Improving the antibody-based evaluation of autoimmune encephalitis

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Lindsey McCracken, MPH
Junxian Zhang, MD
Maxwell Greene, MD
Anne Crivaro, PhD
Joyce Gonzalez, BS, M (ASCP)
Malek Kamoun, MD, PhD
Eric Lancaster, MD, PhD

Correspondence to Dr. Lancaster: Eric.lancaster@uphs.upenn.edu

ABSTRACT

Objective: We tested whether antibody screening samples of patients with suspected autoimmune encephalitis with additional research assays would improve the detection of autoimmune encephalitis compared with standard clinical testing alone.

Methods: We examined 731 samples (333 CSF, 182 sera, and 108 pairs) from a cohort of 623 patients who were tested for CNS autoantibodies by the University of Pennsylvania clinical laboratory over a 24-month period with cell-based assays (CBAs) on commercially obtained slides of fixed cells for antibodies to NMDA receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), γ-aminobutyric acid-B receptor ($GABA_BR$), leucine-rich glioma-inactivated 1 (LGI1), contactin-associated protein-like 2 (Caspr2), and glutamic acid decarboxylase (GAD65). In parallel, our research laboratory screened all samples for reactivity to brain sections and for anti-NMDAR using in-house CBAs. Samples with brain reactivity or positive clinical studies were examined with CBAs for a larger panel of antibodies.

Results: The clinical laboratory reported positive findings for NMDAR (80 samples), GAD65 (8), LGI1 (5), Caspr2 (2), and $GABA_BR$ (4). Sixty-five serum samples and 32 CSF samples were indeterminate for one or more antibodies. In our research laboratory, all but 4 positive results were confirmed, 88 of 97 indeterminate results were resolved, and 15 additional samples were found positive (10 NMDAR, 1 AMPAR, 3 LGI1, and 1 Caspr2). Clinical information supported these diagnoses. Overall, informative autoantibodies were detected in 15.5% of cases.

Conclusions: Standard clinical laboratory kits were specific, but some tests were insensitive and prone to indeterminate results. Screening with immunohistochemistry for reactivity to brain sections, followed by additional CBAs for cases with brain reactivity, improves the diagnostic accuracy of testing for autoimmune encephalitis. ***Neurol Neuroimmunol Neuroinflamm* 2017;4:e404; doi: 10.1212/NXI.0000000000000404**

GLOSSARY

AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; **CBA** = cell-based assay; **$GABA_BR$** = γ-aminobutyric acid-B receptor; **GAD65** = glutamic acid decarboxylase; **IHC** = immunohistochemistry; **IRB** = institutional review board; **LGI1** = leucine-rich glioma-inactivated 1; **NMDAR** = NMDA receptor; **PBS** = phosphate-buffered saline.

Encephalitis is a challenging problem in clinical neurology, having a broad differential diagnosis including a growing list of autoimmune causes. After the initial description of anti-NMDA receptor (NMDAR) encephalitis in 2007,[1] a family of autoimmune diseases has been identified, which is characterized by autoantibodies to surface epitopes of synaptic and cell surface proteins.[2] The other main autoantigens include the GluR1/GluR2 subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR),[3] γ-aminobutyric acid-B receptor ($GABA_BR$),[4] leucine-rich glioma-inactivated 1 (LGI1),[5,6] and contactin-associated protein-like 2 (Caspr2).[7] Standardized cell-based assay (CBA) kits for detecting these antibodies are in widespread use.

From the Department of Neurology (L.M., J.Z., M.G., E.L.), and Department of Pathology (A.C., J.G., M.K.), University of Pennsylvania, Philadelphia.

