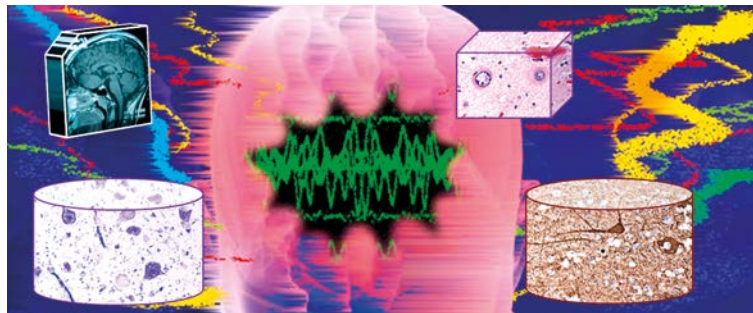


**Abstracts from the Conference
of the Team of Oncologic Neuropathology
of the Committee on Neurological Sciences,
Polish Academy of Sciences**

**“STRUCTURAL AND MOLECULAR BACKGROUND
OF THE DRUG-RESISTANT EPILEPSY”**



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Neuropathology behind symptomatic epilepsy. A bird's eye view over this vast stretch of land of neuropathology with some dives toward elected targets

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Seizures/epilepsy make one of the most significant symptoms of neurological/neurosurgical disorders. Secondary (symptomatic) epilepsy by its definition requires or assumes the presence of morphological pathology of the brain. Though new ILAE (International League Against Epilepsy) proposals of seizures/epilepsy classification discard the term “symptomatic” (epilepsy), this term has very important practical medical meaning, since it, at least implicitly, reminds the physician/neurologist/neurosurgeon, that seizures may have morphological and (supposedly, or hypothetically) operable cause (focal brain lesion). One has to exclude such “simple” (?) lesion first, before starting thinking of genetics or metabolic cause of epilepsy. Yet correlation between neuropathology and secondary epilepsy is complicated. The scope of all conceivable pathologies, that may underlie seizures is enormous and seems to cover almost all, if not verbatim all known disorders with morphological changes in the brain, however frequency of epilepsy/seizures is different in different conditions. Neoplastic, vascular, metabolic, developmental, inflammatory, toxic, and even neurodegenerative diseases, let alone posttraumatic lesions, may manifest with secondary epilepsy. Two main causes of secondary epilepsy may be distinguished: firstly, epilepsy with prevailing genetic and/or developmental background, and secondly epilepsy due to acquired causes (though this division is by far not perfect). From the practical neuropathological point of view, there is definite difference between the way one has to approach autopsy cases (especially those of children, in which developmental or inborn metabolic disturbances play very important role), and biopsy cases, in which tumors or other focal lesions of whatever nature predominate. In “surgical neuropathology” special attention has to be paid to any forms of lesions in which epilepsy is amenable to treatment by neurosurgical methods. This relates first of all to tumors, esp. to some particular tumors typically associated with epilepsy (so called LEATs) and focal malformations of cortical development. For the neuropathologist the information of the type of seizure has relatively limited importance since the phenotype of seizures and a character of pathology in general do not have direct relations. Of much consequence is first of all information on the duration of seizures (epilepsy), and its severity, which may truly help pathologist in difficult cases. He/she

also has to be aware that some changes like Chaslin gliosis, hippocampal sclerosis and other unspecific changes maybe secondary to epilepsy. Nevertheless the general knowledge of epilepsy, at least at relatively basic level and its relations with the particular pathologies of brain is necessary for the neuropathologist. Special attention will be turned to epilepsy in relation to focal dysplasia (including neurofibromatosis), perinatal brain lesions, and Rasmussen's encephalitis, and to secondary changes (due to epilepsy).

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Clinical heterogeneity of West syndrome – the possibilities and limitations of molecular diagnostics

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Background: West syndrome, also described as early infantile epileptic encephalopathy type 1 (EIEE1), is an age-specific, heterogenic epilepsy syndrome characterized by a triad of symptoms: infantile spasms, hypsarrhythmia on EEG, and arrest of psychomotor development. The syndrome has been divided into subtypes with known (symptomatic) and unknown (cryptogenic) aetiology. Mutations in the *ARX* gene are one of the causes of EIEE1, but in most patients with clinical recognition of the syndrome, the genetic background of the disease is very difficult to establish.

Aim: The purpose of the study is to present molecular approaches aiming to establish molecular basis of encephalopathy in patients with clinical recognition of cryptogenic West syndrome.

Material and methods: We used Sanger sequencing of the *ARX* gene and the Next Generation Sequencing using TruSight One sequencing panel (Illumina). The panel consists of 4813 genes with clinical relevance. Obtained data were filtered with custom designed panel containing 405 genes reported as causative for epilepsy using VariantStudio software (Illumina), and visualised with IGV software (Broad Institute). The pathogenicity of obtained variants was predicted using Polyphen2, Sift and Mutation Taster software.

