CLINICAL AND LABORATORY DIAGNOSTIC ASSESSMENT OF IMMUNE-MEDIATED NEUROLOGICAL DISORDERS

Ph.D. Thesis

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INTRODUCTION

Paraneoplastic neurologic syndromes (PNSs) are rare neurological disorders associated with cancer, resulting from a remote effect of a malignant neoplasm. The disease is caused by immune reaction against intracellular antigens (Ma2, Yo, CV2, Hu, amphiphysin, Ri, Tr, GAD65, Zic4, titin, SOX1, recoverin) expressed in tumours, resulting in production of onconeural autoantibodies cross-reacting with neuronal structures and presenting with various neurological symptoms. Onconeural autoantibodies are not likely to be pathogenic, however, their detection in sera can indicate the presence of an underlying tumour, making it possible to conduct targeted cancer screening in affected patients.

During the past few years, it has been recognized that there are central nervous system (CNS) disorders presenting in the form of limbic encephalitis (LE), in which the presence of autoantibodies against the neuronal cell surface receptors such as NMDAR, GABABR, and AMPAR or synaptic proteins, LGI1 and Caspr2, has been documented and shown to be responsible for the development of the symptoms. **Autoimmune encephalitis (AE)** may present with a wide spectrum of clinical symptoms, such as behavioural and psychiatric disorders, cognitive impairment, changes in the level of consciousness, seizures and movement disorders. The distinction between PNSs associated with autoantibodies against intracellular neuronal antigens and neuronal cell surface antibody-mediated AE is crucial. In onconeural antibody-associated PNSs, systemic tumour association is frequent, occurring in >90% of cases, whereas, neuronal cell surface antibody-mediated AE is variably associated with tumours. The better outcome and the effectivity of immunotherapy (steroids, plasmapheresis, immunosuppression, IVIG) in AE, might be explained by reversible neuronal dysfunction, which is caused by pathologic autoantibodies binding to extracellular epitopes of neuronal cell surface proteins, and altering their structure and function. In PNSs, T cell-mediated irreversible neuronal damage is frequently present, thus immunotherapies are generally not effective; however, proper treatment of the tumour can lead to stabilization of the patients. Early clinical diagnosis of these disease groups is important as they can have fatal outcome. Besides clinical features and auxiliary examinations, such as electroencephalography (EEG) and structural magnetic resonance imaging (MRI), accurate diagnosis of PNSs and AE requires detection of characteristic autoantibodies in the serum and/or cerebrospinal fluid (CSF).

Multiple sclerosis (MS) is a chronic, progressive, neuroinflammatory disease, characterized by immune-mediated inflammation, demyelination and axonal damage in the CNS. **Neuromyelitis optica spectrum disorder (NMOSD)** is an inflammatory autoimmune disease of the CNS, primarily affecting the optic nerves and the spinal cord, leading to blindness and paralysis. NMOSD was only recognized as a distinct disease entity and separated from MS over the past 10 years with the discovery of a unique biomarker, autoantibodies against the aquaporin-4 (AQP4) molecule. The clinical success of anti-CD20 antibodies in the treatment of MS and NMOSD underlines the important role of B cells in disease initiation and progression. In addition to antibody production, B cells are important in antigen-presentation and pro-inflammatory cytokine secretion. Studies focusing on B cell subpopulations in MS and NMOSD are limited, and the precise role and changes in naïve and memory B cell distribution, is still unclear in the development of MS and NMOSD.

CD180, or RP105 (radioprotective 105 kDa), is a Toll-like receptor (TLR) homologue molecule, expressed by B cells, monocytes and dendritic cells, and it mediates polyclonal B cell activation, proliferation and immunoglobulin production. The altered expression and functions of CD180 in B cells have been described in autoimmune diseases. CD180-negative B cells were increased in patients with Sjögren's syndrome, and in systemic lupus erythematosus (SLE) patients. Moreover, disease severity in SLE correlated with the amount of CD180-negative B cells in the peripheral blood. It has been reported that non-switched memory (NS) B cells showed the strongest activation after CD180 ligation, and their stimulation via CD180 resulted in enhanced natural autoantibody production.

Natural autoantibodies are low affinity, polyreactive antibodies, which serve as the first line of defense against infections. Anti-citrate synthase (CS) natural IgM/G autoantibodies have been detected in healthy controls (HCs) and patients with systemic autoimmune diseases. Monitoring of anti-CS natural IgM autoantibodies in healthy adults over a five-year period, showed that titer of anti-CS IgM antibodies is constant and characteristic for the given individual. Significantly higher levels of anti-CS IgM autoantibodies were measured in anti-dsDNA IgM-positive SLE serum samples, besides, anti-CS IgM and anti-dsDNA IgM levels also showed correlation, supporting that these IgM autoantibodies are part of the natural immune repertoire in SLE patients. It has been described that the titer of anti-CS IgG antibodies is fluctuating over time, and it shows association with infection-induced antibodies.

