Teaching Course 12

Neuromyelitis Optica Spectrum Disorders

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26 Pathophysiology of NMO spectrum disorders  
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Learning objectives:

1. Understand the neuropathological findings of neuromyelitis optica spectrum disorders (NMOSD) in comparison with multiple sclerosis (MS)

2. Understand the pathogenic potentials of AQP4-IgG to cause astrocytic damage in experimental studies (in vitro and in vivo)

3. Appreciate the presence of AQP4-IgG seronegative NMOSD, especially myelin oligodendrocyte glycoprotein (MOG)-IgG-positive NMOSD which seems to be an inflammatory demyelinating disease.

Overview

In the new international consensus diagnostic criteria, neuromyelitis optica spectrum disorders (NMOSD) are stratified by serostatus of aquaporin 4 (AQP4)-IgG, 1) NMOSD with AQP4-IgG and 2) NMOSD without AQP4-IgG

A major type of lesions in NMOSD is massive astrocyte destruction with deposition of immunoglobulins and activated complements and granulocyte infiltration. Secondarily to these lesions, cystic change with tissue destruction and Wallerian degeneration may occur as well. Studies with cellular damage biomarkers in the cerebrospinal fluids proton magnetic spectroscopy also revealed severe astrocytic damage in NMOSD. Experimental studies have clearly demonstrated the potential of AQP4-IgG to cause astrocyte damage in vitro and in vivo. AQP4 is a transmembrane protein, and AQP4-IgG recognizes extracellular loops of AQP4 expressed on the astrocytic endfeet. AQP4-IgG is mainly IgG1 that can activate complements efficiently at the cell surface, and along the humoral immune factors, granulocytes and macrophages infiltrate into rain parenchyma in animal studies of NMOSD. Thus, AQP4-IgG can destroy astrocytes in antibody-mediated complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, although initial mechanism to trigger AQP4-IgG and other immune components to cross the blood brain barrier remains unknown. Also AQP4-IgG by themselves may internalize aQP4 and affect astrocyte function, and AQP4-reactive T cells seem to play a pathogenetic role. Moreover, there are active lesions with astrocytic clasmatodendrosis without complement deposition, suggesting that diverse mechanisms of tissue injury operate in parallel in patients with NMOSD. Available evidences strongly suggest that NMOSD with AQP4-IgG is an autoimmune astrocytopathic disease.

NMOSD without AQP4-IgG could be a heterogeneous group of diseases, but recent studies have shown that a fraction of AQP4-IgG seronegative NMOSD are positive for
conformation-sensitive myelin oligodendrocyte glycoprotein (MOG)-IgG. Unlike AQP4-IgG-positive NMOSD, MOG-IgG-positive NMOSD appears to be an inflammatory demyelinating disease.

Epidemiology, Genetic and Environmental factors

There are only a small number of epidemiological studies in NMO/NMOSD, but case reports and series have been reported from many countries across the continents. The prevalences of NMO/NMOSD in various parts of the globe are somewhere between 0.5 and 5/100,000 (much lower than the prevalence of MS in Western countries). Unlike MS whose prevalence is higher in white people in Western countries and high-latitude regions, NMO/NMOSD appears to be more evenly distributed in the world. However, the ratios of NMO/NMOSD to MS vary widely in regions (less than 1:10 in American and European cohorts, but close to 1:1~2 in some Asian countries). The ratios also vary in ethnic groups living in the same area. For example, in a survey in Kuala Lumpur, Malaysia, the ratios of NMO/NMOSD to MS were 1:1.4 in Malays, 1:0.6 in Chinese, and 1:4 in Indians. The onset age of NMOSD is around the fourth decade of life, but the onset event may occur at any age from early childhood to elderly patients (3y.o ~ 94y.o. in our series). The female predominance in NMO/NMOSD is observed with a female: male ratio ranging from 3:1 in France to 10:1 in Japan. Although NMO cases are mostly sporadic, familial NMO has been reported in 3% of some cohorts.

Japan started the national registry of MS (including cases with features of NMO) in 1974 when less than 500 cases were registered. But then the cases have increased steadily, and since mid-1980s, increased more rapidly mainly because MRI were widely available and the detection of asymptomatic demyelinating lesions became easier. Despite the improved diagnosis of MS, there has probably been a true rise of MS in Japan because we have not seen many newly diagnosed MS patients in the elderly population. As of 2014, over 18,000 patients are registered. In contrast to a steady increase of MS cases, we suspect the prevalence of cases of NMO/NMOSD is relatively constant. The first Japanese nationwide epidemiological survey of NMO/NMOSD was conducted in 2012, and the preliminary analysis suggests that the prevalence of NMOSD would be around 3/100,000.

Age of onset is about 40 years. Up to 90% of patients of NMOSD with AQP4-IgG are women. Patients with AQP4-IgG-positive NMOSD often have multiple autoimmunities (Sjogren syndrome, lupus, Hashimoto thyroiditis, anti-phospholipid syndrome, myasthenia gravis, etc). Clinical, MRI and laboratory findings of NMOSD reported from various regions and countries are essentially uniform, but the prognosis may be different in countries and ethnic groups. However, the genetic and environmental factors reliably associated with susceptibility to NMOSD are unknown.

Neuropathological findings of NMOSD

5)
Affected optic nerves and spinal cord in NMO are grossly atrophic, necrotic and cavitary. Microscopically, severe necrosis, demyelination, cavities and infiltration of granulocytes, macrophages and lymphocytes, and such vascular pathologies as blood vessel wall thickening and hyalinization are seen in typical NMO lesions.

