

# Intramedullary pilomyxoid astrocytoma with intracerebral metastasis exhibiting oligodendroglioma-like features

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## Abstract

Intramedullary glioma are rare and their biological behaviour can differ from their cerebral counterparts. Pilomyxoid astrocytoma (PMA, WHO grade II), predominantly occur in the hypothalamic/chiasmatic region of infants and children. The few reported cases of pediatric intramedullary PMA displayed a particularly aggressive behavior. Here, we report a diagnostically challenging case of a five year old female patient presenting with intramedullary glioma and local tumor recurrence three years later. Twelve years after the initial manifestation, a second tumor was found intracerebrally. We performed a comprehensive histological, molecular pathological and imaging analysis of the tumors from both localizations. The results revealed a metastasizing PMA with unique histological and genetic features. Our study indicates that PMA comprise a heterogeneous group including aggressive subtypes which may not be compatible with the current classification according to WHO grade II. Furthermore, the case emphasizes the increasing relevance of molecular pathological markers complementing classic histological diagnosis.

## Introduction

Glioma located in the spinal cord (intramedullary glioma, IMG) are rare, accounting for <5% of all central nervous system (CNS) glioma. Histopathological classification and grading of IMG is based on the *WHO classification of Tumors of the Central Nervous System*.<sup>1</sup> Despite a common classification system, the biology of IMG appears to differ from their cerebral counterparts, e.g. IMG do not show the

same correlation between higher grade and age as observed for cerebral glioma. Furthermore, intramedullary high-grade glioma show a tendency to leptomeningeal/ cerebrospinal fluid spread,<sup>2</sup> a behaviour which is uncommon in cerebral high-grade glioma. In 2007, pilomyxoid astrocytoma (PMA) was introduced as new entity in the WHO classification, corresponding to WHO grade II. PMA typically occur in the hypothalamic/chiasmatic region of infants and children. They exhibit morphological features related to pilocytic astrocytoma (WHO grade I) but behave more aggressively with higher rates of local recurrence and cerebrospinal spread.<sup>3,4</sup> Occasionally, PMA also occur outside the hypothalamic/chiasmatic region. Seven cases (2 adults, 5 children) have been reported with a PMA in the spinal cord so far. Interestingly, 2 of the 5 pediatric spinal cord PMA displayed a particularly aggressive behavior with leptomeningeal and peritoneal metastases or rapid progression into glioblastoma.<sup>4,5</sup> Here, we report a case of metastasizing PMA of the spinal cord with unique histological and genetic features in a young female patient.

## Case Report

### Primary manifestation of an unusual intramedullary tumor

In 1999 at the age of 5 years the patient presented with stiffness of the neck and recurrent nuchalgia. The personal history included coxsackie meningitis in 1998 without residual complaints and chickenpox a few months ago. The patient had no family history of neurological or psychiatric illnesses. The neurological examination revealed increased tendon reflexes at the lower limbs without sensorimotor deficits and mild spinal ataxia. A spinal magnetic resonance imaging (MRI) demonstrated an inhomogeneous, intramedullary, partly cystic mass lesion expanding from C1 to Th1 with some focal contrast enhancement. Another circumscribed, contrast-enhancing, partially cystic intramedullary lesion was found at Th9. Additionally, some leptomeningeal contrast enhancement was found extending from Th9 to the sacral region. The serology was negative for coxsackie and borrelia infections. The patient underwent surgical resection of the cervical tumor. Intra-operatively, the tumor exhibited a poorly demarcated growing behavior with broad infiltration of the spinal cord. Intra-operative monitoring allowed for partial tumor resection without postoperative sequelae. No additional treatment was performed after primary surgery. Neuropathological analysis of the tumor specimen demonstrated an unusual glial tumor, which could not be classified unequivocally

