Pediatric glioblastoma with giant cells and “supratentorial” primitive neuroectodermal component – case report and review of the literature

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Abstract

Introduction: The glial differentiation in pediatric “supratentorial primitive neuroectodermal tumors” (sPNET) is occasionally revealed by immunohistochemistry with GFAP (glial fibrillary acidic protein) as isolated positive cells among undifferentiated cells, indicative of divergent cellular phenotypes. Large malignant glial tumors in sPNETs are extremely rare and challenge the neuropathologist by raising the possibility of glioblastomas with sPNET-like features (GB sPNET). The distinction between them is important because of their different treatment and prognosis. Case presentation: A large parieto-occipital tumor with minimal ventricular invasion, in an 11-year-old girl, with a five-month clinical history, was proven to be a highly malignant biphasic tumor, consisting in a glioblastoma with giant cells, representing 75% of the tumor, and sPNET nodules, with one larger dominant nodule. The immunohistochemistry confirmed positivity for synaptophysin, neurofilament, neuron-specific enolase and CD56 in the sPNET compartment and for GFAP, CD56 and vimentin in the glioblastoma. In some parts of the tumor, the two components were well delineated from each other as in a “collision” tumor, but in others, the two different tumors were intermingled. It was histologically diagnosed as sPNET with double differentiation (glial and neural) or glioblastoma with sPNET-like features. Conclusions: These cases are very rare, few reported, especially in the pediatric population, and with high difficulties in histological differential diagnosis, subsequently reflected in the therapeutic decisions.

Keywords: glioblastoma, supratentorial primitive neuroectodermal tumor, pediatric, neuroglial stem cell.

Introduction

Central nervous system (CNS) tumors are the second most common malignancies after leukemia and the most common solid tumors in children (age 0–19 years), having an incidence of 5.26 per 100 000 per year in USA [1].

Glioblastoma (GB), classified as grade IV in the World Health Organization (WHO) classification system, is the most frequent and most aggressive malignant primary brain tumor, 99% of cases appearing in adults [2]. The incidence in children is very low, 0.8 per 100 000 children (before 19 years of age) develop high-grade gliomas each year, second after embryonal brain tumors including medulloblastomas [3]. The treatment for GB consists in local radiotherapy and alkylating chemotherapy such as Temozolomide, but the average survival time is only about one year [4].

Central nervous system/supratentorial primitive neuroectodermal tumors (CNS/s PNETs) are highly malignant grade IV WHO embryonal tumors, accounting for 2–3% of pediatric brain tumors [5], with a mean age at presentation of 5.5 years [2]. According to the 2007 WHO Classification, the term not otherwise specified (NOS) CNS/s PNETs may be used for all undifferentiated or poorly differentiated embryonal tumors in any extracerebellar site of the CNS (cerebral hemispheres, brain stem, spinal cord) [6]. They have the capacity for divergent differentiation along various neuroectodermal cell lines, the antigen expression for neuronal, astrocytic, muscular or melanocytic line being unique for each tumor [2]. These tumors share many morphologic and immunohistochemical features with their cerebellar counterpart, the medulloblastoma, but they differ biologically, and have a worse outcome with little improvement over time [7]. They are usually treated as “high-risk” medulloblastomas with full dose craniospinal radiotherapy and platinum-based chemotherapy [7]. The response to treatment is, however, more frequent and long-term survival is better than in glioblastoma, with an estimated 4-year survival time of 38% [4].

This report presents a clinicopathological description of a pediatric glioblastoma associated with a “supratentorial” primitive neuroectodermal tumor, which most consider now to be a “glioblastoma with PNET-like features” (GB sPNET-like), a very rare, newly proposed entity, and rarely reported in the literature, especially in the pediatric group [8, 9].
**Case report**

An 11-year-old girl presented with a five-month history of neurological disturbances beginning with episodes of motor aphasia and confusion, which was believed at that time to be an anxious and emotional distress syndrome. Five months later, she came back with headaches, three episodes of vomiting and progressive memory disorders, especially in the last three weeks. She did not present any other remarkable findings in her clinical examination. Magnetic resonance imaging (MRI) brain scan revealed a well circumscribed, 6.6/5.2/4.5 cm, heterogeneous intranevraxial lesion, with central necrosis, lacking perilesional vasogenic edema, which was located in the left parieto-occipital lobe (Figure 1a). On T1-weighted coronal section, the lesion displayed heterogeneous contrast enhancement and on axial diffusion sequence presented restriction of diffusion, strongly suggesting a high-grade “supratentorial” PNET (Figure 1, b and c). Subsequently, a craniotomy and gross total resection of the tumor was performed. A postoperative CT brain scan was without residual tumor, and the patient presented full recovery, with a remission of all focal neurological signs and neurological deficits. The pulmonary radiography, abdominal echography and later a complete spinal MRI did not reveal any other lesion. After histological diagnosis, she was further referred to the oncological team for adjuvant chemotherapy (according to HIT 2000 protocol for brain tumors) and craniospinal radiotherapy.

