

**CLINICAL APPROACH TO RARE NEUROLOGICAL  
DISEASES ASSOCIATED WITH B CELL PATHOLOGY**

PhD Thesis

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## **1. INTRODUCTION**

In the countries of the European Union, any disease that affects fewer than five people in 10.000 is considered rare. The present Thesis discusses rare diseases from a pathological and clinical perspective. The Thesis debates on two main disease groups: (i) Among recently described autoimmune limbic encephalitis syndromes, we are primarily focusing on LGI1 (leucine-rich glioma-inactivated 1) encephalitis and GABA<sub>B</sub>R ( $\gamma$ -aminobutyric acid B receptor) encephalitis in terms of clinical approaches and neuroimaging; we also provide the first case presentations in Hungary. (ii) We also discuss the clinical, histological and immunohistochemical data of patients suffering from intravascular lymphoma. The prognosis of both disease groups can be improved by adequate therapy; nevertheless, this requires recognition of these rare entities. Without therapy, these diseases may be fatal.

## **2. ANTIBODY-MEDIATED RARE NEUROLOGICAL DISEASES**

### **2.1 Literature review**

#### ***2.1.1 Paraneoplastic neurological disorders***

Paraneoplastic neurological disorders are neither consequences of direct effects of the tumour, nor results of oncological therapy; they are autoimmune diseases caused by molecular mimicry. Neurological symptoms are precipitated by an immune response initiated against the tumour that cross-reacts with neurological antigens. Tumour-specific antibodies and cytotoxic T cells themselves cause damage to the nervous system, if they gain access to the central nervous system (CNS). The diagnosis requires the consideration of three entities: (i) awareness and recognition of the clinical syndrome; (ii) examination of the antibody most often associated with the syndrome; (iii) screening for the most commonly associated tumour.

Paraneoplastic neurological syndromes (PNS) can be divided into three groups according to how commonly they are associated with a tumour: (i) in classical PNS, the syndrome is so frequently associated with a tumour and antibody that in the presence of the specific antibody the diagnosis can be confirmed even in the absence of the tumour. A CD8<sup>+</sup> cytotoxic T cell response is responsible for the neurological disease; since the antigen is intracellular, antibody responses are considered rather as biomarkers. (ii) In the case of

non-classical PNS, the association with a tumour is weak. Therefore, the definitive diagnosis of paraneoplasia requires the presence of all the three entities (syndrome, antibody, tumour). (iii) Some of these diseases are accompanied by a malignancy; however, they may also occur as a result of autoimmunity without malignancy. These diseases are caused by antibody responses against cellular surface antigens. Such recognitions resulted in a major shift of paradigm of antibody-mediated CNS diseases, including paraneoplastic diseases.

In terms of diagnostics, knowledge regarding the classification of onconeural antibodies is essential. The classic categorisation divides paraneoplastic antibodies into two groups. (i) Well-characterised onconeural antibodies that are commonly associated with tumors and classical paraneoplastic diseases are identified with routine immunohistochemical or immunoblotting methods (e.g. anti-Hu, anti-Yo, anti-CV2, anti-Ri, anti-Ma2, anti-amphiphysin). (ii) Partially-characterised antibodies may associate with tumours, however, the association is not as strong (e.g. anti-Tr, ANNA3, PCA2, Zic4, mGluR1). Besides paraneoplastic syndromes, some onconeural antibodies are also detected in autoimmune syndromes without cancer (e.g. anti-VGKC complex, i.e. voltage-gated potassium channel complex).

Antibodies associated with tumours and neurological diseases can be grouped on the localisation of the antigen as well: intracellular and cell-surface/synaptic proteins can be differentiated. Such classification is also relevant to therapy: antibody-mediated processes against cell-surface/synaptic structures could be influenced easier and have a better prognosis than cytotoxic T cell mediated responses in classical paraneoplastic diseases associated with antibodies against intracellular antigens.

### ***2.1.2 Novel paraneoplastic and autoimmune antibody-mediated encephalitis syndromes***

The recently discovered antibody-mediated processes against cell-surface/synaptic structures are less likely to be associated with tumours. In most cases, the antibody is detectable in the serum and cerebrospinal fluid (CSF), and the antibody titer can be higher intrathecally as would be expected from the serum titer. All these indicate a peripheral antibody response followed by an intrathecal expansion. The exact incidence and prevalence of diseases are unknown. According to a large study, on suspicion of paraneoplastic or autoimmune encephalitis, antibodies against the NMDAR (N-methyl-D-aspartate receptor) antibody can be verified in 67% of the cases, against the VGKC

complex in 7%, against GAD65 (glutamate decarboxylase) in 5%. Antibodies against AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and GABA<sub>B</sub> receptor were found in less than 4%. This Thesis will focus on LGI1 and GABA<sub>B</sub>R encephalitis syndromes.

