The spectrum of autoimmune neurological syndromes has expanded rapidly in recent years due to the discovery of novel neuronal autoantibodies. Many newly identified autoantibodies are directed against cell surface proteins, as opposed to the mainly intracellular antigens targeted by classical neuronal antibodies. Neural surface antibodies are often non-paraneoplastic and are considered to be directly pathogenic in autoimmune encephalopathies. Particularly in unexplained neurological cases, a comprehensive autoantibody screening can help to establish a diagnosis, enabling immediate commencement of life-saving therapy.

**Neuronal Autoantibodies**

Autoantibodies against intracellular neuronal proteins like Hu, Yo, Ri, CV2/CRMP5, GAD65/67, and amphiphysin are well recognised biomarkers in several immune-mediated brain disorders. They are generally considered to be epiphenomena of a T-cell driven paraneoplastic autoimmune reaction. As such they are mere disease markers and probably bear no pathogenic potential in vivo. Experimental transfer of human antibodies into model animals generally has no effects and their removal or down-regulation by treating patients with immunosuppressive drugs does not in most cases lead to lasting improvement, if any.

In contrast, reports on autoantibodies that are often non-paraneoplastic were published in recent years. Most of them are
directed against neural surface-associated receptor and channel proteins including aquaporin-4 (AQP4), N-methyl-D-aspartate receptor (NMDAR), alpha-amino-3-hydroxy-5-methyl-4-isoxozolepropionic acid receptor (AMPAR), gamma-aminobutyric acid receptor type B (GABABR), leucine-rich glioma inactivated protein 1 (LGII), and contactin-associated protein-like 2 (CASPR2), dipeptidyl-peptidase-like protein-6 (DPPX), metabotropic glutamate receptor 5 and IgLON5. Most of the non-paraneoplastic autoantibodies are considered to be pathogenic, generally occur in association with inflammatory damage to the brain and can induce seizure, stroke, impairment of vision, psychosis-like symptoms, and/or movement disorders. Most patients with autoantibodies against neural surface antigens dramatically improve after immunomodulation by plasmapheresis or by treatment with immunosuppressive drugs.

Anti-AQP4
AQP4, the most abundant water channel in the central nervous system (CNS), was identified in 2005 as the target antigen of autoantibodies in neuromyelitis optica (NMO). Prior to that, these antibodies have simply been referred to as “NMO-IgG.” NMO is a severe inflammatory CNS disorder which predominately affects the spinal cord and optic nerves. Symptoms comprise acute visual disorders including blindness, impaired mobility and loss of bladder and bowel control. Without adequate therapy, half of the patients become blind in one or both eyes or cannot walk without support within five years. NMO generally has a worse prognosis for loss of sight and permanent paralysis than classic multiple sclerosis (MS). AQP4 antibodies are highly specific for NMO and are assumed to be involved in the pathogenesis of the disease. The determination of AQP4 antibodies is particularly useful for early serological differentiation of NMO from MS, which is crucial for therapy decision.

Anti-NMDAR
Autoantibodies against glutamate receptors (type NMDA) are highly specific markers for anti-NMDA receptor encephalitis, an inflammatory encephalopathic autoimmune disease which was first described in 2007. Despite many scientific reports it is currently still widely underdiagnosed. Paraneoplastic cases represent 35-40% of the total, among which young women with ovarian teratoma make up the main group. The disease presents with a multi-stage clinical progression with prodromal signs, psychiatric abnormalities, reduced consciousness, epileptic seizures, dyskinesias, and autonomic dysfunction. The determination of NMDAR autoantibodies in serum or CSF is particularly important for differential diagnosis in patients with encephalitis of unknown origin, i.e. with non-infectious etiology, and in young women with de novo epilepsy. In case of a positive serological result, a comprehensive teratoma investigation should follow.

Anti-LGI1 and CASPR2
LGI1 and CASPR2 were recently identified as the main antigenic targets of autoantibodies formerly referred to as “voltage-gated potassium channel (VGKC) antibodies”. LGI1 autoantibodies are found almost exclusively in patients with limbic encephalitis, a disease mainly presenting with seizures, memory deficits, confusion and disorientation and an aggressive progression leading to irreversible hippocampal atrophy. Autoantibodies against CASPR2 are also found in patients with limbic encephalitis, but mostly occur in acquired neuromyotonia and Morvan’s syndrome. Compared with other forms of encephalopathies which are associated with different antibody specificities, the incidence of tumours is quite low (0-10% in case of anti-LGI1 positivity, 0-35% in case of CASPR2 positivity). Differentiation of LGI1 and CASPR2 antibodies is highly important for a fast, directed treatment.

