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# Neuromyelitis optica: clinical features, immunopathogenesis and treatment



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## Summary

**The term 'neuromyelitis optica' ('Devic's syndrome', NMO) refers to a syndrome characterized by optic neuritis and myelitis. In recent years, the condition has raised enormous interest among scientists and clinical neurologists, fuelled by the detection of a specific serum immunoglobulin (Ig)G reactivity (NMO-IgG) in up to 80% of patients with NMO. These autoantibodies were later shown to target aquaporin-4 (AQP4), the most abundant water channel in the central nervous system (CNS). Here we give an up-to-date overview of the clinical and paraclinical features, immunopathogenesis and treatment of NMO. We discuss the widening clinical spectrum of AQP4-related autoimmunity, the role of magnetic resonance imaging (MRI) and new diagnostic means such as optical coherence tomography in the diagnosis of NMO, the role of NMO-IgG, T cells and granulocytes in the pathophysiology of NMO, and outline prospects for new and emerging therapies for this rare, but often devastating condition.**

**Keywords:** aquaporin-4 antibodies (AQP4), Devic syndrome, diagnosis, neuromyelitis optica, NMO-IgG, pathogenesis, pathophysiology, treatment

## Introduction

Neuromyelitis optica (NMO, Devic's syndrome) is an inflammatory disorder of the central nervous system (CNS) that presents typically with relapses of optic neuritis (ON) or myelitis [1–4]. In recent years, the condition has raised enormous interest among scientists and clinical neurologists, fuelled by the detection of a highly specific serum immunoglobulin (Ig)G autoantibody (NMO-IgG) targeting the most abundant astrocytic water channel aquaporin-4 (AQP4) [5–8]. NMO-IgG/AQP4-antibodies are present in up to 80% of patients with NMO [8–11]. This seminal discovery has – together with previous neuropathological work that had already suggested humoral mechanisms to be relevant in the disease pathogenesis [12] – made clear that in most cases NMO is not a subform of multiple sclerosis (MS), as had been assumed for decades, but rather an autoimmune condition with an immunopathogenesis distinct from that of MS despite considerable overlap in clinical presentation and paraclinical findings. AQP4-antibody-positive NMO is part of an expanding spectrum of humorally mediated autoimmune diseases of the CNS that have been identified over the last few years [13,14]. Several studies suggest that optimum treatment options may differ

between NMO and MS, which underscores the necessity for a timely and accurate diagnosis. Another important advance was the discovery that AQP4 autoimmunity is associated with a much broader range of CNS symptoms than just NMO; this prompted the proposal to refer to the condition by terms such as 'NMO spectrum disorder' (NMOSD) [15], 'autoimmune AQP4 channelopathy' [16], 'AQP4 autoimmune syndrome' [17] or 'AQP4 encephalomyelitis' [18].

The aim of this review paper is to summarize current knowledge on the pathogenesis of AQP4-antibody-related NMO and to provide an update on the widening clinical spectrum, relevant paraclinical findings and current treatments.

## History

First reports on patients with myelitis and amaurosis date back to the early 19th century [18–24]. However, neurologists and ophthalmologists only developed sustained interest in this rare syndrome after Eugène Devic and his student Fernand Gault published a review in 1894 [25,26]. Devic and Gault also coined the term *neuro-myélite optique aiguë* [25,26]. In 1907 the Turkish physician Acchioté suggested naming the syndrome after Devic [18].

## Epidemiology

Epidemiological and population-based studies suggest that the prevalence of NMO ranges from <1/100 000 to 4·4/100 000 in Europe and North America [27–31]. However, the true number of cases may be higher, as some studies reported a rate of patients misdiagnosed with MS as high as 30–40%, especially before tests for AQP4 antibodies became broadly available [1,32]. Typical age at onset peaks at approximately 35–45 years, but NMO may also become manifest in children and the elderly [1,33–39]. Female preponderance is substantially higher in seropositive (~9–10:1) than in seronegative patients (~2:1) [1,40]. The majority of NMO cases are sporadic, although rare familial cases indistinguishable from the former with respect to clinical presentation, age and sex distribution have been reported [41].