Results: In one patient we found a deletion of 15 nucleotides (c.451_465delGCGGCCGCGCGGCC) resulting in a loss of 5 alanines in a second polyalanine tract of the *ARX* gene. Although the shortening of polyalanine tracts is still contro-

versial, the X inactivation analysis performed for the family suggests that the deletion might be a pathogenic one. For two patients with West syndrome we obtained several variants in known genes causative for epilepsy. The variants are predicted to be pathogenic. They need to be further confirmed by sequencing analysis of family members.

Conclusions: The analysis of the *ARX* gene should be performed as a first-tier test in West syndrome patients even though exome sequencing is planned, since a high GC content of the *ARX* gene results in a very poor coverage in exome sequencing. The variants obtained by exome sequencing should be further confirmed by sequencing analysis of parents in order to establish *de novo* or inherited character of the variant. In families with a X-linked pattern of inheritance a cosegregation analysis should be performed in order to prove that examined variant segregates with EIEE phenotype.

Mutations in the *SCN1A* gene and what else... analysis of the molecular background of epileptic encephalopathy in Polish patients clinically diagnosed with Dravet syndrome

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Epileptic encephalopathies (EEs) are an intractable group of severe childhood epilepsy disorders in which the epileptic electrical discharges themselves may contribute to progressive psychomotor dysfunctions. Epileptic encephalopathies are genetically heterogeneous diseases that may be caused by mutations in the genes which products are involved in controlling the neuronal excitability. Among the various EEs, Dravet syndrome (DS) has become one of the best-defined phenotype and the highest frequency of mutation detection. 75-80% of the DS cases have a demonstrable mutation in the *SCN1A* gene. Despite this high probability of a mutation identification, in individuals with a typical DS phenotype, up to

25% patients do not carry the *SCN1A* mutations. These cases are described as Dravet syndrome like (DS-like) and suggest the involvement of other genes such as *PCDH19*, *SCN2A*, *SCN1B* and *GABRG2*. About 25% of *SCN1A*-negative female Dravet-like patients carry a mutation in the *PCDH19* gene. Mutations in the other genes have been reported only in single cases with DS-like phenotype.

The aim of this study was to characterize prevalence of mutations in the *SCN1A*, *PCDH19*, *SCN2A*, *SCN1B* and *GABRG2* genes in two groups of patients with epileptic encephalopathies diagnosed as DS or DS-like.

The investigated group comprised Polish patients clinically diagnosed with DS or DS-like. All patients were screened for *SCN1A* point mutations by direct sequencing; subsequent rearrangement analysis (MLPA) was performed only for individuals without an identified point mutation. *SCN1A*-negative patients with phenotypes resembling DS were checked for the *PCDH19*, *SCN2A*, *SCN1B* and *GABRG2* mutations.

In order to identify new genes associated with a DS, affected individuals without *SCN1A* or *PCDH19* mutations were studied by using Next Generation Sequencing (NGS) – Exome Analysis with TrueSight One panel containing the 4,813 genes associated with known clinical phenotypes including epileptic disorders.

In the studied group, *SCN1A* mutations were identified in 85% individuals clinically diagnosed with DS. Mutations in the *PCDH19* gene were identified in 4 patients (females). No mutations within the *SCN2A*, *SCN1B* or *GABRG2* genes were identified, which confirms that mutations in these genes constitute a rare cause of DS-like phenotype.

Angiocentric glioma – a rare intractable epileptogenic tumour with heterogenous histopathology

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Angiocentric glioma (AG) is a rare, low-grade tumour included into the group of “other neuroepithelial tumours”

in the 2007 WHO Classification of Tumours of the Central Nervous System. This is slowly growing neoplasm, classified as WHO grade I, with benign biological behavior. Typically, it occurs in children and young adults with a long-standing, intractable epilepsy.

Angiocentric glioma is typically localized within the cerebral cortex but may extend into the adjacent white matter and is often associated with foci of cortical dysplasia. The typical histopathological features of this tumour is angiocentric growth pattern with the presence of elongated, spindle-shaped cells, arranged around the blood vessels. Such perivascular, pseudorosette-like formations are present also in some other gliomas, particularly in ependymoma, astroblastoma or pilomyxoid astrocytoma. Moreover, AG might display histopathological features with palisading arrangement of tumour cells, resembling schwannoma-like pattern. Infiltration of elongated cells could be also observed in the subpial layers of cerebral cortex. Due to such morphological heterogeneity, the proper diagnosis of AG might be difficult, particularly considering the small biopsy material. Immunohistochemical studies provide the evidence of glial origin of neoplastic cells with expression of glial fibrillary acidic protein and S-100 protein. Moreover, a “dot-like” intracytoplasmic EMA staining, typical for ependymal differentiation of neoplastic cells, might be documented.

We present additional three cases of angiocentric variant of glioma in young adults with documented long follow-up. All tumours displayed characteristic perivascular orientation of neoplastic cells. The distinctive palisading arrangement of tumour cells, resembling schwannoma-like pattern were also documented. The neoplastic cells were positive for GFAP, S-100 protein and vimentin. In numerous cells, a “dot-like” intracytoplasmic EMA staining, were evidenced. The overall MIB-1 labelling index was less than 1%.