*VGKC complex antibodies and LGI1 encephalitis.* Although particular encephalitis cases have been previously mentioned as voltage gated potassium channel encephalitis syndromes, it has been recently recognized that antibodies are not produced against the channel itself, but against closely associated proteins (LGI1, Caspr2, contactin-2). LGI1 is a transsynaptic scaffold-protein expressed by the inhibitor interneurons in the hippocampus. Deficient function may result in hippocampal excitement. LGI1 encephalitis is the most common cause of autoimmune limbic encephalitis (LE) in old age. Male predominance is characteristic. In the pre-encephalitis phase, symptoms of an autoimmune epilepsy syndrome may be present: faciobrachial dystonic seizure (FBDS) characterized by a short, monolateral dystoniform movement of the face, arms, and lower extremities lasting for a few seconds. Without immunotherapy, FBDS may be followed by LE. Hyponatremia can be found in 60% of cases (SIADH – syndrome of inappropriate antidiuretic hormone secretion), and sodium levels may fluctuate throughout the course of the disease even within a lower physiological range. Brain MRI (magnetic resonance imaging) shows T2/FLAIR (fluid-attenuated inversion recovery) temporomedial hyperintensity in more than half of the patients. In approximately one third of the cases, the total protein in the CSF is higher, in one fifth, pleocytosis can be found. Although early immunotherapy results in rapid improvement and good prognosis, functional/cognitive impairment can be detected later in most of the cases.

*GABA<sub>B</sub>R antibody encephalitis.* As GABA is the main inhibitory neurotransmitter of the CNS, it plays a pivotal role in modulating neuronal activity. While GABA<sub>A</sub> receptor functions as an ionchannel (ionotrope receptor), the GABA<sub>B</sub> receptor requires a mediator system, functioning in collaboration with G-protein (metabotrope receptor). Pre- and postsynaptic GABA<sub>B</sub> plays an important role in reducing the duration of intense network processes, and inhibits excessive synchronisation. The first cases of GABA<sub>B</sub>R encephalitis were published in 2010. The clinical symptoms are most probably triggered by synaptic dysfunction. Detection of antibodies in the serum and/or in the CSF and brain MRI play crucial roles in establishing the diagnosis. As GABA<sub>B</sub> receptor encephalitis is often

associated with small cell lung cancer (SCLC), search for malignancy is mandatory. Treatment consists of surgery, oncotherapy and first or second-line immunotherapy.

## **2.2 Clinical approach to LGI1 encephalitis**

### ***2.2.1 Natural course of LGI1 encephalitis***

#### *Aims*

LGI1 LE is a relatively benign disease compared with other antibody-mediated CNS syndromes. Bearing this in mind, we asked, whether there is a spontaneous recovery process that contributes to the benign prognosis. Records of patients with suspected LE but without a previous immunotherapy were reviewed. The possibility of LGI1 LE was retrospectively raised in two cases. Indeed, LGI1 antibody was detected in the serum 36 and 53 months after disease onset. We described the long-term natural course of LGI1 for the first time in these 2 cases.

#### *Case presentations*

*#1.* In February 2009, a previously healthy 50-year-old male developed acute cognitive impairment and anxiety. Addenbrooke's Cognitive Examination (ACE) indicated severe cognitive deficit (61/100) (dementia threshold <83/100 points). He had complex partial seizures and EEG (electroencephalography) revealed epileptic activity in the right temporal area. Valproate was started. CSF showed normal cell count and protein level without oligoclonal bands (OCB). Brain MRI was interpreted as normal, but re-analysis 5 years later disclosed temporomedial edema. Repeated MRI 2 months later detected bilateral atrophy and T2/FLAIR signal hyperintensity of the hippocampal regions. In May 2011, he was seizure-free and could manage simple everyday tasks. Brain MRI demonstrated bilateral hippocampal sclerosis with diffuse brain atrophy. In 2013, LGI1 antibodies were identified in the serum. He did not agree to re-examination of the CSF. He performs well in daily activities, but is unable to work due to deficiencies in short-term memory.

*#2.* In July 2010, a 48-year-old man presented with subacute memory dysfunction. He also had short unilateral tonic movements affecting the left arm and face. EEG indicated right temporal epileptic focus, therefore levetiracetam was started. MRI of the brain was normal. He had severe constant hyponatremia for months, and was even consulted by an endocrinologist, who suggested SIADH. Encephalitis was considered but CSF was normal.

The following weeks, he developed manifest psychosis with hallucinations, but his family did not seek medical help. A few months later, the psychotic episode remitted spontaneously. He stopped antiepileptic treatment in 2011, and has not had seizures since then. In 2013, anti-LGI1 antibodies were present in the serum, but were not in the CSF. Neuropsychological tests indicated mild cognitive impairment without dementia (ACE 84/100), MRI of the brain did not show any pathological signs. He manages his daily life well, and works as a fulltime employee.

