

Original Article

Embryonal tumor with multilayered rosettes, C19MC-altered (ETMR): a newly defined pediatric brain tumor

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Abstract: Embryonal tumor with multilayered rosettes (ETMR), C19MC-altered, is a newly defined and rare pediatric malignant tumor of the central nervous system (CNS) in the 2016 WHO Classification of Tumors of the Central Nervous System. Here we present two cases of ETMR with amplification of the C19MC locus at chromosome 19q13.42. Case 1 is a fifteen-year-old boy, who underwent gamma knife surgery two times three years ago, after presenting with seizures. Magnetic resonance imaging (MRI) identified a large mass in the left frontotemporal lobe. Case 2 is a three-year-old boy who underwent surgery for a right frontal lobe tumor followed by chemotherapy. Eight months later, MRI identified a recurrent tumor in the bilateral frontal lobe. Histologically, cases 1 and 2 exhibited a typical papillary/trabecular and a multilayered rosette pattern resembling medulloepithelioma (ME) and ependymoblastoma (EBL), respectively. Immunohistochemically, CD99, synaptophysin, vimentin, and LIN28A were positive in both cases. Most importantly, both cases displayed amplification in the C19MC locus at 19q13.42 in a fluorescence in situ hybridization (FISH) analysis.

Keywords: Embryonal tumor with multilayered rosettes (ETMR), C19MC locus, LIN28A, chromosome 19q13.42, amplification

Introduction

Embryonal tumor with multilayered rosettes C19MC-altered (ETMR) is a WHO grade IV aggressive embryonal tumor, which is newly defined in the 2016 WHO Classification of Tumors of the Central Nervous System (CNS) [1]. ETMR mainly affects children aged < 4 years old and demonstrates a rapid growth and an aggressive clinical course (the mean survival is 12 months after combination therapies) [1]. Most pediatric CNS embryonal neoplasms were previously diagnosed as embryonal tumor with abundant neuropil and true rosettes (ETANTR), ependymoblastoma (EBL) and medulloepithelioma (ME), and any CNS embryonal tumor with C19MC amplification or fusion were included in this entity [1]. This definition distinguished ETMR from the previously defined CNS primitive neuroectodermal tumors (PNETs), in

which ME and EBL are also included irrespective of the C19MC locus amplification status.

Amplification of the C19MC locus at 19q13.42 was observed in 37/40 (93%) of the tumors morphologically diagnosed as EBL or ETANTR [2]. Nobusawa *et al.* [3] found 19q13.42 amplification in ETANTR, EBL, and ME, but not in AT/RT. Korshunov *et al.* [4] showed that LIN28A, an RNA-binding protein that inhibits the processing of pre-let-7 miRNAs, is a highly specific immunohistochemical diagnostic marker of ETMR [4].

To date, ETMR in the literature remains rare. Here, we present the clinicopathological characteristics of two cases of ETMR (medical history, imaging data, H&E staining, FISH analysis of the C19MC locus, and IHC detection of LIN28A are included) to help provide a better understanding of this new entity.

