



Published in final edited form as:

JAMA Neurol. 2014 January 1; 71(1): 48–54. doi:10.1001/jamaneurol.2013.5055.

Aquaporin 4 IgG Serostatus and Outcome in Recurrent Longitudinally Extensive Transverse Myelitis

Yujuan Jiao, MD

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

James P. Fryer, MS

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

Vanda A. Lennon, MD, PhD

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

Department of Neurology, Mayo Clinic, Rochester, Minnesota
Department of Immunology, Mayo Clinic, Rochester, Minnesota

Andrew McKeon, MD

Copyright 2013 American Medical Association. All rights reserved

Corresponding Author: Sean J. Pittock, MD, Department of Neurology, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (pittock.sean@mayo.edu).

Author Contributions: Dr Pittock had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Jiao, Pittock.

Acquisition of data: Jiao, Fryer, McKeon, Quek, Weinschenker, Wingerchuk, Shuster, Lucchinetti, Pittock.

Analysis and interpretation of data: Jiao, Fryer, Lennon, McKeon, Jenkins, Smith, Pittock.

Drafting of the manuscript: Jiao, Pittock.

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Jiao, Jenkins, Smith.

Obtained funding: Lennon, Pittock.

Administrative, technical, and material support: Pittock.

Study supervision: Pittock.

Conflict of Interest Disclosures: Dr Lennon reported being a named inventor on a patent (patent 7101679; issued in 2006) relating to AQP4 antibodies for diagnosis of NMO and receiving royalties for this technology; being a named inventor on patents (patents 12/678,350 and 12/573,942; filed in 2010 and 2008, respectively) that relate to functional AQP4/NMO-IgG assays and NMO-IgG as a cancer marker; and receiving research support from the Guthy-Jackson Charitable Foundation and the National Institutes of Health (NIH; grant NS065829). Dr Lennon reported that serological testing for neural autoantibodies is offered on a service basis by Mayo Collaborative Service, Inc, an agency of Mayo Foundation, and that neither she nor her laboratory benefit financially from this testing. Dr McKeon reported receiving research support from the Guthy-Jackson Charitable Foundation. Ms Smith reported receiving financial support for research activities from AbbVie. Dr Weinschenker reported serving on data safety monitoring boards for Novartis, Biogen Idec, and Mitsubishi Pharmaceuticals; serving on the editorial boards of the *Canadian Journal of Neurological Sciences*, *Multiple Sclerosis Journal*, and *Turkish Journal of Neurology*; receiving research support from the Guthy-Jackson Charitable Foundation; receiving license royalties for a patent (patent 7101679; details above); and receiving consulting fees from Asahi Kasei Medical Company, Elan Pharmaceuticals, and GlaxoSmithKline Pharmaceuticals. Dr Weinschenker reported that he provided consultation to Alexion Pharmaceuticals but has received no personal fees or personal compensation for these consulting activities. Dr Shuster reported receiving compensation for PRIME CME September 2012. Dr Wingerchuk reported receiving research support from Alexion Pharmaceuticals, Inc, Genzyme, Genentech, TerumoBCT, and the Guthy-Jackson Charitable Foundation. Dr Wingerchuk reported that he has provided consultation to Alexion Pharmaceuticals but has received no personal fees or personal compensation for these consulting activities; all compensation for consulting activities is paid directly to Mayo Clinic. Dr Lucchinetti reported sharing royalties from the marketing of kits for detecting AQP4 autoantibody (patent 7101679; issued in 2006) and from the sale of *Blue Books of Neurology: Multiple Sclerosis 3* (Saunders Elsevier, 2010) and receiving research support from the NIH (grant NS49577-R01), the Guthy-Jackson Charitable Foundation, and the National Multiple Sclerosis Society (grant RG 3185-B-3). Dr Pittock reported being a named inventor on patents (patents 12/678,350 and 12/573,942; details above) and receiving research support from Alexion Pharmaceuticals, Inc, the Guthy-Jackson Charitable Foundation, and the NIH (grant NS065829). Dr Pittock also reported that he provided consultation to Alexion Pharmaceuticals but has received no personal fees or personal compensation for these consulting activities; all compensation for consulting activities is paid directly to Mayo Clinic. No other disclosures were reported.

Additional Contributions: We thank John Schmeling and Eric Jedynak for assistance with serological assays and data analysis and Karen Brekke and Delana Weiss for coordinating the study. No financial compensation was received.

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota
 Department of Neurology, Mayo Clinic, Rochester, Minnesota

Sarah M. Jenkins, MS

Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota

Carin Y. Smith, BS

Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota

Amy M. L. Quek, MBBS

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

Brian G. Weinschenker, MD

Department of Neurology, Mayo Clinic, Rochester, Minnesota

Dean M. Wingerchuk, MD

Department of Neurology, Mayo Clinic, Scottsdale, Arizona

Elizabeth A. Shuster, MD

Department of Neurology, Mayo Clinic, Jacksonville, Florida

Claudia F. Lucchinetti, MD

Department of Neurology, Mayo Clinic, Rochester, Minnesota

Sean J. Pittock, MD

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

Department of Neurology, Mayo Clinic, Rochester, Minnesota

Abstract

IMPORTANCE—Studies focused on recurrent longitudinally extensive transverse myelitis (rLETM) are lacking.

