AQP4 antibody serostatus

Is its luster being lost in the management and pathogenesis of NMO?

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Detecting aquaporin-4 (AQP4) antibody is a key laboratory finding in the diagnosis of neuromyelitis optica (NMO) or its limited forms, known as NMO spectrum disorders (NMOSD).[1,2] It is important to differentiate NMO from multiple sclerosis (MS), as drugs approved for MS (interferon-β, natalizumab, and fingolimod) are ineffective in NMO/NMOSD. AQP4 antibody seropositivity at onset of longitudinally extensive transverse myelitis (LETM), a cardinal feature of NMO, predicts relapses.[3] Moreover, AQP4 antibody can damage astrocytes and cause NMO-like pathology.[4] For these reasons, most neurologists now think that AQP4 antibody seropositivity is critically important in diagnosing NMO/NMOSD.

Neurology® has recently published some key articles on factors related to the AQP4 antibody assay sensitivity[5,6] and the clinical features of seronegative NMO.[6] In this issue of *Neurology*, Jiao and colleagues[7] report their reevaluation of AQP4 antibody serostatus in 163 patients with definite NMO, fulfilling the 2006 criteria by Weinshenker et al.[3] or the 1999 criteria by Wingerchuk et al.[8] (excluding AQP4 antibody serostatus); they used ELISA, prefixed cell-based assay (CBA), and in-house fluorescence-activated cell sorting assay (FACS) to detect AQP4 antibody (table). Fifty-three (33%) were seronegative in their mouse tissue–based indirect immunofluorescence assay (IIF), and sera were available from 49 of them for this study. Based on the combined results from AQP4 antibody assays, they compared the clinical features in seropositive and seronegative NMO.

It is established that CBA and FACS are more sensitive than IIF and ELISA.[5] As expected, two-thirds of the 49 cases previously seronegative by IIF were seropositive in the present study.[7] Consequently, the overall seropositivity in NMO rose to 88% (87% in FACS, 84% in CBA, and 79% in ELISA).

The study by Jiao et al. provides a caveat in judging AQP4 antibody serostatus. By testing serial specimens, they revealed that both positive and negative seroconversions can occur, even with the same assay.[7] In 7 patients, initially seronegative, AQP4 antibody was detected in subsequent specimens by IIF, though the majority of these samples were positive by other assays. Meanwhile, negative seroconversion in blood was possibly attributable to immunosuppression in 5 patients. Therefore, it is important to test samples collected during attacks and before immunosuppressive therapy, and to retest these samples with better assays if initial tests are negative.

Seronegativity in NMO depends heavily on the sensitivity of AQP4 antibody assays. With highly sensitive CBA and FACS, only 12% of the patients of Jiao et al. remained seronegative.[7] The seronegativity was lower than that in a French study (25%).[6] But both studies demonstrated that sexes were equally represented in seronegative NMO (F/M = 1:1 vs 9:1 in seropositive NMO in the study by Jiao et al.) and that simultaneous development of optic neuritis and transverse myelitis at onset was more common in seronegative than in seropositive NMO (32% vs 3.6% in the study by Jiao et al.).[6,7] Interestingly, those 2 features are exactly the ones that characterized monophasic NMO described in Gault's[9] study of 17 cases (14 with monophasic NMO and 3 with relapsing NMO) including the well-known autopsied Devic syndrome case in 1894 and the Mayo Clinic comparison of 23 monophasic and 48 relapsing NMO cases collected from their records (1950–1993).[9] Moreover, none of 9 Dutch monophasic NMO cases was AQP4 antibody positive.[10] In contrast, in the study by Jiao et al., only 1 seropositive and 2 seronegative patients (1.8%) with simultaneous optic neuritis and transverse myelitis who had been followed for less than 3 years had monophasic NMO.[7] Likewise, all patients with seronegative NMO in the French study had relapsing disease.[6] The consistent finding in these 2 recent studies (that monophasic NMO is rare) corresponds with our own clinical experience.

In addition to the relapse likelihood, the analyses by Jiao et al. showed that relapse frequency, severity, or long-term disability outcome were not different between seronegative and seropositive NMO.[7] At 5 years after disease onset, about 1 out of 4 patients required a cane to walk (Expanded Disability Status

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Table Features of AQP4 antibody assays mentioned in the study by Jiao et al.