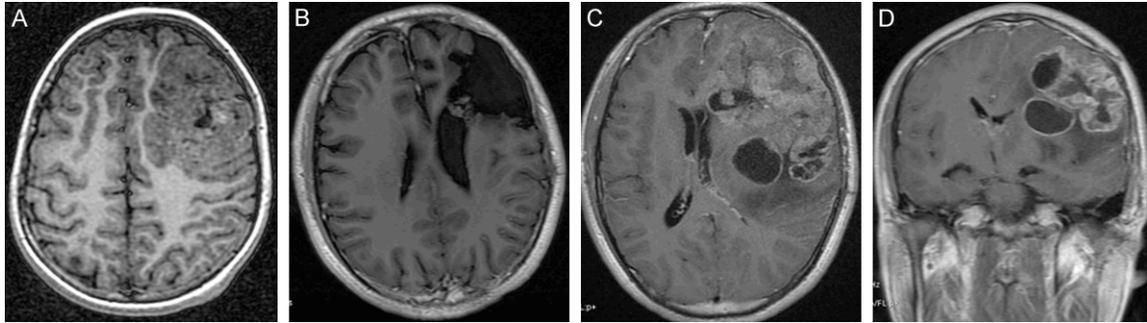


Figure 1. Magnetic resonance (MR) of Case 1: (A) Initial axial T1-weighted image shows an irregular mass in the left frontotemporal lobe; (B) Postoperative Axial Gadolinium (Gd)-enhanced T1-weighted image shows no enhancement, confirming that this mass was gross totally resected; Axial (C) and coronal (D) Gd-enhanced T1-weighted images show a heterogenous enhanced recurrent mass in the primary lesion.

Materials and methods

We reviewed 29 cases of pediatric CNS embryonal tumors diagnosed in three affiliated hospitals of Third Military Medical University (Southwest Hospital, Xinqiao Hospital and Daping Hospital), Chongqing, China, and in the Children's Hospital of Chongqing Medical University, China. We finally identified 2 cases which were initially diagnosed as CNS primitive neuroectodermal tumor (PNETs) with 19q13.42 amplification according to the 2007 WHO classification of CNS tumors. Case 1 was from Xinqiao Hospital, and case 2 was from Children's Hospital of Chongqing Medical University. Written informed consents were obtained from their parents. Formalin-fixed paraffin-embedded tissue sections were first subjected to hematoxylin and eosin (H&E) staining. Then, IHC staining was performed using the standard two-step EnVision method. The antibodies used in this study were vimentin, S-100, GFAP, EMA, CD99, LIN28A, and Ki-67. The antibodies were obtained from Abcam (Abcam, UK), Santa Cruz Biotechnology (Santa Cruz, CA, USA), and ZSGB-Bio (ZhongShan Golden Bridge, Beijing, China).

Dual color fluorescence in situ hybridization (FISH) was performed on formalin-fixed paraffin-embedded tissue sections using ZytoLight SPEC C19MC/19q13 Dual Color Probe (ZytoVision GmbH, Germany), which contains ZyGreen labeled polynucleotides (target sequences mapping in 19q13.42) and ZyOrange labeled polynucleotides (target sequences mapping in 19q13.3). The sections were dewaxed in xylene, dehydrated in ethanol, and pretreated in a 30% sodium bisulfite solution, digested with pepsin, and then dehydrated in

ethanol. Ten microliters of probe were applied on the sections for hybridization at 78°C for 10 min and 37°C for 16 h. Following hybridization, the sections were washed with 2x saline sodium citrate (SSC) and stained with 4'6'-diamino-2-phenylindole (DAPI). The sections were analyzed with a fluorescence microscope with appropriate filters. Cell nuclei with similar sizes, intact borders with no overlap, and with clear dual probe signals were considered effective cell nuclei. Dual color signals in two hundred cell nuclei were randomly counted for each section. The sections were analyzed with a fluorescence microscope with appropriate filters. Cell nuclei with similar sizes, intact borders with no overlap, and with clear dual probe signals were considered effective cell nuclei. Dual color signals in two hundred cell nuclei were randomly counted for each section. The specimens were considered to have 19q13.42 amplification when the Target (Green)/Reference (Red) ratio was > 1.5.

Clinical summary, pathological findings, and FISH analysis

Case 1: In 2012, a 15-year-old boy presented with focal seizures with his limbs twitching, accompanied by urinary incontinence, an upward gaze, and vomiting. Other physical findings and family histories were negative. An unenhanced MRI identified a mass in the left frontotemporal lobe (**Figure 1A**). The patient underwent gamma knife radiosurgery two times, in June and July 2009, but failed to control the seizures. In August 2012, the patient started to complain of headaches and dizziness. Computed tomography (CT) indicated an enlarged mass measuring 90 mm × 60 mm in size, which was removed via total gross resec-