The resection specimen consisted in multiple friable and grey-white tissue fragments, of 1.5/1.2/1 cm to 4/3/3.5 cm, with violaceous/hemorrhagic zones macroscopically. After a thorough microscopic examination of the whole tumor, an unusual biphasic histology was revealed on multiple Hematoxylin and Eosin (HE) stained slides, which required a complex differential diagnosis.

The largest component of the tumor, representing more than 75% of the examined microscopic fields, was composed of glial cells with increasing size and pleomorphism, the dominant histological picture being that of large bizarre cells (50–100 μm), with abundant eosinophilic cytoplasm, atypical pleomorphic nuclei, prominent nucleoli, and frequent multinucleated cells, dispersed in a fine fibrillary background (Figure 2c). Rare foci of different dimensions featuring higher cellularity were observed mainly around capillaries (Figure 2a). These foci contained giant cells interspersed with smaller cells and from these a migration of giant cells could be seen (Figure 2b). The mitotic activity was low, and both coagulative and geographic necrosis were absent. Vascular proliferation was inconspicuous, with fine capillary network, but without microvascular proliferation. Histochemically, the absence of a dense reticulin network between the tumor cells was revealed by Gömöri silver stain (Figure 2c). The histological diagnosis proposed for the glial part of the tumor was that of a glioblastoma with some characteristics of giant cell glioblastoma.

In contrast to the glial bulk of the tumor, the smaller part of the tumor, which represented less than a quarter of the examined microscopic fields, was composed of small nodules (Figure 3a) and one larger dominant nodule (1 cm diameter) (Figure 3b), which were highly cellular, with undifferentiated “small blue cells”, featuring round or oval and slightly irregular basophilic nuclei, high nucleocytoplasm ratios and high mitotic activity (25 mitoses/10 high-power fields) but enclosing no fibrillary cytoplasm or stroma (Figure 3c). These regions were focally well delineated from the glial component as seen in Gömöri silver stain (Figure 2c) but in some parts they slightly merged (Figure 3a); all regions presented isolated giant glial cells scattered between the small cells (Figure 3b). The vascular proliferation in the largest nodule was prominent, with large vessels, but also of some microvascular type with glomeruloid pattern, and associated microscopic areas of coagulative tumor necrosis without real pseudopalisading (Figure 3b). True neuroblasts, Homer Wright rosettes or ganglion-like cells were not seen. However, the cyto-nuclear and architectural patterns raised the possibility of an undifferentiated primitive neuroectodermal tumor, large cell/anaplastic medulloblastoma-like, against a small cell glioblastoma.

Immunohistochemically (IHC), the tumor revealed different immunoreactivity in the two compartments. The glial component was distinctly positive for GFAP (glial fibrillary acidic protein), S100, vimentin and CD56 antigens (Figure 4, a–d). The “small blue cell” component was completely negative for GFAP, S100 and vimentin and intensely positive for CD56 and NSE (neuron-specific enolase), and focally positive for synaptophysin and NF (neurofilament), reflecting a predominantly undifferentiated primitive phenotype and just focally a neuronal
antigen expression (Figure 4, a–d). The Ki67 proliferation index measured from 10% in the glial regions to 40% in the small cell compartment (Figure 3c). The p53 expression was weak, of 10% in the first compartment, pleading against giant cell glioblastoma (p53 positive in 84% of cases) and of moderate intensity in less than 30% of cells in the second compartment.

Sustained by the age of the patient, the relatively short duration of the symptoms, supratentorial location of the tumor, radiological findings and the histological and immunohistochemical features, the initial diagnosis was of a “supratentorial” primitive neuroectodermal tumor (sPNET), with predominantly glial but also neural differentiation (as revealed by immunohistochemistry). However, the glial compartment being the predominant one, was also raised a second possibility of “glioblastoma with PNET-like features”.

