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Malignant gliomas, the most common primary brain tumors in adults, are genetically heterogeneous and their tumor microenvironment (TME) consists of various stromal and immune cells playing an important role in tumor progression and responses to therapies. Clinical and experimental studies demonstrated the presence of both resident microglia and peripheral monocytes/macrophages in the TME of malignant gliomas, however failed to dissect their functional phenotypes due to a lack of reliable markers. Single-cell RNA sequencing (scRNA-seq) and cytometry by time-of-flight (CyTOF) are powerful techniques allowing quantification of transcriptomes or >30 protein targets in individual cells. Those studies demonstrated the diversity of myeloid and lymphoid infiltrates in the glioma TME and various contributions of immune cell populations in gliomas with different genetic alterations. A ratio of microglia and monocytes-derived macrophages differs in IDH-wt and IDH-mut gliomas, with the predominance of microglia and lower infiltration of lymphocytes in the later. Across molecular glioblastoma subtypes, the mesenchymal (MES)-like state was associated with increased myeloid cells and T cell accumulation. The studies have identified cell subpopulations and signaling pathways that promote tumor progression, influence patient survival or make tumors vulnerable to immunotherapy. The accumulating results show that a precise definition of functional phenotypes of myeloid and lymphoid cells in gliomas might be essential for designing effective immunotherapies.
of the young (often abbreviated as PLNTY); and Diffuse low-grade glioma, MAPK pathway-altered. The high-grade group also comprises 4 types: Diffuse midline glioma, H3 K27-altered; Diffuse hemispheric glioma, H3 G34-mutant; Diffuse pediatric-type high-grade glioma, H3-wild-type and IDH-wildtype; and Infant-type hemispheric glioma. WHO CNS5 now lists 2 molecularly defined types of supratentorial ependymoma: one with ZFTA fusion and another with YAP1 fusion. It also now includes 2 molecularly defined types of PF ependymoma, group PFA and group PFB, as well as a spinal tumor defined by the presence of MYCN amplification. In contrast to previous WHO classifications, the myxopapillary ependymoma is now considered CNS WHO grade 2. The embryonal tumors (aside from Medulloblastoma) are AT/RT; Embryonal tumor with multilayered rosettes (ETMR); CNS neuroblastoma, FOXR2-activated; and CNS tumor with BCOR internal tandem duplication.

Reference

Cerebral angiopathy and the diffuse inflammatory process accompanying COVID-19 disease
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The world is struggling with pandemic since 2019 caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing the disease COVID-19. During the ongoing global pandemic of COVID-19 with recurrent waves in most countries, more than 130 million people were infected and there were about three million deaths worldwide. The disease COVID-19 produces varying symptoms, with malaise, fever headache, olfactory disorder, cough, and difficulty of breath being common. The respiratory system is affected the most but clinically manifest also damage to the central nervous system is manifested. However, the detailed neuropathological sequelae remains largely unknown. We report neuropathological changes in 52 patients with COVID-19 disease aged between 22 years and 88 years. Most patients had different comorbidities and died under different circumstances. Samples from all brain structures and lung specimens were taken for histopathological, immunohistochemical and some samples for ultrastructural examinations. The numerous neuropathological changes in the brains of the patients with COVID-19 disease were caused by pre-existing diseases of patients and/or by necessary treatment.

However, irrespective of coexisting diseases, disease duration and type of death, changes in the wall of vessels with coexisting microbleeds/petochial hemorrhages as well as perivascular and diffuse inflammatory processes of varying severity were observed in the brains of all COVID-19 patients.

Methods to support peripheral nerve regeneration

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Neuropathy, or damage to peripheral nerves, is usually manifested not only by sensory disturbances but also by movement problems. The most common symptoms are pain, hypersensitivity, and paresthesia. Neuropathy can be limited to a single nerve (mononeuropathy) or multiple peripheral nerves (polyneuropathy). The muscles innervated by the damaged nerves undergo gradual atrophy due to loss of impulses. There are several degrees of nerve damage. These are neuropraxia, axonotmesis, and neurotmesis. Only in the first case is a spontaneous return of function possible. A number of methods have been developed to aid in the regeneration of peripheral nerves. Starting from surgical techniques, supplementation with appropriate preparations to modern methods supporting transplantation and reaching for discoveries in the field of nanotechnology.
Molecularly targeted therapies in adult type gliomas

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Currently only minority of patients with brain tumours benefit from molecularly targeted therapies, but identification of new molecular targets and development of new treatment strategies will hopefully improve efficacy and lead to their wider use.