## Clinical presentation, disease course and prognosis

In more than 90% of patients, NMO is a relapsing disease with attacks of ON, myelitis or both, occurring unpredictably [1]. A monophasic course accounts for the remaining 10% and is more often associated with simultaneous ON and myelitis [1,36], while a progressive course seems to be extremely uncommon [42]. Attacks of ON and myelitis are often more disabling and, if untreated, remission is poorer than in MS, which leads to a faster accrual of irreversible neurological disability. Following older studies, approximately 60% of patients exhibited severely impaired ambulation [expanded disability status scale (EDSS) [43] ≥6] or blindness in at least one eye after a disease course of 7–8 years [36]. Five-year survival rate was reported to be as low as 68% in a North American study on patients seen between 1977 and 1997, which is in strong contrast to more recent studies that report 5-year survival rates of more than 90% [1,44]. In a small subset of patients the disease may follow a benign course, with only minor disability after up to 10 years [1,45]. The majority of NMO-related deaths result from severe ascending cervical myelitis or brainstem involvement leading to respiratory failure [1,36]. The discrepancy between older and newer studies with respect to prognosis and survival rate may be explained by increased awareness of the disease subsequent to the detection and availability of AQP testing in clinical routine. Thus, it can be assumed that the rate of misdiagnoses may have dropped and that more patients are diagnosed and treated earlier. Moreover, treatment options have improved. Nevertheless, NMO remains a potentially life-threatening and severely disabling condition that usually requires prompt and consequent immunosuppressive treatment. Clinical decision-making with respect to diagnosis and treatment initiation remains challenging when a patient presents with ON or myelitis only, or with other clinical symptoms, such as brainstem encephalitis with intractable hiccups and vomiting or a syndrome of inappropriate anti-diuretic hormone secretion [1,46–50]. In such cases, testing for AQP4-antibody by means of a both highly sensitive and highly specific assay can be essential [51]. Other symptoms and syndromes that have occasionally been reported in association with AQP4 autoimmunity include seizures [52], posterior reversible encephalopathy syndrome [53], myeloradiculitis [54], meningoencephalitis [55], findings related to brainstem involvement, such as hearing loss, diplopia, olfactory dysfunction and other cranial nerve palsies, or endocrinological abnormalities due to diencephalic lesions [1,56–58]. Moreover, pain syndromes [1,59,60] and cognitive dysfunction [61–63] seem to develop more frequently than appreciated previously.

In contrast to MS, a higher proportion of NMO patients (30–50%) exhibit laboratory findings or clinical signs of other systemic or organ-specific autoimmunity, such as systemic lupus erythematosus, Sjögren's syndrome, autoimmune thyroid disease, myasthenia gravis or, possibly, autoimmune-mediated vitamin $B_{12}$ deficiency [64–74]. The invariable association with myelitis and/or ON suggests that AQP4 antibodies in patients with rheumatic diseases do not represent an unspecific epiphenomenon, but rather points to the existence of two concomitant autoimmune conditions.

Two studies found an increase in relapse rate in the first or the first and second trimenon, respectively, after delivery [75,76]. Preliminary data suggest that AQP4-antibodies might also be capable of causing damage in AQP4-expressing organs and tissues outside the CNS (e.g.

placentitis with the risk of miscarriage [77–79], myositis [80–83], internal otitis [56] or gastritis [74]).

## Diagnostic criteria

In 2006, the diagnostic criteria for NMO were revised after NMO-IgG were detected. In addition to including this novel and highly specific marker, the absolute restriction of CNS involvement beyond the optic nerves and spinal cord was removed and the specificity of longitudinally extensive spinal cord lesions emphasized [84,85]. In addition to the two clinical index events of ON and acute myelitis, a diagnosis of NMO now requires that two of the three following supportive criteria be fulfilled:

- Contiguous spinal cord magnetic resonance imaging (MRI) lesion extending over three or more vertebral segments;
- Brain MRI not meeting diagnostic criteria for MS according to Paty [86]; and
- NMO-IgG seropositive status.

Given the rapid expansion of our knowledge on NMO, it is to be expected that these diagnostic criteria may be modified or replaced in the nearer future.

## Immunopathogenesis

Several lines of evidence from clinical, pathological and immunological studies indicate that AQP4-antibodies have a decisive role in the pathogenesis of NMO [87–90]:

(a) NMO-IgG/AQP4-IgG is highly specific for NMO and its limited forms [9,51,88]. The largest study performed thus far found the antibody in only 0·6% of 1672 controls using a tissue-based assay (TBA) [29]. Similarly, specificity rates as high as 99·83% ($n = 604$; TBA) [91], 99·57% [$n = 234$; cell-based assay (CBA)] [92], 99·27% ($n = 137$; TBA) [7], 99·71% ($n = 695$, TBA) [93], 98·69% [$n = 153$, enzyme-linked immunosorbent assay (ELISA)] [10], 100% ($n = 100$, CBA [9], $n = 85$, CBA [11], $n = 114$, fluorescence activated cell sorter (FACS) [94], $n = 178$, ELISA [94], $n = 85$, immunoprecipitation [11]) were reported in a number of recent studies (see references [88] and [51] for a comprehensive summary).