### *Discussion*

Long-term follow-up of LGI1 encephalitis without immunotherapy has not been described. The two retrospectively identified LGI1 LE cases were examined before the description and international recognition of the disease itself.

Psychosis and epilepsy spontaneously recovered in both cases, but both patients remitted with moderate/mild cognitive dysfunction. Recent data suggest that only around one third of patients can achieve full recovery even on immunosuppressive treatment, and treated patients may also show disturbed spatial orientation and apathy. In one of our patients, no antibodies were detected in the CSF 3 years after the acute LE, suggesting that exclusive presence of LGI1 antibodies in the sera may not be enough for disease evolution if the blood-brain barrier is intact. It is well-known however, that antibodies may persist in the serum. In about 8% of the cases, antibodies can only be detected in the CSF, supposedly due to serum titer below a measureable threshold. Early immunotherapy is crucial, and cognitive deterioration can be prevented by early treatment of FBDS.

To summarise, our study has been the first to discuss clinical data with respect to the long-term natural course of LGI1 encephalitis. In some cases, the disease is benign and shows a monophasic course; nevertheless mild functional/cognitive deficit remains several years after disease onset. Therefore, early immunotherapy is necessary in all cases. Besides immunotherapy, a tendency of spontaneous recovery may play a role in the relatively fast improvement, when compared with the other recently discovered antibody-mediated encephalitis syndromes.

### ***2.2.2 A prospective multimodal MRI study of LGII encephalitis***

#### *Introduction*

Cases discussed in the previous chapter indicated a benign course of LGII encephalitis. Nevertheless, one of our patients developed global cerebral atrophy and hippocampal sclerosis despite spontaneous improvement in the clinical picture. It indicated that LGII encephalitis may affect the entire brain and not only the limbic structures. As some data in the literature also suggest global cerebral involvement, we planned an international (Hungarian and Danish) prospective MRI study investigating global and regional brain volumes, structural alterations and metabolic dysfunction.

#### *Aims*

We investigated 2 major groups of questions: (1) Can visual assessment of conventional MRI sequences at different time points during the clinical course identify changes? How these changes are associated with the clinical outcomes? Can they help better understanding of the underlying pathomechanism? (2) Can a prospectively planned multimodal MRI identify structural and metabolic changes? Can they help better understanding of the underlying pathomechanism? Are these predictive of cognitive impairment functional outcomes?

Therefore, conventional and prospective multimodal MRI scans were compared with each other, and correlated with short- and long-term clinical/paraclinical parameters, respectively.

#### *Patients and Methods*

Nine patients (four females), diagnosed with LGII LE participated in this study. Three patients were treated in Hungary (Department of Neurology, Kaposi Mor Teaching Hospital, Kaposvar). Six patients were treated in Denmark (Department of Neurology, Odense University Hospital, Odense and Department of Neurology, Rigshospitalet, Copenhagen University Hospital, Copenhagen). We also included nine Hungarian age- and sex-matched healthy volunteers (mean age:  $62.00 \pm 13.4$  years) in the prospective MRI study. Cognitive and functional disability scores (Mini-Mental State Examination: MMSE and modified Rankin scale: mRS) were evaluated at the onset,  $2.4 \pm 1.5$  months after onset (nadir) and  $23.4 \pm 7.6$  months after the onset. Results of ACE test were available  $23.4 \pm 7.6$

months after onset of LGII LE. The study procedures were approved in Hungary and Denmark by the local ethics committees.

Magnetic resonance imaging:

The initial MRI examinations were performed as part of the clinical diagnostic procedure within one month after disease onset. A prospective multi-modal MRI investigation was performed  $33.1 \pm 18$  months after the disease onset.

1. Acute phase clinical MRI acquisitions: The initial clinical MRI examinations included axial- and coronal T2-weighted Turbo Spin Echo (TSE), axial- and coronal FLAIR, sagittal T1-weighted TSE, three-dimensional T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE) and single-shot diffusion weighted spin-echo echo planar imaging.
2. Prospective image acquisition for multi-modal analysis: The patients were examined at two neuroimaging sites applying identical scanning protocols (Pécs, Siemens Magnetom® Trio™ 3T és Hvidovre, Siemens Magnetom® Verio™ 3T). Besides sequences used in acute examinations MRS (magnetic resonance spectroscopy) and DTI (diffusion tensor imaging) were also taken.