Encouraging results of early phase trials with drugs using isocitrate dehydrogenase (IDH) mutations as potential targets in patients with IDH mutant recurrent or progressive glioma were reported.ivosidenib selective IDH1 inhibitor and Vorasidenib (AG-881) first-in-class, dual inhibitor of mutant IDH1/2, both showed activity in non-enhancing tumours. This led to a phase 3, randomized study of Vorasidenib (AG-881) in patients with residual or recurrent Grade 2 glioma with an IDH1 or IDH2 mutation (INDIGO trial) which is now open for recruitment.

Another approach was used in NOA-16 first-in-human study of an IDH1(R132H)-specific peptide vaccine (IDH1-vac) inducing specific therapeutic T helper cell responses. In 33 patients with newly diagnosed grade 3 and 4 IDH1- (R132H)+ astrocytomas. Vaccine-induced immune responses were observed in 93.3% of patients, three year progression-free and death-free rates were 0.63 and 0.84 respectively.

BRAFV600 mutated brain tumours are rare in adult patients and data from cases treated with BRAF Inhibitors alone or in combination with anti MEK therapy show high response rates and warrant further research.

In rare (less than 2% of brain tumours) diffuse gliomas with NTRK fusions, first generation NTRK inhibitors were found to induce responses to treatment in majority of patients. Efficacy of second-generation of NTRK inhibitors is being explored in clinical trials to compare their efficacy with first-generation drugs and, to overcome tumour resistance to first line compounds.

Current techniques in the surgical treatment of gliomas

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Gliomas are the most frequent primary brain tumors in adults comprising 30-40% of all intracranial neoplasms. Besides histopathology and molecular characteristics, the key prognostic factor is localization of a tumor. Eloquent and peri eloquent areas of the brain responsible for speech, somatosensory, motor, and other neurophysiological functions are frequently invaded by a growing tumor. The gold standard for treatment of brain tumors is a maximal surgical resection with preservation of cortical areas and white matter pathways. Maximal cytoreduction combined with adjuvant therapy significantly increases progression-free and overall survival (PFS and OS, respectively) concurrently preserving the quality of life after the surgery. Moreover, harvesting of adequate amount of tissue samples is essential for correct diagnosis that must include genetic profile of a tumor. Significantly improved survival rate was noted for the extent of resection (EOR) higher than 90% and < 10 ml of residual tumor volume. Since visual delimitation of tumor margins is challenging and hardly possible, achievement of EOR can be facilitated by the awareness of central nervous system plasticity and implementation of modalities for pre- and intraoperative planning. Eloquent areas can be mapped with several imaging techniques such as functional MRI (fMRI), diffusion tensor imaging fiber tracking (DTI), transcranial magnetic stimulation (nTMS), and evaluation of linguistic performance during awake surgery. Furthermore, the enhancement of tissue differentiation can be achieved with the application of fluorescent dye and blue light during a surgery.

The authors review recent advancements in surgical management, the impact of preoperative planning, and cooperation in a multidisciplinary team on treatment outcomes of gliomas located in brain eloquent areas.

Key words: glioma, brain mapping, DTI, fMRI.
Genetically determined cerebral microangiopathies

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Genetically determined microangiopathies belong to a group of vascular disorders called cerebral small vessel disease which affect the small arteries, arterioles, veins and capillaries of the brain, but often also the eye and internal organs. Cerebral microangiopathies can be asymptomatic but they usually cause strokes, cognitive dysfunction, mood disorders, and white matter hyperintensities on brain imaging. Research in humans has identified several genetically determined microangiopathies such as CADASIL, CARASIL, CARASAL and others, but the relationships between mutations, lesions and symptoms are poorly understood. Diagnostics is based on skin-muscle biopsy examination and genetic tests, and can often be difficult. Nevertheless, early identification of cerebral microangiopathies is important as it allows patients to avoid further unnecessary tests and modify the treatment of comorbidities, and possibly identify potential targets for intervention in the future and prevent disease progression before symptoms develop.

New approach to the classification of idiopathic inflammatory myopathies

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Since seminal papers of A. Bohan and J.B. Peter in 1975 [1] which paved a way to understanding of pathology of myositis establishing the distinction between polymyositis and dermatomyositis lots of new discoveries have been made elongating and broadening the “way” substantially, yet its final destinations obviously lie somewhere ahead. Later on, myositis that form separated nosological entities have been named collectively as idiopathic inflammatory myopathies (IIM), which term encompasses: dermatomyositis (DM), polymyositis (PM), inclusion body myositis (IBM), and immune mediated necrotizing myopathy (IMNM). Recently, following new discoveries and clinical observations which resulted especially in understanding of the crucial role of so called “myositis specific antibodies” (MSA) the time has ripen to re-evaluate our views on IIMs. The accumulated data tend to convince to the new approach to idiopathic myositis and to their new division [2]. It is proposed that the following four main entities should replace the “old” constituents of IIMs, i.e.: DM (in itself perceived as a “spectrum”), IMNM (also as a sort of “spectrum”), antisynthetase syndrome (similarly as spectrum ASS), and (sporadic) IBM. In the offing there are some other entities as myopathy associated with antimitochondrial M2 antibody (AMA), myositis associated with anti-PD-1/ PD-L1 inhibitors (see review [3]). However it seems disputable whether they are truly “idiopathic”. Moreover the “scene” of myositis is further complicated if one considers many “comorbidities” with extra-muscle pathology that seem to justify the term “overlap myositis”. One of many practical, clinical and neuropathological consequences of the new approach to myositis is “disappearance” of PM from IIMs. The short review of the new theoretical and practical-neuropathological approach to IIMs will be presented. Supposedly new discoveries may narrow indications for the biopsy, all the same the task of neuropathologist becomes more complicated demanding not only to consider clinico-pathologic but also clinico-serological-pathological (and even genetical) correlations.
Breathing impairments in neurodegenerative disorders