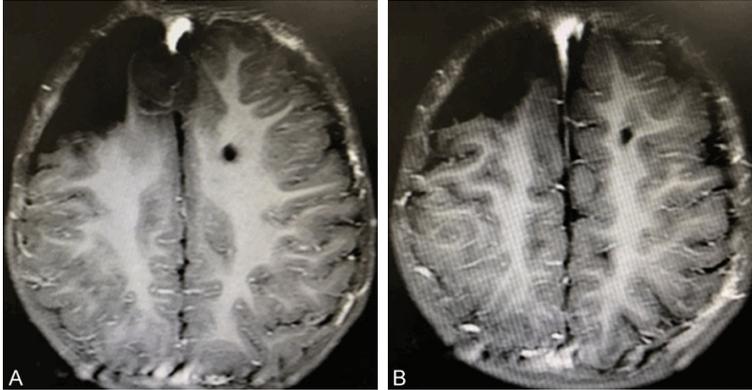


Figure 2. Gadolinium (Gd)-enhanced magnetic resonance (MR) images at preoperative and postoperative presentation for Case 2, who underwent tumor resection followed by combined chemotherapy: (A) Axial Gadolinium (Gd)-enhanced T1-weighted image shows a recurrent solid and mixed cystic lesion in the bilateral frontal lobe, with heterogenous ring-enhancement; (B) Axial Gadolinium (Gd)-enhanced T1-weighted image shows that the recurrent lesion was gross totally resected and no enhancement was observed.

tion (**Figure 1B**). One year later, MRI revealed a large gadolinium-enhanced mass at the primary lesion, indicating a recurrence (**Figure 1C, 1D**). The patient's parents did not allow further treatment, and the patient was lost for follow-up.

Histologically, the tumor consisted of spindle shaped cells arranged in clusters tending to form a typical papillary or trabecular structure (**Figure 3A, 3B**). Occasionally, deeply stained nuclei and mitotic figures can be found. Abundant blood vessels, necrosis and calcification were observed (**Figure 3A, 3B**). Immunohistochemically, the tumor cells were positive for CD99 (**Figure 3C**), EMA (**Figure 3E**), Syn (**Figure 3F**) and LIN28A (**Figure 3I**). Vimentin was strongly expressed (**Figure 3G**), but CK expression was absent (**Figure 3D**). The Ki-67 labeling index was approximately 60% (**Figure 3H**). The FISH analysis showed amplification at the 19q13.42 locus, with an amplification index (Green/Red) of 1.55 (**Figure 5A**).

Case 2: A three-year-old boy was admitted to Children's Hospital of Chongqing Medical University to undergo chemotherapy following a tumor resection 8 months earlier. The physical findings were negative. However, MRI identified a recurrent tumor mass (27 mm × 22 mm × 28.5 mm in size) in the bilateral frontal lobe with mild enhancement (**Figure 2A**). The patient first received combined chemotherapy with the following regimen: methotrexate, intraventricular injection, 2 mg/day for 2 days; carboplatin,

200 mg/m²/day, 146 mg for 3 days; etoposide, 150 mg/m²/day, 109.6 mg for 3 days. After combined chemotherapy, the recurrent masses were gross totally resected (**Figure 2B**). The patient is currently being followed-up.

Histologically, the tumor was presented with sheets and clusters of round/oval-shaped cells incorporating numerous multilayered rosettes lacking a neuropil-like matrix (**Figure 4A, 4B**). In these cells, deeply stained nuclei, dispersed chromatin, and mitoses figures were observed (**Figure 4A, 4B**). Immunohistochemistry showed the expressions

of CD99 (**Figure 4C**), Syn (**Figure 4F**) and LIN28A (**Figure 4I**). The immunoreactivity to vimentin was intense (**Figure 4G**), but the expressions of CK (**Figure 4D**) and EMA (**Figure 4E**) were negative. The Ki-67 labeling index was approximately 70% (**Figure 4H**). The FISH analysis showed amplification of the 19q13.42 locus, with an amplification index (Green/Red) of 2.8 (**Figure 5B**).