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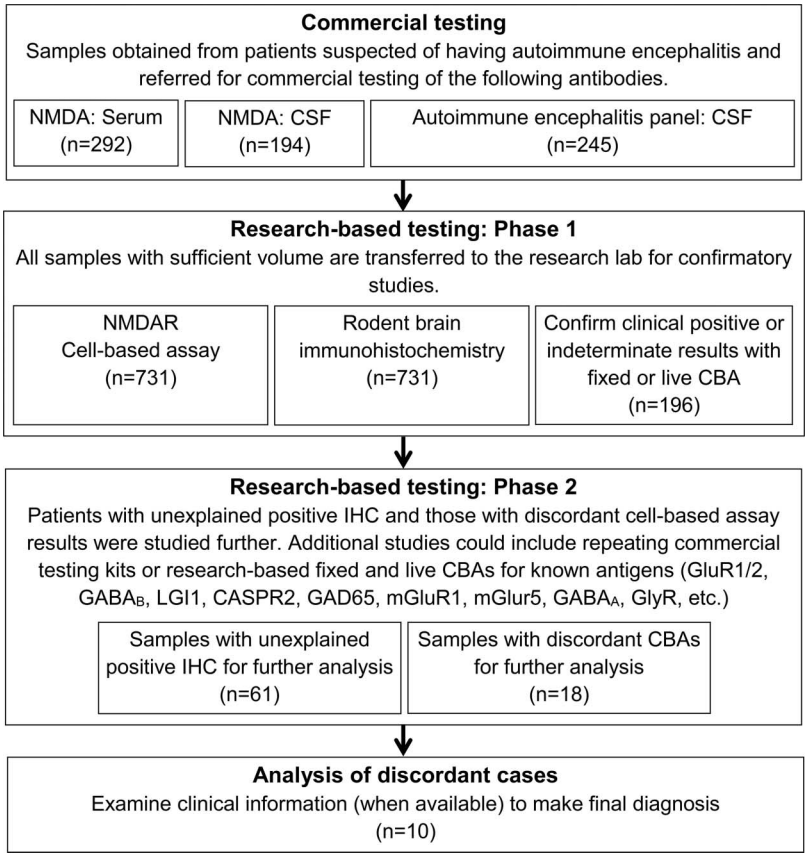
Most of these autoimmune synaptic diseases were initially identified by finding patients whose CSF or serum samples showed immunohistochemical (IHC) reactivity with rodent brain sections. In our practice, we have encountered patients with positive reactivity to brain sections but negative testing with commercial kits. We hypothesized that additional cases of autoimmune encephalitis may be detected using additional research methods beyond the commercially available CBA kits used in the clinical laboratory. We therefore have studied samples that had been sent for autoimmune encephalitis testing to the University of Pennsylvania clinical laboratory, which uses a commercial CBA kit for a series of antigens. In our research laboratory, we performed rodent brain IHC and additional CBAs on all samples with brain reactivity to determine whether the research studies would detect additional cases with brain antibodies not found in the clinical laboratory and to resolve the discordant cases with negative clinical laboratory testing but reactivity to brain sections.

Figure 1 Flow of samples through the clinical and research laboratories



All 731 samples referred to the Hospital of the University of Pennsylvania for antibody testing were examined using commercial testing kits and in our research laboratory as described in the methods section. CBA = cell-based assay; $GABA_B$-R= γ-aminobutyric acid-B receptor; GAD65 = glutamic acid decarboxylase; IHC = immunohistochemistry; LGI1 = leucine-rich glioma-inactivated 1; NMDAR = NMDA receptor.

METHODS

Case identification and clinical information. We analyzed all serum samples that had been sent for clinical laboratory testing to the Hospital of the University of Pennsylvania clinical laboratory for the autoimmune encephalitis panel (which includes the NMDAR, AMPAR, $GABA_BR$, LGI1, Caspr2, and glutamic acid decarboxylase [GAD65] assays), as well as a CSF NMDAR test and/or the serum NMDAR, over a 24-month period (January 2015 to December 2016). The flow of samples through the clinical and research laboratories is shown in figure 1. Under our institutional review board (IRB) protocol, we had limited clinical information on some patients, but detailed clinical information was collected for most patients, including those treated at the Hospital of the University of Pennsylvania.

Clinical laboratory assays. CBAs for clinical laboratory studies were conducted using Euroimmun IIFT kits: Autoimmune Encephalitis Mosaic 1 (FA 1121-1005-1), GAD65 (FA 1022-1005-50), and/or NMDAR kits (FA112d-1005-51 or FA112d-1010-51) according to the manufacturer's instructions. For cases in which there was a discrepancy with the research results, the same methods were repeated in the research laboratory.

