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LECTURES

TDP-43 pathology in the pathogenesis of different neurological diseases

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Transactive response DNA binding protein of 43 kDa (TDP-43) is a highly conserved RNA/DNA-binding protein involved in multiple cellular functions, including regulation of RNA splicing, stability, maturation and trafficking. Under pathological conditions it moves from nucleus to cytoplasm and forms neurotoxic neuronal cytoplasmic inclusions.

TDP-43 inclusions are now considered as a major pathological hallmark of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Although, growing body of evidence indicate that TDP-43 pathology play an important role in the pathogenesis of other neurodegenerative diseases, i.e. Alzheimer's disease, Parkinson's disease, multiple system atrophy, progressive supranuclear palsy, and is identified in the diseases with distinct pathological mechanisms, i.e. post-traumatic (chronic traumatic encephalopathy), neoplastic (pilocytic astrocytoma), post-infectious (post-encephalitic parkinsonism).

The mechanisms of TDP-43 induced neurodegeneration are still not fully understood. Here, we would like to discuss the incidence, morphology and role of TDP-43 pathology in the pathogenesis of different neurological diseases with particular emphasis on diseases with various, non-degenerative etiologies.

Small fiber neuropathy

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The diagnosis of small fiber neuropathy includes impairment of the function of small fibers without symptoms from the large fibers. The main complaining are paresthesias, pain of various modalities, disturbed temperature sensation, and autonomic dysfunction with impaired thermoregulation, skin changes, as well as sexual and gastrointestinal dysfunction. A typical length-dependent pattern with symptoms being pronounced in the distal parts of the extremities can be found in the majority of patients. Others, present a non-length-dependent pattern, where symptoms are distributed in an irregular, patchy way or are limited to a certain area of the body e.g., to the area of innervation of a single peripheral nerve. The incidence varies between 1.3 and 4.4 per 100,000 and the morbidity between 13.3 and 131.5, depending on the studied population. The etiology is recognized in about two third of cases and in the most of them, the neuropathy is of diabetic origin. Other factors include renal and thyroid dysfunction, autoimmunologic conditions like Sjögren syndrome, sarcoidosis or monoclonal gammopathy, toxicity related to ethanol, drugs, and other substances. Rarely, hereditary ethology like Fabry disease can be found. Skin biopsy with counting of the linear density of intraepidermal nerve fibers remain the gold diagnostic standard. Other diagnostic methods include confocal corneal microscopy, quantitative sudomotor axon reflex test, quantitative sensory testing, and others. Treatment should be oriented towards the symptomatic pain relief. Causal therapy includes tight glycemia control and immunomodulation in respective etiologies.

Imaging and analysis of digital slides in neuropathology – virtual microscopy and artificial intelligence systems

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Modern methods of microscopic imaging enable the digitization of microscopic specimens regardless of their type, size, or observation technique. Virtual microscopy systems are increasingly being used, allowing the digitization of images using techniques such as multiplexing, which involves multiple staining. This results in images containing a huge amount of information.

Quantitative analysis for such images is possible through computerized image analysis systems assisted by artificial intelligence (AI). Training such systems, i.e., creating neural networks responsible for detecting specific structures, opens up enormous possibilities for classical neuropathological diagnostics and scientific research.

The combination of data from virtual microscopy images with clinical data from patients sourced from hospital information systems (HIS) or laboratory information systems (LIS) provides the opportunity to create powerful diagnostic tools. The main challenges associated with creating neural networks using virtual slides include file size and the ability to store them, as well as limited interpretability. However, advances in scanning

systems and AI software analysis are addressing these issues.

In summary, artificial intelligence algorithms will play a crucial role in the automatic recognition and classification of pathological changes, expediting the diagnostic process. The tight integration of imaging data with clinical information will allow for a more comprehensive analysis of cases, supporting personalized patient care. Cloud platforms will facilitate access to vast datasets, enabling collaboration among experts worldwide and accelerating progress in the field of neuropathology.

Effect of extracellular vesicle secretome on glioblastoma heterogeneity

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The global transcriptome data is widely used to develop new cancer therapies and biomarkers, including for glioblastoma. However, despite decades of research on protein-coding transcriptome, only incremental improvements have been made in outcomes. As protein-coding sequences account for less than 2% of the genome, it has become increasingly apparent that aberrations of non-protein-coding genes drive important cancer phenotypes. Small non-coding RNA, such as microRNA, functions in RNA silencing and post-transcriptional regulation of gene expression and has organ, tissue, and cell-specific expression. However, subtype-specific microRNA signatures in glioblastoma tumors and stem-like cells can also be found in extracellular vesicles. By tracing small molecules such as microRNAs from bulk glioblastoma *via* single glioblastoma stem-like cell and single extracellular vesicle approaches, the microRNAome signature explains the co-existence of both subtypes within individual tumors and the dynamic evolution of the transcriptome as the disease progresses, explaining why any tissue sample or section is only a snapshot of the disease at a given moment.

Non-coding RNA in the brain tumor microenvironment

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The concept of tumor microenvironment describes a complex ecosystem surrounding a tumor, composed of various non-malignant cells, including blood vessels, immune cells, stroma cells, signaling molecules, and the extracellular matrix. Within the glioblastoma tumor mass, cancer cells frequently experience adverse conditions that arise from distorted vasculature, resulting in dynamic fluctuations in the availability of oxygen and nutrients. To cope with these challenges, glioblastoma cells enforce multiple adaptative mechanisms controlled by diverse classes of non-coding RNAs, including long non-coding RNAs, microRNAs, and circular RNAs. The presentation focuses on the roles of these molecules in shaping complex phenotypes that enable cancer cells with plasticity and adaptability to manage metabolic stressors, resulting in strengthened survival. Therefore, non-coding RNAs are promising candidates for therapeutic targets and biomarkers currently tested in experimental therapeutic approaches.

Spinal bone and soft tissue tumors

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Primary bone and soft tissue tumors of the spine belong to a group of very rare tumors. The lecture presents the characteristics of these tumors in the different locations of intradural, intradural extradural and extradural. The occurrence of tumors in anterior and posterior vertebral elements was also presented. Special attention was paid to chordomas in the context of the new WHO classification of Soft Tissue and Bone Tumours of 2020 and Central Nervous System Tumours of 2021. The metastatic capacity of chordomas was highlighted. A new disease entity Poorly Differentiated Chordoma characterized by epithelioid cells with necrosis and nuclear expression with brachyury antibody with differential diagnosis approach was presented. The heterogeneity of the tumor and brachyury expression in dedifferentiated chordoma (DC) is important in the acquisition of diagnostic material by the surgeon

and in pathological diagnosis. Prior radiotherapy was noted in 25% of DC cases. Two clinical faces of Osteoid Osteoma and Osteoblastoma with identical morphological and molecular characteristics are important entities. Osteosarcoma of the spine accounts for 3.6-14.5% of all malignant tumors occurring most often in the vertebral bodies of the lumbosacral region. Listed are the subtypes of osteosarcoma according to 5th ed. WHO. Infectious, degenerative, metabolic and inflammatory diseases should be considered in the differential diagnosis of spinal tumors based on imaging and laboratory diagnostics. Undifferentiated small round cell sarcomas of bone and soft tissue are diagnosed with the support of molecular diagnostics. Spinal tumors require a multidisciplinary approach at every stage of diagnosis. They pose a particular challenge to pathologists because of the sparse diagnostic material.

Ultrastructural image of neuronal ceroid lipofuscinoses (NCL) type 2

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Neuronal ceroid lipofuscinoses type 2 (CLN2 disease) is an autosomal recessive neurodegenerative disorder generally with onset at 2-4 years of age, characterized by seizures, loss of vision, progressive motor and mental decline, and premature death. CLN2 disease is caused by loss-of-function mutations in the tripeptidyl peptidase 1 (*TPP1*) gene leading to deficiency in TPP1 enzyme activity. Approximately 60% of patients have one of two pathogenic variants (c.509-1G > C or c.622C > T). CLN2 disease belongs to the group of the neuronal ceroid lipofuscinoses (NCLs) characterized by massive accumulation of autofluorescent lipopigments in storage bodies in neurons, and in extraneuronal cells. Ultrastructurally, lysosomal inclusions are seen as granular osmiophilic deposits (GRODs), curvilinear (CVPs), fingerprint (FPPs), or rectilinear profiles (RLPs).

We present the case of an almost four-year-old girl with clinical suspicion of NCL. The development of the patient was disharmonious. EEG revealed focal temporal sharp and slow waves bilaterally with tendency to generalization. The MRI showed no abnormalities. Quick dry blood spot detection CLN2 test revealed decreased TPP1 activity of 0.9 umol/l/h (cut-off value > 4.5). The whole exome sequencing isolated from the blood revealed a mutation c.622C > T/c.509-1G > C in the *TPP1* gene mostly detected in the CLN2 disease. Additionally a biopsy of the rectal mucosa was performed, where the muscularis mucosa, connective tissue, nerve fibers and small blood vessels were examined. Numerous deposits of CVPs were found in the analyzed cells and few deposits of mixed CVPs in association with FPPs. Rectal biopsy is a useful method of obtaining cells with high morphological and functional diversity, and can be used to detect pathological ultrastructural lipopigment deposits occurring in CLN2 disease.