Discussion

CNS embryonal tumors include several groups of pediatric brain tumors with typical multilayered rosettes. These are supratentorial primitive neuroectodermal tumors (PNET), medulloblastoma (infratentorial location), medulloepithelioma, neuroblastoma, ependyoblastoma, and atypical teratoid/rhabdoid tumors (AT/RT) [7]. The 2016 WHO Classification proposed a new integrated diagnostic criterion for C19MC-altered ETMR [1]. If ETMR diagnosis is suggested histologically, then 19q13.42 amplification should be assessed using FISH. If ETMR is diagnosed on the basis of histology alone, the tumors may be diagnosed as ETMR, NOS (not otherwise specified) [1].

Most C19MC-altered ETMRs have been reported as case reports (**Table 1**) [8-15]. ETMRs may develop in both the supratentorial and infratentorial compartments. The most common site is the cerebral hemisphere, with a frequent involvement of the frontal and parietotemporal regions [16]. In addition to the supratentorial

ETMR

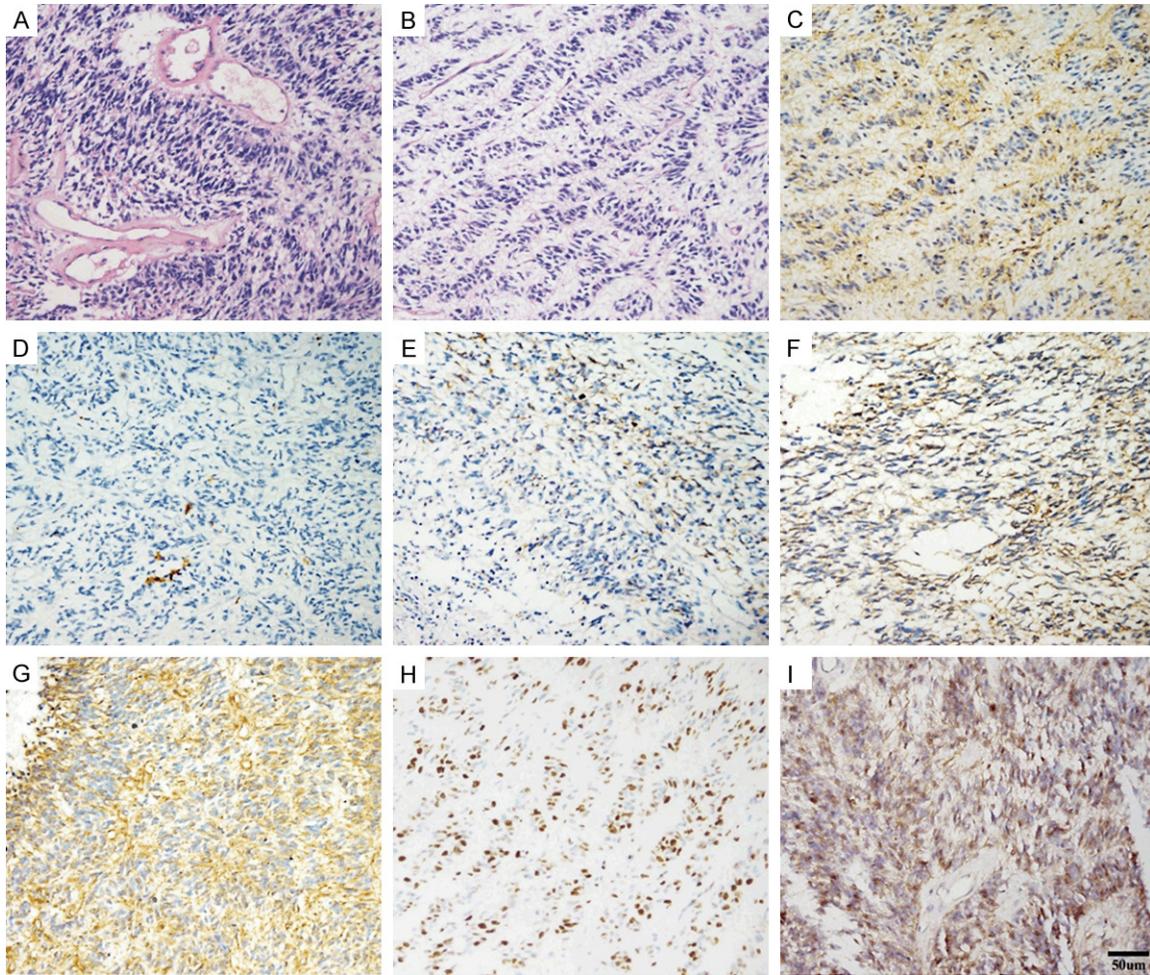


Figure 3. Microscopic appearance of Case 1: (A, B) Spindle shaped cells are arranged in clusters tending to form a typical papillary or trabecular structure (H&E); (C-I) Immunohistochemistry shows the expression of CD99 (C), epithelial membrane antigen (EMA, E), synaptophysin (Syn, F), vimentin (G) and LIN28A (I). The immunoreactivity of cytokeratin was negative (CK, D); the Ki-67 labeling index was approximately 60% (H). Scale bar 50 μm: (A-I).

compartment, they can also originate in the cerebellum, brainstem, and spinal cord [17, 18]. For the radiological features, the head computed tomographic (CT) image shows a hyper attenuating mass in the cerebral hemisphere. MRI shows well-defined margins, minimal vasogenic edema, and subtle enhancement lesions [18]. There are no specific radiological features distinguishing ETMR and other brain tumors [19].

In the present report, the two cases were located in the frontal lobes (Figures 1, 2). The patients had a smooth recovery after tumors were gross totally resected. Like other brain tumors, gross total resection and radiation may provide some benefit for ETMR cases [1]. However, ETMR demonstrates rapid growth

and is associated with an aggressive clinical course after combination therapies. Tumor recurrence, wide-spread leptomeningeal dissemination, and extraneural metastases are frequent in the terminal stages of the disease [1]. So, it is important to elucidate the tumorigenesis well and to seek an effective therapy for this aggressive disease.

In our clinic, we also treated a 12-year-old girl with ETMR in the right frontal lobe. The tumor was total resected, and the histopathological features included multilayered and mitotically active structures consisting of pseudostratified neuroepithelium with a central, round, or slit-like lumen. IHC staining showed expressions of synaptophysin, vimentin and LIN28A. Unfortunately, since it was not possible to assess the