Standard protocol approvals, registrations, and patient consents. This study was approved by the IRB of the University of Pennsylvania. Participants provided informed consent for collection of relevant clinical information. Samples from participants whose consent was not obtained were de-identified and retained for research without clinical information. No participants approached for consent declined or withdrew. Those enrolled were studied rapidly so there was no loss to follow-up.

Rodent brain IHC. As described previously,[8] adult female Wistar rats were anesthesized and decapitated. Brains were removed and washed in 0.1 M phosphate-buffered saline (PBS) in a dish. Brains were bisected sagitally at the midline and placed in cold (4°C) 4% paraformaldehyde in PBS for 1 hour. Brains were transferred to 40% sucrose in 0.1 M PBS for 48 hours then snap frozen in 2-methylbutane cooled with liquid nitrogen. Sections were cut at 7 μm, mounted on glass slides, and stored at −20°C until use. Slides were defrosted for 15 minutes at room temperature and washed with PBS (5 minutes, 2×), and endogenous peroxidase was quenched with 0.3% hydrogen peroxide in PBS for 15 minutes. Slides were washed with PBS (5 minutes, 3×), then blocked with 5% normal goat serum (Jackson ImmunoResearch, West Grove, PA) in PBS for 1 hour at room temperature. CSF was applied at 1:2 or serum at 1:200 in buffer overnight at 4°C. Slides were washed with PBS (5 minutes, 3×). A secondary biotinylated goat anti-human immunoglobulin G (Vector BA-3000; Vector Laboratories, Inc., Burlingame, CA) was diluted 1:2,000 in the block and incubated at room temperature for 2 hours. Slides were washed with PBS (5 minutes, 3×), and the ABC Vectastain Elite kit and then diaminobenzidine EqV (Vector SK-4105) were used to demonstrate reactivity. Slides were lightly co-stained with 50% hematoxylin for 1 minute, washed 3× with ddH_2O, dehydrated sequentially with ethanol then xylene substitute (Thermo 9990505; Thermo Fisher Scientific, Waltham, MA), and mounted with Permount SP15-100. Those with a characteristic staining pattern were scored as positive.

Research CBAs. HEK293 cells were plated on 12-mm glass coverslips in 24 well plates then transfected with the appropriate plasmids (see below) using the jetPRIME reagent (Polyplus, Illkirch, France) or Lipofectamine 3000 (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. Transfection was calibrated to provide 5%–20% transfection, generating sufficient numbers to transfected cells to analyze and abundant untransfected cells as controls. After 4 hours, cells were changed to fresh culture media if jetPRIME reagent was used and incubated for 18–24 hours to allow expression at 37°C. For live staining, cells were treated with case sera (1:10) or CSF (1:1) in a culture medium for 1 hour at 37°C. Cells were washed with PBS (3×), fixed with 4% paraformaldehyde in PBS for 10 minutes at room temperature, and then washed with PBS (3×). Cells were blocked in 5% normal goat serum in PBS for 1 hour at room temperature. For staining of fixed cells, the CSF (1:2–1:5) or serum (1:100–1:200) samples were applied for 1 hour at room temperature, and then cells were washed with PBS 3×. Cells were then labeled with the appropriate primary commercial antibody (see below) for 1 hour at room temperature then washed with PBS (3×). Secondary fluorescent antibodies were applied (Alexa 488- or 594-conjugated anti-human, and an appropriate commercial secondary); the coverslips were washed with PBS (3×) and then with ddH2O (3×); the coverslips were mounted using Fluoromount-G with 4',6-diamidino-2-phenylindole (Southern Biotech, Birmingham, AL). Slides were visualized under a fluorescence microscope (Leica, Wetzlar, Germany) and scored as positive or negative. For experiments involving the NMDAR, the NMDAR antagonist MK801 (10–20 nM) was applied after transfection to prevent toxicity from NMDAR calcium influx.