OBJECTIVES—To determine the aquaporin 4 (AQP4) IgG detection rate using recombinant human AQP4-based assays in sequential serum specimens collected from patients with rLETM categorized as negative by first-generation tissue-based indirect immunofluorescence (IIF) assay and to define the clinical characteristics and motor disability outcomes in AQP4-IgG-positive rLETM.

DESIGN, SETTING, AND PARTICIPANTS—A search of the Mayo Clinic computerized central diagnostic index (October 1, 2005, through November 30, 2011), cross-linked with the Neuroimmunology Laboratory database, identified 48 patients with rLETM, of whom 36 (75%) were positive and 12 (25%) negative for neuromyelitis optica (NMO) IgG (per IIF of serial serum specimens). Stored serum specimens from “seronegative” patients were retested with recombinant human AQP4-based assays, including enzyme-linked immunosorbent, transfected cell-based, and fluorescence-activated cell-sorting assays. Control patients included 140 AQP4-IgG-positive patients with NMO, of whom a subgroup of 20 initially presented with 2 attacks of transverse myelitis (rLETM-onset NMO).

MAIN OUTCOMES AND MEASURES—AQP4-IgG serostatus, clinical characteristics, and Expanded Disability Status Scale score.

RESULTS—Six patients with negative IIF results were reclassified as AQP4-IgG positive, yielding an overall AQP4-IgG seropositivity rate of 89%. Fluorescence-activated cell-sorting, cell-based, and enzyme-linked immunosorbent assays improved the detection rate to 89%, 85%, and 81%, respectively. The female to male ratio was 2:3 for AQP4-IgG-negative rLETM and 5:1 for AQP4-IgG-positive patients. The AQP4-IgG-positive patients with rLETM or rLETM-onset NMO were similar in age at onset, sex ratio, attack severity, relapse rate, and motor disability. From Kaplan-Meier analyses, 36% of AQP4-IgG-positive patients with rLETM are anticipated to

need a cane to walk within 5 years after onset. For patients with rLETM-onset NMO, the median time from onset to first optic neuritis attack (54 months) was similar to the median disease duration for AQP4-IgG–positive patients with rLETM (59 months). The median number of attacks was 3 for AQP4-IgG–positive patients with rLETM (range, 2–22), and the first optic neuritis attack for those with rLETM-onset NMO followed a median of 3 myelitis attacks (range, 2–19). Immunosuppressant therapy reduced the relapse rate in both AQP4-IgG–positive and AQP4-IgG–negative patients with rLETM.

CONCLUSIONS AND RELEVANCE—Recombinant antigen–based assays significantly increase AQP4-IgG detection in patients with rLETM, and AQP4-IgG–negative adults with rLETM are rare. Evolution to NMO can be anticipated in AQP4-IgG–positive patients. Early initiation of immunotherapy may result in a more favorable motor outcome.

Aquaporin 4 (AQP4) IgG is validated as a clinical biomarker of neuromyelitis optica (NMO) spectrum disorders.^{1,2} Longitudinally extensive transverse myelitis (LETM) is incorporated into contemporary diagnostic criteria for NMO.^{2,3} When results are positive for AQP4-IgG, LETM is classified as an NMO spectrum disorder.^{4–6} The “longitudinally extensive” designation indicates that sagittal spinal magnetic resonance images have an abnormal T2-weighted signal extending across at least 3 vertebral segments.^{2,4,5} The outcomes of LETM include poor recovery, severe disability, and mortality, especially when diagnosis and immunotherapy are delayed.^{2,5} In single-attack LETM, AQP4-IgG seropositivity predicts recurrence or conversion to NMO.⁴

Reported AQP4-IgG seropositivity rates are underestimated owing to assay insensitivity and immunotherapy effects.¹ A blinded international collaborative comparison of the sensitivities of currently used assay methods (indirect immunofluorescence [IIF], cell-based assay [CBA], enzyme-linked immunosorbent assay [ELISA], immunoprecipitation, and fluorescence-activated cell sorting [FACS]) confirmed that assays using recombinant antigen are more sensitive than IIF assays.¹ The AQP4-IgG detection rate in recurrent LETM (rLETM) has not been studied systematically with recombinant antigen–based assays, nor have clinical and demographic characteristics associated with rLETM been clearly defined. In this article, we report an updated estimate of the AQP4-IgG positivity rate for Mayo Clinic patients with rLETM, 25% of whom were categorized as NMO-IgG negative with first-generation IIF testing. We retested stored serum specimens using 3 recombinant antigen–based assays.