ETMR

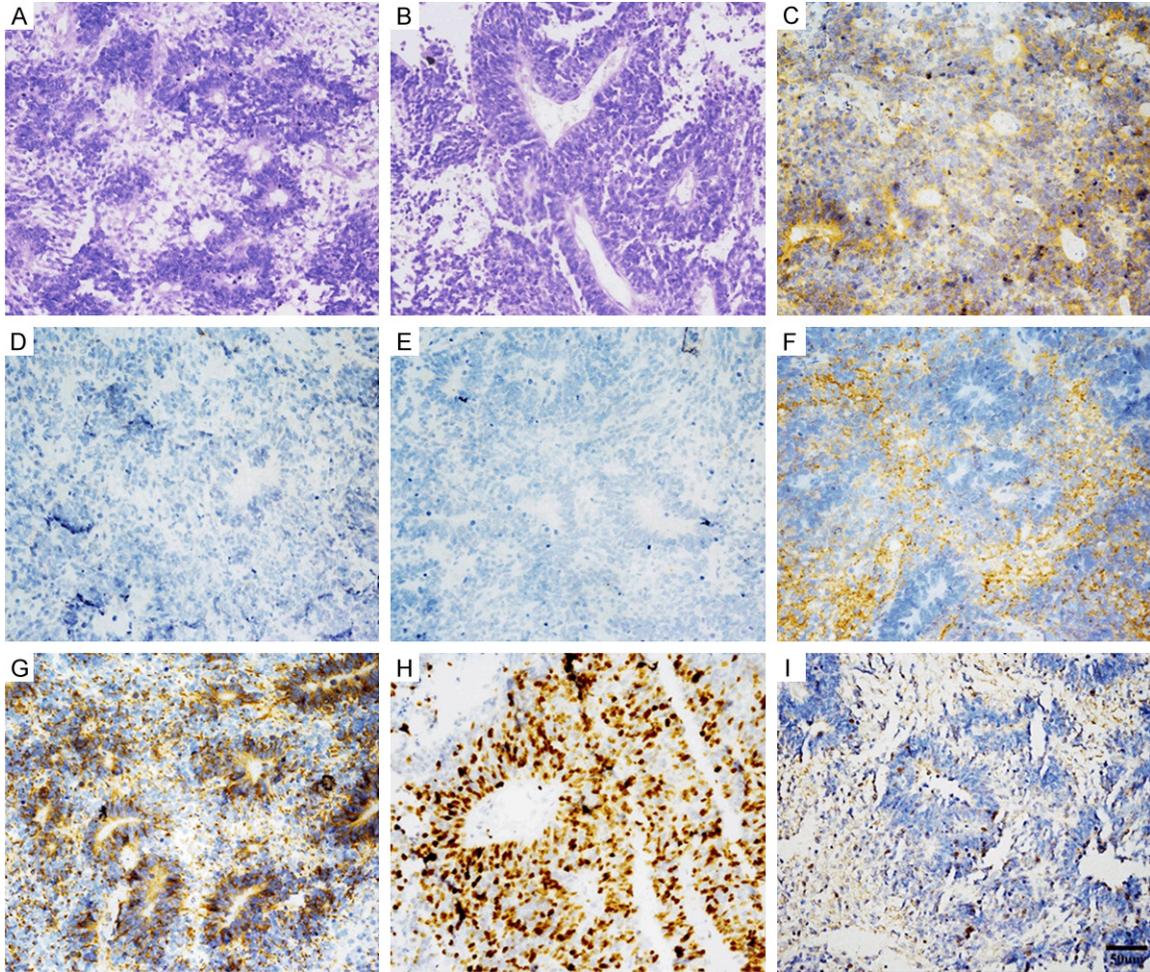


Figure 4. The microscopic appearance of Case 2: (A, B) Sheets and clusters of round/oval-shaped cells incorporate numerous multilayered rosettes lacking a neuropil-like matrix; (C-I) Immunohistochemistry shows the expression of CD99 (C), synaptophysin (F), vimentin (G) and LIN28A (I), but an absence of the expression of cytokeratin (D) and epithelial membrane antigen (EMA, E); the Ki-67 labeling index was approximately 70% (H). Scale bar 50 µm: (A-I).

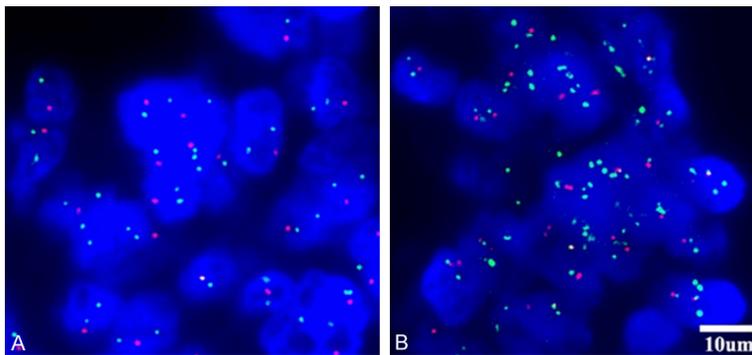


Figure 5. Fluorescence in situ hybridization (FISH) analysis detects the amplification of the 19q13.42 locus of Case 1 and Case 2: the amplification index (Green/Red) for Case 1 is 1.55 (A) and for Case 2 is 2.8 (B). The green lights represent polynucleotides targeting sequences mapping in 19q13.42; the red lights represent polynucleotides targeting sequences mapping in 19q13.3 as an internal reference.

status of 19q13.42, the tumor was diagnosed as ETMR, NOS. Two years later, the tumor recurred, and she underwent a second surgery. One year later, she died of a recurrent tumor with widespread leptomeningeal dissemination (unpublished data).