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Alzheimer's disease (AD) and Parkinson's disease (PD) are most common age-related, brain neurodegenerative disorders. AD is recognized as a most prevalent reason for dementia affecting more than 45 million worldwide. PD mainly impacts the motor system and affects an estimated 10 million human population. In addition to their characteristic symptoms, both disorders present with respiratory problems, which are often underdiagnosed and overlooked in treatment. Regarding AD, the most common breathing impairments are respiratory dysrhythmias, shortness of breath, bronchitis, pneumonia, and obstructive sleep apnea that impair cognitive function. In PD respiratory disturbances include dysrhythmic breathing pattern, tachypnea, dyspnea, decreased respiratory pressure, sleep disordered breathing and reduced exercise tolerance. Respiratory impairments have been associated with the process of aging, such as postural changes, decreased lung capacity and respiratory muscles weakness, however they are exacerbated in neurodegenerative disorders compared to healthy adult controls. It is hypothesized that some of the respiratory problems appearing in neurodegenerative diseases may arise from pathological changes occurring in the areas of the brainstem responsible for breathing control. This lecture discusses the most common respiratory disorders associated with AD and PD, their likely causes, and treatment attempts.

Exosomes and immune response in neurological paraneoplastic syndromes

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Neurological paraneoplastic syndromes (NPS) are remote effects of cancer, which may lead to disturbances in central nervous system, peripheral nervous system, neuromuscular junction and skeletal muscles. NPS are immune-mediated. Cytotoxic T lymphocytes play a crucial role in NPS pathophysiology. However, a humoral response is initiated as well and leads to the production of onconeural antibodies. The pathophysiological role of onconeural antibodies is still under discussion, but there are no doubts about their role in NPS diagnosis.

Extracellular vesicles are defined as cell membrane-bound structures that separate from the cell and transport its contents. Extracellular vesicles include exosomes, microparticles, microvesicles, and apoptotic bodies. Exosomes and microvesicles diameter ranges from 30-200 nm. They derive from eukaryotic cells following direct damage, apoptosis, or activation. Exosomes contain elements retained from their precursor cells and carry a variety of molecular cargo, including proteins, receptors, and nucleic acids.

Moreover, exosomes provide an alternative yet crucial pathway for intercellular communication in addition to soluble mediators and direct cell-to-cell contact and have thus been implicated in the pathophysiology of various inflammatory diseases, immune response, and cancer-host tissues interplay. Exosomes are able to modify the neoplastic microenvironment by angiogenesis regulation and communication with healthy cells. Exosomes exhibit immunosuppressive effects and lead to neoplasm “escape” from immune system control. Thus the studies on the role of exosomes in anti-cancer immune response may elucidate pathomechanisms that underly NPS.

We have found that exosomes isolated from cancer patients with NPS exhibit higher activities of Na⁺/K⁺-ATPase than cancer patients without NPS or without onconeural antibodies (Michalak et al., J Clin Lab Invest Update 2013). The expression of perforin, a biomarker of cytotoxic response, is lowered in exosomes isolated from ovarian cancer patients compared to endometrial cancer (Wyciszkievicz et al., Sci Rep 2019). Moreover, only in exosomes we have found strong positive correlations between small heat shock proteins like alpha-B Crystallin and perforin in ovarian and endometrial cancer patients, Hsp20 and perforin in ovarian cancer, and Hsp22 and perforin in ovarian cancer patients. A marker of cytotoxicity – gran-
zyme B also correlated with alpha-B Crystallin in ovarian cancer and Hsp 20 both in ovarian and endometrial cancer. In another study (Zaborowski et al., Cancer Immunother 2021), we observed increased expression of granzyme B in peripheral blood mononuclear cells (PBMC) in NPS patients with onconeural antibodies compared to seronegative NPS patients.