Several pathogens, including Chlamydia pneumoniae, Chlamydia trachomatis, Mycoplasma pneumonia, Helicobacter pylori and Borrelia burgdorferi have been described to be possibly involved in the development of NMOSD or MS.

AIMS

1. To analyse the characteristics of onconeural and neuronal cell surface autoantibody testing in Hungary:

We retrospectively analyzed the results of serum and CSF samples of patients with suspected PNSs and AE, obtained by the Department of Immunology and Biotechnology, Clinical Center, University of Pécs, Medical School, Pécs, Hungary from 2010 until 2018. In the study, we aimed to determine national 1) prevalence, 2) incidence, and 3) age- and sex-based distribution of twelve types of onconeural autoantibodies (anti-Hu, anti-Yo, anti-Ri, anti-Ma2, anti-CV2/CRMP5, anti-amphiphysin, anti-Tr/DNER, anti-GAD65, anti-Zic4, anti-titin, anti-SOX1 and anti-recoverin), and six types of autoantibodies directed against neuronal cell surface receptors or their associated proteins (anti-NMDAR, anti-LGI1, anti-Caspr2, anti-GABABR, anti-AMPAR1, and anti-AMPAR2) in the Hungarian population. We further aimed to determine 4) whether neuronal cell surface autoantibody types were detected in serum and/or CSF.

2. To investigate the clinical characteristics and outcome of neuronal cell surface antibody-mediated autoimmune encephalitis patients in a multicentre cohort in Hungary:

We retrospectively determined national 1) demographics, 2) prodromal symptoms, 3) clinical features, 4) tumour associations, 4) CSF findings, 5) EEG and 6) brain MRI results, 7) therapy and 8) prognosis of 30 patients diagnosed with neuronal cell surface autoantibody positivity (anti-NMDAR, anti-LGI1, anti-GABABR, anti-Caspr2) in the Department of Immunology and Biotechnology, Clinical Center, University of Pécs, Medical School, Pécs, Hungary from 2012 until 2018. We further aimed to compare AE patients based on 9) the presence of signs of CNS inflammation, and 10) neuronal cell surface autoantibody types.

3. To analyse the distribution and CD180 expression of naïve and memory B cell subsets in patients with autoimmune CNS disorders:

The aim of the study was to determine 1) distribution and 2) CD180 expression of peripheral blood naïve, double negative (DN), switched (S) and non-switched (NS) memory B cell subsets defined by CD19/CD27/IgD staining in NMOSD, and compare with MS and HCs. We further aimed to measure 3) anti-CS natural IgM/G autoantibody levels, and 4) infection-induced

antibody levels, including anti-Chlamydia pneumoniae, anti-Chlamydia trachomatis, anti-Mycoplasma pneumonia, anti-Helicobacter pylori and anti-Borrelia burgdorferi antibodies in sera, which might be involved in the development of NMOSD or MS. We aimed to 5) assess the correlation between anti-CS natural IgG autoantibody levels and infection-induced antibody levels.

MATERIALS AND METHODS

1. Single-center study of onconeural and neuronal cell surface autoantibody testing in Hungary

For onconeural autoantibody detection in sera, line-immunoblot assays with six and twelve recombinant protein antigens were applied. For neuronal cell surface autoantibody testing from sera and/or CSF, cell-based indirect immunofluorescence BIOCHIP assays were used. Samples were obtained by the Department of Immunology and Biotechnology, Clinical Center, University of Pécs, Medical School, Pécs, Hungary, from various Hungarian neurological clinics from 2010 (onconeural autoantibody testing) and 2012 (neuronal cell surface autoantibody testing) until 2018 as part of a nationwide program. Retrospective evaluation of 2543 serum samples from 2362 patients with suspected PNSs and 1247 sera and/or CSF samples from a total of 1034 patients with suspected AE was performed.

2. Clinical characteristics of neuronal cell surface antibody-mediated autoimmune encephalitis in a Hungarian cohort

We retrospectively identified 30 definite AE patients tested positive for at least one neuronal cell surface autoantibody (anti-NMDAR, anti-LGI1, anti-GABABR, anti-Caspr2) in sera, CSF or both in sera and CSF at the Department of Immunology and Biotechnology, Clinical Center, University of Pécs Medical School, Pécs, Hungary. Clinical data were collected using an online questionnaire in collaboration with neurologists specialized in neuroimmunology from four major clinical centers of the region in Hungary. Statistical evaluation of clinical data was performed with the SPSS IBM version 26 (IBM, Armonk, NY, USA).