Methods

The study protocol was reviewed and approved by the Mayo Clinic Institutional Review Board (IRB 08-006647). Only patients providing written informed consent for research studies were included.

Detection of NMO/AQP4-IgG

Serum samples were collected at clinic visits, particularly at acute exacerbations. All testing was performed under blinded conditions. The IIF substrate was a composite cryosection of normal adult mouse brain, kidney, and gut tissues.⁷ Patients whose serum samples tested positive at IIF were not retested with other assays owing to the 99% specificity of IIF for NMO. All serial samples yielding a negative IIF result were retested sequentially with assays using recombinant human AQP4. The M1 isoform of AQP4 was the antigen in commercial ELISA kits (RSR [Kronus, Ltd]; results ≥ 5 U/mL were categorized as positive) and in the transfected immunofluorescence CBA (Euroimmun; each sample was categorized as positive or negative by 3 experienced readers).¹

The in-house FACS assay used both M23 and M1 isoforms of AQP4 (unless serum volume was insufficient to analyze in independent assays). For the FACS assay, human embryonic kidney cells (HEK 293) were transfected transiently with a plasmid (pIRES2-AcGFP) encoding both green fluorescent protein (GFP) and either AQP4-M1 or AQP4-M23. After 36 hours, FcR Blocking Reagent (Miltenyi Biotech catalog No. 130-059-901) was added to the mixed population of cells (transfected expressing AQP4 on the surface and GFP in the cytoplasm and nontransfected lacking AQP4 and GFP). Patient serum (heat-inactivated at 56°C for 30 minutes) was added to 100 000 cells at a 20% concentration in a final volume of 100 μ L. After incubation (at 4°C for 30 minutes) and washing, the cells were resuspended with 100 μ L of AlexaFluor 647–conjugated goat anti-human IgG (Invitrogen catalog No. A21445, 1:500 dilution), held on ice for 30 minutes, washed, fixed with 4% paraformaldehyde, and examined with flow cytometry (BD FACS LSRII; Becton, Dickinson and Co).

Results were obtained with acquisition/analysis software. Binding of a patient's IgG to the AQP4-transfected (GFP-positive) cells was measured in terms of the intensity of AlexaFluor 647 fluorescence. The median fluorescence intensity (MFI) value for AlexaFluor 647 corresponding to IgG bound to AQP4-transfected cells was expressed as a ratio of the MFI value for that patient's IgG binding to nontransfected control cells in the same aliquot (MFI AQP4-transfected cells/MFI nontransfected cells = AQP4-IgG binding index). The AQP4-IgG binding index values were considered positive if they were 3 or higher for assays using AQP4-M23–transfected cells or 2 or higher for those using AQP4-M1–transfected cells.

Patients and Samples

A search of the Mayo Clinic computerized central diagnostic index (October 1, 2005, through November 30, 2011) identified 699 patients tested with IIF for NMO-IgG and assigned a diagnosis of NMO, NMO spectrum disorder, Devic disease, myelitis, myelopathy, optic neuritis (ON), optic neuropathy, clinical isolated syndrome, acute disseminated encephalomyelitis, or central nervous system demyelinating disease. The clinical course in 48 patients was relapsing transverse myelitis with at least 1 radiologically confirmed LETM episode (rLETM). Of 163 patients fulfilling diagnostic criteria for NMO described by Wingerchuk in either 1999⁸ or 2006³ (excluding antibody status), 140 were AQP4-IgG seropositive by at least 1 assay: IIF, ELISA, CBA, or FACS.

All patients were clinically evaluated at Mayo Clinic locations in Rochester, Minnesota; Scottsdale, Arizona; or Jacksonville, Florida. We compared clinical characteristics of the rLETM group, the 140 AQP4-IgG–positive patients with NMO, and a subgroup of 20 patients with NMO whose course started with 2 or more attacks of transverse myelitis (rLETM-onset NMO). The following clinical data were collected: date of birth, sex, ethnicity, age at disease onset, coexisting autoimmune disorders, disease duration, onset symptom, attack severity (with *severe* defined as unable to walk at nadir for spinal or brain attack), attack type and frequency, and visual acuity. We recorded times to permanent motor disability (Expanded Disability Status Scale score ≥ 6 [intermittent or unilateral assistance required to walk 100 m with or without resting] or ≥ 8 [restricted to bed or chair or perambulated in wheelchair for ≥ 6 months]). Data were collected retrospectively through review of case records.

Statistical Analysis

Characteristics were compared between patient groups by using χ^2 (or Fisher exact) tests for categorical data and 2-sample *t* (or Wilcoxon rank sum) tests for continuous data. Disease characteristics over the course of all attacks per patient were compared using regression models with generalized estimating equations, using logistic regression for binary and linear

regression for continuous data. Therapy “intervals” were defined for each patient to correspond to periods during which long-term immunosuppressant therapy was (or was not) being used. Relapse rates were compared between on-therapy and off-therapy intervals using linear regression models with generalized estimating equations. Disability outcomes were compared between groups using log-rank tests, and the risk of these outcomes was estimated using the Kaplan-Meier method. Adjusted associations of patient characteristics with these outcomes were assessed with Cox proportional hazards regression models. All statistical testing was considered as exploratory data analysis. Thus, no adjustments were made for multiple testing. Data were analyzed using SAS statistical software (version 9; SAS Institute Inc), and figures were created using R software (<http://www.R-project.org/>). Differences were considered statistically significant at $P < .05$.