To conclude, the expression of small heat shock proteins is associated with a cytotoxic response without such relation in ovarian patients serum or peritoneal fluid. Sero-positive NPS are associated with up-regulation of exosomal Na⁺/K⁺-ATPase and granzyme expression in PBMC. Thus exosomes studies can currently be used not only for “liquid biopsy” but also for monitoring of anti-cancer immune response and NPS evaluation.

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**New biochemical and neuropathological markers of traumatic lesions of the central nervous system in postmortem examination**

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Among the biochemical and neuropathological markers of traumatic changes, there are proteins derived from neurons and glial cells. In studies identifying markers of traumatic lesions, we compared two groups – a control group (sudden cardiac deaths) and a study group (potentially fatal head injuries). The groups included deaths directly at the scene, without CPR and hospitalization. The study groups did not differ in age statistically, and the groups excluded changes and neurodegenerative diseases of the central nervous system. Biochemical studies included ELISA tests determining the concentration of selected biomarkers in body fluids. Neuropathological studies included, among others, immunohistochemical staining for these markers to verify the observed changes. The material collected for biochemical tests was cerebrospinal fluid, serum, urine, saliva, and the vitreous body of the eye. The material collected for neuropathological examinations was the frontal lobe with the corpus callosum. The material was collected up to 24 hours after death. In summary, selected neuropeptides from the central nervous system are “early” markers of experienced head injuries in post-mortem biochemical and neuropathological examinations. The cytoskeleton proteins involved in axonal transport are “early” markers of experienced head injuries in neuropathological studies, and may also indicate functional disorders of the central nervous system following head injuries. Head injuries (even if they are not fatal) result in structural and functional damage to the blood-brain barrier (not visible in macroscopic examination), which can be documented by immunohistochemical staining. Phagocytic microglia may be involved in the “transition” of neuropeptide markers from the brain to body fluids.

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**Molecular factors important for the exosomes functioning in patients suffering from Alzheimer’s disease**

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**Introduction:** In the modern era predicted life expectancy increases which leads to higher percentage of elderly in population. As the aging process progress in the society there is an increasing risk of neurodegenerative disorders. Especially the Alzheimer’s disease (AD) which is one of the most frequent pathology affecting patients worldwide. Recent findings may suggest the exosomes as one of the potential factor taking part in AD pathology. Expression products of genes APOE, RAB11A and TSG101 may have a potential role in AD neuropathology. In our study we have focused on analysing genetic variants of APOE (E2/E3/E4), RAB11A (exon 2) and TSG101 (exons 8 and 9) as well as RAB11A protein plasma levels.

**Material and methods:** Blood and plasma samples have been used: 50 samples from AD patients, 55 samples from patients that have AD history in the family, 51 samples from control group. Mismatch primer qPCR method has been used for APOE (E2/E3/E4) analysis. Genetic variants of RAB11A (exon 2) and TSG101 (exons 8 and 9) have been analysed with HRM and confirmed by sequencing. RAB11A protein levels have been measured with ELISA kit.

**Results:** There are no signs of genetic variants in analysed exons of RAB11A and TSG101. APOE E4 variant has been found in significantly more AD patients comparing to control group. Moreover, the AD patients have shown a trend of higher RAB11A protein level median, especially in APOE E4 patients.

**Conclusions:** Possibly the RAB11A protein levels that influence exosome functioning may be the future marker of exosomal dysfunction. However the exact role of APOE and RAB11A interactions in AD needs further studying.
Nocardia brain abscess – presentation of an unusual case requiring a broad differentiation spectrum

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58-years-old man diagnosed due to the first epileptic seizure in his life. The imaging studies (CT and MRI with contrast) revealed the presence of a focal lesion in the frontal lobe of the right brain hemisphere. The patient was qualified for neurosurgical treatment. During the surgery, the tumor of astonishingly increased cohesion with the cystic part was removed. Intraoperative histopathological evaluation did not indicate neoplastic infiltration, instead the suggestion of the abscess was stated. The microbiological diagnosis of Nocardia abscessus was based on the culture of the explant from tissue retrieved intraoperatively. This microbiological diagnosis seemed to be congruent to the neuropathological picture especially regarding conspicuous extremely dense mixed fibrotic/glial reaction, though the fungi could not be directly visualized in tissue.

A case of PLNTY associated with ICH and FCD

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15-years-old boy experienced first in life absence epileptic attacks and was diagnosed with small focal hemorrhage in the left temporal lobe. MRI appearance of the lesion was suggestive of cavernous malformation. After institution of antiepileptic medication he was operated on, and standard lesionectomy was performed.