3. Investigation of B cell abnormalities in patients with autoimmune CNS disorders

Twelve patients with NMOSD, fifteen patients with RRMS (relapsing-remitting multiple sclerosis), and six age- and sex-matched HCs were enrolled in the study. All NMOSD patients were diagnosed based on the 2015 new diagnostic criteria for NMOSDs and all MS patients met the revised McDonald criteria. We performed four-color flow cytometric analysis using anti-human CD19-FITC, anti-human CD27-APC, anti-human IgD-PerCP and anti-CD180-PE antibodies to investigate the distribution and the CD180 expression at protein level in peripheral blood B cell subsets in NMOSD and MS patients. To analyse the CD180 expression at mRNA level in peripheral blood B cell subsets in NMOSD and MS patients, we separated naive and memory B cells using S3e Cell Sorter (Life Science Research/Bio-Rad Hercules, CA, USA), which was followed by RNA isolation, cDNA generation and CD180 mRNA expression of naïve and memory B cells was determined by qPCR. The mRNA expression of CD180 was normalized to GAPDH (a "housekeeping" gene) as reference. An in-house ELISA method was applied to measure anti-CS natural IgM/G autoantibody levels in sera of NMOSD and MS patients. Commercial ELISA kits were used to detect infection-induced antibodies, including anti-Chlamydia pneumoniae IgM/G/A, anti-Chlamydia trachomatis IgM/G/A, anti-Mycoplasma pneumonia IgM/G/A, anti-Helicobacter pylori IgG/A and anti-Borrelia burgdorferi IgM/G. Statistical evaluation of data was performed with the SPSS IBM version 26 (IBM, Armonk, NY, USA).

RESULTS

1. Single-center study of onconeural and neuronal cell surface autoantibody testing in Hungary

Upon analysis of 2543 serum samples from 2362 patients with suspected PNSs, we found 235 positive samples (9.2%) belonging to 190 patients (8%). Since the introduction of the onconeural autoantibody test in 2010, an overall increase was observed in the number of tested samples. We found an overall 1.9/100,000/year incidence of onconeural autoantibody positive cases in the Hungarian population. In the onconeural autoantibody positive patient group (n=190) the following autoantibody frequencies were found: anti-Yo (24.7%), anti-Hu (20%), anti-Ma2 (18.9%), anti-CV2 (12.1%), anti-titin (9.4%), anti-Zic4 (8.9%), anti-amphiphysin (7.4%), anti-Ri (2.6%), anti-GAD65 (2.6%), anti-Sox1 (2.6%) and anti-recoverin (1.1%). Age- and sex-based distribution of the onconeural autoantibody positive patient group (n=190),

revealed higher proportion of affected females (*n*=120) compared to males (*n*=70), with a median age of 62 years (range: 16-88 years). During the analysis of 1247 test requests (sera and/or CSF samples) from a total of 1034 patients with suspected AE, we found 98 positive samples belonging to 60 patients (5.8%). We found an overall 0.6/100,000/year incidence of neuronal cell surface autoantibody positive cases in the Hungarian population. The frequency of autoantibodies was the following: anti-NMDAR autoantibody was present in 70%, anti-LGI1 in 15%, anti-GABABR in 12%, and anti-Caspr2 in 7% of patients. Anti-NMDAR encephalitis mostly affected females: of the 42 anti-NMDAR positive patients, 28 were female, median age 25 years. Anti-LGI1 encephalitis most frequently occurred in middle-aged males: of the 9 anti-LGI1 positive patients, 6 were male, median age 51 years. Anti-GABABR encephalitis affected elderly males: of the 7 anti-GABABR positive patients, 4 were male, median age 58 years. Anti-Caspr2 encephalitis occurred in male adults: of the 4 anti-Caspr2 positive patients, 3 were male, median age 52 years. Half of the patients had repeated tests at different time points, which resulted in autoantibody positivity multiple times in 17/30 cases. In most anti-NMDAR patients, autoantibodies were detected only or with stronger positivity in CSF, in anti-LGI1 and anti-Caspr2 patients, serum was the adequate sample type, and in anti-GABABR patients autoantibodies were present both in sera and CSF.