Data processing:

1. Visual evaluation of the MRI: Upon assessing global cortical atrophy, a qualitative scale (0-3) was used. Atrophy of the hippocampus and the temporal lobe were assessed according to Scheltens criteria (score 0-4).
2. Volumetric analysis: MPRAGE images were evaluated by FreeSurfer v5.3.
3. DTI and Tract-Based Spatial Statistics (TBSS): After brain extraction of the diffusion, data images of fractional anisotropy (FA) and mean diffusivity (MD) were generated. Voxel-wise statistical analyses of FA and MD data were performed using TBSS v1.2 part of FMRIB's Software Library (FSL).
4. MR Spectroscopy: Tissue type segmentation of T1-weighted MPRAGE was performed using the FAST software implemented in FSL, the voxels were constructed using Gannet software suite.

Statistical analyses:

For TBSS analysis, the voxel-wise statistics were performed on the skeletonized data using a permutation-based non-parametric analysis ( $p < 0.05$ ). All other statistical analyses were performed using SPSS 20.0 (IBM Corp., Armonk, NY).

### *Results*

*Clinical data:* Mean age at diagnosis was  $59.9 \pm 14.5$  years. Transient epilepsy developed in eight patients. Seven patients received immunotherapy in the acute stage. Immunosuppressive agents were orally administered over  $15.2 \pm 8.0$  months in six cases. Cognitive abilities measured by MMSE at 24 months (median 27; range: 21-30) showed a five-point improvement compared to nadir at  $2.4 \pm 1.5$  months (median 22; range: 10-28). The functional status evaluated by mRS at  $23.4 \pm 7.6$  months (median 2; range: 0-3) showed a two-point improvement compared to nadir (median 4; range: 3-5). Five patients showed cognitive decline at 24 months tested by ACE.

*MRI findings in the acute stage of limbic encephalitis:* Diagnostic MRI scans were performed within less than one month after disease onset in eight patients. FLAIR images revealed hyperintensities in the hippocampus in all the eight patients. Seven of the eight patients showed hyperintense signal changes in the hippocampus on T2-weighted images, whereas enhancing hippocampal lesions were only seen in one out of four patients on contrast-enhanced T1-weighted images. Seven out of eight patients showed edema in the hippocampus as revealed by FLAIR and diffusion-weighted imaging. Five patients displayed edema in the amygdala and the temporal cortex was affected in two cases (temporopolar and temporomesial). None of the patients had extratemporal lesions. Global cortical atrophy was found in six patients. Only a few patients showed atrophy of the hippocampus ( $n=3$ ), corpora mamillaria ( $n=3$ ) or amygdala ( $n=1$ ).

*Visual comparison of acute and follow-up MRI findings:* MRI data obtained in the subacute stage were compared with those prospectively acquired  $33.1 \pm 18$  months after disease onset. The number of patients identified with edema in the hippocampus decreased from seven to three. Additional edema of the temporal cortex and amygdala on the FLAIR and diffusion-weighted scans was present in one case. Hippocampal T2 hyperintensity persisted in six patients and FLAIR hyperintensity in seven cases. Eight of nine patients

developed hippocampal sclerosis. All nine patients showed global cortical and hippocampal atrophy (bilateral n=4).

*Long-term volumetric changes 33.1±18 months after disease onset:* Patients had significantly smaller volume in several brain areas outside the limbic structures (total segmented brain volume, brainstem, right thalamus, left n. accumbens, right hippocampus, mid posterior and central part of corpus callosum, bilateral cerebellar cortex, right cerebellar white matter, bilateral cortical white matter). Comparing clinical features with volumetric data, the following correlations were found: mRS score at onset significantly correlated with the volumes of cerebellar cortex and white matter ( $p=0.002$   $r=-0.932$  and  $p=0.02$   $r=0.842$ ) respectively, corrected for multiple comparisons. ACE test at  $23.4\pm 7.6$  months positively correlated with the volumes of putamen ( $p=0.004$   $r=0.911$ ).

*Widespread changes in white-matter microstructure 33.1±18 months after disease onset:* FA analysis showed widespread reductions in patients (corrected  $p<0.05$ ) diffusely across the entire cerebellar and cerebral white matter. Areas showing highly significant differences (corrected  $p<0.01$ ) include: medial lemniscus; superior and inferior cerebellar peduncle; pontine crossing tract; corticospinal tract; anterior corona radiate; fornix; retrolenticular-, anterior- and posterior parts of internal capsule; genu, splenium and body of corpus callosum; posterior thalamic and superior longitudinal radiation. MD analysis revealed widespread differences between patients and controls (corrected  $p<0.05$ ) diffusely across the entire cerebellar and cerebral white matter (MD was higher in patients).

*Metabolic changes in the white and grey matter 33.1±18 months after disease onset:* According to single-voxel proton MRS, only the reduced glutamine/glutamate concentration measured in the white matter voxel remained significant after correcting for multiple comparisons.