Inflammatory myopathies in clinical and neuropathological practice

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Idiopathic inflammatory myopathies (IIM) are a heterogeneous group of disorders that can affect muscles, skin and other organs. According to the current classification, dermatomyositis, polymyositis, immune mediated necrotizing myopathy and overlap myositis with antisynthetase syndrome are distinguished. Inclusion body myositis has different pathophysiology and lack the effective pharmacological treatment. In most cases, IIM are characterized by a chronic, relapsing and remitting course. The response to treatment is generally good, however, there is a group of patients who require intensive therapy for many years. Recently, the association of specific auto-antibodies with different IIM has limited the role of muscle biopsy. Nevertheless, in sero-negative patients and in doubtful cases neuropathological assessment is very important. Dermatomyositis is characterized by perivascular, perimysial and perifasciucular inflammation of B-cells and $CD4^+$ T-cells. Polymyositis and overlap myositis have mainly $CD8^+$ T-cells and expression of MHC class I. In necrotizing myopathy there are numerous necrotic fibers with macrophages, and almost no other inflammatory infiltrates. Inclusion body myositis presents $CD8^+$ T-cells, rimmed vacuoles, inclusions and accumulation of various proteins. We present the data about 13 patients with immune mediated necrotizing myopathy from the Department of Neurology, Medical University of Warsaw. There were 12 women aged 41-85 years and

one man aged 56 years. 9 patients had anti-HMGCR antibodies and 2 anti-SRP antibodies. In 10 cases the muscle biopsy presented scattered necrotic fibers with minor inflammatory changes. All patients received immunosuppressive treatment for many years, nevertheless in most cases the course of the disease was serious with significant neurological impairment.

Hematopathological conditions in central nervous system

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Central nervous system (CNS) involvement by hematolymphoid diseases is an uncommon phenomenon, presenting as CNS infiltration of a systemic condition (secondary involvement) or isolated CNS engagement of the disease, the latter more seldom. While symptom constellation may vary, neuroradiological investigation remains the baseline of the diagnostic procedure. Combination of morphological, immunophenotypical and molecular analyses enables identification and detailed characterization of the entity according to current classifications. In the presented cases, cerebrospinal fluid (CSF) or brain tissue biopsies were used for diagnostic evaluation. CSF based diagnostics enabled detection of an isolated relapse of precursor T-cell acute lymphoblastic leukemia and of acute myeloid leukemia, as well as diagnosis of local relapse and transformation of a small B-cell lymphoma, the latter presenting mainly as spinal nerve root thickening. Another patient group are persons after a specific previous treatment, who present with lymphoproliferative disorders (LPD) involving CNS. Of the two presented cases, one patient had a fatal tumour-forming infiltration of Epstein-Barr virus positive diffuse large B-cell lymphoma (EBV+ DLBCL) following allogeneic stem cell transplantation for malignant T-cell lymphoma (previously classified as post-transplant lymphoproliferative disorder, monomorphic variant). The other case concerned a self-resolving classic Hodgkin lymphoma-like LPD following methotrexate and infliximab treatment for neurosarcoidosis. To sum up, awareness of hematolymphoid diseases as differential diagnoses in CNS lesions is crucial for adequate and timely diagnosis, which can be reached using a multimodal diagnostic approach.

H2A.Z histone variants facilitate HDACi-dependent removal of H3.3K27M mutant protein in paediatric high-grade glioma cells

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Patients with pediatric high-grade gliomas (pHGGs) have dismal prognosis of 9-15 months of survival and no effective therapy. The DIPG subtype of pHGG (diffuse intrinsic pontine gliomas) occurs in the brainstem, where resection is unattainable, leaving palliative but not curative radiotherapy as the major standard of care. Over 80% of DIPGs confer a mutation in histone 3 (H3.3 or H3.1) resulting in lysine to methionine substitution (H3K27M). H3K27M causes global epigenetic alterations (a loss of H3K27 trimethylation and an increase H3K27 acetylation) and a consequent oncogenic change in gene expression. To date, no therapeutic strategy exists that would aim to suppress the levels the oncogenic H3K27M.

Using multiple cellular models expressing H3.3K27M histone variant, we show that pan-HDAC inhibition leads to the temporary but significant reduction in the H3.3K27M protein (up to 80%), which is independent of the H3F3A mRNA expression. ChIPseq analysis confirms a global genome-wide drop in the H3.3K27M occupancy at the chromatin upon SB939 treatment (HDACi). H3.3K27M loss is most striking at SB939-upregulated genes with minimal basal expression in untreated samples. Also, some of the H3K27M-dependent genes become downregulated in response to SB939 treatment. Mechanistically, the SB939-mediated loss of H3.3K27M partially depends on chloroquine (CQ) – the DNA-intercalating agent and lysosomal inhibitor. Moreover, the loss of H3.3K27M is facilitated by co-occurrence of H2A.Z, as evidenced by the knock-down of H2A.Z histone iso-

forms. ChIPseq analysis confirms similar occupancy of H3.3K27M and H2A.Z at the same SB939-inducible genes.

We provide a new insight into disease-specific mechanism of HDAC inhibition and are the first to show a possibility of pharmacological manipulation of the H3.3K27M protein levels. Our work opens new options to directly target H3.3K27M oncohistone, which may have implications in future therapies. (Supported by the grant UMO-2017/27/B/NZ2/02827).

Tight junction proteins and their reorganization in primary brain tumors and cerebrovascular diseases

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Tight junction (TJ) proteins occludin, claudins, and ZO-1 (zona occludens) are essential cell adhesion components maintaining the integrity of blood-brain barrier (BBB). Experimental hypoxia has been shown to cause BBB disruption and rearrangement of occludin and ZO-1. Although they are likely closely associated, principal TJ proteins have different localization patterns; claudin-5 and occludin are expressed more externally, closer to the extracellular space in cerebral endothelial TJs, whereas ZO-1 is expressed submembraneously in the deeper layers. Claudin-5 is involved in forming TJs comprising very long, branched strands, whereas occludin forms short ones. ZO-1 is a membrane-associated guanylate kinase. Associations between the principal TJ molecules and their rearrangements during ischemia may be responsible for the changes in serum levels. In clinical settings, the higher CLN-5/ZO-1 ratio and increased circulating claudin-5 and occludin concentrations associated with clinically evident hemorrhagic transformation in ischemic stroke patients. Moreover, the analyzes of autopsy brains of ischemic stroke patients and intracerebral hemorrhage show the modulated expression of TJ proteins. In low-grade gliomas (LGG), the structure and function of the blood-brain tumor barrier (BBTB) depends on its morphology. In high grade gliomas (HGG), the surface of vessels responsible for microcirculation and their diameter is more extensive than in low-grade gliomas. In HGG, BBB disruption may be associated with brain edema and gadolinium enhancement in neuroimaging. In the plasma of brain tumor patients, claudin 5 concentration is higher (2.81 ng/ml, 0.3065-0.4065 [$p < 0.0001$]) than in CSF (0.0; 0.0-0.0). ZO-1 level is increased in plasma (0.3510; 0.3065-0.4065R U/ml [$p = 0.0001$]) compared to CSF (0.1180; 0.04496-0.1792). Different levels of claudin in plasma were found depending on histological diagnosis, with the highest diversity in glioblastoma patients.

To conclude, tight junction proteins may be applied as biomarkers of BBB disruption and analyzed in living patients and autopsy samples. The reorganization of TJ proteins is observed in primary brain tumors as well as in cerebrovascular diseases.

Modern therapies for malignant gliomas

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The International Boards (EANO, SNO/ASCO, NCCN) provide guidelines for the diagnosis and management of malignant gliomas based on the results from the latest clinical trials. Treatment decisions should be made based on several factors: clinical, radiological, pathological and molecular. Patients performance status, neurological function, age and individual risks are important. Younger age (< 40 years) and better performance status at diagnosis are associated with favorable outcomes. The extent of resection is a prognostic factor, the goal of surgery is to remove as much tumor as safely possible without compromising neurological function. Some patients could be offered the watch-and-wait option. The molecular markers have strong clinical implications: IDH-mutated (IDH-mt) tumors are associated with higher response rates to chemotherapy than the wild-type; 1p19q codeletion is linked to longer survival and better response towards cytotoxic agents. *MGMT* status can guide treatment decisions on the use of alkylating agents for glioblastoma patients. Several new markers are under estimation. All malignant gliomas recur, so the main goal of adjuvant treatment is to improve local control and survival without inducing neurotoxicity and clinical deterioration. According to the results of clinical trials (RTOG 9802, CATNON, EORTC 26951, RTOG 9402) radiotherapy and chemotherapy increase survival. Modern, conformal radiation techniques with better target coverage and sparing of normal brain tissue are widely used. The dose and schedule of radiotherapy depends on

tumor pathology, clinical prognostic factors and tumor volume. Most of patients should receive chemotherapy concurrently with radiotherapy or in postradiotherapy manner taking into account molecular characteristics. Temozolomide is the most commonly used oral alkylating agent. PCV polychemotherapy is recommended for patients with IDH-mutant 1p/19q-codeleted oligodendrogliomas. There is no standard of care for patients with recurrent tumors, with the second surgery, reirradiation and/or systemic treatment as the options. Several molecular targets are tested in clinical trial and IDH targeting is an interesting option. $BRAFV^{600E}$ mutations is another possible target. Immunotherapy has recently have been actively tested to treat primary and recurrent malignant gliomas but with limited efficacy. There is a strong need to develop novel therapies in adult type gliomas.