Results

“Seronegative” Patients With rLETM Reclassifiable as AQP4-IgG Positive

Indirect immunofluorescence assay testing of serial specimens from 48 patients with rLETM yielded a cumulative seropositivity rate of 75% (Figure 1). Stored specimens were available to retest 11 of the 12 patients classified as seronegative. Six specimens yielded a positive result with at least 1 recombinant antigen–based assay. This increased the cohort’s AQP4-IgG–positive rate to 89% (42 of 47 patients): 89% (42 of 47 patients) for FACS, 85% (40 of 47) for CBA, and 81% (38 of 47) for ELISA (Table 1). Testing a patient serially in the disease course enhanced AQP4-IgG detection (eg, Figure 2A). Of the 5 patients who were persistently AQP4-IgG negative, 3 had only 1 available specimen for retesting, and 4 were receiving immunotherapy when the specimen was obtained.

Serostatus Conversion From Positive to Negative With Immunotherapy

Of the 6 patients reclassified as AQP4-IgG positive after retesting with recombinant antigen–based assays, 1 converted to seronegative after rituximab treatment (Figure 2B).

Demographic Differences Between AQP4-IgG–Negative and AQP4-IgG–Positive Patients With rLETM

Among the 5 AQP4-IgG–negative patients, men outnumbered women (3:2 ratio), but women outnumbered men (5:1 ratio) among the 42 AQP4-IgG–positive patients with rLETM. The AQP4-IgG–negative patients were on average younger at disease onset than the AQP4-IgG–positive patients. No other demographic or clinical differences were observed between AQP4-IgG–positive and AQP4-IgG–negative patients with rLETM (Table 2).

Demographic and Phenotypic Similarities Between AQP4-IgG–Positive Patients With rLETM or rLETM-Onset NMO

Patients positive for AQP4-IgG with rLETM or rLETM-onset NMO did not differ significantly with respect to sex ratio, ethnicity, age at disease onset, frequency of coexisting autoimmune diseases, annualized relapse rate, severity at onset, or subsequent attacks (Table 2), and Kaplan-Meier analysis revealed no significant differences in time to motor disability (Figure 3 and Table 2). In AQP4-IgG–positive patients at 5 years from disease onset, 36% with rLETM and 41% with rLETM-onset NMO would be expected to require a cane to walk (Figure 3). In contrast, at 5 years after disease onset, the outcome for the whole AQP4-IgG–positive NMO group appeared more favorable with respect to motor disability, with 22% expected to require a cane to walk (Figure 3 and Table 2). Small numbers precluded comparison with the 5 patients with AQP4-IgG–negative rLETM, although all 5 in this group remained ambulatory at a median follow-up interval of 30 months after onset. Kaplan-

Meier analysis revealed no significant differences in the percentage of patients expected to need a wheelchair at 5 years after onset (8% for AQP4-IgG–positive rLETM, 5% for AQP4-IgG–positive rLETM-onset NMO, and 8% for the whole AQP4-IgG–positive NMO group; Kaplan-Meier curve, not shown; Table 2).

NMO Evolution for AQP4-IgG–Positive Patients With rLETM

Patients who were AQP4-IgG positive had a shorter median disease duration (first attack to last follow-up) than those with rLETM-onset NMO (59 vs 133 months; $P = .001$). The median interval from disease onset to first attack of ON in patients with rLETM-onset NMO (54 months) was similar to the median disease duration in AQP4-IgG–positive patients with rLETM (59 months). Furthermore, the median attack number for the patients with rLETM was 3 (range, 2-22). For the patients with rLETM-onset NMO, the first ON occurred after a median of 3 transverse myelitis attacks (range, 2-19).

Relapse Rate in rLETM After Immunosuppressant Therapy

A total of 192 attacks were recorded for the 47 patients with rLETM whose serostatus was known. Immunosuppressant therapy (azathioprine, mycophenolate mofetil, oral prednisone, monthly intravenous methylprednisolone, rituximab, eculizumab, or combinations) was administered for half the cumulative disease duration (mean, 61% and 52% of disease duration for AQP4-IgG–positive and AQP4-IgG–negative patients, respectively). Similar to results in patients with NMO, the mean (SD) annualized relapse rate for AQP4-IgG–positive patients with rLETM was 2.5 (2.5) when they were not receiving immunosuppressant therapy but 0.4 (0.5) when they were ($P < .001$); the comparable rates for seronegative patients were 1.4 (1.4) vs 0.3 (0.3) ($P < .001$). Interpretation is limited by small numbers (Table 2).