The histogenesis of AG is controversial. Angiocentric growth, “dot-like” EMA positivity and ultrastructural features might suggest ependymal differentiation. The appropriate diagnosis of this peculiar type of glial tumour is important for successful treatment without application of aggressive therapy.

Histopathology of brain tumours associated with epilepsy

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The neoplasms associated with epilepsy present with a broad spectrum of low-grade glial and glio-neuronal tumours, mostly corresponding to WHO grade I. These tumours usually manifest with epilepsy with early seizure onset, which is poorly controlled by antiepileptic drugs. Recently, the name “long-term epilepsy associated tumours” (LEATs) or epileptomas was introduced for these particular neoplastic lesions. Long-term epilepsy associated tumours are likely appear during brain development and they are preferential localized in the temporal lobe. The most frequent histopathological types of epileptogenic tumours include ganglioglioma (GG), dysembryoplastic neuroepithelial tumour (DNT), pleomorphic xanthoastrocytoma (PXA), pilocytic astrocytoma (PA), oligodendroglioma, and angiocentric glioma (AG). These tumours are frequently accompanied by focal cortical dysplasia (FCD) type IIIb. Blumcke *et al.* has proposed a novel, clinically useful, so-called A-B-C terminology to classify the large spectrum of epileptogenic brain tumours (Acta Neuropathol 2014; 128: 39-54). This classification recommends to use the standardized parameters based on selected immunohistochemical markers i.e. CD34, MAP2 and IDH1. The A-B-C terminology of neuropathological diagnosis of epileptomas includes: 1) “BNET” (basic neuroepithelial tumor) with CD34 expression, proposed to define the broad spectrum of GGs with dysplastic neuronal and neoplastic glial components; 2) “GNET”, without CD34 expression, proposed to describe gangliocytic neuroepithelial tumor; 3) “DNET”, exhibiting the multinodular appearance and specific glio-neuronal element, remains unchanged, as originally described by Dumas-Duport; 4) “CNET”, proposed to define composite neuroepithelial tumours, characterized by the co-existence of at least two distinct LEAT entities; 5) “ANET”, proposed as synonymous to the AG with ependymoma-like features; 6) “INET”, which lacks any CD34 and glial MAP2 labeling, considered as an isomorphic neuroepithelial tumor, previously described as isomorphic astrocytoma corresponding to WHO Grade I; 7) “ENET-Epileptoma/NOS”, proposed to describe any other epileptogenic neuroepithelial tumor that does not match the proposed A-B-C terminology.

Surgical resection of LEATs may be directed to oncological issue and/or to prevent epilepsy progression.

The perturbations in the neuronal interaction – a possible cause of the epileptic encephalopathy related to mutations in the *PCDH19* gene

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Epilepsy and mental retardation limited to females (EFMR), caused by mutations in the X-linked *PCDH19* gene, is classified as an early infantile encephalopathy. It is characterized by a wide spectrum of phenotypes, varying from the severe cases resembling Dravet syndrome to more benign ones with normal intelligence. The *PCDH19* gene mutations in most cases arise *de novo*, but if not, the EFMR is characterized by an unusual mode of X-linked inheritance. Heterozygous females are generally affected, but hemizygous male carriers are not. Currently, this pattern of inheritance is explained by the model of “cellular interference” and tissue mosaicism due to the presence of the normal and mutated allele of the *PCDH19* gene in heterozygous females/mosaic males.

Mutations in the *PCDH19* gene are mainly localized in the extracellular cadherin domain (> 90%) building the homophilic interactions between the protocadherin 19 molecules. Deletions of the part or the whole gene are less common.

Three cases of the molecularly confirmed EFMR are presented. We identified mutations in the *PCDH19* gene – missense and nonsense in the region encoding cadherin domain and microdeletion of the X chromosome encompassing the whole *PCDH19* gene. Analysis of the phenotype-genotype correlations has shown that the mutation's type may influence the disease course but only in the relation to developmental delay and intellectual disability. Loss of one copy of the gene, or lack of protein synthesis (due to a nonsense mutation) may be less harmful than expression of two different forms of the protocadherin 19 interacting on the neuronal surface (missense mutation).

New concepts in definition and classification of the epilepsies. Proposal of International League Against Epilepsy

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Over the past several decades, significant advances in neuroimaging, genomic technologies, and molecular biology have improved the understanding of the pathogenesis of seizures and epilepsy. In addition, many epilepsy syndromes have been delineated. As a result, the International League Against Epilepsy (ILAE) Commission on Classification and Terminology has prepared a new manuscript on the organization of the epilepsies, based on the 2010 proposal. Epilepsies can be organized in a flexible, multidimensional way depending on the purpose eg. by age, etiology, seizure type, EEG abnormality. A quick overview will be presented. It is unfortunate that most of the proposals in this report are modified interpretations and nomenclature of previous ILAE classifications; new terms are not better than the old ones, and recent advances have not been incorporated. Hence, the new ILAE report met with considerable protest from several expert epileptologists (*Panayiotopoulos. The new ILAE report on terminology and concepts for organization of epileptic seizures: A clinician's critical view and contribution. Epilepsia 2011; 52: 2155-60*). New terminology and concepts update the classification to be consistent with current understanding of the epilepsies in clinical practice. Currently there is no biologically based classification of the epilepsies.