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cally according to the 1999 edition of the *WHO classification of Tumors of the Central Nervous System*. In detail, hematoxylin and eosin (HE) staining showed a glial tumor with moderate pleomorphism and heterogeneous growth patterns. In some areas long cytoplasmic processes formed a dense glial fibrillary matrix, while other areas displayed a looser texture. Within the latter, regions with an angiocentric growth pattern and perivascular pseudorosettes reminiscent of ependymoma could be observed next to areas of lower cell density consisting of predominantly bipolar cells in a mucoid background. Focally, small concentric microcalcifications were detected. Rosenthal fibers or eosinophilic granular bodies were absent. Mitoses were found occasionally and vascular endothelial proliferation was moderate, occasionally with fibrotic vessel walls. Necrosis was absent. Proliferation activity determined by Ki67 staining was 10%.

Immunohistochemical analysis revealed a cytoplasmic positivity for glial fibrillary acidic protein (GFAP), with a perivascularly accentuated staining. Tumor cells also displayed a strong cytoplasmic expression of MAPII. Furthermore, >90% of nuclei showed a positive

staining for Olig2. Immunohistochemical reactions with an antibody directed against the neuronal marker synaptophysin were negative. Histological diagnosis was descriptive, namely an unusual astrocytic tumor corresponding to WHO grade III (Figure 1A-H).

### Recurrence of the intramedullary tumor after 3 years

In 2002, a control MRI of the spinal axis revealed progression of the residual intramedullary tumor showing a circumferential enlargement of the spinal cord with solid as well as cystic components from C1 to C7. Focal contrast enhancement could be seen around the cystic compartments while plain T1 weighted images showed a hypo- to isointens pattern. No additional changes were found at Th9 and the lumbosacral region. Neurological examination of the ambulatory patient only showed minor worsening of spinal ataxia and again no sensorimotoric or vegetative deficit. Surgical resection confirmed tumor recurrence with histopathological features that were in part different to those seen at the time of initial manifestation (Figure 1I-P): in the recurrent tumor sample, a biphasic growth pattern was observed consisting of compact areas with numerous bipolar cells associated with Rosenthal fibers as well as loose-textured areas with multipolar cells and microcysts. The histological features seen in the primary tumor sample, such as mucoid matrix and angiocentric/pseudorosette like formations, were less prominent in the recurrent tumor.

Vessels often showed moderate proliferation and hyalinized walls. Although mitotic figures were not prominent, proliferation activity determined by Ki67 staining varied from 1% to focally 20%. GFAP was strongly expressed in the compact areas, while it was focally absent in the loose-textured areas. MAP2 showed a moderate expression in the compact areas and a strong staining in most of the loose-textured areas. Expression of Olig2 was observed in <50% of nuclei in the compact areas, while it was present in >90% of nuclei in the loose-textured areas. Similar to the primary tumor, immunohistochemical reactions for Synaptophysin were negative. In addition, the histological slides from the initial manifestation 1999 were re-evaluated, and the diagnosis of an *anaplastic variant of a pilocytic astrocytoma (PA) WHO grade III* was favored (Figure 1I-P).

Again, partial tumor removal was well tolerated and the patient did not receive adjuvant therapy.

### Additional cerebral tumor manifestation after 12 years

In 2011, the patient suffered from a tonic-clonic seizure. Neurological examination did not reveal any new deficit. Imaging studies