On the 60th anniversary of journal „Folia Neuropathologica". „Neuropatologia Polska" – the analysis of the journal's first decade (1963–1972)

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The journal "Neuropatologia Polska" (since 1994: "Folia Neuropathologica") was established in 1963. The vast material analysed from the consecutive issues of the journal in the years from 1963 till 1972 was subjected to statistical and content analysis. It outlines the circumstances surrounding the creation of the magazine and shows how it evolved in the first years. From its first year, the magazine has included works of a very high substantive level and a wide range of topics. The journal "Neuropatologia Polska" set paths for the development of neuropathology in clinical and experimental aspects. What is very important it created a platform for international cooperation in many fields, included researchers and scientists from Western countries and foreign academic centers in difficult times.

The perineural invasion in cancer

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The nerves with a complex structure, including the epineurium, perineurium, and endoneurium, create a sanctuary for cancer cells, promoting their growth and proliferation. Perineural invasion (PNI) is one of the ways of spreading cancer, which commonly occurs in head and neck, pancreatic, and prostate cancer. Although the phenomenon of PNI has been described for many years, there are doubts about the definition, clinical value, and assessment method. Usually, it is defined as the encirclement of at least 33% of the nerve or the presence of cancer cells within layers of the nerve. Many studies showed contradictory results concerning the importance of PNI as a prognostic factor in overall survival, risk of relapse, and pain. It is suggested that in prostate and hepatocellular cancer, the presence of PNI increases the risk of bone metastases. The method of PNI assessment includes studies *in vitro* (ex. transwell model, dorsal root ganglia co-culture model), *ex vivo* (ex. explanted nerve model, organoids), and *in vivo* (ex. orthotopic or heterotopic xenografts). PNI may also be observed in cancer patients as the perineural spread in radiological imaging and be symptomatic (ex. plexopathy).

Brain Bank. Biobanking. Digital Brain

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Biobanking entails collecting and storing human biological material and related medical data to advance scientific research and medical diagnostics, ranking among the top ten world-changing ideas. Biobanks can be broadly classified into two categories: those targeting specific diseases like cancer and population-based biobanks. Population-based biobanks are typically established outside of healthcare and academic institutions, amassing biological material along with donor metadata. Additionally, virtual biobanks preserve dig-

itized data, including images, DNA/RNA sequencing results, and more. In Poland, the history of biobanking is rich, with universities, research institutes, and hospitals taking a lead role. The largest neuropathological biobank is situated at the Institute of Psychiatry and Neurology in Warsaw, housing brain fragments, diagnostic samples, paraffin-embedded materials, histological preparations, and digital copies stored within the Digital Brain platform. Safeguarding donor privacy and establishing rights over biological samples and their transfer are paramount. An emerging idea is for donors to actively engage in biobanking and reap the benefits of research conducted on their biological material. Human biological material and associated data in biobanks are invaluable resources for biomedical research. Essential elements include efficient management structures, transparent access procedures, fair compensation, and priority setting, with ongoing discussions underscoring the practical implementation challenges. The future of medical research is intricately linked to biobanking, contingent on the quantity and diversity of available biological samples and bioinformatics data, cost-effective strategies, and the participation of patients and citizens. Effective biobanks should provide high-quality biological samples at an affordable cost, facilitating research programs that benefit all.

5-ALA fluorescence-guided surgery of high-grade gliomas

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High-grade gliomas (HGGs) are characterized with poor prognoses. Surgical resection is the standard first line treatment. Patient outcomes depend heavily on the extent of surgical resection (EOR).

Several methods such as neuronavigation and intraoperative magnetic resonance imaging (iMRI) were applied for enhancement of HGG visualization, especially at tumor borders. Conventional image-guided surgery is limited by brain shift in case of neuronavigation and high cost and time-consuming use in case of iMRI. Other techniques to maximize the EOR include visualization of the tumor with fluorescent dyes.

5-aminolevulonic acid (5-ALA) is an increasingly utilized intraoperative fluorescent imaging agent for patients with HGG. It enhances visualization of HGG tissue. 5-ALA is a natural metabolite in the human body that is produced with the hemoglobin metabolic pathway. Exogenous 5-ALA acts like a prodrug that is administered orally and accumulates in brain tumor cells. Then it is metabolized to protoporphyrin IX (PpIX) which florescent after exhibition with blue light. That red light fluorescence of tumor can be observed by the surgeon via operating microscope and guide through the resection. This guidance could be especially useful at the tumor boundaries because of infiltrative-growth pattern of HGGs.

Utilization of 5-ALA was found to be associated with a greater extent of resection in HGG surgeries, as well as longer OS and PFS.

Authors report utility of 5-ALA in clinical setting, future perspectives for 5-ALA as diagnostic agent and intraoperative photodynamic therapy with 5-ALA as a promising therapeutic approach in HGGs.

Mitochondrial diseases

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Mitochondrial diseases are rare genetic disorders caused by dysfunction of the respiratory chain in mitochondria (RCD). They manifest themselves with multi-organ damage and a very diverse phenotype. Symptoms often begin in childhood and early childhood. Diagnostics requires the cooperation of many specialists (including pathologist, geneticist, biochemist). The diagnostic material that is valuable from the pathologist's point of view is the brain and muscle. The following disease entities were discussed: Leigh disease, MELAS, Alpers disease and mitochondrial DNA deletions.

Neuropathological description of child with biallelic BUB1 mutation

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BUB1 gene is responsible for maintaining chromosome stability during mitosis. We present the case of child with biallelic BUB1 mutation, who was diagnosed with numerous brain lesions. Macroscopically: microcephaly and agenesis of corpus callosum was found. Microscopically disturbed organization of cortical layers in the cerebral and cerebellar hemispheres was stated. In the cerebellar white matter heterotopic neurons were observed. Wide band of Purkinje cells were found beyond the cortex. Heterotopic Purkinje cells disseminated in the deep white matter were also observed. The dentate olivary dysplasia was diagnosed. BUB1 mutation cause a neurodevelopmental disturbances including microcephaly and migrational abnormalities in the cerebellar and cerebral hemispheres.

POSTERS

Mechanisms of dysfunction of the rat brain parenchymal arterioles in experimental acute AVP-associated hyponatremia

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Introduction: Hyponatremia, defined as a decrease in plasma Na^+ concentration below 135 mM, is associated with a significant morbidity and mortality. In patients with neurological diseases, hyponatremia often results from an excessive secretion of vasopressin (AVP). One of the causes of neurological symptoms in AVP-associated hyponatremia may be a disturbed regulation of cerebral microvessels. Our previous *in vitro* studies showed that the decreased Na^+ concentration in the media in the presence of AVP leads to the constriction and impaired response of parenchymal arterioles (PA) to the endothelium dependent adenosine triphosphate (ATP). The aim of present study was to disclose the mechanisms of these abnormalities.

Methods: Studies were performed in the organ chamber on the isolated, pressurized, and perfused rat PAs using *in vitro* model of acute AVP-associated hyponatremia (121 mM Na^+ + 15 pg/ml AVP). In this model: a selective antagonist of V_{1a} vasopressin receptor – SR49059 (10^{-7}M), 20-hydroxyeicosatetraenoic acid synthesis inhibitor (HET0016, 10^{-6} M), peroxynitrite decomposition catalyst FeTMPyP (10^{-5} M), and a precursor of nitric oxide (NO) – L-arginine (10^{-10} M) were tested for the normalization of PA tone and responses.

Results: Vasoconstriction of PA was abolished by V_{1a} receptor antagonist and diminished by FeTMPyP. HET0016 did not affect this response. FeTMPyP, but not L-arginine, restored the response of parenchymal arterioles to ATP.

Conclusions: Dysfunction of parenchymal arterioles in the *in vitro* model of acute AVP-associated hyponatremia depends on nitrosative stress and stimulation of vasopressin V_{1a} receptor.

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Effects of serotonergic system arousal by 5-HTP and fluoxetine on breathing in a model of Parkinson's disease with bilateral 6-OHDA damage

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Parkinson's disease (PD), in addition to dopaminergic neurodegeneration, exhibits deficits in the serotonergic system (5-HT) responsible for respiratory impairment.

To model PD, we performed bilateral injection of 6-hydroxydopamine (6-OHDA) into both striata. The purpose of the study was to analyze respiratory impairments in response to hypercapnia before and after arousal of the serotonergic system by increasing endogenous serotonin level with fluoxetine (5-HT reuptake inhibitor) and by 5-HTP (5-hydroxy-L-tryptophan, a precursor of 5-HT biosynthesis) in male Wistar rats. We analyzed respiratory disturbances in response to 7% hypercapnia (CO_2 in O_2) in the plethysmographic chamber before and after stimulation of the serotonergic system in awake rats 5 weeks after 6-OHDA or vehicle injection.

In 6-OHDA rats, levels of serotonin (5-HT), dopamine (DA), and noradrenaline (NA) in the striatum and brain stem were decreased, which influence the attenuation of the respiratory response to hypercapnia, compared to sham-operated rats. Fluoxetine treatment increased respiration during hypercapnia in 6-OHDA and sham animals, but this effect in PD rats was greater than in sham animals. 6-OHDA rats treated by fluoxetine achieved values similar to represented by sham operated untreated rats. Administration of 5-HTP augmented the hypercapnic respiratory response resulting from an increase in the frequency of breathing in 6-OHDA and sham rats.

