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RESEARCH ARTICLE

"Obvious" indications for Neural antibody testing in Epilepsy or Seizures: The ONES checklist

Yiu-Chia Chang[1] | **Maryam Nabavi Nouri**[1,2] | **Seyed Mirsattari**[1,3] | **Jorge G. Burneo**[1,4] | **Adrian Budhram**[1,5]

[1]Department of Clinical Neurological Sciences, London Health Sciences Centre, Western University, London, Ontario, Canada

[2]Department of Paediatrics, London Health Sciences Centre, Western University, London, Ontario, Canada

[3]Department of Medical Biophysics, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada

[4]Neuroepidemiology Unit, Schulich School of Medicine & Dentistry, Western University, London, Ontario, Canada

[5]Department of Pathology and Laboratory Medicine, London Health Sciences Centre, Western University, London, Ontario, Canada

Correspondence
Adrian Budhram, Department of Clinical Neurological Sciences, London Health Sciences Centre, 339 Windermere Road, N6A 5A5 London, ON, Canada.
Email: adrian.budhram@lhsc.on.ca

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Abstract

Objective: Numerous predictive scores have been developed to help determine which patients with epilepsy or seizures of unknown etiology should undergo neural antibody testing. However, their diagnostic advantage compared to only performing testing in patients with "obvious" indications (e.g., broader features of autoimmune encephalitis, characteristic seizure semiologies) requires further study. We aimed to develop a checklist that identifies patients who have "obvious" indications for neural antibody testing and to compare its diagnostic performance to predictive scores.

Methods: We developed the "Obvious" indications for Neural antibody testing in Epilepsy or Seizures (ONES) checklist through literature review. We then retrospectively reviewed patients who underwent neural antibody testing for epilepsy or seizures at our center between March 2019 and January 2021, to determine and compare the sensitivity and specificity of the ONES checklist to the recently proposed Antibody Prevalence in Epilepsy and Encephalopathy (APE2)/Antibodies Contributing to Focal Epilepsy Signs and Symptoms (ACES) reflex score.

Results: One-hundred seventy patients who underwent neural antibody testing for epilepsy or seizures were identified. Seventy-four of 170 (43.5%) with a known etiology were excluded from sensitivity/specificity analyses; none had a true-positive neural antibody. Of the 96 patients with an unknown etiology, 14 (15%) had a true-positive neural antibody. The proportion of false-positives was significantly higher among patients with a known etiology (3/3, 100%) compared to an unknown etiology (2/16, 13%; $p = .01$). There was no significant difference of the APE2/ACES reflex score compared to the ONES checklist with regard to sensitivity (93% for both, $p > .99$) or specificity (71% vs. 78%, $p = .18$) for true-positive neural antibodies.

Significance: Compared to only performing neural antibody testing in patients with epilepsy or seizures of unknown etiology who have "obvious" indications, predictive scores confer no clear diagnostic advantage. Prespecified definitions of

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what constitutes a true-positive neural antibody is required in future studies to avoid false-positives that can confound results.

KEYWORDS

acute symptomatic seizures secondary to autoimmune encephalitis, autoimmune encephalitis, autoimmune epilepsy, autoimmune seizures, autoimmune-associated epilepsy

1 | INTRODUCTION

Among patients with epilepsy, there has been increasing interest in the detection of neural antibodies that indicate an immune etiology.[1,2] The Antibody Prevalence in Epilepsy score, later revised to the Antibody Prevalence in Epilepsy and Encephalopathy (APE2) score, was developed to help determine which patients benefit most from neural antibody testing to diagnose "autoimmune epilepsy."[3–6] However, the appropriateness of the term "autoimmune epilepsy" has been questioned, and the need for its conceptual distinction from autoimmune encephalitis has been emphasized.[7–9] Many clinical and neuroimaging items included in predictive scores that are intended to be applied to patients with epilepsy or seizures of unknown etiology are derived from features of autoimmune encephalitis, for which dedicated diagnostic criteria exist.[8,10] This is reflective of the finding that whereas some patients with neural antibody-associated disease may present with seizures in relative isolation, a substantial proportion ultimately develop broader features of autoimmune encephalitis.[8,10,11] It has recently been suggested that patients who have features that are clearly suspicious for neural antibody positivity, such as those indicative of a broader encephalitis, should be excluded from investigations of neural antibody testing in epilepsy or seizures of unknown etiology.[7] This was attempted in the Antibodies Contributing to Focal Epilepsy Signs and Symptoms (ACES) study, which excluded patients who had features suggesting an immune etiology that were recognized by the referring clinician.[12] Expectedly, the rate of neural antibody positivity in the ACES study was low, at 3.4%, which is in contrast to other studies reporting neural antibody positivity in up to 31.5% of patients with seizures of unknown etiology that did not have this exclusion criterion.[12,13] Remarkably, however, among the patients in the ACES study who were neural antibody-positive, virtually all of them had neuroimaging findings, clinical symptoms, seizure semiologies, or biochemical abnormalities that were characteristic of neural antibody-associated presentations, but went unrecognized by the referring clinician, which led to study inclusion.[12] This finding highlights the need for an assessment tool that can be used to effectively identify patients with epilepsy or seizures of unknown etiology who have presentations that should raise suspicion for neural antibody positivity, and thus "obviously" merit neural antibody testing. It also lends credence to the hypothesis that after systematic identification of such patients for testing, there may be no additional diagnostic utility of predictive scores.

