OBSERVATION

Prediction of Neuromyelitis Optica Attack Severity by Quantitation of Complement-Mediated Injury to Aquaporin-4–Expressing Cells

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Background: Recent reports support a pathogenic role in neuromyelitis optica (NMO) for the aquaporin-4 (AQP4)–specific autoantibody (NMO-IgG). Neuromyelitis optica is an inflammatory demyelinating central nervous system disease, usually relapsing, that causes variable degrees of attack-related disability. The NMO-IgG binds in vitro to the extracellular domain of AQP4, activates complement, and causes astrocyte lesioning.

Objective: To compare the prognostic utility of NMO-IgG titer and quantitative measures of complement-mediated injury to AQP4-expressing cells in NMO attacks.

Design, Setting, and Participants: A retrospective clinical-serological correlative study at Mayo Clinic’s Neuroimmunology Laboratory was undertaken. Over an 18-month period, we identified NMO-IgG–seropositive patients in whom sufficient serum and adequate clinical information pertaining to NMO attacks (6 severe, 6 mild) were available to analyze clinical-serological correlations. Sera from 9 patients with multiple sclerosis and 9 healthy subjects (all NMO-IgG seronegative) served as controls. Complement activation was measured by quantifying the number of green fluorescent protein–AQP4–transfected HEK 293 cells permeable to the viability dye propidium iodide after exposure to patient serum and active complement.

Main Outcome Measures: Attack severity (mild or severe), percentage of AQP4-transfected cells lesioned, and NMO-IgG titer.

Results: The median percentage of AQP4-transfected cells lesioned by complement in the presence of serum from patients with NMO was 14% for patients with mild attacks and 54% for patients with severe attacks (P = .005). Median complement activation values for sera from healthy subjects and patients with multiple sclerosis were 8% and 12%, respectively. Patients with mild NMO attacks and patients with severe NMO attacks did not differ significantly with respect to NMO-IgG titer (P = .089).

Conclusions: A laboratory measure of complement-mediated cell injury may serve as a prognostic biomarker in NMO. Larger prospective studies are required to validate this observation.

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NEUROMYELITIS OPTICA (NMO) comprises a spectrum of relapsing autoimmune, inflammatory, demyelinating disorders of the central nervous system (CNS) and predominantly affects the optic nerves and spinal cord. The NMO-IgG autoantibody, detected by a tissue-based immunofluorescence assay, is 58% to 73% sensitive and 91% to 100% specific for clinically defined NMO. Its detection allows the distinction of NMO from multiple sclerosis (MS) and other idiopathic inflammatory CNS disorders. Patients who are NMO-IgG seropositive generally have a severe course, with rapid accrual of attack-related disability (typically blindness and paraplegia). Recovery is complete or nearly complete in milder NMO cases, even in the setting of frequent attacks.

There is growing evidence that NMO-IgG is a pathophysiological effector in NMO through its binding to the astrocytic transmembrane protein aquaporin-4 (AQP4), which is the predominant water channel in the CNS. A pathogenic role for NMO-IgG has been demonstrated in vitro and is strongly implied by immunohistochemical findings in patients’ lesional CNS tissues. The NMO-IgG binds to the extracellular domain of the AQP4 water channel and initiates complement lesioning of astrocytes. In the absence of complement, NMO-IgG downregulates astrocyte surface expression of both AQP4 and the major CNS glutamate transporter, excitatory amino acid...
transporter 2, which is coupled to AQP4. Perturbation of water and glutamate homeostasis likely contributes to the pathogenesis of NMO. A loss of AQP4 from polarized end-feet of astrocytes where they contact endothelial cells of the blood-brain barrier could adversely alter osmotic regulation, blood-brain barrier function, glial cell recruitment, and maintenance of the neural microenvironment.

The NMO-IgG antibody titers may correlate with disease activity in NMO attacks in some patients followed longitudinally, and they may also be directly proportional to transverse myelitis lesion length and attack nadir severity. To date, these findings have not been reproduced in large prospective studies.

Functional autoantibody assays are rare in neurologic practice. A singular example is the muscle acetylcholine receptor modulating antibody assay used in evaluation of myasthenia gravis. It measures endocytosis and degradation of acetylcholine receptors initiated through cross-linking of cell surface acetylcholine receptors by bivalent antibodies. Acetylcholine receptor loss values of 90% and higher correlate significantly with weakness severity and the probability of an associated neoplasm.