(b) AQP4-IgG serum levels were found to correlate with NMO disease activity in several independent studies [10,95–97], serum levels increasing shortly before relapse and declining during recovery [95–97].

(c) Similarly, AQP4-antibody-positive plasmablasts are selectively increased in the blood of NMO patients and peak at relapse [98].

(d) In patients with isolated ON and in patients with isolated longitudinally extensive transverse myelitis (LETM), AQP4-antibodies have been shown to predict conversion to NMO, i.e. the development of additional LETM or additional ON, respectively [10,99–101].

(e) In patients with NMO or its limited forms, the presence of AQP4-antibodies predicts future relapse [1,99–101].

(f) Clinically, the presence of AQP4-antibodies in patients with NMO is associated with distinct phenotypic features. AQP4-antibody-positive patients are more often female (~10:1), show a relapsing disease course more often than seronegative patients and frequently exhibit signs of co-existing autoimmunity [10,102,103]. By contrast, seronegative NMO is more frequently monophasic and shows only a slight female preponderance (~1:2) [10,102].

(g) Some studies found a correlation between disease severity and AQP4-antibody status and/or titres [95–97,102]: the presence of AQP4-antibodies may be associated with more extensive spinal involvement as assessed by MRI [1,97], more severe attack-related optic and/or motor disability [1,97] and, possibly, more severe disability in the long-term course [102]. Seronegative NMO was also reported to take a milder disease course in paediatric patients [35]. In addition to AQP4-antibodies, genetic background and age at onset also seem to determine prognosis [103].

(h) B cell or antibody-targeted treatments have been found to be effective in NMO, including plasma exchange (PE) [104–109], B cell depletion by rituximab [87,110–114] and the interleukin (IL)-6 receptor inhibitor tocilizumab [115–117].

(i) Incomplete B cell depletion or recurrence of B cells is associated with breakthrough attacks [95,114].

(j) Successful treatment is usually associated with a decline in AQP4-antibody serum concentrations and stable suppression during remission [10,95–97].

(k) AQP4 is expressed at highest levels in opticospinal tissues, which also appear to contain higher amounts of supramolecular AQP4 aggregates [118]. An immune response targeting AQP4 could thus well explain why the optic nerve and the spinal cord are predilection sites in NMO.

(l) NMO lesions are characterized by a marked loss of astrocytic AQP4 and by prominent deposits of IgG and IgM concentrated around blood vessels, i.e. at the main sites of AQP4 expression [12,119–121].

(m) In some lesions astroglial loss is not associated with loss of myelin and neuronal axons [119,120,122], suggesting that the initial immune response in NMO is directed against astrocytes, a cell population that expresses AQP4 at high levels.

(n) While AQP4 is lost in parallel with glial fibrillary acidic protein (GFAP), indicating astrocyte loss, GFAP is preserved in other lesions, indicating that AQP4 is the primary target of the anti-astrocytic immune

response in NMO [119,120,122]. This initial loss of AQP4 might reflect internalization (and possibly endolysosomal degradation [123]) of AQP4 (either of M1-AQP4 [124] or of both isoforms [125]), as demonstrated both in transfected cell lines and in cultured astrocytes [123,124,126–128], and might thus still be reversible. However, as a limitation, a more recent study could not find evidence of AQP4 endocytosis *in vivo* after injecting fluorescent AQP4-antibodies [129].

(o) Most importantly, passive transfer animal experiments using IgG from AQP4-antibody-positive patients were able to reproduce the neuropathological features of NMO. Intracerebral injection of IgG from AQP4-antibody-positive patients, together with human complement, caused a marked loss of astrocytes [130]. However, the fact that pretreatment with complete Freund's adjuvant or pre-existing experimental autoimmune encephalomyelitis (EAE) was required for inducing tissue damage in studies administering IgG intravenously or intraperitoneally suggests that a disrupted blood–brain barrier (BBB) and, possibly, an inflammatory environment is necessary for AQP4-IgG to exert its pathogenic effects *in vivo* [131–134]. As in human lesions, AQP4 preceded astrocyte loss, demyelination and neuronal necrosis in those models [130,133].

