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Review

# Paediatric-type diffuse low-grade gliomas: a clinically and biologically distinct group of tumours with a favourable outcome

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**Summary**

The WHO 2021 classification of central nervous system cancers distinguishes diffuse gliomas that arise in adults (referred to as the "adult type") and those that arise in children (defined as "paediatric") based on clinical and molecular characteristics."). However, paediatric-type gliomas may occasionally be present in younger adults and occasionally adult-type gliomas may occur in children. Diffuse low-grade paediatric glioma includes diffuse astrocytoma altered by MYB or MYBL1, low-grade polymorphic juvenile neuroepithelial tumour, angiocentric glioma, and diffuse low-grade glioma with an altered MAPK pathway. Here, we examine these newly recognised entities according to WHO diagnostic criteria and propose an integrated diagnostic approach that can be used to separate these clinically and biologically distinct tumor groups.

**Key words:** paediatric-type diffuse low grade glioma, diffuse astrocytoma altered by MYB or MYBL1, low-grade polymorphic juvenile neuroepithelial tumour, angiocentric glioma, diffuse low-grade glioma with an altered MAPK pathway

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## Introduction

Low-grade gliomas (together with glioneuronal tumours) represent over 30% of paediatric CNS neoplasms, rendering them the most frequently encountered brain tumours in children, even if they remain relatively rare [1].

In the recently revised edition of the classification of the central nervous system (CNS) tumours published by the World Health Organization (WHO) [2] four distinct histomolecular entities, namely diffuse astrocytoma MYB or MYBL1 altered, angiocentric glioma, polymorphous low-grade neuroepithelial tumour of the young (PLNTY) and diffuse low-grade glioma MAPK pathway-altered are come together in the group of paediatric-type diffuse low-grade gliomas.

Here we review these newly recognised entities and consider some strategies for an integrated diagnosis, highlighting the importance of immunohistochemical and molecular testing and interpretation.

## Diffuse astrocytoma, MYB- or MYBL1-altered

The diffuse astrocytoma, IDH- H3-wildtype and MYB- or MYBL1-altered, is a diffusely infiltrative glial neoplasm, composed of monomorphic cells

with genetic alterations in MYB or MYBL1, grade 1, according to CNS WHO 2021 [1].

This entity is a rare tumour, accounting for about 2% of paediatric low-grade gliomas (pLGGS) [3]. When associated with epilepsy surgery, it is present in about 0.3% of cases [4].

Clinically, they are supratentorial located (42.5% in temporal, 27.5% in frontal, 20% occipital and 10% in parietal lobes) and are associated with seizures (patients showed seizure onset at a mean age of 14.6 years) [3,5,6].

At imaging, diffuse astrocytoma, MYB- or MYBL1-altered, is typically hypointense on T1, shows mixed signal or hyperintensity on T2-FLAIR and is non-enhancing mass. It does not show restricted diffusion [5,7,8]. Tumours are well defined, but they focally show diffuse growth patterns [5,7].

The entity of "diffuse astrocytoma, MYB or MYBL1-altered" includes a subset of pLGGS not leading the own histologic features of angiocentric glioma but having recurrent amplifications and structural variants of MYB and MYBL1 [3,9,10] including fusions with various gene partners such as PCDHGA1, MMP16, MAML2 [5,10].

Even if "diffuse astrocytoma, MYB or MYBL1-altered" falls under paediatric diffuse gliomas, there appears to be a related group of diffuse gliomas that occur predominately in adults, the so-called "isomorphic glioma". The "isomorphic subtype of diffuse astrocytoma" was identified histologically in 2004 as a supratentorial, highly differentiated glioma with low cellularity, low proliferation, and focal diffuse brain infiltration [6]. An isomorphic subtype of long-term epilepsy-associated astrocytoma associated with benign prognosis. Patients typically had seizures since childhood, and all were operated on as adults.

Morphologically, diffuse astrocytoma, MYB or MYBL1-altered, including isomorphic astrocytoma, is typically well-differentiated, low to moderately cellular glial neoplasm, comprised of astrocytes with small, rounded nuclei and regular chromatin structure, with low proliferative activity. Immunohistochemically, GFAP-positive, MAP2-, OLIG2-, IDH1 p.R132H-, CD34-negative, were detected and nuclear ATRX-expression was retained. The proliferation index was low. A prominent accumulation of the p53-protein was not found [5]. The vasculature was inconspicuous. Necrosis was not observed.

The molecular profiles of both paediatric MYB/MYBL1- altered gliomas and isomorphic gliomas display features associated with alterations of MYBL1, rather than MYB, including gene fusions [5]. Despite these similarities, it should be noted that analysis of the methylation profiles of a group of isomorphic diffuse gliomas found these to form a distinct cluster, albeit one closely related to MYB/MYBL1- altered diffuse astrocytoma occurring in children, as well as angiocentric gliomas [5].

## Differential diagnosis

Angiocentric gliomas have the same architectural and cytological features that characterise MYB- or MYBL1-altered diffuse astrocytoma, but all angiocentric gliomas have a rearrangement of MYB, most commonly associated with an MYB:QKI fusion.

It is more important to distinguish MYB- or MYBL1-altered diffuse astrocytoma from adult-type IDH-mutant or IDH-wildtype diffuse astrocytic gliomas, given their distinct biological behaviours.

In 2016 WHO classification, diffuse gliomas with MYB/MYBL1 alterations could be simply categorised as diffuse astrocytoma, IDH-wildtype, in the absence of a broad genomic characterisation. Because the diffuse astrocytoma IDH-wildtype is composed of tumours that are molecularly high-grade gliomas (i.e. glioblastoma, WHO grade 4), such classification would be misleading; MYB/MYBL1 altered diffuse gliomas in both children and adults seem to be generally indolent and usually behave in WHO grade 1 way.