Key Points

- We developed the ONES checklist through literature review
- There was no difference in sensitivity or specificity of the ONES checklist compared to the recently proposed APE2/ACES reflex score
- False-positive neural antibody results can occur in patients with epilepsy or seizures, the proportion of which is higher in those with known etiology
- Studies are needed to clarify which patients, if any, do not have "obvious" indications for testing but would benefit from predictive scores
- Defining what constitutes a true-positive neural antibody is needed in future studies to avoid false-positives that can confound results

To evaluate this hypothesis, we developed the "Obvious" indications for Neural antibody testing in Epilepsy or Seizures (ONES) checklist through literature review. It consists of presentations that are individually suspicious for neural antibody positivity, and should therefore prompt consideration of testing. The phrase "epilepsy or seizures" was chosen to highlight the uncertainty surrounding the most appropriate terminology prior to completion of neural antibody testing, the result of which can provide insight into underlying seizure pathophysiology and the likelihood of enduring seizure predisposition.[8,9] To evaluate the diagnostic performance of the ONES checklist, we then retrospectively reviewed patients assessed at our center to determine

and compare the sensitivity and specificity of the ONES checklist to the APE2/ACES reflex score, which was recently proposed to optimize the performance of predictive scores.[7]

2 | MATERIALS AND METHODS

2.1 | "Obvious" indications for neural antibody testing in patients with epilepsy or seizures: Development of ONES checklist

One of the authors with formal training in autoimmune neurology (A.B.) drafted the ONES checklist, based on review of the literature pertaining to neural antibody-associated disease and alternative diagnostic considerations. Details regarding the rationale behind each item are provided in Appendix S1 with references. Although paired serum and cerebrospinal fluid (CSF) testing is generally recommended once the decision is made to pursue neural antibody testing,[2] CSF profile (e.g., white blood cell count, protein, oligoclonal bands) was not incorporated in the ONES checklist because of the infrequency of lumbar puncture in epilepsy evaluations.[14] The checklist was brought forth to three of the other authors with formal training in epilepsy (M.N.N, S.M., J.G.B.), and each item was confirmed for inclusion after discussion to achieve consensus. The ONES checklist is shown in Table 1. A guide to its operationalization is provided in Table 2.

Special consideration was given to testing for antibodies against myelin oligodendrocyte glycoprotein (MOG) and glycine receptor (GlyR). Although some have recommended expanded neural antibody testing that includes testing for anti-MOG and anti-GlyR routinely in patients with epilepsy or seizures,[13] there are potential issues with this approach. The positive predictive value of anti-MOG has been shown to decrease substantially when testing patients with atypical phenotypes for MOG-associated disease.[15] Meanwhile, anti-GlyR has specificity for stiff-person spectrum disorders (SPSD)/progressive encephalomyelitis with rigidity and myoclonus (PERM), but low levels have been found in diverse syndromes, and false-positivity has been reported in up to 4% of healthy controls.[16,17] Anti-GlyR has also been detected in a variety of epileptic presentations, sometimes without features of SPSD/PERM and with variable response to immunotherapy, raising further questions regarding its clinical significance in this context.[18,19] For these reasons, the ONES checklist restricts testing for anti-MOG and anti-GlyR to patients with characteristic features of these antibodies, which is intended to avoid false-positives associated with more indiscriminate testing.

2.2 | Evaluating diagnostic performance of ONES checklist compared to APE2/ACES reflex score

Two authors (A.B. and Y.-C.C.) independently reviewed the electronic medical records (EMRs) of all patients at London Health Sciences Centre who had serum and/or CSF neural antibody testing ordered as part of neurological evaluation for epilepsy or seizures in the outpatient clinic, elective admission (epilepsy monitoring unit [EMU]) setting, inpatient ward, or intensive care unit (ICU) between March 2019 and January 2021, inclusive. Patients with a more likely nonimmune etiology (e.g., idiopathic generalized/genetic epilepsy, toxic/metabolic derangement, malformation of cortical development), or with a more likely immune etiology not associated with neural antibody positivity (e.g., Rasmussen encephalitis, multiple sclerosis), were classified as having a known etiology and excluded from sensitivity/specificity analyses as shown in Figure 1. The number of true-positive neural antibody results and the proportion of false-positive results (described further below) in patients with a known etiology were compared to those with an unknown etiology to assess for any significant difference. Clinical data required to complete the APE2 score, ACES score, and ONES checklist were independently extracted from the EMR of each patient with epilepsy or seizures of unknown etiology by A.B and Y.-C.C., with discussion to achieve consensus in discrepant cases. A "Yes" to one or more items was classified as positive for the ONES checklist, and an APE2 score of $\geq$4 or an APE2 score of $\leq$3 followed by a ACES score $\geq$2 was classified as positive for the APE2/ACES reflex score.[7] Each patient's neural antibody status classification, ONES checklist classification, and APE2/ACES reflex score classification as positive or negative (Figure 1) allowed for sensitivity and specificity calculations.

2.3 | Neural antibody test methodologies employed

Patients underwent comprehensive panel-based testing that included composite mouse brain/nonbrain tissue indirect immunofluorescence (TIIF) to screen for neural-specific antibodies against intracellular and extracellular antigens as previously described,[20] fixed cell-based assays (CBAs) for anti-N-methyl-D-aspartate receptor (NMDAR), leucine-rich glioma-inactivated 1 (LGI1), contactin-associated protein-like 2 (CASPR2), dipeptidyl-peptidase-like protein 6, γ-aminobutyric acid type B receptor ($GABA_BR$), and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (EUROIMMUN), and testing for high levels of antibodies against glutamic acid decarboxylase

TABLE 1 ONES checklist[a]