(p) Strong direct evidence for a pathogenic role of AQP4-IgG comes from the finding that AQP4-antibody belongs mainly to the complement-activating IgG1 subclass [11,94,135,136]. In line with this finding, the presence of the terminal membrane attack complex, indicating complement activation, at sites of AQP4 loss has been described as a key feature of NMO lesions in humans [12,119,120]; anaphylatoxin C5a levels in the cerebrospinal fluid (CSF) of AQP4-antibody-seropositive patients have been found to be elevated [137]; and eculizumab, a C5 inhibitor, was recently shown to substantially reduce the relapse rates in patients with NMO [138,139]. In several independent *in-vitro* studies it was shown that sera from NMO-IgG-positive patients, but not from controls, can induce (according to some studies, titre-dependent) death of AQP4-transfected cell lines in the presence of human complement [11,123,136,140,141] (possibly more effectively after transfection with M23-AQP4 than M1-AQP4 [142]). One of these studies even reported a correlation between the percentage of damaged cells by AQP4-IgG-positive sera and the severity of clinical relapses [140]. Similarly, co-administration of (human) complement was necessary to induce lesion pathology in AQP4-IgG-driven animal models of NMO, whereas a C1 complement inhibitor prevented tissue damage [130]. As in human lesions, complement deposits have been found within spinal cord lesions in these animal models [130,132,133]. This observation is corroborated by *ex-vivo* and animal models of NMO. Exposure to AQP4-antibody-positive NMO sera or recombinant NMO antibody in the presence of human complement reproduced the loss of AQP4, GFAP and myelin that characterizes human NMO lesions in cultured mouse spinal cord slices or optic nerves [143]. Lesions were not seen in spinal cord slices from AQP4 null mice [143]. Verkman and colleagues performed a number of sophisticated experiments that provide further strong evidence for an essential role of AQP4-antibody- and complement-dependent cytotoxicity (CDC): a high-affinity monoclonal antibody (termed aquaporumab) from recombinant monoclonal antibodies derived from AQP4-IgG-positive CSF plasmablasts of a patient with NMO and rendered non-pathogenic by introducing IgG1Fc mutations at locations required for the induction of CDC [144], cleavage of IgG from NMO patients by means of an IgG-degrading enzyme of *Streptococcus pyogenesto* (IdeS) to yield Fc and $F(ab')_2$ fragments [145], selectively deglycosylating the heavy chain of natural AQP4-IgG with bacteria-derived endoglycosidase S to render it non-pathogenic [146], and preincubation with small molecules (identified by automated high-throughput screening) that sterically block interaction between AQP4-antibody and its target antigen [147,148] have all been shown to prevent lesion formation in both slice cultures and mice exposed to AQP4-antibody-positive sera and human complement.

While AQP4-antibody-mediated CDC may play a major role in the pathogenesis of NMO, there is abundant evidence suggesting that additional immunological players are involved:

(a) *NMO lesions* have been shown to contain large numbers of macrophages, eosinophils and neutrophils, which often display signs of degranulation, as well as a few T cells [12,149].

(b) Numerous *proinflammatory cytokines* have been found to be elevated in the serum and CSF of patients with NMO: serum levels of IL-6 are increased significantly and have been implicated in the maintenance of AQP4-antibody-positive plasmablasts in the blood [98,150]. IL-6 is also elevated in the CSF [150,151], as are the B cell recruiting and activating factor (BAFF), a proliferation-inducing ligand (APRIL) and C-X-C motif chemokine 13 (CXCL13) [152–154], indicating the presence of a B cell-friendly environment in the CNS as well. However, IL-6 also promotes development and maintenance of IL-17-producing T helper type 17 (Th17) cells by inhibiting the conversion of conventional T cells to forkhead box protein 3 $(FoxP3)^+$ T regulatory cells [155,156]. Whether Th17 cells contrib-

ute to NMO pathogenesis is currently being studied [157–161]. IL-17 levels are indeed increased in patients with NMO during acute attacks [157,162], and an IL-17 gene polymorphism has been reported recently in Chinese patients with NMO [162]. Intrathecal activation of the IL-17/IL-8 axis is thought to promote recruitment of neutrophils [163]. IL-4, a major secreted cytokine of eosinophils, is known to cause a shift towards a type 2 helper T cell (Th2) response [164]. Both neutrophils and eosinophils have been found in the CSF and are present in NMO lesions [12,165]. In spinal cord slice cultures a number of proinflammatory cytokines, including IL-6, were shown to enhance AQP4-antibody-mediated cytotoxicity [143].

