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# NEUROIMMUNOLOGY DIAGNOSTICS

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## ABSTRACT

Neuroimmunology has led to advanced diagnostics of previously cryptic disorders, using autoantibody testing against neurological targets. Neuropsychiatric syndromes and autoimmune encephalitis can now be routinely diagnosed using specialized antibody tests such as immunofluorescence and immunoblot assays in specialized laboratories. This helps in early and accurate diagnosis, leading to favorable patient prognosis. Here, we briefly review the diagnostics for Neuroimmunologic and related disorders including autoimmune encephalitis, demyelinating diseases, neuropathies, paraneoplastic syndromes, stiffperson syndrome, inflammatory myopathies as well as Alzheimer's disease.

## INTRODUCTION

Autoantibody testing is becoming increasing relevant for Neurologists due to the rapidly growing list of novel autoantibodies against neurological targets. These are detectable by several major methods: Immunofluorescence (IIFT), Immunoblot (IB), Bioluminescence (BL) and ELISA (Tozzoli et al., 2002; Westgeest et al., 1988). IIFT is the GOLD standard for the diagnosis of auto-antibodies (Meroni and Schur, 2010; Tozzoli et al., 2002; Westgeest et al., 1988). IB is a very sensitive test designed as a modification of the classic western blot on a pre-manufactured strip (Westgeest et al., 1988). IB tests have the advantage of being able to detect multiple specific auto-antibodies in a single test. Due to their high sensitivity, low titre results are prone to false positivity and clinical correlation is advised. ELISA is a more specific, but less sensitive test than IB (Copple et al., 2011; Meroni and Schur, 2010). ELISA is useful when high titre antibodies of unique specificity are present. BL is more sensitive than ELISA and has good specificity but has limited availability. In this primer, we briefly review the various Neuroimmunologic disorders and their diagnostic antibody testing for the busy Neurologist.

## AUTOIMMUNE ENCEPHALITIS

Autoimmune encephalitis is treatable and patients frequently present with acute or subacute onset of any of the following signs: seizures, psychosis, confusion, dementia, abnormal movements or neuropsychiatric manifestations (Shin et al., 2017). MRI brain and CSF analysis may be normal or more typically, show subtle abnormalities such as increased protein and leukocytosis. Several antibodies are detectable in autoimmune encephalitis by an IIFT based assay in serum or CSF, with over 85% sensitivity. These include antibodies against NMDA, AMPA, GABA receptors, DPPX, LGI1 and CASPR2. As shown in Figure 1, neuronal antigens are of functional significance. Binding of auto-antibodies results in internalization and degradation of these cell surface receptors and proteins, leading to neuronal dysfunction. NMDA and AMPA are involved in long term potentiation and depression and their disruption leads to cognitive impairment, neuropsychiatric manifestations and seizures (Shin et al., 2017; Joubert and Honnorat. 2015). LGI1 and CASPR2 are part of the voltage-gated-potassium-channel complex (VGKC) on the pre-synaptic membrane in glutamergic neurons (Figure 1). Antibody binding to LGI uncouples AMPA

receptors and leads to their endocytosis and functional disruption. Anti-GABA antibodies, if present in high titers, lead to seizures and encephalopathy in concordance with GABA disinhibition. Overlap syndromes with NMDA-receptor and anti-GAD antibodies also exist with variable symptomatology (Joubert and Honnorat. 2015).

## DEMYELINATING DISEASES

Neuro-myelitis Optica Spectrum Disorders (NMOSD) are a differential diagnosis in multiple sclerosis, transverse myelitis, optic neuritis and investigation of CNS lesions (Dos Passos et al., 2018). NMOSD typically suffer transverse myelitis or optic neuritis and sometimes may also develop cerebral lesions.