2. Clinical characteristics of neuronal cell surface antibody-mediated autoimmune encephalitis in a Hungarian cohort

Among the 30 definite AE patients, the most commonly identified antibody was anti-NMDAR (19/30, 63.3%), followed by anti-LGI1 (6/30, 20%), anti-GABABR (3/30, 10%) and anti-Caspr2 (3/30, 10%). One patient showed positivity for both anti-LGI1 and anti-Caspr2 antibodies. Most patients were males (19/30, 63.3%) with a median age of 39.3 years (range: 1-75 years). The most common prodromal symptoms were fever or flu-like symptoms (10/30, 33.3%), occurring mainly in anti-NMDAR encephalitis patients. In one case herpes simplex virus (HSV) encephalitis preceded the development of anti-NMDAR encephalitis. Median time to diagnosis was 2 months (range 1-53 months). The most common initial presentations were psychiatric symptoms (17/30, 56.7%), which were present in almost all cases during the disease course (25/30, 83.3%). Other clinical features included seizures (22/30), memory loss (15/30), insomnia (7/30), speech disorders such as dysarthria or aphasia (5/30), status epilepticus (4/30) and ataxia (3/30). Involuntary movements, such as dyskinesia, dystonia or choreoathetosis were the most common in anti-NMDAR encephalitis (9/19). Hyponatraemia (5/6) and faciobrachial

dystonic seizures (FBDS; 3/6) were the most frequent in the anti-LGI1 encephalitis patient group. Eight patients (26.7%) had associated tumours, most commonly lung carcinoma. Abnormal CSF findings, such as pleocytosis (white blood cell count > 5 cells/mm^3), the presence of OCB, increased protein level (> 450 mg/L) and/or elevated IgG index (> 0.65) were observed in 13/24 of the tested AE patients, most commonly in anti-NMDAR and anti-GABABR patients. EEG abnormalities, most frequently focal slowing (6/30) and interictal epileptiform discharges (4/30), were observed in 14/29 AE patients. Abnormal brain MRI results, most commonly, unilateral or bilateral lesions in the insula/hippocampus were detected in 14/27 AE patients. First-line immunotherapy (24/30), mainly the combination of methylprednisolone pulse and plasmapheresis (12/30) was applied. Second-line therapy (azathioprine or rituximab) was introduced in four anti-NMDAR encephalitis patients, not responding to first-line therapy. The median hospital stay of AE patients was 23 days. Patients were severely impaired on admission, with a median mRS score of 4 (range 2-5). Most AE patients (25/30, 83.3%) achieved a good outcome (mRS ≤ 2) following treatment. Median follow-up duration was 33 months (range 1-77). AE patients with associated tumour (*n=8*) had a significantly higher mRS score at the time of the last visit (median: 2.5, range: 6) compared to AE patients without tumour (*n=22*; mRS at the last visit median: 0, range: 3) (p = 0.045). Most AE patients had favorable prognosis (mRS score of 0 at the last visit following treatment ;20/30). Relapses (1/30) and death (3/30) were uncommon. 36.7% (11/30) of AE patients lacked presence of both CSF inflammatory markers and brain MRI abnormalities. Significantly higher age at onset of the disease was observed in AE patients with inflammatory changes (p = 0.024). Anti-NMDAR encephalitis patients (*n=19*) were in more severe condition at the onset of the disease with significantly higher mRS score at the time of diagnosis (median: 5, range: 3) compared to anti-LGI1, anti-GABABR and anti-Caspr2 encephalitis (*n=11*; mRS score at the diagnosis median: 3, range: 3) (p = 0.028). A trend of longer time to diagnosis was observed in the non-NMDAR patient group compared to NMDAR positive patients (p = 0.063).

3. Investigation of B cell abnormalities in patients with autoimmune CNS disorders

Analysis of percentages of total CD19$^+$ B cells in NMOSD and MS showed no significant differences compared to HCs. In NMOSD, frequency of naïve B cells was significantly lower, and percentage of memory B cells was significantly higher compared to MS, however, did not differ compared to HCs. In NMOSD, the percentage of naïve B cells was significantly lower and the frequency of non-switched memory (NS), switched memory (S) and double negative

(DN) B cells was significantly higher compared to MS. Immunomodulatory therapy did not alter distribution of B cell subsets in MS. CD180 expression at protein and mRNA levels in separated naïve and memory B cell subsets did not differ among NMOSD, MS patients and HCs, whereas, CD180 expression at protein level was significantly decreased in NS memory B cells of both NMOSD and MS patients compared to HCs. Anti-CS natural IgM autoantibody titer was significantly decreased in NMOSD and MS sera compared to HCs, but no differences were found in anti-CS IgG levels. We found higher tendency of anti-CS IgG levels in the anti-Chlamydia pneumoniae IgG positive patients compared to the anti-Chlamydia pneumoniae IgG negative patients.