Diffuse astrocytoma, MYB- or MYBL1-altered are paediatric low-grade glioma, usually epilepsy-associated tumours, with a good prognosis, even if available outcome data are limited. In the large series of paediatric patients reported in the literature, most patients had stable disease or no evidence of disease after long follow-up (range of follow-up from 2.5 to 12 years). Of patients with epilepsy, about 90% became seizure-free after resection and the remainder had a reduction in seizure frequency [5].

## Illustrative case

A 3-year-old boy, presented with a new onset seizure, described as a 'limp in the limbs', lasting for about 5-10 minutes. None of these episodes was associated with any gross neurological deficits, loss of consciousness or difficulty in breathing.

MRI was performed and he was found to have a large right frontoparietal tumour, hypointense on T1 (Fig. 1), and hyperintense on T2-FLAIR (Fig. 1A) but without enhancement, It does not show restricted diffusion. Tumours are well defined, but they focally show diffuse growth patterns.

A biopsy was obtained and found to show a diffuse low-grade glioma with moderate cellularity: astrocytes were small with rounded nuclei and regular chromatin (Fig. 1B).

Immunohistochemically neoplastic cells were positive for GFAP but negative for Olig2 (Fig. 1C) and IDH1

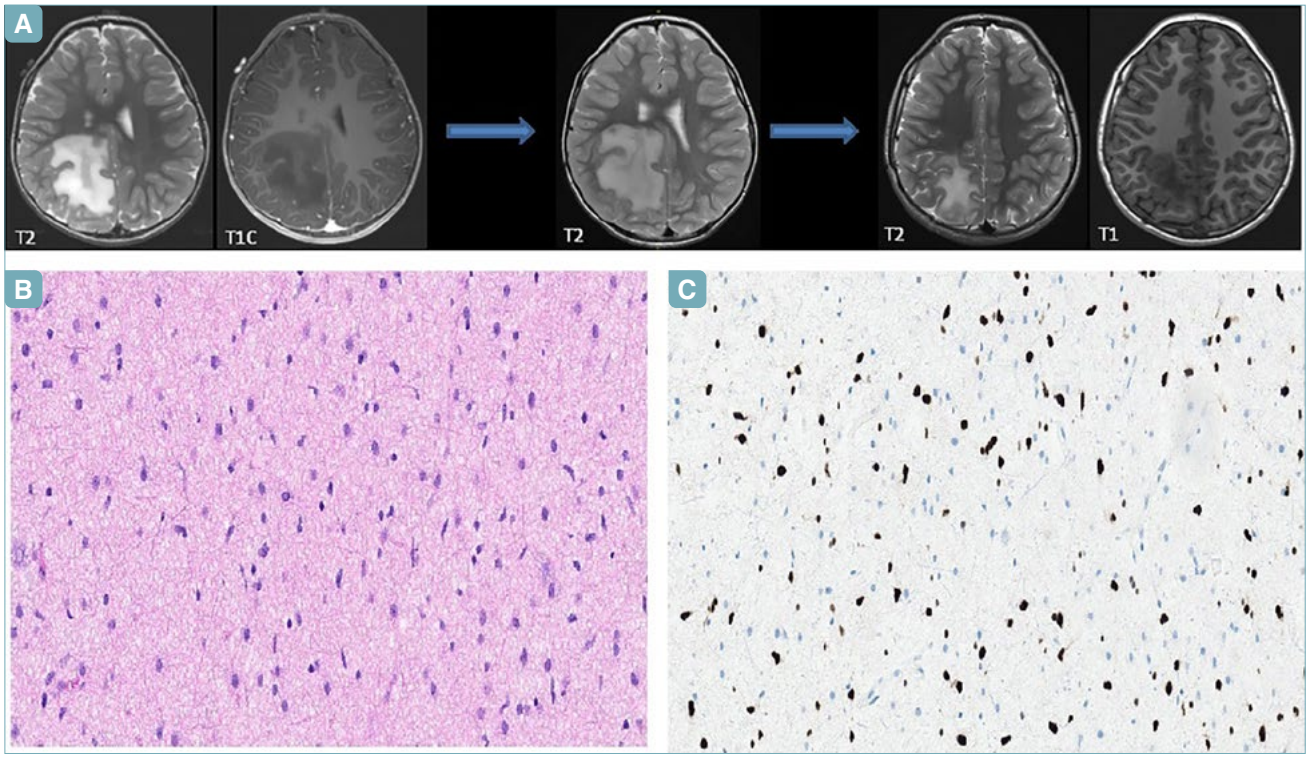


**Figure 1.** Diffuse astrocytoma MYB- or MYBL1-altered shows hyperintensity in T2 and hypointensity in T1 at MRI (A). Histologically, the tumour is a moderately cellular, well-differentiated astrocytoma (B: H&E, original magnification 20x). Immunohistochemical staining for Olig2 is negative in neoplastic cells (C: Olig2, original magnification 20x).

R132H. The proliferation index was low.
An abnormal chromosomal microarray was found, with gain of 9p24.3p21.1 (including PTPRD, CDKN2A, and CDKN2B), loss of 21p11.2q22.3, and gain of chromosome X, but not specific (c/w a clonal neoplastic process). Next generation sequencing panel detected the MYB-PCDHGA1 (exon14:exon 2) fusion. No pathogenic mutations were detected.
All features were suggestive of diffuse astrocytoma, MYB- or MYBL1-altered and the diagnosis was also confirmed by methylation array.
Per family wishes, he did not receive any chemotherapy radiation or surgical resection.
Over time these seizures were fairly well controlled, since diagnosis of the brain tumour, with a stable neurologic course without new neuro deficits.