Neuropathological examination revealed features of focal cortical dysplasia, past cerebral hemorrhage and infiltration of oligodendroglioma like neoplastic cells with numerous microcalcifications. Immunohistochemical studies showed low mitotic index and strong CD34 expression. This picture was consistent with diagnosis of PLNTY – polymorphous low-grade neuroepithelial tumor of the young, an epilepsy associated neoplasm recently included in the WHO 2021 classification of CNS tumors. This is the first known case of PLNTY which presented not only with epilepsy but also hemorrhage.

Primary isolated angiitis manifesting as a tumor of temporal lobe in a woman 40 years old

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Introduction: Primary central nervous system (CNS) vasculitis is uncommon disorder restricted to the brain and spinal cord. The neurological manifestations include headache, altered cognition, focal weakness, or stroke. The histopathological examination of the lesion is the only way that allows to make a definite diagnosis.

Case description: 40-year-old woman with increasing headache, dizziness, episode of speech disorder and a drop of the corner of her mouth and later seizures. MRI of the head showed the presence of a nonspecific ring enhancing lesion in the fronto-temporal region of the right cerebral hemisphere. The lesion was surrounded by a finger like edema. In the middle of the lesion, vascular structures could be observed.

Subsequent follow up examinations suggested an inflammatory lesion (for instance sarcoidosis) or, less likely, lymphoma. Further radiological examinations suggested even the possibility of glioblastoma.

Neuropathological examination showed: Features of gliosis with hypertrophic gemistocyte like astrocytes incl. so called Alzheimer type I cells, numerous perivascular and intraparenchymal lymphocytic, infiltrates mostly of CD3+ cells (CD20+ cells only scarce), mitoses absent, LI Ki-67 approx. 3. Especially conspicuous were numerous vessels with obliterated lumen. Clinical and radiological hypothesis of glioma was rejected with suggestion of vasculitis as an essential pathology.

The biopsy was repeated and in the second operation the tissue material was much more ample and once again
revealed strikingly conspicuous vascular pathology (features of vasculitis, thrombosis, intima and media proliferation).

Interestingly, inflammatory infiltrations were not limited to vessels but also dispersed among parenchyma necessitating the exclusion of systemic lymphoproliferative disorder. In the follow up (6 months), the patient feels well and is under periodical control.

**Conclusions:** Primary CNS vasculitis is a well-established pathological entity [1], but the differentiation is extremely difficult since it must include not only glioma, lymphoma but also particular forms of CNS inflammation like neurosyphilis and giant cell arteritis, Whipple disease and many other conditions. Unfortunately clinical and radiological data are not very helpful [2]. As the result, the diagnosis burdens neuropathologist with extreme responsibility necessitating especially cautious and meticulous workup.

**References**

### Brain morphological changes in COVID-19

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**Introduction:** COVID-19 is a global disease and was characterized as a pandemic by the WHO. According to WHO as of 15 September 2021, there have been 226 897 779 confirmed cases of COVID-19, including 4 666 804 deaths in the world. The last updated on September 15 2021 for Ukraine indicates that coronavirus cases were 2 325 796 and deaths were 54 550. The coronavirus can damage the lungs, heart and also brain. COVID-19 related CNS manifestations include anosmia or ageusia, encephalopathy, encephalitis and acute cerebrovascular disease. Notable clinical symptoms in COVID-19 patients were CNS manifestations, mostly in the presence of comorbidities such as type 2 diabetes mellitus and hypertension.

**Purpose:** To investigate the brain tissue of patients who died from COVID-19.

**Methods:** All patients died in Lviv regional and city hospitals from complications of COVID-19 and had a positive test for SARS-CoV-2 detected by qRT-PCR reaction. Patients were autopsied between March 2020 and September 2021 and information from the autopsy protocol included macroscopic and microscopic characteristics of brain and general autopsy findings. We used common histological methods of staining with hematoxylin and eosin, immunohistochemistry staining for activated astrocytes (GFAP, Thermo scientific), activated microglia (CD68, Clone Ab-4, Thermo scientific) and T lymphocytes (CD3, Clone SP7, Thermo scientific) in the cortex, basal ganglia, brainstem and cerebellum. The study was conducted in accordance with research regulations and conformed to the Declaration of Helsinki.

**Results:** This post-mortem study included 94 cases. The patients had a median age of 68 years (range, 21-97 years), 54% men. The majority of patients (59.57%, \( n = 56 \)) had hypertension, while 31 (32.98%) had type 2 diabetes mellitus and hypertension, 7 (7.45%) had hypertension and obese or diabetes mellitus, hypertension and obese. Ischemic lesions in the brain with focal encephalolysis were documented in 15 (39.47%) out of 38 patients with type 2 diabetes mellitus, obese and hypertension. In most cases the arteriolosclerosis with perivascular rarefaction was present. Hemorrhagic infarctions were rare. The astrogliosis with positive GFAP was seen in all cases but showed variable degrees. The perivascular activation of microglia and the microglial nodules with CD68 positive cells were in the studied regions of the brain, but less in cerebellum, and perivascular infiltration by CD3 was most pronounced in the brainstem.