Activation of the serotonergic system reversed the compromised respiratory response to elevated CO_2 levels found in PD and appears to be a promising therapeutic approach for respiratory impairments in PD.

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Neuroepithelial tumor with Patz1 fusion – a case report

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A 17-year-old girl was admitted due to recurrent headaches since several months. The neuroimaging showed a solid-cystic lesion of the left frontal lobe up to 70 mm, radiologically described as a parasitic infection. The girl underwent surgical resection of the lesion. Histopathologically, the tumor was a polyphenotypic glio-neuronal neoplasm with regional dedifferentiation within both components, and anaplasia inside glial part with focal necrosis, high mitotic index and high cellularity – this gliosarcomatous component. Areas of oligodendroglioma-like differentiation and scattered giant cells were noticeable. The immunohistochemical profile was: GFAP+, Olig2+, CD34+ focally, EMA (–), Vimentin (+), Ki67 up to 20%, SMA (–), Synaphisin+ in part, S100+, NeuN & NFP in part+, p53 (–) wt, IDH1R132 (–), ATRX+, BRAFV600 (–), pan TRK (–), H3G34 (–). The initial diagnosis of the anaplastic ganglioglioma was made, after the consultation it was changed into high-grade glioma, NOS. The analysis of the methylation profile of the tumor was performed, which finally changed the diagnosis to Neuroepithelial Tumor with Patz1 fusion. PATZ1 fusions define a very rare molecular class of histologically polyphenotypic neuroepithelial tumors, showing diverse morphologic features, they may be low- or high-grade. This emerging histologic and molecular entity of childhood brain tumors is not included in the latest WHO CNS classification.

The oxygen enrichment in tumor biology – a preliminary study

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Introduction: Evaluation of stable isotopes of elements reveals rapidly developing area of cancer studies. Oxygen plays a key role in cancer metabolism and hypoxia is known to be associated with therapeutic failures in solid tumors. The assessment of isotope ratio of oxygen is unique in the literature and its potential relation with tumor biology appear unknown.

Aims: The comparison of oxygen isotopic profile in benign and malignant entity with the same histological components.

Material and methods: Oxygen isotopic composition in ganglioneuroma and ganglioneuroblastoma tissue samples was determined by Thermo Scientific MAT 253 following pyrolysis at 1350°C and chromatographic separation (70°C) of H_2 and CO in a He gas stream. Measured values were calibrated to repeat analyses of gelatine (certified reference material provided by Elemental Microanalysis) and caffeine (measured and published by various research groups) standards and are reported vs. VSMOW on the VSMOW-SLAP scale. RESULTS: The difference in isotopic composition between the examined tumors was revealed and appeared as much as 1.3‰ ($\delta^{18}O$ 12.6 ±0.1 in ganglioneuroma vs. 13.9 ±0.3 in ganglioneuroblastoma).

Conclusions: Revealed different profiles of oxidation may suggest the separate oxygen metabolic pathway in malignant tumors and an oxygen enrichment as a potential source of the observed therapeutic failures.

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Methylcathinone – "the king of legal highs" and manganese encephalopathy

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Methylcathinone is a psychostimulant that increases the release of dopamine and norepinephrine in the brain. Ephedrone – methylcathinone obtained using "home" methods – is produced by the oxidation of drugs containing ephedrine. Methylcathinone itself is relatively low toxic, while the side effect of ephedrone is dementia, and parkinsonian and pyramidal syndromes.

Case report: After 3 months of intravenous use of ephedrone, a 25-year-old woman developed gait disturbances with falls, followed by dysphagia, dysarthria, symptoms of multifocal dystonia and parkinsonian syndrome. Despite discontinuing the use of ephedrone, the symptoms gradually worsened, which led to the

patient's immobilization in a wheelchair and her death 8 years later. Microscopic examination of the patient's brain was performed using routine histopathological methods, TUNEL method, and immunohistochemical reactions with antibodies against: ubiquitin, β-amyloid, α-synuclein, tau protein, GFAP, ferritin, C4β complement, and CD3, CD45, CD68 antigens. Histopathological examinations revealed changes already described previously with manganese poisoning, such as: loss of neurons in the globus pallidus, reactive gliosis in the substantia nigra and excessive accumulation iron deposits in astroglia in the basal ganglia. In addition, abnormalities not occurring in manganism were also found, such as: generalized inflammation of small cerebral vessels of moderate severity and the presence of unknown material in the lumen of microvessels. Some astrocytes and neurons showed immunoreactivity in the TUNEL method. No signs of neurodegenerative process were found in the structures of the nigro-striatal system and striatum.

Conclusions: Ephedrone's neurotoxicity is only partially due to manganese poisoning and it may also cause vasculitis, possibly following intravenous administration.

Astrocyte-oligodendrocyte crosstalk in the *in vitro* rat model of neonatal hypoxia-ischemia

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Intercellular communication may influence the cell response triggered by pathophysiological conditions. One of them is neonatal hypoxia-ischemia (HI), which leads to many abnormalities as a result of oxygen and glucose deficiency. Therapeutic hypothermia is the only therapy available for neonatal HI. In the absence of effective therapy, there is a need to search for new therapeutic strategies. Modulation of the interactions between glial cells might be one of them.

Primary mixed glial cultures were established from the brains of Wistar rat pups and after 12 days the individual glial fractions (oligodendrocyte progenitors/OPCs, astrocytes) were isolated and subjected to the oxygen-glucose deprivation (OGD) procedure, mimicking HI. The cells were cultured as monofractions or their co-cultures. After 24 hours, cells and culture media were collected and analyzed with ELISA kits. The CXCL12 secretion by control OPCs is 3-fold higher than that released by astrocytes and 4-fold higher than measured in co-culture. Expression of CXCR4 in OPCs is 3-fold lower after OGD than in control cells and after OGD in co-culture. ELISA assay indicated that after OGD the CXCR2 expression by control astrocytes is 4-fold higher than in co-culture and 9-fold higher after OGD versus co-culture. The performed study also indicated that the BDNF expression is highly modulated by cell interactions in co-cultures. In conclusion, the co-culture experiments showed that the crosstalk between neonatal macroglial cells significantly influences the expression of the selected active compounds (like chemokines and their receptors, trophic factors) and thus may be potential targets for future therapeutic strategies.

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In search of traces of altered autophagy in glial cells after hypoxic-ischemic injury *in vitro*

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Perinatal asphyxia is a frequent complication during labor and may lead to neonatal brain damage resulting in hypoxic-ischemic (HI) encephalopathy. The pathophysiological pattern of damage mainly involves the white matter of the central nervous system. The following have been identified as potential mechanisms involved in the progress of damage: astrogliosis, oligodendrocyte cell death from oxidative stress or excitotoxicity and activation of immunological response. An important pathway, recently proven to be upregulated in neuronal cells in this condition and contribute to neuronal cell death, is autophagy. Currently little is known about the role of this process in an altered phenotype of glial cells, especially activated astrocytes and microglia and oligodendrocyte progenitor cells (OPCs) inhibited to differentiate.

The aim was to investigate, whether autophagy is activated in OPCs, astrocytes and microglia, after mimicking HI injury in the *in vitro* conditions with the procedure of temporary oxygen-glucose deprivation (OGD). The evaluation of the level of LC3 and p62 by WB revealed, that autophagy can be activated 6h after OGD in OPCs and astrocytes and remained at higher levels in the latter for a further 18 hours. Immunofluo-

rescent labelling of cells fixed 24 h after OGD revealed changes in the morphology of astrocytes and microglia, followed by increased expression of Lamp1, a marker of lysosomes involved in degradation of autophagosome cargo. An injury also affected cell viability.

Ultimately, it is hoped that the results of ongoing research will identify the best therapeutic approach targeting autophagy in this condition.

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Clinical, neuropathological and neuroradiological presentation in an immuno-deficiency patient with relapsing Burkitt lymphoma invading the central nervous system: a case report

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Purpose: Burkitt lymphoma (BL) is a specific, rare and aggressive type of non-Hodgkin lymphoma. It accounts for 1-2% of all cases of non-Hodgkin lymphoma in the general population.

Case description: A case of a relapsing Burkitt lymphoma in a 27 year-old HIV-positive patient is presented. The radiological and neuropathological examinations with subsequent specialized neurooncological treatment are discussed.

Comment: Nowadays with antiretroviral therapy CNS relapse of Burkitt lymphoma in immunocompromised patients is very rarely encountered. The MRI findings and laboratory tests are unspecific as shown in the presented case. The final diagnosis can be established properly by obtaining the brain tissue by stereotactic biopsy. Even the neuropathological examination requires high skills to provide final diagnosis and subsequent neurooncological treatment.

Involvement of proinflammatory cytokines IL-1β and TNF-α to hypoglossal nerve activity in obesity

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Introduction: Remodeled adipose tissue in obesity produces pro-inflammatory cytokines that promote inflammatory responses in the body, which can lead to the development of metabolic disorders, obstructive sleep apnea (OSA) and carotid artery (CB) dysfunction. The role of inflammatory mediators in chemoreception and how they affect the respiratory response at the level of respiratory nerves is unknown. The objective of this study was to determine the effects of the pro-inflammatory cytokines IL-1β and TNF-αα on the activity of nerves controlling upper airway patency during inspiration – the hypoglossal nerve (HG) and the phrenic nerve (PHR) in obese and control rats during acute hypoxia.