Figure 2 – The glial component of the tumor presents: (a) A small focus of higher cellularity around capillaries with a background of giant glial cells (HE staining, ×40); (b) Giant cells interspersed with smaller cells in the nodule and apparent giant cell “migration” (HE staining, ×100); (c) Bizarre, multinucleated giant cells in a fine fibrillary stroma outside the nodule (HE staining, ×200); (d) Absence of dense reticulin stroma between all types of tumor cells, the left inferior corner of the image presenting small “primitive” cells (Gömöri silver staining, ×100).

Figure 3 – The primitive sPNET compartment of the tumor: (a) As a small cell area in the inferior left corner of the image merging slightly with the glial component (HE staining, ×100); (b) or as a large field from the dominant nodule of the tumor, with conspicuous vascular proliferation and tumor necrosis (HE staining, ×100).
Figure 3 (continued) – The primitive sPNET compartment of the tumor: (c) Composed by undifferentiated primitive “small blue cells” (HE staining, ×40); (d) With moderate expression of p53 in less than a third of the small cells (IHC, ×200). The p53 positivity in immunohistochemistry means brown staining of the nuclei using 3,3′-Diaminobenzidine chromogen, contrasting with the blue staining of the background (Hematoxylin).

Figure 4 – The immunohistochemistry (IHC) revealed the differences between the two compartments of the tumor, the left inferior corner of each image comprising the primitive compartment and the upper right the glial compartment: (a) Positive GFAP expression in the glial compartment and in isolated glial cells interspersed with the “small cells” in the primitive compartment (IHC, ×200); (b) Positive synaptophysin expression exclusively in the small cell compartment (IHC, ×200); (c) Positive vimentin expression in the glial cells, with the same pattern of distribution as GFAP (IHC, ×200); (d) Positive CD56 expression in both cell types (IHC, ×200). The positivity immunostaining means brown staining of the cytoplasm and/or nuclei using 3,3′-Diaminobenzidine chromogen, contrasting with the blue staining of the background (Hematoxylin).

Discussion

Given their rarity, pediatric glioblastomas are difficult to study, but it is becoming obvious that they are widely different from their histological adult counterpart, presenting specific biology and genetics, in spite of the same clinical behavior and resistance to the most aggressive treatment regimens [10]. As glioblastomas, the CNS/sPNETs, tumors primarily of the childhood, are difficult
to study being extremely rare and have poor prognosis (the poorest among embryonal brain tumors), but somewhat better prognosis than glioblastomas [4]. They are different in genetics and biology from their cerebellar counterpart, the medulloblastomas [2]. Both pediatric glioblastomas and CNS's PNETs reflect well the differences between adult and pediatric tumors, and also the fact that pathologically identical tumors from different CNS compartments represent divergent diseases, with various genetics, biology and clinical behavior [10].

This case report is focused on the rarest association between two highly malignant tumors, one glial and one neuroectodermal, which usually are considered distinct in clinical behavior and therapeutic response. This association is described now as a variant of glioblastoma, that is not yet included in the WHO Classification system, named “glioblastoma with PNET-like features” (GB sPNET) or more accurately “malignant glioma with PNET-like features” (MG sPNET) [4, 8, 11]. The malignant glioma component may be a glioblastoma or gliosarcoma, but may present foci of a lower grade glioma as in secondary glioblastoma [4, 12].

Most of the cases described in the literature as MG sPNETs were isolated case reports, with very few series reported. The largest series, published by Perry et al. [4], described 53 cases clinicopathologically and genetically [4, 9]. The majority of reported cases were encountered among adults (median age 54 years), and very rarely in children such as the current case [4, 8, 13]. The occurrence of symptoms was usually of short duration (commonly suggesting a primary glioblastoma in adults), less than six months in 85% of cases, similar to the described case [4, 9]. The most frequent location was in the temporal lobe (52% of cases), the tumor being radiologically well circumscribed, with significant mass effect, and presenting diffuse perilesional edema and rarely (15% of cases) intralesional hemorrhage, necrosis and cystic spaces [9]. Similar features were present in the current case, except for the parieto-occipital location and secondary changes. In another series reported by Song et al. [13], the majority of patients presented ring-enhancing lesions on MRI, consistent with a classic glioblastoma, but also restricted diffusion in diffusion-weighted imaging (DWI), which suggested highly cellular PNET-like components [13].