Discussion

This study, the first to our knowledge to focus on patients with relapsing LETM, had several important observations. First, recombinant antigen–based assays significantly increase the AQP4-IgG detection rate in patients with rLETM; AQP4-IgG–negative adults with rLETM are rare. Second, AQP4-IgG–positive patients with rLETM are phenotypically similar to AQP4-IgG–positive patients with NMO with rLETM onset. Third, immunosuppressant therapy reduces the relapse rate in both AQP4-IgG–positive and AQP4-IgG–negative patients with rLETM.

The various assays currently used for clinical detection of AQP4-IgG differ in sensitivity. Using 3 different recombinant antigen–based assays, we detected AQP4-IgG in half of the patients with NMO initially deemed seronegative by IIF, reducing the seronegativity rate from 25% to 11%. Assays using recombinant antigen also enabled AQP4-IgG detection in the retesting of archival serum specimens collected serially and initially reported as negative (service IIF evaluation). Three of the 5 patients with rLETM who remained classified as seronegative had only a single specimen, and 4 were receiving immunotherapy when the sample was obtained. Thus, the timing of blood sample collection and the use of immunosuppressant therapy may influence AQP4-IgG detection.

When optimized assays were used for AQP4-IgG detection, the seronegative rate in this adult rLETM cohort of 43 patients was only 7%, much lower than that reported elsewhere.^{1,7,9} As is true for patients with NMO, the sexes were similarly represented in AQP4-IgG–negative patients with rLETM, with a 5:1 female to male ratio in AQP4-IgG–positive patients.^{10,11} For patients with disease onset before age 18 years, 2 of 4 were AQP4-IgG positive and female (age at onset, 15 and 17 years), and 2 were AQP4-IgG

negative and male (age at onset, 3 and 6 years). In contrast to adults with multiple sclerosis, 14% of children with multiple sclerosis have longitudinally extensive spinal cord lesions.¹² Therefore, the 2 seronegative boys in this report may have had multiple sclerosis, with initial attacks being rLETM.

Our study suggests that a proportion of seropositive patients with rLETM may develop ON with time and thus fulfill Wingerchuk et al³ NMO criteria because (1) patients with rLETM are similar to those with rLETM-onset NMO with respect to sex ratio, age at onset, and frequency of coexisting autoimmune diseases and clinical characteristics (relapse rate, attack severity, and disability outcome); (2) they had a shorter duration of follow-up than NMO and rLETM-onset NMO groups; and (3) their duration of follow-up is similar to the median time from onset to first ON for patients with NMO with initial rLETM onset. Furthermore, the first ON attack in patients with rLETM-onset NMO occurred after a median of 3 LETM attacks, and the median number of LETM attacks in the rLETM group was 3.

As is true for AQP4-IgG–positive patients with NMO, immunosuppressant therapies significantly reduce the relapse rate in AQP4-IgG–positive patients with rLETM. Because these patients, like those with rLETM-onset NMO, have a worse motor outcome than the entire AQP4-IgG–positive NMO cohort, early initiation of attack prevention therapies is even more critical in this group.

This study and other recent reports from the United States and Europe indicate that assays detecting IgG binding to cells expressing recombinant AQP4 with quantitative flow cytometry (FACS) or visual observation (CBA) are more sensitive than ELISA and significantly more sensitive than immunoprecipitation and tissue-based immunofluorescence assays in aiding the diagnosis of NMO and NMO spectrum disorders.^{1,11,13-17} For patients considered at high risk for AQP4 autoimmunity (including those with rLETM), testing for AQP4-IgG by more sensitive CBA or FACS assays is recommended if less sensitive assays yield negative results. Retesting a later sample is warranted if initial test results are negative in a high-risk patient.¹⁷

Acknowledgments

Funding/Support: This work was supported by grant R01 NS065829 from the NIH and by the Guthy-Jackson Foundation.

Role of the Sponsors: The funding agencies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES

1. Waters PJ, McKeon A, Leite MI, et al. Serologic diagnosis of NMO: a multicenter comparison of aquaporin-4–IgG assays. *Neurology*. 2012; 78(9):665–671. discussion 669. [PubMed: 22302543]
2. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG. The spectrum of neuromyelitis optica. *Lancet Neurol*. 2007; 6(9):805–815. [PubMed: 17706564]
3. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology*. 2006; 66(10):1485–1489. [PubMed: 16717206]
4. Weinshenker BG, Wingerchuk DM, Vukusic S, et al. Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis. *Ann Neurol*. 2006; 59(3):566–569. [PubMed: 16453327]
5. Pittock SJ, Lucchinetti CF. Inflammatory transverse myelitis: evolving concepts. *Curr Opin Neurol*. 2006; 19(4):362–368. [PubMed: 16914974]
6. Mealy MA, Wingerchuk DM, Greenberg BM, Levy M. Epidemiology of neuromyelitis optica in the United States: a multicenter analysis. *Arch Neurol*. 2012; 69(9):1176–1180. [PubMed: 22733096]

7. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet*. 2004; 364(9451):2106–2112. [PubMed: 15589308]
8. Wingerchuk DM, Hogancamp WF, O'Brien PC, Weinshenker BG. The clinical course of neuromyelitis optica (Devic's syndrome). *Neurology*. 1999; 53(5):1107–1114. [PubMed: 10496275]
9. Pittock SJ, Lennon VA, de Seze J, et al. Neuromyelitis optica and non organ-specific autoimmunity. *Arch Neurol*. 2008; 65(1):78–83. [PubMed: 18195142]
10. Quek AM, McKeon A, Lennon VA, et al. Effects of age and sex on aquaporin-4 autoimmunity. *Arch Neurol*. 2012; 69(8):1039–1043. [PubMed: 22507888]
11. Marignier R, Bernard-Valnet R, Giraudon P, et al. NOMADMUS Study Group. Aquaporin-4 antibody-negative neuromyelitis optica: distinct assay sensitivity-dependent entity. *Neurology*. 2013; 80(24):2194–2200. [PubMed: 23658379]
12. Banwell B, Tenenbaum S, Lennon VA, et al. Neuromyelitis optica-IgG in childhood inflammatory demyelinating CNS disorders. *Neurology*. 2008; 70(5):344–352. [PubMed: 18094334]
13. Kalluri SR, Illes Z, Srivastava R, et al. Quantification and functional characterization of antibodies to native aquaporin 4 in neuromyelitis optica. *Arch Neurol*. 2010; 67(10):1201–1208. [PubMed: 20937947]
14. Waters P, Jarius S, Littleton E, et al. Aquaporin-4 antibodies in neuromyelitis optica and longitudinally extensive transversemyelitis. *Arch Neurol*. 2008; 65(7):913–919. [PubMed: 18625857]
15. Apiwattanakul M, Asawavichienjinda T, Pulkes T, et al. Diagnostic utility of NMO/AQP4-IgG in evaluating CNS inflammatory disease in Thai patients. *J Neurol Sci*. 2012; 320(1-2):118–120. [PubMed: 22831763]
16. Sato DK, Nakashima I, Takahashi T, et al. Aquaporin-4 antibody-positive cases beyond current diagnostic criteria for NMO spectrum disorders. *Neurology*. 2013; 80(24):2210–2216. [PubMed: 23677744]
17. Jiao Y, Fryer JP, Lennon VA, et al. Updated estimate of AQP4-IgG serostatus and disability outcome in neuromyelitis optica. *Neurology*. 2013; 81(14):1197–1204. [PubMed: 23997151]

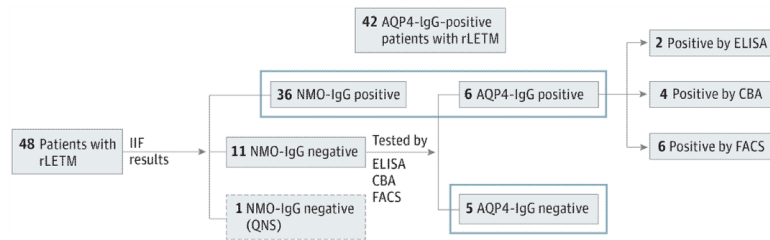


Figure 1. Flowchart for Recurrent Longitudinally Extensive Transverse Myelitis (rLETM) Cohort

Of 48 patients whose serum was tested (1 sequential specimen) with tissue-based indirect immunofluorescence (IIF), 12 were categorized as negative. Of 11 with available stored specimens, 6 (55%) were positive for aquaporin 4 (AQP4) IgG when retested with recombinant antigen–based assays. CBA indicates cell-based assay; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell-sorting assay; NMO, neuromyelitis optica; and QNS, quantity not sufficient or no sample for further evaluation.

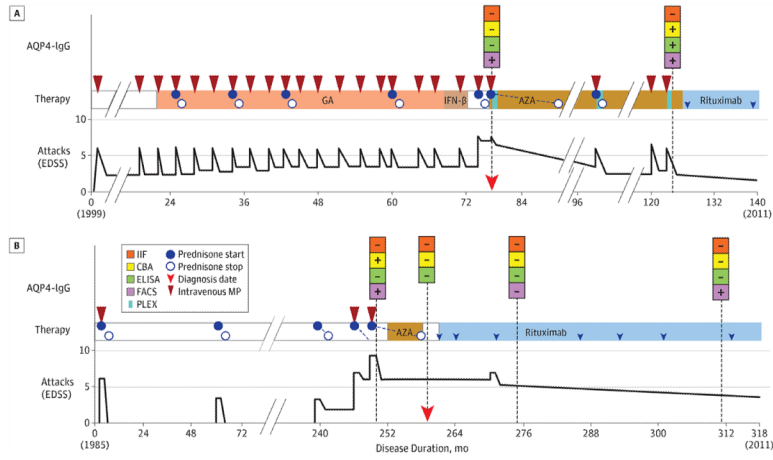


Figure 2. Clinical Course, Therapy, and Results of Aquaporin 4 (AQP4) IgG Testing by Multiple Assays in Serial Serum Samples From 2 Patients With Recurrent Longitudinally Extensive Transverse Myelitis

