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Reactive astrogliosis or astrocytoma: in the quest of assistance in solving well-known neuropathologist's dilemma

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It is very well known, that reactive astrocytic response manifesting in hyperplasia and hypertrophy of astrocytes (named gliosis or astrogliosis) may be seen in variety of pathologies, and may be extremely polymorphic what results in a risk of misdiagnosis of glioma. The risk is especially high in cases of oligobiopsy, where the amount of tissue material is very limited, sometimes scarce. In many cases knowledge and experience of neuropathologist and her/his cautiousness in interpretation of biopsy material, also in confrontation with results of neuroimaging , may turn out to be insufficient to make correct diagnosis. The GFAP immunostaining, which is a standard and paragon method used in examination of brain biopsy material as well as other methods are notoriously insufficient and more or less worthless in differentiation of astrocytoma and astrogliosis. New hope arose with the introduction of antibody that may specifically detect a product of mutated gene of isocitrate dehydrogenase (IDH-1) (Dianova, Hamburg, Germany). The R132H mutation of IDH-1 occurs probably in most so called secondaryglioblastomas and their lower-grade precursors (diffuse astrocytomas and anaplastic astrocytomas). We tried to check whether antibody against mutated IDH-1 may be helpful in diagnosis of glioma and especially in differentiation between glioma and reactive gliosis. Moreover, following mostly incidental observations with strong immunoexpression of cytokeratins detected by AE1/AE3 antibody in reactive astrocytes, we decided to include assessment of AE1/AE3 immunopositivity (along with that of IDH-1) in gliomas and in cases of reactive gliosis i.e. around craniopharyngioma, haemangioblastoma, primary lymphoma, metastatic tumors, vascular malformations, in inflammatory, necrotic and demyelinating lesions (50 cases in all). The preliminary results indicate, that in no cases of bona fide reactive astrogliosis expression of IDH-1 was observed, however in some cases IDH-1 immunopositivity (probably unspecific) was detected in macrophages, and pyramidal neurons. IDH-1 positivity was found in 2/13 glioblastomas, 6/6 oligodendrogliomas, 2/7 astrocytomas gr. II, 3/9 astrocytomas gr. III and 1/5 metastatic carcinomas. AE1/AE3 immunoexpressionin gliosis was semiquantitatively weaker than that of GFAP and marked only fibrillary hypertrophic astrocytes but not gemistocytic forms. In gliomas AE1/AE3 positivity was scarce and probably, at least in considerable proportions, limited also to reactive (not neoplastic) astrocytes, especially around vessels. These preliminary results indicate that both AE1/AE3 antibody and against mutated IDH-1 can be helpful in differentiation between gliosis and glioma, however further studies are necessary.

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Cytotoxic effect of arvanil on glioma C6 cells *in vitro*

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Cannabinoids are defined as ligands of the cannabinoid receptors. Two types of these receptors are known, CB1 located preferentially on immune cells and CB2 located preferentially on the neurons. Two major groups of naturally occurring cannabinoids are distinguished: exogenous phytocannabinoids of plant origin and endogenous endocannabinoids encountered mainly in the central nervous system. The first discovered endocannabinoid anadamide (AEA), which is a ligand of the CB1 receptors, is also the agonist of the the transient receptor potential cation channel subfamily V member 1 (TRPV1). AEA is endowed with anticancer properties, manifested by induction of apoptosis. Our previous experiments have suggested that the fast (observed within 24 h) cytotoxic effects of AEA on C6 rat glioma cells cells are related to the interaction with the TRPV1 receptor.

Arvanil is a hybrid between anadamide and capsaicin (capsicum alkaloid), the model TRPV1 agonist. The aim of present study was to evaluate cytotoxicity of arvanil on glioma C6 cells *in vitro*. The effect of arvanil on cell viability was quantitated with the use

of a colorimetric MTT assay. Induction of apoptosis was visualised by a Hoechst-33342 dye.

Our results indicated that arvanil is cytotoxic toward C6 glioma cells after 24 h exposure in concentrations 15 μM and 30 μM. The hybrid compound induced chromatin condensation and apoptosis. This antiglioma effect was more pronounced than that of equimolar concentration of a conventional anticancer drug 5-fluorouracil.