The glial compartment described in this case presented unusual histological features, between giant cell glioblastoma and classic glioblastoma, which highlighted the more complex nature of the tumor. In spite of the predominance of giant bizarre glial cells, a diagnosis of true giant cell glioblastoma or a pleomorphic xanthoastrocytoma was excluded histochemically because of the absence of a dense reticulin network between the tumor cells, which is one of the hallmarks of these glial tumors [2]. Similarly, a classic glioblastoma was excluded because of the absence of microvascular proliferation and pseudopalisading necrosis [2]. The rare foci with smaller cells around the vessels, from which giant cells apparently migrated, made the diagnosis more difficult. The differential diagnosis of these foci with the small cell GBM was simple, in the last one the small cell pattern being more monomorphic, with bland oval nuclei, more likely to be confused with an oligodendroglioma [4]. The proposed histological diagnosis was glioblastoma with some features of giant cell glioblastoma, because only in giant cell variant of glioblastoma a histological picture dominated by giant cells may be associated with the absence of microvascular proliferation and necrosis [2].

The presence of small sPNETs elements in a glial tumor is always a diagnostic challenge for the pathologist. Conversely, in sPNETs the diagnosis appear easier, since according to WHO Classification “immunohistochemical techniques occasionally reveal GFAP to be expressed among the undifferentiated cells, indicative of divergent cellular phenotypes” [2]. However, in the context that “the antigen expression is unique for each tumor” [2], the extent of glial differentiation in a primary neuroectodermal tumor was never specified. The present case being a pediatric one and with fulfilled immunohistochemical criteria, the first diagnosis was that of CNS’s PNET with double differentiation (glial and neural). However, the presence of extensive glial differentiation raised also a second possibility of glioblastoma with sPNET-like features (GB sPNET), with somewhat different treatment and prognosis.

Histologically, the GB sPNETs consist of undifferentiated hypercellular areas with eventual neuronal differentiation alongside with large classic GB areas [9]. However, the immunohistochemical evaluation, now improved by certain specific markers, is mandatory for diagnosis. As highlighted by Song et al. [13], the use of vimentin and CD56 makes the diagnosis of GB PNET much easier. The GFAP, S100, vimentin and CD56 expression is almost always widespread in the glioma components. Vimentin is strongly and diffusely expressed in the astrocytic components and is negative in PNET components, while CD56 is strongly and diffusely positive in both, astrocytic and PNET components [13]. The primitive components lack the expression of GFAP or present a slight expression, in fewer cells [4]. For differential diagnosis, the GFAP and CD56 positivity will differentiate a small cell glioblastoma from the PNET component [4]. The neural differentiation is highlighted focally by synaptophysin and by NSE expression (less specific) [4], and more specifically by presence of NF between undifferentiated cells. The nuclear NeuN expression is described in primitive foci [4], alongside with the cytoplasmic staining for nestin, an intermediate filament expressed by immature cells, attesting for the primitive nature of the tumor [14]. Nuclear p53 expression is also a frequent feature (85% of cases) in both components, compatible with a “secondary” malignant glioma in most adult GB sPNETs, lower grade of glioma being also found in the tumors [4]. The very low expression of the p53 protein in our case was more consistent with a “primary glioblastoma”, which is contrasting with the “secondary” nature of the most “adult” GB sPNETs.

In spite of the apparently easier histological and immunohistochemical diagnosis, the clinical reality reflects a different situation [7]. In a clinical study of Jakacki et al. [7] pediatric sPNETs treated with Carboplatin during radiotherapy, some discordances between institutional and central pathologic review were highlighted. In this study, the central pathologic review reclassified 37 cases of sPNETs in: “classic” sPNET in 14 cases, “undifferentiated” sPNET in eight cases (which displayed an infiltrative growth and
little synaptophysin immunoreactivity), “malignant gliomas” in 13 cases, and glioblastomas in the last two cases (at the time of second-look surgery or autopsy) [7]. “Malignant gliomas” were defined by the authors as “highly cellular GFAP-positive tumors with absent or only focally present synaptophysin staining”, and the “undifferentiated” tumors as “very dense cellular tumors that were only focally if at all immunoreactive for synaptophysin and GFAP” [7]. They did not find any significant differences between the subgroups, although survival distributions approached significance when the combined “classic” and “undifferentiated” group was compared to the “malignant glioma”, the survival being slightly better for the first group [7]. Their findings highlighted the large number of tumors with extensive glial antigenic expression diagnosed within sPNETs, and the significance of these findings in the prognosis. The study reflected the real situation and the authors recognized that “the pediatric small cell supratentorial malignancies can be notoriously difficult to categorize, particularly in cases without specific histological or immunohistochemical features” [7]. In other studies, gliomatous and lipomatous degenerations of sPNET tumors has been also reported [15, 16]. The heterogeneity described in sPNETs was in concordance with the great potential of the tumor cells to display divergent histological differentiation and antigen expression for neuronal and astrocytic lines, and also for muscular/mesenchymal or melanocytic lines, corresponding to the three molecular stratifications described in these tumors (primitive neural, oligoneural and mesenchymal lineage), with probable prognostic and treatment impact [3, 7, 15, 17].