1a) Perform panel-based neural antibody testing including anti-MOG if any of the following are present:			
Are any of the following present?	Yes	No	Antibody/antibodies of most relevance
Brain magnetic resonance imaging[b]			
Cortical T2-FLAIR hyperintense lesion(s) with or without involvement of the underlying white matter, in temporal relation to seizure onset			Anti-MOG, NMDAR, $GABA_AR$, mGluR5
Large (>1–2 cm) T2-FLAIR hyperintense lesion(s) involving the white matter suggestive of non-MS demyelination, in temporal relation to seizure onset			Anti-MOG, may overlap with anti-NMDAR
Clinical			
Optic neuropathy or myelopathy of unknown etiology, in temporal relation to seizure onset			Anti-MOG, may overlap with anti-NMDAR
1b) Perform panel-based neural antibody testing including anti-GlyR if the following is present:			
Are any of the following present?	Yes	No	Antibody/antibodies of most relevance
Clinical			
Prominent stiffness, spasms, rigidity, and/or hyperekplexia of unknown etiology, in temporal relation to seizure onset			Anti-GlyR, amphiphysin, DPPX, GAD65
2) If all of the above features are absent, perform panel-based neural antibody testing excluding anti-MOG and anti-GlyR if any of the following are present:			
Are any of the following present?	Yes	No	Antibody/antibodies of most relevance
Brain magnetic resonance imaging[b]			
T2-FLAIR hyperintensity restricted to the medial temporal lobe(s) without atrophy, in temporal relation to seizure onset			Various
Linear radial perivascular enhancement, in temporal relation to seizure onset			Anti-GFAP, may overlap with anti-NMDAR
Biochemical			
New (within 1 year), refractory, temporal lobe or presumed temporal lobe seizures, with serum sodium < 130 mEq/L of unknown etiology[c,d]			Anti-LGI1
Clinical/semiological/electroencephalographical			
Distinguishable central or peripheral nervous system dysfunction of unknown etiology, in temporal relation to seizure onset[e]			Various
Musicogenic seizures			Anti-GAD65
Faciobrachial dystonic seizures[f]			Anti-LGI1
New (within 1 year), refractory, temporal lobe or presumed temporal lobe seizures, with pilomotor seizures[d]			Anti-LGI1
New (within 1 year), refractory, temporal lobe or presumed temporal lobe seizures, with paroxysmal dizziness spells[d]			Anti-LGI1
New (within 1 year), refractory, temporal lobe or presumed temporal lobe seizures, beginning after 50 years of age[d]			Anti-LGI1, CASPR2
Refractory temporal lobe or presumed temporal lobe seizures, with anti-GAD65-associated systemic autoimmunity[d,g]			Anti-GAD65
Historical			
New (within 1 year) seizures, beginning within 2 years of tumor diagnosis[h]			Various
New (within 1 year) seizures, beginning within 1 year of last immune checkpoint inhibitor treatment			Various

(Continues)

TABLE 1 (Continued)

2) If all of the above features are absent, perform panel-based neural antibody testing excluding anti-MOG and anti-GlyR if any of the following are present:	
New (within 1 year) seizures, beginning or worsening within 3 months of last antiviral treatment for herpes simplex virus encephalitis[i]	Anti-NMDAR

Abbreviations: CASPR2, contactin-associated protein-like 2; DPPX, dipeptidyl-peptidase-like protein 6; FBDS, faciobrachial dystonic seizures; FLAIR, fluid-attenuated inversion recovery; $GABA_AR$, γ-aminobutyric acid type A receptor; GAD65, glutamic acid decarboxylase 65; GFAP, glial fibrillary acidic protein; GlyR, glycine receptor; HSV, herpes simplex virus; LGI1, leucine-rich glioma-inactivated 1; mGluR5, metabotropic glutamate receptor 5; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NMDAR, N-methyl-D-aspartate receptor; ONES, "Obvious" indications for Neural antibody testing in Epilepsy or Seizures.

[a]The ONES checklist should only be used in patients with epilepsy or seizures of unknown etiology. When pursuing neural antibody testing, serum and cerebrospinal fluid testing is generally recommended to maximize sensitivity and specificity.

[b]All listed neuroimaging abnormalities presume the imaging appearance is not more suggestive of an alternative etiology (e.g., tumor, infection, toxic/metabolic, hypoxic/ischemic, seizure-related change).

[c]Serum sodium < 130 mEq/L of unknown etiology requires exclusion of competing etiologies (e.g., hypovolemia, medication effect including antiseizure medications and diuretics, heart failure, liver or renal disease, spurious laboratory result).

[d]Temporal lobe seizures with involvement of adjacent regions (e.g., temporoperisylvian) are included.

[e]Examples include cognitive impairment, behavioral changes, psychiatric symptoms, aphasia/speech disturbances, sleep disturbances, movement disorders, brainstem/cerebellar dysfunction, dysautonomia, radiculopathy/neuropathy, neuropathic pain, and peripheral nerve hyperexcitability. For optic neuropathy, myelopathy, or features of stiff-person spectrum disorders/progressive encephalomyelitis with rigidity and myoclonus, see preceding items.

[f]Includes FBDS with or without basal ganglia T1/T2 hyperintensity, the finding of which on neuroimaging should prompt careful case review for FBDS in the appropriate clinical context.

[g]Examples of anti-GAD65-associated systemic autoimmunity include type 1 diabetes mellitus, autoimmune thyroid disease, pernicious anemia, vitiligo, celiac disease, and Addison disease.

[h]Excluding keratinocyte carcinoma, and primary or secondary brain tumor. [Correction added on 17 May 2022, after first online publication: In the preceding sentence, the phrase "nonmelanoma skin cancer" has been replaced with the term "keratinocyte carcinoma".]

[i]Documentation of negative cerebrospinal fluid polymerase chain reaction for HSV is required. The phrase "beginning or worsening" of seizures is intended to acknowledge that patients may also have seizures related to initial HSV encephalitis, which must be distinguished from new or worsening seizures potentially attributable to post-HSV autoimmune encephalitis.

