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Brief Communication

Low Yield of Routine Cerebrospinal Fluid Analysis to Assess for An Autoimmune Etiology in Patients with Chronic Seizures of Unknown Cause

Meaghan Daub[1], Kamala Sangam[1], Jorge G. Burneo[1,2] and Adrian Budhram[1,3]

[1]Department of Clinical Neurological Sciences, London Health Sciences Centre, Western University, London, ON, Canada, [2]Neuroepidemiology Unit and Epilepsy Program, Schulich School of Medicine & Dentistry, Western University, London, ON, Canada and [3]Department of Pathology and Laboratory Medicine, London Health Sciences Centre, Western University, London, ON, Canada

ABSTRACT: In this study, we examined the yield of routine cerebrospinal fluid (CSF) analysis to assess for an autoimmune etiology in patients with chronic seizures of unknown cause. Forty-seven patients were included. Six of 47 (13%) had inflammation on routine CSF analysis, none of whom were diagnosed with seizures related to autoimmune encephalitis (AE). Meanwhile, 2/47 (4%) were diagnosed with seizures related to AE, neither of whom had inflammation on routine CSF analysis. Routine CSF analysis to assess for an autoimmune etiology in patients with chronic seizures of unknown cause is low yield, and has suboptimal specificity and sensitivity for seizures related to AE.

RÉSUMÉ : Faible performance de l'examen du liquide céphalorachidien d'usage pour la recherche d'une cause auto-immune de crises d'épilepsie chronique d'origine inconnue. L'étude visait à examiner la performance de l'examen du liquide céphalorachidien (LCR) d'usage chez des patients souffrant de crises d'épilepsie chronique d'origine inconnue. Ont participé à l'étude 47 patients, dont 6 (13 %) présentaient de l'inflammation à l'examen habituel du LCR, mais aucun diagnostic d'épilepsie liée à une encéphalite auto-immune (EAI) n'a été posé chez l'un d'eux. Par contre, des crises d'épilepsie en lien avec une EAI ont été diagnostiquées chez 2 patients sur 47 (4 %), mais aucun ne présentait d'inflammation à l'examen courant du LCR. Il ressort donc de l'étude que l'examen du LCR d'usage pour la recherche d'une cause auto-immune de crises d'épilepsie chronique d'origine inconnue a une faible performance ainsi qu'une spécificité et une sensibilité sous-optimales en ce qui concerne les crises d'épilepsie liées à une EAI.

Keywords: autoimmune disease; epilepsy; neuroimmunology; seizures

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Among patients with seizures of unknown cause, the search for an autoimmune etiology has intensified in recent years. Numerous tools have been developed to help select who should be tested for neural antibodies to diagnose seizures related to autoimmune encephalitis (AE).[1–4] One such tool is the Antibody Prevalence in Epilepsy and Encephalopathy (APE2) score, which includes lumbar puncture (LP) for routine cerebrospinal fluid (CSF) analysis in its calculation.[1] Yet, while the role of routine CSF analysis is established for patients with new-onset seizures alongside other neuropsychiatric symptoms raising suspicion for AE,[5] its role when assessing for an autoimmune etiology in patients with chronic (≥1 year) seizures is unclear. To address this knowledge gap, we examined the yield of routine CSF analysis to assess for an autoimmune etiology in patients with chronic seizures of unknown cause who were admitted to our Epilepsy Monitoring Unit (EMU) and underwent LP.

We identified all patients admitted to our EMU between January 2012 and July 2023 who underwent LP and had at least one tube of CSF submitted for cell count determination. Electronic medical records of patients with chronic (≥1 year) seizures at time of LP were reviewed for inclusion. Patients were included if (1) brain MRI did not show findings to indicate a specific seizure etiology, and (2) LP was performed to assess for an autoimmune seizure etiology. Brain magnetic resonance imaging (MRI) showing only nonspecific white matter abnormalities, medial temporal lobe signal abnormality with or without atrophy, or possible seizure-related change was not grounds for exclusion. Evidence of possible inflammation on routine CSF analysis was defined by one or more of: (1) CSF protein > 50 mg/dL with CSF RBC count of < 1000 cells/μL; (2) CSF WBC count > 5 cells/μL with CSF RBC count of < 1000 cells/μL; (3) elevated CSF/serum