DISCUSSION AND CONCLUSIONS

1. Single-center study of onconeural and neuronal cell surface autoantibody testing in Hungary

Due to the discovery of neuronal cell surface autoantibodies in the past decade, AE has been recognized as a distinct disease entity from onconeural autoantibody-associated PNSs. PNSs and AE are diverse groups of diseases, presenting with various clinical symptoms, tumour associations, and treatment responses. We report the first comprehensive study on national prevalence, incidence and age and sex-based distribution of onconeural and neuronal cell surface autoantibodies in Hungary to date. We found similar incidence of onconeural (1.9/100,000/year) and neuronal cell surface (0.6/100,000/year) autoantibody positivity in the Hungarian population to data previously reported. Onconeural autoantibodies, most commonly anti-Yo, anti-Hu and anti-Ma2 antibodies were detected in older individuals (median age: 62 years), and mainly occurred in females. Among neuronal cell surface autoantibodies: anti-NMDAR was the first most frequent in young females, anti-LGI1 was the second most frequent in middle-aged males, followed by anti-GABABR in elderly males, and anti-Caspr2 in male adults, similarly to other national data reported in the literature. Our data show an increasing tendency in the number of onconeural and neuronal cell surface autoantibody positive patients, making it important to employ reliable laboratory tests that allow accurate diagnosis to be made. It is important to note that different methods are used for testing the autoantibodies in the two disease groups. Highly sensitive and specific multiplex cell-based assay is available for AE diagnostics, in which HEK293 cells expressing high levels of antigens of interest are used. For PNSs diagnostics, recombinant antigens are used in an immunoblot assay. The evaluation of patients with suspected AE should include testing for autoantibodies in both serum and CSF

simultaneously. In patients with PNSs, testing of serum alone might be adequate for establishment of precise diagnosis. Finally, early recognition of these diseases is important because without proper treatment they can have fatal outcome.

2. Clinical characteristics of neuronal cell surface autoantibody-mediated autoimmune encephalitis in a Hungarian cohort

We report the first comprehensive multicenter retrospective study of characteristics of 30 definite AE patients with neuronal cell surface antibody positivity (anti-NMDAR, anti-LGI1, anti-GABABR, anti-Caspr2) in Hungary to date. In accordance with previous publications, the most common AE type was anti-NMDAR encephalitis, followed by anti-LGI1 encephalitis. Characteristics of neuronal cell surface autoantibody-mediated AE patients in the Hungarian cohort was similar to other previously reported national cohorts. Prodromal symptoms were the most common in anti-NMDAR encephalitis. HSV infection, which is considered to be the most common viral trigger and also a possible trigger of anti-NMDAR encephalitis, occurred in one patient. Similar to data reported, most common symptoms included psychiatric symptoms, seizures, involuntary movements, memory loss and sleep disorders. FBDS occurred with lower prevalence in our cohort, but exclusively in anti-LGI1 encephalitis. Anti-NMDAR and anti-GABABR encephalitis were frequently associated with CSF inflammatory changes, such as pleocytosis and/or the presence of OCB, whereas, in anti-LGI1 and anti-Caspr2 positive patients, mainly normal CSF results were found, or increased total protein levels were detected in a few cases. Tumour association was the most common in anti-NMDAR and anti-GABABR encephalitis. Ovarian teratoma is considered to be the most frequent tumour type in anti-NMDAR encephalitis, but no association was found in our cohort. In anti-GABABR encephalitis, the dominant tumour type was small-cell lung carcinoma. In 63.3% of AE patients in our cohort, signs of inflammation were detected in CSF and/or brain MRI, but no significant correlation was found between inflammatory markers and prognosis. Similar to published data, we also reported an overall significantly higher age of onset in AE patients presenting with inflammatory changes.

In conclusion, characteristics of AE patients in our Hungarian multicenter retrospective study are in agreement with previous findings. In addition, anti-NMDAR encephalitis patients presented with more severe disability at admission compared to anti-LGI1, anti-GABABR and anti-Caspr2 encephalitis patients. Presence of tumour was associated with worse outcome

compared to those AE patients without cancer. However, none of the anti-NMDAR encephalitis female patients had ovarian teratoma. 37% of patients lacked presence of both CSF inflammatory markers and brain MRI abnormalities. This observation, in addition to the role of auxiliary examinations (CSF analysis, EEG, brain MRI), emphasizes the importance of clinical presentation and autoantibody testing in diagnostic workflow. Our findings also highlight the significance of early introduction of first-line immunotherapy that resulted in favorable outcome in most AE patients in our cohort.