**Conclusions:** In summary, the morphological changes of the central nervous system associated with COVID-19 include ischemic infarction with encephalolysis, astrogliosis, microgliosis, perivascular infiltration by CD3 in different regions of the brain.

### ABCB1/MDR1 genetic variants in patients with migraine treated with antiepileptic drugs

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Migraine is a chronic disease that significantly reduces the quality of life of patients. There are currently no drugs that can effectively treat migraine attacks. Failure to respond to treatment may be due to altered expression of P-GR a product of the ABCB1/MDR1 gene. The study aimed to analyze the frequency of the C1236T, G2677 (A/T), and C3435T polymorphisms in the
ABC1/MDR1 gene in patients with migraine treated with antiepileptic drugs (AEDs).

**Material and methods:** 146 people were examined, including 46 people diagnosed with migraines without aura (MO) and 27 with diagnosed migraines with aura (MA), and 73 controls. DNA was isolated from the blood of the test subjects. Genotyping of polymorphisms was performed using HRM point analysis of PCR-HRM products and confirmed by sequencing.

**Results:** The study shown that the homozygous TT C1236T the genotype of the ABC11/MDR1 gene seems to be associated with MO (p < 0.05). It was also found that the TT G2677AT genotype of the ABC1/MDR1 gene reduces the risk of aura in migraine patients (p < 0.05). The C allele of C1236T predisposes to aura in patients with migraines (p < 0.05). In addition, a tendency was observed in patients with migraine treated with AEDs to a more frequent occurrence of the C allele of C1236T, the G allele of G2677AT, and the C allele of C3435T, which may predispose to the use of AEDs in patients with migraine.

**Conclusions:** Further studies of ABC1/MDR1 gene polymorphisms could in the future be used for personalized design migraine pharmacotherapy.

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**The role of BET proteins in controlling the phagocytic activity of microglia**

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The proper function of microglia is a prerequisite of maintaining homeostasis in the brain, and its dysregulation may significantly contribute to neurodegenerative processes. Especially, the deregulation of microglia-mediated phagocytosis may have detrimental effects on the brain tissue in the course of chronic neuroinflammation. In search of an effective and specific method of regulating microglial activity, we focused on the bromodomain and extraterminal domain (BET) proteins: Brd2, Brd3, and Brd4 that, in cooperation with transcription factors, control microglial gene expression. In this study, we used pharmacologic (cell-permeable inhibitor JQ1) and genetic (siRNA-mediated gene silencing) inhibition of BET in immortalized murine microglial cell line BV2. Our data demonstrated that the expression of BET proteins did not change in activated microglia. However, inhibition of BET proteins significantly reduced the expression of several inflammation-related genes, including IL-1β, TNF-α, Nos2 in BV2 cells treated with lipopolysaccharide (LPS). Also, suppression of BET attenuated LPS-induced phagocytizing activity of microglia. Our results indicate that inhibition of BET proteins may be the specific and efficient method of modulating microglial activity that could offer a beneficial treatment strategy in neurodegenerative disorders.

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**Graphene in neuroscience – presence and future**

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This is a brief review to address the accomplishments and perspectives of graphene and its compounds application in neuroscience. Non-invasive and innovative treatments for many neurological disorders remain a major challenge. The key seems to be the development of nanoparticles capable of crossing the blood-brain barrier and the delivery of therapeutic compounds. The development of imaging technology and attempts to regenerate neurons is also a target for neuroscience.

Thanks to their properties, graphene and graphene-derived materials have been tested and applied in some biomedical nanotechnological fields for few years. Due to its chemical composition, the surface of graphene enables its non-toxic influence also at the cellular level. Therefore, in neuroscience, graphene and its derivatives can be used as nanocarriers for drug delivery in neurooncology and neurodegenerative diseases. Graphene nanocomposite tags are suitable for various types of bioimaging. The ability to stimulate the growth and differentiation of neurons allows the use of graphene materials also in regenerative medicine and even for the treatment of brain developmental disorders. Conductivity as an important property of graphene biomaterials enables their use as biocompatible substrates for tissue engineering, as well as conductive electrodes for response profiling and the study of neural networks.

The material composition of graphene compounds, its functionalization and hybrids, as well as the dimension of particles are an important aspect because of their pivotal diversity of biocompatibility, safety, toxicity and activity. Graphene may be a promising material for the exploration of the nervous system and future therapy modality.

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Age dependent alterations of transcription of genes encoding proteins related to mitochondria biogenesis in animal model of Alzheimer’s disease

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Alterations of mitochondria function are proposed to play a crucial role in pathomechanism of Alzheimer disease (AD). Peroxisome proliferator activated receptors (PPARs) act as a potent transcription factor and a regulator of mitochondria metabolism and function.