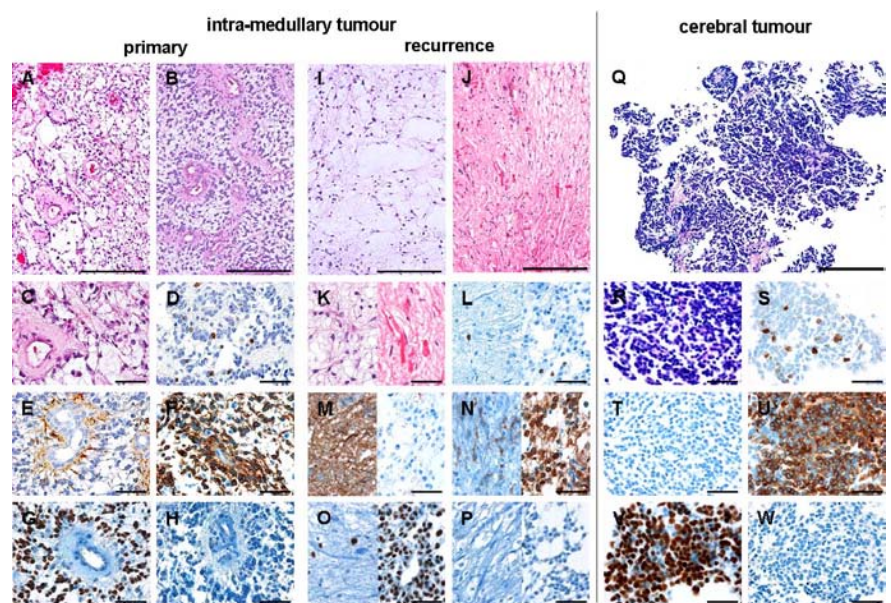
revealed manifestation of an additional tumor located intracranially alongside the parahippocampal gyrus. MRI demonstrated a multilobular cystic architecture including solid contrast enhancing nodules and lamellar enhancements. The imaging features of this new tumor manifestation resembled the initial spinal tumor.

Furthermore, assessment of the known intra-medullary tumors showed a transformation to a more solid cervical tumor with less cystic compartments but clearly progressive contrast enhancement. No additional changes were found on spinal MRI. In a short term 2 months follow-up, the intracranial tumor showed a quick progression of the cystic compartments.

Positronemissiontomography (PET) of the brain and the spine with [<sup>18</sup>F] fluoroethyltyrosine (FET) revealed both an intense pathological FET-uptake in the cervical intramedullary tumor as well as in the newly detected cerebral tumor manifestation (Figure 2). An additional kinetic analysis was only feasible for the supratentorial lesion and showed decreasing time-activity-curves suggesting higher malignancy. Histopathological evaluation of the cerebral lesion was performed by stereotactic biopsy. Histological examination of the intracerebral lesion showed a tumor of high cellular-

ity consisting of monomorphous tumor cells with chromatin dense nuclei and scant cytoplasm without prominent cytoplasmic processes. Mitotic activity was high. Vascular proliferation or necrosis was not present in the small stereotactic specimen.

Immunohistochemical reactions using an antibody against GFAP were negative. Further analysis clearly demonstrated a neuroectodermal differentiation of the tumor cells by showing a strong cytoplasmic expression of MAP2. Furthermore, immunohistochemical analysis of Olig2 demonstrated staining in >90% of the nuclei. Synaptophysin was not expressed. Ki67 staining indicated a proliferation activity of 10% (Figure 1Q-W). Based on the 2007 edition of the *WHO classification of tumors of the nervous system*<sup>1</sup> the histological picture seemed to be compatible with an anaplastic oligodendroglioma WHO grade III (O III) (Figure 1Q-W). However, in the context of the known intramedullary lesion which was re-evaluated in parallel and now found to be most likely compatible with the new entity of pilomyxoid glioma (PMA) WHO grade II, a genetic analysis of both tumors was conducted for further characterization of this unusual case. The case was transferred to our interdisciplinary neuro-oncology board. Treatment decision was in favour of external beam radia-



**Figure 1.** Histological and immunohistochemical findings. Histological and immunohistochemical findings of the intramedullary primary tumor (A-H), the local recurrence (I-P) and the cerebral tumor (Q-W). HE stained sections showing the morphology of the tumors in low (A-B, I-J, Q) and high (C, K, R) magnification. Proliferative activity determined by Ki67 (D, S, L). Illustration of further immunohistochemical marker expression as follows: GFAP (E, M, T), MAP2 (F, N, U), Olig2 (G, O, V) and synaptophysin (H, P, W). For the recurrent tumor, harbouring a biphasic pattern with loose (I) and compact (J) architecture, each picture (K-P) shows a representative loose area (left) and compact area (right). Scale bar 200 μm (A, B, I, J, Q), 50 μm (C-H, K-P, R-W).