65 (GAD65). Between March 2019 and September 2020, high levels of anti-GAD65 were determined by fixed CBA (EUROIMMUN), whereas from October 2020 onward this was replaced by enzyme-linked immunosorbent assay (ELISA) using a serum cut-off of >10 000 IU/ml and a CSF cutoff of >100 IU/ml (KRONUS).[21,22] Only high levels of anti-GAD65 were considered positive. Patients with TIIF staining indicative of anti-Hu, Yo, Ri, Ma2, amphiphysin, collapsin response mediator protein 5/CV2, or SOX1 were considered positive only if confirmed by immunoblot (EUROIMMUN). Patients with TIIF staining indicative of antibodies against $GABA_AR$ or metabotropic glutamate receptor type 5 were planned to be reflexed to confirmatory fixed CBA (EUROIMMUN), whereas patients with TIIF staining indicative of anti-glial fibrillary acidic protein were planned to be sent out for confirmatory CBA (Mayo Clinic).[23–26] Any patient with neural-specific staining on TIIF that was not indicative of any of the aforementioned antibodies was considered to have an unclassified neural-specific antibody. Panel-based testing therefore incorporated use of TIIF, CBAs, assays to determine high levels of anti-GAD65, and demonstration of paraneoplastic antibody positivity by two assays, in keeping with recommended best practices.[7,22,27,28] All fixed CBAs were performed at a dilution of 1:10 for serum and undiluted for CSF. Isolated serum weak positivity for anti-NMDAR or anti-CASPR2 by CBA at 1:10 dilution was considered a false-positive and thus classified as a negative neural antibody result for the purposes of sensitivity/specificity analyses, given previous findings by us and other groups that suggest false-positivity/clinical irrelevance when detecting only low serum levels of these antibodies by CBA.[29–31] All other neural antibody results determined as outlined above were considered true-positive results. Separate from panel-based neural antibody testing, testing for anti-MOG was performed by fixed CBA (EUROIMMUN), and anti-GlyR was planned to be sent out for CBA (Mayo Clinic)[17] in patients with characteristic features of these antibodies.

2.4 | Statistical methodologies employed

Sensitivities, specificities, and their 95% confidence intervals of the APE2 score, ACES score, APE2/ACES reflex score, and ONES checklist for true-positive neural antibodies were determined. The McNemar test was used to compare significance of differences in sensitivity and specificity between the APE2/ACES reflex score and ONES checklist.[32] Categorical variables were compared using Fisher exact test. Probability

TABLE 2 Guide to operationalization of the ONES checklist

1. The ONES checklist should only be used in patients with epilepsy or seizures of unknown etiology, after appropriate evaluation (e.g., clinical history, physical examination, brain magnetic resonance imaging, electroencephalography) to exclude more likely alternative diagnoses.
2. The ONES checklist restricts anti-MOG and anti-GlyR testing to patients with typical disease phenotypes. The tiered approach to the checklist should be followed, with progression from one tier to the next only if the answer is "No" to all preceding items. In the rare patient with seizures and features of both anti-MOG and anti-GlyR, panel-based testing that includes both of these antibodies should be performed, hence their listing as (1a) and (1b) on the ONES checklist.
3. Nervous system dysfunction or neuroimaging findings that are ictal or postictal phenomena should not be the reason for answering "Yes" to relevant items on the ONES checklist. Close clinical and/or neuroimaging follow-up can aid in making these distinctions and is encouraged.
4. The phrase "in temporal relation to seizure onset" used throughout the ONES checklist emphasizes the importance of evaluating clinical symptoms and neuroimaging findings as they relate to seizure onset, because a temporal relationship supports a shared etiology.
5. The term "distinguishable" and the phrase "of unknown etiology" used throughout the ONES checklist are intended to emphasize the importance of distinguishing dysfunction possibly attributable to neural antibody associated-disease not only from alternative diagnoses, but also from neuropsychiatric symptoms that are common among patients with epilepsy. Inquiry into the impact of such symptoms on activities of daily living, collection of ancillary clinical history from friends or relatives, and formal cognitive assessment/neuropsychometric testing can help make these determinations and are encouraged.
6. The term "refractory" refers to failure of two or more antiseizure medications (either as monotherapies or in combination). In patients with high seizure frequency, timely identification using the ONES checklist relies on expedient determination of seizure refractoriness.
7. Medial temporal lobe T2-FLAIR hyperintensity with atrophy suggestive of MTS is not included in the ONES checklist, because of the frequency of MTS in nonimmune temporal lobe epilepsy. In patients with MTS, however, it is critical to review any previously available neuroimaging to look for T2-FLAIR hyperintensity restricted to the medial temporal lobe(s) without atrophy that is suggestive of autoimmune limbic encephalitis in temporal relation to seizure onset, which is included in the ONES checklist.
8. Where "temporal lobe" seizure localization is specified, review of clinical information (e.g., seizure semiology) and ancillary test data (e.g., electroencephalography) is critical to identify supportive evidence for this localization. Temporal lobe seizures with involvement of adjacent regions (e.g., temporoperisylvian) are included. Thorough review is particularly important for patients with recurrent generalized tonic–clonic seizures, in whom temporal lobe seizure origin may not be immediately apparent. (Continues)

TABLE 2 (Continued)

9. The term "presumed temporal lobe seizures" is intended to identify rare patients with recurrent seizures for whom there are no clinical or ancillary test data that definitively aid in seizure localization. These are patients who could, however, reasonably be presumed to have temporal lobe seizures in the absence of evidence to suggest otherwise. For this reason, patients with non-temporal lobe symptoms/semiologies, or electroencephalographic findings suggesting exclusively extratemporal/independent extratemporal multifocal spike foci should not be considered to have "presumed temporal lobe seizures."
10. In patients with seizures and one or more historical features, clinicians are likely to pursue neural antibody testing even prior to definitive determination of seizure localization or refractoriness, so no qualifiers regarding these aspects are included. However, the use of the word "seizures" (plural) should be kept in mind, to avoid incorrect application of these items to single provoked seizures that may occur in this setting. Patients with a single seizure and historical feature(s) may still be considered for neural antibody testing, but often have other items on the checklist that raise suspicion for neural antibody positivity (e.g., other nervous system dysfunction, neuroimaging abnormalities). Thorough review to exclude more likely alternative diagnoses is particularly important in these medically complex patients.

