The Canadian Journal of Neurological Sciences (2023), **50**, 766–768
doi:10.1017/cjn.2022.276

Canadian Journal of Neurological Sciences
Journal Canadien des Sciences Neurologiques

Brief Communication

Positive Predictive Value of Anti-GAD65 ELISA Cut-Offs for Neurological Autoimmunity

Adrian Budhram[1,2], Erin Freeman[2], Vipin Bhayana[2] and Liju Yang[2]

[1]Department of Clinical Neurological Sciences, London Health Sciences Centre, Western University, London, Ontario, Canada and [2]Department of Pathology and Laboratory Medicine, London Health Sciences Centre, Western University, London, Ontario, Canada

ABSTRACT: High anti-GAD65 levels associate with core manifestations of GAD65 neurological autoimmunity. ELISA cut-offs for high anti-GAD65 levels (>10,000 IU/ml in serum, >100 IU/ml in CSF) have been proposed that merit further evaluation. We reviewed patients who underwent anti-GAD65 ELISA for suspected autoimmune encephalitis and found values above these cut-offs to have a positive predictive value (PPV) for neurological autoimmunity of 88%. Anti-GAD65 values above proposed ELISA cut-offs have a reasonably high PPV for neurological autoimmunity in patients with suspected autoimmune encephalitis. Consideration of alternative diagnoses and corroboration with CSF can help flag potentially clinically irrelevant results and avoid patient misdiagnosis.

RÉSUMÉ : La valeur prévisionnelle positive des seuils d'anti-GAD65 selon la méthode ELISA dans les maladies auto-immunes neurologiques. Des taux élevés d'anti-GAD65 sont associés aux principales manifestations d'affections auto-immunes neurologiques. Des seuils de taux élevés d'anti-GAD65 (> 10 000 UI/ml dans le sang; > 100 UI/ml dans le liquide céphalorachidien [LCR]) selon la méthode ELISA ont été proposés et ils méritent d'être étudiés davantage. Aussi avons-nous examiné des dossiers de patients soumis à des tests de détection d'anti-GAD65 selon la méthode ELISA pour présomption d'encéphalite auto-immune, et, ce faisant, nous avons constaté que des valeurs supérieures à ces seuils avaient une valeur prévisionnelle positive (VPP) de 88 % à l'égard d'une auto-immunité neurologique. Ainsi, des valeurs d'anti-GAD65 supérieures aux seuils ELISA proposés ont une VPP raisonnablement élevée à l'égard d'une auto-immunité neurologique chez les patients souffrant d'une encéphalite auto-immune présumée. La prise en considération d'autres diagnostics possibles et la corroboration de leur existence par des analyses du LCR peuvent aider à attirer l'attention sur des résultats cliniques potentiellement aberrants, et à éviter la pose de diagnostics erronés.

Keywords: Autoimmune disease; Neuroimmunology

(Received 16 April 2022; final revisions submitted 11 July 2022; date of acceptance 12 July 2022; First Published online 21 July 2022)

Antibodies against glutamic acid decarboxylase-65 (anti-GAD65) are associated with systemic autoimmune diseases such as type 1 diabetes mellitus (T1DM), autoimmune thyroid disease and pernicious anemia.[1,2] High levels of anti-GAD65, which refers to levels that can be over 1000-fold higher than levels found in patients with systemic autoimmune disease, have been linked to discrete core manifestations of GAD65 neurological autoimmunity that include stiff-person spectrum disorders (SPSD), cerebellar ataxia, temporal lobe epilepsy and limbic encephalitis.[1-3] Less common presentations of neurological autoimmunity associated with high levels of anti-GAD65, such as paraneoplastic encephalomyelitis, encephalitis following stem cell transplantation, and multifocal neurological dysfunction after immune checkpoint inhibitor (ICI) therapy, have also been described.[4-6] Published cut-offs as to what constitutes high levels of anti-GAD65 have previously been reported for radioimmunoassay (RIA).[2,7] However, the need for radioactive reagents is a disadvantage to RIA that has spurred interest in alternative antibody detection methodologies. Brain tissue-based assays such as indirect immunofluorescence or immunohistochemistry, line blots and cell-based assays that only detect relatively high levels of anti-GAD65 are available and can be helpful in patients with suspected GAD65 neurological autoimmunity.[1] Practically, however, their lower analytical sensitivity may be a limiting factor to clinical chemistry laboratories that are interested in implementing a single assay that can detect both low levels of anti-GAD65 (for suspected systemic autoimmune disease) and high levels of anti-GAD65 (for suspected neurological autoimmunity).