In summary, C19MC-altered ETMR is a new entity that has a poor outcome in children. The incidence of ETMR remains unclear, because only single cases reports have become available so far.

ETMR

Table 1. Summary of cases of the C19MC-altered intracranial embryonal tumor with multilayered rosettes in recent years

Age	Sex	Initial symptoms	Location	Resection	Chemotherapy	Immunohistochemistry	Prognosis	Ref.
2 ys	M	Personality changes and ataxia for 1 month, morning headache, nausea and vomiting for 2 weeks	Cerebellum vermis	Sub-total	Yes	Positive for CD99, GFAP, INI1, NeuN, NF, Syn, Vimentin; Negative for Actin, CK, EMA; Ki67 index: 47%	> 17 months of PFS	8
33 ms	F	Episodic headaches, increased head circumference and mild gait disturbance for 6 months	Left parieto-occipital lobes	Near-total	Yes	Positive for EMA, INI1, NF and Syn	10 months of PFS	9
29 ms	M	Progressive visual disturbance for 1 months; headache, nausea and vomiting for 1 weeks	Bilateral parieto-occipital lobes	Sub-total	No	Positive for CD99, CgA, EMA, INI1, MAP2, Nestin, NeuN, NF, p53, S-100, Syn and Vimentin; Negative for Actin, CK, Desmin, Germ cell markers and Olig2; Ki67 index: 70% and 58%	NA	10
4 ys	M	Headache, nausea, vomiting, gait and balance disturbances for 2 months, more recently strabismus and left hemiplegia	Right mid-pons, mesencephalon	Sub-total	No		NA	
2 ys	M	Ataxic gait, dysarthria, and dysphagia	Basilar part of the pons	Sub-total	Yes	Positive for GFAP, INI1, LIN28A, Syn	Died 7 months after surgery	11
33 ms	M	Vomiting and gait disturbance for 3 months	Intramedullary mass, right dorsal part of the pons	Sub-total	Yes	Positive for EMA, INI1, LIN28A, NF, NeuN, Syn; Negative for GFAP; Ki67 index: 70%	Died 6 months after surgery	12
8 ms	F	Complaints of vomiting and drooping of left eyelid for 3 weeks	Left cerebellar hemisphere	Sub-total	No	Positive for CD99, CgA, EMA, INI1, NF, p53, Syn and Vimentin; Negative for CK, Desmin, GFAP and NSE; Ki67 index: 70%-80%	Expired 1 week after diagnosis because of widespread CNS drop metastases	13
2 ys	F	Two episodes of seizure, multiple episodes of vomiting, and weakness of the left side of the body for 7 days	Right parieto-occipital lobes	Total	Yes	Positive for Syn, vimentin; Negative for CK	> 6 months of no evidence for recurrence	14

However, epidemiological data may be obtained in the future, as a new ICD-O code (9478/3) has been assigned to this new entity (2016 WHO Classification). The most common clinical manifestations are symptoms and signs of increased intracranial pressure and focal neurological signs. The radiological features are similar to other brain tumors. The integrated diagnosis should be based on histology (CNS embryonal tumor with multilayered rosettes), immunoreactivity (synaptophysin and vimentin, and the specific biomarker LIN28A), and genetics (amplification of C19MC locus at 19q13.42 by FISH) to reliably diagnose this novel aggressive pediatric brain tumor.

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Disclosure of conflict of interest

None.

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