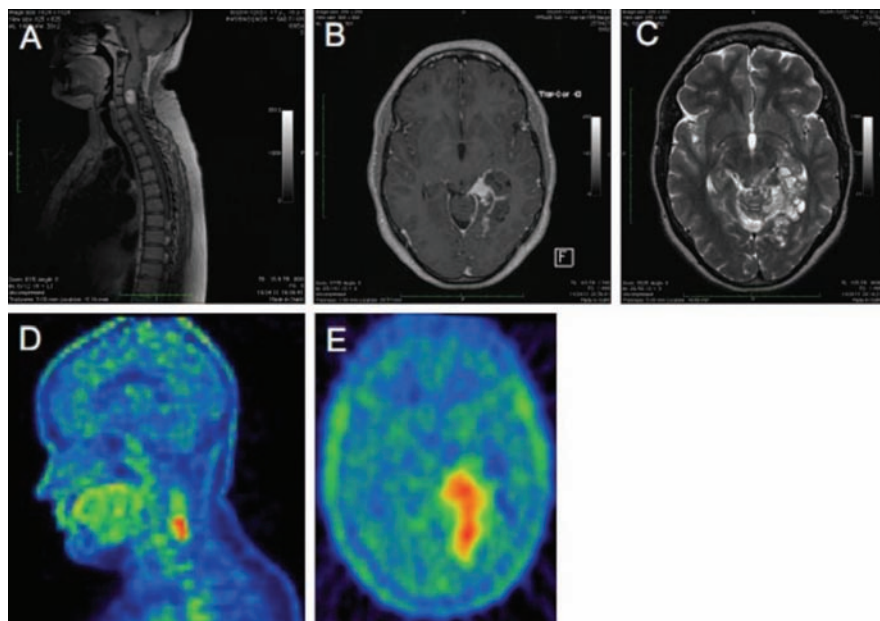
tion for the cerebral metastasis and careful observation of the currently stable spinal tumour status. At last follow-up evaluation in October 2011 the ambulatory patient presented without any new neurological deficit. Control MRI investigation of entire neuroaxis revealed regressive alterations of the cerebral tumor and a stable status of the intramedullary tumors.