Corresponding author: Adrian Budhram; Email: adrian.budhram@lhsc.on.ca

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IgG index; (4) unmatched CSF oligoclonal bands. Cell count and protein cutoffs aligned with the APE2 score.[1] Elevated CSF/serum IgG index and unmatched CSF oligoclonal bands were included as evidence of possible inflammation on routine CSF analysis because of their role in the diagnostic evaluation of AE.[5,6] CSF neural antibody testing was reviewed but not incorporated in yield calculation, which was intended to examine yield of routine CSF analysis that could potentially help select patients for neural antibody testing.[1] In patients with repeated LPs, only the initial LP was reviewed. Yield of routine CSF analysis was calculated as the proportion of patients in whom evidence of possible inflammation on routine CSF analysis contributed to a final diagnosis of seizures related to AE. Final diagnosis was based on last clinical documentation. This study was approved by the Western University Health Science Research Ethics Board.

Forty-seven patients met inclusion criteria. The median age at time of LP was 35 years (range: 19–72 years) and 29/47 (62%) were female. The median time from seizure onset to LP was 4.6 years (range: 1.0–48.5 years). Forty-five of 47 patients (96%) had focal-onset seizures, and 40/47 (85%) were medically refractory. With respect to routine CSF analysis, 47/47 (100%) had cell counts and protein tested, and 34/47 (72%) had IgG index and oligoclonal bands tested. Thirty-seven of 47 (79%) underwent CSF testing for one or more neural antibodies, although only 21/47 (45%) underwent more comprehensive CSF neural antibody testing as described previously.[4] Six of 47 (13%) had evidence of possible inflammation on routine CSF analysis that was typically mild (mild protein elevation < 55 mg/dL, mild pleocytosis < 10 cell/ μL, "faint" oligoclonal bands; see Table 1).

The 6 patients with evidence of possible inflammation on routine CSF analysis are summarized in Table 1. None had documented comorbidities known to associate with mild CSF abnormalities (e.g. diabetes mellitus). Five of 6 (83%) had a documented seizure less than 24 hours before LP. Documented reasons for performing LP to assess for an autoimmune seizure etiology included seizure refractoriness, presurgical epilepsy evaluation, nonspecific MRI findings and nonspecific serum antibodies. Five of 6 (83%) underwent testing for one or more neural antibodies, and all were negative. One of 6 (17%) underwent an immunotherapy trial without sustained benefit. The final diagnosis in all 6 was idiopathic focal-onset epilepsy. Because no patient had evidence of possible inflammation on routine CSF analysis that contributed to a diagnosis of seizures related to AE, the yield of routine CSF analysis was calculated to be 0/47 (0%; 95% CI [0%, 8%] by Wilson score interval). Meanwhile, 2/47 patients (4%) were diagnosed with seizures related to AE (anti-leucine-rich glioma-inactivated 1 [LGI1] encephalitis, anti-contactin-associated protein-like 2 [CASPR2] encephalitis). These two patients are summarized in Table 2. Routine CSF analysis showed no evidence of possible inflammation in either case. Both exhibited serum antibody positivity, while only one exhibited CSF antibody positivity.

On routine CSF analysis performed to assess for an autoimmune etiology in patients with chronic seizures of unknown cause, we found that evidence of possible inflammation was uncommon. When present, it was typically mild and thus relatively nonspecific. As would be anticipated among patients with predominantly medically refractory seizures who underwent LP while admitted to an EMU, recent seizure activity prior to CSF collection was frequently documented; this could have contributed to the presence of such CSF abnormalities (e.g. mild pleocytosis, mildly elevated protein),[7] emphasizing the importance of considering this potentially confounding factor when interpreting CSF results. Furthermore, while not present in this study, consideration should also be given to patient comorbidities that have been associated with mild CSF abnormalities (e.g. mildly elevated protein with diabetes mellitus) when interpreting CSF results.[8] Among the minority with evidence of possible inflammation on routine CSF analysis in our study, none were diagnosed with seizures related to AE. Of the two patients who were diagnosed with seizures related to AE, neither had evidence of possible inflammation on routine CSF analysis. Taken together, our findings indicate that routine CSF analysis to assess for an autoimmune etiology in patients with chronic seizures of unknown cause is low yield.