Abbreviations: FLAIR, fluid-attenuated inversion recovery; GlyR, glycine receptor; MOG, myelin oligodendrocyte glycoprotein; MTS, mesial temporal sclerosis; ONES, "Obvious" indications for Neural antibody testing in Epilepsy or Seizures (ONES).

values of <.05 were considered statistically significant. Analyses were performed using SAS Studio.

3 | RESULTS

3.1 | True-positive neural antibody results were not observed in patients with epilepsy or seizures of known etiology

One-hundred seventy patients underwent neural antibody testing for epilepsy or seizures. Seventy-four of 170 (43.5%) who were classified as having a known etiology were excluded from sensitivity/specificity analyses (Figure 1), none of whom had a true-positive neural antibody result.

3.2 | Patients with epilepsy or seizures of unknown etiology who underwent neural antibody testing were predominantly adults evaluated in outpatient/elective admission (EMU) setting

Of the remaining 96 patients with epilepsy or seizures of unknown etiology, 77 (80%) were adults (age ≥ 18 years)

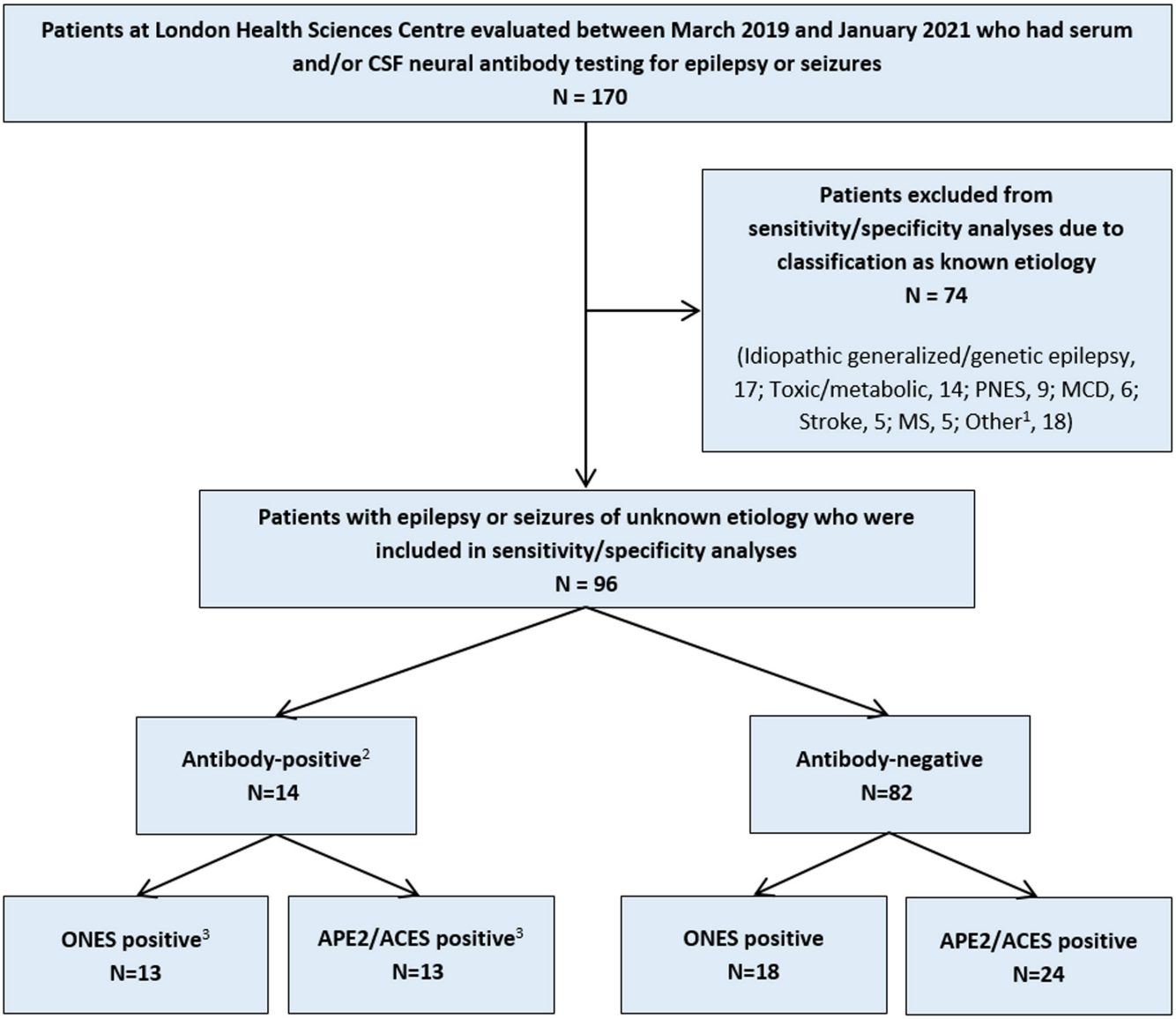

FIGURE 1 Identification of patients for inclusion in specificity/sensitivity analyses and their classifications.
[1]Other known etiologies included Rasmussen encephalitis ($n = 3$), vasculitis ($n = 2$), neurodegenerative ($n = 2$), infectious ($n = 2$), developmental/epileptic encephalopathy ($n = 2$), posterior reversible encephalopathy syndrome ($n = 2$), posttraumatic ($n = 1$), cavernous malformation ($n = 1$), glioma ($n = 1$), delayed radiation-induced leukoencephalopathy (1), and chronic-appearing frontal lesion not otherwise specified ($n = 1$).
[2]Only patients with true-positive neural antibody results (see text) were classified as neural antibody-positive for sensitivity/specificity analyses. True-positive neural antibody results consisted of anti-leucine-rich glioma-inactivated 1 ($n = 5$), anti-glutamic acid decarboxylase 65 (GAD65; $n = 3$), anti-myelin oligodendrocyte glycoprotein ($n = 2$), anti-contactin-associated protein-like 2 (CASPR2; $n = 2$), anti-N-methyl-D-aspartate receptor ($n = 1$), and unclassified neural-specific antibody ($n = 1$). False-positive neural antibody results (classified as neural antibody-negative for sensitivity/specificity analyses) consisted of isolated weak serum positivity for anti-CASPR2 ($n = 2$).
[3]One anti-GAD65 patient who was negative by both the "Obvious" indications for Neural antibody testing in Epilepsy or Seizures (ONES) checklist and the Antibody Prevalence in Epilepsy and Encephalopathy (APE2)/Antibodies Contributing to Focal Epilepsy Signs and Symptoms (ACES) reflex score is described in the text.
CSF, cerebrospinal fluid; MCD, malformation of cortical development; MS, multiple sclerosis; PNES, psychogenic nonepileptic seizures

at time of seizure onset and 44 (46%) were female. The median age of seizure onset was 28 years (range = 1–76 years). Seventy-one of 96 (74%) were evaluated in the outpatient/elective admission (EMU) setting, whereas 25 of 96 (26%) were evaluated in the inpatient ward/ICU setting. The median time from seizure onset to neural antibody testing was 2 years (range = 0–41 years), and 36 of 96 (38%) were tested within 1 year of seizure onset. Forty

of 96 (42%) underwent serum and CSF testing, 54 of 96 (56%) underwent serum testing only, and two of 96 (2%) underwent CSF testing only.

3.3 | True-positive neural antibody results were observed in 15% of patients with epilepsy or seizures of unknown etiology

