences, and no specific location in the spinal cord (4). Just few published cases reported PNETs of other localizations such as the kidneys, upper-urinary tract (9), orbit (10), chest wall (11), and others (17).

Nowadays, PNET diagnostics is generally based on MRI and histological/immunohistochemical findings. Demonstrating the expression of MIC2 glycoprotein (CD99) by immunohistochemical staining showing the specific EWS-FLI1 chimeric gene presence in pPNET and PNET with mixed differentiation by other specific stainings (CD34, CD45, CD20, etc.) offers an easy way of making a differential diagnosis within and between CNS PNET and pPNET (12). These tumors, which express MIC2 gene (CD99), seem to be least aggressive after complete resection (10). Pfister et al. (2007) were interested in molecular genetic status of pediatric supratentorial PNETs and medulloblastomas (18). They found that *CDKN2A* deletions tended to be associated with metastatic disease at diagnosis (Fisher exact test,  $P=0.07$ ). Heterozygous deletions of 9p21.3 were detected in 3.5% of medulloblastomas and in 14% of supratentorial PNETs, whereas homozygous deletions were not observed in any medulloblastoma, but in 19% of supratentorial PNETs (Fisher exact test,  $P<0.001$ ). All patients with heterozygous deletions of 9p21.3 died within 4 years after surgical resection (18). Inda et al. reported no homozygous deletion of either *p16INK4* or *p14ARF* in any of the PNET samples (19). Like in previously reported cases, no homozygous deletions of either *p16INK4a* or *p14ARF* were observed in the present case of PNET. In addition, the expression of genes *p16INK4a* and *p14ARF* can be measured at the protein level by immunohistochemistry (in this case, immunochemistry has not been performed because of lack of certain resources and possibilities). Pfister et al. (2007) identified other aberrations such as a copy-number gain of chromosome region 20q13.33 and *UNC5C* or *UNC5B* loci, and large-scale losses on chromosome arms 1p, 9p, 16q, and 17p, and others by applying fluorescence in situ hybridization and array-based comparative genomic hybridization. With reference to these findings, they suggested genetic differences existing between supratentorial PNETs and medulloblastomas, which will be an important line of future research to identify the cell origin for both the tumor entities and the molecular pathways in their development (18).

The algorithm of PNET treatment has not been

created yet. In accordance with patient's clinical status, complete tumor excision, chemotherapy, and radiotherapy are performed as standard procedures. Usually, the chemotherapy protocol (vincristine, cisplatin or carboplatin, methotrexate, and other medications) before, during, or after radiotherapy is chosen by a treating physician (20). In the present case, only partial tumor resection and radiotherapy to the brain and the spinal cord were performed resulting in the 3.6-year relapse-free survival. Prognoses of patients with PNET are different. In the pediatric population, PNET prognosis is worse in comparison with medulloblastoma (21). Prognosis of purely intramedullary spinal cord PNET usually is very poor, and most patients die within 2 years despite treatment (17). Older age appears to be prognostically more favorable (5). Unfortunately, the 5-year survival in case of CNS PNET remains to be less than 50% in all age groups (5). To date, only 2 cases have been reported with an exclusively long-term relapse-free survival, i.e., males with 18-year (22) and 17-year (5) relapse-free survival. Good prognostic factors seem to be early diagnosis of PNET, complete tumor resection combined with chemotherapy and radiotherapy (5, 23, 24), and some tumor signs such as intratumoral calcifications and Ki-67 labeling index ( $<30\%$ ) as well (5, 14, 24). More studies are needed to identify molecular PNET status and verify good prognostic factors.

### Conclusions

Primitive neuroectodermal tumors originate from primitive or undifferentiated neuroepithelial cells that usually occur in pediatric patients and are extremely rare in adult patients worldwide. Therefore, for the first time in Lithuania, one case of rare adult PNET of the central nervous system with typical immunohistochemical findings has been reported.

The diagnostics of primitive neuroectodermal tumors does not differ from other types of the central nervous system tumors. In accordance with patient clinical status, complete tumor excision, chemotherapy, and radiotherapy are performed as a treatment standard with different survival prognosis.

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### Statement of Conflict of Interest

The authors state no conflict of interest.

## Primityvus neuroektoderminis centrinės nervų sistemos navikas, diagnozuotas 51 metų pacientei

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**Raktažodžiai:** primityvus neuroektoderminis navikas, centrinė nervų sistema, branduolinis magnetinis rezonansas, imunohistochemija, *CDKN2A* geno delecija.

**Santrauka.** Primityvus neuroektoderminis navikas (PNET) yra vienas iš retų ir itin agresyvių nežinomos etiologijos centrinės ir periferinės nervų sistemos navikų. Šiuo terminu apibūdinami navikai sudaryti iš apvalių, smulkių ląstelių, kilusių iš pirminių neuroektoderminių vaisiaus audinių. PNET navikai dažniausiai diagnozuojami jaunesniems nei 15 metų vaikams, ypač retai suaugusiesiems.

Straipsnyje aptariamas itin retas centrinės nervų sistemos primityvaus neuroektoderminio naviko, diagnozuoto 51 metų pacientei, klinikinis atvejis bei pateikiama trumpa literatūros apžvalga.