Molecular prognostic markers in gliomas, in practice

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Gliomas are among the most extensively studied human tumors. Numerous studies focused on their biological background provided a great evidence of different molecular aberrations involved in glioma pathogenesis. Large comprehensive studies revealed different molecular profiles of low grade gliomas and five different glioblastoma (GBM) molecular subtypes. These comprise glioblastoma characterized by CpG methylator phenotype (G-CIMP), *IDH1* mutations and "proneural" expression signature, the molecular profile related closely to low grade gliomas and associated with good prognosis, as well as other glioblastomas that may be classified as "mesenchymal", "neural", "classical" and "proneural/without G-CIMP/*IDH1* mutations". Those others GBMs share also distinct profile of genomic mutation: low frequency of *TP53* mutations, *EGFR* amplifications and common *PTEN* mutations, and are associated with poor outcome.

As a result of expanding knowledge of glioma biology a number of potential prognostic markers have been proposed. These involve various genomic mutations: particular gene mutations or chromosomal aberrations, mRNA expression based multiple genes profiles and signatures and epigenetic aberrations that utilises analysis of DNA methylation of multiple or single loci.

The usefulness of most extensively validated and best described molecular markers: *IDH1* mutation, *MGMT* promoter methylation an loss of heterozygosity (LOH) of 1p/19q will be discussed with respect to recently published clinical results and author's own experience.

IDH1 mutations provide an attractive prognostic and diagnostic marker detectable with PCR and DNA sequencing, as well as immunohistochemical staining, whereas *MGMT* promoter methylation and LOH 1p/19q assessment with PCR based methods are still not uncontroversial due to discrepancy of different studies results and lack of methodological standards.

Multiparametric MR imaging of cerebral glioma

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MR imaging is the preferred technique for the diagnosis, treatment assessment and monitoring of patients with CNS glioma. Conventional MR imaging with gadolinium contrast enhancement is currently combined with advanced multiparametric MR imaging techniques to obtain morphologic and metabolic informations. The purpose of the lecture was to present the value of structural MR imaging and MR measurements of and spectroscopic parameters for glioma characterization. Multiparametric MR assessment of glioma based on ^{1}H-MR spectroscopy, diffusion weighted imaging (DWI) with diffusion tensor imaging (DTI), apparent diffusion coefficient (ADC) and fractional anisotropy (FA) techniques and dynamic contrast enhanced (DCE) perfusion weighted imaging (PWI) can classify the type of glioma and discriminate high from low grade gliomas and infiltrating tumor from surrounding vasogenic edema or normal tissues. The application of comprehensive MR protocol enables grading glioma with almost 100% accuracy. The use of high-concentration contrast media and advanced MR imaging techniques provide reliable lesion visualization to direct biopsy. DT tractography is a promising for localizing tumor infiltration in 3D and may have applications for intraoperative surgical planning avoiding eloquent white matter tracts. The advanced techniques have also the ability to predict progression-free survival glioma patients.

Angiocentric glioma – histopathology and immunohistochemistry of a rare epilepsy-associated tumour

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Angiocentric glioma (AG) is a low-grade, slowly growing tumour corresponding to WHO grade I that occurs in children and young adults presenting with longstanding epilepsy. It is usually located superficially in the fronto-parietal, temporal and hippocampal region. The morphology of tumour is characterized by perivascular orientation of the spindle-shaped cells forming so called angiocentric pattern accompanied by features of ependymal differentiation. Sometimes the tumour cells exhibit schwannoma-like areas or palisading growth pattern, especially at the brain surface.

We present two examples of angiocentric variant of glioma in children (15-year-old girl and 14-year-old boy) with chronic and intractable epilepsy. The tumours were located in the right temporal lobe and left parieto-occipital area. In both cases the total tumour excision was performed and the patients were discharged home in a good condition without seizures.

In both cases the histopathological findings revealed tumours composed predominantly of uniform elongated glial cells exhibiting perivascular pseudorosette-like pattern and spindle-shaped or schwannoma-like areas. The subpial neoplastic infiltration with palisading of tumour cells at the brain surface was also seen. The neoplastic cells displayed immunoreactivity for GFAP, S-100 protein and vimentin. Moreover, a slight "dot-like" EMA staining, suggested ependymal differentiation, were detected. The tumour showed low MIB1 labeling index below 1%. These findings allow to establish the diagnosis of angiocentric gliomas.