Assay	Methods	Factors related to assay sensitivity	Sensitivity
IIF	Mouse tissue–based IIF	Different amino acid sequences in extracellular domains of transmembrane AQP4 between mouse and human	+
ELISA (commercial kit)	AQP4-M1 coated onto ELISA plate wells	AQP4 antibodies are less likely to bind to membrane-unbound AQP4; low-positive results can be false-positives	++
CBA (commercial kit)	Ready-to-use kit with prefixed human AQP4-M1–transfected cells	Higher nonspecific binding in prefixed cells than in living cells; AQP4-M1 does not form OAP	+++
In-house FACS	Flow cytometric analysis of human AQP4-M23–transfected cells	AQP4 antibodies are more likely to bind to AQP4-M23–forming OAP than AQP4-M1	++++

Abbreviations: AQP4 = aquaporin-4; CBA = cell-based assay; FACS = fluorescence-activated cell sorter; IIF = indirect immunofluorescence; OAP = orthogonal arrays of particles.
AQP4, a predominant water channel in the CNS, has 6 transmembrane portions and 3 extracellular loops, and exists as 2 isoforms (heterotetramers), M1 and M23, due to different start codons (M23 is shorter). AQP4 is abundantly expressed on astrocytic endfeet.

Scale score 6.0) and half of patients were legally blind (visual acuity <20/200) in at least one eye in both groups. Therefore, the authors concluded that seronegative NMO is clinically similar to seropositive NMO.

What, then, is the importance of AQP4 antibody serostatus in the management of NMO? Do the data contradict the previous report on prediction of future relapse in cases with initial LETM by seropositivity?[3] In other words, is the AQP4 antibody serostatus no longer useful in deciding whether or not start long-term immunosuppressive therapy without delay after diagnosis? We had probably better not make a snap judgment. For example, relapses may be more likely in definite NMO with 2 or more lesions than in LETM, even in seronegative patients. Immunosuppressive therapy reduced the average annualized relapse rate (ARR) in both groups. However, the reduction was highly significant in seropositive cases (2.2→0.7), and the posttreatment ARR was lower in seropositive cases (0.7 vs 1.0).[6] Meanwhile, median attack numbers were lower in seronegative cases than in seropositive cases (4 vs 7). Perhaps the type and amount of drugs the patients received were not the same in the 2 groups (most patients treated with rituximab and eculizumab were seropositive in published reports). Hence, further studies in acute exacerbation as well as long-term prophylaxis including both seronegative and seropositive NMO may reveal different therapeutic responses between these 2 groups.

Current theories of immunopathogenesis and molecular target therapies in NMO owe much to neuropathologic findings of autopsied cases, analyses of cytokines and CSF cell damage markers, and experimental results on AQP4 antibody and astrocytes.[4] So, if there are truly seronegative NMO cases, research may open a new page in our understanding of this unique disease. The International Panel on Diagnosis of NMO is currently trying to establish new clinically relevant criteria for NMO/NMOSD incorporating AQP4 antibody serostatus.

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K. Fujihara serves on scientific advisory boards for Bayer Schering Pharma, Biogen Idec, Mitsubishi Tanabe Pharma Corporation, Novartis Pharma, Chugai Pharmaceutical, Ono Pharmaceutical, Nihon Pharmaceutical, Merck Serono, and Alexion Pharmaceuticals; has received funding for travel and speaker honoraria from Bayer Schering Pharma, Biogen Idec, Eisai Inc., Mitsubishi Tanabe Pharma Corporation, Novartis Pharma, Astellas Pharma Inc., Takeda Pharmaceutical Company Limited, Asahi Kasei Medical Co., Ltd., and Daiichi Sankyo; serves on the editorial board of *Clinical and Experimental Neuroimmunology*; and has received research support from Bayer Schering Pharma, Biogen Idec Japan, Asahi Kasei Medical Co., The Chemo-Sero-Therapeutic Research Institute, Teva Pharmaceutical K. K., Mitsubishi Tanabe Pharma Corporation, Teijin Pharma, Eisai Inc., and Kowa Pharmaceuticals America, Inc. and Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Technology and the Ministry of Health, Labor and Welfare of Japan. D.K. Sato receives scholarship from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan and has received research support from Ichiro Kanehara Foundation. Go to Neurology.org for full disclosures.

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