(c) AQP4-IgG could act on *macrophages*, *neutrophils and eosinophils* by binding to Fc receptors. Direct evidence for a role of neutrophils in the pathogenesis of NMO comes from the finding that neutrophil elastase inhibitors can ameliorate lesion formation in mouse models of NMO [166], and from the observation that disease was exacerbated both in a mouse model of NMO and in an NMO patient treated with granulocyte colony-stimulating factor [166,167]. In spinal cord slice cultures treated with NMO-IgG and human complement, the severity of lesions is increased markedly by including macrophages, neutrophils or eosinophils (or their granule toxins) and could be ameliorated by antihistamines such as cetirizine and ketotifen, which have eosinophil-stabilizing effects [143,168]. Antihistamines were also effective in an animal model of NMO [168], and lesion severity has been shown to be increased in transgenic hypereosinophilic mice. By contrast, reduced lesion severity was observed in mice rendered hypoeosinophilic by anti-IL-5 antibody or by gene deletion [168]. Neutrophil and eosinophil infiltration has also been noted in animal models of NMO following peripheral or intracerebral injection of AQP4-IgG and complement [133,168]. Complement-dependent attraction has been suggested to be involved in granulocyte trafficking through the BBB [128]. Granulocyte trafficking is not inhibited by natalizumab, a drug employed successfully in MS; accordingly, natalizumab does not seem to be effective in NMO [169–171].

(d) The contribution of *natural killer (NK) cells* to NMO pathology is controversial. In one study, NMO-IgG binding to human fetal astrocytes was found to result in NK cell degranulation and astrocyte killing by antibody-dependent cellular cytotoxicity (ADCC) [128]. NK cell-mediated ADCC following exposure to AQP4-antibodies was also observed in mouse astrocyte and spinal cord slice cultures and in a mouse passive transfer model [143,172]. An AQP4-antibody mutant lacking ADCC effector function and Fc fragments generated by IdeS-mediated cleavage of recombinant AQP4-specific IgG significantly reduced tissue damage [145,172]. However, the relevance of these findings in human disease is somewhat doubtful, given the rarity of granzyme $B^+$ and perforin$^+$ NK cells and cytotoxic T cells in active human NMO lesions reported in a recent study [149].

(e) *B cells* are surely involved in the pathophysiology of NMO as progenitors of AQP4-antibody-producing plasma cells. In addition, B cells could contribute to the pathogenesis of NMO by producing IL-6 and as antigen-presenting cells for AQP4-specific T cells. BAFF, APRIL and CXCL13 levels are elevated in the CSF and the percentage of BAFF-R- and CXCR5-expressing peripheral B cells is higher in NMO [152–154]. A recent study reported possibly impaired immunoregulatory B cell properties, as indicated by lowered $CD19^+CD24^{high}CD38^{high}$ regulatory B cell levels and reduced B cell expression of regulatory IL-10 [154].

(f) *T cells* are also certainly relevant, because T helper cells (including Th17 cells [173]) are involved in B cell isotype switching and affinity maturation. Furthermore, $CD3^+$ and $CD8^+$ T cells were detected directly within NMO lesions, albeit at low numbers [12]. Moreover, NMO has been reported to be associated with human leucocyte antigen D-related (HLA-DR)B1*03 in Brazilians [174]; this allele group has also been associated with other autoimmune disorders, including systemic lupus erythematosus, a disease which frequently co-exists with NMO [64–67]. Similarly, a French group found NMO-IgG-positive NMO to be associated with a high frequency of HLA-DRB1*01*03 alleles, mainly of the DR3 pattern [175]. HLA-DR3 heterodimers enhance T cell stimulation and stabilize T cell/CD4/class II interaction [175]. Like DR1, DR3 has been reported to increase the T cell response by enhancing B and T cell co-operation [175]. The exact role of T cells in NMO is currently being investigated intensively, and several studies have attempted to identify immunodominant (T cell-activating) determinants of human AQP4 [157,159,160,176–180]. T cells have also been proposed to be involved in molecular mimicry (e.g. involving bacterial aquaporins), which is currently discussed as a potential trigger of NMO attacks [160,181–184].

(g) In addition, a role for *glutamate-mediated excitotoxicity* has been discussed. While one study reported that membrane AQP4 in human embryonic kidney (HEK) cells transfected with human AQP4 is endocytosed together with the excitatory amino acid transporter 2 (EAAT2) following incubation with NMO-IgG in the absence of complement, resulting in disruption of glutamate homeostasis, another study could not confirm these findings in cultures of mouse astrocytes [126,129]. An increase in extracellular glutamate could result in overstimulation of neurones and

oligodendrocytes, and could render the latter more susceptible to Ig-independent complement attack [126,185].