Assays to predict functional outcome in NMO might facilitate therapeutic decision making. Here we demonstrate the potential for a quantitative measure of complement activation by NMO-IgG to predict the clinical severity of an NMO attack.

### METHODS

Between January 1, 2007, and July 31, 2008, we retrospectively identified 12 NMO-IgG–seropositive patients (by tissue-based immunofluorescence assay) for whom detailed information was available. This information included clinical and radiological data, NMO-IgG titer, and timing of immunosuppressive therapies in relation to blood draw dates (Table). Attacks of NMO were mild in 6 patients (complete or near complete clinical recovery) and severe in 6 patients (blind or paraparetic with little or no clinical recovery). High-titered NMO-IgG–positive sera (pooled from a minimum of 50 patients) were used as positive controls; sera from 9 healthy subjects and 9 patients with classical MS served as disease specificity controls.

Complement activation was measured (blinded) by flow cytometric analysis of the percentage of green fluorescent protein–AQP4–transfected HEK 293 cells permeable to the viability dye propidium iodide after exposure to patient serum (20% serum for 30 minutes at room temperature) and active complement (20% complement for 45 minutes at 37°C; Low-Tox-H rabbit complement; Cedarlane Laboratories Ltd, Burlington, North Carolina) as previously described. Permeability to propidium iodide indicates a potentially lethal compromise in plasma membrane integrity. The total number of cells counted per condition was 10 000. We have previously demonstrated that untransfected HEK 293 cells (cells lacking AQP4 expression) are unaffected by exposure to NMO-IgG in the presence of active or inactive complement and that the increase in membrane permeability in AQP4–expressing cells exposed to NMO-IgG is dependent on active complement. To control for the requirement of active complement in this study, we included as both positive and negative controls pooled NMO-IgG–positive sera and normal sera as well as active or heat-inactivated complement. These experiments validated that NMO-IgG and active complement were both necessary for efficient membrane lesioning (data not shown).

The NMO-IgG titer (reciprocal of the final dilution scored positive in doubling serum dilutions by standardized immunofluorescence assay) and degree of complement activation (percentage of propidium iodide–positive [lesioned] cells) were compared for patients with NMO who had mild and severe attack.

### Table. Demographic, Clinical, and Serological Data for 12 NMO-IgG–Seropositive Patients

<table>
<thead>
<tr>
<th>Patient No./Sex/Age at Blood Draw, y</th>
<th>Ethnicity</th>
<th>Attack Severity</th>
<th>Course to Date</th>
<th>Interval From Attack Onset to Blood Draw</th>
<th>Treatment at Blood Draw</th>
<th>Attack-Related Disability</th>
<th>NMO-IgG Titera,b</th>
<th>AQP4-Transfected Cells Lesioned by Complement, %b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/71 White</td>
<td>Severe</td>
<td>ON (first attack)</td>
<td>≤4 wk</td>
<td>IVMP No light perception OU</td>
<td>61 440</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2/F/24 African American</td>
<td>Severe</td>
<td>TM (first attack)</td>
<td>≤3 wk</td>
<td>IVMP Paraplegia</td>
<td>15 360</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/F/42 African American</td>
<td>Severe</td>
<td>ON and TM (recurrent)</td>
<td>6 mo (blood draw during remission)</td>
<td>None No light perception OU; wheelchair-bound</td>
<td>960</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/F/43 Arab</td>
<td>Severe</td>
<td>ON and TM (recurrent)</td>
<td>≤2 wk</td>
<td>IVMP Moderate paraparesis; finger counting OU</td>
<td>15 360</td>
<td>52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5/F/16 African American</td>
<td>Severe</td>
<td>ON and TM (recurrent)</td>
<td>1 d</td>
<td>IVMP Moderate paraparesis; perceives hand waving OU</td>
<td>15 360</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/F/46 White</td>
<td>Severe</td>
<td>ON and TM (recurrent)</td>
<td>6 mo (blood draw during remission)</td>
<td>Azathioprine, 50 mg/d</td>
<td>7680</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/M/11 Unknown</td>
<td>Mild</td>
<td>ON (recurrent)</td>
<td>1 d</td>
<td>IVMP Full recovery</td>
<td>240</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8/F/46 White</td>
<td>Mild</td>
<td>TM (recurrent)</td>
<td>1 d</td>
<td>IVMP Mild monoparesis</td>
<td>1920</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/F/63 White</td>
<td>Mild</td>
<td>TM (first attack)</td>
<td>≤3 wk</td>
<td>None Mild monoparesis</td>
<td>960</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10/F/35 African American</td>
<td>Mild</td>
<td>ON (first attack)</td>
<td>≤1 wk</td>
<td>None Full recovery</td>
<td>3840</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/F/78 Unknown</td>
<td>Mild</td>
<td>TM (first attack)</td>
<td>≤2 wk</td>
<td>None Full spontaneous</td>
<td>30 720</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/M/48 Unknown</td>
<td>Mild</td>
<td>TM (recurrent)</td>
<td>≤4 wk</td>
<td>IVMP Full recovery</td>
<td>240</td>
<td>19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AQP4, aquaporin-4; IVMP, intravenous methylprednisolone; NMO, neuromyelitis optica; ON, optic neuritis; TM, transverse myelitis.