Material and methods: The experiments were completed on Wistar rats fed a high-fat (HF) or standard (CTL) diet for 10 weeks. Thereafter, electrophysiological recordings of PHR and HG nerve activity were taken on anesthetized, paralyzed and ventilated rats during normoxic breathing and after hypoxic stimuli (8% O_2 in N_2). Each hypoxia was separated by cytokine administration.

Results: The length of hypoxia leading to apnea was significantly shorter in HF animals after IL-1β injection. Both cytokines tend to increase basal neural HG activity in HF rats. In response to hypoxia, HG amplitude in HF rats was slightly reduced after IL-1αadministration in both CTL and HF rats. TNF-αsignificantly reduced HG amplitude in HF rats by 28% after hypoxia administration.

Conclusions: Among the studied cytokines, TNF-α mainly affects the mechanism of the respiratory response to hypoxia.

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Future directions of MR imaging markers of multiple sclerosis activity

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The poster presents the future directions of development for MR imaging markers of multiple sclerosis (MS) activity. The proper imaging of MS patient is crucial for putting the proper diagnosis, for monitoring the effectiveness of treatment. Therefore, there is still a need for finding new imaging markers of disease activity.

The poster shows the comparison off the most popular and indicated by authors imaging markers of MS activity in MR images, that are most mentioned: the central vein sign, the chronic active lesions, the leptomeningeal enhancement, and the choroid plexus volume.

We present briefly meaning of markers, their MR images, and their appliance in routine practice as well as their disadvantages. The central vein sign is detected on SWI sequence (susceptibility weighted images), where appears as linear hypointensity, which means the vein in the central part of demyelinating lesion. Chronic active lesions – these are demyelinating plaques, which do not show the postcontrast enhancement, but a hypointense rim on SWI images. Leptomeningeal enhancement means the postcontrast enhancement of the meninges, resulting from local inflammation and accumulation of immune cells. Choroid plexus volume due to its well-known immunological function correlates with the inflammatory process in the brain.

Circulating miRNA expression profiling in patients with migraine and healthy controls – a preliminary report

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Although a common and well-studied disorder with available treatment options, migraine is still among the ones causing the highest number of years lived in disability, according to the WHO Global Burden of Disease Study report. This is not only because of its high prevalence but also due to misdiagnoses and diagnostic delays. Currently diagnosis is established solely on patient interview, which is subject to communication errors. Therefore, attempts have been made to find an objective biomarker of migraine. Among the candidates are circulating miRNAs. In recent years, numerous studies have been conducted on the potential of these molecules in the diagnosis and therapy of human diseases. So far, studies on migraine have yielded promising, if inconclusive results.

The aim of our preliminary study was to select a number of miRNAs able to differentiate patients with migraine (n = 13) from sex and age matched controls (n = 13). We used qRTPCR (miRCURY LNA miRNA Focus PCR Panel, Qiagen) to measure expression of 179 miRNAs most commonly found in serum. Among those, a number have a minimum two fold difference in expression between the research and control group. Nevertheless, with stricter selection criteria these differences have decreased. In our exploratory work we consider the possibilities of further research, including verification of the preliminary results, as well as general challenges related to working on that are circulating miRNAs.

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PGC-1α activator ZLN005 enhances neurogenesis during the early stage of human dorsal forebrain organoid development

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PGC1α protein is a mitochondrial biogenesis activator encoded by the *PPARGC1A* gene. PGC1α is essential for proper differentiation and maturation of neuronal cells, but the molecular mechanism remains elusive. ZLN005 is a small molecule, which stimulates transcription of PGC1α *via* AMP-activated protein kinase. In our study, we investigated the effects of ZLN005 on neuronal differentiation in dorsal forebrain organoids derived from human induced pluripotent stem cells (iPSCs) treated from day 40 to day 54.

First, the cytotoxicity of ZLN005 was assessed in organoids at concentrations ranging between 1 and 10 μM. The Alamar Blue and ATP assays indicated that cell viability was not diminished. Next, the effect of ZLN005 on the expression of PGC1α was investigated using qRT-PCR and immunohistochemistry. Compared to the control group, PGC1α was observed in the nuclei of cells outside of the neural rosettes in the treated organoids. Surprisingly, there was no increase in mRNA levels for *PPARGC1A* and other genes linked to the PGC1α pathway. In order to determine the effect of PGC1α stimulation on neurogenesis we examined the expression of selected neuronal markers after ZLN005. In the treated group, the gene expression analyses revealed a significant upregulation of *NESTIN*, *TUBB3, FABP7*, and *NEFL*.

In summary, our study demonstrates a PGC1α-dependent enhancement of neurogenesis in dorsal fore-

brain organoids. Interestingly, our preliminary results suggest that ZLN005 exerts effects on PGC1α expression exclusively at the translational level, however, the underlying mechanism still requires further studies.

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Results of treatment of congenital central hypoventilation syndrome (Ondine's curse) in Poland

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Congenital central hypoventilation syndrome (CCHS) is a rare genetic disorder with an estimated incidence of 1 in 200,000 births. A mutation in the *PHOX2B* gene was described, responsible for 91% of cases. 90% of cases are spontaneous mutations. In Poland, the number of patients is estimated to be around 50. The symptoms that define the course of the disease and its diagnosis are associated with respiratory failure due to impaired autonomic respiratory control in the central nervous system. Establishing the diagnosis requires respiratory criteria and genetic diagnostic work-up. There are 3 pathogenic types of the PHOX2B gene: PARM, NPARM, deletions of the entire gene or exon 3. There is no curative treatment for the cause and leads to death if untreated. The aim of treatment is to provide ventilation during sleep. New in Poland is the implantation of diaphragmatic nerve stimulators. Between 2021 and 2023, nine children (age 3-17) underwent such surgery. All are sleeping using pacemakers. Parents of 7 pacemaker patients were asked to complete questionnaires containing the tools: the Implantation Satisfaction Survey and the Cantril Ladder. 5 out of 7 families report an increase in quality of life after pacemaker implantation and 6 out of 7 predict a further increase. 6 out of 7 children sleep better, 4 out of 7 have more energy, 6 out of 7 parents are satisfied with the results of the operation. Implantation of diaphragmatic nerve stimulators allows a return to a physiological breathing pattern. The quality of life for families and the quality of the child's sleep improves.

Evaluation of diffusion tensor imaging and magnetic resonance spectroscopy for use in the assessment of brain tumour malignancy

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Introduction: Diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS) are advanced radiological methods used in the imaging of tumours and other pathological lesions of the brain. As an extension to the standard MR protocol they are an important adjunct to it but are rarely the decisive factor regarding the lesions malignancy or final diagnosis. These are important issues not only in regards to the assessment of the patient's prognosis itself, but are a critical aspect defining the strategy and development of a treatment plan. The key method of answering the above questions remains biopsy. The aim of this study is to analyse the potential of DTI and MRS imaging as a non-invasive, fast and reliable alternative to mechanical biopsy for the assessment of tumour malignancy.

Methods: This paper presents a retrospective analysis of selected patients undergoing MRI examinations with DTI- and MRS-enhanced protocols that had tumours and their degree of malignancy confirmed by mechanical biopsies. DTI-derived parameters (mean diffusivity MD, fractional anisotropy FA, axial diffusivity AD, radial diffusivity RD) and ratios of observed metabolite concentrations with MRS (NAA/Cr, Cho/Cr, Cho/NAA, presence of lactates and lipids) were analysed. The results obtained were compared with measurements for healthy tissues from the same patients.

Conclusions: The methods described above offer a potential alternative to the invasive diagnostic method of biopsy for the assessment of tumour malignancy. The parameters determined show relationships to WHO grade, but more detailed studies are needed.

Streamlined CRISPR/Cas9-mediated generation of dual-reporter *PPARGC1A* human induced pluripotent stem cell line

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CRISPR/Cas9 system utilized for the precise integration of fluorescent proteins into target genes in induced pluripotent stem cells (iPSCs) significantly facilitates disease modeling and study of organ formation. Current approaches for gene editing in iPSCs suffer from multiple limitations, including labor-consuming, and low editing efficiency. Moreover, investigating gene promoter activity may present challenges due to developmental-dependent low expression.

In our study, we aimed to establish a reporter cell line that exhibits green fluorescent protein (GFP) expression under the control of *PPARGC1A* (encodes PGC1α protein) gene promoter to explore its relevance during brain organoid development. However, the basic level of *PPARGC1A* expression in iPSCs is low. To overcome these obstacles, we constructed a plasmid encoding GFP fused to *PPARGC1A* promoter and NLS_BFP (nuclear localization signal combined with blue fluorescent protein) tag expressed from the constitutive active CAG promoter. A donor DNA template containing sequences encoding PGC1α_GFP and CAG_NLS_BFP, positioned within the AAVS1 homology arms, was used to ensure accurate genome integration. Next, iPSCs were transfected using donor and CRISPR/Cas9 plasmids. After 5 days, cells expressing weak GFP signal along with NLS_BFP were isolated by fluorescence-activated cell sorting.

Expanded cell clones, analyzed by fluorescence microscopy, displayed GFP and BFP signals while maintaining morphology features. Furthermore, the insertion of the BFP sequence into the AAVS1 locus was confirmed through PCR analysis.

Application of the nuclear BFP reporter system enhanced the utility of CRISPR-mediated gene editing in human iPSC cells. Our model of the reporter human iPSC line, although challenging to work with, demonstrate important preclinical value.