The plasmids used for the respective research assays were published previously: NMDAR (gift of Dr. David Lynch),[9] AMPAR,[3] $GABA_BR$,[4] LGI1/Caspr2,[5] mGluR1 and mGluR5,[10] $GABA_AR$,[11] glycine receptor,[12] and GAD65.[12]

The following primary commercial antibodies were used for the respective assays: NMDAR (Rb/NMDAR1, clone 1.17.2.6, 1:1,000; Millipore, Billerica, MA), Caspr2 (Rb/Caspr2, Abcam 33994, 1:2,000; Abcam, Cambridge, United Kingdom), GAD65 (Ms/GAD65, Abcam 26113, 1:1,000), AMPAR (Rb/GluR1, Abcam 31232, 1:6,000), and $GABA_BR$ (Ms/GABAb R1, Abcam 55051, 1:2,000). For CBAs involving LGI1, ADAM23 was coexpressed to bind LGI1 to the cell membrane; for these experiments, the commercial antibody was rabbit anti-ADAM23 (ab28304, Abcam, 1:3,000). Additional research studies were conducted for $GABA_A$ receptor (Ms/GABA(A)α1, Chemicon/Millipore, MAB339, 1:1,000), glycine receptor (Rb/GlyRα1, Sigma HPA016502, 1:2,000; Sigma-Aldrich, St. Louis, MO), mGluR1 (green fluorescent protein–tagged plasmid), and mGluR5 (Rb/mGluR5, Abcam 76316, 1:200). Goat secondary fluorescent antibodies to rabbit, mouse, or human were purchased from Jackson Immunoresearch (goat anti-human Alexa Fluor 488, 109-545-088, 1:2,000; goat anti-mouse Alexa Fluor 488, 115-545-116, 1:2,000) or Invitrogen (goat anti-Rb, Alexa Fluor 488, A-11034, 1:2,000; goat anti-Rb Alexa Fluor 594, A-11037; goat anti-human Alexa Fluor 594, A-11014).

Standard for determining which cases were true positive for different forms of autoimmune encephalitis. Following the standard proposed by Gresa-Arribas et al.,[13] cases having both characteristic rodent brain IHC reactivity and specific reactivity to a CBA were judged as true positives. In addition, cases with a positive CBA and clinical information strongly supporting the diagnosis (e.g., meeting the criteria for definite anti-NMDAR encephalitis proposed by Graus et al.[16]) were also judged as true positives.

RESULTS

Clinical laboratory findings. Of 623 cases, the clinical laboratory reported 67 patients with NMDAR antibodies (24 CSF, 33 sera, 10 pairs), and 18 positive results for other tested antigens GAD65 (8), LGI1 (4), Caspr2 (2), and $GABA_BR$ (4). One case had both GAD65 and $GABA_BR$. The clinical laboratory reported 65 serum samples as indeterminate for NMDAR antibodies, and 32 CSF had one or more indeterminate results (24 NMDAR, 11 GAD65, 6 LGI1, 3 AMPAR, 3 Caspr2, and 2 $GABA_BR$). Indeterminate results were largely due to excessive nonspecific labeling of the cultured cells (nuclear and/or cytoplasmic constituents) using the clinical laboratory kits according to the manufacturer's recommendations, particularly with sera at 1:10 dilution.

All but 4 positive clinical laboratory findings, 2 CSF NMDAR and 2 sera NMDAR, were replicated in the research laboratory, using both fixed and live CBAs, demonstrating a high specificity for clinical laboratory kits. One CSF not replicated was likely a true positive since it was a repeat sample on a known anti-NMDAR encephalitis patient; the other CSF was likely a false positive because he did not have clinical symptoms of anti-NMDAR encephalitis, and for the other 2 sera, there was no clinical information. In addition, except for 9 sera that produced excessive background labeling, all indeterminate CSF and serum samples were resolved as positive or negative in the research laboratory by screening for IHC reactivity to brain sections and additional CBAs in reactive samples. Seven samples labeled indeterminate by the clinical laboratory were found to be positive (table 1) and the others were determined to be negative. Live CBAs for NMDAR or other antibodies were helpful for resolving cases with indeterminate results due to sera that produced excessive background labeling of fixed cells.

