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Neuromyelitis Optica Spectrum Disorders:

Critical Role of Complement-Dependent Cytotoxicity

DEAN M. WINGERCHUK, MD
Professor of Neurology | Mayo Clinic | Scottsdale, Arizona | Neurologist | Scottsdale, Arizona

Introduction to NMOSD

Neuromyelitis optica spectrum disorders (NMOSD) are a group of uncommon inflammatory syndromes that affect the central nervous system with symptoms that predominantly involve the optic nerves and spinal cord,[1] potentially causing paralysis and blindness.[2] What was once thought to be a single disorder consisting of bilateral optic neuritis and transverse myelitis, referred to as neuromyelitis optica (NMO), has now been shown to consist of a broader spectrum of disorders that can be further defined by the presence or absence of serum aquaporin-4 immunoglobulin G antibodies (AQP4-IgG).[1] NMOSD is also distinct from multiple sclerosis (MS).

The evolution in diagnostic criteria for NMOSD mirrors the gradual appreciation of the varied clinical presentations that occur and the role of pathogenic AQP4-IgG in many, but not all, patients.[1] The French version of the name "neuromyelitis optica" was first used by Devic in an 1894 description of a syndrome marked by acute myelitis and optic neuritis.[3] Lennon and colleagues discovered AQP4-IgG in patients who had NMO and determined that these antibodies were pathogenic.[3-5] Furthermore, these antibodies were not present in patients with MS.[4] This finding clarified the fact that NMO is a separate entity distinct from MS[3] and led to revised criteria requiring optic neuritis, myelitis, and at least 2 of 3 additional supportive criteria, one of which was the presence of AQP4-IgG.[6]

Yet some patients presenting with NMO did not have AQP4-IgG, and a review of patients' clinical, neuroimaging, serological, and pathological characteristics made it clear that NMO was not a single disease entity, but rather a spectrum of disorders.[7,8] International consensus diagnostic criteria were published in 2015 to define a new unifying term (NMOSD), set out clinically-based criteria to define NMOSD, and incorporate both positive and negative results from serologic testing for AQP4-IgG.[1]

Diagnosing NMOSD

For an NMOSD diagnosis, the International Panel for NMO Diagnosis (IPND) recommends that at least one discrete clinical attack of central nervous system (CNS) symptoms must have occurred and that alternative diagnoses have been excluded.[1] The IPND includes separate recommendations for patients with a positive serum test for AQP4-IgG and those whose AQP4-IgG status is either negative or unknown. The complete criteria for including and excluding patients from the diagnosis of NMOSD are quite extensive,[1] and only key points are summarized here.

Diagnostic Criteria for NMOSD in Adults With AQP4-IgG

The current diagnostic criteria for adult patients with AQP4-IgG include a positive serum test for the antibodies plus at least one of the following "core clinical characteristics"[1]:

- Optic neuritis
- Acute myelitis
- Area postrema syndrome, defined as an episode of otherwise unexplained hiccups or nausea and vomiting
- Acute brainstem syndrome
- Symptomatic narcolepsy or acute diencephalic clinical syndrome with NMOSD-typical diencephalic magnetic resonance imaging (MRI) lesions
- Symptomatic cerebral syndrome with NMOSD-typical brain lesions.

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Diagnostic Criteria for NMOSD in Adults Without AQP4-IgG or in Whom AQP4-IgG Status Is Not Available

According to the IPND, these patients should have at least 2 of the core clinical characteristics "occurring as the result of one or more clinical attacks" and the following[1]:

- One of the core clinical characteristics must be optic neuritis, acute myelitis with longitudinal extensive transverse myelitis (LETM), or area postrema syndrome
- Dissemination in space of 2 or more different core clinical characteristics
- Fulfillment of additional MRI requirements as applicable.