We and others conducted immunopathological studies of NMO lesions and demonstrated an extensive loss of immunoreactivities to AQP4 and glial fibrillary acidic protein (GFAP), another astrocytic protein, especially in the perivascular regions with deposition of immunoglobulins (IgG and IgM) and activated complements, and a relative preservation of the staining of myelin basic protein (MBP) in acute NMO lesions. In those lesions, areas of AQP4 loss were larger than those of GFAP loss. Compared with the losses of astrocytic proteins, areas of MBP loss were much smaller. At higher magnification, numerous GFAP-positive astrocytic cell debris and “clasmatodendrosis” defined as cytoplasmic swelling and vacuolation of astrocytes, with beading of their dendrites were seen at the periphery of NMO lesions. Most of these pathological analyses were done with spinal cord lesions, but similar findings were reported in a biopsied cerebral periventricular lesion of an NMO-IgG-positive patient with confusion. Those characteristic pathological findings were not seen in MS, infarction or control samples, and suggest that massive astrocytic damage associated with humoral immunity may be of primary importance in the development of NMO lesions and that myelin damage might be a secondary phenomenon.

Meanwhile, Popescu et al reported that medullary lesions in the AQP4-rich area postrema commonly involved in patients with intractable hiccup, nausea and vomiting were characterized by inflammation (cellular infiltrate and complement deposition), and a preservation of GFAP staining and myelin an axons despite AQP4 loss. Thus, the area postrema lesions appear be inflammatory, but non-destructive, and non-demyelinating, and the symptoms related to respiration and vomiting might be attributable to the local astrocytic dysfunction in those cases.

Sharma et al showed that damage of astrocytes and subsequent demyelination can occur following injection of lipopolysaccharide into the white matter. Initially microglia were activated and then retraction of astrocytic foot processes at the glia limitans and loss of AQP-4 and connexins to form gap junctions of astrocytes and oligodendrocytes occurred, suggesting a functional astrocytic disturbance. In this model, demyelination followed astrocyte pathology. Similar pathological findings were seen in active Pattern III MS lesions. However, this astrocyte pathology in MS mainly affected the cell processes and destruction or loss of astrocytes was minor, while astrocytes are much more widely destroyed in NMO lesions. Matsuoka et al also reported that antibody-independent AQP4 loss (with preservation of GFAP staining) can occur in MS and Baló’s disease as well as NMO but they did not analyze the lesions with GFAP loss which are the most characteristic in NMO. These studies revealed that astrocytes can be affected in MS as well as NMO, but extensive astrocytolytic lesions are characteristic of NMO.

Six different lesion types of NMOSD\(^6\)
Active lesions in NMO display a wide spectrum of pathology even within a single tissue block of an individual patient. We have distinguished six different lesion types. The first reflects complement deposition at the surface of astrocytes, associated with granulocyte infiltration and astrocyte necrosis and followed by demyelination, global tissue destruction and the formation of cystic, necrotic lesions (lesion type 2). Such destructive lesions lead to Wallerian degeneration in lesion-related tracts (lesion type 3). Around active NMO lesions AQP4 may selectively be lost in the absence of aquaporin 1 (AQP1) loss or other structural damage (lesion type 4). Another pattern is characterized by clasmatodendrosis of astrocytes, defined by cytoplasmic swelling and vacuolation, beading and dissolution of their processes and nuclear alterations resembling apoptosis, which was associated with internalization of AQP4 and AQP1 and astrocyte apoptosis in the absence of complement activation. Such lesions give rise to extensive astrocyte loss, which may occur in part in the absence of any other tissue injury, such as demyelination or axonal degeneration (lesion type 5). Finally, lesions with a variable degree of astrocyte clasmatodendrosis are found, which show plaquelike primary demyelination that is associated with oligodendrocyte apoptosis, but with preservation of axons (lesion type 6). In active multiple sclerosis (MS) lesions astrocytes reveal changes of reactive protoplasmatic or fibrillary gliosis. Only in a subset of lesions, in patients with aggressive disease, loss of AQP4 is observed in the initial stage of their formation, which is associated with retraction of astrocyte processes in the absence of complement deposition, granulocyte infiltration or loss of AQP1 or astrocytes. Our data underline the primary assault of astrocytes in NMO lesions, but also indicate that different mechanisms of tissue injury operate in parallel in the same patient and even within the same lesion.

**Experimental studies**

a) **In Vitro Studies**

Hinson et al demonstrated that AQP4 antibody, predominantly IgG1 in IgG subclasses, bound to ectodomain of AQP4, and induced AQP4 endocytosis and degradation. AQP4 antibody also activated complements and destroyed AQP4-transfected cells in culture. Control sera without AQP4 antibody did not induce such changes. Interestingly, there was a high expression of AQP4 in the paranodal astrocytic endfeet. Vincent et al showed that NMO-IgG-positive patients’ sera but not MS patients’ sera reacted with human fetal astrocytes, and that NMO-IgG binding to astrocytes altered AQP4 polarized expression and increased permeability of a human blood-brain barrier (BBB) endothelium/astrocyte barrier. They also found that NMO-IgG binding to human fetal astrocytes induced NK cell degranulation, astrocyte killing by antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent granulocyte attraction through the BBB model. Kinoshita et al used astrocytes with and without transfected AQP4-specific small interfering RNA as target cells in culture and proved that AQP4 antibody-positive sera bound to AQP4 and induced necrosis.