Our study has limitations. As illustrated among the patients who had evidence of possible inflammation on routine CSF analysis, documented reasons for pursuing LP may not have been particularly suspicious for an autoimmune etiology in isolation. Poor patient selection for LP could therefore have been contributory to the low yield of routine CSF analysis. However, these reasons for pursuing LP should be considered in the context of the study population more generally, which consisted primarily of patients with medically refractory, focal-onset seizures of unknown cause. If there were value to LP in patients with chronic seizures, one would intuitively expect this to be most evident in this population. Notably, neural antibody testing was limited for the majority of patients. Comprehensive neural antibody testing for AE has only become available at our institution in recent years, and one could argue that the low calculated yield of routine CSF analysis was due to missed diagnoses of AE as a result of incomplete neural antibody evaluations. Yet, while comprehensive serum and CSF neural antibody testing has been recommended in patients with new-onset seizures alongside other neuropsychiatric symptoms typical of AE, the need for such comprehensive neural antibody testing in patients with chronic seizures potentially occurring in relative isolation has not been established. A recent study consisting primarily of patients with chronic focal-onset seizures in relative isolation found only anti-LGI1, anti-CASPR2 and anti-glutamic acid decarboxylase-65 (GAD65) in a small minority, suggesting that testing for this more limited repertoire of antibodies as clinically indicated in this patient population may be sufficient.[3] Serum testing is adequate to screen for these three antibodies and was performed in most patients with evidence of possible inflammation on routine CSF analysis in our study,[9–11] making it unlikely that low yield of routine CSF analysis was a result of limited neural antibody testing. Similarly, while CSF neural antibody testing was not incorporated in our yield calculation, the only two patients diagnosed with seizures related to AE had neural antibodies that are more sensitively detected in serum by commercially available assays.[11] Both lacked evidence of possible inflammation on routine CSF analysis, as is often the case for patients with these antibodies.[12] No patients with anti-GAD65-associated seizures were identified in this study, although routine CSF analysis in these patients may similarly be relatively non-inflammatory.[13]

We acknowledge that, following serum detection of a neural antibody that has been associated with chronic seizures in relative isolation (anti-LGI1, anti-CASPR2, anti-GAD65), CSF testing can still be valuable. Evidence of possible inflammation on routine CSF analysis may have prognostic significance, while demonstration of neural antibody positivity in CSF (as well as intrathecal synthesis

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Table 1. Summary of six patients with chronic seizures and evidence of possible inflammation on routine cerebrospinal fluid analysis

Seizure duration (Years)	Evidence of possible inflammation on routine CSF analysis	Time from last documented seizure to LP	Documented reason for performing LP	Documented comorbidities	Serum neural antibodies tested[1]	CSF neural antibodies tested	Immunotherapy	Final diagnosis	Seizures related to AE?
17.1	Elevated protein (51.7 mg/dL)	2.5 weeks	Nonspecific subcortical white matter hyperintensities	None	Anti-GAD65, VGKC (Negative)	None	None	Idiopathic focal-onset epilepsy (frontal)	No
15.6	Elevated WBC count (6 cells/μL)	<24 hours	Not explicitly stated	Anxiety/depression	Anti-NMDAR, GAD65, VGKC, PNA (Negative)	Anti-NMDAR (Negative)	None	Idiopathic focal-onset epilepsy (temporal)	No
12.2	Elevated protein (54.7 mg/dL)	<24 hours	Positive antiphospholipid antibodies	Dyslipidemia	None	None	None	Idiopathic focal-onset epilepsy (temporal)	No[2]
10.8	Elevated WBC count (7 cells/μL), unmatched CSF OCB ("faint")	<24 hours	Positive thyroid antibodies	Anxiety	Anti-NMDAR, GAD65, VGKC, PNA (Negative)	Anti-NMDAR, PNA (Negative)	Steroids, without sustained benefit	Idiopathic focal-onset epilepsy (temporal)	No[3]
4.6	Elevated protein (50.6 mg/dL)	<24 hours	Seizure refractoriness	None	Anti-GAD65 (Negative)	Anti-GAD65 (Negative)	None	Idiopathic focal-onset epilepsy (temporal)	No
2.4	Unmatched CSF OCB	<24 hours	Presurgical epilepsy evaluation	Mitral valve prolapse	Anti-NMDAR, GAD65, VGKC, PNA (Negative)	Anti-NMDAR, LGI1, CASPR2, PNA (Negative)	None	Idiopathic focal-onset epilepsy (temporal)	No