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Pre-, intra- and post-neurosurgery language assessment in patient with astrocytoma, IDH1-mutant G3

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Preoperative functional magnetic resonance imaging (fMRI) and neuropsychological testing allow for the safest planning and execution of neurosurgery for patients with brain tumors in eloquent areas. Neurosurgeons can perform the most aggressive and secure tumor removal by combining preoperative fMRI with intraoperative language assessment.

We describe our experience planning and directing an awake craniotomy for a tumor impinging on the language area utilizing fMRI. Female patient with astrocytoma IDH1-mutant G3 had preoperative neuropsychological language evaluation. The patient did not present any language skills disturbances before surgery. Speech arrest, comprehension disruption, and paraphasic errors were discovered during intraoperative awake language mapping near the tumor boundary, linking to functional regions that explained the findings preoperatively validated by fMRI. Specific phonemic paraphasic mistakes and discrete anomic abnormalities were found during the post-neurosurgical neuropsychological testing.

This instructive instance demonstrates the potential advantages of combining awake surgery and fMRI in the neurosurgical treatment of eloquent brain tumors.

Inhibitor of bromodomain and extraterminal (BET) proteins OTX-015 affects amyloidogenesis in aged mice

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The major neuropathological hallmarks of Alzheimer's disease (AD) are the accumulation of senile plaques and neurofibrillary tangles, consisting of aggregated amyloid-beta (Aβ) and hyperphosphorylated tau protein, respectively. Thus, preventing the formation of those deposits in the brain is commonly recognized as a potentially beneficial strategy in the treatment of AD. One of the recent therapeutical approaches is the modulation of gene expression evoked by epigenetic signaling mediated by a variety of factors, including bromodomain and extraterminal (BET) proteins-the readers of epigenetic acetylation code.

Our study aimed to analyze the impact of BET inhibition on AD-related alterations in the aged brain. OTX-015, an inhibitor of BET, was orally given to 12-month-old mice C57BL6 for 2 weeks, and then behavioral, genetic (qPCR), and immunochemical (ELISA, WB) analyses of the brain cortex were performed.

We observed that OTX-015 restored age-related decline in cognitive function. Moreover, it significantly reduced the level of Aβ1-40 and Aβ1-42 in the brain cortex of aged mice, concomitantly with the changes in the expression of the enzymes involved in Aβ precursor protein processing (e.g. Psen1 and Psen2). On the other hand, OTX-015 had a negligible effect on the immunoreactivity of tau and phospho-tau (Ser199/202, Ser396, Ser416) as well as the expression of inflammation-related genes.

Our study demonstrated that inhibitors of BET proteins significantly affect amyloidogenesis, and further studies should be conducted to reveal their potential role in prevention or in slowing down the progression of AD.

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The Neuropathology of Köhlmeier-Degos disease – case report and literature review

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Degos disease (malignant atrophic papulosis [MAP]/Kohlmeier-Degos disease) is a rare vasculopathy described by Austrian pathologist Walter Köhlmeier in 1941 and reported one year later by French dermatologist Robert Degos. The eponym used currently involves only the contribution of Robert Degos, an eponym Köhlmeier-Degos disease is also used.

Köhlmeier-Degos disease is a rare vasculopathy of unknown origin, in many cases – fatal, characterized by infarcts in the skin, gastrointestinal tract, and central nervous system (CNS). The involvement of CNS was reported in 20-60% of cases. Currently, 200 reports Köhlmeier-Degos disease are available in the literature.

The most common neuropathological findings included narrowing or occlusion of small cerebral arteries. Subtle fibrous plaques with subendothelial localization are observed in carotid, cerebral, and vertebrobasilar arteries. A more pronounced accumulation of collagen was observed in the meninges and sulci.

Our case was a 27-year-old female admitted to the Stroke Unit due to multifocal neurological deficits. Neuroimaging revealed multifocal ischemic lesions localized subcortically and in the brain stem. The diagnosis was established based on skin changes. Skin rash appeared as white papules with erythematous circumferential border on the trunk, thighs, and lower legs. The clinical course of the disease was fatal. Neuropathological examination revealed occluded small cerebral arteries in hemispheric white matter, brain stem, and spinal cord. Necrosis and demyelinating lesions were found in white matter, brain stem, and spinal cord.

To conclude, we present a fatal course of Kohlmeier-Degos disease with the first manifestation of the central nervous system lesion.

Primary adult sellar SMARCB1/INI1-defficient tumor – a case study

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We describe a case of a rare primary sellar region tumor in a 42-years-old woman with visual impairment, periodic headaches for few months, and isolated hyperprolactinaemia. The neuroimaging showed infiltrative suprasellar tumor, compressing the optic chiasm. The transsphenoid resection of tumor in two steps was performed, but the first post-operative material presented mainly normal pituitary gland tissue and small foci of spindle cells neoplasm. In the second step the material revealed the neoplasm made of small and epithelioid cell malignant neoplasm with myxo-fibrous stroma. In addition, the elements of Rathke's cleft cyst were found. The immunohistochemistry showed: INI-1 expression loss, CD34+, cMyc+, FLI-1+, EMA+ and Vimentin+ in part of cells, Cam 5.2+ in single cells, WT1 weak+, p53 +mut, Ki67 up to 50%. The rest of many examined antigens was not detected. The tumor sections underwent two external consultations. Finally, the adult sellar SMARCB1/INI1-defficient tumor was recognized. The post-operative status of patient was complicated with coma, the patient died three months later.

Primary sellar SMARCB1/INI1-deficient tumors in adults are extremely rare and classified as a part of wide, very heterogenous group of SMARCB1/INI1-defficient neoplasms, with the most common intracranial atypical teratoid/rhabdoid tumor (AT/RT) – a highly malignant pediatric neoplasm. Rare reported cases of SMARCB1/INI1-defficient neoplasms in adult population occur mostly in the sellar region, belonging to ATRT-MYC subgroup. Differential diagnosis of this rare entity can be very difficult.

The role of ceramide/sphingosine kinase 1 and sphingosine-1-phosphate receptors signalling in α-synuclein transduced cells

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The interplay between α-synuclein (α-syn) – protein overexpressed in Parkinson's disease (PD) and pro-apoptotic ceramide/pro-survival sphingosine-1-phosphate (S1P) homeostasis, called 'sphingolipid rheostat' is poorly understood. The current study aimed to investigate expression of proteins engaged in sphingolipids signalling in the PD cellular model, i.e. SH-SY5Y cells with lentiviral-mediated transfer of gene for the human α-syn – SH-SNCA and empty vector transduced cells, as an appropriate control. On the other hand, we evaluated the level of α-syn in the above model under stress conditions, evoked by pro-apoptotic cell permeable C2-ceramide and inhibition of sphingosine kinase 1 (Sphk1), being an enzyme crucial for S1P synthesis. PCR-RT analysis indicated, that α-syn overexpression reduced the mRNA level of S1P receptor 1 and 3, which are crucial in S1P pro-survival signalling. Furthermore, treatment with Sphk1 inhibitor (SK1-I) markedly increased the expression of α-syn in SH-SNCA cells, both at mRNA and protein level, which was assessed by PCR-RT and Fluorescence-Activated Cell Sorting technique, respectively. Moreover, flow cytometry analysis of dead cells, as well as MTT assay indicated that incubation with SK1-I and C2-ceramide dramatically reduced cell viability/mitochondrial function of both SH-SNCA and control cells. Finally, S1P receptors modulators such as: SEW2871, ponesimod and siponimod protected cells against C2-ceramide toxicity. In summary, our results suggest that the imbalance in sphingolipid ratio in favour to pro-apoptotic ceramide and disturb Sphk1/S1P receptor signalling may be associated with α-syn overexpression/stress. On top of that, S1P receptors activation seems to be valuable target under ceramide toxicity in α-syn-overexpressed cells.

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Creutzfeldt-Jakob disease: clinical cases

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Creutzfeldt-Jakob disease (CJD) is a transmissible spongiform encephalopathy that manifests as a rapidly progressive dementia.

Clinical case 1. 68 y.o. woman delivered to the emergency department with severe speech impairment in a somnolent state – 13 points Glasgow Coma Scale. Neurological examination showed horizontal nystagmus with a right side gaze fixation, facial asymmetry, left side tongue deviation, extrapyramidal signs, tendon and periosteal reflexes D>S, subcortical signs. MRI findings: cortical hyperintensity in right temporal and occipital lobes (DWI), hyperintensity in right nucleus caudatus, putamen and thalamus. EEG investigation: periodic sharp wave complexes in the left parieto-temporal lobe.

Clinical case 2. 67 y.o. man delivered to the emergency department with headache, vertigo, abnormal gait and coordination, progressive extremities weakness, disorientation, memory and concentration impairment. Neurological status: conscious – 15 points Glasgow Coma Scale, periodic time and place disorientation, horizontal nystagmus, cerebellar ataxia, left side pyramidal signs. MRI findings: cortical hyperintensity in frontal, temporal, parietal and occipital lobes bilaterally, mostly on the left hemisphere without damage of basal ganglia (DWI).

Conclusions: Described clinical cases correspond to the criteria of possible CJD according to CDC's Diagnostic Criteria for Creutzfeldt-Jakob Disease (2018). First clinical case presents a Heidenhain variant, second case – Brownell-Oppenheimer variant. The most recommended approaches to *in vivo* diagnosis of CJD are strict application of diagnostic criteria, careful interpretation of neurovisualization, EEG.