Fourteen of 96 patients with epilepsy or seizures of unknown etiology (15%) had a true-positive neural antibody result (anti-LGI1, $n = 5$; anti-GAD65, $n = 3$; anti-MOG, $n = 2$; anti-CASPR2, $n = 2$; anti-NMDAR, $n = 1$; unclassified neural-specific antibody, $n = 1$). The patient with an unclassified neural-specific antibody met diagnostic criteria for definite autoimmune limbic encephalitis.[10] The number of true-positive neural antibody results was significantly higher among patients classified as having an unknown etiology for epilepsy or seizures (14/96, 15%) compared to a known etiology (0/74, 0%; $p = .0003$).

3.4 | Proportion of false-positive neural antibody results was significantly higher in patients with epilepsy or seizures of known etiology

Three patients with epilepsy or seizures of known etiology were considered false-positives based on isolated weak serum staining for anti-NMDAR or anti-CASPR2 by CBA (malformation of cortical development, $n = 1$; neurodegenerative, $n = 1$; delayed radiation-induced leukoencephalopathy, $n = 1$). Meanwhile, two patients with epilepsy or seizures of unknown etiology were considered false-positives based on isolated weak serum staining for anti-CASPR2 by CBA (medically controlled temporal lobe epilepsy without other clinical features of autoimmune encephalitis, $n = 2$). The proportion of positive results that were considered false-positives was significantly higher among patients classified as having a known etiology for epilepsy or seizures (3/3, 100%) compared to an unknown etiology (2/16, 13%; $p = .01$).

3.5 | No significant difference was found when comparing sensitivity and specificity of APE2/ACES reflex score to ONES checklist

There was no statistically significant difference found when comparing the sensitivity of the APE2/ACES reflex score to the ONES checklist for true-positive neural antibodies (93%, 95% confidence interval [CI] = 79%–100% for both, $p > .99$). One patient with new onset temporal lobe seizures, who was unable to undergo brain magnetic resonance imaging due to deep brain stimulator implantation for anorexia nervosa, had high levels of anti-GAD65 and was missed by both the APE2/ACES reflex score and ONES checklist. Her APE2 score was 3 (1 point for new onset seizure activity, 2 points for elevated CSF protein) and her ACES score was 1 (1 point for autoimmune diseases, vitiligo). This patient had ongoing seizures despite a therapeutic dose of lacosamide and monthly intravenous immunoglobulin, but deferred further antiseizure medication or immunotherapy. If the patient had trialed and failed another antiseizure medication (thus classifying her as refractory), she would have been captured by both the APE2/ACES reflex score (APE2 score = 5) and the ONES checklist (refractory temporal lobe seizures with anti-GAD65-associated systemic autoimmunity). There was also no statistically significant difference found when comparing the specificity of the APE2/ACES reflex score (71%, 95% CI = 61%–81%) to the ONES checklist (78%, 95% CI = 69%–87%) for true-positive neural antibodies ($p = .18$). Sensitivities and specificities are provided in Table 3. For each item of the ONES checklist, the proportion of patients with a positive checklist item who had true-positive neural antibodies is shown in Table 4.

TABLE 3 Sensitivities and specificities of predictive scores and the ONES checklist for neural antibody positivity

	APE2 score	ACES score	APE2/ACES reflex score	ONES checklist	p^a
Sensitivity, % (95% CI)	86 (67–100)	64 (39–89)	93 (79–100)	93 (79–100)	>.99
Specificity, % (95% CI)	72 (62–82)	90 (84–97)	71 (61–81)	78 (69–87)	.18

Abbreviations: ACES, Antibodies Contributing to Focal Epilepsy Signs and Symptoms; APE2, Antibody Prevalence in Epilepsy and Encephalopathy; CI, confidence interval; ONES, "Obvious" indications for Neural antibody testing in Epilepsy or Seizures.

aProbability values are for comparisons of sensitivity and specificity of APE2/ACES reflex score to ONES checklist.