### *Discussion*

The most characteristic alterations in the acute LE phase were edema and T2/FLAIR hyperintensity of the hippocampus typical of LE in general, but we did not find extratemporal lesions. FLAIR hyperintensity of the hippocampus was always followed by hippocampus atrophy on the same side. A recent study suggested that persistent inflammation or epileptic activity may contribute to such radiological progression in patients with VGKC/LGI1 encephalitis. Presence of edema, despite clinical remission for

years in our seizure-free three patients may indeed indicate persistent inflammation similar to autoimmune encephalitis of different etiologies. However, epilepsy could also contribute to the progressive temporomesial MRI pathology: all eight patients with initial epilepsy developed hippocampal sclerosis, although the site of epileptic focus and hippocampal sclerosis did not correlate.

Follow-up volumetry showed a significant volume decrease in total segmented brain volume, white matter, corpus callosum, hippocampus, n. accumbens, brainstem and cerebellum. The volume reduction is most likely due to atrophy. The expression pattern of LGI1 with prominent staining in the hippocampus, the neocortex, thalamic nuclei and cerebellum corresponds to this observed atrophy.

FA analysis also indicated diffuse deterioration of cerebral and cerebellar white matter integrity. Our data also suggest axonal damage in frontal areas despite the lack of extratemporal lesions on conventional MRI during the acute stage; the most significant changes were measured in the anterior corona radiata, the anterior half of the capsula interna and the anterior 1/3 of the corpus callosum. MRI data regarding the frontal areas have been described only in individual cases; however patients with VGKC complex encephalitis may also have symptoms similar to frontotemporal dementia.

Lower glutamine/glutamate concentrations were found in the white matter compared to controls. Decreased glutamine/glutamate ratios were similarly observed in patients with Alzheimer's disease and patients suffering from major depression. Although our patients were not tested for such disorders, this may suggest the need for increased vigilance for affective disturbances in patients following LGI1 encephalitis. Considering structural MRI data indicating global atrophy, we expected a significant tNAA (total N-acetylaspartate) difference between the LGI1 patients and control subjects. Although the mean tNAA was somewhat lower in LGI1 group in both grey and white matter, the difference was not significant. Since the mean age of the patients was 60 years, it is possible that decrease of tNAA due to aging masks loss of tNAA related to the pathological condition.

We also correlated MRI outcomes with clinical data at onset, at nadir ( $2.4 \pm 1.5$  months) and at 2-year follow-up. Functional outcome (mRS) at onset negatively correlated with volumes of the cerebellar grey matter. Such correlation in LGI1 encephalitis has not been described, but visually assessed irreversible cerebellar atrophy was associated with poor

outcome in a recent study of NMDAR encephalitis. ACE test at 24 months positively correlated with the volumes of putamen. The putamen is actively involved in a variety of cognitive functions such as episodic memory, cognitive control and category learning. It receives input from frontal areas and becomes connected to other subcortical structures during learning, such as the hippocampus.

In conclusion, our results indicate (1) development of global brain atrophy in patients with LGII LE, despite early immunotherapy. (2) Besides alterations in the temporal limbic structures, we would like to call attention to progressive changes in the frontal lobe, basal ganglia and the cerebellum. These changes may contribute to persistent cognitive and functional deficits. (3) Metabolic changes detected with MR spectroscopy indicate neuroinflammation and abnormal glutamine/glutamate levels, however, they do not prove direct neuronal damage (i.e. significantly lower tNAA).

### **2.3 Clinical approach to GABA<sub>B</sub>R encephalitis**

#### *Aims*

GABA<sub>B</sub>R encephalitis is rare even among autoimmune encephalitis syndromes. This chapter intends to highlight novel characteristics, which may affect diagnosis and therapy based on reviewing and analysing clinical data of patients suffering from GABA<sub>B</sub>R encephalitis. Due to the rare occurrence of the disease, our retrospective analysis included data of Hungarian and Danish patients, and reported the first Hungarian cases.

#### *Case presentations*

#1. The 67-year-old, heavy smoker man was admitted to a department of neurology because of severe disorientation and short-term memory problems, which developed within days. Vascular dementia was suspected. His symptoms started to improve spontaneously after six weeks. ACE showed 80/100 points two months later. Brain MRI indicated atrophy and sclerosis of the left hippocampus. Autoimmune encephalitis was suspected: GABA<sub>B</sub>R antibodies were found in both the serum and CSF. All tumour-screening examinations proved negative; nevertheless, GABA<sub>B</sub>R antibodies persisted in the serum. Eighteen months after the onset of symptoms, FDG-PET CT (18F-fluorodeoxyglucose - positron emission tomography - computed tomography) showed enhanced activity in enlarged

mediastinal and retroperitoneal lymph nodes: cytology proved SCLC. The patient has been treated with chemotherapy. At the time of writing the Thesis, survival was 34 months.