### Molecular pathological characterization of the histological distinct tumors

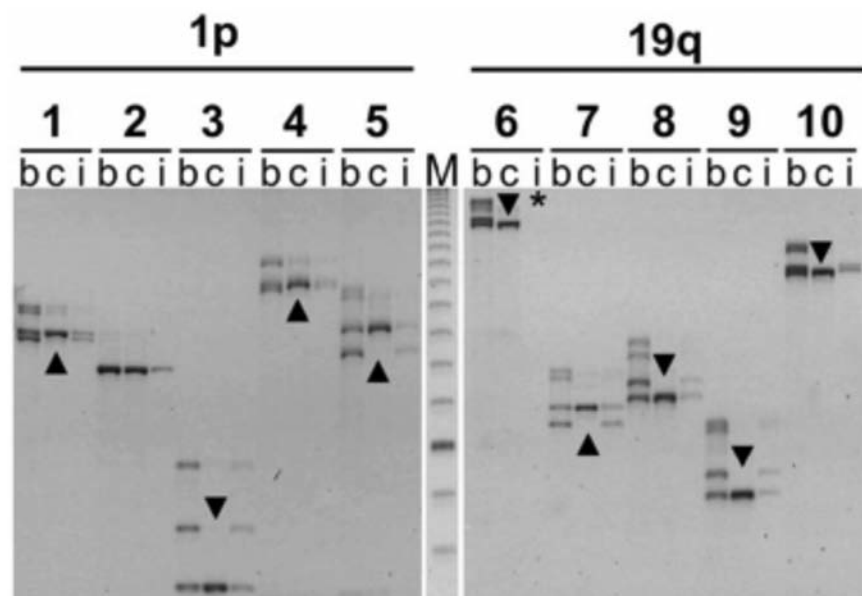
Frozen tissue of the cerebral tumor and formalin-fixed, paraffin-embedded (FFPE) tissue of the intramedullary tumor were subjected to molecular pathological analysis. Determination of 1p/19q deletion, MGMT promotor methylation and IDH1/2 mutation status was performed as described.<sup>6-8</sup> In the cerebral tumor, PCR-based microsatellite analysis clearly showed a loss of chromosomal material on the short arm of chromosome 1p and the long arm of chromosome 19q, which is typically observed in oligodendroglial tumors. In contrast, the intramedullary tumor did not harbour the 1p19q co-deletion (Figure 3). Combined methylation specific (MSP)-PCR and capillary sequencing revealed that the MGMT promotor sequence was not methylated. Moreover, no mutations of IDH1 or IDH2 were detected by pyrosequencing - both in the intramedullary and the intra-cerebral tumor. As recent studies detected the BRAF<sup>V600E</sup> mutation in a subset of predominantly extra-cerebellarly localized pilocytic astrocytoma WHO grade I,<sup>9</sup> as well as in pediatric glioma of WHO grade II-IV,<sup>10</sup> we also tested both tumors regarding this mutation. Pyrosequencing analysis was performed on a PyroMark Q24 system with a commercial certified kit (both: Qiagen, Hilden, Germany) according to the manufacturer's instructions and excluded a BRAF<sup>V600E</sup> mutation in both samples (*data not shown*).

### Discussion

Here, we describe the case of a female patient who initially presented with an unusual intramedullary glial tumor at the age of five years in 1999 and local tumor recurrence in 2002. In 2011, follow-up examination revealed a second tumor located cerebrally. A close review of the literature regarding PMA reveals that there is a relatively wide range of the histological picture including intermediate forms between PMA and PA. Importantly, PMA have been reported to occasionally undergo maturation resulting in the histological characteristics of PA.<sup>11</sup> This feature of PMA is not included in the current WHO classification, but is likely to be the explanation for the histological distinct



**Figure 2.** Magnetic resonance imaging and fluoroethyltyrosine-positronemissiontomography Magnetic resonance imaging (MRI) and fluoroethyltyrosine-positronemissiontomography (FET-PET) taken at the time of diagnosis of the cerebral tumor in 2011: (A) T1-weighted sagittal MRI with gadolinium administration showing the intramedullary lesion. (B) T1-weighted axial MRI with gadolinium administration showing the cerebral tumor. (C) T2-weighted MRI showing the intracerebral tumor in the left parahippocampal gyrus. (D) 15 corresponding FET-PET scan of the cerebral tumor revealing intensive FET-uptake. (E) FET-PET scan of the earlier intramedullary tumor also presenting with high FET-uptake.



**Figure 3.** Microsatellite analysis of the cerebral tumor and the intramedullary tumor Markers for chromosome 1p (left gel): (1) D1S608, (2) D1S1592, (3) D1S548, (4) D1S1161, (5) D1S1184. Markers for chromosome 19q (right gel): (6) D19S718, (7) D19S433 (8)D19S601, (9) D19S559, (10) D19S431. M: 20 bp ladder with prominent 200 bp band. For each marker, PCRs derived from blood (b), cerebral tumor (c) and intramedullary primary tumor (i) were loaded on the left, middle and right lane, respectively. Nine of ten markers were informative and clearly show the 1p19q co-deletion (arrows) in the cerebral tumor, but not in the intramedullary primary tumor. (\*): not detectable.

appearance of the local recurrence compared to the primary intramedullary tumor (Figure 1). Of note, there was a relatively high proliferation activity (Ki67 up to 20%).