TABLE 4 Proportion of patients with a positive antibody for each ONES checklist item

ONES checklist item	Proportion of patients with item who had a positive antibody[a]
Cortical T2-FLAIR hyperintense lesion(s) with or without involvement of the underlying white matter, in temporal relation to seizure onset	3/7 (43%)[b]
Large (>1–2 cm) T2-FLAIR hyperintense lesion(s) involving the white matter suggestive of non-MS demyelination, in temporal relation to seizure onset	0/0 (-)
Optic neuropathy or myelopathy of unknown etiology, in temporal relation to seizure onset	0/0 (-)
Prominent stiffness, spasms, rigidity, and/or hyperekplexia of unknown etiology, in temporal relation to seizure onset	0/0 (-)
T2-FLAIR hyperintensity restricted to the medial temporal lobe(s) without atrophy, in temporal relation to seizure onset	4/8 (50%)[b]
Linear radial perivascular enhancement, in temporal relation to seizure onset	0/0 (-)
New (within 1 year), refractory, temporal lobe or presumed temporal lobe seizures, with serum sodium < 130 mEq/L of unknown etiology	0/0 (-)
Distinguishable central or peripheral nervous system dysfunction of unknown etiology, in temporal relation to seizure onset	11/21 (52%)[c]
Musicogenic seizures	0/1 (0%)[d]
Faciobrachial dystonic seizures	2/2 (100%)
New (within 1 year), refractory, temporal lobe or presumed temporal lobe seizures, with pilomotor seizures	0/0 (-)
New (within 1 year), refractory, temporal lobe or presumed temporal lobe seizures, with paroxysmal dizziness spells	0/0 (-)
New (within 1 year), refractory, temporal lobe or presumed temporal lobe seizures, beginning after 50 years of age	1/1 (100%)
Refractory temporal lobe or presumed temporal lobe seizures, with anti-GAD65-associated systemic autoimmunity	2/3 (67%)
New (within 1 year) seizures, beginning within 2 years of tumor diagnosis	0/0 (-)
New (within 1 year) seizures, beginning within 1 year of last immune checkpoint inhibitor treatment	0/0 (-)
New (within 1 year) seizures, beginning or worsening within 3 months of last antiviral treatment for herpes simplex virus encephalitis	0/0 (-)

Abbreviations: FLAIR, fluid-attenuated inversion recovery; GAD65, glutamic acid decarboxylase 65; MS, multiple sclerosis; ONES, "Obvious" indications for Neural antibody testing in Epilepsy or Seizures.

aEleven patients were positive for more than one item on the ONES checklist. Only true-positive neural antibodies are considered positive antibodies for the purposes of this table (see text for more information).

bOne patient with cortical T2-FLAIR hyperintensity and two patients with medial temporal lobe T2-FLAIR hyperintensity without atrophy had neuroimaging abnormalities that were possibly seizure-related changes, but lack of close (e.g., within 4 weeks) neuroimaging follow-up to assess for resolution resulted in findings being considered of unknown onset. None of these three patients had a true-positive neural antibody result.

cIncluded cognitive impairment (13 patients), behavioral change/cognitive impairment (two patients), aphasia (two patients), behavioral change/cognitive impairment/dysautonomia (one patient), behavioral change (one patient), psychosis (one patient), and visual field deficit (one patient).

dOne patient had musicogenic seizures as well as seizures triggered by tactile stimuli, suggesting a broader reflex epilepsy.

4 | DISCUSSION

We found no clear diagnostic advantage of the APE2/ACES reflex score when compared to only performing neural antibody testing in patients who had "obvious" indications for testing as identified by the ONES checklist, with no significant difference in sensitivity or specificity for true-positive neural antibodies. Benefits of the ONES checklist are its relative ease of use compared to sequential predictive scores and its highlighting of presentations suspicious for neural antibody positivity that may go unrecognized by clinicians.[12] Our study suggests that there is no additional diagnostic utility of currently available predictive scores after the systematic identification of patients with "obvious" indications for neural antibody testing. This finding should be incorporated into the design of future studies that aim to investigate neural antibody prevalence, predictive scores for neural antibody

testing, and novel immunologic biomarkers in patients with epilepsy or seizures.[7]

It should be emphasized that neural antibody positivity is not the diagnostic gold standard for all neuroinflammatory diseases that can cause epilepsy or seizures, some of which may have immune mechanisms independent from autoantibody production that underpin their pathogenesis.[33] Therefore, patient exclusion by the ONES checklist does not exclude the possibility of an immune etiology for epilepsy or seizures. As an example, concern for an immune etiology may be raised in patients who present with epilepsia partialis continua (EPC), given the association of EPC with neuroinflammatory diseases such as Rasmussen encephalitis and, rarely, multiple sclerosis.[34,35] However, outside of patients who have cortical T2-fluid-attenuated inversion recovery hyperintensity, which is included in the ONES checklist primarily because of its association with anti-MOG, the presentation of EPC in isolation has not been reproducibly associated with neural antibody positivity and is thus not included in the ONES checklist.[2,35–37] This example highlights the primary intent of the ONES checklist, which is to identify patients with epilepsy or seizures of unknown etiology who have neural antibody-associated disease specifically, and not all immune causes more generally; it for this reason that patients with non-neural antibody-associated forms of neuroinflammatory disease (e.g., Rasmussen encephalitis, multiple sclerosis) were classified as having epilepsy or seizures of known etiology in this study and excluded from sensitivity/specificity analyses. Because a negative neural antibody result in isolation cannot definitively exclude an immune etiology for epilepsy or seizures, consideration of this diagnostic possibility should persist in patients in whom there is a high index of suspicion clinically; in such patients, the judicious use of immunotherapy trials may have both therapeutic and diagnostic utility, bearing in mind when interpreting the outcomes of such trials that some non-immune epilepsies may respond to immunotherapy as well.[38]