#2. The 82-year-old, non-smoking man was admitted to the department of neurology due to repeated secondary generalized focal motor seizures. The sodium concentration of the peripheral blood was constantly low (<135 mmol/l). Brain MRI did not show specific alterations. CSF was characterized by mild mononuclear pleocytosis. Seizures persisted despite carbamazepin combined with levetiracetam and methylprednisolone, and coma developed. He needed respiratory support. After improvement of consciousness, his cognitive abilities progressively decreased. Chest X-ray and abdominal ultrasound examinations were negative. Due to the persistent hyponatremia, encephalitis antibodies were examined, revealing GABA<sub>B</sub>R antibodies in both the serum and CSF. The patient died within three months and no autopsy was performed.

#3. The 62-year-old, heavy smoker man was seen due to focal seizures with secondary generalisation. Brain CT scan was normal, and he was treated with levetiracetam and sent to the outpatient clinic for follow-up. Due to reoccurrence of seizures, he was re-admitted. Brain MRI was normal. CSF revealed mononuclear pleocytosis and normal protein level; viral PCRs (polymerase chain reaction) and flow cytometry were normal. Despite antiepileptic treatment, the patient suffered several generalized seizures and developed rapid cognitive deterioration with confusion and memory loss. GABA<sub>B</sub>R antibodies were elevated in both the serum and CSF. Whole body FDG-PET CT revealed slightly increased metabolic activity in mediastinal lymph nodes, and biopsy confirmed the diagnosis of SCLC. Chemotherapy was started with repetition of plasma exchange, but his cognitive abilities remained severely impaired, and the patient died after six weeks due to infection.

#4. A 60-year-old, regular smoker male patient was admitted to a town hospital due to refractory generalized tonic-clonic seizures. He had amnesia and was confused. CT of the brain without contrast was considered normal. CSF analysis revealed normal protein and cell content, but positive OCBs and a slightly increased IgG index. Biopsy of a right-sided lung density (detected by chest CT) revealed SCLC. High titer of IgG GABA<sub>B</sub>R antibodies were found in the CSF and serum. Control MRI examinations with two months interval showed subtle changes consistent with edematous mesial temporal lobes on FLAIR. Following IvIg and iv/oral steroids, the patient made an uneventful cognitive recovery. He was managed with oral prednisolone and levetiracetam for two years without reoccurrence

of seizures or cognitive deficits. Twenty-two months after disease onset he passed away due to metastasized lung cancer.

### *Discussion*

Besides already known characteristics, our cases highlight novelties in connection with GABA<sub>B</sub>R encephalitis that have not been discussed in the literature to date. GABA<sub>B</sub> receptor encephalitis usually develops in elderly men and women. Epileptic seizures are very common (90%); however, seizures may sometimes be absent, as indicated by Case 1. Since the pathogenesis involves inflammation, immunotherapy should be part of the treatment protocol; otherwise the prognosis of epilepsy is poor. Severe memory loss, especially short-term memory problems indicate LE. Rarely, the disease can present without LE, and has to be considered in unexplained subacute cerebellar ataxia, opsoclonus and brainstem encephalitis.

We found two peculiar features, which have not been previously reported. (i) Spontaneous improvement of the severe cognitive deficit and short-term memory loss were observed in Case 1. This improvement suggested an autoimmune origin rather than paraneoplastic disease. Nevertheless, SCLC was detected 18 months later in lymph nodes. It is possible that the occult tumor and the low or changing antigen load might play a role in the spontaneous improvement. This case indicates that spontaneously remitting GABA<sub>B</sub>R encephalitis may precede detection of SCLC by one and a half years. (ii) We found consistently decreased sodium levels in the serum of Case 2, which may also be a characteristic feature of LGI1-encephalitis. No anti-LGI1 antibodies were found in our cases.

CSF was abnormal in three out of four cases compatible with previous data indicating pathological findings in about 70-90% of cases. Of note, the patient with spontaneous improvement and long survival had normal findings in the CSF. GABA<sub>B</sub>R antibodies were present in both the serum and CSF in all four cases. Previous data indicated no correlation between antibody levels and severity of the disease. In Case 1, antibodies persisted in the sera despite the spontaneous improvement of encephalitis. No additional antibodies against surface or intracellular antigens were found in our cases, nevertheless screening for other surface and onconeural antibodies may be important: antibodies against voltage-gated calcium channels, thyroid peroxidase, anti-GAD65, anti-amphiphysin, anti-SOX1, anti-NMDAR, anti-Hu and anti-CV2 have been described. Association with additional

antibodies may indicate paraneoplastic etiology; in 50% of the cases SCLC could be found. If LE is associated with SCLC and anti-Hu antibodies are not present, GABA<sub>B</sub>R antibodies have to be examined. The associated antibodies may also modify the clinical picture: e.g. psychiatric symptoms may be dominant if GABA<sub>B</sub>R antibodies co-associate with NMDAR antibodies, while refractory status epilepticus may be characteristic in the co-presence of anti-GAD65.