In 2005 a Task Force of the ILAE formulated conceptual definitions of “seizure and epilepsy” (Fisher *et al.* 2005). The definitions were not sufficiently detailed to indicate in individual cases whether a person did or did not have epilepsy. Therefore, the ILAE commissioned a second task force to develop a practical (operational) definition of epilepsy, designed for use by doctors and patients. The results of several years of deliberations on this issue have now been published (Fisher *et al. A practical clinical definition of epilepsy. Epilepsia 2014; 55: 475-82*) and adopted as a position of the ILAE. This practical definition is designed for clinical use.

A person is considered to have epilepsy if they meet any of the following conditions:

1. At least two unprovoked (or reflex) seizures occurring greater than 24 hours apart.
2. One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over 10 years.

3. Diagnosis of an epilepsy syndrome. Epilepsy is considered to be resolved for individuals who had an age-dependent epilepsy syndrome but are now past the applicable age or those who have remained seizure-free for the last 10 years, with no seizure medicines for the last 5 years. The revised definition is not perfect. It will become more useful over time as we gain better information on seizure recurrence risks. But for now, the new definition better reflects the way clinicians think about epilepsy.

Progress in epilepsy treatment in tuberous sclerosis complex (TSC) – prevention is better than treatment

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Introduction of new antiepileptic drugs (AEDs) in the last decades was intended to reduce the number of patients with drug-resistant epilepsy. However, new agents have been characterized by better safety and lower ratio of interactions, but their efficacy remained at the similar level. The search of newer and more efficacious therapeutic interventions resulted in the concept of earlier, preventative treatment of epilepsy.

The search for new therapeutic approaches led to a renewed interest in the process of epileptogenesis. This cascade of molecular and cellular alterations (including changes in expression and function of receptors and ion channels) begins with an insult, brain injury, or genetic predisposition. Beginning from the insult there is often a latent period lasting weeks to months prior to the onset of seizures, followed by the development of clinical epilepsy and its comorbidities. This latent period of epilepsy development may offer a time window when an appropriate medication may stop or modify the epileptogenic process.

With improvement of tuberous sclerosis complex (TSC) diagnostics we are currently able to diagnose the disease prenatally or during the early infantile period. During the recent years in these patients treated in the Children's Memorial Health Institute in Warsaw carried out EEG studies every 4 weeks in the first 6 months of life or every 6 weeks in the next months until 24 months of age. Those with permanently normal EEG did not require any intervention. These patients whose EEG demonstrated paroxysmal activity received antiepileptic treatment with vigabatrin. The treatment has been continued until 24 months of age unless the patients developed clinical seizures.

Such approach has been assessed in 14 patients and resulted in significantly lower incidence of drug-resistant epilepsy. We have not observed any severe forms of mental retardation at 24 month of age comparing to 38% in the control group treated with standard approach.

Our results confirm that epilepsy prevention is possible and that antiepileptogenic (not antiepileptic) treatment may provide a new strategy for preventing epilepsy in susceptible individuals. Currently we conduct the EPIS-TOP project within 7th Framework Programme of the European Commission, which is intended to discover changes in the molecular biomarkers within the epileptogenic process leading to the clinical seizures.

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The role of mTOR pathway in epilepsy treatment

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Epilepsy affects about 1% of the general population and in one third of patients the seizures are refractory to medical treatment. Most currently available antiepileptic drugs suppress seizures but do not influence the process of epileptogenesis, thus change neither the natural history of the disease nor the burden of epilepsy-related neuropsychiatric problems. Recently, much attention is paid to the research on the mechanisms of epileptogenesis. It has been showed both in animal models and clinical settings that Mammalian Target of Rapamycin (mTOR) pathway is involved in development of wide spectrum of epilepsies. mTOR is a serine-threonine kinase regulating cell growth, differentiation, proliferation, and metabolism. Up-regulation of mTOR pathway is a key finding in many disorders associated with epilepsy, like tuberous sclerosis complex (TSC), cortical dysplasias, Cowden syndrome, and others. It has also been reported in brain tissue obtained from epileptogenic foci. Many studies showed that in animal models, mTOR inhibitor, rapamycin, or its derivate, may ameliorate the development of seizures and reduce the risk of epilepsy comorbidities. In mouse model of TSC, rapa-

mycin introduced after the onset of seizures reduced epilepsy severity and prolonged survival, whereas introduced before the onset of clinical seizures prevented epilepsy and premature death of the animals.

mTOR inhibitor, everolimus, was approved by EMA and FDA for the treatment of brain and kidney tumors associated with TSC. Accumulating data indicate that mTOR inhibitors may alleviate epilepsy in TSC patients. Clinical studies aimed to investigate the impact of everolimus on drug-resistant focal epilepsy in TSC are currently ongoing.

In conclusion, mTOR pathway presents a promising possible target for antiepileptogenic treatment, however, further studies are needed.