Research laboratory findings. We labeled sections of the rat brain with the CSF and sera of all patients tested in the clinical laboratory. The patterns of reactivity with rodent brain IHC are shown in figure 2. Rodent brain IHC was positive for 92% of the 99 samples with positive findings using the clinical testing kits. In addition, 25 other samples had IHC reactivity with a clear positive finding later established in the research laboratory. Further analysis of these samples with research CBAs detected additional positive samples: 10 NMDAR (7 sera and 3 CSF), 1 AMPAR, 3 LGI1, and 1 Caspr2. All positive results were repeated 3 times to confirm. An additional 10 samples (4 CSF and 6 sera) had positive brain section reactivity that could not be classified further, and some of these samples are undergoing additional research studies to identify novel potential antigens. These cases, if

Table 1 Characteristics of samples judged positive by the research laboratory but negative in the clinical laboratory

Diagnosis/clinical result/sample type	Positive; research tests	Clinical information
NMDAR positive/indeterminate/CSF	Brain IHC; live and fixed CBA	20-y-old, female. Initial episode with psychosis, memory failure, decreased responsiveness, and dystonia. Full recovery with immune therapy. CSF obtained during relapse with psychosis.
NMDAR positive/indeterminate/CSF	Brain IHC; live and fixed CBA	21-y-old, female. Psychosis, memory failure, decreased responsiveness, respiratory failure, and coma. Recovered with IVIg, steroids, and rituximab over 8 mo.
NMDAR positive/indeterminate/CSF	Brain IHC; fixed CBA	60-y-old, male. Personality change, pressure speech, paranoid delusions, forgetfulness, and emotional lability. Recovery over 6 mo with steroids, IVIg, rituximab.
Caspr2 positive/negative/CSF	Brain IHC; live CBA	43-y-old, female. No clinical information due to regulatory limitations.
AMPAR positive/negative/CSF	Brain IHC; live and fixed GluR1,2 CBA	77-y-old, female. Patient reported in original case series of AMPAR patients. CSF was obtained during relapse with ongoing memory and behavioral problems.
LGI1 positive/negative/CSF	Brain IHC; live CBA	52-y-old, male. Acute disorientation to space and time, generalized convulsion, insomnia, poor memory for new events, and FBDS. Improved on PLEX and steroids.
LGI1 positive/negative/CSF	Brain IHC; LGI1 CBA	65-y-old, male. New falls, confusion, poor memory for new events over several mo. Mild hyponatremia. Recovered over 2 mo with steroids.
LGI1 positive/indeterminate/CSF	Brain IHC; live CBA	72-y-old, male. Emotional lability, poor memory for new events, confusion, FBDS, and a few generalized convulsions. Recovery over 3 mo with steroids, IVIg, and rituximab.
NMDAR positive/indeterminate/serum	Brain IHC; live CBA	18-y-old, female. No clinical information due to regulatory limitations.
NMDAR positive/indeterminate (2×)/serum	Brain IHC; fixed and live CBA; CSF positive	59-y-old, female. Known anti-NMDAR encephalitis. Relapse with compulsive journal keeping, shoplifting, profound confusion, and decreased responsiveness. CSF mild lymphocytic pleocytosis. Recovery with steroids, IVIg, and rituximab.
NMDAR positive/negative/serum	Brain IHC; fixed and live CBA; CSF positive	11-y-old, female. No clinical information due to regulatory limitations.
NMDAR positive/indeterminate/serum	Brain IHC; live CBA; positive CSF	11-y-old, male. Positive CSF. No clinical information due to regulatory limitations.
NMDAR/negative/serum	Brain IHC; live CBA; positive CSF	15-y-old, female. Initial episode with seizures and behavioral problems, diagnosed with ant-NMDAR encephalitis. Prolonged hospitalization complicated by autonomic storming and seizures. Responded to IVIg, steroids, and rituximab. Persistent mild psychiatric symptoms.

Abbreviations: AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Caspr2 = contactin-associated protein-like 2; CBA = cell-based assay; FBDS = faciobrachial dystonic seizures; Ig = immunoglobulin; IHC = immunohistochemistry; LGI1 = leucine-rich glioma-inactivated 1; NMDAR = NMDA receptor; PLEX = plasma exchange.
Those with clinical information were all diagnosed by expert treating physicians.

sufficient volume was obtained, were screened with research tests for NMDAR, GluR1/GluR2, $GABA_BR$, Caspr2, LGI1, mGluR5, and $GABA_AR$ antibodies.

As outlined in the Methods section, cases with a positive CBA supported by either brain section IHC reactivity or clinical characteristics strongly suggesting the diagnosis were judged as true positives. These clinical characteristics and test results are included in table 1. The relative sensitivity of the clinical laboratory kits for the various diseases is shown in table 2.