This study focused on age dependent alterations of transcription of genes encoding PPARα, and proteins involved in mitochondria biogenesis in AD mice model.

The study was carried out using 3-, 6-, 12-months old FVB mice with London mutation (V7171), AD Tg mice and control mice (without transgene). Brain cortex was used for analysis. Biochemical, immunochemical and qPCR methods were applied.

Our data indicated age dependent regulation of transcription of gene coding PPAR-α in the brain cortex of AD Tg mice. Transcription of gene coding PPAR-α was downregulated in 12 month old AD Tg mice. Furthermore, we have observed that mutant transgene alters in the same manner transcription of gene coding PGC-1α, a transcriptional coactivator, and crucial regulator of mitochondria biogenesis. Moreover, our data indicated transient enhancement of mRNA level for Transcription Factor A Mitochondrial (TFAM) and Neuronal Respiratory Factors (NRF1, NRF2) in 3 months old AD Tg mice and significant downregulation of these genes in 12 months old AD mice. Additionally, we found decrease of genes expression encoding enzymes of anti-oxidative defence in mitochondria. Summarizing this study provide novel data on alterations of transcription of genes responsible for regulation of mitochondria biogenesis in AD and suggested PPAR-α as the promising molecular target for neuroprotection in AD.

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Rudolph Virchow – different approach towards the brain dissection

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This year we celebrate 200th birth anniversary of Rudolph Virchow. This scientist, though small in posture was a real giant in achievements in pathology, but neuropathology as well. When he got to Charite Hospital Prosecutor’s Department in 1842 he probably did not know what legacy would be left after him. Of course now everyone will quote his immense input in development of pathology with Cellular Pathology theory or understanding of circular disorders and its consequences, especially thrombosis and cerebral strokes. But we have to remember that he also changed autopsy as a procedure totally. By this we mean the approach (why it is performed), technique which he tried to standardize and succeeded (how), what can be gained from autopsy (standardisation of the post mortem report and obligatory microscopic analysis of the obtained tissue samples). He opposed the Rokitansky’s school by treating the disease as a full body/system malfunction, hence obligatory analysis of all internal organs, even those which might have seemed unaffected. It also included central nervous system. He acknowledged the autopsy skills of his teacher, Professor Froirep, but disagreed in the technique. Publishing “Die Sections-Technik im Leichenhause des Charite-Krankenhauses” Virchow clarified step by step how organs should be assessed. His method of brain dissections differed from older techniques. Virchow suggested firstly opening the ventricles by cuts done from internal side of the hemisphere to external parts, and later analysing the symmetry of the hemispheres and the subcortical structures by paralel frontal cuts dividing the hemispheres to two parts (frontal and posterior), later cutting those parts in the middle into smaller parts, and so on. As the future has proved this method was more useful in forensic pathology. Nowadays Spielmeyer’s method of brain analysis is preferred, especially in specimens formaline fixed prior to examination.
Isomorphic astrocytoma/glioma: a contribution to considerations on this little known nosological entity based on own cases

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The isomorphic astrocytoma/isomorphic diffuse glioma is a prototypical diagnosis, first proposed by Blümcke et al. in 2004, that combines the characteristics of pilocytic astrocytoma and diffuse astrocytoma. As of 2021, this entity is not incorporated into the WHO classification of tumors of the central nervous system, and as such, this supposed gap in the diagnostic armamentarium of neuropathologists and neurosurgeons creates conditions for misdiagnoses, potentially leading to overtreatment of tumors. In consequence, a case for the official recognition of this subtype is possibly warranted.

We present a short summary on this little-known condition, accompanied by a case series of 3 neurooncological patients, whose resected neoplasms fitted the diagnosis of this subtype.

Evaluation of relationship between Health Assessment Questionnaire Disability Index and pain Visual Analog Scale, Disease Activity Score of 28 joints in patients with rheumatoid arthritis

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Rheumatoid arthritis (RA) is one of the most common chronic inflammatory diseases of unknown etiology, affecting approximately 1% of the population, and leading to functional limitations and premature death [Lancet 2016; 388: 2023; Best Pract Res Clin Rheumatol 2018; 32(2): 174]. RA is characterized by persistent synovitis, systemic inflammation, and autoantibodies (particularly to rheumatoid factor and citrullinated peptide) that mainly affect joints with some extra-articular involvement. Inadequately treated, it usually causes persistent joint pain, irreversible deformities and functional disability leading to a poor quality of life (including mood disturbances such as anxiety and depression) [BMC Rheumatol 2019; 3: 34; 2019; 3: 43]. The aim of the study was to evaluate the Health Assessment Questionnaire Disability Index (HAQ-DI) score and related factors such as the pain Visual Analog Scale (VAS), Disease Activity Score of 28 joints (DAS28) in RA patients before and after 4-weeks comprehensive rehabilitation program [Rheumatol Int 2021; 41(4): 781]. The study demonstrated a statistically significant reduction in DAS28 and VAS scores, as well as an improvement in self-assessment of Health-Related Quality of Life (HRQoL) in patients with moderate to high disease activity, after rehabilitation. In conclusion, the research have shown not only positive rehabilitation effects, but also improved HRQoL of RA patients, regardless of the level of disease activity.