Angiocentric glioma represents a distinctive clinico-pathological entity among gliomas developing in children and young adults. This tumour displays features typical for both an infiltrating astrocytoma and ependymoma.

New metabolic signature of glioblastoma

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Glioblastoma is the most common, the most malignant and generally uncurable type of glioma in adults. In recent years, attention was drawn to the metabolic processes involved in the pathogenesis and progression of glioblastoma.

Rapidly proliferating cancer cells are dependent on the energy sources, especially glucose. Based on the aerobic glycolysis – so called Warburg effect, cancer cells produce energy, nucleotides, amino acids and lipids required for growth, survival and new cell mass building. "Reprogrammed" metabolic pathways are closely associated with the occurrence of hypoxia within the tumor, which results in multiple alterations of signaling and biochemical pathways. Such changed metabolism creates susceptibility to new oncological therapies.

The main player of tumor hypoxia signaling is driven by hypoxia-inducible factor 1α (HIF1α). However, recently some alterations in several metabolism-related enzymes required for aerobic glycolysis – isocitrate dehydrogenase 1 (IDH-1), pyruvate kinase M2 (PKM2), pyruvate dehydrogenase kinase 1 (PDK1), lactate dehydrogenase A (LDHA), and CAIX (carbonic anhydrase IX) have been described in glioblastoma cells. Glioblastoma shows important metabolic differences (*cell reprogramming*), that determine its biological malignancy.

The exact functions of these enzymes and their alterations need careful investigation to precise the metabolic signature of glioblastoma. Further identifications of the metabolic enzymes critical for glioblastoma cells growth appears to be an interesting new approach for the tailored therapy of this neoplasm.

Neuroimaging in pediatric gliomas

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One of the objectives of neurooncologic imaging is try to correctly classify and improve ability to preoperative assessment of glioma grading.

Combining conventional and advanced magnetic resonance (MR) techniques can give important morphologic and physiologic datain the characterization of brain neoplasms and increase the sensitivity and predictive value in predicting intracranial tumor grade.

The essential MR imaging sequences are: T1- and T2-weighted images, contrast-enhanced T1-weighted images, diffusion weighted imaging – DWI. A number of advanced MRI techniques such as: diffusion tensor imaging – DTI, perfusion weighted imaging – PWI, proton magnetic resonance spectroscopy – HMRS, susceptibility weighted imaging – SWI have been included to routine protocol to provide information for the assessment of brain neoplasms.

Based on MR imaging can be used to suggest a tumor's classification to the group of tumors with low- (WHO I, II) or high- (WHO III, IV) degree of histological malignancy.

On the basis of conventional magnetic resonance imaging (T1-, T2-weighted images, DWI with ADC maps) can be analysed important tumors' features such as the presence or lack of contrast enhancement and peritumoral edema. Low-grade tumors mostly revealed hyperintensity on T2- and hypo- or isointensity on diffusion-weighted images. Signal intensity is connected with tumors' cellularity. Brain tumors with high hypercellularity and high nuclear-to-cytoplasmic ratio typical showed decreased T2-signal with diffusion restriction seen as high signal on DWI. Additionally, assessment of apparent diffusion coefficient – ADC values of intracranial neoplasms play significant role in differentiation between high- and low-grade tumors. Tumor cellularity determinated ADC values.The value of contrast enhancement and peritumoral edema in pediatric group of patients is limited. Some of the low-grade tumors may mimic the appearance of high-grade neoplasms. Pilocytic astrocytomas and desmoplastic gangliogliomas show strong enhancement of the solid part of the tumor. Other tumors like for example high-grade medulloblastomas may not enhanced.

Recently, susceptibility weighted imaging – SWI is a technique supporting the differential diagnosis of brain neoplasms. In brain tumors, SWI has been used to assess tumor visibility, small tumor vessels and for depicting and the evaluation of the neovascularity and angiogenesis. Intratumoral susceptibility signal: linear or dotlike indirectly suggest the presence of new, abnormal blood vessels within high-grade tumors.

The combination of routine and advanced sequences and techniques may be useful for diagnosis of brain neoplasms and may help to differentiate between low and high grade intracranial gliomas.