The IPND concluded that the diagnostic criteria were also applicable to children with some caveats, including that LETM lesions may occur in pediatric MS as well as NMOSD and that cerebral presentations are more common in children than in adults.[1]

No clinical characteristic is considered to be pathognomonic of NMOSD, and the authors emphasize that the criteria now allow for a diagnosis of NMOSD in patients without clinical involvement of either the spinal cord or the optic nerves.[1]

The consensus guidelines also address the need to carefully distinguish between NMOSD and MS, pointing out that "detection of a LETM spinal cord lesion associated with acute myelitis is the most specific neuroimaging characteristic of NMOSD and is very uncommon in adult MS."[1] The guidelines also list several neuroimaging results as "red flags" for clinicians to consider MS and other diseases in a differential diagnosis of NMOSD.

Considerations for AQP4-IgG Testing

The international panel recommends cell-based serum assays because these methods currently have the best results for autoantibody detection, citing a false positive rate of 0.1% in a MS cohort and a mean sensitivity of 76.7% in a pooled analysis of individuals with NMOSD.[1] A more recent review of AQP4-IgG testing also concludes that cell-based assays are the most sensitive and specific but that specialist centers are able to achieve comparable results using immunohistochemistry or flow cytometry.[9] Sensitivities, measured using AQP4-IgG seropositive samples, ranged from 51.5% to 100% in 21 different assays. Specificities, measured using samples from controls and seronegative patients with NMOSD, ranged from 85.8% to 100%.

Epidemiology

The epidemiology of NMOSD is often based on studies that used earlier definitions of the disorders, so the numbers are evolving as the newer criteria are incorporated. Recent, population-based prevalence estimates in whites are about 4 per 100,000. The prevalence also varies depending on the ethnicity; for example, in Martinique, black Afro-Caribbeans have a disease prevalence of about 10 per 100,000.[10,11] The 2015 IPND criteria should expand the numbers of those correctly diagnosed with NMOSD by "identifying individuals who would have been diagnosed with idiopathic transverse myelitis, idiopathic optic neuritis, or atypical MS" using earlier criteria.[1] NMOSD occurs more frequently in women than in men, with a median age of onset of 39 years.[12,13] The diagnosis has also been made in both children and the elderly.[12] Some familial cases have been identified, but account for only a very small percentage of patients.

Patients who are seropositive for AQP4-IgG differ from those who are seronegative for the antibodies in a few respects; the former are more likely to be female and have coexisting autoimmunity, more severe clinical attacks, and a higher total load of spinal cord lesions.[12] Those who are seronegative are more likely to present with bilateral optic neuritis.[12] While some researchers have reported a lower rate of relapse among seronegative patients, others have found no difference in relapse rate.[12] Several factors seem to be very similar in the 2 groups, including age of onset, time to relapse, mortality, brain lesions as visualized on MRI, cerebrospinal fluid (CSF) finding, and relapse outcome.[12]

Pathophysiology

Aquaporins are a family of proteins that are involved in transporting water through cell membranes.[14] Aquaporins are also strongly expressed in certain tumors, including astrocytomas and glioblastomas, and may facilitate tumor angiogenesis, tumor growth, and metastasis.[14] AQP4 (one of the family of aquaporins) form the most abundant water channels in the CNS and are found in astrocyte membranes facing blood-brain and blood-CSF interfaces, where they facilitate water transfer across those membranes.[14,15] AQP4 has also been shown to play a role in neural signal transduction and neuroinflammation in studies of AQP4 knockout mice.[14]

The primary form of IgG found in AQP4-IgG is IgG1, a subtype of IgG that both strongly activates complement and binds to Fc receptors.[15] So, when AQP4-IgG bind to AQP4, the antibodies activate complement, which leads to complement-dependent cytotoxicity. AQP4-IgG can also produce antibody-dependent cellular cytotoxicity by binding to the Fc receptors on natural-killer cells (NK cells) when those cells are present.[14,15] Both complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity contribute to NMOSD pathogenesis, although complement-dependent cytotoxicity seems to be the primary mechanism.[14,15]