Characteristics of positive cases. Taken together, these studies detected known autoantibodies against neuronal cell surface or synaptic proteins in 96 of 623 cases (15.4%) referred for testing due to suspected autoimmune encephalitis. NMDAR antibodies were far more common than the other antibodies in this group, particularly in younger adults. NMDAR antibodies were the only antibodies detected in patients younger than 30 years. The median age of patients with NMDAR antibodies was 19 years compared with a median age of 59 years for patients with the other antibodies. This is consistent with prior case series of patients with LGI1, Caspr2, AMPA, and GABA-B antibodies, all of which tend to occur in middle aged and older adults.[3–5,7]

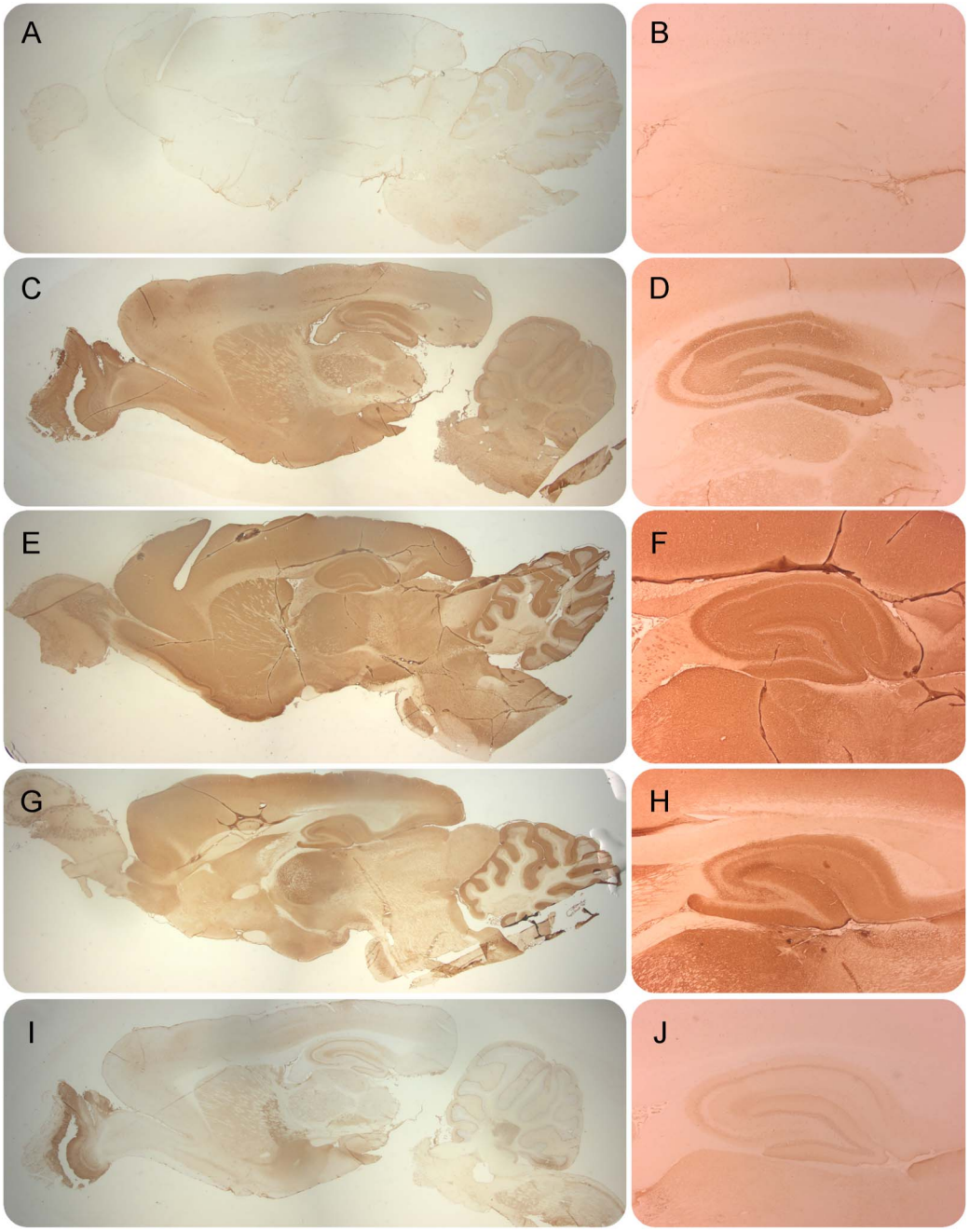
Patterns of reactivity with rodent brain IHC. The cases with reactivity on brain section IHC showed characteristic staining of the synaptic layers of the hippocampus. The patterns of NMDAR, LGI1, AMPAR, $GABA_BR$, and Caspr2 are shown in figure 2. The pattern of staining seen with GAD65 antibodies is particularly characteristic and easily differentiated from the others (figure 2). The 8 patients with positive reactivity but without known antigens showed a pattern of brain reactivity similar to the pattern of NMDAR or LGI1, but were negative for all tested antibodies, as noted above.