a Tested at 1:120 dilution. A negative value would indicate normal.
b The degree of linear association between the NMO-IgG titer and the percentage of cells lesioned by complement was moderate (Pearson correlation coefficient, \( r = 0.52 \)). The NMO-IgG titer weakly predicted the percentage of cells lesioned by complement (measured by regression analysis; \( R^2 = 0.25; P = .10 \)).
Our preliminary analysis of data from testing sera of patients with NMO and control participants indicates a statistically significant association between NMO attack severity and measures of complement-mediated injury to AQP4-expressing cells. The results support our hypothesis that binding of NMO-IgG to AQP4 initiates an inflammatory cascade resulting in potentially irreversible neurological injury. Control sera did not significantly activate complement, and we did not find a statistically significant association between NMO-IgG titer and attack severity or between NMO-IgG titer and degree of complement activation.

Limitations of this study include the retrospective design and small numbers of patients tested, which may have precluded detection of a positive association between NMO-IgG titer and attack severity. Also, the technique used to measure the percentage of HEK 293 cells permeable to propidium iodide has not been validated as a quantitative measure of complement-mediated cell lesioning in a clinical service laboratory setting. Larger prospective studies correlating NMO-IgG characteristics (including antibody titers and quantitative functional effects) with clinical course or attack severity may provide prognostic serological profiles. For the individual patient, these profiles might identify novel therapeutic targets and direct therapeutic decision making.

No data are currently available to support the efficacy of a specific therapeutic modality for NMO. Current recommendations are based on small case numbers and anecdotal experience. Nevertheless, it has been established that disability in NMO is attack related and that a secondary progressive course is uncommon. This contrasts with MS, where the main risk factor for developing permanent disability is the onset of a secondary progressive course, apparently independent of superimposed relapses. Thus, successful attack prevention therapy, if initiated early, should have a greater effect on the natural history of NMO than on that of MS. Therapies to date are limited to immunosuppressive agents (prednisone, azathioprine, mycophenolate mofetil, rituximab, and mitoxantrone hydrochloride) for attack prevention and to intravenous methylprednisolone and plasmapheresis for treatment of acute attacks. Our preliminary data may support consideration of complement-inhibitory therapies in patients with severe attacks.

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**RESULTS**

Patients with mild and severe attacks of NMO did not differ significantly with respect to age, sex, ethnicity, and therapy at the time of blood draw (Table). All patients with mild attacks of NMO and 4 of 6 patients with severe attacks of NMO had blood drawn within 4 weeks of attack onset. The median percentages of AQP4-transfected cells lesioned by complement in the presence of sera from patients with mild and severe NMO attacks were 14% (range, 8%-19%) and 54% (range, 21%-60%), respectively (P = .005) (Figure). The NMO-IgG titers for patients with mild NMO attacks and patients with severe NMO attacks did not differ significantly (P = .089). Median complement activation values for sera from healthy subjects and patients with MS were 8% (range, 8%-12%) and 12% (range, 9%-15%), respectively. The positive control NMO-IgG–positive serum pool had a mean value of 55% complement activation (data not shown).
Financial Disclosure: Drs Hinson, Lennon, and Pittock are named inventors on a filed patent relating to functional assays for detecting AQP4-IgG. In accordance with the Bayh-Dole Act of 1980 and Mayo Foundation policy, Dr Lennon stands to receive royalties for intellectual property related to the AQP4 autoantigen. This intellectual property is licensed to a commercial entity for development of a simple antigen-specific assay to be made available worldwide for patient care. The test will not be exclusive to Mayo Clinic. To date, the authors have received a total of less than $10,000 in royalties. Mayo Clinic offers the test as an indirect immunofluorescence assay to aid the diagnosis of NMO, but the authors do not benefit personally from the performance of the test. The other authors have no conflicting financial interests.

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REFERENCES