Corresponding author: Dr. Adrian Budhram, Department of Clinical Neurological Sciences, London Health Sciences Centre, 339 Windermere Rd., London, Ontario, N6A 5A5, Canada. Email: Adrian.budhram@lhsc.on.ca

Cite this article: Budhram A, Freeman E, Bhayana V, and Yang L. (2023) Positive Predictive Value of Anti-GAD65 ELISA Cut-Offs for Neurological Autoimmunity. *The Canadian Journal of Neurological Sciences* **50**: 766–768, https://doi.org/10.1017/cjn.2022.276

Anti-GAD65 enzyme-linked immunosorbent assay (ELISA) is an assay that can detect low levels of anti-GAD65 and is more easily implemented in many clinical laboratories than RIA. Recently, cut-offs for high levels of anti-GAD65 by ELISA, which were shown to have excellent concordance with immunohistochemistry and cell-based assay for the detection of high levels of anti-GAD65, have been proposed to help diagnose GAD65 neurological autoimmunity.[8] Using these proposed anti-GAD65 ELISA cut-offs of >10,000 IU/ml in serum and >100 IU/ml in CSF, it was reported that 34/36 patients (94%) with neurological symptoms and anti-GAD65 values above these cut-offs had core manifestations of GAD65 neurological autoimmunity.[8] Two patients, however, had atypical presentations for GAD65 neurological autoimmunity in the form of pseudo-orthostatic tremor and optic neuropathy. It is possible that anti-GAD65 values above proposed ELISA cut-offs in these two patients represented results that lacked clinical relevance to their neurological presentation (simply referred to hereafter as clinically irrelevant results), highlighting the need for further evaluation of these cut-offs.[8] Importantly, in this context, the concern is not one of inaccurately measuring anti-GAD65 (which would suggest imperfect analytical specificity of the test), but rather one of incorrect determination of GAD65 neurological autoimmunity when this diagnosis is based solely on these cut-offs (which would suggest imperfect clinical specificity of test cut-offs); it is for this reason that the term "clinically irrelevant" rather than "false-positive" is used in this context. Evaluations of the potential for diagnostic tests to generate clinically irrelevant or false-positive results are critical to avoid "phenotype creep", whereby the neurological features of alternative diagnoses are mistakenly assumed to broaden the clinical spectrum of a neural antibody based solely on seropositivity by an imperfectly specific assay or, in this context, cut-off.[9] Positive predictive value (PPV), which is the probability that a patient with a positive test result truly has the disease of interest, is a diagnostic measure that informs the potential for clinically irrelevant or false-positive results in clinical practice.[10] We therefore evaluated the PPV of anti-GAD65 values above ELISA cut-offs for neurological autoimmunity, after 1 year of their implementation as part of neural antibody testing for suspected autoimmune encephalitis at at London Health Sciences Centre (LHSC).

We reviewed all patients who underwent anti-GAD65 ELISA at LHSC Clinical Immunology laboratory as part of neural antibody testing for suspected autoimmune encephalitis between October 1, 2020 and November 30, 2021. Testing was performed using KRONUS anti-GAD65 ELISA kits, with serial dilutions performed to determine the end concentration in patients with values of >250 IU/ml. Those with an anti-GAD65 value of >10,000 IU/ml in serum and/or >100 IU/ml in CSF were included in this study. Their available clinical information was reviewed, which was obtained through clinical questionnaires and telephonic correspondence as quality assurance measures, as well as through electronic medical record review of patients evaluated at LHSC. Patients with anti-GAD65 values above the aforementioned cut-offs with a compatible clinical phenotype and no more likely alternative diagnosis were classified as a clinically relevant result, while those with an incompatible clinical phenotype and/or a more likely alternative diagnosis were classified as a clinically irrelevant result. Classifications were performed by a neurologist with fellowship training in autoimmune neurology (A.B.). The PPV of anti-GAD65 values above ELISA cut-offs for neurological autoimmunity was then calculated as the proportion of all patients with anti-GAD65 values above ELISA cut-offs that were classified as clinically relevant. This study was approved by the Western University Health Science Research Ethics Board (No. 120281).

Between October 1, 2020 and November 30, 2021, 16 patients who underwent neural antibody testing for suspected autoimmune encephalitis had an anti-GAD65 value above ELISA cut-offs (12 serum, 1 CSF, 3 serum and CSF). The median age was 62 years (range: 15–81 years) and 11/16 (69%) were female. The median serum anti-GAD65 value was 182,090 IU/ml (range: 13,576–685,000 IU/ml), and the median CSF value was 350.5 IU/ml (range: 196–9788 IU/ml). Of these patients, 14 were classified as having a clinically relevant anti-GAD65 result: 10 had core manifestations of GAD65 neurological autoimmunity (cerebellar ataxia, 4; temporal lobe epilepsy, 3; SPSD, 2; limbic encephalitis, 1), while four had presentations that, while less typical, were still compatible with neurological autoimmunity associated with high levels of anti-GAD65 (post-stem cell transplantation encephalomyelitis, 1; encephalitis following ICI therapy, 1; paraneoplastic encephalomyelitis, 1; encephalitis with seizures not otherwise specified, 1). Meanwhile, two patients were classified as having a clinically irrelevant anti-GAD65 result: one patient with end-stage renal disease secondary to T1DM was diagnosed with uremic encephalopathy that improved with dialysis (serum anti-GAD65 value 204,620 IU/ml, no CSF submitted), and one patient with a history of hypothyroidism and chronic, severe nausea/vomiting was diagnosed with gait unsteadiness secondary to presumed nutritional deficiency that resolved after nourishment (serum anti-GAD65 value 13,576 IU/ml, no CSF submitted). Based on these classifications, the PPV of anti-GAD65 values above ELISA cut-offs for neurological autoimmunity was calculated as 14/16 (88%).