a) **EAE Studies**

By administering purified IgG derived from AQP4 antibody-positive NMO patients to rats with experimental autoimmune encephalomyelitis (EAE) induced by MBP-reactive T cell transfer or MBP immunization, clinical disease became more severe and CNS lesions resembling NMO developed. In those lesions, there were loss of AQP4 and
GFAP, infiltration of granulocytes, T cells, activated macrophages and microglia cells, an extensive deposition of immunoglobulins and activated complements on astrocyte processes of the perivascular and superficial glia limitans. Compared to obvious astrocytic damage, changes of myelin and neurons were subtle. Neither injection of IgG purified from AQP4 antibody-negative cases to the EAE nor injection of AQP4 antibody to rats without EAE induced those NMO-like pathological changes. Furthermore, Bradl et al demonstrated that absorption of AQP4 antibodies from purified IgG with AQP4-transfected cells reduced the AQP4 antibody titers and resulted in much less astrocyte pathology, supporting the direct role of AQP4 antibody in the development of the NMO-like CNS lesions. They also showed that injection of AQP4 antibody into naïve rats, young rats with leaky BBB, or after non-encephalitogenic T cell line did not induced the NMO-like lesions. Bennett et al detected plasma cells producing pathogenic AQP4 antibodies in the CSF of a patient with early-stage NMO and proved that the recombinant monoclonal antibody to AQP4 derived from the CSF cells mediated complement-dependent cytotoxicity (CDC) and ADCC against AQP4-expressing glioblastoma cells. Taken together, AQP-4 antibody can induce NMO-like CNS lesions in animals with T-cell-mediated brain inflammation.

b) Studies on T cell requirement

Later, Saadoun et al successfully induced NMO-like CNS lesions in mice by intracerebral injection of AQP4 antibody and human complements, suggesting T cell-mediated CNS inflammation is not required for the lesion formation if sufficient amounts of immunoglobulins and complements reach the CNS parenchyma and react to AQP4 molecules expressed on astrocytes. Recently they further demonstrated that T cells are not required in the NMO lesion formation using the same intracerebral injection technique and T cell deficient mice. Kinoshita et al showed that injection of AQP4 antibody and complete Freund’s adjuvant alone could induce NMO-like lesions in vivo, suggesting non-specific inflammatory stimulation in the presence of AQP4 antibody may be enough to induce the NMO-like CNS lesions.

c) AQP4 antibody seropositivity before NMO onset

NMO patients may be seropositive for AQP4 antibody years before the onset, and thus it is important to identify immunological events in humans that increase the blood brain barrier (BBB) permeability to allow AQP4-antibody to cross the BBB and bind to AQP4 expressed on astrocytes.

d) AQP4 antibody binding sites

Details of binding of AQP4 antibody to ectodomain of AQP4 is crucial to develop highly sensitive AQP4 antibody detection assays and to elucidate how the autoantibody damages AQP4-expressing astrocytes in CDC. Pisani et al identified loop C and loop A as two major conformational AQP4 epitopes for AQP4 antibody binding, but loop E also contributed to the binding to some extent. Additionally, there was direct evidence that transition of AQP4 tetramer to AQP4-orthogonal array of particles (OAP) involves conformational changes of the extracellular loops.

e) M1-AQP4 vs. M23-AQP4

There are two major isoforms of AQP4, M1-AQP4 and M23-AQP4. They have identical extracellular domain residues, but M1-AQP4 has 22 more amino acid in the cytoplasmic C-terminus. The plasma membrane form OAP. M23-AQP4 is the OAP-forming isoform, whereas M1-AQP4 alone is unable to form OAP. Hinson et al found
that the binding of NMO-IgG to the ectodomain of astrocytic AQP4 is isoform-specific, e.g. M1-AQP4 is completely internalized, but M23-AQP4 resists internalization and is aggregated into larger-order OAP that activate complement more effectively than M1 when bound by AQP4 antibody. As the second finding, they showed that AqP4 antibody binding to either isoform impairs water flux directly, independently of AQP4 down-regulation, but this is not a direct proof of the water flow through the water channel and confirmatory studies are needed. Phuan et al also demonstrated that CDC was greatly (>100-fold) reduced in M1-AQP4 expressing cells compared with M23-AQP4 expressing ones indicating AQP4 antibody-dependent CDC requires AQP4 OAP assembly, but that ADCC produced by natural killer cells did not depend on AQP4 OAP assembly. Therefore, the ratios of M1-AQP4 to M23-AQP4 in various CNS tissues could influence vulnerability of local astrocytes to AQP4 antibody’s CDC but not to ADCC in NMO. As a potential protective autoantibody for treatment of NMO, Tradtrantip et al generated non-pathogenic recombinant monoclonal AQP4 antibody that blocks the binding of AQP4 antibody to AQP4 selectively. These antibodies are anti-AQP4 Fab with mutated Fc lacking that do not activate complement or induce CDC. This AQP4 antibody successfully and safely prevented the NMO lesion development in cell culture, ex vivo spinal cord slices and in vivo mouse model. This study is a direct proof of the pathogenicity of AQP4 antibody in causing astrocytic damage.