## Polymorphous low-grade neuroepithelial tumour of the young (PLNTY)

Polymorphous low-grade neuroepithelial tumour of the young (PLNTY) is a rare and indolent neoplasm recently introduced in the World Health Organization (WHO) classification of central nervous system tumours and originally described and named by Huse et al. in 2017 [11].
PLNTY mainly affects the temporal lobe (80%), though lesions in the parietal, frontal, and occipital lobes have also been reported. It preferentially occurs in children and young adults (range 4-57 years, mean age of 20,6 years), with a slightly female predominance [11].
PLNTYs belong to the wide category of LEATs (long-term epilepsy-associated brain tumours)which typically cause seizures and are associated in many cases with early-onset and antiepileptogenic drugs resistant epilepsy. However, PLNTYs may also present with headaches, dizziness or visual disturbance.
Johnson et al., in the largest imaging review conducted on PLNTYs, reported that such tumours are generally well-circumscribed mass with a peripheral cystic component (in 89% of cases) located in cortical or subcortical areas. On magnetic resonance images (MRI) most of these lesions show FLAIR hyperintensity, hyperintensity in T2WI, and iso- or hypointensity in T1WI with slight or no enhancement after contrast-enhanced. A constant finding is the presence of centrally located grit calcifications that cause a heterogeneous

intralesional signal in TC imagines [12].
PLNTY has a distinct DNA methylation profile and almost invariably presents genetic features involving mitogen-activated protein kinases (MAPK) pathway constituents such as BRAF proto-oncogene or fibroblast growth factor receptors 2 and 3 (FGFR2, FGFR3). BRAF-V600E mutations or FGFR3-TACC3, FGFR2-KIAA1598, and FGFR2-CTNNA3 fusions occur in a mutually exclusive fashion [13]. Up to now, about 45% of the genetically investigated lesions have a BRAF-V600E mutation which is prominently found in young adults (mean age: 24.2 years), whereas FGFR2 fusions are more likely to be observed in younger patients (mean age: 8.5 years) [14]. The specific mechanisms by which these alterations contribute to the pathogenesis of PLNTY are not clear [11].
Histologically, though a certain variability was observed, an oligodendroglioma-like component is characteristic. Intra-tumoral heterogeneity ranges from cells having uniformly small rounded nuclei with perinuclear haloes to elements exhibiting significant anisonucleosis with spindled, wrinkled, or grooved nuclear membranes and intranuclear pseudo-inclusions. Foci of vague or more fully developed perivascular pseudorosetting can be present. Diffuse calcifications are also frequently seen. Mitosis, necrosis, vascular proliferation, inflammation, gemistocytes elements, Rosenthal fibres, and prominent cytonuclear atypia are not reported [11].
The tumour cells show immunopositivity for glial markers (GFAP and Olig2), though GFAP expression may be weak, focal or patchy. A constant and striking feature is the intense, frequently widespread expression of CD34 by tumour cells and by ramified neuronal elements in the peripheral cerebral cortex. Immunoreactivity for BRAF p.V600E may be encountered, reflecting the presence of these mutations in a subset of PLNTY. The proliferation rate quantified by the Ki-67 index is generally very low (< 1%), but higher values (up to 5%) have been reported. The tumour cells are typically negative for neuronal markers (synaptophysin, chromogranin, NeuN), EMA, and IDHp. R132H. PLNTYs do not harbour ATRX mutation and do not exhibit chromosome 1p/19q codeletion.

## Differential diagnosis

From the histopathological point of view, the differential diagnosis includes oligodendrogliomas, diffuse astrocytomas, gangliogliomas, and clear cell ependymoma. However, the combination of morphological, immunohistochemical and molecular features can help to reach the correct diagnosis.
PLNTYs exhibit an almost invariably benign clinical course (CNS WHO grade 1) and appear to be well controlled by gross total resection [11]. Seizure freedom is frequently achieved after surgical treatment and in the follow-up of 8 patients up to 89 months, only one case reports a breakthrough seizure with evidence of possible local recurrence at the base of the resection site [15]. The current available English literature report only one case of a paediatric patient who experiences recurrent glioblastoma-like histology within < 18 months after a diagnosis of PLNTY with FGFR-TACC3 fusion [16]. Comprehensive genomic analysis, additional case identification, and follow-up are required to aid in prognostication and to determine the long-term risk of recurrence and biological progression.

### Illustrative case

Here we report an example of PLNTY occurring in a 3 years old female with some chronic refractory seizures since she was 11 months old. MR imaging revealed a solitary mass located in the left temporal lobe which appears hypointense in T1WI and hyperintense in T2WI with a “salt and pepper signal” (Fig. 2A). The patient underwent surgery, and the mass was removed completely. Histologic examination (Fig. 2B and 2C) showed a neoplasm that involve the white matter and the overlying cortex composed of roundish cells with peri-nuclear halos, in an oligodendroglioma-like fashion. In addition, calcifications, ranging from small calcospherules to larger psammomata’s bodies, were present. No Rosenthal fibres or eosinophilic granular bodies were found. Neither necrosis, mitosis, nor microvascular proliferation was identified.
Immunohistochemically, neoplastic cells were strongly positive for oligodendrocyte transcription factor 2 (Olig2) and showed a more variable expression of GFAP. Strong and diffuse positivity for CD34 was present throughout the lesion and some neuronal cells with ramifying CD34 staining were seen in the peripheral cortex (Fig. 2D). The tumour cells were also positive for BRAFV600E (Fig. 2E). Ki-67 proliferation index was unremarkable (1%). Additional immunohistochemical studies showed no staining for neuronal markers, like NeuN and synaptophysin, IDH1 (R132H), no overexpression of p53 as well as retained ATRX expression. Morphological and immunohistochemical features were strongly suggestive of the diagnosis of polymorphous low-grade neuroepithelial tumour of the young).

## Angiocentric glioma

Angiocentric glioma (AG) is a diffuse glioma composed mainly of thin, cytologically bland, bipolar cells aggregating at least partly in perivascular spaces [1].