3. Investigation of B cell abnormalities in patients with autoimmune CNS disorders

Several studies focus on the distribution of B cell subpopulations in MS and NMOSD, whereas, the functional characterization of B cell subsets in these disorders is limited. Similar to previous findings, we found no significant differences in the percentage of total $CD19^+$ B cells and distribution of naïve and memory B cell subsets in NMOSD or MS compared to HCs. We detected significantly lower percentage of naïve and higher frequency of NS, S memory and DN peripheral blood B cells in NMOSD compared to MS. We did not observe any significant differences related to the type of disease modifying therapies (DMT) MS patients received. Observations similar to our data have been reported, however, altered distribution of B cell subsets in MS patients have also been described due to the different therapies. Increased proportion of memory B cells was described in MS patients treated with natalizumab, whereas, reduced proportion of memory B cells were reported in MS patients treated with dimethyl fumarate, interferon ß, glatiramer acetate, fingolimod or alemtuzumab. The TLR homologue CD180 molecule activates the majority of B cells, resulting in phenotypic and functional alterations. Increased proportion of CD180-negative B cells was described in SLE and Sjögren's syndrome, and significantly decreased expression of CD180 in peripheral blood B cells of diffuse cutaneous systemic sclerosis (dcSSc) patients was reported. We found that the CD180 expression was exclusively decreased in NS memory B cells in NMOSD and MS compared to HCs. It has already been described in SLE that the CD180-negative B cells are highly activated cells, and anti-CD180 antibody ligation resulted in decreased CD180 expression, thus the diminished CD180 expression of NS memory B cells in NMOSD and MS might be a result of B cell activation via CD180. NS memory B cells resemble B1 B cells and have innate-like features, suggesting their potential role in natural autoantibody production. It was described that NS memory B cells are highly activated by CD180 ligation resulting in the enhancement of natural IgM autoantibody production. According to our results, diminished

CD180 expression of NS memory B cells could contribute to the lower anti-CS natural IgM levels found in NMOSD and MS compared to HCs. Correlation was reported between anti-CS natural IgG levels and cardiovascular-disease associated pathogens, including Chlamydia pneumoniae in coronary artery bypass grafting patients, and higher anti-CS natural IgG levels were detected in anti-measles IgG positive SLE patients, indicating a connection between natural IgG autoantibodies and infection-induced antibodies. We investigated the relationship between anti-bacterial antibodies possibly involved in the development of NMOSD or MS and natural autoantibodies, and found higher tendency of anti-CS natural IgG levels in anti-Chlamydia pneumoniae IgG positive NMOSD and MS patients than in anti-Chlamydia pneumoniae IgG negative patients.

In conclusion, our results support the role of B cell subsets in the fine tuning of the immune homeostasis. We highlight the importance of natural autoantibodies, these first-line components of the adaptive immune response in the balance of self-tolerance and anti-microbial immunity, and in the development of autoimmune diseases of the CNS.

SUMMARY OF THE NEW SCIENTIFIC RESULTS

1. We report the first comprehensive report on national prevalence, incidence and age and sex-based distribution of onconeural and neuronal cell surface autoantibodies, and characteristics of AE patients diagnosed with neuronal cell surface antibody positivity (anti-NMDAR, anti-LGI1, anti-GABABR, anti-Caspr2) in Hungary to date.

2. We reported 1.9/100,000/year incidence of onconeural autoantibody positivity and 0.6/100,000/year incidence of neuronal cell surface autoantibody positivity in the Hungarian population. Onconeural autoantibodies, most commonly anti-Yo, anti-Hu and anti-Ma2 antibodies were detected in older individuals (median age: 62 years), and mainly occurred in females. Among neuronal cell surface autoantibodies: anti-NMDAR was the first most frequent in young females, anti-LGI1 was the second most frequent in middle-aged males, followed by anti-GABABR in elderly males, and anti-Caspr2 in male adults, similarly to other national data reported in the literature.

3. Our data show an increasing tendency in the number of onconeural and neuronal cell surface autoantibody positive patients, emphasizing the role of autoantibody testing in accurate diagnosis of PNSs and AE, which based on our data should be performed in both serum and CSF simultaneously.

4. Characteristics of neuronal cell surface autoantibody-mediated AE patients in the Hungarian cohort was similar to other previously reported national cohorts, however, differences were observed in anti-NMDAR encephalitis that showed no association with ovarian teratoma, occurred more frequently among young males, and presented with a more severe disability at the onset of the disease compared to anti-LGI1, anti-GABABR and anti-Caspr2 encephalitis. Besides, presence of tumour was associated with worse outcome in AE patients compared to those patients without cancer.

5. One-third of neuronal cell surface autoantibody-mediated AE patients in the Hungarian cohort lacked signs of inflammation both in CSF and brain MRI, which, in addition to the role of auxiliary examinations (CSF analysis, EEG, brain MRI), emphasizes the importance of clinical symptoms and autoantibody testing in diagnostic workflow for early introduction of immunotherapy, which can lead to favorable outcome in AE patients.

6. Investigation of B cell distribution in autoimmune CNS disorders revealed significantly lower percentage of naïve and higher frequency of NS, S memory and DN peripheral blood B cells in NMOSD compared to MS.