f) Complement-independent pathogenicity of AQP4 antibody

Complement-independent effects of AQP4 antibody on astrocytes have been investigated as well. Marignier et al analyzed how AQP4 antibody altered the morphology and function of rat astrocyte and oligodendrocyte from primary culture and rat optic nerves, and showed that in the absence of complement, AQP4 antibody reduced membrane expression of AQP4 and glutamate transporter type 1 on cultured astrocyte. This treatment also reduced oligodendrocytic cell processes and induced their cell death both in vivo and ex vivo. They further showed some experimental evidences to suggest astrocytic dysfunction and support the hypothesis of a glutamate-mediated excitotoxic death of oligodendrocytes. When taken together, these findings suggest a direct, complement-independent effect of AQP4 antibody on astrocytes, with secondary damage to oligodendrocytes possibly resulting from glutamate-mediated excitotoxicity in NMO. Hinson et al previously reported down-regulation of AQP4 and excitatory amino acid transporter 2, and disruption of glutamate homeostasis as well, but Ratelade et al did not confirm the findings in an in vivo model.

g) T cell response to AQP4

Matsuya et al detected significant T cell responses to some AQP4 peptides exclusively in patients with NMO, and Bradl et al demonstrated that AQP4-specific T cells induce brain inflammation with particular targeting of the astrocytic glia limitans and permit the entry of pathogenic AQP4 antibodies to induce NMO-like CNS lesions. Rituximab severs B cell-T cell interaction and suppresses T cell proliferation in response to antigen stimulation. The therapeutic efficacy of rituximab in NMO may at least in part be attributable to suppression of AQP4-specific T cells.

h) Pathogenic potential of neutrophils

Neutrophils often infiltrate into NMO lesions, but their pathogenetic role has not been clear. Saadoun et al found that neutropenia reduced neurinflammation while neutrophilia induced by granulocyte-colony stimulating factor (G-CSF) increased
severity of NMO lesions following intracerebral injection of AQP4 antibody and human complement into rats. Administration of neutrophil protease inhibitor Sivelestat ameliorated pathological findings of early NMO-like lesions. The pathogenetic implication of neutrophils in NMO was confirmed in a recent report of a patient whose first NMO episode was exacerbated by inadvertent administration of G-CSF. The expression of G-CSF was definitely increased in neurons in and around the NMO lesions, but there was little or no expression in MS lesions and control brains.

i) Disruption of BBB by NMO sera

Shimizu et al demonstrated that NMO patients’ sera reduced the expression of tight junction proteins (claudin-5) and disrupted BBB (a significant decrease of transendothelial electrical resistance) through upregulation of autocrine vascular endothelial growth factor in human brain microvascular endothelial cells (BMEC). They also found that autoantibody against BMEC in the NMO sera but not AQP4 antibody mediated these changes. This study suggests that autoantibodies in NMO sera other than AQP4 antibody may also contribute to the pathogenesis of NMO.

Upregulation of IL6 in NMOSD

Some cytokines (IL6, IL8, IL13, G-CSF, etc) are upregulated in CSF during relapse of NMOSD. IL6 promotes production of AQP4-IgG from plasmablasts of NMOSD. CSF-IL6 significantly correlates CSF-GFAP, and CSF-AQP4-IgG titers correlates CSF-GFAP in NMOSD. Thus, the amount of pathogenic AQP4-IgG is associated with severity of inflammation and astrocyte damage.

Seronegative NMOSD and MOG-IgG-positive NMOSD

AQP4 is a transmembrane protein with 3 extracellular loops, and AQP4-IgG in NMOSD recognize the conformational epitopes of the AQP4 loops. Amino acid sequences of the extracellular domains of AQP4 are somewhat different in humans and rodents. Therefore, human AQP4-transfected cell-based assays are more sensitive than human AQP4-ELISA and mouse brain tissue-based indirect immune fluorescence assay.

Despite the use of the most sensitive cell-based AQP4-IgG assay, some patients clinically diagnosed with NMO/NMOSD are consistently seronegative. French and Mayo groups reported such unique features of “seronegative NMO” as no female preponderance, simultaneous development of optic neuritis and myelitis at onset, and less frequent severe visual impairment.

More recently, two reports (Brazilian-Japanese collaboration and Oxford group) on myelin oligodendrocyte glycoprotein (MOG)-IgG-positive cases of NMOSD have been published. Both studies employed cell-based assays to detect conformation-sensitive MOG-IgG (In contrast, the results in previous studies with ELISA and Western blot did not reveal unique clinical features probably due to non-specific binding.). The patients with MOG-IgG-positive NMOSD were all AQP4-IgG-negative. MOG-IgG-positive NMOSD were characterized by no female preponderance, simultaneous development of optic neuritis and myelitis or bilateral optic neuritis, myelitis preferentially involving the lumbosacral region, and less relapse and better functional recovery.

Interestingly, bilateral visual impairment was due to optic chiasmal involvement in
AQP4-IgG-positive cases while in MOG-IgG-positive patients, such an ocular symptom was caused by bilateral optic nerve lesions. Neuronal damage of optic neuritis detected by optical coherence tomography was milder in MOG-IgG-positive cases than in AQP4-IgG-positive ones.