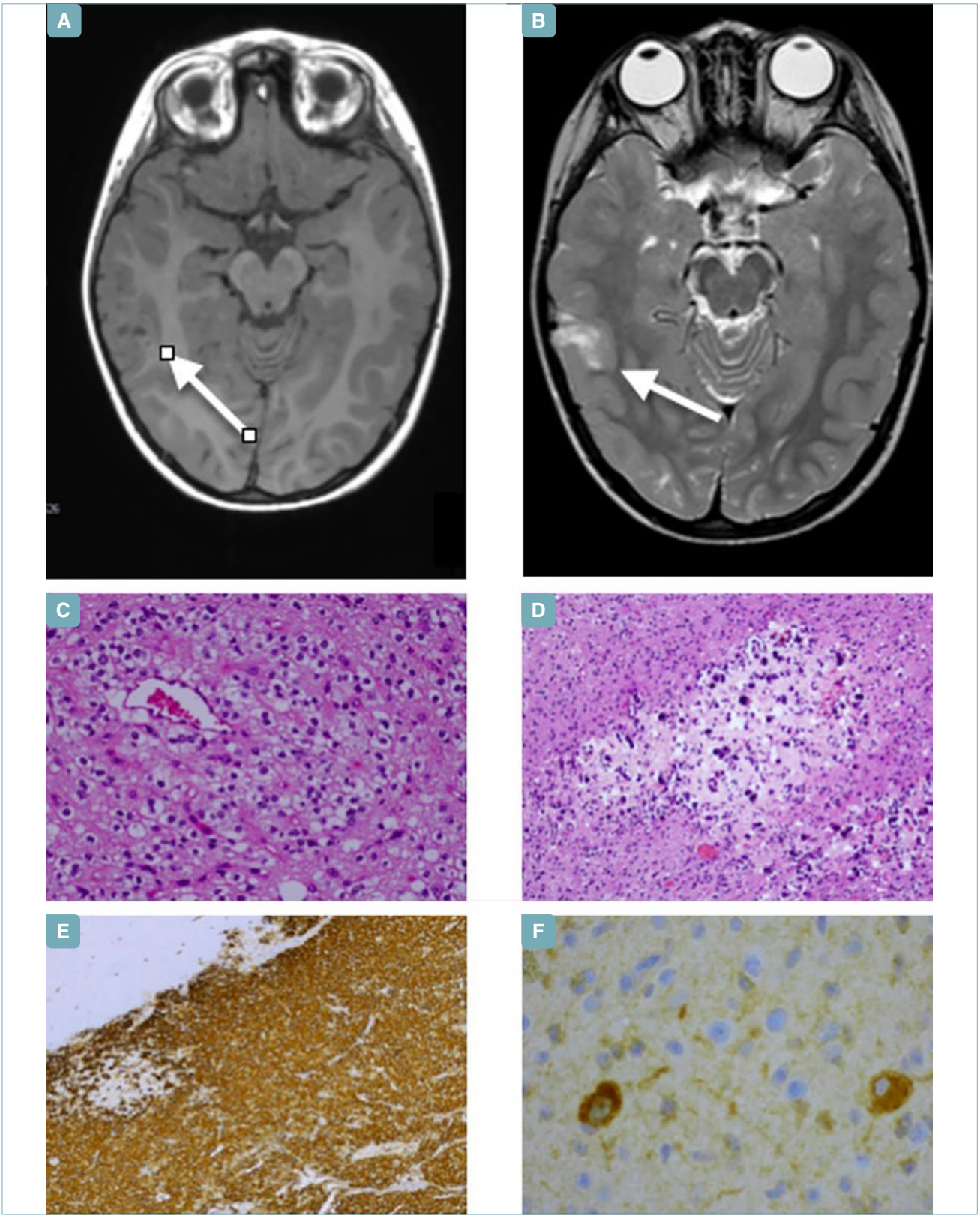


**Figure 2.** Polymorphous low-grade neuroepithelial tumour of the young (PLNTY) shows hypointensity in T1WI (A) and hyperintensity with a "salt and pepper sign" in T2WI (B). Morphologically the tumor is composed by oligodendroglioma-like cells with interspersed thin-walled capillary channels (C: H&E, original magnification 20x). Abundant micro-calcification within the lesion (D: H&E, original magnification 10x). Intense and widespread CD34 positivity in tumour cells (E: CD34, original magnification 4x). Expression of BRAF V600E in neoplastic cells (F: BRAF V600E, original magnification 40x).

Identified for the first time in 2005 [17,18] and recognised as a distinct entity since 2007 [19], in 2016 WHO edition it belonged to the section "other glioma" (together with astroblastoma and chordoid glioma of the third ventricle) [20], whereas in the new classification it has been classified among "paediatric-type low-grade diffuse gliomas".

The rarity of AG limits the understanding of this entity; nevertheless, 2021 WHO Classification assigned grade 1.

AG is often detected in the setting of childhood long-term and drug-resistant epilepsy surgery and therefore it has always been considered a low-grade epilepsy-associated brain tumour (LEAT), representing 0.8% (13 cases) of 1680 tumours collected from the German Neuropathology Reference Center [21]. In a recent series published by Kurokawa et al. median age was 13 years old, range 2-83 years [22].

Supratentorial, cortical location is typical, with a slight temporal lobe predominance 21 but brainstem lesions have also been described [23].

Magnetic Resonance Imaging (MRI) exhibits unifocality, T2 hyperintensity, no enhancement and often a cortical rim of hyperintensity on T1-weighted images and a stalk-like extension to the adjacent ventricle on T2-weighted images [17].

Histopathologically, AG shows an infiltrative appearance with monomorphic, bipolar spindled cells trapping pre-existing neurons. The hallmark features of this entity are the perivascular arrangement of neoplastic cells around cortical blood vessels (ependymoma-like appearance) and the aggregation beneath the pia-arachnoid structures in horizontal streams (palisades).Schwannoma-like nodules, solid regions and epithelioid appearance can be encountered.

Mitoses, microvascular proliferation and necrosis are virtually absent. The infiltrating tumour cells are GFAP positive whereas Olig2 is negative.