### References

- Hart MN, Earle KM. Primitive neuroectodermal tumors of the brain in children. *Cancer* 1973;32:890-7.
- Visee S, Soltner C, Riolland X, Machet MC, Loussouarn D, Milinkevitch S, et al. Supratentorial primitive neuroectodermal tumors of the brain: multidirectional differentiation does not influence prognosis. A clinicopathological report of 18 patients. *Histopathology* 2005;46:403-12.
- Kim UR, Arora V, Devanand J, Khazei HM. Multimodality treatment approach in management of primary peripheral primitive neuroectodermal tumor of the orbit. *Indian J Ophthalmol* 2009;57(5):395-8.
- Otero-Rodríguez A, Hinojosa J, Esparza J, Muñoz MJ, Iglesias S, Rodríguez-Gil Y, et al. Purely intramedullary spinal cord primitive neuroectodermal tumor: case report and review of the literature. *Neurocirugia (Astur)* 2009;20(4):381-7.
- Krampulz T, Hans VH, Oettel F, Dietrich U, Puchner MJA. Long-term relapse-free survival with supratentorial primitive neuroectodermal tumor in an adult: a case report. *J Neurooncol* 2006;77:291-4.
- Majo C, Alonso J, Aguilera C, Serrallonga M, Acebes JJ, Aru C, et al. Adult primitive neuroectodermal tumor: proton MR spectroscopic findings with possible application for differential diagnosis. *Radiology* 2002;225:556-66.
- Gilbertson RJ, Ellison DW. The origins of medulloblastoma subtypes. *Annu Rev Pathol* 2008;3:341-65.
- Rorke LB. The cerebellar medulloblastoma and its relationship to primitive neuroectodermal tumors. *J Neuropathol Exp Neurol* 1983;42:1-15.
- Ammani A, Ghadouane M, Hajji F, Janane A, Ameer A, Abbar M. Primitive neuroectodermal tumor (PNET) of the upper-urinary tract. *Prog Urol* 2009;19(8):579-81.
- Das D, Kuri GC, Deka P, Bhattacharjee K, Bhattacharjee H, Deka AC. Primary primitive neuroectodermal tumor of the orbit. *Indian J Ophthalmol* 2009;57(5):391-3.
- Demir MK, Koşar F, Sanli Y, Esmaeilzadeh S, Urer HN. 18F-FDG PET-CT features of primary primitive neuroectodermal tumor of the chest wall. *Diagn Interv Radiol* 2009;15(3):172-5.
- Kampman WA, Kros JM, De Jong TH, Lequin MH. Primitive neuroectodermal tumors (PNETs) located in the spinal canal; the relevance of classification as central or peripheral PNET: case report of a primary spinal PNET occurrence with a critical literature review. *J Neurooncol* 2006;77(1):65-72.
- Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO Classification of Tumours of the Central Nervous System. *Acta Neuropathol* 2007;114:97-109.
- Ohba S, Yoshida K, Hirose Y, Ikeda E, Kawase T. A supratentorial primitive neuroectodermal tumor in an adult: a case report and review of the literature. *J Neurooncol* 2008;86:217-24.
- Deme S, Ang LC, Skaf G, Rowed DW. Primary intramedullary primitive neuroectodermal tumor of the spinal cord: case report and review of the literature. *Neurosurgery* 1997;41(6):1417-20.
- Musahl V, Rihn JA, Fumich FE, Kang JD. Sacral intraspinal extradural primitive neuroectodermal tumor. *Spine J* 2008;8(6):1024-9.
- Saada E, Thariat J, Follana P, Birtwisle-Peyrottes I, Haudebourg J, Trojani C, et al. Primitive neuroectodermal tumor of the pelvis in an elderly patient. *Onkologie* 2009;32(8-9):499-502.
- Pfister S, Remke M, Toedt G, Werft W, Benner A, Mendrzyk F, et al. Supratentorial primitive neuroectodermal tumors of the central nervous system frequently harbor deletions of the *CDKN2A* locus and other genomic aberrations distinct from medulloblastomas. *Genes Chromosomes Cancer* 2007;46(9):839-51.
- Inda MM, Muñoz J, Coullin P, Fauvet D, Danglot G, Tuñón T, et al. High promoter hypermethylation frequency of p14/ARF in supratentorial PNET but not in medulloblastoma. *Histopathology* 2006;48(5):579-87.
- Herrlinger U, Steinbrecher A, Rieger J, Hau P, Kortmann RD, Meyermann R, et al. Adult medulloblastoma: prognostic factors and response to therapy at diagnosis and at relapse. *J Neurol* 2005;252:291-9.
- McNeil DE, Coté TR, Clegg L, Rorke LB. Incidence and trends in pediatric malignancies medulloblastoma/primitive neuroectodermal tumor: a SEER update. *Surveillance Epidemiology and End Results. Med Pediatr Oncol* 2002;39(3):190-4.
- Louis DN, Hochberg FH. Cerebral primitive neuroectodermal tumor in an adult, with spinal cord metastasis after 18-year dormancy. *J Neurooncol* 1990;9(1):77-80.
- Yang HJ, Nam DH, Wang KC, Kim YM, Chi JG, Cho BK. Supratentorial primitive neuroectodermal tumor in children: clinical features, treatment outcome and prognostic factors. *Childs Nerv Syst* 1999;15(8):377-83.
- Kim DG, Lee DY, Paek SH, Chi JG, Choe G, Jung HW. Supratentorial primitive neuroectodermal tumors in adults. *J Neurooncol* 2002;60(1):43-52.

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