Role of Complement-dependent Cytotoxicity

The complement system was once thought to have a fairly limited role in the immune system, recognizing and eliminating pathogens. Now, however, complement proteins are known to have a much broader role in immunity, and dysregulation of the complement system has been shown to affect the pathogenesis and clinical picture of several autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and systemic sclerosis.[16] Complement, through complement-dependent cytotoxicity, also plays a key role in the pathophysiology of NMOSD.[12]

In NMOSD, AQP4-IgG is thought to enter the CNS through endothelial transcytosis or an increased permeability of the blood-brain barrier.[12] Once AQP4-IgG binds to AQP4, complement produced locally by astrocytes is activated, leading to a cascade of events that convert a series of inactive complement components to active forms, including the precursors C3 and C5 to C3a and C3b and C5a and C5b, respectively.[17] C5b in turn initiates the development of the membrane attack complex (MAC), which intercalates into astrocyte cell membranes and causes astrocyte damage (**Figure**).[12,14,15]

C3a and C5a recruit inflammatory cells, including neutrophils, eosinophils, and macrophages, which leads to cytokine and chemokine release.[14,15,17] This process also increases the permeability of the blood-brain barrier, allowing more AQP4-IgG to enter the CNS.[14]

Astrocytes are the primary cell type damaged by complement-dependent cytotoxicity, but the inflammatory reaction can also damage oligodendrocytes and neurons,[14,15] leading to secondary demyelination.[12] Demyelination has been found in NMOSD lesions in the gray and white matter and across multiple spinal cord segments.[12]

Role of Antibody-dependent Cellular Cytotoxicity

Antibody-dependent cellular cytotoxicity has been shown to cause NMO-like lesions in experimental studies, indicating its potential role in the development of NMOSD.[12] In particular, when NK cells are injected together with AQP4-IgG into astrocyte cell cultures and animal models of NMOSD, antibody-dependent cellular cytotoxicity develops and contributes to astrocyte injury.[15]

Two different mechanisms may explain antibody-dependent, cellular, cytotoxicity-related astrocyte injury, one of which also involves complement. Injection of AQP4-IgG plus NK cells into mouse brain caused antibody-dependent cellular cytotoxicity with the development of NMO-like lesions but with little myelin loss.[15] In a separate, ex vivo, spinal cord slice model of NMOSD, lesions with myelin loss were first created by the addition of AQP4-IgG plus complement. Adding NK cells to these lesions produced antibody-dependent cellular cytotoxicity with additional myelin loss. These results suggest that antibody-dependent cellular cytotoxicity may be a possible second mechanism that can injure astrocytes independent of direct complement cytotoxicity.[15]

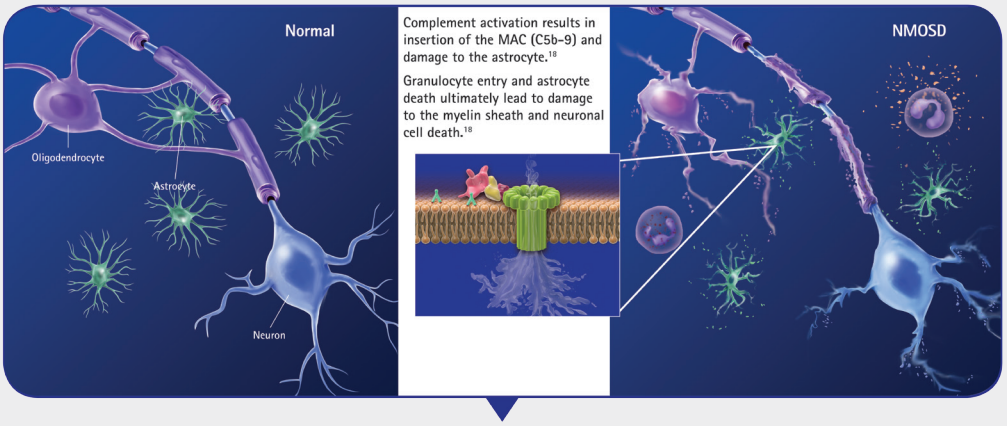
In addition to NK cells, macrophages, neutrophils, and eosinophils, which are often found in NMO lesions, expressed Fc receptors may also play a role in the development of antibody-dependent cellular cytotoxicity.[15]