Prominent microcolumnar cortical architecture in the case of pontocerebellar hypoplasia

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Pontocerebellar hypoplasia is congenital neurodevelopmental disorder characterized by hypoplasia of the cerebellar cortex, dentate nuclei, pontine and olivary nuclei. This report presents a case of 22-week-old infant born at 25 gestational weeks by cesarean section (adjusted age 7 weeks). During hospitalization the neonate underwent sepsis and surgery due to meconium ileus. The infant had seizures treated with phenobarbital and phenytoin. He clinically presented failure to thrive, hypotonia, episodes of irritability. An ultrasonography of the brain revealed intraventricular hemorrhage. Magnetic resonance imaging (MRI) examination of the head showed posthemorrhagic changes in the cerebellar vermis and cerebellar hemispheres with destruction of cerebellar hemispheres. Child died suddenly due to respiratory insufficiency. Postmortem examination of the brain revealed microencephaly, hypoplastic cerebellum and narrow basal part of the pons. Neuropathological abnormalities included hypoplasia of

the cerebellar hemispheres with the exception of the vermis and flocculi. Complete loss of Purkinje and granule cells in the cerebellar hemispheres cortex with astrocytic reaction, and a normal microscopic appearance of the floccular and vermal cortex were stated. Loss of the majority of neurons in the dentate nucleus with remaining dentate neurons clustered in islands, loss of pontine nuclei with near absence of transverse pontine fibers and dysplastic olivary nuclei with reactive changes were noted. The morphological picture of brain abnormalities correspond with the diagnosis of pontocerebellar hypoplasia. The changes within brain stem and cerebellum in the presented case coexisted with generalised persistent fetal radial columnar architecture. The minicolumnar arrangement was visible in all regions of the cerebral hemispheres with predominance in the temporal and frontal lobes. Prominent microcolumnar cortical architectonic pattern is regarded as separate type Ia of focal cortical dysplasias according to International League Against Epilepsy classification. There is a dilemma whether persistent fetal cerebral cortical circuit is the result of maturational arrest in histogenesis of neocortex or primary cortical dysplasia. Sarnat concludes that columnar architecture is a maturational arrest in histogenesis of the neocortical plate and becomes a component of cortical dysplasia in the perinatal period. It seems that in the presented case the persistent prominent columnar organization of neurons is a result of maturational delay in the cortical development accompanying pontocerebellar hypoplasia.

State-of-the-art imaging of patients with refractory epilepsy

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The crucial issue in surgical treatment of patients with refractory epilepsy is an accurate detection, localization and characterization of brain lesion. There is significant improvement in clinical status after surgery in over 70% of patients with refractory epilepsy if abnormal focal lesions were correctly diagnosed.

The diagnosis is difficult, due to a broad spectrum of underlying pathologies (e.g. hippocampal sclerosis, congenital abnormalities, epileptomas) which are often very subtle or invisible in available neuroimaging techniques.

The imaging procedure of choice in the investigation of patients with epilepsy is magnetic resonance imaging (MRI) performed on high-field systems (1.5 T and 3.0 T)

using dedicated protocol. Acquired images should be evaluated by experienced neuroradiologists.

Besides the standard sequences, such as T1, T2, FLAIR, DWI in axial plane, T2 in coronal and sagittal planes (4-5 mm slice thickness), applied in brain imaging, it is vital to use higher spatial resolution sequences like FLAIR in a plane perpendicular to the long axis of the hippocampus (2-3 mm), 3D T1-weighted inversion recovery (3D T1 IR) with isotropic voxel (1 mm) and sequence sensitive for the detection of hemosiderin and calcium (T2*/SWI).

Contrast enhanced images can be helpful in characterization of epileptogenic lesions. Furthermore, functional MRI and MR tractography is useful for mapping of the speech-eloquent areas and preoperative planning.

Magnetic resonance imaging allows morphologic evaluation of the brain, whereas positron emission tomography (PET) is a functional imaging technique which enables, by visualization of the regional glucose uptake, to assess the metabolic activity of the brain. Another valuable method is computed tomography, which allows detection of calcifications and hemorrhage.

The important issue in the identification of focal epileptogenic lesions is the correlation of radiological findings with clinical status and the results of other tests, including electroencephalography (EEG). Such analysis facilitates accurate characterization of abnormalities detected on imaging, helping in discrimination of epileptogenic and non-epileptogenic lesions.

Material and methods: Analysis included 71 patients aged 20-62 years (mean = 35) with right ($n = 31$) and left ($n = 36$) temporal lobe epilepsy, right ($n = 1$) and left ($n = 1$) frontal lobe epilepsy, left parietal lobe epilepsy ($n = 1$), gelastic epilepsy ($n = 1$). MRI (1.5 T) was performed using following sequences: T1SE (axial-5 mm), T2TSE (axial-5 mm), FLAIR (axial-5 mm), DWI (axial-5 mm), T2TSE (sagittal-5 mm), T2TSE (coronal-5 mm), IR (coronal-2 mm), FLAIR (coronal-2 mm) and contrast-enhanced T1SE (axial-5 mm), 3DT1GRE (1 mm). MRI findings were correlated with histopathology results.