EMA demonstrates surface or dot-like, microlumen-type cytoplasmatic labelling, suggesting an ependymoma-like differentiation [17].

Ependymal features have also been confirmed by electron microscopy: elongated intermediate junctions, micro lumina and microvilli have been described in neoplastic cells of AG [23].

Some authors suggested that AG derived from the bipolar radial glia during embryogenesis, sharing ependymal features [18].

IDH1-R132H, BRAF V600E and neuronal antigens (Chromogranin A, Synaptophysin and NeuN) are negative.

Ki-67 proliferation index is low (ranging from 1% to 5%).

Rare cases with anaplastic features (i.e., multiple mitoses, high proliferative index) have been described but the clinical significance is uncertain.

Almost all AGs have an MYB: QKI gene fusion (or another MYB alteration) but 2021 WHO Classification considers this genetic alteration only a desirable diagnostic criterion.

### Differential diagnosis

Differential diagnosis includes low-grade diffuse-type paediatric gliomas, low-grade adult-type diffuse glioma, astroblastoma MN1-altered and ependymoma.

Considering the family of low-grade diffuse-type paediatric gliomas, astrocytoma MYB or MYB1-altered and diffuse low-grade glioma MAPK pathway-altered lack the perivascular arrangement, the palisade disposition beneath the pia-arachnoid complex and the EMA dot-like immunoreactivity of neoplastic cells typical of AG.

Polymorphous low-grade neuroepithelial tumour of the young (PLNTY) shows oligodendroglioma-like components, sparse calcification, CD34 abnormal expression and genetic anomalies involving the MAPK pathway (BRAF and FGFR).

Regarding low-grade adult-type diffuse glioma, AG lacks the IDH mutation harboured by diffuse astrocytoma and oligodendroglioma.

Astroblastoma MN1-altered has the same ependymoma-like immunophenotype as AG, but it is characterised by circumscribed borders, epithelioid elements, prominent perivascular sclerosis and MN1 rearrangement.

Cortical supratentorial ependymomas are generally enhancing mass with discrete borders; histologically, neoplastic cells are round or oval rather than the spindle and in most cases show true ependymal rosettes, lumina and fibrillar areas. In addition, ependymoma generally lacks MYB: QKI gene fusion.

### Illustrative Case

A 3-year-old boy presents with a new onset seizure.

On imaging a large T1 hypointense and T2 hyperintense (Fig. 3A) mass was detected, involving the right frontal-parietal lobe and extending to the corpus callosum and septum pellucidum.

Histopathologically, the lesion shows an infiltrative pattern of growth with a perivascular arrangement of neoplastic cells around vessels (Fig. 3B).

Neoplastic cells were GFAP positive, Olig2 negative; EMA demonstrated dot-like immunoreactivity (Fig. 3C). Ki-67 labelling index was very low (2%).

NGS panel detected the MYB-PCDHGA1 (exon 14:exon 2) fusion.

All features are suggestive of the diagnosis of Angiocentric Glioma.