While these studies in experimental models are suggestive of the importance of antibody-dependent cellular cytotoxicity in the development of NMOSD lesions, the precise role and extent of impact of antibody-dependent cellular cytotoxicity in NMOSD has not yet been completely defined.[12]

Pathogenesis of NMOSD in Patients Who Are AQP4-IgG Seronegative

A few different hypotheses have been proposed to explain the development of NMOSD in patients who are seronegative for AQP4-IgG.[12] One suggestion is that current assays may not be sensitive enough to identify AQP4-IgG in all patients. Another is that AQP4-IgG seronegative patients may have antibodies directed against other antigens present in astrocytes. A third is that the AQP4-IgG levels in serum may increase with clinical relapses and decrease with immunosuppressive therapy and may only be detectable at certain times in some patients.[1] Or, NMOSD in patients who are seronegative may have a different pathogenesis.[1] There is a subset of NMO IgG+ patients who have been reported to serorevert on immunosuppressive therapy but still have attacks, but the mechanism for this is unclear.

FIGURE. Complement activation leads to the production of MAC, which causes astrocyte injury[18]



Adapted from Papadopoulos MC, Verkman AS. Aquaporin 4 and neuromyelitis optica. *Lancet Neurol.* 2012;11(6):535-544.

Unmet Needs in NMOSD

The goal in treating NMOSD is to reduce neurological disability by stopping or lessening acute attacks and preventing additional exacerbations.[2] Intravenous steroids and plasma exchange have formed the standard approach to treating acute attacks, while various types of immunosuppressive therapy have been used to

try to prevent further exacerbations and, most importantly, avoid the significant morbidity that is associated with exacerbations.[2,19] Immunosuppressive therapies come with serious risks of adverse effects, however, including malignancy, infertility, cytotoxicity, myelotoxicity, and infections.[19] The need to clearly distinguish between NMOSD and MS is of particular concern when trying immunosuppressive therapies, as some treatments that are successful in MS can exacerbate NMOSD, including interferon, natalizumab, and oral fingolimod.[2]

There is still a considerable need for additional evidence-based, prospective, randomized clinical trials (RCTs) to determine optimal approaches to treating acute attacks and preventing exacerbations of NMOSD.[2,19] Much of the current clinical data comes from limited retrospective and prospective case series rather than RCTs.[2] Additional information is also needed about the best approaches for treating women during pregnancy and for pediatric NMOSD.[2] Newer treatment strategies that are more specific for NMOSD may also be developed based on research into its immunopathogenesis, including the roles of complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity.[12]

Summary and Future Directions for Research

Neuromyelitis optica spectrum disorders are a group of inflammatory syndromes with symptoms that predominantly involve the optic nerves and spinal cord and can lead to paralysis and blindness. International consensus diagnostic criteria published in 2015 delineate clinically-based criteria to better define NMOSD. These criteria recognize the presence of AQP4-IgG in most patients with NMOSD but include the subset of patients who are seronegative for AQP4-IgG. Current estimates of NMOSD prevalence may expand with the adoption of 2015 criteria that now identify individuals who may previously have been misdiagnosed with other conditions.

The pathogenesis of NMOSD involves primarily AQP4-IgG-mediated complement-dependent cytotoxicity that injures and kills astrocytes and produces secondary demyelination. AQP4-IgG can also trigger antibody-dependent cellular cytotoxicity, which can also injure astrocytes. Interestingly, one of the pathways of antibody-dependent cellular cytotoxicity is linked to the presence of complement. The exact role that antibody-dependent cellular cytotoxicity plays in NMOSD is not completely understood at present.

While some approaches to long-term treatment of recurrent NMOSD have been developed based on immunosuppression, evidence-based, prospective RCTs are needed to determine optimal approaches to treat acute attacks and prevent exacerbations. Newer treatment strategies that are more specific for NMOSD may also be developed based on research into the immunopathogenesis of these disorders.

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