A, Retesting may increase the AQP4-IgG detection rate. Initial samples were negative at tissue-based indirect immunofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA), and AQP4-transfected cell-based assay (CBA) but positive at fluorescence-activated cell-sorting (FACS) assay; subsequent samples converted to positive at CBA and ELISA but not IIF. The frequency of attacks decreased with immunosuppressant therapy. Numbers on the y-axis represent Expanded Disability Status Scale (EDSS) scores. B, Sensitivity improved with recombinant antigen-based assays; serial serum specimens were persistently classified as AQP4-IgG negative with IIF but reclassified as positive with use of multiple recombinant antigen-based assays. Serostatus commonly converts from positive to negative with immunotherapy. The frequency of attacks decreased with immunosuppressant therapy. Numbers on the y-axis represent EDSS scores. AZA indicates azathioprine; GA, glatiramer acetate; IFN-β, interferon beta; MP, methylprednisolone; PLEX, plasma exchange; +, positive; and -, negative.

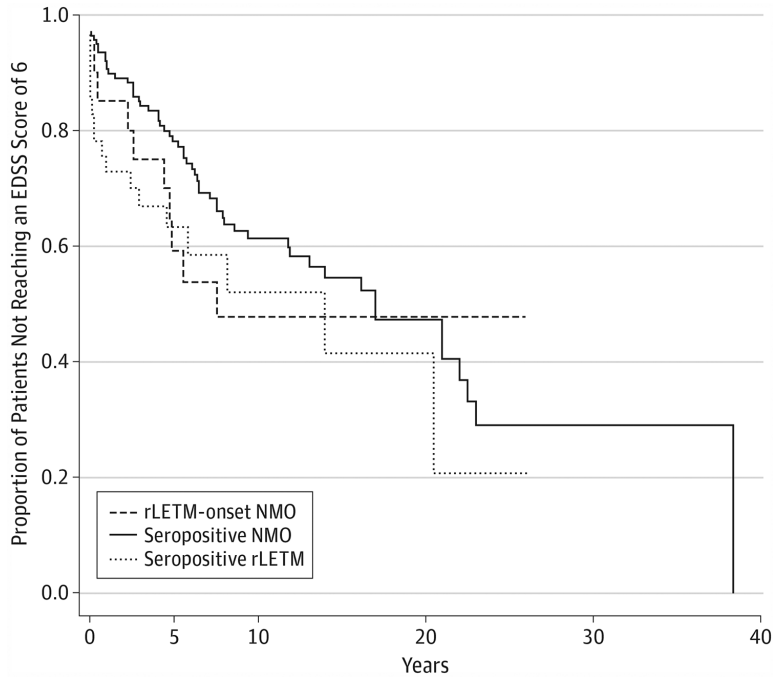


Figure 3. Kaplan-Meier Estimates of Time to Motor Disability in Aquaporin 4 (AQP4) IgG–Positive Patients With Recurrent Longitudinally Extensive Transverse Myelitis (rLETM), rLETM-onset Neuromyelitis Optica (NMO), or NMO

Major disability outcomes were similar in AQP4-IgG–positive patients with rLETM and those with rLETM-onset NMO. At 5 years after onset, 36% of patients with rLETM and 41% with rLETM-onset NMO were expected to need a cane (Expanded Disability Status Scale [EDSS] score, 6; intermittent or unilateral assistance [canes, crutches, or braces] required to walk 100 m with or without resting) ($P = .94$); a lower frequency (22%) was expected for the total AQP4-IgG–positive NMO group. The rLETM-onset NMO group included AQP4-IgG–positive patients with NMO who initially presented with 2 attacks of transverse myelitis.

Table 1

AQP4-IgG Detection Rate by Assay Type in Patients With rLETM Categorized as Seronegative by IIF Assay

Assay Type	Positive for AQP4-IgG, No. (%) ^a		
	Positive on First Available Sample	Positive on Serial Samples	Overall Detection Rate
ELISA	0/11	2/11 (18)	38/47 (81)
CBA	1/11 (9)	4/11 (36)	40/47 (85)
FACS	5/11 (45)	6/11 (55)	42/47 (89)
Any assay or combination of assays	5/11 (45)	6/11 (55)	42/47 (89)

Abbreviations: AQP4, aquaporin 4; CBA, cell-based assay; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell-sorting assay; IIF, indirect immunofluorescence; rLETM, recurrent longitudinally extensive transverse myelitis.

^aSerum samples were available for 11 of 12 patients initially classified as seronegative at IIF assay.