Results: In 57 of 71 patients MRI detected focal lesions (80% sensitivity), failing to demonstrate abnormalities in 14 patients: FCDIIA ($n = 10$), "dual pathology" ($n = 3$), FCDIB ($n = 1$). Of 74 abnormalities revealed by histopathology, MRI correctly characterized 42 lesions (57% sensitivity), including all of FCDIIB, FCDIIIA, FCDIIIB, FCDIIID, double pathologies, DNTs, gangliogliomas, hamartomas (100% sensitivity), 80% of glial scars and post-infarct malacia, 68% of "dual pathologies", 17% of FCDIIA. None of FCDIA, FDAIB, oligodendrogliomas and tuberous sclerosis were correctly characterized by MRI.

Conclusions: MRI is feasible for the detection and characterization of some lesions in refractory epilepsy, including FCDIIB, FCDIIIA, FCDIIIB, FCDIIID and epileptomas. The demonstration and characterization by MRI of more than one lesion is often not possible.

Refractory epilepsy: radiologic – pathologic correlation

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Aims and objectives: We aimed to assess the sensitivity of magnetic resonance imaging (MRI) for the detection and characterization of focal brain lesions in patients who subsequently underwent operation due to refractory epilepsy. MRI findings were correlated with histopathology results based on a new classification of focal cortical dysplasia (FCD) proposed by ILAE (International League Against Epilepsy) Diagnostic Methods Commission in 2011.

The current histopathological classifications of cortical dysplasia

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There is a wide variability of pathological presentation of cerebral cortex lesions, associated with pharmaco-resistant epilepsy, that linked to cortical dysplasia. The first description of epilepsy-related cortical abnormality was reported by Taylor *et al.* (1971). Since then, the term "focal cortical dysplasia" (FCD) has been widely accepted to describe a large spectrum of lesions characterized by disorganization of cortical lamination and various cytoarchitectural abnormalities. During the last years, numerous FCD classifications have been proposed. The scheme introduced by Palmini *et al.* (2004), dividing FCD into distinct histopathological type I and type II, have appeared to be most acceptable in international community. According to

Palmini's classification, FCD type I included type IA with architectural disturbances of cortical lamination, and type IB with additional hypertrophic pyramidal neurons outside layer 5. Focal cortical dysplasia type II was also divided into type IIA with dysmorphic neurons and type IIB with dysmorphic neurons and large, eosinophilic balloon cells.

A revised clinicopathologic classification system was proposed by ILAE (International League Against Epilepsy) Task Force in 2011 (Blümcke *et al.*, 2011). This is a three-tiered classification system of focal cortical dysplasia that distinguishes isolated forms of FCD (FCD types I and II) from variants associated with another epileptogenic lesions (FCD type III). This scheme seems to provide a better characterization of clinical, imaging, pathologic and genetic features of distinct FCD subtypes and is helpful for clinical practice. According to ILAE histopathological classification, focal cortical dysplasia type I is a malformation presenting with abnormal radial (FCD type Ia) or tangential (FCD type Ib) cortical lamination. Moreover, the combination of both this variants is considered as FCD type Ic. Focal cortical dysplasia type II represents a malformation with disrupted cortical lamination and specific cytologic abnormalities, which differentiates FCD type IIa with dysmorphic, bizarre neurons in grey and/or white matter from FCD type IIb with dysmorphic neurons and so-called "balloon cells", similar to those observed in tuberous sclerosis. Cells with an intermediate phenotype between dysplastic neurons and balloon cells could be also encountered. Immunohistochemical studies reveal expression of both, neuronal and glial markers in dysmorphic and some balloon cells. The neuronal anomalies are usually accompanied by advanced astroglial gliosis in surrounding tissue. Focal cortical dysplasia type III refers to cortical lamination abnormalities associated with a principal lesion, usually adjacent to or affecting the same cortical area/lobe. Four variants of FCD type III are distinguished: FCD type IIIa – associated with hippocampal sclerosis (HS); FCD type IIIb – associated with glioneuronal tumours (ganglioglioma, dysembryoplastic neuroepithelial tumor – DNT) or other epilepsy-associated neoplasms; FCD type IIIc – associated with vascular malformations (cavernous or arteriovenous malformations, leptomeningeal vascular malformations, telangiectasias, meningioangiomas) and FCD type IIId – associated with any other principal lesion, acquired during early life (traumatic brain injury, perinatal ischemic injury, inflammatory or infectious diseases i.e. Rasmussen encephalitis, bacterial or viral infections). Moreover, so-called FCD type III not otherwise specified (NOS) is introduced to identify a clinically/radiologically suspected principal lesion in cases when tissue is not available for microscopic analysis.

The rare association between FCD types IIa and IIb with hippocampal sclerosis, tumour, or vascular malfor-

mation should be classified as "double pathology", not as FCD type III variant. Such pathology are suspected to be association of two epileptogenic lesions with independent pathogenesis.

It is worth noting, that any classification system using histopathologic examination requires representative surgical biopsy material and standardized laboratory techniques.