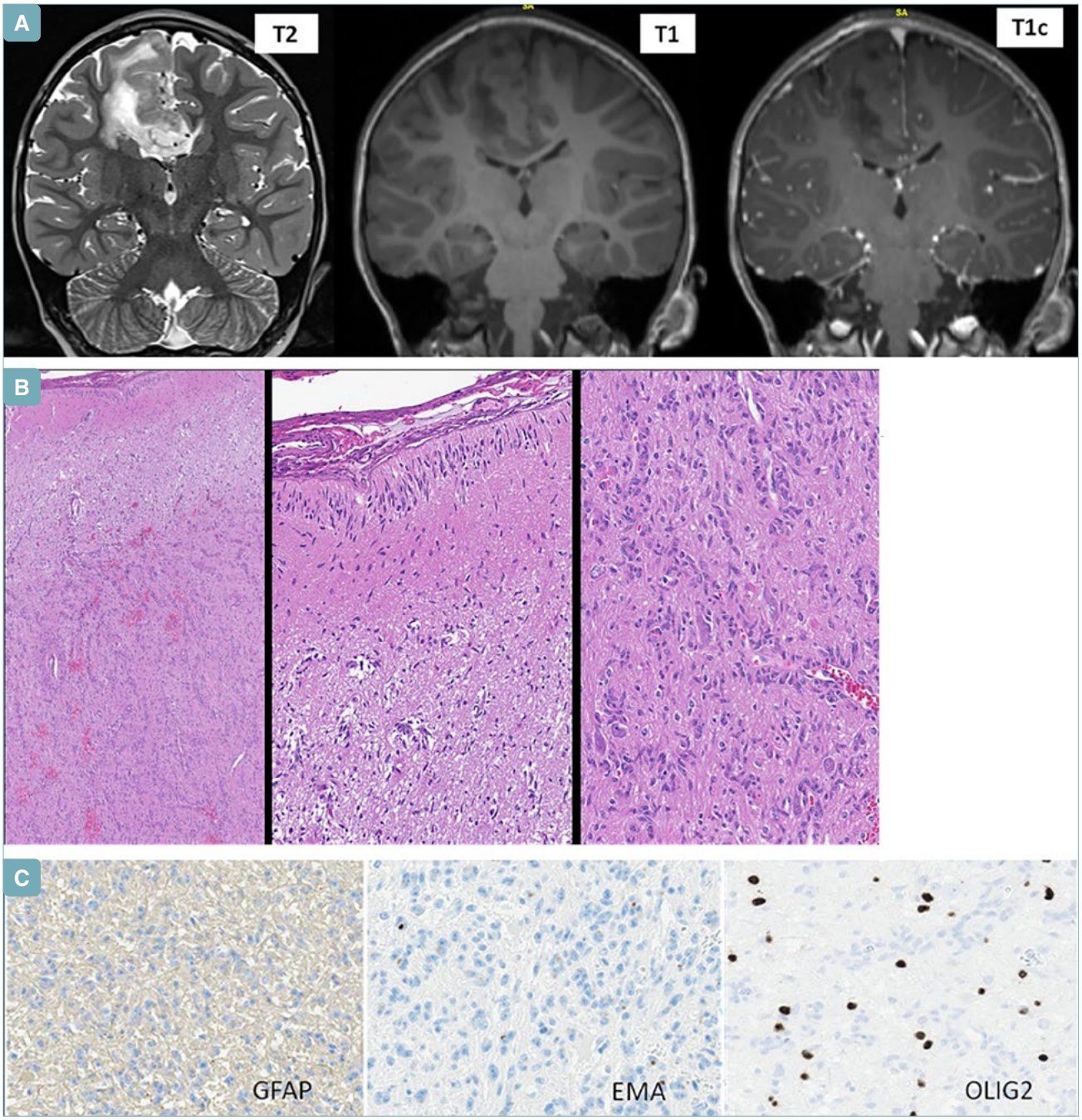


**Figure 3.** At MRI Angiocentric Glioma is a lesion with T1 hypointensity and T2 hyperintensity (A). The tumour shows ependymoma-like appearance and palisades beneath the pia-arachnoid (B: H&E, original magnification 4x and 10x). The infiltrating tumour cells are GFAP positive and Olig 2 negative; EMA demonstrates dot-like, microlumen-type cytoplasmatic immunoreactivity (C: GFAP, Olig2, EMA, original magnification 20x).

## Diffuse low-grade gliomas MAPK pathway-altered

Mitogen-activate protein kinase (MAPK) cascades are important signalling pathways that regulate many crucial cellular processes including cell growth and differentiation, apoptosis, angiogenesis wound healing and tissue repair, integrin signalling, cell migration and inflammatory and stress responses [24]. These cascades start when signalling molecules, i.e. fibroblast growth

factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and transforming growth factor (TGF), bind their receptors on the cell surface. This bind determines the sequential activation of three to five layers of cytoplasmic protein kinases that catalyse the phosphorylation of numerous cytosolic proteins and nuclear transcription factors with consequent regulation of the gene expression [25].

The MAPK signalling pathway is de-regulated in various diseases including immunological, inflammatory and degenerative syndromes. It is also related to the development, progression and metastatic potential of a wide range of neoplasms via multiple mechanisms, including abnormal expression of pathway receptors and/or genetic mutations that lead to activation of receptors and downstream signalling molecules. Among these neoplasms, there are several CNS tumours such as pilocytic astrocytoma, pleomorphic xanthoastrocytoma, gangliogliomas, and dysembryoplastic neuroepithelial tumour (DNT)WHO [26,27].

In addition to the well-known tumour entities mentioned above, the latest edition of the WHO classification of the CNS tumours in the group of paediatric-type diffuse low-grade gliomas includes the new category of diffuse low-grade gliomas MAPK pathway-altered [1].

These tumours generally occur in childhood, arise anywhere in CNS and particularly in the cerebral hemispheres and are frequently epilepsy-associated.

Their exact incidence is not yet available due to specific molecular tests required for the diagnosis. However, they are believed to be rare [1].

On neuroimaging, diffuse low-grade gliomas MAPK pathway-altered often appear as inhomogeneous enhancing masses with cystic elements.

Histopathologically they are morphologically heterogeneous and infiltrating tumours showing astrocytic, oligodendroglial or sometimes mixed features.

In the majority of the cases, atypia is inconspicuous and cellular density low. Mitoses are absent or very rare and there is no vascular proliferation and necrosis. The pattern of infiltration is not extensive and consists of an excess of incorporation of normal cells at the periphery of the lesion [1].

The molecular characterisation of these tumours is pathogenetic alterations in the genes coding a MAPK pathway protein. In all cases, there are no IDH1/2 and H3F3A mutations and deletion of CDKN2A [1].

Based on the specific molecular alteration some subtypes have been identified [28,29]. The most frequent are diffuse low-grade glioma FGF receptor 1 (FGFR1) tyrosine kinase domain-duplicated, diffuse low-grade glioma FGFR1-mutant and diffuse low-grade glioma BRAF-mutant [1].

FGFR1 is a receptor tyrosine kinase (RTK) playing an important role in signal transduction via activation of its intramembranous tyrosine kinase domain (TKD). All FGFR1 alterations lead to FGFR1 autophosphorylation with consequent up-regulation of the MAPK pathway. BRAF gene is a proto-oncogene coding a protein also called BRAF. BRAF is a member of the rapidly accelerated fibrosarcoma (RAF) kinase family, which transduces signals downstream of rat sarcoma viral oncogene homolog (RAS) via the MAPK pathway playing a role in cell growth. Mutation in BRAF, more often consisting in a replacement of valine with glutamic acid at position 600 (p.V600E), acts as phosphomimic within the RAS/MAPK pathway rendering it constitutively active [28,29].

In addition to these alterations other MAPK signalling alterations involving NTRK1/2/3, MET, FGFR2, MAP2K1, RAF, ALK and ROS have been identified in a small number of cases.

All these molecular alterations are not specific to diffuse low-grade gliomas-MAPK pathway-altered and may be documented in different CNS tumour types. Thus, an integrated clinical, morphological and molecular diagnostic approach is needed for the correct diagnosis

Remarkably, molecular alteration and morphology are related: FGFR1 altered tumours tend to have nodular architecture and oligodendrocyte-like features showing morphological overlap with DNT. BRAFp.V600E altered tumours instead tend to have astrocytic features in absence of Rosenthal fibres and eosinophilic granular bodies.