**Astrocytic damage in AQP4-IgG-positive NMOSD vs. Demyelination in MOG-IgG-positive NMOSD**

There are four lines of evidence of severe astrocytic damage in AQP4-IgG-positive NMOSD.\(^6,7,15\)

1) Extensive loss of AQP4 and GFAP in the NMO lesions
   Immunopathological studies clearly demonstrated an extensive loss of immunoreactivities to AQP4 and GFAP, especially in the perivascular regions with deposition of immunoglobulins and activated complements, and a relative preservation of the staining of MBP in acute NMO lesions. A recent in-depth analysis revealed six different lesion types in NMO/NMOSD including typical activated complement deposition at the surface of astrocytes, associated with granulocyte infiltration and astrocyte necrosis and followed by demyelination and global tissue destruction (Type 1) and apoptosis-like clasmatroclendrosis of astrocytes without complement deposition (Type 5).\(^6\)

2) Remarkable elevation of CSF-GFAP levels in relapse\(^7\)
   The CSF-GFAP levels in relapse in NMO were significantly higher than those in MS or controls, and far beyond the levels in ADEM and spinal infarction. The CSF-GFAP levels correlated with disability status scales or spinal lesion length in NMO.

3) Pathogenicity of AQP4-IgG in vitro and in vivo\(^7\)
   AQP4-IgG and complement damage astrocytes or AQP4-transfected cells in culture. Injection of AQP4-IgG into rat’s blood in EAE induced by MBP-reactive T cell transfer or MBP immunization, clinical disease is augmented and CNS lesions resembling those of NMO develop. Those lesions consist of AQP4 and astrocyte loss, granulocytic infiltrates, T cells and activated macrophages and microglia cells, and an extensive deposition of immunoglobulins and activated complements on astrocyte processes of the perivascular and superficial glia limitans. Neither injection of AQP4-IgG-negative IgG to the EAE nor injection of AQP4-IgG to rats without EAE causes such pathological changes.

4) Low myoinositol/creatine value on \(^1\)H-MR Spectroscopy\(^15\)
   Myoinositol is considered to be a marker of astocytic activation and proliferation. On \(^1\)H-MR Spectroscopy, the myoinositol/creatine values in cervical cord NMO lesions were lower than in control and MS.
These findings strongly suggest that unlike MS, a prototypic demyelinating disease, astrocytes are the primary target in NMO and massive astrocytolysis occurs in acute exacerbations of NMO. NMO should now be classified as an astrocytopathic disease rather than a demyelinating disease, and thus the term “OSMS” is not suitable for this disease.

On the other hand, we recently reported that CSF-MBP levels in a MOG-IgG-positive NMO was significantly elevated but CSF-GFAP was undetected, suggesting that MOG-IgG-positive NMOSD is a demyelinating disease. Neuropathological findings of MOG-IgG-positive brain lesions are characterized by Pattern II MS pathology (demyelination associated with deposition of immunoglobulins and activated complements).

**Pregnancy in NMOSD**

AQP4 is expressed in the syncytiotrophoblast of human and mouse placenta. Placental AQP4 expression is high during mid-gestation and progressively decreases with advancing pregnancy. Intraperitoneally injected AQP4-IgG binds mouse placental AQP4, activates coinjected human complement, and causes inflammatory cell infiltration into the placenta, placental necrosis and fetal death, suggesting that AQP4-IgG can cause miscarriage. In fact, miscarriage is significantly increased in AQP4-IgG-positive pregnant women.

NMOSD attacks do not increase or decrease during pregnancy, but a significant increase occurs 3–6 months after delivery. The influences of delivery-related hormonal changes and physical and mental stress on the disease activity are yet to be clarified, but breastfeeding does not change the number of attacks. Pregnancy is a relevant issue in the management of patients with NMOSD, as the majority (90%) of AQP4-IgG-positive NMOSD patients are females, and many of them are in the childbearing age. Despite potential risks to the fetus, using corticosteroid or immunosuppressive drugs in pregnant women with NMOSD should be considered to prevent relapse. Babies born to AQP4-IgG-positive mothers are seropositive at birth, but convert to seronegativity after a month or so.

**Pain in NMOSD**

NMOSD lesions often develop in the ascending and descending pathways of nociception. Pain is highly prevalent (>80%) in NMOSD and often severe. Painful tonic spasm is a paroxysmal symptom usually seen in the recovery phase of LETM of
NMOSD and is treated with sodium channel-blocking antiepileptic agents such as carbamazepine, gabapentin, clonazepam and phenytoin sodium.

On the other hand, chronic neuropathic pain and paresthesia of LETM often involving the trunk and both legs is severe (terrible, agonizing, unbearable, etc) and persistent (although it may fluctuate daily), and responds only partially to drug therapy (analgesics, antiepileptic agents, antidepressants, etc. Among them, pregabalin is relatively effective.). This type of neuropathic pain interferes with NMOSD patient’s daily life (especially walking ability and enjoyment of life) and reduces the health-related quality of life. In theory, inhibition of excess glutamergic signals and inflammatory mediators of nociception like IL1β, IL6, IL17, tumor necrosis factor, and high-mobility group protein B1 and enhancement of GABAergic inhibition may be efficacious to relieve pain in NMOSD,\(^\text{21}\) and in fact, tocilizumab, an IL6 receptor monoclonal, reduced neuropathic pain as well as annual relapse rate in some patients with NMOSD.\(^\text{22}\)

References


Learning objectives:

1. Understand the evolution of neuromyelitis optica (NMO) diagnostic criteria in a historical context

2. Know syndromes and radiological findings of NMO spectrum disorder (NMOSD) and how they differ from those characteristic of MS.

3. Appreciate the pitfalls of NMOSD diagnosis, including potential for false positive and false negative serology, and how to differentiate from other diseases that mimic NMOSD.

