Antibody Investigations in 2,750 Children With Suspected Autoimmune Encephalitis

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Abstract

Objectives
To assess the frequency and types of neuronal and glial (neural) antibodies in children with suspected autoimmune encephalitis (AE).

Methods
Patients younger than 18 years with suspected AE other than acute disseminated encephalomyelitis, whose serum or CSF samples were examined in our center between January 1, 2011, and April 30, 2022, were included in this study. Samples were systematically examined using brain immunohistochemistry; positive immunostaining was further investigated with cell-based assays (CBA), immunoblot, or live neuronal immunofluorescence.

Results
Of 2,750 children, serum or CSF samples of 542 (20%) showed brain immunoreactivity, mostly (>90%) against neural cell surface antigens, and 19 had antibodies only identified by CBA. The most frequent targets were N-methyl-D-aspartate receptor (NMDAR, 76%) and myelin oligodendrocyte glycoprotein (MOG, 5%), followed by glutamic acid decarboxylase 65 (2%) and γ-aminobutyric acid A receptor (2%). Antibodies against other known cell surface or intracellular neural antigens (altogether 6% of positive cases) and unknown antigens (9%) were very infrequent.

Discussion
The repertoire of antibodies in children with AE is different from that of the adults. Except for NMDAR and MOG antibodies, many of the antibodies included in diagnostic panels are rarely positive and their up-front testing in children seems unneeded.

Introduction
Neural antibodies play a central role in the diagnosis of autoimmune encephalitis (AE). Antibody testing panels have been developed in parallel to the discovery of novel autoantigens, but limited attention has been paid to their suitability for children. Although some studies examined the frequency of one or a few neural antibodies in children, a comprehensive assessment of the frequency and repertoire of these antibodies in pediatric AE has not been reported. Knowing the frequency of neural antibodies in children is important because it can reduce unnecessary testing and
potential misdiagnoses. Here, we assessed the frequency of neural antibodies in 2,750 children with suspected AE.

Methods

Study Design and Participants

Patients younger than 18 years with suspected AE and whose samples were examined in our center between January 1, 2011, and April 30, 2022, were included in this study. Patients with acute disseminated encephalomyelitis (ADEM) or neuromyelitis optica spectrum disorders were excluded.

Serum and CSF samples were examined using rat brain immunohistochemistry (eMethods, links.lww.com/NXI/A934). If positive, samples were further examined with an array of cell-based assays (CBAs) or immunoblot depending on the pattern of immunostaining. If CBAs were negative, samples were then examined with live neuronal immunofluorescence to determine whether the target antigen was on the cell surface. Antibodies against myelin oligodendrocyte glycoprotein (MOG), glycine receptor (GlyR), and dopamine 2 receptor (D2R) were directly assessed with CBAs in selected patients according to the syndrome. Since 2014, all pediatric patients in our prospective studies are assessed with CBAs for MOG and GlyR antibodies.

Literature Review on Antibodies in Pediatric AE

We searched MEDLINE and PubMed for studies published in English since January 1, 2007, to December 31, 2022,
using the MeSH terms encephalitis, autoimmune, child, and pediatric.

**Standard Protocol Approvals, Registrations, and Patient Consents**

This study was approved by the Ethical Board Committee of Hospital Clinic de Barcelona. Written informed consents were obtained from patients or proxies.

**Data Availability**

Anonymized data are available by request from qualified investigators.

**Results**

In total, 2,750 children (median age 9 years, IQR 9 years; 52% female) with suspected AE were included in this study. Paired serum/CSF samples were available from 2,161 (79%), only CSF from 317 (11%), and only serum from 272 (10%).

The process of antibody detection and antigen specificity confirmation is shown in Figure 1. Samples of 542 (20%) patients showed reactivity with brain tissue: 497 (92%) had a neuropil pattern of immunostaining suggesting antibodies against neuronal cell surface antigens, 20 (4%) showed intracellular staining, and 25 (4%) showed other patterns (see below).

Among the 497 cases with neuropil immunostaining, CBAs identified the antigen in 457 (92%). The other 40 (8%) cases with negative CBA were further investigated with live neuronal immunofluorescence, showing antibody cell surface reactivity in 11 (unknown antigen) and absent cell surface reactivity in the remaining 29 (intracellular membrane antigens).

Among the 20 patients whose samples showed intracellular staining, 7 had antibodies against known onconeuronal antigens (Hu, Ma2, Yo, Kelch-like protein 11 [KLHL11]) and 13 against unknown antigens. Of the 25 patients whose samples showed other patterns of brain reactivity, all had antibodies against well-defined antigens (confirmed by CBA), including MOG, glutamic acid decarboxylase 65 (GAD65), aquaporin 4 (AQP4), and glial fibrillary acidic protein.