In principle, two possible explanations for the simultaneous presence of the glioma in different localizations can be considered: either both tumors are distinct entities or the cerebral tumor is a metastasis of the intramedullary tumor. In the current case, the available evidence favors the *metastasis*-hypothesis. The occurrence of distinct primary brain tumors within one and the same patient in the absence of prior radiation is very rare. The reports in the literature mostly refer to cases of multicentric glioblastoma or glioblastoma paralleled by the occurrence of meningioma, which can occur simultaneously by chance as these are the most frequent primary brain tumors in adults.<sup>12,13</sup> In pediatric patients, multicentric glioma are even rarer and limited to only few case reports.<sup>14-16</sup> A genetic predisposition such as neurofibromatosis or Li-Fraumeni syndrome in which brain tumors occur frequently is unlikely in this case, as an unremarkable family history and the absence of any clinical symptoms suspicious of these diseases argue against this possibility.

Interestingly, the cerebral tumor showed histological aspects of an O III. Moreover, it harbors a 1p19q co-deletion. Thus, at first glance the genetic evidence favors the existence of distinct tumors. However, the overall constellation of molecular pathological markers (MGMT promoter not methylated and no IDH1/2 mutation) provides strong evidence for the *metastasis-hypothesis*. It has been demonstrated that virtually all 1p19q co-deleted oligodendroglial tumors also harbor IDH1/2 mutations as well as a methylated MGMT promoter sequence<sup>17</sup> - a finding which is in line with our own experience: 47/47 (100%) consecutively examined (October 2009 to August 2011) 1p19q co-deleted tumors displayed this genetic signature. Interestingly, the cerebral tumor reported here neither shows IDH1/2 mutations nor a methylation of the MGMT promoter sequence. This indicates that the cerebral tumor is a metastasis of the intramedullary PMA which gained the 1p19q co-deletion resulting in an oligodendroglia-like phenotype. Of note, both the results of the additional genetic marker analysis (no BRAF<sup>V600E</sup> mutation) as well as the immunohistochemical profile (MAPII and Olig2 positive, synaptophysin negative) are compatible with this interpretation. The loss of GFAP expression is not contradictory in this context, as GFAP expression gradually is reduced over time (primary intramedullary tumor > recurrent intramedullary tumor > cerebral tumor) and is thus compatible with a de-differentiation process.

Importantly, MRI features of the cerebral tumor resembled the initial spinal tumor, sug-

gesting metastatic spread. Also, PET-scan analysis revealed a high tracer uptake in both lesions. To evaluate the probability of metastatic spread in PMA, we conducted a Medline/PubMed search and found 7 reports (2 adults, 5 children) of PMA located in the spinal cord (reviewed in Paraskevopoulos D *et al.*).<sup>5</sup> Two of the 5 pediatric patients presented with a particular aggressive tumor biology comparable to that seen in our case, showing leptomeningeal and peritoneal metastasis via a ventriculoperitoneal shunt in the one case and rapid progression into glioblastoma in the other case.<sup>4,5</sup>

## Conclusions

In conclusion, our unusual case indicates that PMA constitute a heterogeneous group which may include aggressive subtypes not compatible with the current classification according to WHO grade II. Furthermore, the case emphasizes the increasing relevance of molecular pathological markers complementing classic histological diagnosis.

## Note added in proof

After submission of this manuscript, the patient underwent surgery again due to enlargement/progression of the intramedullary tumor. Histopathological examination now revealed areas very similar to that seen in the previous samples obtained from this localization. In addition, a smaller area with increased cell density and oligodendroglial-like appearance similar to the picture of the cerebral lesion was observed. This area was sampled for molecular pathological analysis and showed a 1p19q co-deletion. These new findings indicate that the cerebral lesion was indeed a metastasis of the intramedullary tumor.

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