Brain MRI indicated abnormal signal changes in three out of the four cases, which corresponds to other case series, where brain MRI is uninformative only in about one-fourth or one-third of the cases. Mediotemporal or cortical T2/FLAIR hyperintensities, signal changes in the corpus callosum, or leptomeningeal enhancement can usually be seen. In Case 2, MRI was interpreted as normal, but re-evaluation revealed bilateral FLAIR hyperintensities in the hippocampal area. Indeed, alterations in limbic structures may be subtle on MRI and have to be looked for.

The low number of cases complicates prognostic knowledge. Spontaneous improvement in paraneoplastic cases has not been reported: this is unique in Case 1. Two of the four patients with paraneoplastic disease died despite chemotherapy. Mortality is high especially in paraneoplastic cases, but 80% responds well to immunotherapy. Long-term prognosis depends on the associated cancer, therefore patients should be followed.

To summarise, our cases point towards the bad prognosis associated with a GABA<sub>B</sub>R encephalitis. Hyponatremia may also characterise this disease, not only LGII encephalitis. Despite spontaneous remission, LE may precede cancer diagnosis with more than a year, therefore cases which may appear to have an autoimmune etiology should also be followed. The disease should also be considered in cases of therapy-resistant epileptic attacks in the elderly in the absence of a known etiology.

### **3. INTRAVASCULAR LYMPHOMA AND THE CENTRAL NERVOUS SYSTEM**

#### **3.1 Literature review**

Non-Hodgkin lymphomas affecting the CNS are rare. They may develop in three different ways:

*A. Primary CNS lymphoma.* The CNS parenchyma is primarily affected. Based on localisation, they can be primary cerebral, leptomeningeal, vitreoretinal or spinal with corresponding symptoms.

*B. Secondary CNS lymphoma.* Metastasis of lymphoma outside the CNS.

*C. Intravascular lymphoma (IVL).* IVL is a subtype of extranodal large B cell lymphoma restricted to the lumen of vessels. The Asian type: is characterised by hepatosplenomegaly and haemophagocytosis syndrome. The Western type (cutaneous variant) is accompanied by fever, neurological and dermatological symptoms. Investigation guidelines of lymphomas may give false negative results in IVL. The triad of anemia, increased LDH (lactate dehydrogenase) and erythrocyte sedimentation rate (ESR) are the typical laboratory findings. In the case of CNS involvement, brain MRI may show aspecific white matter T2/FLAIR hyperintensities, but extracerebral manifestations should be always investigated in order to find a biopsy site. Two main groups of lymphomas can be distinguished based on the gene-expression profile: (i) centrum germinativum B cell type (GCB), which expresses genes characteristic of physiological centrum germinativum, and (ii) activated B cell type (ABC or non-GCB), which expresses genes characteristic of activated B cells. The ABC phenotype has much worse prognosis. In IVL, 20% are of the GCB, and 80% of the non-GCB types. Treatment evidences based on randomised studies are not available for IVL. In case series, R-CHOP therapy has been shown to have good results; positive effects have been also reported with autologous stem cell transplantation. In the case of CNS involvement, intrathecal chemotherapy may also be required.

#### **3.2 Clinical and pathological characteristics of intravascular lymphoma**

##### *Aims*

Regarding the rare incidence of intravascular lymphoma, our prior aim was – for the first time in our country – to analyse and report the clinical and immunohistochemical data of several patients, relate them to disease progression, outcome and therapeutic options.

### *Case presentations*

#1. A 53-year-old male patient was examined because of temporary aphasia followed by progressive gait ataxia, dementia and seizures. Laboratory results showed decreased hemoglobin, increased LDH and elevated ESR. Brain MRI revealed multifocal confluent cortical and subcortical T2 hyperintensive lesions without contrast enhancement. CSF showed albumino-cytological dissociation. IVL was suspected. Biopsy taken from the brain tissue confirmed the suspected diagnosis. Immunostaining characterised the lymphoma as an ABC phenotype. The patient received a 4-month R-CHOP chemotherapy, and subsequently underwent autologous stem cell transplantation. Eighteen months after the treatment he was symptomfree, and he has been in remission for more than two years.

#2. A 62-year-old male patient developed acute, painful, ascending paresis. Based on clinical signs and albumino-cytological dissociation in the CSF, Guillain-Barre syndrome was suspected. Anemia and increased ESR were noted. The patient's condition progressed, multifocal neurological signs appeared. Thoracal MRI revealed intramedullar T2 hyperintensity suggestive of longitudinal transverse myelitis. Brain MRI showed multifocal T2, FLAIR hyperintense cortical and subcortical lesions. Chest and abdominal CT scans were negative. On suspicion of IVL, brain biopsy was performed, but histology only showed haemorrhagic transformation. Fifty days after the appearance of symptoms, the patient died amid symptoms of respiratory failure. Autopsy confirmed the diagnosis of IVL with an ABC phenotype.