Our study demonstrates that anti-GAD65 values above proposed ELISA cut-offs have a reasonably high PPV for neurological autoimmunity in patients with suspected autoimmune encephalitis, thereby supporting their use in clinical practice. Importantly, however, we found that clinically irrelevant serum anti-GAD65 results above these ELISA cut-offs could still occur. This suggests that high serum anti-GAD65 levels are best viewed as necessary but not sufficient to make a diagnosis of neurological autoimmunity, and that alternative diagnoses should still be thoroughly considered in patients with this finding.[2]

There are several limitations to our study. Our sample size is small, which is an inherent challenge to the investigation of rare diseases. However, our findings surrounding PPV are in keeping with previous investigations into patients with high levels of anti-GAD65 determined by other methodologies.[2,7] The distribution of core manifestations of anti-GAD65 neurological autoimmunity differed somewhat from previous reports in the literature, with cerebellar ataxia and temporal lobe epilepsy being more commonly found than SPSD.[2,7] This could reflect test ordering practices leading to sample submission; clinical presentations involving the cerebellum or cerebrum may be more likely to undergo neural antibody testing for suspected autoimmune encephalitis, while for patients with suspected SPSD clinicians may order anti-GAD65 alone at one of numerous institutions that offer this testing. Only abbreviated clinical information was available for some patients, which could hinder identification of overlap syndromes (e.g. overlap of SPSD and cerebellar ataxia). While this would be problematic for a clinical characterization study, even abbreviated clinical information can facilitate the classification of patient test results as clinically relevant or clinically irrelevant for the purposes of PPV determination. In the majority of patients, including the two patients classified as having clinically irrelevant

https://doi.org/10.1017/cjn.2022.276 Published online by Cambridge University Press

serum anti-GAD65 results, no CSF was submitted for anti-GAD65 testing. Although only submitting serum to perform neural antibody testing for suspected autoimmune encephalitis is common, paired serum and CSF is generally recommended[3]; with regard to patients with high levels of serum anti-GAD65 specifically, determination of high anti-GAD65 levels in CSF as well as calculation of anti-GAD65 intrathecal synthesis rate may inform the likelihood of neurological autoimmunity and should be incorporated in future studies of diagnostic test performance.[1] Only patients with anti-GAD65 values above ELISA cut-offs were included in this study of PPV, and so determination of the negative predictive value of these cut-offs for neurological autoimmunity was not possible. Finally, it should be emphasized that the PPV of anti-GAD65 values above ELISA cut-offs for neurological autoimmunity in this study was determined in those undergoing testing for suspected autoimmune encephalitis; because PPV decreases as disease prevalence in the tested population decreases, the PPV of anti-GAD65 values above ELISA cut-offs for neurological autoimmunity in patients undergoing testing for non-neurologic disease (e.g. suspected T1DM) would expectedly be substantially lower than was found in this study.

Despite these study limitations, our findings have a clear practical benefits to both laboratorians and clinicians who are implementing or interpreting anti-GAD65 ELISA testing for suspected neurological autoimmunity. Anti-GAD65 levels above the proposed ELISA cut-offs should raise suspicion for neurological autoimmunity in the appropriate clinical context, as indicated by their reasonably high PPV in patients with suspected autoimmune encephalitis. However, even in patients with high serum anti-GAD65 levels by ELISA, consideration of alternative diagnoses and corroboration with CSF can help to flag potentially clinically irrelevant results and avoid patient misdiagnosis.

Acknowledgements. None.

Funding. No funding was received for this manuscript.

Disclosures. Adrian Budhram reports that he holds the London Health Sciences Centre and London Health Sciences Foundation Chair in Neural Antibody Testing for Neuro-Inflammatory Diseases, and receives support from the Opportunities Fund of the Academic Health Sciences Centre Alternative Funding Plan of the Academic Medical Organization of Southwestern Ontario (AMOSO).

Erin Freeman has no disclosures to report.

Vipin Bhayana has no disclosures to report.

Liju Yang has no disclosures to report.

Statement of Authorship.

Adrian Budhram analyzed the data and drafted the manuscript.

Erin Freeman reviewed the manuscript for intellectual content.

Vipin Bhayana reviewed the manuscript for intellectual content.

Liju Yang reviewed the manuscript for intellectual content.

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https://doi.org/10.1017/cjn.2022.276 Published online by Cambridge University Press