The prolonged hyperbaric oxygen as a component of the combination therapy with CK2 kinase inhibitors against malignant glioma cells *in vitro*

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Treatment options for glioblastoma remain limited and unfortunately even with maximal resection and postoperative adjuvant treatments, the median overall survival of patients remains approximately 15 months. In the present study we investigated the effect of HBO (2ATA – atmospheres absolute) prolonged to 2 hours on antitumor efficacy of selected CK2 kinase inhibitors in two distinct human malignant glioma cell lines, T98G and U87MG. Tested compounds were administered in increasing concentrations within the range of 5-100 μM either under normoxia or with the addition of HBO at the beginning of experiments. After 24 hour incubation, 2 hour HBO decreased the viability of T98G cells in response to CK2 kinase inhibitors 2-aminoetylo-amino-4,5,6,7-tetrabromo-1H-benzimidazol (TBIAEA), 2/ 4,5,6,7-tetrabromoN^{2}metylo-N^{2}hydroksyetylo 2 aminobenzimidazol (TMHA), and 3/ 1-(β-D-2′-deoxy-ribofuranosyl)-4,5,6,7-tetrabromo-1H-benzimidazol(TDB), as compared to treatments with these inhibitors under normoxia. The 2 hour HBO-induced reductions of viability amounted up to 36% over pharmaceutics alone and occurred at 5-100 μM of their concentration range. Notably, as compared with 1 hour HBO sessions combined with CK2 kinase inhibitors , 2 hour HBO sessions combined with these pharmaceuticals produced greater reductions of viability of T98G cells, resulting in the lowest numbers of surviving tumor cells in the whole experiment. In T98G cells, as compared with U87MG cells, 2 hour HBO caused more potent reductions over pharmaceuticals alone.

Thus, the prolonged dosing of HBO may increase tumoricidal effect of antiglioma agents and hence deserves further study. Investigating even more prolonged HBO exposures and other kinase inhibitors combined with HBO against glioblastoma cells would be worth a while.

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Case studies of AI segmentation and MRI spectroscopy of gliomas

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In this project we wanted to check the usefulness of AI segmentation in voxel selection in magnetic resonance spectroscopy (MRS). We wanted to show the advantage of an AI solution in reporting tumor size.

Six cases of brain gliomas were analyzed based on MRI examination. Glioma segmentation was performed in DeepBraTumIA software based on T1w, T2w, FLAIR and T1w post contrast images. Brain metabolite concentration rates were calculated in syngo. *via* software based on chemical shift imaging. The voxel was selected based on glioma segmented maps (edema, tumor, contrast-enhancing tumor compartment). We prepared an automatic measurement of the longest diameter of glioma. The results of segmentation were used for precise voxel selection in MR spectroscopy. See graphical abstract of one of the cases. In each case it was easier to differentiate between edema or tumor. Based on segmentation we could separate the cases with a contrast-enhancing compartment.

Automating calculation helps to improve the quality of volumetric measurements. Our findings may contribute to the adoption of AI glioma segmentation in clinical settings and advanced brain research.

Incidence of perineural invasion in resected non-small cell lung cancer – preliminary study

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Introduction: Increasing data suggests a pivotal role of neural microenvironment in cancer development and progression. Perineural invasion (PNI) is defined as neoplastic infiltration of any nerve layer, epineurium, endoneurium or perineurium. In a set of malignancies it was proved to affect patients outcome usually as a predictor of poor prognosis. PNI incidence is various in different tumor types. Little is known about its incidence and role in non-small cell lung cancer (NSCLC).

Material and methods: The study included 124 resected NSCLC cases from the patients operated between 2011 and 2012. Clinicopathological features were reviewed retrospectively. All available H&E sections taken during routine examination from the tumors were thoroughly examined to established pathologic characteristics such as histologic type, grade, pTNM, size of the tumor or vascular involvement.

Results: Out of the all analyzed cases 57 (46.0%) were squamous cell carcinoma and 67 (54.0%) adenocarcinomas. PNI was identified in 17 (13.7%) tumors with at least one nerve affected. In one case more than 10 nerves were infiltrated. Additionally, vascular invasion (VI) was found in 39 (31.5%), spreading through air spaces (STAST) in 36 (29,0%) and pleural involvement (PI) in 66 (23.4%) cases. In a group of PNI positive cases most were squamous cell carcinomas (12/17, 70.6%) from which 5 (5/17, 29.4%), were poorly differentiated tumors (grade 3). In 9 cases (9/17, 53.0%) PNI coexisted with vascular invasion, in 3 (3/17, 17.6%) with STAS and in 8 (8/17, 47.1%) with PI.

Conclusions: The study shows that PNI occurs in these type of tumor together with other features known as the indicators of worse outcome. The role of PNI is NSCLC is still unknown and needs careful investigation.

The promising pathophysiological aspects to better understand and manage the risks of rupture of unruptured cerebral aneurysms

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Introduction: Actuality to deeply understand the underlying mechanisms in the aneurysm wall launching the progression and potentially rupture may be helpful to optimize the clinical decision making process among patients with unruptured cranial aneurysms (totally fast 18 million in Europe), especially asymptomatic with low score according to specific scales such as PHASES und UIATS.

Aim of the study was to gather and analyze the current relevant scientific achievements describing the pathophysiology of inflammatory remodeling of aneu-

rysm wall resulting in the degeneration und poor clinical outcome.

Material and methods: 529 patients with unruptured saccular intracranial aneurysms are being observed and treated during 2013-2023. 19.1% have already been operated (98% endovascular, 2% transcranial approach). Among them 19.8% had multiple, 4.95% – mirror intracranial aneurysms. Primarily we used PHASES and in the last years UIATS Score to estimate and individualize the risk of aneurysm rupture. We conducted also the literature review using the PubMed service.

Results and discussion: Our findings correlate with literature data confirming the thickening of aneurysm wall, myointimal hyperplasia und hypocellularity with accelerated collagen breakdown. The high wall shear stress activates pro-inflammatory signaling thorough macrophage chemoattractant protein 1 (MCP1) promoting the smooth muscle cell proliferation being thus a promising target for drug therapy. This process increases the aneurysm wall permeability, which can be detected using the dynamic contrast-enhanced MR perfusion.

Conclusions: The detection of biochemical markers of aneurysm wall remodeling with modern radiological correlates looks promising to improve the early diagnosis, treatment and prevention of rupture of the cerebral aneurysms.

AI neuroscientist: Current and future prospects of virtual reality environments with machine learning (ML) and artificial intelligence (AI) in neuroscience

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Innovative technologies such as extended reality (XR) in general, and virtual/augmented reality (VR/AR) in particular offer tremendous opportunities for neuroscience research in both healthy and disease conditions. With the extremely rapid development of IT/ICT, various unusual computer (bio)science solutions are being proposed, for example, such as the Metaverse concept for future generations and their activities. This modern approach is based primarily on ML models and AI techniques. ML is a key element of AI that allows computers to learn from and make decisions based on data, and constantly update their "skills" based on new data sets. AI encompasses many different disciplines, from computer science, (intelligent) data analysis/search and (bio)statistics, (bio)(neuro) hardware and software engineering, linguistics, medicine and neuroscience, to philosophy and psychology. This report presents and discusses the prospects and some of the limitations of applications of machine learning and artificial intelligence in the field of basic and clinical neuroscience, particularly neuropsychology, neurology and neurogeriatrics. Achievements to date have shown that ML/AI are promising and effective tools for AI researchers, especially AI neuroscientists, e.g., for diagnosis and prognosis, development of biomarker arrays and evaluation of treatment strategies. Artificial intelligence is expected to be one of the most important technologies in the near future, and thus is likely to have an increasing impact on our lives (e.g., see [1]). This impact includes, but is not limited to, the use of ever-increasing amounts of information (big data), the fusion of AI and VR/AR, and then the emergence of the future Metaverse equipped with AI. The creation and exploration of these new digital worlds and their impact on human societies will be a great challenge for neuroscience.

1. https://www.europarl.europa.eu/news/en/headlines/society/20230601STO93804/eu-ai-act-first-regulation-on-artificial-intelligence

Relevant recent research findings on the relationship between breathing and neural and/or mental activity

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Research on the functioning and plasticity of the body's systems has been conducted on animals, also through model (computational) and experimental studies with humans, as well as clinical observations. These findings, among others, point to the vital importance and connection between respiratory rhythms and brain activity. The current results of these studies demonstrate that breathing can play a pivotal role in coordinating neuronal activity, behavior and emotion. In fact, it has already been shown that: (i) breathing affects neural activity of many different regions in the brain,

(ii) respiration modulates different frequency ranges in brain dynamics, (iii) various breathing protocols produce different neuronal and psychological effects, (iv) effects of breathing on the brain are related to the simultaneous modulation of biochemical and physiological parameters (and the synchronization of internal body signals), and (v) there is an as yet unknown but important the link between respiration pattern/activity and mind in both health and disease. The report presents relevant recent findings on the relationship between breathing and neural/mental activity, new perspectives on cognition and emotion, interoceptive rhythms in the brain, and possible promising areas of application. For example, breathing protocols are already being developed to manipulate mental states for therapeutic goals (e.g., breathing brain and respiratory perception models). On the other hand, induced (neuro) (respiratory) plasticity changes play an important role, especially in various deficit states and dysfunctions. Respiratory-neuro-mental connections and neuroplasticity phenomena offer hope for brain-based therapeutic uses of breathing in mental, neurological, respiratory or neuromuscular disorders.