Ceramide-mediated insult is potentiated by sphingosine kinase 1 inhibition and mitigated by sphingosine-1-phosphate receptors activation in alpha-synuclein transduced cells

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Disturbed equilibrium between pro-apoptotic ceramide and pro-survival sphingosine-1-phosphate (S1P) in favour of ceramide is noted in many neuronal diseases, while activation of S1P receptors signalling is recognized as efficient in their therapeutic strategy. In this study, SH-SY5Y cells transduced with lentiviruses carrying a gene for the human alpha-synuclein (α-syn), called further SH-SNCA were treated with cell-permeable ceramide analogue. This performs a double hit model of Parkinson disease, evoked both by α-syn loading and sphingolipid homeostasis disruption. In these conditions, the relative level of sphingosine kinase 1 (Sphk1), one of the enzymes essential for maintaining S1P/ceramide homeostasis was examined. Flow cytometry analy-
Metformin modulates gene expression of inflammatory proteins and sphingosine kinase 2 in diabetic mice hippocampus

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Alterations of bioactive sphingolipids are evident during obesity and type 2 diabetes mellitus (T2DM), that affects millions of people around the world and represents a growing socioeconomic problem. Moreover, the role of bioactive sphingolipids as well as molecular mechanisms by which metformin (first drug of choice for the management of T2DM) exerts its anti-inflammatory effects in brain still remains unknown. In present study we examined the effect of metformin on diabetic mice hippocampal mRNA levels of sphingosine kinase SPHK2, sphingosine-1-phosphate receptors: S1PR1, 3 and pro-inflammatory TNF and IL-6.

10-12 week old male C57BL/6J mice were given high-fat diet (HFD) for 16-weeks. Control group received standard diet (SD). After 8 weeks on HFD, mice were given low doses of streptozotocin (STZ, 15 mg/kg i.p.) to create T2DM. SD mice received appropriate control. After 96 days on HFD, animals started receiving metformin by gavage (250 mg/kg) in water for 2 weeks, then hippocampus was isolated for qPCR analysis. All experiments were analysed using one-way ANOVA followed by Tukey’s post-hoc test.

We observed significant increase of Spk2, S1pr3, Tnf and Il6 gene expression as well as downregulation of S1pr1 in HFD+STZ mice hippocampus. Metformin significantly reduced elevated mRNA levels of Spk2, Tnf and Il6.

Observed results demonstrate important role of metformin as a regulator of genes that encodes pro-inflammatory cytokines (Tnf and Il6) and sphingosine kinase 2 as well as suggest their involvement in inflammatory process that occurs in brain of diabetic mice.

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Diffuse glioneuronal tumour with oligodendroglioma-like features and nuclear clusters (DGONC) – report of a case with molecular diagnostics in progress

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We present a special case of a recurring left temporal lobe tumor in a 32-year-old woman. In 2009, the primary tumour of 65 × 45 × 33 mm was subtotally resected, diagnosed as anaplastic oligodendroglioma (III WHO) due to mitotic activity and microvascular proliferation, and treated with chemo- and radiotherapy. The patient was reoperated twice between 2010 and 2020, showing the same histopathology, but that time not consistent with WHO 2016 oligodendroglioma criteria, being IDH1-wildtype and 1p19q not-codeleted, moreover showing focal neuronal differentiation. The recent regrowth in 2021 examination revealed again oligodendroglioma-like tumor cells with scattered multinucleated cells and atypical neurons. IHC revealed: IDH1R132H negativity, Olig2 (+), ATRX (–), p53 wildtype, GFAP (focally +), synaptophysin (focally +), NeuN (focally +), Ki67 – up to 25%. Based on morphology, immunophenotype and clinical history, the tumour was classified as Diffuse Glioneuronal Tumour with Oligodendroglioma-like features and Nuclear Clusters (DGONC). DGONC is a newly emerging entity of brain tumors in the WHO 2021 classification, showing both glial and neuronal differentiation. It occurs mainly in the pediatric and young adult population, but can occur at any age. DGONC displays monosomy of chromosome 14, but methylation profiling is necessary for the unequivocal diagnosis. The genetic analysis to finally prove our patient’s diagnosis is in progress.