The differential diagnosis primarily includes DNT, PLNTY, and adult-type IDH-mutant oligodendrogliomas for tumours with FGFR1 alterations and pilocytic astrocytoma, ganglioglioma, and pleomorphic xanthoastrocytoma for tumours with BRAF mutations. DNT and gangliogliomas can be distinguished by the presence of neuronal neoplastic cells and, for DNT, also for the microcystic and nodular architecture; PLNTYs show a characteristic and widespread strong expression of extravascular CD34; adult-type oligodendrogliomas are IDH mutant and 1p/19q; co-deleted pilocytic astrocytoma frequently displays granular bodies and Rosenthal fibres; pleomorphic xanthoastrocytomas typically have pleomorphic cytology with giant and xanthomatous cells. Furthermore, IDH-mutant gliomas and H3 K27-altered diffuse midline gliomas may sometimes show the same histopathological features of a BRAF p.V600E–mutant diffuse low-grade glioma so the ascertain of IDH1/2 and H3F3A status might be needed in some cases [1].

Diffuse low-grade gliomas MAPK pathway-altered are believed to have a favourable outcome even if a CNS WHO grade has yet to be assigned [1]. The availability of targeted therapies for MAPK-altered tumours can positively improve the outcome of these tumours [1].

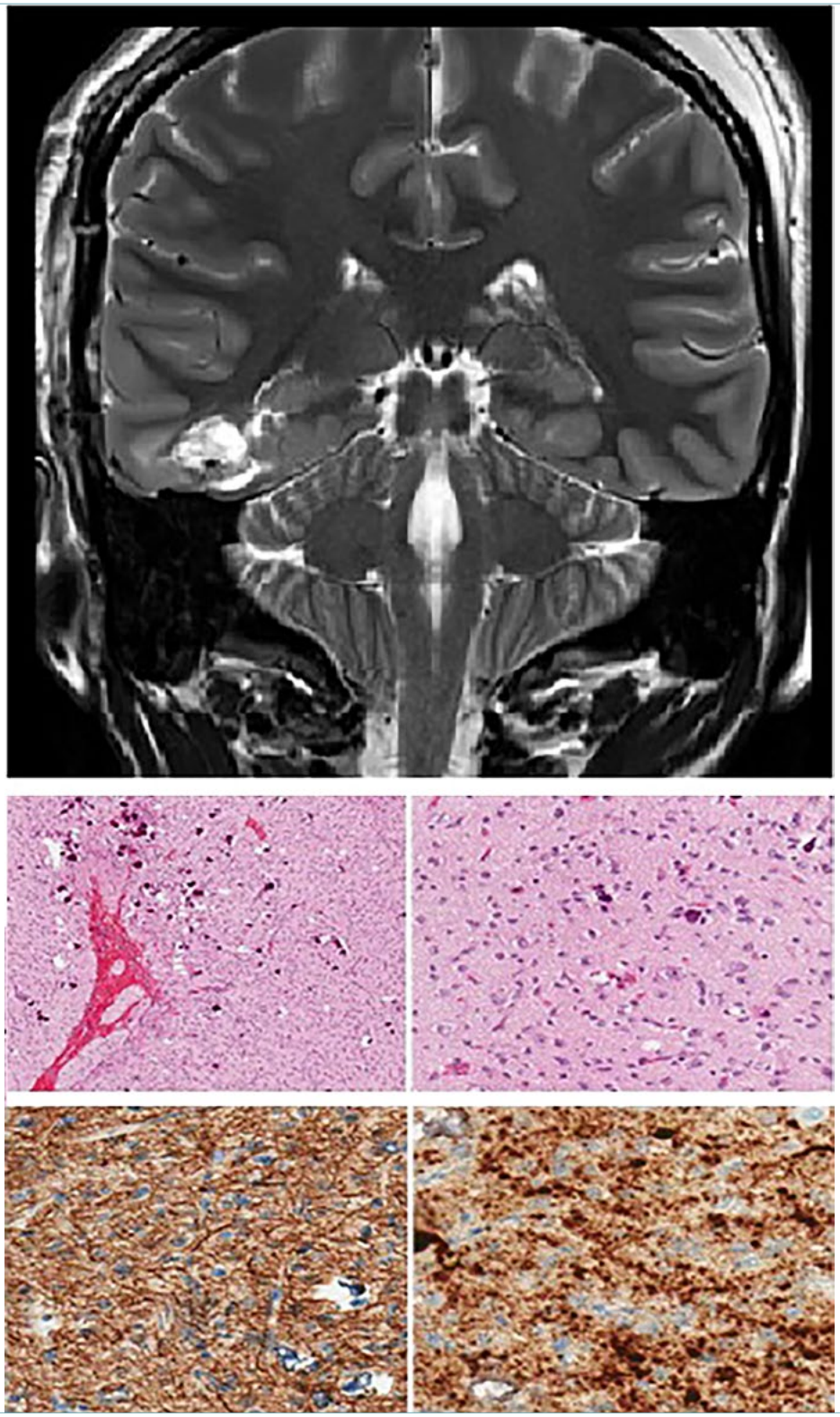

**Figure 4.** Diffuse low-grade gliomas MAPK pathway-altered is a subcortical inhomogeneous lesion with poorly defined margins at MRI (A). Histologically, neoplastic astrocytes entrap cortical neurons and microcalcifications (B: original magnification 10x) and show GFAP (C: GFAP, original magnification 20x) and BRAFV600E positive immunostaining (D: BRAF, original magnification 20x).

### Illustrative case

The patient was a 21-years-old woman with pharmacoresistant mesial temporal epilepsy. MRI showed a right temporal cortico-subcortical lesion with poorly defined margins. The lesion was inhomogeneous (due to the presence of calcifications and small cystic components). (Fig. 4A) She, therefore, underwent a surgical procedure with gross total resection of the tumour. Histologically, the lesion was composed of glial cells with an astrocytic morphology entrapping normal cortical neurons (Fig. 4B). The tumour cells had a bland appearance and were mildly atypical. Psammoma bodies were present. No Rosenthal fibres or eosinophilic granular bodies were found. Neither necrosis, mitosis, nor microvascular proliferation was identified. Immunohistochemically, neoplastic cells were diffusely positive for GFAP and (Fig. 4C). Neuronal markers neurofilaments and synaptophysin were negative. Ki-67 proliferation index was unremarkable (1%). IDH1 (R132H) was negative and ATRX expression was retained. There was no p53 overexpression. The tumour cells were positive for BRAFV600E immunostaining. (Fig. 4D) BRAFV600E mutation was confirmed by the molecular test.