Anti-Aquaporin-4 (AQP4-Ab) antibodies are a hallmark of NMO and are detectable in blood with 80% sensitivity and nearly 100% specificity using IIFT (Figure 2). Antibodies against myelin oligodendrocyte glycoprotein (MOG) are associated with better prognosis of acute disseminated encephalomyelitis (ADEM) and are also found in 25% of AQP4-Ab negative NMOSD (Dos Passos et al., 2018). Both these antibodies are useful in diagnosing the underlying cause of ADEM and Optic neuritis. NMOSD are frequently misdiagnosed as isolated transverse myelitis or optic neuritis or multiple sclerosis. NMOSD are treatable with long term immunomodulation.

## NEUROPATHIES

Common forms of acute and peripheral neuropathies are now being recognized as autoimmune in etiology and many are responsive to treatment if diagnosed. Anti-ganglioside antibodies have been found in autoimmune neuropathies, especially in axonal variants of Guillain-Barré syndrome (GBS), acute motor axonal neuropathy (AMAN) and acute motor-sensory neuropathy (AMSAN), chronic inflammatory demyelinating polyneuropathies (CIDP) and Miller Fisher syndrome and multifocal motor neuropathy (MMN) (Plomp and Willison.2009). The following antibodies are part of the IB assay for neuropathies: GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b (Plomp and Willison.2009). Approximately 60% of patients with GBS have anti-ganglioside antibodies in sera during the acute clinical phase of the disease.

Recently, novel auto-antibodies were found in CIDP and GBS, directed against membrane proteins at and around the nodes of Ranvier (Burnor et al. 2018).

Prominent amongst these were auto-antibodies against paranodal proteins neurofascin-155 (NF155), contactin-1 (CNTN1) and Contactin-associated protein 1 (Caspr) (Doppler et al. 2016; Manso et al. 2016). Caspr is attached to NF155 and CNTN1. Autoimmunity against these proteins is most commonly mediated by IgG4 with a severe phenotype resistant to IV immunoglobulin (IVIG) treatment but responsive to Rituximab (Burnor et al. 2018).

## PARANEOPLASTIC SYNDROMES (PNS)

PNS are autoimmune mediated complications of cancer. These can present when cancer is small and curable and sometimes years before appearance of malignancy, therefore diagnosis allows significant opportunity for early therapeutic intervention. PNS may present with cerebellar degeneration (subacute), limbic encephalitis, brainstem encephalitis, and sensory neuronopathy (Höftberger et al., 2015). In PNS, the tumor cells express antigens that normally only occur in neurons, inducing an auto-antibody response leading to neuronal injury. PNS develop in ~15% of malignant cancers, occurring most frequently in small-cell lung carcinoma, neuroblastoma, thymoma, and cancers of the ovary, breast, uterus and testis. Onco-neuronal antibodies in PNS are directed against intracellular neuronal antigens: Amphiphysin, CV2, PNMA2/Ta, Ri/Anna2, Yo/Pca-1, Recoverin, Hu/Anna, SOX, Titin (Höftberger et al., 2015). A positive result has a high probability of a tumor. In concordance with clinical data, detection of anti-neuronal antibodies is considered sufficient for a definitive diagnosis of PNS.

## STIFF-PERSON SYNDROME (SPS)

SPS is a rare neurological disease, which can be paraneoplastic or non-paraneoplastic in origin. The disease manifests with severe progressive muscle stiffness, typically in the spine and lower extremities and may be confused with Parkinson's Disease. Paraneoplastic cases are associated with antibodies against amphiphysin. Non-paraneoplastic cases are characterised by autoantibodies against glutamate acid decarboxylase (GAD), which are found in 60-90% of patients (Rakocevic et al., 2012). However, anti-GAD antibodies are not specific markers for stiff-person syndrome as they also occur in other neuronal diseases and, in particular, diabetes mellitus type I, albeit in lower titres (Rakocevic et al., 2012).