Congenital glioblastoma multiforme – case report

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Congenital central nervous tumours form a unique group among neoplasms. They are different from other tumour groups not only due to the onset time but also to their histopathology, anatomic location, biologic behaviour. Congenital glioblastoma is among the rarest types of congenital brain tumours and is uncommon in the prenatal period.

We report a rare case of congenital brain neoplasm detected by prenatal ultrasound examination and magnetic resonance imaging at 26 gestational weeks. In the MRI rapidly growing and devastating brain tumour suspected of being malignant was stated. Based on MRI findings and consultation with specialists team, pregnancy was terminated at 28 weeks. Newborn presented hydrops foetalis. The child died shortly after birth due to cardiorespiratory insufficiency. At autopsy a large tumour with a spongy-like appearance was found. Tumour involved nearly the whole right cerebral hemisphere and led to marked hydrocephalus. In the histological and immunohisto-

chemical examination tumour presented features of glioblastoma multiforme. Neoplastic cells were immunopositive for GFAP, S-100 protein and negative for neuronal markers. Frequent mitoses and high MIB-1 labelling index were seen in the tumour areas. The coexistence of tumour and vascular developmental anomaly was stated. The conglomerates of numerous, distended, thin walled foetal-like blood vessels were located beside the tumour tissue, which presented disturbance in differentiation and maturation of vascular net.

How the pathologic report (with or without molecular biology findings) impacts the decisions on the use of radio(chemo)therapy in brain gliomas?

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Currently, the clinical practice in adults' brain gliomas is based mainly on the results of prospective trials from the XX century and has remained unchanged from the 90s. The one exception is the routine treatment of patients with glioblastoma (GBM) in good performance status and younger than 70 with radio-chemotherapy (RT-CHT) based on the temozolomide (TMZ) (Stupp schema).

Surgery remains the main therapeutic modality of low grade gliomas (WHO GII); the use of postoperative radiotherapy is related to the presence of prognostic factors, such as age, extent of surgery and histological type (oligodendroglioma). Presence of recognized molecular prognostic factors, such as the O-6-methylguanine-DNA methyltransferaze (MGMT) promoter methylation, isocitrate dehydrogenase (IDH)1 or IDH2 mutation, and 1p/19q codeletion in case of oligodendrogliomas and oligoastrocytomas is currently not taken into account in therapeutic decisions, because their validation in larger prospective trials is still required. IDH1 mutation has recently become a focal point for research aimed at understanding the biology of gliomas; however, still the impact of the detection of this mutation on the clinical practice is limited.

For GBM, the MGMT promoter methylation is a predictive factor related to the improved survival after RT-CHT with TMZ in comparison with RT alone. In two randomized trials that compared in elderly GBM patients the use of TMZ alone with postoperative RT, the predictive value of MGMT promoter methylation for better outcome after CHT was demonstrated. Detection of 1p/19q codeletion should be performed for anaplastic oligodendrogliomas and oligoastrocytomas, because it was demonstrated in randomized studies that this chromosomal alteration is related to the improved survival after RT followed by adjuvant CHT in comparison with RT alone.

Molecular characteristics of paediatric gliomas

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Gliomas are the most common brain tumours both in childhood and adults. They are divided into four grade groups (WHO 2007 classification), however an incidence is remarkably associated with patients age, with grade I pilocytic astrocytoma being typical for children and grade IV glioblastoma being typical for adult age. Importantly, recent molecular investigations indicate that childhood gliomas are biologically different from their adults counterparts.

Cancer Genome Atlas (TCGA) pilot project assessed the value of large-scale molecular analysis in adult glioblastoma revealing that more than 70% of tumours have aberrations in three signalling pathways RTK/RAS/PI(3)K, p53 and RB, with over 40 different mutations detected.

By contrast, recent comprehensive analyses based on microarray and the next generation sequencing techniques uncovered remarkably different genetic landscape in childhood glioblastoma, with somatic mutations in the chromatin remodelling pathway genes *H3F3A, ATRX* and *DAXX* identified in 44% of tumours. Also *IDH1* mutations and *EGFR* amplification frequently found in adults were rarely detected in children. Altogether these findings led to recognition of six molecular subgroups of glioblastomas, which were associated not only with patients age but also with the location of tumour.