Neuromyelitis optica was a term that was widely adopted to refer to a syndrome described by Devic (although others had recognized it previously) in 1894 in a publication and review of the literature by his colleague, Gault. It was typically defined as sequential bilateral optic neuritis and myelitis over a short interval in time, and was believed to usually be “monophasic” although relapsing forms were described. Based on the work of a few groups who conducted clinical reviews with broader inclusion criteria, the spectrum was widened to embrace individuals with unilateral rather than bilateral optic neuritis and to allow for relapses. The first diagnostic criteria to diagnose NMO and differentiate it from MS were proposed in 1999. A disease specific diagnostic antibody, NMO-IgG, was identified in 2004 in patients diagnosed as having neuromyelitis optica in the United States and the “pure” form of Asian opticospinal MS from Japan. The antibody was shown to target aquaporin-4 (AQP4-IgG) in 2005. Shortly thereafter, the diagnostic antibody, confirmed by several groups to be a moderately sensitive and highly specific marker, was incorporated into revised and simplified diagnostic criteria.

As of 2006, criteria for diagnosis of neuromyelitis optica (NMO) required both optic neuritis and myelitis and 2 of 3 additional specificity characteristics: normal brain MRI at initial presentation; T2-weighted spinal cord MRI lesion at the time of a myelitis episode that was at least 3 vertebral segments long; autoantibodies targeting aquaporin-4 (AQP4-IgG). However, it quickly became apparent that these criteria that would have previously been considered very liberal by allowing for unilateral optic neuritis, relapses and cerebral lesions, were too conservative. AQP4-IgG was also detected in many patients with recurrent myelitis or recurrent optic neuritis only; the clinical course of these patients resembled NMO more closely than MS. Some cerebral syndromes not previously recognized to be associated with NMO were found to occur in AQP4-IgG seropositive patients and patients with otherwise typical NMO. The most specific brain lesions of NMO are lesions of the area postrema or hypothalamus, longitudinally extensive corpus callosum lesions, and long corticospinal tract lesions in
the corona radiata and brainstem. Collectively, syndromes of optic neuritis and myelitis not satisfying traditional NMO criteria and NMO-typical brain syndromes and lesions were referred to as NMO spectrum disorders (NMOSD).

Studies from service testing laboratories reveal that the vast majority of AQP4-IgG seropositive patients have had optic neuritis and myelitis or a well-accepted signature syndrome of NMOSD, such as intractable vomiting or hiccup. Referral bias for serological testing undoubtedly influences this conclusion. Recent population-based studies of AQP4-IgG reveal confirm that the antibody is highly specific, but not entirely specific. In low prevalence/low pre-test probability situations, the issues of specificity are highlighted as shown in a recent study of approximately 1000 patients in the Kaiser Permanente system, 2 (0.2%) of whom had clinically unrecognized NMOSD; both had positive serologic tests for AQP4-IgG, but an additional 5 had false positive tests using a commercial ELISA assay, but only 1 (0.1%) had a positive result with a cell-binding assay. It is generally accepted that cell-based assays have the best current sensitivity and specificity to detect AQP4-IgG and should be requested in any case where there is clinical uncertainty.

An International Panel constituted by 18 experts from 9 countries on clinical, serological and imaging issues pertinent to NMO met over a 2 year to develop new diagnostic criteria recognizing the growing spectrum of the disease, potential for early diagnosis facilitated by serologic data and confusing issues about seronegative NMO. Among the key recommendations of the Panel are the following:

1. Eliminate distinctions between NMO and NMOSD; NMOSD is the preferred “umbrella” term for both what was previously known as NMO and NMOSD, regardless of the detection of AQP4-IgG
2. Stratify the diagnosis based on whether or not AQP4-IgG is detected.
3. It is possible to diagnose NMOSD after any single compatible clinical syndrome, either “signature” syndrome of NMO (optic neuritis, myelitis or intractable vomiting/hiccup) or “atypical” (e.g. brainstem lesion, tumefactive brain lesion, hypothalamic lesion) in an AQP4-IgG seropositive patient.
4. It is possible to diagnose a patient who is AQP4-IgG seronegative as having NMOSD, but the requirements are more rigorous and include evidence of dissemination in space; at least one signature syndrome of NMOSD and additional MRI requirements for many of these syndromes. Recurrent myelitis or recurrent optic neuritis in an AQP4-IgG seronegative patient cannot currently qualify for a diagnosis of NMOSD.

Seronegative NMOSD is difficult and likely heterogeneous entity, the characteristics of which will likely become better defined with time. Seronegative NMOSD could include patients who

1. currently seronegative for AQP4-IgG, but will eventually prove to be seropositive
2. have MS but some “NMO-like characteristics” (e.g. severe optic neuritis; myelitis with a long spinal cord lesion)
3. have other illnesses that may mimic NMO (e.g. sarcoidosis and paraneoplastic syndromes)

4. have other autoantibodies (e.g. anti-myelin oligodendrocyte glycoprotein, MOG) who may have somewhat distinctive clinical characteristics.

The Panel cautioned clinicians to avoid including those with other diseases that could mimic NMOSD, although no criteria were felt to be absolutely exclusionary.

In the setting of a syndrome that is not characteristic or convincingly associated with NMO, caution is necessary. False positive results may occur with AQP4 assays, and patients should not be diagnosed with NMOSD in the absence of clinical and radiological characteristics that are compatible with NMOSD.