#3. A 46-year-old female had had intermittent fever for weeks, when she developed a temporary right-sided hemiparesis. LDH and ESR were increased. CSF showed albumino-cytological dissociation. The first brain MRI showed no significant pathology. She developed multifocal neurological signs. Repeated MRI revealed blurred, tiny T2 hyperintensities in both thalami and the pons. Steroid bolus therapy resulted in temporary improvement. Abdominal CT performed with the aim to screen for tumour showed an enlarged adrenal gland, which was subsequently removed. Postoperative pneumonia and septic shock developed, and the patient died. Histology of the adrenal gland verified IVL of ABC phenotype, postmortem.

## *Discussion*

Due to its varied clinical appearance, diagnosis of IVL is difficult, and a large variety of potential diagnoses should be considered and excluded. Obliteration of vessels by lymphoma cells can recanalise spontaneously or as a result of steroid therapy (Case #3), raising the possibility of further disorders. Without treatment, the initial fluctuation of neurological symptoms is invariably followed by a progression. Acute polyradiculopathy developing due to IVL (Case #2) should not be confused with neurolymphomatosis, a condition of lymphomatous infiltration of nerves. The typical laboratory triad of anemia, increased LDH and elevated ESR was present in two of our patients. Our cases also exemplify the wide and varied neuroradiological spectrum. As fluctuating morphology may be typical in the clinical presentation, so can dynamic morphology be characteristic in a neuroradiological perspective, depending on the actual occlusion and recanalisation of vessels (Case #3). MRI is positive in most cases; however, if no changes are shown, the symptoms may be explained by the incomplete or transient occlusion of the vessels ('TIA mimics'). The dynamic occlusion/recanalisation of vessels can result in negative histology (Case #2), or the parenchyma shows necrosis or haemorrhage without lymphoma cells. When searching for other clues, the common affection of the skin should be also kept in mind as biopsy site. Immunohistochemistry verified ABC phenotype in all three cases (MUM1+, Bcl-6±, CD10-), similar to tendencies mentioned in the literature: about 80% of patients with IVL belong to this category. As regards to therapy, there are no uniform guidelines. Poor prognosis is not only due to the aggressive nature of the disease, but also to late diagnosis. In the case of our first patient, early R-CHOP therapy – often considered effective in publications – combined with autologous stem cell transplantation resulted in relapse-free status for at least 24 months.

As the first such study in Hungary, our data are in line with international results. Our Western type patients showed fluctuating, wide-spectrum neurological symptoms. Although MRI findings are aspecific, dynamic morphological presentation is of great importance. Since histology is the only method that can establish definite diagnosis, it is worthwhile to search for extracerebral (e.g. cutaneous or adrenal gland) manifestations as potential biopsy sites. We presented the first patient in the literature, who was treated with a combination of R-CHOP and autologous haemopoetic stem cell transplantation.

#### 4. SUMMARY OF THE THESES

- The natural course of LGI1 limbic encephalitis can be monophasic and spontaneously remitting. This could partially explain the rapid response to immunotherapy and the relatively benign prognosis contrary to other antibody-mediated encephalitis syndromes.
- Based on the visual assessment of longitudinal brain MRI changes in LGI1 encephalitis, persistence of autoimmune inflammation and/or the pathogenic role of epilepsy can be suspected.
- Our results indicate global cerebral atrophy, structural and metabolic changes in patients with LGI1 limbic encephalitis irrespective of the immunotherapy, i.e. despite its name the disease is not a 'limbic' encephalitis.
- Besides lesions in temporal structures, damage of frontal areas, the basal ganglia and the cerebellum may also play a role in the persistence of cognitive and functional deficits.
- Chronic metabolic changes point toward the necessity to examine patients for mood disorders.
  
- The spontaneous remission of GABA<sub>B</sub>R encephalitis may precede cancer diagnosis with more than a year, thus cases which may appear autoimmune relapsing/remitting in origin have to be closely followed.
- Persisting hyponatremia may occur in GABA<sub>B</sub>R encephalitis.
- Absence of epilepsy and normal cerebrospinal fluid may indicate a more benign prognosis of GABA<sub>B</sub>R encephalitis.
  
- The ABC phenotype of intravascular lymphoma is the most common.
- The combination therapy of R-CHOP and autologous stem cell transplantation may result in good prognosis in intravascular lymphoma.

## PUBLICATIONS

### Publications related to the Thesis:

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