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A non-specific histopathological form of dysembryoplastic neuroepithelial tumour – diagnostic dilemmas

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Dysembryoplastic neuroepithelial tumour (DNT) is a distinct clinic-pathological entity related with a drug resistant, intractable epilepsy with the onset in childhood or young adolescence. The tumour was originally described as a benign lesion with characteristic histopathological features, including intracortical topography, nodular structure and presence of unique glial and neuronal components, called "specific glio-neuronal element". Such lesion was often accompanied by cortical dysplasia of the adja-

cent cerebral cortex and/or bone deformity. However, DNT might present a large spectrum of morphological picture that results in some difficulties in differential diagnosis.

Three different histopathological subtypes of DNT could be identified: 1) simple form, that is composed exclusively of specific glioneuronal element, 2) complex form with nodular architecture, including nodules that resemble conventional gliomas, accompanied by specific glioneuronal element and 3) non-specific form of DNT that lacks the specific glioneuronal element and resemble other conventional types of glial or glioneuronal neoplasms. The non-specific histopathological form of DNT is controversial. Occasionally, tumors exhibiting a mixed pattern, with features of DNT and additional neuronal or glioneuronal components. All subtypes of DNT are characterized by clinical stability with long-follow up with a normal life without recurrences and seizures.

The aim of this study was to present an unique morphological patterns of non-specific form of DNT composed of multinodular intracortical lesions with various histological components. Considering the non-specific form of DNT, other tumours presenting with epilepsy must be considered in differential diagnosis i.e.: low grade glial tumours as pilocytic astrocytoma, oligodendroglioma, astrocytoma or mixed oligoastrocytoma and high-grade glial neoplasms as anaplastic astrocytoma, anaplastic oligoastrocytoma or astroblastoma. Moreover, mixed glioneuronal tumour as ganglioglioma ought to be taken into consideration. The distinction of DNT lesion from other forms of gliomas allows to avoid unnecessary aggressive radio- or chemotherapy in children or young adults.

Children may also have other types of seizures, including tonic-clonic and atonic seizures. Gelastic epilepsy is slightly more common in boys than in girls. It is, however, very rare and of every 1,000 children with epilepsy, only one – two children will have gelastic epilepsy. In the clinical features they can be found also signs of precocious puberty, learning and behavioural problems.

The most common areas of the brain which give rise to gelastic seizures are the hypothalamus, the temporal lobes and the frontal lobes. A common cause of gelastic epilepsy is a small tumor in the hypothalamus. This tumor may be either a hamartoma or an astrocytoma. A hamartoma is a benign tumor mass, made up of an abnormal mixture of cells. The majority of these tumors are benign. This means that they may grow only very slowly, and do not spread to other parts of the brain or body.

The aim of the presentation is the case of 21 yrs old woman with drug resistant gelastic epileptic seizures, treated with many different antiepileptic drugs for 17 years. The diagnostic MRI scans were performed four times, and described as “normal”. In repeated MRI of the brain in 2013 the tumor mass in hypothalamus was diagnosed, what was proved in PET CT. The patient was surgically treated. The neuropathological examination revealed the typical features for hypothalamic hamartoma. The postoperative course complicated by paresis and impairment of memory functions. After intensive rehabilitation lasting for several months, she has no neurological problems. From the operation the gelastic seizures don't exist any longer. She suffers from complex partial seizures, 2-3/moth, and still needs the antiepileptic pharmacological treatment. Surgery, or a special form of radiotherapy, is only available in the high specialized neurosurgical centres.

Gelastic epilepsy as a result of hypothalamic hamartoma

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Gelastic epilepsy is a type of epilepsy in which seizures may begin at any age, but usually before three or four years of age. The seizures usually start with laughter, often described as ‘empty’ and not very pleasant. The laughter occurs suddenly, comes on for no obvious reason.

Reactive gliosis – in the search of new elements of its immunohistochemical signature

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Reactive gliosis is unspecific response of astrocytes in many different conditions. Reactive gliosis of astrocytes is possibly is most often unspecific morphological change observed in central nervous system in neoplasms, inflammations, malformations, vascular pathologies, demyelinations, toxic conditions, metabolic disturbances and neurodegeneration. Though having the same name and similar morphology, supposedly molecular and metabolic properties of gliosis differ with regard to its underlying condition. There is still need to find ways for more specific immunohistochemical characterization of gliosis. The aim of the study was the estimation of relatively little known proteins.

Astrocyte elevated gene 1 (AEG-1)/LYRIC/MTDH and Mesencephalic astrocyte-derived neurotrophic factor (MANF)/ARMET. Astrocyte elevated gene 1, also known as metadherin, regulates key cells processes in oncogenesis like angiogenesis, migration, invasion and metabolism of tumour cells. MANF belongs to evolutionary conservative group of neurotrophic factors. Moreover we tested expression of cytokeratins and mutated IDH1. Material: 96 cases of gliosis concomitant to following pathologies: primary and secondary tumours, abscesses, infarcts, vascular malformations. The regions of special interest were areas of gliosis in aforementioned lesions. The immunoexpression of the proteins was investigated using the following antibodies: Anti-human cytokeratin clone AE1/AE3, Rabbit monoclonal anti-Lyric clone EP 4445, Mouse monoclonal anti IDH1 R132H, Rabbit anti-human polyclonal MANF LS-B2688, Anti-human monoclonal antibody GFAP. AEG-1 immunopositivity was noted in 28/46 cases of gliosis and in 14/50 gliomas. MANF immunopositivity was noted in 20/46 cases of gliosis and in 15/50 gliomas (mostly glioblastomas). Moreover expression of MANF and AEG-1 was observed in cytoplasm and dendrites of neurons. Almost all cases of gliosis showed distinct expression of cytokeratins AE1/AE3 in astrocytes which was always limited to