7. Diminished CD180 expression of NS B cells was detected both in NMOSD and MS patients, which might be a result of B cell activation via CD180. We suggest that altered CD180 expression of NS memory B cells could contribute to the lower anti-CS IgM natural autoantibody production found in NMOSD and MS compared to HCs.

8. We reported significantly higher anti-CS natural IgG levels in anti-Chlamydia pneumoniae IgG positive NMOSD and MS patients than in anti-Chlamydia pneumoniae IgG negative patients, indicating the possible connection between natural IgG autoantibodies and infection-induced antibodies.

9. Our results suggest the role of B cell subsets in the fine tuning of the immune homeostasis. We highlight the importance of natural autoantibodies, these first-line components of the adaptive immune response in the balance of self-tolerance and anti-microbial immunity and in the development of autoimmune diseases of the CNS.

PUBLICATIONS

1. Publications related to the thesis:

Böröcz, K., **Hayden, Z.**, Mészáros, V., Csizmadia, Z., Farkas, K., Kellermayer, Z., Balogh, P., Nagy, F., and Berki, T. (2018). "Autoimmune encephalitis: possibilities in the laboratory investigation." *Orv Hetil, 159*(3), 107-112. doi: 10.1556/650.2018.30951. Quartile Ranking: Q3 Impact Factor: 0.564 (2018)

Berki, T., **Hayden, Z.**, Böröcz, K., Csizmadia, Z., Kellermayer, Z., and Balogh, P. (2018). "Immunmediált kórképek diagnosztikája: laboratóriumi szakemberek és klinikusok együttműködése." *Neurológiai Praxis*, *1*(4), 14-15.

Hayden, Z., Böröcz, K., Csizmadia, Z., Balogh, P., Kellermayer, Z., and Berki, T. (2019). "A paraneoplásiás neurológiai szindrómák diagnosztikája és immunológiai vonatkozásai." *Magy Onkol, 63*(3), 261-267. Quartile Ranking: Q4 Impact Factor: 0.197 (2019)

Hayden, Z., Böröcz, K., Csizmadia, Z., Balogh, P., Kellermayer, Z., Bodó, K., Najbauer, J., and Berki, T. (2019). "Single-center study of autoimmune encephalitis-related autoantibody testing in Hungary." *Brain Behav*, *9*(12), e01454. doi: 10.1002/brb3.1454. Quartile Ranking: Q2 Impact Factor: 2.091 (2019)

Hayden, Z., Bóné, B., Orsi, G., Szots, M., Nagy, F., Csépány, T., Mezei, Z., Rajda, C., Simon, D., Najbauer, J., Illes, Z., and Berki, T. (2021). "Clinical characteristics and outcome of neuronal surface antibody-mediated autoimmune encephalitis patients in a national cohort." *Front. Neurol.* https://doi.org/10.3389/fneur.2021.611597 Quartile Ranking: Q2 Impact Factor: 2.889 (2020)

Hayden, Z., Erdő-Bonyár, S., Bóné, B., Balázs, N., Bodó, K., Illes, Z., Berki, T., and Simon, D. (2021). "Toll-like receptor homolog CD180 expression is diminished on natural

autoantibody producing B cells of patients with autoimmune CNS disorders." *Journal of Immunology Research*. Accepted for publication. Quartile Ranking: Q1 Impact Factor: 3.327 (2020)

2. Publications not related to the thesis:

Orsi, G., Cseh, T., **Hayden, Z.**, Perlaki, G., Nagy, A. S., Giyab, O., Olsen, A. D., Madsen, S. J., Berki, T., and Illes, Z. (2021) "Microstructural and functional brain abnormalities in multiple sclerosis predicted by osteopontin and neurofilament light." *Mult Scler Relat Disord.* https://doi.org/10.1016/j.msard.2021.102923 Quartile Ranking: Q2 Impact Factor: 2.889 (2020)

Cummulative impact factor of publications related to the thesis: 9.068

Cummulative impact factor of all publications: 11.957

3. Oral presentations:

Hayden Z., Böröcz K., and Berki T. „Autoimmun encephalitisek laboratóriumi diagnosztikája." DKK17 (Doktoranduszok a klinikai kutatásokban) Konferencia, Pécs, Magyarország, 2017.

Hayden Z., Böröcz K., and Berki T. „Autoimmune encephalitis: possibilities in the laboratory investigation." IDK 2018 (Interdiszciplináris Doktorandusz Konferencia), Pécs, Magyarország, 2018.

Berki T., Böröcz K., Csizmadia Z., **Hayden Z.**, and Balogh P. „Novel strategies to detect neurological ion-channel specific autoantibodies." MLDT (Magyar Laboratóriumi Diagnosztikai Társaság) 59. Naggyűlése, Pécs, Magyarország, 2018.