(h) Similarly, it is still controversial whether AQP4-antibodies exert some of their pathogenic effects by compromising the water homeostatic function of AQP4 either by blocking the water pore or by endocytosing the protein. According to a time-to-lysis assay using AQP4-transfected *Xenopus* oocytes, one study reported that NMO-IgG impairs water influx [124]; however, other studies which utilized cultured astrocytes [127] or plasma membrane vesicles isolated from AQP4-expressing Chinese hamster ovary (CHO) cells did not find an effect on the water transport capability of AQP4 [90,186,187].

(i) As mentioned above, IgG deposits in NMO lesions are accompanied by prominent IgM deposits [12]. IgM is an even more efficient activator of complement than IgG. Serum *AQP4-IgM* antibodies can be detected in approximately 10% of NMO patients [188].

Depending on the detection method used, 10–50% of patients with NMO are negative for AQP4-IgG [51]. Insufficient assay sensitivity is certainly a common cause of AQP4-IgG seronegativity, as shown in a number of recent comparative studies [9,10,51,189–191]. Moreover, AQP4-antibody titres have been shown to vary strongly over the course of disease depending, among other factors, on disease activity and treatment status. Retesting in a second, more sensitive assay and at follow-up visits, in particular during acute relapses, is thus advisable in seronegative cases (see reference [51] for a comprehensive overview and comparison of the currently available assays and a discussion of diagnostic pitfalls). However, the fact that approximately 10–20% of patients are seronegative even in the most up-to-date assays, as well as the recent demonstration of significant epidemiological and clinical differences between seropositive and seronegative patients [1,102,189], suggests that NMO might indeed be an aetiologically heterogeneous syndrome, i.e. a common phenotype shared by various autoimmune, (para)infectious [183,192,193] and metabolic diseases affecting the optic nerve and spinal cord. Indirect evidence for a role of so far unknown autoantibodies in seronegative NMO comes from reports that PE also has a therapeutic effect in some seronegative NMO patients [104] and from studies finding that complement-dependent astrocyte cell death induced by serum from AQP4-IgG-seronegative patients with NMO is more pronounced than that induced by serum from patients with MS or healthy donors [141]. Recently, antibodies to myelin oligodendrocyte glycoprotein (MOG) have been identified in a subset of patients with seronegative NMOSD [194–197]; the pathogenic, prognostic and therapeutic relevance of these antibodies is currently being investigated. Moreover, anti-CV2/CRMP5 and, possibly, NMDA receptor autoimmunity have been shown to mimic NMO in single patients [198,199]. In addition, connective tissue disorders (CTD), in particular systemic lupus erythematosus and Sjögren's syndrome, have been implicated in the pathogenesis of NMOSD in some patients [64,65,67]. A broad summary of the differential diagnosis of NMO is provided in the reference list [200–202]. It should be kept in mind that a lack of NMO-IgG/AQP4-antibody seropositivity does not rule out a diagnosis of NMO, according to the currently most widely adopted diagnostic criteria [84]. As will be discussed in the following sections, CSF analysis and spinal cord and brain imaging can facilitate the differential diagnosis of seronegative NMO and MS.

## Paraclinical findings

### Cerebrospinal fluid

CSF findings in NMO and MS differ markedly. CSF-restricted oligoclonal bands (OCB), a diagnostic mainstay in MS, are present in only approximately 18% of AQP4-antibody-positive cases and frequently disappear during remission [1,165]. Similarly, quantitative evidence for intrathecal IgG synthesis, i.e. an elevated IgG CSF/serum ratio, is only present in approximately 8% of CSF samples and exclusively during relapse [165]. By contrast, OCB are present in far more than 90% of cases in classical MS [203,204] and can be detected over the entire course of the disease [205]. A positive, polyspecific, intrathecal immune reaction to measles, rubella and varicella zoster virus (also termed MRZ reaction [206–208]) – as defined by at least two out of three positive antibody indices – is present in 60–80% of MS patients, but absent in approximately 97% of NMO patients [1,209]. CSF white cell counts (WCC) are often normal or only mildly elevated in NMO (median 19/μl during acute disease, 3/μl during remission [165]). However, cell counts >100/μl are possible [1,165], especially during relapse [165]. In addition to lymphocytes and monocytes, cytology often reveals neutrophilic and eosinophilic granulocytes [1,36,165], cell types which are usually absent in MS. An elevated albumin CSF/serum ratio, indicating blood–CSF barrier (BCB) disruption, and an increase in total protein is present in approximately 50% of cases, more often during acute attacks. CSF lactate levels are elevated during acute myelitis in approximately 40%, but normal during remission [165,210]. In rare AQP4-antibody-positive NMOSD patients, elevated lactate with marked neutrophilic pleocytosis may be taken falsely as bacterial CNS infection, all the more if the meninges are also involved [55,165,211]. A positive correlation was reported between QAlb values, CSF total protein levels and CSF L-lactate levels, on one hand, and the spinal cord lesion load as determined by MRI, on the other hand [165]. Importantly, CSF findings in AQP4-antibody-positive NMOSD vary significantly both between relapse and remis-