unequivocally smaller number of astrocytes when compared with GFAP what confirms that AE1/AE3+ cytokeratins are much more selective marker of reactive astrocytes than GFAP. No cases of gliosis showed positivity mutated IDH1, though 26% of gliomas (80% of oligodendrogliomas) were IDH1 positive. Only part of gliosis and of gliomas was AEG-1 and/or MANF positive. Further research is necessary to find the answer for the cause of these differences.

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Current recommendations in cavernoma related epilepsy – diagnostic evaluation and management

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Cerebral cavernous malformations (CCMs) are well-defined lesions occur in 0.4-0.9% of the population. Seizures and epilepsy are frequent clinical manifestations, represent the most common symptomatic presentation of supratentorial lesions and have a great impact on social function and quality of life. Patients with CCM-related epilepsy (CRE) who undergo surgical resection achieve postoperative seizure freedom in only about 75% of cases, because insufficient efforts are made to adequately define and resect the epileptogenic zone. The authors present current pathophysiological concepts related to epilepsy associated with CCMs, definitions of definitive and probable CRE, and recommendations regarding the diagnostic evaluation. They also discuss the spectrum of disorders associated with these lesions and review the natural history, prognosis, and options for etiology-specific management of patients with CRE. At the time being published data do not permit the proposal of an evidence-based treatment guideline with regards to the resection of CCM in patients with CRE, although authors recommend excision of at least the cortical parts of the surrounding gliotic hemosiderin stained tissue.

A case of tuberous sclerosis complex associated with hemimegalencephaly in an infant of 32 weeks gestational age

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Hemimegalencephaly (HME) and tuberous sclerosis complex (TSC) are distinct and unrelated conditions that both are characterized by an early abnormality in cortical development, responsible for the intractable epilepsy. The association of hemimegalencephaly and TSC is extremely rare, with only a few cases published in the literature so far.

This report presents a case of a preterm male infant born at 32 week of gestational age by emergency cesarean section because of intrauterine asphyxia danger. Examination by ultrasonography disclosed cardiomegaly, multiple cardiac tumors corresponding to rhabdomyoma, cysts in the kidneys and the brain abnormality exhibiting marked enlargement and pachygyria of the left hemisphere. The baby died in the second day of age. Postmortem gross examination of the brain showed left hemimegalencephaly with features of pachygyria and agyria, hardened centrum semiovale and dilated left lateral ventricle, whereas contralateral hemisphere, brain stem and cerebellum appeared unaffected. By microscopic study of the brain the lesions were characteristic for TSC and consisted of cortical and subcortical tubers and subependymal nodules in both hemispheres and subependymal giant cell astrocytoma in the left hemisphere. The changes in the left hemimegalencephalic hemisphere were more extensive, with massive aggregates of gemistocyte-like balloon cell, extending from the cortical surface into the subcortical white matter and the subventricular germinal matrix. Some of the alterations and cellular abnormalities in hemimegalencephalic hemisphere had common characteristics for both TSC and HME and were difficult to separate between them and to consider, whether they represent two separate malformations or one process connected with the early development of TSC with extraordinarily extensive cerebral lesions.

Focal cortical dysplasia in patients with drug resistant epilepsy. Neuropathological features

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Focal cortical dysplasia (FCD) is very common in patients with drug refractory epilepsy in both children and adult patients. The ILAE (International League Against Epilepsy) Diagnostic Methods Commission, proposed a three-tiered classification system to characterize clinicopathological FCD entities.

We analyzed the neuropathological changes in two females (one aged 28; epilepsy diagnosed at the age of 10 years and the other 59 years old; epilepsy since the age of 12) treated neurosurgically because of drug-resistant epilepsy.

A neuroimaging study, showed in both cases focal cortical thickening and/or blurring of the grey-white matter interface in frontal lobes. The neuropathological findings (using histochemical and immunohistochemical methods) in cortical dysplasia showed cortical laminar disorganization and various pathological forms of neurons. Immature neurons were usually seen as round homogeneous cells with large nuclei and dysmorphic neurons with distorted cell body and pathological accumulation of neurofilaments. Immature neurons were sometimes immunoreactive to neuronal antibodies. The observed giant cells had large cytoplasm, usually normal in shape, sometimes had multiple nuclei. Balloon cells, considered the most characteristic of FCD, occurred in both cases. These cells showed large body size, eosinophilic cytoplasm and eccentric, sometimes multi-lobed nucleus. They had both neuronal and glial features like-intermediate cells. In the younger woman hypertrophy and proliferation of astroglia in areas of dysplasia were observed. Focal cortical dysplasia type II b with dysmorphic neurons and balloon cells was diagnosed in both described cases. The frequency of seizures decreased after neurosurgery.