Hayden Z., Böröcz K., and Berki T. „New laboratory diagnostic methods in autoimmune encephalitis patients." MedPÉCS 2018 (Medical Conference for PhD Students and Experts of Clinical Sciences) Konferencia, Pécs, Magyarország, 2018.

Hayden Z. „Az autoantitest meghatározás jelentősége az autoimmun encephalitis és a paraneoplasiás neurológiai szindróma differenciál diagnosztikájában." III. Idegtudományi

Centrum PhD és TDK Konferencia, Pécs, Magyarország, 2018. - PhD IV. section **prize for 2nd place**

Hayden Z. „Autoimmune encephalitis and paraneoplastic neurologic syndromes: importance of autoantibody detection in the differential diagnosis.” XVI. János Szentágothai Multidisciplinary Conference, Pécs, Magyarország, 2019. - Medical sciences section I. for PhD students **prize for 3rd place**

Hayden Z., Böröcz K., Csizmadia Z., Kellermayer Z., Balogh P., and Berki T. „Autoantitestek diagnosztikus jelentősége az idegrendszer autoimmun ioncsatorna betegségeiben.” 49. Membrántranszport Konferencia, Sümeg, Magyarország, 2019.

Hayden Z., Böröcz K., Csizmadia Z., Kellermayer Z., Balogh P., and Berki T. „A paraneopláziás neurológiai szindróma laboratóriumi diagnosztikájának egycentrumos retrospektív vizsgálata.” Magyar Neuroimmunológiai Társaság VI. Kongresszusa, Visegrád, Magyarország, 2019.

<u>Topic related Student Research oral presentations</u>

Hayden Z. „Transzfektált sejt alapú autoantitest meghatározási módszer az autoimmun encephalitisek laboratóriumi diagnosztikájában.” PTE ÁOK Tudományos Diákköri Konferencia, Pécs, Magyarország, 2016.

Hayden Z. „Új módszerek az autoimmun encephalitisek laboratóriumi diagnosztikájában.” PTE ÁOK Tudományos Diákköri Konferencia, Pécs, Magyarország, 2017. – Immunology section **prize for 2nd place**

Hayden Z. „Új módszerek az autoimmun encephalitisek laboratóriumi diagnosztikájában.” PTE ÁOK 11. Tudományos Diákköri Szalon, Pécs, Magyarország, 2017.

Hayden Z. „Új módszerek az autoimmun encephalitisek laboratóriumi diagnosztikájában.” 33. Országos Tudományos Diákköri Konferencia, Pécs, Magyarország, 2017.

4. Poster presentations:

Hayden Z., Böröcz K., and Berki T. „ Statistical and epidemiological study of autoimmune encephalitis patients' samples in the past 6 years." MLDT (Magyar Laboratóriumi Diagnosztikai Társaság) 59. Naggyűlése, Pécs, Magyarország, 2018.

Hayden Z., Böröcz K., and Berki T. "Statistical analysis of autoimmune encephalitis patients' samples." MedPECS 2018 (Medical Conference for PhD Students and Experts of Clinical Sciences) Konferencia, Pécs, Magyarország, 2018.

Hayden Z., Böröcz K., Csizmadia Z., Kellermayer Z., Balogh P., and Berki T. „Single-center retrospective study of paraneoplastic neurologic syndrome-related autoantibody testing in Hungary." MedPECS 2019 (Medical Conference for PhD Students and Experts of Clinical Sciences) Konferencia, Pécs, Magyarország, 2019.

Hayden Z., Böröcz K., Csizmadia Z., Kellermayer Z., Balogh P., and Berki T. „Autoantitestek diagnosztikus jelentősége az idegrendszer autoimmun ioncsatorna betegségeiben." 49. Membrántranszport Konferencia, Sümeg, Magyarország, 2019. – **prize for poster presentation**

Hayden Z., Bóné B., Orsi G., Szots M., Nagy F., Csépány T., Mezei Z., Rajda C., Simon D., Najbauer J., Illes Z., and Berki T. „Clinical characteristics, treatment and outcome of anti-NMDAR encephalitis patients in Hungary: A multicenter retrospective study." MedPECS 2020 (Medical Conference for PhD Students and Experts of Clinical Sciences) Konferencia, Pécs, Magyarország, 2020.

Hayden Z., Böröcz K., Csizmadia Z., Kellermayer Z., Balogh P., and Berki T. „Autoimmun encephalitis és paraneopláziás neurológiai szindróma pathomechanizmusának és laboratóriumi diagnosztikájának összehasonlító elemzése." MIT (Magyar Immunológiai Társaság) Vándorgyűlés 2020. Online Konferencia, Magyarország, 2020.

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