Anti-GABABR
Autoantibodies against GABAB receptors were first identified in a group of patients with paraneoplastic or immune-mediated limbic encephalitis. Patients with these autoantibodies show epileptic seizures, confusion and memory deficits, with the seizures constituting the primary clinical symptom in most cases. Around 50% of patients have a tumour, mostly small-cell lung carcinoma. Additional autoantibodies, e.g. against GAD, TDPO, VGCC or SOX1, are detected in almost half of patients, suggesting a generalised autoimmune disorder. Antibodies against the GABA B1 subunit are present in all patients, while antibodies against the B2 subunit are only found in isolated cases, indicating that the relevant epitopes are localised primarily in the B1 subunit. Anti-GABABR autoantibodies should be determined in all patients with encephalitis but no evidence of a causative organism and in suspected cases of limbic encephalitis.

Anti-AMPAR
Autoantibodies against the GluR1/GluR2 subunits of glutamate receptors (type AMPA) are found in patients with a special form of autoimmune-mediated limbic encephalitis. Associated symptoms include progressive memory deficits, confusion, disorientation, lethargy, aggressive behaviour, hallucinations, epileptic fits and nystagmus. Around 70% of affected individuals have bronchial carcinoma, breast carcinoma or thymoma. Consequently, the detection of AMPAR antibodies can be the first indication of an underlying tumour. An overlap with other systemic autoimmune diseases (e.g. stiff-person syndrome, diabetes mellitus, Raynaud’s syndrome, hypothyroidism) is observed in about 50% of cases. The majority of patients investigated so far demonstrate an isolated reactivity to GluR1 (30%) or GluR2 (60%), with only 10% showing the simultaneous presence of antibodies against both subunits. Therefore, both types of antibody should be determined.

Anti-DPPX
DPPX was reported in 2013 as a novel cell-surface autoantigen of encephalitis. DPPX is mainly produced in brain tissue and interacts with the voltage-gated potassium channel Kv4.2. It is an important regulator of membrane excitability in hippocampal CA1 pyramid cells. The main symptoms of anti-DPPX associated encephalitis are restlessness, forgetfulness, confusion, hallucinations, muscle spasms and tremor. Severe diarrhoea and weight loss also frequently occur. A positive serological result should not exclude a tumour investigation.

Anti-IgLON5
In 2014, IgLON5 was discovered as target antigen of autoantibodies in patients with a unique tauopathy presenting with parasomnia and sleep breathing dysfunction. Mortality in the index patients was dramatically high. Due to the heterogeneous clinical presentation, determination of IgLON5 antibodies may...
be considered in the differential diagnosis of autoimmune encephalitis, Creutzfeldt-Jakob disease or rapidly progressive neurodegenerative dementia.

**Detection by IFA**

For the monospecific determination of autoantibodies directed against neural surface antigens, recombinant cell-based indirect immunofluorescence assays (RC-IFA) are available that present the relevant antigens in their authentic form. Differently transfected cell substrates can be combined in multiplex-RC-IFA (Figure 1). Used in conjunction with cryosections of e.g. hippocampus, cerebellum or optic nerve in the form of Biochip mosaics they yield maximal diagnostic sensitivity (Figures 2 and 3). Even more important, this comprehensive approach greatly assists the differentiation of clinically similar encephalitis subtypes (autoimmune, infectious, paraneoplastic) and thus supports fast decision-making on oftentimes life-saving treatment options.

**Multiplex Application**

The value of multiparametric testing for anti-neural antibodies can be illustrated by a study on over 16,000 samples sent over a time period of one year to a routine clinical immunology laboratory for analysis of neural antibodies. The sera were subjected to multiplex testing regardless of the test request. Of the positive sera submitted for monospecific analysis, 56% contained the requested antibody, while 49% showed a different relevant antibody (5% as secondary finding). The majority (61%) of antibody findings were directed against cell surface antigens, with antibodies against classic intracellular targets being less prevalent (39%). The most common surface targets were NMDAR (26%), AQP4 (14%), LG11 (7%), CASPR2 (7%) and GABABR (2%). Antibodies against AMPAR and DPPX each occurred in 1% of samples. Overall, the multiplex testing nearly doubled the relevant serological diagnoses.

**Summary**

The recent identification of novel neuronal autoantibodies has considerably enhanced the diagnosis of autoimmune neurological diseases. Antibodies against AQP4, NMDAR, LG11, CASPR2, AMPAR, GABABR, DPPX and IgLON5 are among the newly identified specificities. Antibody detection can secure a diagnosis, enabling prompt treatment to preserve the patient’s neurological function and even life. Since some neuronal antibodies occur only relatively rarely, multiparametric testing is favoured over selective or sequential analysis to avoid diagnostic gaps and shorten the time to diagnosis. It is anticipated that current research will uncover further autoantibody specificities which will broaden diagnostic capabilities still further.