## MYOSITIS

Differentiating inflammatory from degenerative myopathies is a challenge at initial presentation. Moreover the clinical spectrum of several inflammatory myopathies also overlaps making exact diagnosis difficult. IB based assay for myositis can detect a range of antibodies to accurately diagnose inflammatory myositis which help in prognosis and therapeutics (Figure 3). Antibodies directed against MDA5, Mi-2, SRP and tRNA synthetases (OJ, EJ, PL-12, PL-7, Jo-1), PMScl100, Ku antigens can be tested in a single blood test using the IB assay. MDA5 is a special syndrome resembling SLE and dermatomyositis (DRM) with rapidly progressive respiratory failure (Saeed, 2017). TIF1γ is associated with DRM with ~ 60% frequency of malignancy. Both MDA5 and TIF1γ therefore require urgent diagnosis (Betteridge et al., 2011).

## ALZHEIMER'S DISEASE AND DEMENTIA

Though dementias are neurodegenerative disorders, there is evidence that they may be a consequence of immune mediated injury as well (Long and Day, 2018). Furthermore, it was recently shown in a large study of about 0.8 million patients that autoimmune diseases increase risk of dementia and Alzheimer's Disease (AD) (Li et al, 2018). Therefore early detection of AD is important. AD is the most common form of Dementia and affects 10% of population above 65 years of age. Majority of patients are left undiagnosed which is unfortunate as now there is treatment available to slow down the progression of dementia and improve cognition and quality of life. Clinical diagnosis is unreliable. The neuropathology of AD starts decades before the onset of clinical disease and is reflected in the concentrations of beta- amyloid 1-42 and total Tau protein in the CSF.

Beta amyloid alone and tau alone are sensitive but not specific for AD but when both assays are used together, they greatly improve sensitivity and specificity for diagnosis of AD. In AD the CSF beta-amyloid 1-42 levels are low (<50%) and CSF total Tau protein is high (>300%), and this combination forms the "AD signature" (Blennow et al., 2015). These changes in beta- amyloid 1-42 and total Tau protein levels occur in the preclinical phase and persist through the disease course and therefore are an early finding to assist diagnosis of AD. Low beta-amyloid 1-42 and high total Tau protein levels (AD Signature) used together may allow discrimination from healthy controls with level of sensitivity and specificity over 85% (Blennow et al., 2015).

## CONCLUSIONS

Neuroimmunology has emerged as a major subspecialty of Neurology overlapping significantly with Rheumatology (Saeed and Ahmad, 2017). Neuropsychiatric syndromes and autoimmune encephalitis can now be routinely diagnosed using specialized antibody tests such as IIFT and IB assays in specialized laboratories. This helps in early and accurate diagnosis which allows early institution of immunomodulatory treatments, making a marked difference in patient prognosis.

**Figure 1. Physiological functioning of GABAergic and Glutamatergic neurons**
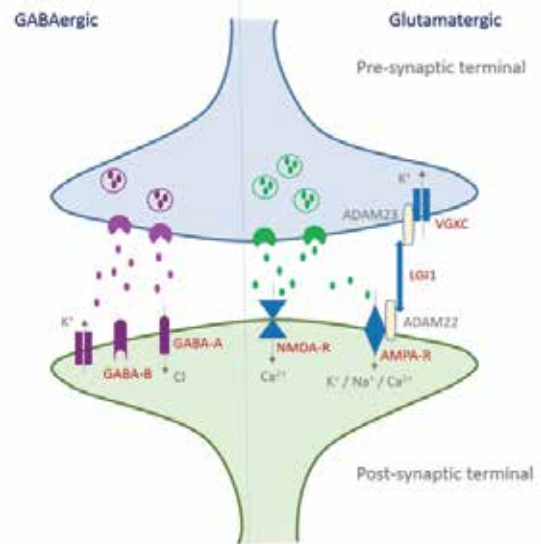


This figure shows the normal functioning of variousreceptors and proteins whose disruption by auto-antibodies leads to autoimmune encephalitis