Spatiomolecular recognition of tau prion-like propagation in tauopathies combined with cellular vulnerabilities identification – study design

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Prion-like propagation of pathological tau is currently a highly argued hypothesis. Mechanisms involved include tau secretion and uptake *via* direct membrane translocation or extracellular vesicles, direct cell-to-cell contact with nanotubes, and transsynaptic transport.

Tauopathies differ among the specific brain regions involved in tau propagation, leading to a variety of phenotypes. For example, specific tau strains influenced the range of neuropathologic changes observed in patients with Alzheimer's disease (AD); however, the hippocampal neurons were vulnerable in each case independently of the strain. Some factors of this cell vulnerability have been identified (i.e., genetic predisposition, familial and somatic, epigenetic modification, the stressor-threshold hypothesis, calcium homeostasis, or regional physiological proteome). Although the susceptibility to tau propagation and resulting degeneration has been extensively studied the lack of suitable technologies has left this territory mostly unexplored. The molecular characteristics of the tau-seeding resistant cell types compared to the vulnerable ones have not been well defined.

In this study, we propose using a novel methodology of high-resolution molecular imaging techniques, specifically Raman hyperspectral mapping (RHM) combined with surface-enhanced Raman scattering (SERS). The latter allows for multiplexing by specific protein antibodies attached to Au-nanoparticles. These methods allow spatial recognition with detailed molecular content mapping of studied samples. The characteristics of highly vulnerable cells will be compared with those of the resistant cells. Tissue sections of patients with tauopathies, specifically AD, frontotemporal lobar degeneration, and progressive supranuclear palsy will be examined, additionally comparing to non-degenerative age-matched control tissues. All data will be analyzed using the artificial neural network-based approach and the differentiating molecular characteristics identified.

The first nitrogen, carbon and oxygen comparative isotopic profiling in cancer – a pilot data

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Introduction: Isotope ratio mass spectrometry evaluates naturally occurring stable isotopes of elements. Although the isotopic studies in oncology highly develop, the oxygen assessment is a rarity and comparative nitrogen-carbon-oxygen profiling haven't been found in the literature. Aim of the study was the assessment of isotope ratio of nitrogen, carbon and oxygen in cancer tissue.

Material and methods: Undifferentiated neuroblastoma tissue samples collected for nitrogen and carbon isotope evaluation were lyophilized and placed in tin capsules with 1 mg of vanadium pentoxide as an oxidant and combustion catalyst. The isotopic composition of carbon and nitrogen was determined with the use of Sercon 20-22 CF-IRSM coupled with an elemental analyzer, according to Fry. Oxygen isotopic composition was determined with the use of EA-Py-CF-IRMS.

Results: Isotopic signals of all the examined elements were identified. The values of carbon and nitrogen isotope ratio isotopes in cancer tissue presented as follow: $\delta^{15}N$ appeared 7.27‰, $\delta^{14}C$ –23.30‰ and $\delta^{18}O$ = 13.7‰.

Conclusions: Comparative determining the isotope composition of nitrogen, carbon and oxygen reveals the final isotopic profile of tumor metabolism. The achievement of complex of three different isotopic data types opens the biochemical possibility to search for a metabolic pathway which characterized undifferentiated neuroblastoma and may appear universal for the aggressive cancers.

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Morphological features of cerebral stroke in diabetes mellitus

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Diabetes mellitus (DM) leads to a significant increase in mortality and a deterioration in the quality of life of patients, due to the development of cardiovascular complications. Ischemic stroke in diabetic patients occurs 2-4 times more often than in the general population. Mortality in ischemic stroke is 50-60%, in hemorrhagic form 70-95%. The prognosis for stroke patients with diabetes is more pessimistic than for those without diabetes.

The aim of this study was to identify the morphological features of cerebral stroke in diabetes mellitus.

Seventeen cases of ischemic stroke on diabetes mellitus background were selected from the archive of Pathology Department. Medical records and histories of diseases were reviewed to retrieve information regarding patient and the cause of death. Information obtained from the autopsy protocol included macro- and microscopic characteristics, status of intracranial arteries and general autopsy findings.

In the cortical-medullary arteries of the zone of ischemic necrosis of the brain tissue segmental fibrinoid necrosis, parietal and occlusive thrombi, perivascular fibrosis and hyalinosis were present. In most of the cases were the presence of focal perivascular encephalolysis and lacunar infarcts, the fusion of small foci with the formation of large ischemic foci of colliquation necrosis. The shadow cells, pyknosis, compaction of the Nissl tigroid, lipofuscin in neurons, atrophy of the neuronal cytoskeleton, intraneuronal inclusions, single neuronal plaques were found outside the stroke zone.

The most significant morphological features in cerebral stroke are a combination of pathology of the microvasculature with a dominant damage to the intracerebral arteries.

The influence of histone deacetylase inhibitor – sodium butyrate – on complement system activation in a rat model of neonatal hypoxic-ischemic brain injury

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Introduction: Pathogenesis in hypoxic-ischemic brain injury (HI) is a complex process. One of the mechanisms that play an important role in neonatal asphyxia is the inflammatory response. The complement system, which is one of the inflammatory factors, can play a dual role in brain injury. Complement normally protects against infection and promotes tissue repair, but it can also contribute to damage when it is over-activated. Sodium butyrate (SB) – histone deacetylase inhibitor (HDACi) – provides an inflammatory reduction.

The main purpose of this study was to investigate the effect of SB treatment on complement system activation and elimination of synaptic connections after HI.

Material and methods: Cerebral HI was induced in Wistar rat pups by permanent unilateral ligation of the common carotid artery, followed by 60 minutes of hypoxia. SB (300 mg/kg b.w.) was administered in a 5-day regimen, the first injection administered immediately after exposure to hypoxia.

Results: After HI, we observed an increase in complement system gene expression, which was significantly reduced after SB treatment. In addition, after HI synaptic proteins gene expression decreased and returned to control levels after SB administration. HI induced brain tissue damage and disruption of synaptic structures. SB treatment diminished tissue degradation. In addition, behavioural tests were performed on rat pups, but no effect of HI or SB on locomotor or cognitive function was demonstrated.

Conclusions: The decrease in complement system activity, as well as the protection of brain tissue and

synaptic structures, suggests a neuroprotective effect of SB.

Age related alterations in mRNA level of genes related to redox state, mitochondria function and amyloid β homeostasis in animal model of Alzheimer's disease. Effect of fenofibrate

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Alzheimer's disease (AD) is the most common neurodegenerative disease characterized by synaptic and neuronal cell loss, higher expression of amyloid precursor protein (APP) and liberation of Aβ peptides. Subsequently, Aβ neurotoxicity may lead to alterations of mitochondria functions, redox state and of nuclear receptors including peroxisomes proliferator activated receptors (PPARs). Despite intensive studies, pathogenesis of AD is unknown and therapy is unsuccessful.

The aim of this study was to investigate the age related changes in transcription of genes related to redox state, mitochondria. Aβ metabolism and the effect of PPAR-α ligand, fenofibrate (FF) in the AD Tg mice.

We have used the familial model of AD with the V717I "London" mutation of APP, 3, 6 and 12-month-old mice FVB-Tg. Mice were injected i.p. with fenofibrate (30 mg/kg b.w.) in an appropriate solvent for 14 days. The controls mice were treated ip. with the solvent only. Brain cortex was used and qPCR was performed.

Our study focused on age related alterations in expression of genes engaged in mitochondria fusion and fission (*Mfn1*, *Mfn2*, *Opa1*, *Dnm1*, *Fis1*) and biogenesis (*PPARα/Ppargc1α*, *Tfam*, *Nrf1*, *Nrf2*) in AD Tg mice. The effect of FF was determined. Additionally, the expression of genes and the engagement of FF in regulation of the mRNA level of genes involved in APP/Aβ degradation (*Bace1*, *Psen1,2*, *Ide*, *Mme*, *Mm2,9,10,11*) and cells fate: (*Sirt1,3,4,5*, *Bcl2*, *Bax*, *Bad*) was investigated.

Our results indicated down regulation in expression of *Sirt1* in AD mice and the protective effect of FF. Moreover, at 12 months old, AD Tg mice lower mRNA level of genes coding NRF2, PGC1α, PPARα was indicated and FF enhanced transcription of *Nrf-2* and *Bcl2*.

Our data indicated age dependent alterations of genes expression involved in redox state and mitochondria biogenesis, which could be promising targets in delaying progression of pathology in AD.

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LECTURES without abstracts

Neuropathological brain tumor assessment – Dr. Robot taking over?

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The 5th edition of the WHO classification of lymphoid tumors – what is new

German Ott

IKP Robert-Bosch Krankenhaus, Stuttgart, Germany

Neuroimaging of gliomas

Barbara Bobek-Billewicz

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Current WHO classification of adult type gliomas – the presence and future

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Current WHO classification of pediatric type gliomas. Methylation profiling in CNS tumor diagnostics

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New classification of pituitary tumors

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Neuroimaging of cerebral lymphomas

Edyta Szurowska, Anna Jankowska

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Tripeptidylpeptidase 1 (TPP1) deficiency causes cerebellar ataxia and dilated cardiomyopathy

Małgorzata Bednarska-Makaruk

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Rare head and neck tumors in the new WHO classification

Monika Durzyńska

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