The most frequent in children low grade pilocytic astrocytoma is characterized by activation of MAPK pathway. The mechanisms of MAPK pathway activation include a presence of *KIAA1549*:*BRAF* fusion gene (~60% of cases) and other *BRAF, RAF1* and *NF1* aberrations. Similarly to the findings in glioblastoma, type of mutations, global methylation and expression profiles in pilocytic astrocytomas were distinct in different tumour locations.

The biological differences described in gliomas suggests that therapeutic targets may differ according to tumour site and therefore tumour location should be considered in future clinical trials.

The spectrum of pathomorphology in astroglial tumours

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Gliomas are the most common brain tumours. The current WHO classification of astroglial tumours is based on histological similarities to different subtypes of glial cells. Generally, gliomas are divided into two groups of different biological behavior: 1) well-circumscribed (grade I-II) and 2) diffusely infiltrative (grade II-IV). The advanced diversity of histological appearance of gliomas and/or inadequate biopsy tissue sampling available for histological examination might be related with misdiagnosis and overtreatment. Various lesions including non-neoplastic and neoplastic processes of different histogenesis and grading ought to be considered in the differential diagnosis of glial tumours. Reactive gliosis with astrocytic hypertrophy appearing in many non-neoplastic reactive lesions related with infarction, hemorrhage, metastasis, inflammatory and demyelinating processes might exhibit morphological features typical for low-grade or diffuse astrocytomas. The diagnostic difficulties of diffuse gliomas is related with cytologic pleomorphism and heterogeneity of cellular compartments including giant, spindle, epithelioid, lipid-rich, granular and small undifferentiated cells. Extreme cytologic pleomorphism might be seen in both, low-grade astrocytic lesions as pleomorhic xanthoastrocytoma as well as in high-grade glioblastoma. The combination of astroglial tumour and preserved, normal neuronal cells should not be mistaken as a ganglioglioma. Moreover, cells of ganglion appearance may be present in well-differentiated and malignant gliomas but immunoreactivity for GFAP often evidences their astroglial nature. Gemistocytic and vacuolated tumour cells might resemble mixture of reactive astrocytes and macrophages in demyelinating diseases. Oligodendroglial-like cells with perinuclear halos and round nuclei might be seen in low-grade pilocytic astrocytoma and malignant gliomas. Glioblastoma exhibits multiple tissue pattern with small or giant cells, oligodendroglial component, PNET-like tissue, mesenchymal phenotype, glandular differentiation, epithelioid or rhabdoid components or perivascular formation. Differential diagnosis includes a variety of primary and metastatic lesions including anaplastic oligodendroglioma, anaplastic ependymoma, medulloblastoma (PNET, rhabdoid) teratoid tumour, metastatic carcinoma, melanoma or primary lymphoma. Moreover, due to necrosis and acute inflammation in some glioblastomas the infarct, post-radiation ischemic lesions, inflammatory process and abscesses might be also considered.

Nowadays is obvious that management of gliomas depends on histological diagnosis but genetic studies might provide a useful genetic background of the tumors that could help to establish the treatment procedures.

Anti-tumour effect of novel pentabromobenzylisothioureas studied on human T98G glioblastoma cell lines and SEGA-derived cultures

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Tumours of astroglial origin are the most frequent primary brain malignancies. The prognosis of malignant gliomas remains very poor as they are charac-

terized by infiltrative growth and resistance to current anti-tumour therapy. Progression of malignant gliomas growth is thought to be related with intracellular signal transduction pathways involving activation of protein kinases that are considered to play an important role in differentiation, proliferation and cell survival. The specific novelty inhibitors of constitutively active serine/threonine kinase (CK2) i.e. isothiourea derivatives have been suggested to induce apoptosis and affect proliferation in some human cancer cells.

In this study we examined the cytotoxic and proapoptotic activity of a number of selected modified isothiourea derivatives – pentabromobenzylisothioureas bromides (ZKKs) against the adult human glioblastoma T98G cell line and cell lines derived from rare, low-grade pediatric brain tumour of a mixed glioneuronal lineage (subependymal giant cell astrocytoma – SEGA), and normal human cultured astrocytes. All tested ZKKs compounds revealed cytostatic effect towards glioma cells but two of them: ZKK-3 and ZKK-2 appeared to be most effective and showed a strong anti-proliferative activity and high apoptotic effect in neoplastic astroglial cells lines. Treatment of T98G and SEGA-derived cell cultures with M ZKK-3 and ZKK-2 at concentration of 50 μ for 48 h induced apoptosis and strong inhibition of cell proliferation up to 60% and 50% respectively (determined by flow cytometry analysis and Multisizer3 Beckman Coulter).