The histological differentiation is usually the reflection of different genetic alterations in the phenotype. However, an unusual case of pediatric sPNET with malignant astrocytic transformation, reported by Kuhn et al. [8], revealed a clonal origin for both components by analysis of loss of heterozygosity. The deletions found were present either in both components or in the glial component only, deletion specific for sPNET being not found. Moreover, in a case series including 40 GB sPNETs, Perry et al. (2007) [4] demonstrated by fluorescence in situ hybridization that glioma-associated alterations involved both components, 10q loss being present in 50% of cases, and N-myc or c-myc gene amplifications being seen only in the primitive elements, in 43% of cases [4]. They concluded that, in GB sPNETs, the primitive component arises within a pre-existing malignant lesion (most often a “secondary” glioblastoma) and often shows histological anaplasia and N-myc or (c-myc) amplification [4].

The biology of these biphasic tumors revealed an intriguing complexity enabling different possible approaches. The first possible way of double differentiation may be the extensive glial differentiation from a primitive component [2], but this conception, which is more compatible with the multi-lineage potential of sPNETs, needs a more precise definition of the extension, type and grade of the astrocytic differentiation acceptable in sPNETs. The second possible way is metaplasia of the glial component, which was proposed by Perry et al. [4] in the case of fully developed glial malignant tumors associated with sPNET-like small nodules. This may be also the truth in our case, suggested by some unexplained small hypercellular nodules in the glial mass, with mixed phenotype, of small and large giant cells, beside the primitive ones. However, the presence of one significantly larger nodule comprised of primitive cells raises further questions concerning the biology of this tumor and places it closer to their second hypothesis of a tumor stem/progenitor cell clone expansion [4]. The third, most improbable way is that of a “collision” tumor. In spite of the focal “collision” pattern in our case, this may be explained by the different subclones formed in the tumor, the primitive one having a more expansive growth and subsequently being focally well delineated from the glial one.

Concerning the treatment possibilities, the concurrent presence of the two components requires special attention to both pathological entities. The primitive component suggests a faster grow and the possibility of invasion of the arachnoid space and cerebrospinal fluid dissemination, with ependymal seeding, intracranial meningeal deposits and drop metastases [4, 9]. The glial component suggests a more invasive growth in the brain along classic secondary structures, and frequent local recurrences [2, 4]. Standard protocols of treatment have not been established. However, a gross total tumor resection remains the most important primary step. The well-circumscribed character of the lesion facilitates resection and explains the slightly lower local recurrence rate than in classic glioblastoma [4, 9]. The adjuvant oncological treatment in adults is usually focused on the glial compartment, but when a sPNET-like behavior is seen, especially CSF dissemination, a switch to platinum-based therapy must be considered [4]. However, the prognosis remains poor, almost similar to that of glioblastoma.

## Conclusions

We described a highly malignant pediatric brain tumor, with a biphasic histological structure and a difficult differential diagnosis between “sPNET with double differentiation (glial and neural)” and “glioblastoma with sPNET-like features”, a new variant of glioblastoma that is not yet included in the WHO Classification system. The main part of the tumor being a “giant cell-like glioblastoma”, the definitive diagnosis needed a thorough microscopic examination of the whole tumor that revealed the smaller, more primitive sPNET component. The phenotype of the two components was established immunohistochemically with GFAP, S100, vimentin and CD56 for the glial component and CD56, synaptophysin, NSE for the neuro-ectodermal/neural one. The large extension of the glial component, the presence of small hypercellular nodules with mixed phenotype, besides the primitive ones, sustained the theory of metaplasia or the hypothesis of a tumor stem/progenitor cell clone expansion in a glial component, and the diagnosis of “glioblastoma with giant cells and sPNET component”. These cases are very rare, few reported, especially in the pediatric population, and with high difficulties in histological differential diagnosis, subsequently reflected in the therapeutic decisions.

### Conflict of interests

The authors declare that they have no conflict of interests.
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