ANTIBODIES TO AQUAPORIN-1 ARE NOT PRESENT IN NEUROMYELITIS OPTICA
OPEN

Although more than 70% of all patients with neuromyelitis optica (NMO) are seropositive for aquaporin-4 (AQP4) antibodies, a substantial proportion of patients fulfilling the clinical criteria for NMO or limited forms of the disease (NMO spectrum disorders [NMOSD]) are negative for these antibodies. This raises the possibility of other autoantibodies in these patients. One putative target, the ubiquitously expressed water channel aquaporin-1 (AQP1), which is partially lost or internalized in certain NMO lesions, was described in a subset of patients with NMOSD. However, AQP1 antibodies were also found in patients with multiple sclerosis (MS), thus raising concerns about the specificity of these findings. Therefore, we developed a recombinant live cell immunofluorescence assay (CBA) for AQP1 antibodies based on our AQP4 antibody assay. We analyzed 176 serum samples from Austrian patients with NMOSD (n = 67), Austrian patients with MS (n = 31), and controls (n = 78) for the presence of IgG AQP1 antibodies and AQP4 antibodies. Furthermore, both assays were validated in a blinded cohort of 58 patients with NMOSD (n = 36) or MS (n = 22) from Lyon (France) and Oxford (United Kingdom). Clinical and demographic data of patients and controls are shown in the table.

Methods. Analysis of AQP1 antibodies and AQP4 antibodies was performed using a live CBA described previously. Briefly, HEK293A cells were transiently transfected using the pcDNA6.2C-EmGFP-GW/TOPO plasmid (Invitrogen, Carlsbad, CA), expressing AQP4 (isoform M23) or AQP1 (isoform 1) fused C-terminally to emerald green fluorescence protein. Transfected cells were blocked with goat IgG in phosphate-buffered saline (PBS)/10% fetal calf serum (FCS) (Sigma-Aldrich, St. Louis, MO) followed by serum diluted 1:20 and 1:40 in PBS/FCS for 1 hour at 4°C. Serum preabsorption with liver powder was not performed because a previous report indicated loss of AQP1 antibodies after pretreatment. Bound antibodies were detected using Cy3Tm-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) for 30 minutes at room temperature. Bound antibodies were determined using a fluorescence microscope (Leica DMI 4000B). All samples were evaluated by 2 independent, clinically blinded investigators who agreed on all samples.

Results. Expression of AQP1 was verified by staining with a commercial AQP1-specific antibody recognizing full-length AQP1. However, the antibody only recognized the target after fixation of AQP1-transfected HEK293A cells with paraformaldehyde (figure e-1A at Neurology.org/nn). In order to prove surface expression and correct topology of AQP1, a myc-tag was inserted at position T120 (extracellular loop C) by site-directed mutagenesis. Staining with an anti-myc-tag monoclonal antibody clearly showed the surface expression of AQP1 in live HEK293A cells (figure e-1B).

As seen in the table, AQP4 antibodies were detected in 81 of 103 (79%) patients with NMOSD in the combined cohorts. AQP4 antibodies were absent in 53 patients with MS and 78 controls; therefore, the specificity of the AQP4 antibody assay was 100%. In contrast, AQP1 antibodies were absent in all 234 serum samples from patients with NMOSD and MS and controls. Although the AQP4 antibody CBA showed high sensitivity and specificity, a comparable AQP1 antibody CBA did not detect any antibodies in 234 serum samples (figure e-1C).

Discussion. This finding is in contrast to previous studies by 2 groups reporting the presence of AQP1 antibodies in 17%–74% of patients with NMOSD, but also in controls. In our opinion, these differences could be explained by methodologic differences between the studies (using radioimmunoprecipitation, ELISA with peptides, or fixed CBAs might have exposed intracellular epitopes, whereas our assay only detects antibodies to extracellular epitopes). Methodologic differences have proven to be a constant problem in this field for decades. For example, there is now clear evidence that antibodies to the myelin oligodendrocyte glycoprotein are specific
for a subset of demyelinating diseases only if appropriate CBAs are used (these antibodies are detected at similar frequencies in patients and controls using ELISA). Similarly, although numerous studies confirm the importance of AQP4 antibodies as diagnostic biomarkers for NMOSD, these are usually tested as core antigens in CBAs used (these antibodies are detected at similar frequencies in patients and controls using ELISA). A possible limitation of our AQP1 assay is the use of HEK293A cells, because astrocytic AQP1 might be expressed in a complex against which the immune system could react.

However, the absence of AQP1 antibodies in NMOSD and controls fits very well with AQP1’s role in hematology. AQP1, also known as channel-forming integral protein, is well-known in transfusion medicine because it contains the Colton blood group antigen expressed on erythrocytes. Anti-Colton antibodies are very rare and lead to significant delayed or acute transfusion reactions or hemolytic disease, a clinical phenotype absent in NMOSD. To conclude, our study failed to confirm the presence of AQP1 antibodies in NMOSD.

**Table**

<table>
<thead>
<tr>
<th>NMOSD</th>
<th>NMO</th>
<th>LETM/ON</th>
<th>MS</th>
<th>CTRL</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>45</td>
<td>58</td>
<td>53</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>40 (89)</td>
<td>40 (69)</td>
<td>32 (63)</td>
<td>61 (78)</td>
<td>0.017&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age, y, median (range)</td>
<td>49 (18-83)</td>
<td>51 (13-78)</td>
<td>40 (19-66)</td>
<td>44 (18-84)</td>
<td>0.007&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AQP4-IgG, n (%)</td>
<td>40 (89)</td>
<td>41 (71)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AQP4-IgG titer, median (range)</td>
<td>640 (0-20,480)</td>
<td>320 (0-10,240)</td>
<td>0</td>
<td>0</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AQP1-IgG, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AQP1-IgG titer, median (range)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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</table>

Abbreviations: AQP1 = aquaporin-1; AQP4 = aquaporin-4; CTRL = controls (23 patients with other neurologic diseases, 25 patients with systemic lupus erythematosus, and 30 healthy controls); LETM/ON = patients with longitudinally extensive transverse myelitis (n = 45) or optic neuritis (n = 13); MS = multiple sclerosis according to the 2005 revisions to the McDonald criteria (n = 53); NMO = clinically definite neuromyelitis optica according to the Wingerchuk 2006 criteria; NMOSD = NMO spectrum disorders (NMO + LETM/ON).

<sup>a</sup> Groups were compared using the χ² test.

<sup>b</sup> Groups were compared using the Kruskal-Wallis test.
antibody validation experiments organized by Euroimmun. Go to Neurology.org/nn for full disclosure forms. The Article Processing Charge was paid by the Austrian Science Funds (project I916, Erare EDEN).

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