The specific diagnostic criteria proposed by the Panel are summarized in the table below:

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**Table: Diagnostic Criteria for Neuromyelitis Optica Spectrum Disorders**  (International Panel for NMO Diagnosis, 2015)

<table>
<thead>
<tr>
<th>NMOSD with AQP4-IgG</th>
<th>NMOSD without AQP4-IgG or Unknown AQP4-IgG Status</th>
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<tbody>
<tr>
<td>1. At least 1 core clinical characteristic</td>
<td>1. At least 2 core clinical characteristics resulting from 1 or more clinical attacks and satisfying all of the following requirements:</td>
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<tr>
<td>2. Positive test for AQP4-IgG*</td>
<td>a) At least one of: ON, acute myelitis with LETM, or APS</td>
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<tr>
<td>3. Exclusion of alternative diagnoses**</td>
<td>b) Dissemination in space (&gt;2 different core characteristics)</td>
</tr>
<tr>
<td></td>
<td>c) MRI requirements, if applicable (see below)</td>
</tr>
<tr>
<td>2. Negative test(s) for AQP4-IgG* or testing unavailable</td>
<td></td>
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<tr>
<td>3. Exclusion of alternative diagnoses**</td>
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</table>

* Using best available detection method (cell-based assay strongly recommended)

** Evaluation for alternative diagnoses guided by “red flags”

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**Core Clinical Characteristics of NMOSD**

<table>
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<th>Most common:</th>
<th>Less common:</th>
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</thead>
<tbody>
<tr>
<td>1. Optic neuritis (ON)</td>
<td>4. Acute brain stem syndrome</td>
</tr>
<tr>
<td>2. Acute myelitis</td>
<td>5. Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic MRI lesions</td>
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</table>
Supporting MRI Requirements for NMOSD without AQP4-IgG

1. Acute optic neuritis: brain MRI normal or demonstrating only nonspecific white matter lesions; OR
   optic nerve MRI with T2-hyperintense lesion or T1-weighted gadolinium-enhancing lesion extending over >1/2 optic nerve length or involving optic chiasm

2. Acute myelitis: spinal cord MRI showing attack-associated lesion extending >3 contiguous segments (LETM); OR >3 contiguous segments of focal cord atrophy in patients with prior history of acute myelitis

3. Area postrema syndrome: dorsal medulla/area postrema MRI lesion

4. Acute brain stem syndrome: peri-ependymal brain stem lesions


Seronegative NMOSD remains to be characterized, and the current definition is meant to reflect the clinical similarity of seronegative NMOSD to seropositive NMOSD. The clinical and radiologic criteria are similar, but more rigorous. About 20-30% of patients with a NMOSD-compatible clinical syndrome and no viable alternative diagnosis are AQP4-IgG seronegative. The reasons for this are largely unknown for a given individual but may include: imperfect test sensitivity; treatment effects that interfere with antibody detection; presence of alternative antibodies that lead to a similar syndrome, and incorrect diagnosis. Furthermore, occasionally the antibody may be detected by one of the AQP4-IgG tests even though a patient does not have compatible clinical features with NMOSD. Although retrospectively presymptomatic seropositive individuals have been identified, it is safest to assume that such patients are false positive until clinical symptoms emerge and the Panel indicated that until studies of the natural history of such individuals establish that they are at high risk for emergence of NMOSD, a diagnosis of NMOSD is not warranted exclusively based on AQP4-IgG seropositivity.

Currently, the best defined subgroup of seronegative NMOSD appears to be a group of patients with MOG autoantibodies. This subgroup appears to constitute less than half of the seronegative group, but in comparison with AQP4-IgG seropositive NMOSD, they are more commonly male, younger, more likely to have optic neuritis rather than myelitis or myelitis and optic neuritis, and when they have myelitis, more likely to have lower
thoracic cord/conus lesions\textsuperscript{12}. However, no individual characteristic has yet defined this syndrome nor distinguishes it from AQP4-IgG seropositive NMOSD, and serological methods to detect these antibodies have not yet been adequately standardized. Furthermore, MOG antibodies seem to be present in other conditions including acute disseminated encephalomyelitis and pediatric optic neuritis\textsuperscript{13}. The separation of these syndromes from MOG-autoantibody-associated seronegative NMO is an evolving story. The IPND criteria can easily be modified to accommodate accepted variants of NMOSD based on serology as serology is the principal stratification factor.

The IPND criteria align formal criteria with contemporary expert practice for patients with NMOSD, which now include and treat patients who have more limited syndromes of NMOSD with AQP4-IgG as they do patients defined by former more conservative criteria. They are a tool to educate clinicians and clinical trainees as to the current standards for diagnosis of NMOSD. They facilitate treatment and clinical research by allowing for accurate diagnosis of patients with NMOSD at an early point in the disease. And they expand the pool of patients potentially suitable for clinical trials for NMOSD.

However the criteria do not provide a diagnosis for every potential presentation of NMOSD; detailed workup is essential for atypical cases and alternative diagnoses, such as paraneoplastic diseases, sarcoidosis and rarely neoplasia may mimic NMOSD in rare patients.
References

Treatment of the neuromyelitis spectrum disorders

The neuromyelitis optica spectrum disorders (NMOSD) are relapsing conditions that rarely lead to the non-relapse progressive disability which is common in multiple sclerosis (MS). Thus the main focus of NMOSD management is to treat and prevent relapses.