Sensitivity of rodent brain IHC for anti-NMDAR encephalitis. Rodent brain IHC and NMDAR CBA

on CSF have been reported to be 100% concordant in a series of 250 patients, but 15% of serum samples are negative on one or both tests.[13] In our cohort, all samples were screened directly for NMDAR antibodies using research CBAs. Of the 90 samples positive for NMDAR antibodies on CBA (clinical or research), 85 were judged as positive on rodent brain IHC, yielding a sensitivity of 94% for rodent brain IHC in detecting NMDAR antibodies. Rodent brain IHC is, as discussed above, not specific to NMDAR encephalitis and may show similar abnormalities with other synaptic CNS autoimmune conditions. Since it was impractical to perform CBAs for all antigens on every sample, the sensitivity of rodent brain IHC for the other antibodies could not be determined.

Sensitivity of the LGI1 clinical laboratory assay in CSF and serum. As noted above, only 4 of 7 LGI1 positive

Figure 2 **Patterns of reactivity observed with rat-brain IHC**



Immunohistochemistry was performed with rat brain sections using human CSF. Whole brains (left) and higher power views of the hippocampus (right) are shown for control (A, B), anti-NMDAR encephalitis (C, D), anti-LGI1 encephalitis (E, F), anti-GABA-B encephalitis (G, H), and anti-GAD65 (I, J). The cases with reactivity on brain section IHC showed characteristic staining of the synaptic layers of the hippocampus. GABA= γ-aminobutyric acid; GAD65 = glutamic acid decarboxylase; IHC = immunohistochemistry; LGI1 = leucine-rich glioma-inactivated 1; NMDAR = NMDA receptor.

Table 2 Number of true-positive and true-negative cases (as determined by the clinical testing kits and research laboratory, or research laboratory alone) along with corresponding sensitivity and specificity measures of the commercial testing kits

	True positive	True negative	Sensitivity	Specificity
NMDAR serum	50	240	86% (n = 43)	99.2% (n = 238)
NMDAR CSF	38	399	92.1% (n = 35)	99.8% (n = 398)
LGI1 CSF	8	237	62.5% (n = 5)	100% (n = 237)
Caspr2 CSF	3	242	66.7% (n = 2)	100% (n = 242)
AMPA CSF	1	422	0% (n = 0)	100% (n = 244)
$GABA_B$	4	241	100% (n = 4)	100% (n = 241)
GAD65	7	238	100% (n = 7)	100% (n = 238)

Abbreviations: AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Caspr2 = contactin-associated protein-like 2; LGI1 = leucine-rich glioma-inactivated 1; GABA= γ-aminobutyric acid; GAD65 = glutamic acid decarboxylase 65 kDa; NMDAR = NMDA receptor.

CSF samples were detected in the clinical laboratory. The clinical characteristics of these patients were consistent with anti-LGI1 encephalitis (table 1). To determine the sensitivity of this assay with serum samples, we tested 10 LGI1 positive serum samples, including 1 from a patient whose CSF was not identified using the commercial kits. Using the commercial kits, all 10 serum samples could be identified as LGI1 positive.

Sensitivity and specificity of the NMDAR serum live CBA. We examined 290 serum samples for NMDAR antibodies. Forty-one were determined positive using the commercial testing kits, and another 7 were detected using a combination of our research methods including brain tissue IHC and CBAs with live and fixed cells. Of these 48 positive samples, 37 were studied using a live cell CBA, and 34 (92%) were found to be positive with this assay.