The data obtained *in vitro* documented that novel pentabromobenzylisothioureas (ZKKs) might potently inhibit cell proliferation and induce apoptosis in cultured neoplastic astroglial cells in tumours of various malignancy. These results might suggest their potential efficacy as anti-tumour agents against glioma-derived neoplasms.

Why do glioblastoma cell lines represent only minor fraction of these tumors?

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Glioblastoma cell lines showing EGFR amplification are unique. Moreover, stable cell lines of glioblastoma present genetic patterns, which are considered are extremely rare in tumors specimens. Our results indicate that classical monolayer conditions select normal glioma associated stromal cells versus tumor cells showing EGFR amplification, due to a complex reasons: senescence, proliferation arrest, mitotic catastrophe, and occasionally apoptosis. Additionally, amplicons extrusion and low adhesion ability can be partially responsible for difficulties in culturing glioma cells with EGFR amplification.

Glioblastoma cells showing features of stemness are affected by those phenomena as any glioblastoma cells. Novel monolayer serum free conditions did not protect glioblastoma cells from aforementioned processes. Importantly, all subpopulations of glioblastoma cells derived from tumor specimens with the EGFR amplification proliferate in 3D conditions for no less than 4 months.

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Malignant astroblastoma. Case report

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Astroblastoma is a rare neuroepithelial tumor of uncertain histogenesis and malignancy, without established grading in the current WHO Classification of Tumours of the CNS. Astroblastoma is defined by distinctive microscopic pattern of pseudorosette arrangement of tumour cells around hyalinized blood vessels and by low-grade or anaplastic (high-grade) histopathological characteristics, while its clinical and biological behavior and mode of therapy remain still unsettled.

We report the clinicopathologic feature of malignant astroblastoma in a case of 26-year-old woman, followed for 6 years. The patient presented for the first time with a short history of headache, nausea, vertigo and slight left hemiparesis. MRI revealed a well-circumscribed cystic tumour of 70 ×

26 × 60 mm, with a solid contrast enhancing mass of 38 × 36 × 26 mm. Tumor was located in peripherally in the right parieto-temporal region. Surgical total resection of the tumor, adjuvant postoperative radiotherapy and chemotherapy with Temozolamide were performed. Subsequent twofold reoperations for local tumor recurrence were carried out 3 and 4 years after the first surgery. The last operation was subtotal and was followed by rapid tumour recurrence and progression. The patient died in the 6-th year after detection of the first lesion.

Histological examination of the primary and recurrent tumours revealed combined pattern of typical astroblastic pseudorosettes and sheaths of anaplastic tumour cells, respectively in cystic and solid tumour areas. Neoplastic cells were immunopositive for GFAP, S-100 protein, vimentin and negative for EMA and neuronal markers. Frequent mitoses and high MIB-1 labelling index were seen in the compact tumour areas with high cellular density. Mitoses were absent and MIB-1 labelling index was low in the tumour areas characterized by perivascular pseudorosettes. The presented case is considered as malignant astroblastoma exhibiting features characteristic for both anaplastic (high-grade) as well as low-grade astroblastoma in the primary and recurrent tumors.

