Appendix 1

Case 1

A 4-year-old boy presented with photophobia, abdominal pain, vomiting, and anorexia with severe weight loss in the last 2 months. Craniospinal magnetic resonance imaging (MRI) revealed communicating hydrocephalus associated with multiple small cysts scattered on the surface of the cerebellum, brainstem, mesial temporal lobes, and spinal cord. Diffuse gadolinium enhancement involving the leptomeninges surrounding the brain and spinal cord as well as the roots of the cauda equina was noted without evidence of an intraparenchymal mass. Head computed tomography (CT) showed absence of evident intracranial calcifications. The patient underwent ventriculoperitoneal shunt surgery. Infectious cerebrospinal fluid markers were negative. A biopsy of the pathological tissue at the level of the caudal roots was performed and a diagnosis of diffuse leptomeningeal glioneuronal tumor (DLGNT) was reached through anatomopathological and immunohistochemical analysis. The child was treated with systemic chemotherapy, at first with 3 consecutive cycles of high-dose Methotrexate, and subsequently according to a modified International Society of Pediatric Oncology-Low Grade Glioma (SIOP-LGG) protocol (Vincristine and Cyclophosphamide alternating with Vincristine and Cisplatin) for 1 year. The response was good with improvement of cachexia and weight recovery. The patient then presented phases of stability alternated with phases of mild progression. About 2 years after diagnosis, craniospinal MRI showed an increase in size and number of the cysts and revealed linear hypointensities on T2* gradient echo (GE) images along some anterior mesial frontal and inferior cerebellar gyri and in the basal cisterns, that corresponded to subtle hyperdensities on CT scan. These signal and density alterations progressively increased in the following years, with clear formation of calcifications at the level of cerebellar vermis, basal cisterns, sylvian fissures and bilateral frontal sulci (*Figure 4A-4D*). During progression phases, the patient was treated with oral Temozolomide and Etoposide, followed by Vinblastine, Vinorelbine and finally TPCV regimen (Thioguanine, Procarbazine, Lomustine, and Vincristine). Two and a half years after stop therapy, at the age of 10, the patient presented with occipital pain, stiffness and lack of appetite. Craniospinal MRI showed increase of the leptomeningeal contrast enhancement. Due to both clinical and radiological disease progression, wanting to avoid craniospinal radiotherapy and considering the reported efficacy in an adult with primary diffuse leptomeningeal glioneuronal tumor (34), we decided to treat the patient with Bevacizumab (10 mg/Kg/dose biweekly), that was administered for 15 months, with regression of neurological symptoms. Craniospinal MRI studies revealed initial reduction of leptomeningeal contrast enhancement, followed by stabilization of the neuroimaging features, while head CT demonstrated increased leptomeningeal calcifications (*Figure 2*). At present, the child is 12.4 years old and remains asymptomatic.

Case 2

A 2-year-old girl was admitted to our institution because of progressive paraparesis, fecal/urinary incontinence, photophobia, vomiting and bilateral clonus. On admission, head CT revealed communicating hydrocephalus without visible intracerebral masses nor calcifications (*Figure 4E*). Craniospinal MRI showed cystic lesions diffusely scattered throughout the cerebellum, basal cisterns and on the surface of the brainstem. Contrast-enhanced T1-weighted images revealed diffuse marked thickening and enhancement of the leptomeninges, particularly in the basal cisterns, sylvian fissures, interhemispheric fissure, and spinal cord. There were also multiple non-enhancing cystic lesions along the spinal cord associated with focal dilatations of the ependymal canal. This patient was initially diagnosed with tuberculous meningitis despite absence of laboratory evidence. A ventriculoperitoneal shunting was placed and empirical treatment against tuberculosis was started, which proved ineffective. Therefore, a biopsy of the pathological tissue at the level of the cauda roots was performed and a diagnosis of DLGNT was made. The patient was treated with systemic chemotherapy according to the International SIOP-LGG with vincristine and carboplatin for 18 months, with clinical improvement and radiological stability. Craniospinal MRI performed every 3 months revealed progression of the cystic lesions and leptomeningeal enhancement. Moreover, head CT documented subtle calcified depositions in the basal cisterns (*Figure 4F*). Therefore, 18 months after the end of first line therapy, we treated the patients with oral Temozolomide and Etoposide, without radiological improvement, and we proposed to proceed with craniospinal radiotherapy, but parents strongly refused it. Therefore, we continued chemotherapy with Vinblastine for 6 months, but after an initial radiological stability, the disease continued to progress. Subsequently, the parents asked to be referred for a second opinion to another hospital, where craniospinal tomotherapy (IMRT-Tomo) was administered with clinical and radiological improvement. The initial dose was 32.4 Gy in 18 fractions