sion and – probably reflecting both differences in lesion volume and the rostrocaudal CSF gradient – between acute myelitis and acute ON [165]; in fact, normal CSF findings are not unusual in patients presenting with acute AQP4-antibody-positive ON [165]. No significant differences were found between seropositive and seronegative patients with regard to OCB, MRZ reaction and WCC in a recent multicentre study [1].

AQP4-antibodies are produced mainly by plasma cells in the peripheral blood. The trigger underlying AQP4-antibody production is unknown, although molecular mimicry has been suggested [160,181–184,212–215]. By contrast, intrathecal synthesis to an extent detectable by antibody index calculation is very rare [131,136,216,217]. AQP4-antibodies may enter the CNS by passive diffusion and, in addition, at sites lacking a proper BBB, such as the area postrema [47], or through a disrupted BBB, caused possibly by acute infections, which were shown to precede NMO attacks in 15–35% of patients [1,36,44,103,218]. Notably, AQP4, the target antigen of NMO-IgG, is itself an integral constituent of the BBB.

### Magnetic resonance imaging

Spinal MRI is crucial for diagnosis and differential diagnosis. Long cord lesions extending over three or more vertebral segments, often with patchy and inhomogeneous contrast enhancement over weeks or even months or, less frequently, central necrosis and cavitation, are characteristic features and highly suggestive of an NMOSD [1,37,84,219]. However, it is important to keep in mind that, depending on the timing of spinal MRI to onset of clinical symptoms, NMOSD patients may well exhibit shorter spinal lesions [1,32] and that other, mostly rare differential diagnoses of long cord lesions need to be considered, including spinal ischaemia, neurosarcoidosis and others [201,202]. Despite their often dramatic appearance, cord lesions in NMO may improve substantially upon treatment and even recover fully. Conversely, severe inflammation may cause irreversible cord atrophy, which may be a negative predictive factor for response to PE in case of subsequent attacks [220]. Recently, so-called spinal 'bright spotty lesions' have been suggested as an additional criterion to distinguish NMOSD from MS [221]. Moreover, advanced imaging techniques such as magnetic resonance spectroscopy and diffusion tensor imaging that are not applied regularly in clinical routine have confirmed severe spinal tissue injury and also suggest astrocytic damage that may help to distinguish NMO from MS [222–224].

Substantial new insights on brain involvement in NMO result from a multitude of MRI studies performed in the past few years. These studies were encouraged by the seminal work by Pittock and colleagues who showed that, contrary to previous thinking, the majority of NMO patients (up to 60%) exhibit (mostly unspecific) lesions on serial cranial MRI during the course of the disease. Some of these lesions are typical of MS and may even fulfill the so-called 'Barkhof criteria' [1,225]. Similar findings have been reported by other groups, with approximately 15% of patients fulfilling the Barkhof criteria [1,226]. Thus, it is widely accepted nowadays that, although many patients have normal cranial MRI findings at disease onset, brain lesions – including even those resembling typical MS lesions – do not rule out an NMO diagnosis [227]. However, ultrahigh-field imaging studies reported that, in contrast to MS, NMO lesions do not typically show central veins and a hypointense rim and lack visible cortical lesions [228,229]. This is in line with other imaging and neuropathological reports that indicate the absence of cortical demyelination in NMO [63,230,231]. Brain lesions tend to be located at sites of high aquaporin-4 expression, such as the diencephalon, the hypothalamus and the aqueduct [232–234], and may also appear large and oedematous in the corpus callosum [235,236]. Contrast enhancement on brain MRI with a cloudlike shape and pencil-thin ependymal enhancement were reported to be typical of NMO [237,238]. Recent diffusion, perfusion and brain volume studies, including voxel-based morphometry, revealed diffuse and widespread white matter and grey matter alterations in NMO [239–243]. Thus, brain damage is probably more severe than can be estimated from conventional MR images.