Symptom management is also an important part of care because many patients are disabled by visual impairment, mobility problems, sphincter and erectile difficulties, and importantly, chronic pain syndromes secondary to transverse myelitis. However symptomatic management is not specific for NMOSD, but similar to that for MS, and thus will not be covered here.

There are no RCTs in NMOSD and thus these treatment recommendations are based upon lower level evidence. The principle of using immunosuppressive agents has been based on anecdotal reports even before the NMO antibody was discovered that this condition responded to corticosteroids and is backed by experience in other antibody mediated conditions now we recognise the relevance of the aquaporin 4 antibodies. Since many observational studies have been published but comparisons of pre and on-treatment relapse rates will be confounded by ‘regression to the mean’ and a natural history of reducing relapses over time, whereas reports comparing treatments may be subject to ‘indication bias’. Changes in the natural history over time such as the reduction in mortality rates may be attributed to earlier and more appropriate treatment regimes, however comparisons across studies are complicated by the different inclusion criteria of the cohorts, the development of more sensitive NMO antibody assays over time and changes in diagnostic criteria. Reviews or guidelines on the treatment of NMO have been published involving National1,2, European3 and International4 experts which all recommend similar treatment algorithms.
**Treatment of relapses**

The underlying diagnosis at the time of the onset attack is usually not clear and so all attacks no matter what the underlying antibody status are treated the same. In contrast to MS, where relapses are often mild, rarely leave severe permanent impairment, and are not the main contributor to long-term disability, NMOSD relapses are treated more urgently and aggressively. Because all relapses in AQP4 antibody positive patients have a greater risk of causing residual disability it is reasonable to treat subsequent relapses in NMOSDs in a similar manner and not stratify them based upon guesses about potential untreated outcomes.

Most relapses respond to prompt high dose intravenous prednisolone for 3-5 days, however for those where disability is severe and/or response is slow, early plasma exchange or IVIG (where PLEX is not available or contraindicated) should be tried.

Once the acute attack has been treated and while awaiting the serum antibody results, oral prednisolone cover is recommended (doses around 30mg daily) to reduce the risk of rebound and early relapses, and if the serum AQP4 antibodies are negative this cover can be limited to 3-6 months. Furthermore, if long term immunosuppressive treatments are instituted such as azathioprine or mycophenolate mofetil, it usually takes up to 6 months to achieve adequate efficacy of these agents. Those patients with MOG antibodies may relapse beyond 6 months and thus a longer period of prevention (up to 12 months) seems reasonable if the antibodies remain.
Relapse Prevention

Long-term immunomodulation is recommended in all AQP4 antibody positive patients, and in AQP4 antibody negative patients with a similar phenotype who have relapsing disease (and in whom MS is unlikely). Standard oral immunosuppressant’s (IS) such as azathioprine (standard dose of 2.5mg/kg, mycophenolate mofetil (standard dose 1g-1.5g bd), methotrexate (standard dose 12.5-20mg ow) as well as prednisolone are options. The non-steroidal IS are increased in a graded fashion with regular blood monitoring, whereas prednisolone is usually started at higher doses (between 30-60mg od) and reduced slowly over time.

This authors experience suggests that the addition of low dose prednisolone to other steroid sparing agents maybe more effective than single agent therapy alone. Regular rituximab infusions (usually 6 monthly or when the serum CD19/20 B cell population starts to rise) is accepted to be an effective relapse prevention strategy although due to its cost, access may be limited in some countries. Non-randomised, observational studies suggest that mycophenolate and Rituximab may be more effective than azathioprine.\(^7,8\)
Relative benefits and advantages

The choice of immunosuppressive regime will depend on many factors in addition to local health service commissioning arrangements or health insurance cover. Prednisolone may be associated with weight gain, mood changes and insomnia and can disrupt diabetic control. Azathioprine, methotrexate and mycophenolate may be poorly tolerated due to malaise and gastric side effects and can be associated with abnormal full blood counts and liver function tests. Rituximab is commonly associated with mild infusion related events which may be reduced by pre-infusion methylprednisolone (with antihistamines), and occasional transient early post-infusion relapses have been reported. Infections are a fairly commonly reported side effect. Methotrexate and mycophenolate are contra-indicated in pregnancy although the FDA does not recommend any of the IS NMO treatments currently. Guidelines from a myasthenia gravis workshop recommended prednisolone and azathioprine as safe in pregnancy.

AQP4 antibody negative cohorts

It can be challenging to distinguish antibody negative NMOSD from MS in some patients, and yet it is important to do so because different disease modifying therapies are recommended for these two conditions. Of even more importance are the observations that many MS drugs appear to exacerbate NMO such as interferon-beta, natalizumab, and fingolimod, and because of its predisposition to cause other antibody mediated conditions alemtuzemab is also not advocated for NMO. For conditions with indeterminate diagnosis between MS and NMO where there is still significance concern about a diagnosis of NMOSD, the use of Rituximab or immunosuppressive agents such as azathioprine and mycophenolate may be safer options and likely are efficacious for both MS and NMOSD.
Future Directions

Our greater understanding of the pathogenic immunological network in NMOSD has highlighted many potential therapeutic targets. Some of these targets are already in clinical trial or in development such as complement inhibitors, anti-B cell monoclonals and drugs that act on B cell differentiation, and blocking therapies of AQP4 antibody binding. Therapeutic progress is challenged by the rarity of NMOSD limiting the availability of sufficient patients to test so many different treatments and the likely high cost of new treatments which could limit their availability in many countries.
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