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to the craniospinal axis, followed by a first boost of 9 Gy in 5 fractions to a reduced volume of brain and spine, and a second boost of 12.6 Gy in 7 fractions on a further reduced volume involving brain, brainstem, and spinal cord. However, after about 1.5 years, she returned to our institution for the appearance of acute repetitive seizures and left hemiplegia. Head CT demonstrated a hypodensity in the right fronto-temporo-parietal regions, increased calcifications in the basal cisterns, and new widespread calcifications at the level of medial temporal lobes, sylvian fissure, interhemispheric fissure, and frontal-parietal gyri (*Figure 4G,4H*). Craniospinal MRI revealed a subacute arterial ischemic infarct in the right middle cerebral artery territory associated with diffuse supratentorial white matter T2-hyperintensity, likely related to radiation-induced leukoencephalopathy. Conversely, the cystic lesions and leptomeningeal enhancement were reduced. In the following weeks, thanks to antiepileptic and rehabilitative treatment the neurological conditions improved and the patient was discharged home. Unfortunately, in the following months, the patient worsened again and underwent a devastating clinical course characterized by an arterial ischemic infarct in the left middle cerebral artery territory (*Figure 5*), progressive neurological deterioration, consciousness alteration, repetitive seizures, central panhypopituitarism, and need for enteral nutrition. Craniospinal MRI showed no signs of disease progression. Finally, the child died of bronchopneumonia at the age of 9.4 years, 7 years after clinical onset. The parents did not authorize autopsy.

Case 3

A previously healthy 13-year-old boy presented to another hospital with sudden onset of awakening at night, confusional state, dysarthria, and dyslalia. Dysgraphia and difficulty in concentration in the previous weeks were also reported by the parents. Contrast-enhanced craniospinal MRI depicted some cystic lesions over the cerebellar surface and diffuse leptomeningeal enhancement particularly in the cerebral and cerebellar subarachnoid spaces, pontine and ambient cisterns, and spinal cord, with no evidence of intraparenchymal involvement. Moreover, linear hypointensities on T2-weighted images were observed along the posteromedial surface of the thalami with corresponding calcifications on CT scan (*Figure 4I*). A small calcification was retrospectively noted also within the left sylvian fissure. The search of most common infectious markers in the cerebrospinal fluid was negative. A diagnosis of DLGNT was made through a biopsy of the pathological tissue at the spinal level, and a wait-and-see approach was maintained for the first 22 months. At age 14, as described in (25), the boy presented to our Institution due to slight clinical worsening with seizures, character changes (hyperactivity, mood imbalance) and one episode of partial epilepsy; neurological examination remained normal without focal deficits. Craniospinal MRI showed worsening of the neuroimaging features. Therefore, a chemotherapy treatment with vincristine and carboplatin was started according to the international SIOP-LGG protocol. After a few months, due to the onset of neurotoxicity 1 mainly consisting of a peripheral neuropathy, this chemotherapy regimen was interrupted and a second line chemotherapy was started with oral temozolomide (5 cycles) without apparent radiological benefit. Parents thus asked to interrupt chemotherapy. Following the appearance of seizures secondary to the disease, we decided to prescribe valproic acid (15 mg/kg twice a day) also for its autophagic and apoptotic mechanism in central nervous system malignancies, with both clinical and radiological improvements after 3 and 6 months. In the following years, during a long and essentially stable clinical course, multiple MRI examinations showed reduction of the leptomeningeal enhancement and progressive appearance of diffuse supra- and infratentorial cortical and leptomeningeal hypointensities on T2* GE images. Conversely, CT scans documented an increase of calcifications only at the level of the leptomeninges over the posteromedial surfaces of the thalami, right frontal and bilateral temporal sulci (*Figure 4J-4L*). At 23 years of age, due to a slow and progressive cognitive decline characterized by spatial and temporal disorientation, attention and memory disorders, together with a slight neuro radiological worsening, a therapy with Vinblastin (6 mg/m^2 weekly) was started, but then stopped after only 2 months for further clinical deterioration in terms of walking disorders and cognitive impairment. We thus decided to treat the patient with Bevacizumab (10 mg/Kg/dose biweekly). In the first 6 months of treatment, the patient showed a slight neurological improvement with diffuse reduction of leptomeningeal contrast enhancement on MRI followed by radiological stabilization (*Figure 3*). However, after 20 months, at the age of 25.8 years, the neurological conditions heavily worsened, and, in agreement with parents, we decided to stop the treatment with Bevacizumab. The patient is still alive, at home.

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