One could argue that restricting the development of the ONES checklist to described presentations of established neural antibodies limits its ability to identify novel disease phenotypes of as yet undiscovered neural antibodies. Although this is a valid theoretical concern, comprehensive tissue-based neural antibody testing in studies of patients with epilepsy or seizures of unknown etiology have not robustly demonstrated novel neural antibodies of clear clinical relevance in this patient population.[12,13] This is in keeping with our study, in which only one patient had an unclassified neural antibody by TIIF; this patient met criteria for definite autoimmune limbic encephalitis and was captured by the ONES checklist. Meanwhile, an opposing and often underappreciated concern is that broadly performing neural antibody testing in hopes of identifying novel disease phenotypes can dramatically increase the proportion of false-positive results; this is due to the lowering of positive predictive value that occurs when performing testing in low-probability scenarios with assays in widespread clinical use that have high but imperfect specificity.[15,17,30,39] This issue is exemplified in our study by the significantly higher proportion of false-positive results in patients who were classified as having epilepsy or seizures of known etiology, and who thus had an intuitively lower probability of neural antibody-associated disease. The possibility of false-positives takes on particular importance when attempting to interpret previous studies of neural antibody testing in patients with epilepsy or seizures, which have reported patients with isolated serum positivity for certain neural antibodies (e.g., anti-NMDAR, CASPR2, GlyR) and atypical disease phenotypes.[19,40,41] The risk of false-positives is increasingly being recognized when neural antibody testing is performed in low-probability scenarios,[15,17,28–30,39,42–45] highlighting the importance of appropriate patient selection. To this end, tools like the ONES checklist can serve not only to enhance clinician recognition of neural antibody-associated presentations, but also to trigger scrutiny of possible false-positive results in patients with atypical disease phenotypes who may have indiscriminately undergone neural antibody testing. Improved neural antibody reporting practices by the testing laboratory can also help to avoid misinterpretation of clinically irrelevant or false-positive results in patients with epilepsy or seizures; as an example, the inclusion of interpretative comments when reporting isolated low serum levels of certain antibodies, such as serum anti-GAD65 detected by ELISA at values < 10 000 IU/ml or serum anti-NMDAR as well as anti-CASPR2 detected by CBA with only weak positivity at 1:10 dilution, can serve to educate clinicians that these results typically lack relevance in patients with neurological symptoms.[21,29,30,46]

4.1 | Limitations

This was a retrospective study, and lack of systematic data collection at the time of patient assessment could have impacted the performance of a checklist or predictive score. However, the high sensitivity of both the ONES checklist and APE2/ACES reflex score is reassuring in this regard. Because of the retrospective nature of data collection, blinding to the neural antibody result during EMR review was not possible, given that neural antibody status was highlighted across clinical notes. However, independent review by two clinicians was performed to minimize bias. The ONES checklist was found to have high sensitivity in

our patient cohort that consisted primarily of patients in the outpatient/elective admission (EMU) setting, but it does place emphasis on seizure refractoriness. Its sensitivity could therefore be lower when applied to patients who first present with seizures to hospital. It is, however, well suited for an epilepsy clinic, where patients are more likely to have failed antiseizure medications, prompting referral. Anti-MOG and anti-GlyR were not tested routinely in patients who did not have typical disease features, so some patients who would have been positive for these antibodies could theoretically have been missed. However, our tiered approach minimizes the possibility of false-positives that can occur when testing for these antibodies in low-probability scenarios. There is an element of subjectivity to some items included in the ONES checklist that aim to distinguish relevant findings from those due to other disease etiologies, epilepsy comorbidities, and ictal or postictal phenomena. In particular, distinguishing the neuropsychiatric symptoms secondary to neural antibody-associated disease from those that are common among patients with epilepsy can be exceptionally challenging.[47] Although formal cognitive assessment can be useful in this regard, validated instruments to aid in this differentiation are lacking. This represents a knowledge gap that could be addressed through future study, as improved ability to make this distinction clinically would be advantageous to the diagnostic evaluation of patients with epilepsy and directly result in increased specificity of the ONES checklist.

5 | CONCLUSIONS

Compared to only performing neural antibody testing in patients with epilepsy or seizures of unknown etiology who had "'obvious" indications for testing as identified by the ONES checklist, we found that predictive scores conferred no clear diagnostic advantage. The diagnostic utility of predictive scores should be examined critically in future prospective studies to systematically determine which patients, if any, do not have "obvious" indications for neural antibody testing but would still benefit from their use. Prespecified definitions of what constitutes a true-positive neural antibody, particularly if testing patients with atypical disease phenotypes or if using neural antibody tests with imperfect specificity, is required in such studies to avoid false-positives that can confound results.

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CONFLICT OF INTEREST

S.M. reports that he is on the advisory boards and speaker bureaus for UCB Canada, Eisai, and Sunovion Pharmaceuticals Canada. He is in the editorial board of *Epilepsy & Behavior Reports* and *Frontiers in Neurology*. J.G.B. reports that he holds the Jack Cowin Endowed Chair in Epilepsy Research at Western University. A.B. reports that he holds the London Health Sciences Centre and London Health Sciences Foundation Chair in Neural Antibody Testing for Neuro-Inflammatory Diseases. Neither of the other authors has any conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Yiu-Chia Chang: Conceptualization, investigation, writing–review & editing. Maryam N. Nouri: Conceptualization, writing–review & editing. Seyed Mirsattari: Conceptualization, writing–review & editing. Jorge G. Burneo: Conceptualization, writing–review & editing. Adrian Budhram: Conceptualization, methodology, investigation, formal analysis, writing–original draft, supervision.

ORCID

Maryam N. Nouri https://orcid.org/0000-0001-6317-4890
Adrian Budhram https://orcid.org/0000-0003-4860-0470

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