While there is now compelling evidence that AQP4-Ab-positive 'Asian opticospinal MS' (OSMS) is identical to Western NMO, a small proportion of Asian patients still cannot be easily classified as NMO or MS, e.g. seronegative patients presenting with LETM and a secondary progressive course or OSMS patients with LETM and peripheral spinal cord lesions [244,245]. However, re-evaluation using more up-to-date assays, together with strict MRI criteria distinguishing between confluent (as sometimes seen in MS) and contiguous (as typically seen in NMO) longitudinal lesions, may help to clarify the nosological status of those patients.

### Optical coherence tomography and visual-evoked potentials

Optical coherence tomography (OCT) is a non-invasive technique by which unmyelinated retinal CNS axons (the so-called retinal nerve fibre layer RNFL) and their neurons, the retinal ganglion cells, can be visualized. Neuroaxonal retinal damage has been shown widely in MS and ON and is currently under investigation in many other neurological conditions [246–254]). In NMO, OCT studies have been consistent with the clinical experience of a more severe visual dysfunction and poorer visual outcome than for MS and more profound damage to the RNFL [246,255–257]. Whereas progressive reduction of the RNFL, independent of clinical attacks of ON, has been reported in MS, retinal axonal loss in NMO is probably associated predominantly with clinical relapses [258–261]. The utility of OCT for

distinguishing NMO from MS and other inflammatory conditions with ocular involvement is currently being investigated. Visual evoked potentials show either reduced amplitudes or prolonged latencies, or both; in more severe cases there may be no response at all [262]. Delayed P100 latencies may indicate that the optic nerve is subclinically affected in patients presenting with LETM, but with no history of clinically apparent ON.

## Treatment

NMO is still an incurable disease. The goal of treating acute NMO events is to improve relapse symptoms and restore neurological functions; long-term immunosuppression aims to prevent further attacks [4,263,264]. Any treatment recommendations are limited by the small size of most studies, which were mostly retrospective case-series. No prospective controlled trials in NMO have been conducted, and most study designs with long placebo treatment would probably be considered unethical. Relapses are treated with high-dose intravenous methylprednisolone; if response is insufficient, patients may benefit from PE [265]. If a patient has previously responded well to PE, PE may be considered as initial treatment in case of another relapse. In patients in whom both steroids and PE do not improve symptoms, treatment with intravenous immunoglobulins [266] or an escalation to cytoablative therapy such as cyclophosphamide may be considered [264].

For long-term immunosuppression, patients usually receive either B cell-targeted therapies such as intravenous rituximab or oral azathioprine and/or prednisone [87,110,113,267–272]. Other possible options include mycophenolate mofetil [273], methotrexate [274] or mitoxantrone which, however, is limited by major side effects such as cardiotoxicity or leukaemia and thus generally not considered as initial treatment [264,275–280]. It is beyond the scope of this paper to provide details on dosing schemes and monitoring of the various NMO drugs, and therefore we refer the reader to two recent, excellent overviews on treatment recommendations [264,281]. However, one aspect deserves mention: less severe lesions have been found in type I interferon (IFN) receptor-deficient mice, suggesting that type I IFNs might be involved in the pathogenesis of NMO. Accordingly, IFN-β, a therapeutic mainstay in MS, has been repeatedly reported to exacerbate disease or to be ineffective in patients with NMO. The use of IFN-β in the treatment of NMO is therefore strongly discouraged. Similarly, lack of efficacy or disease exacerbation has also been reported following treatment with other typical MS drugs such as natalizumab and, in single cases, also fingolimod and alemtuzumab [169–171,282–290].

## Further prospects

A recent, small, open-label study with the monoclonal antibody eculizumab, an inhibitor of the complement component C5 approved for the treatment of paroxysmal nocturnal haemoglobinuria, reported an impressive reduction of relapse rates in 14 NMO patients with disease activity [138,139]. This finding has stimulated a larger trial that is expected to begin in late 2013 or early 2014. Given the role of IL-6 in NMO, IL-6-targeted therapy with the monoclonal anti-IL-6-receptor antibody tocilizumab might represent another future treatment strategy, following encouraging case reports [115–117]. Further preliminary but intriguing experimental approaches are competitive, non-pathogenic, AQP4-specific antibodies, neutrophil elastase inhibitors or antihistamines with eosinophil-stabilizing properties [144,166,168,291].

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