Gluamine, glutamate and glutaminase in gliomas

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Elevated glutamine (Gln) metabolism provides energy and biomass required for glioblastoma cell growth. In the first step of glutaminolysis, catalyzed by phosphate-activated glutaminase (GA), Gln is converted to glutamate (Glu) and ammonia. Glu released from glioma cells plays a crucial role in tumor growth and invasion. In mammals, there are two genes coding for GA: the *GLS* gene encodes kidney-type (K-type) isozymes, whereas the *GLS2* gene encodes liver-type (L-type) isozymes. Deregulated expression and/or activity of GA isoforms is a hallmark of different tumors and neoplastic cell lines. It has been speculated that GA isoforms play opposite roles in tumorigenesis: expression of *GLS* is correlated with high rate of cell proliferation, whereas expression of *GLS2* is associated with low proliferation rates and is characteristic for resting or quiescent cells. In malignant glia-derived tumors, GA isoforms coded by the *GLS* gene are overexpressed, whereas *GLS2*-coded isoforms are hardly detectable in there. Silencing of *GLS* reduces growth of glioblastoma cells *in vitro* and *in vivo*. Overexpression of *GLS2* gene decreases proliferation and migration of glioblastoma cells and sensitized them to the alkylating chemotherapeutics. Moreover, *GLS2* isoforms play significant role in controlling redox status in glioblastoma cells. These findings demonstrate the critical role of Gln metabolism in the manifestation of aggressive glial tumor phenotype.

The importance of immunohistochemical expression of new markers in the diagnosis of diffuse gliomas

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Over the past 25 years, there has been an increasing use of immunohistochemical markers in the assessment and management of adult gliomas. The availability of poly- and later monoclonal antibodies that selectively bind to epitopes on formalin-fixed paraffin embedded tissue has dramatically improved routine neurooncology (Table I). Some molecular signatures are used diagnostically to help neuropathologists classify brain tumours, whereas others are used to estimate prognosis for patients. Most crucial, however, are those markers that are used to predict response to certain therapies, thereby directing clinicians to a particular treatment while avoiding other potentially deleterious therapies. In the last decade, there have been considerable improvements in the way that brain tumours are characterised. Knowledge of genomics, proteomics and the molecular level has matured and the demand for new diagnostic markers and biomarkers in human gliomas has increased. Immunohistochemistry is an attractive tool that may allow conclusions with regard to the origin of the tumour

tissue and to prognosis by means of protein expression without the need for a sophisticated molecular laboratory to test for genetic markers.

Astrocytomas, oligodendrogliomas, and oligoastrocytomas, collectively referred to as diffuse gliomas, are the most common primary brain tumours. These tumours are classified by histologic similarity to differentiated astrocytes and oligodendrocytes, but this approach has major limitations in guiding modern treatment and research. This review focus on the role of immunohistochemistry in the routine diagnosis of such diffuse gliomas using selected new markers which seem useful in assisting with the diagnosis (Table I). Diffuse gliomas are a very heterogeneous group of glial tumours in terms of pathological features and clinical outcome. In addition, the diagnosis of these tumours on the basis of morphological criteria is challenging in daily practice. Over the last few years, several molecular markers with clinical significance have been identified in diffuse gliomas. The most relevant molecular biomarkers are: 1p19q codeletion associated with oligodendroglial phenotype and better prognosis; isocitrate dehydrogenase (IDH) 1 and 2 mutations associated with better prognosis; TP53 mutations associated with astrocytic phenotype; and MGMT promoter methylation associated with better prognosis. Our study showed that some immunohistochemical tests have proven to be useful in the stratification of diffuse gliomas, whereas the utility of others for the day-to-day practice of pathology awaits further study and validation. The importance of critical literature review and careful consideration of practical issues such as test standardisation, compliance, cost-effectiveness, and availability must all be considered before implementing any new immunohistochemical test in his own laboratory. Most of the new markers are transcription factors which show nuclear positivity. This makes possible quantitive evaluation with a high degree of consistency and reproducibility. It could be helpful to overcome a high inter-laboratory and inter-observer variability which seriously hampers comparison of results from different studies.

Taken together new immunohistochemical markers are contribute greatly to the classification of diffuse gliomas and should be tested routinely as diagnostic markers. We are currently evaluating the clinical relevance, and preliminary results show the additional clinical value next to the histopathological and immunohistochemical classification. The small sample size of patients and the prospective nature of this study is major limitations of this report. Further studies are warranted to validate these interesting results in larger patient populations.

Table I. Immunohistochemical markers used or intend to use routinely in the diagnosis of brain tumours.

	Before 1980	1980-2000	"New" markers
Glial markers	S100	GFAP Vim	IDH1 IDH2 Olig2
Neuronal markers	NSE	NF/NFF PGP9.5 Syn ChrA	NeuN INA
Proliferation markers	–	Ki67 PCNA	P53 MCM2
Other markers		Ker Des SMA EMA	CK group CD group EGFR MGMT