In addition, 19 patients had antibodies difficult to visualize with brain tissue assays (i.e., a subset of MOG antibodies, GlyR, and D2R) and were directly demonstrated with CBAs: 15 had MOG antibodies (among 894 patients tested), 3 had GlyR antibodies (731 tested), and 1 had D2R antibodies (148 tested).

The relative frequencies of neural antibodies (including patients with more than 1 antibody) are shown in Figure 2. N-methyl-d-aspartate receptor (NMDAR) (439, 76%) and MOG (31, 5%) accounted for 81% of all antibodies. After excluding 53 (9%) cases with antibodies against unknown antigens, the remaining 10% of antibody frequencies comprised γ-aminobutyric acid A receptor (GABA_A), GAD65, AQP4, GlyR, Hu, and metabotropic glutamate receptor 5 (mGluR5).

The clinical features of pediatric AE associated with NMDAR, non-ADEM MOG, GAD65, GABA_A, AQP4, GlyR, Hu, and mGluR5 antibodies are shown in Table 1, and those with antibodies against unknown antigens are presented in eTable 1 (links.lww.com/NXI/A934).

The characteristics of previous studies examining neural antibodies in children or mixed populations of children and adults are presented in eTable 2 (links.lww.com/NXI/A934).

**Discussion**

In this study of pediatric patients with suspected AE other than ADEM, one-fifth had serum or CSF immunoreactivity...
with rat brain tissue, among which more than 90% had antibodies against neuronal cell surface proteins. The 2 most frequent neuronal or glial targets were NMDAR (76%) and MOG (5%), followed by GAD65 (2%), GABA_{A}R (2%), and several other cell surface or intracellular antigens (altogether comprising 6%).

We used a diagnostic approach that combines clinical information and brain tissue assays adapted to recognize most neural antibodies except for D2R, GlyR, and a subset of MOG antibodies.\textsuperscript{9,10} Thus, excluding these 3 antibodies which were not investigated in all patients, we are able to provide the relative frequency of most antibodies.

In children, MOG antibodies usually associate with demyelinating disorders, such as ADEM, which were not part of this study.\textsuperscript{8} However, even with the exclusion of ADEM and considering that before 2014, MOG antibodies were not routinely examined in children with suspected AE, these antibodies were the second most common after NMDAR antibodies. Most patients with MOG antibodies had cortical encephalitis, highlighting the importance of MOG antibody testing in this clinical context.\textsuperscript{8}

The small number of cases with antibodies other than NMDAR and MOG is noteworthy: they represented 10% of all positive cases and 2% of the entire series (2,750 patients). Two of 8 (25%) patients with isolated GABA_{A}R antibodies had predominant cerebellar symptoms, which is unusual in adult patients.\textsuperscript{11} Antibodies against intracellular or onconeural antigens were extremely uncommon; the most frequent were directed against GAD65 (usually nonparaneoplastic) and Hu, which included 3 opsoclonus-myoclonus-ataxia associated with neuroblastoma and 1 brainstem encephalitis without tumor. Given that in the context of opsoclonus-myoclonus-ataxia with neuroblastoma, the Hu antibodies form part of the antitumor response rather than the neurologic syndrome,\textsuperscript{3} only 4 patients (one each: Hu, Yo, Ma2, and KLHL11) among 2,750 (0.1%) were diagnostically assisted by onconeural antibodies. These findings warn against the excessive use of paraneoplastic panels in children with potential AE.

We identified 53 (9%) patients with antibodies against unknown antigens that deserve future investigations to characterize novel clinical-immunological phenotypes.

A limitation of our study is that until 2014, not all patients were routinely tested for MOG and GlyR antibodies; therefore, some patients with these antibodies could have been missed. A similar problem applies to D2R antibodies, but their association with AE is extremely rare.\textsuperscript{12} By contrast, the number of patients with GABA_{A}R antibodies may reflect a referral bias due to the absence of commercial testing until recently.