AE = autoimmune encephalitis; CASPR2 = contactin-associated protein-like 2; CSF = cerebrospinal fluid; GAD65 = glutamic acid decarboxylase-65; LGI1 = leucine-rich glioma-inactivated 1; LP = lumbar puncture; NMDAR = N-methyl-D-aspartate receptor; OCB = oligoclonal bands; PNA = paraneoplastic antibody panel; VGKC = voltage-gated potassium channel; WBC = white blood cell.

[1]Anti-VGKC performed as serum screening test for anti-LGI1 and CASPR2, in line with previously implemented testing algorithms for these antibodies.

[2]Initial concern was of temporal lobe seizures possibly related to antiphospholipid syndrome (APS). However, the patient was assessed by a hematologist who noted that he had had no venous or arterial thrombotic events to suggest APS, and stated that neither anticoagulation nor immunotherapy was indicated. At last epilepsy clinic follow-up, documented diagnosis was temporal lobe epilepsy of unknown etiology.

[3]Initial concern was of temporal lobe seizures possibly related to steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT). However, the patient was neither encephalopathic nor steroid-responsive like would be expected of SREAT. At last epilepsy clinic follow-up, documented diagnosis was temporal lobe epilepsy of unclear etiology.

Table 2. Summary of two patients with chronic seizures related to autoimmune encephalitis

Seizure duration (Years)	Evidence of possible inflammation on routine CSF analysis	Positive neural antibody	Features characteristic of neural antibody positivity	Immunotherapy
1.9	None (CSF WBC count: 0 cells/μL; CSF protein: 18.1 mg/dL; no elevation of CSF/serum IgG index; no unmatched CSF OCB)	Anti-LGI1 (Positive in serum only by CBA)[1]	Faciobrachial dystonic seizures and cognitive impairment	Steroids and IVIG, with sustained benefit Decrease in seizures from multiple daily to infrequent, brief, focal aware seizures every few weeks
1.4	None (CSF WBC count:1 cell/μL; CSF protein: 35.7 mg/dL; no elevation of CSF/serum IgG index; no unmatched CSF OCB)	Anti-CASPR2 (Positive in serum and CSF by TIIF and CBA)[1]	Cerebellar ataxia, peripheral neuropathy, weight loss, mild cognitive impairment	Steroids, IVIG and rituximab, with sustained benefit Decrease in seizures from multiple daily to seizure freedom

CASPR2 = contactin-associated protein-like 2; CBA = cell-based assay; CSF = cerebrospinal fluid; IgG = immunoglobulin G; IVIG = intravenous immunoglobulin; LGI1 = leucine-rich glioma-inactivated 1; LP = lumbar puncture; OCB = oligoclonal bands; TIIF = tissue indirect immunofluorescence; WBC = white blood cell.
[1]Serum and CSF neural antibody testing including TIIF and CBA performed as part of comprehensive autoimmune encephalitis panel testing for both patients.

for anti-GAD65) can help confirm antibody specificity in patients with equivocal disease phenotypes.[5,10,11] We also recognize that LP to perform CSF neural antibody testing can be useful in rare patients who are assessed for chronic seizures following an initially undiagnosed encephalitic presentation, because they may harbor neural antibodies that are more sensitively detected in CSF such as anti-N-methyl-D-aspartate receptor.[11] Nonetheless, our study demonstrates that routine CSF analysis to assess for an autoimmune etiology in patients with chronic seizures of unknown cause is low yield, and has suboptimal specificity and sensitivity for seizures related to AE. Further research is needed to better determine which patients with chronic seizures may benefit from CSF testing to help diagnose an autoimmune etiology.

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