Table 2

Demographic and Clinical Features in AQP4-IgG-Positive and AQP4-IgG-Negative Patients With rLETM and AQP4-IgG-Positive Patients With NMO and rLETM-Onset NMO

Characteristic	Negative rLETM (n = 5)	Positive rLETM (n = 42)	Positive rLETM-Onset NMO (n = 20)	Positive NMO (n = 140)	P Value ^a
Sex, No. of females to No. of males (female to male ratio)	2:3	35:7 (5:1)	18:2 (9:1)	126:14 (9:1)	NS
Ethnicity, white to nonwhite ratio	0.7:1	1.8:1	1.9:1	1.6:1	
Age at onset, median (range), y	30 (3-52)	50 (15-75)	48 (21-68)	39 (5-71)	NS
Duration of follow-up after onset, median (range), mo	30 (11-152)	59 (10-318)	133 (41-311)	107 (5-535)	.001
Coexisting autoimmune diseases, %	20 ^b	38 ^c	30	33	NS
Frequency of severe onset, %	40	43	40	45	NS
Frequency of severe attacks in first 3 attacks, %	31	42	43	43	NS
Head MR imaging findings, No. of patients					
Available	5	39
Normal	2	10
Nonspecific	3	22 ^d
Multiple sclerosis-like	0	7
CSF findings, No. of patients ^e					
Sample available	5	27
WBC count >5/mL	2	12
WBC count, median (range), cells/mL	... (9-27)	29 (6-290)
Neutrophils >40%	0	3
4 Oligoclonal bands	0	5
Elevated protein (>45 mg/dL)	2	13
Protein, median (range) mg/dL	... (47-85)	84 (61-198)
Elevated IgG index (>0.85)		4
Relapse characteristics					
No. of attacks, median (range)	3 (2-6)	3 (2-22)	7 (4-20)	7 (1-40)	<.001
Relapse within 1 y of onset, %	80	60	40	76 (55)	NS
Relapse within 2 y of onset, %	100	74	55	92 (66)	NS
Time to first relapse, median (IQR), mo	3 (3-9)	7 (3-24)	19 (6-42)	10 (3-32)	.03
Relapse rate, times/patient/y, median (IQR)	1.1 (0.5-2.2)	0.8 (0.4-1.8)	0.7 (0.5-1.1)	0.9 (0.5-1.4)	NS
Disability					
Outcome at last follow-up, No. (%)					
EDSS score 6 ^f	1 (20)	17 (41)	10 (50)	57 (41)	...
EDSS score 8 ^f	0	4 (10)	5 (25)	29 (21)	
Kaplan-Meier estimate of expected outcome 5 y after disease onset (95% CI), %					
EDSS score 6	...	36 (20-51)	41 (19-63)	22 (15-29)	NS

Characteristic	Negative rLETM (n = 5)	Positive rLETM (n = 42)	Positive rLETM-Onset NMO (n = 20)	Positive NMO (n = 140)	P Value ^a	
EDSS score	8	...	8 (0-16)	5 (0-15)	8 (3-13)	NS
Response to long-term immunosuppressant therapy ^g						
Annualized relapse rate without therapy, mean (SD)	1.4 (1.4)	2.5 (2.5)	...	2.2 (2.7)	...	
Annualized relapse rate with therapy, mean (SD)	0.3 (0.3)	0.4 (0.5)	...	0.7 (0.9)	...	
P value ^h	.001	.001001	...	

Abbreviations: AQP4, aquaporin 4; CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; ellipses, data were not provided; IQR, interquartile range; MR, magnetic resonance; NMO, neuromyelitis optica; NS, not significant; rLETM, recurrent longitudinally extensive transverse myelitis; WBC, white blood cell.

^a Comparison between seropositive patients with rLETM or rLETM-onset NMO (NMO presenting with 2 attacks of transverse myelitis).

^b Autoimmune thyroid disease (n = 1).

^c These 16 patients may have more than 1 autoimmune condition, including autoimmune thyroid disease (n = 10), antiphospholipid syndrome (n = 1), Sjögren syndrome (n = 2), rheumatoid arthritis (n = 3), thrombotic thrombocytopenic purpura (n = 1), and type 1 diabetes mellitus (n = 1).

^d Posterior medullary lesion in 1 patient; 1 patient ultimately developed progressive multifocal leukoencephalopathy after prolonged immunosuppression.

^e Where not otherwise indicated, values for CSF findings indicate numbers of patients.

^f An EDSS score of 6 indicates that the patient requires intermittent or unilateral assistance (canes, crutches, or braces) to walk 100 m, with or without resting. An EDSS score of 8 indicates that the patient is restricted to bed or chair or perambulated in a wheelchair but may be out of bed much of the day, retains many self-care functions, and generally has effective use of his or her arms.

^g Long-term immunosuppressant therapy included azathioprine, mycophenolate mofetil, oral prednisone, monthly intravenous methylprednisolone, rituximab, eculizumab, or combinations.

^h Comparison between annualized relapse rate with and without therapy in each subgroup.