The described morphological, immunohistochemical and molecular features were diagnostic for diffuse low-grade glioma MAPK pathway-altered (BRAFV600E mutant).

## Discussion

The 5th edition of WHO 2021 classification of central nervous system cancers divide diffuse gliomas that occur in adults ("adult type") and those that occur in children ("paediatric") based on clinical and molecular characteristics. However, paediatric-type gliomas may sometimes arise in younger adults and occasionally adult-type gliomas may arise in children. Diffuse low-grade paediatric glioma includes diffuse astrocytoma, low-grade polymorphic juvenile neuroepithelial tumour altered by MYB or MYBL1, angiocentric glioma, and diffuse low-grade glioma with an altered MAPK pathway [30]. It is particularly important to recognise those tumours in adolescents and young adult patients because all gliomas with alterations in the MAPK pathway or MYB and MYBL1 have distinctly different prognoses from other glioma subtypes and offer possibilities for targeted therapy [9].

Molecular tests are increasingly needed for the complete definition of CNS neoplasms, but a clinicopathological approach is necessary for everyday practice and can lead to the diagnosis of pLGG since the preoperative studies.

Herein, we propose a step-integrated diagnostic approach that can be used to separate these clinically and biologically distinct tumor groups (Tab. I).

The first step is the clinico-radiological setting: pLLGs are infiltrative supratentorial tumours, typically associated with long-term and drug-resistant epilepsy in children and young adults.

**Table I.** Immunohistochemical and molecular features of paediatric-type diffuse low-grade gliomas.

| | Paediatric-type diffuse low-grade gliomas | | | | |
|---|---|---|---|---|---|
| | **MAPK-altered** | **MYB or MYBL1-altered** | **PLNTY** | **AG** | **DA/O** |
| ***IHC*** | | | | | |
| GFAP | + | + | + | + | + |
| OLIG2 | + | - | + | - | + |
| CD34 | + (ramified cortical cells) | - | + (neoplastic cells) | - | - |
| EMA | - | - | - | + | - |
| IDH1 *(R132H)* | - | - | - | - | + (90%) |
| BRAF *(V600E)* | + | - | + | - | - |
| | | | | | |
| ***Molecular*** | | | | | |
| IDH | - | - | - | - | IDH1/2 mutations |
| MAPK pathway | BRAF, FGFR other MAPK alterations | MYB – MYBL1 altertions | BRAF, FGFR | MYB:QKI gene fusion, other MYB alterations | - |

Abbreviations. LGG low-grade glioma, PLNTY Polymorphous low-grade neuroepithelial tumour of the young, AG Angiocentric glioma, DA diffuse astrocytoma, O oligodendroglioma IHC immunohistochemistry.

The second step is the histological evaluation: despite pLGGs sharing overlapping morphologic features, they can show different morphologies.
Diffuse astrocytic pattern is typical of glioma MAPK-alterated and MYB or MYBL1-alterated, polymorphic and oligodendroglial pattern of PLNTY, ependymoma-like pattern of AG.
The histological assessment will be simpler in surgical specimens than in biopsy.
The third step is the immunohistochemical analysis: a small panel of six antibodies (GFAP, Olig2, CD34, EMA, IDH1 R132H and BRAF V600E) could help in the differential diagnosis also in the preoperative specimen pLGGs require invariably immunoreactivity for GFAP and negativity for IDH1 R132H (detected in adult-type glioma).
Astrocytic pattern, positivity for Olig2 and BRAF V600E in neoplastic cells with scattered ramified elements CD34+, suggest the diagnosis of diffuse low-grade glioma MAPK pathway-altered.
Astrocytic pattern, negativity for Olig2, CD34 and BRAF V600E, rise the hypothesis of diffuse low-grade glioma MYB or MYBL1-altered.
Oligodendroglial phenotype, and positivity for Olig2 and CD34 are typical of PLNTY (regardless of the BRAF status).
Ependymomatous phenotype, negativity for Olig2, CD34, BRAF V600E with a dot-like immunoreactivity for EMA are consistent with AG.
It is also useful for the immunohistochemical evaluation of H3.3K27M, to rule out the possibility of H3-altered glioma.
This practical algorithm can be extremely useful both in bioptic and surgical specimens, keeping molecular tests for specific cases, such as the study of low-grade diffuse glioma Olig2+ with negativity for BRAF V600E (to evaluate MAPK pathway, IDH status, H3 status, CDKKN2A deletion) or the research of MYB-MYBL1 alterations in diffuse low-grade glioma Olig2-.
In addition, targeted treatments with FGFR or pan-RAF inhibitors represent viable approaches in the context of recurrent or residual disease that may decrease the need for other treatment modalities such as radiation therapy.
The molecular approach alone is not recommended without clinicopathological background, because MAPK alterations are neither specific to paediatric-type diffuse glioma (for example they can be encountered in Pylocitic Astrocytoma) nor to a single entity of this group (BRAF mutation or FGFR alteration are present both in PLNTY and in diffuse low-grade glioma MAPK pathway-altered).
In conclusion paediatric-type, diffuse gliomas are a new intriguing family of the 2021 WHO Classification of Tumors of Central Nervous System, originating from a better molecular comprehension of different entities. A clinicopathological matrix approach is necessary (and many times sufficient) to reach an integrated, tiered diagnosis.

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### Conflict of interest

The authors declare no conflict of interest.

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### Ethical consideration

This article does not contain any studies involving human partecipants performed by any of the authors.
Illustrative cases have been reported respecting patient anonymity.

### Authors' contributions

All the authors (VPF, CC, AS, AB) contributed equally to the conception, desing, acquisition of data, drafting and critically revising the article.

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