DISCUSSION Testing in the clinical laboratory using commercially available kits detected most cases of autoimmune encephalitis due to neuronal cell surface antibodies. The tests, which could be performed rapidly, were specific but lacked sensitivity for some antigens. These kits were also prone to indeterminate results, especially with sera. Overall, 12% of all positive cases were missed by the commercial testing kits; these cases were detected only through screening with rodent brain IHC and then research-based CBAs. The validity of our diagnoses is based on the clinical information, their prior test results, the correspondence of sera/CSF results, and the concordance of brain reactivity with CBAs. The research studies are slower, more labor intensive, and more difficult to standardize but have value in evaluating these cases.

LGI1 is a particular concern, being relatively common in older adults, since 3 of 7 cases were missed in CSF (57% sensitivity). This is consistent with a prior study, reporting 53% sensitivity for LGI1 antibodies in CSF using the kits.[14] Despite multiple attempts by 2 laboratory teams knowing the expected results, these results were negative repeatedly using commercial kits, indicating that the problem is sensitivity of the test kits and not operator error. Of interest, the kits were more sensitive for LGI1 serum samples, detecting 10 of 10 samples tested. Testing both sera and CSF for LGI1 antibodies may therefore help with sensitivity, although this could introduce more indeterminate results.

Rodent brain IHC was a useful test for screening for the group of synaptic autoimmune diseases, having good specificity and sensitivity for the CNS autoantibodies included in the kits. Of interest, the cases with positive brain staining but negative laboratory test results using commercial kits consisted mostly of cases with autoantibodies that were missed using the kits, rather than patients with antibodies to rare antigens not included in the panel (mGluR5, $GABA_A$, etc.) or patients with reactivity to unknown brain antigens. In the setting of a positive IHC and negative clinical laboratory results, the most likely explanation is that the patient has one of the common diseases, but this was missed by the clinical CBAs. The smaller number of cases with reactivity to brain sections but no antigens detected on research CBAs may eventually be classified as having specific autoimmune disorders, as new antigens are discovered, or they could represent false positives of the assay. Performing rodent brain IHC may therefore be a useful complementary test to standard clinical laboratory studies. Cases with negative clinical laboratory studies but a positive IHC can then undergo additional targeted CBAs to obtain clear diagnoses and to identify other patients who may be studied to define novel synaptic autoimmune diseases.

It is well known that the average age of patients with anti-NMDAR encephalitis (about 20) is much younger than those with LGI1, Caspr2, or $GABA_BR$ antibodies (about 60 years for each). This study supports the concept that anti-NMDAR encephalitis, in children and young adults (under 30 years old), is far more common than the other neuronal cell surface antibody-associated diseases combined. Although other neuronal cell surface autoantibodies have been rarely reported in children, the primary step needed to improve diagnosis in children is therefore to use the best possible methods to detect NMDAR antibodies.

An important limitation of this study is that it focused on autoimmune encephalidities with antibodies against neuronal cell surface proteins. In this group of diseases, glycine antibodies, a synaptic cell surface antigen associated with stiff-person syndrome and progressive encephalomyelitis with rigidity and myoclonus, may not be detected well by rodent brain

IHC and specific CBAs are necessary when this disorder is suspected. Of course, other types of immune mechanisms may also cause encephalitis but not be detected by any of these methods. In addition, we studied patients whose treating physicians suspected autoimmune encephalitis and ordered testing through our laboratory. Patients with atypical presentations may therefore be under-represented.

Clinicians should bear in mind the limitations of the various antibody tests for autoimmune encephalitis. Negative results on the standard kits, even with CSF, cannot, unfortunately, be taken as definitive evidence against the synaptic autoimmune diseases. Repeat studies using brain IHC and, in cases with brain reactivity, additional CBAs may therefore be useful for evaluating cases in which there is a strong suspicion of the disorders but confirmation is not obtained. Clinicians should consider referral to a reference laboratory in these cases.[15]

AUTHOR CONTRIBUTIONS

L.M.: analysis of data and drafting the manuscript. J.Z., M.G., A.C., and J.Z.: analysis of data. M.K. and E.L.: analysis of data and drafting the manuscript.

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DISCLOSURE

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