**Figure 2. Immunofluorescence testing for autoimmune neurologic disorders**



Anti-Aquaporin (AQP4)     Anti-GABA     Anti-NMDA
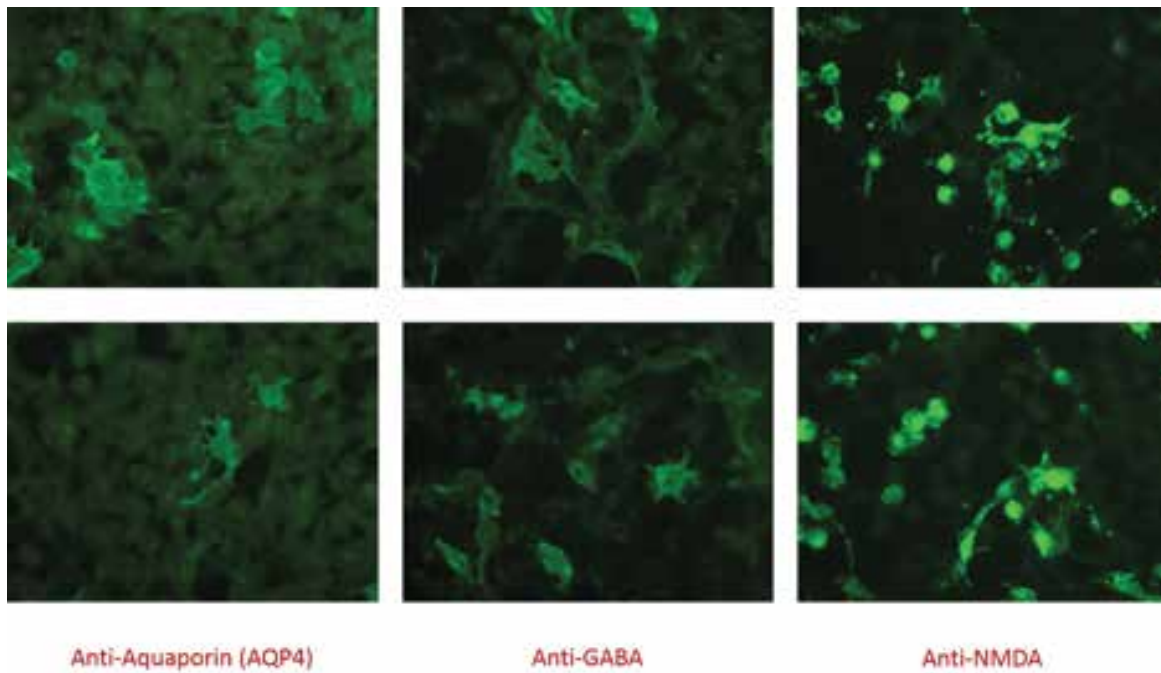
Photographs of IIFT in patients with NMOSD (AQP4 antibodies) and autoimmune encephalitis (anti-GABA and anti-NMDA antibodies).

**Figure 3. Immunoblot for inflammatory myositis**



| Antigen | Intensity | Class |
|---|---|---|
| Mi-2alpha (Mi-2a) | 20 | + |
| Mi-2beta (Mi-2b) | 30 | ++ |
| TIF1gamma (TIF1g) | 1 | o |
| MDA5 (MDA5) | 1 | o |
| NXP2 (NXP2) | 1 | o |
| SAE1 (SAE1) | 0 | o |
| Ku (Ku) | 10 | (+) |
| PM-Scl100 (PM100) | 0 | o |
| PM-Scl75 (PM75) | 4 | o |
| Jo-1 (Jo-1) | 1 | o |
| SRP (SRP) | 17 | + |
| PL-7 (PL-7) | 2 | o |
| PL-12 (PL-12) | 9 | (+) |
| EJ (EJ) | 2 | o |
| OJ (OJ) | 2 | o |
| Ro52 (Ro52) | 4 | o |
| Control (Co) | 126 | +++ |

Immunoblot scan (EUROIMMUN) of a patient with inflammatory myopathy.
Multiple antibodies are positive including Mi-2a, Mi-2b and SRP.

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