These limitations, however, do not affect the implications of our findings, which suggest that the current approach to neural antibody testing in children is inadequate. In these patients, testing should be based on the clinical picture focusing mainly

### Table 1

<table>
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<tr>
<th>Antibody; number of patients(^b)</th>
<th>Age in years, median (range); male/female</th>
<th>Syndrome/symptoms</th>
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<tr>
<td>NMDAR; 437</td>
<td>9 (0.2–17); 169/268</td>
<td>≤3 major symptoms(^a) 180/404 (45%); ≥4 major symptoms 224/404 (55%); information not available 33. Overlapping syndrome with concurrent antibodies: MOG (7); GABA_{A}R (1); GlyR (1); GABA_{A}R &amp; GlyR (1); GFAP (1); GABA_{A}R (1)</td>
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<tr>
<td>Non-ADEM MOG; 24</td>
<td>7.5 (2–16); 11/13</td>
<td>Cortical encephalitis (20/24); polyfocal encephalitis (3/24); brainstem/cerebellar encephalitis (1/24)</td>
</tr>
<tr>
<td>GAD65; 10</td>
<td>12.6 (1–15); 4/6</td>
<td>Limbic encephalitis (7/10, one of them with concurrent NMDAR antibody); encephalitis with seizures (2/10); cerebellitis with seizures (1/10)</td>
</tr>
<tr>
<td>GABA_{A}R; 8</td>
<td>13 (0.8–16); 6/2</td>
<td>Encephalitis with seizures (7/8, 2 of them with status epilepticus). MRI assessable in 6 patients: 4 cortical/subcortical lesions; 2 cerebellar lesions</td>
</tr>
<tr>
<td>AQP4; 5</td>
<td>12, (5–14); 2/3</td>
<td>Encephalitis with diencephalic-brainstem-spinal cord lesions (2/5); limbic encephalitis (2/5); basal ganglia encephalitis (1/5)</td>
</tr>
<tr>
<td>Hu; 4</td>
<td>4.5 (2–8); 2/2</td>
<td>Opsoclonus-myoclonus-ataxia syndrome (3/4, all with neuroblastoma, one of them with concurrent NMDAR antibody); brainstem encephalitis (1/4, no evidence of tumor)</td>
</tr>
<tr>
<td>GlyR; 3</td>
<td>14, (5–17); 1/1, sex unknown 1</td>
<td>Encephalitis with seizures (2/3); stiff-person spectrum disorder (1/3)</td>
</tr>
</tbody>
</table>
| mGlur5; 3                       | 14, (6–16); 2/1                          | Encephalitis with seizures and normal brain MRI (2/3, 1 with Hodgkin lymphoma); limbic encephalitis (1/3, with Hodgkin lymphoma)

Abbreviations: GABA_{A}R = γ-aminobutyric acid A receptor; GFAP = glial fibrillary acidic protein; GlyR = glycine receptor; MOG = myelin oligodendrocyte glycoprotein; NMDAR = N-methyl-D-aspartate receptor.

\(^{a}\) Antibodies with ≤3 patients are not included in the table.

\(^{b}\) Patients with ≥2 antibodies were classified according to the antibody most frequently associated to the indicated clinical manifestations.

\(^{c}\) Symptoms include psychiatric, behavioral, or cognitive alterations; speech dysfunction; seizures; movement disorder, dyskinesias, or rigidity/abnormal postures; decreased level of consciousness; autonomic dysregulation or central hypoventilation.

\(^{d}\) Patients with ≥2 antibodies were classified according to the antibody most frequently associated to the indicated clinical manifestations.

\(^{e}\) Symptoms include psychiatric, behavioral, or cognitive alterations; speech dysfunction; seizures; movement disorder, dyskinesias, or rigidity/abnormal postures; decreased level of consciousness; autonomic dysregulation or central hypoventilation.
on NMDAR and MOG antibodies that by far are the most frequently involved (81%), followed by GAD65 and possibly GABA<sub>R</sub> antibodies, which are much less common (4%). In adults, commercial antibody testing frequently associates with false-positive or misinterpretation of results. Therefore, the implementation of the same tests to children, who have a different repertoire of diseases with more restricted antibody associations, may amplify the number of diagnostic errors. In children, many antibodies are so rare that their detection outside the expected syndrome requires confirmation with other tests before characterizing the disorder as antibody-associated or escalating treatment with immunotherapy. Thus, in children with suspected AE, upfront testing for many of these antibodies seems unneeded.

Acknowledgement
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Disclosure
J. Dalmau holds patents for the use of Ma2, NMDAR, GABABR, GABAAR, DPPX and IgLON5 as autoantibody tests. J. Dalmau receives royalties related to autoantibody tests from Athena Diagnostics and Euroimmun, Inc. M. Guasp and G. Olivé-Cirera are recipients of a Rio Hortega grant (CM21/00016 and CM22/00066, respectively) from the Instituto de Salud Carlos III (ISCIII), Spain, cofunded by Fondo Social Europeo Plus (FSE+). The rest of the authors have no conflicts of interest related to the submitted work. Go to Neurology.org/NN for full disclosures.

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### Appendix Authors

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