

Prospective Quantification of CSF Biomarkers in Antibody-Mediated Encephalitis

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Abstract

Objective

To determine whether neuronal and neuroaxonal injury, neuroinflammation, and synaptic dysfunction associate with clinical course and outcomes in antibody-mediated encephalitis (AME), we measured biomarkers of these processes in CSF from patients presenting with AME and cognitively normal individuals.

Methods

Biomarkers of neuronal (total tau, VILIP-1) and neuroaxonal damage (neurofilament light chain [NfL]), inflammation (YKL-40), and synaptic function (neurogranin, SNAP-25) were measured in CSF obtained from 45 patients at the time of diagnosis of NMDA receptor ($n = 34$) or *LGII/CASPR2* ($n = 11$) AME and 39 age- and sex-similar cognitively normal individuals. The association between biomarkers and modified Rankin Scale (mRS) scores were evaluated in a subset ($n = 20$) of longitudinally followed patients.

Results

Biomarkers of neuroaxonal injury (NfL) and neuroinflammation (YKL-40) were elevated in AME cases at presentation, whereas markers of neuronal injury and synaptic function were stable (total tau) or decreased (VILIP-1, SNAP-25, neurogranin). The log-transformed ratio of YKL-40/SNAP-25 optimally discriminated patients from cognitively normal individuals (area under the receiver operating characteristic curve 0.99; 95% confidence interval 0.97, >0.99). Younger age ($\rho = -0.56$; $p = 0.01$), lower VILIP-1 ($\rho = -0.60$; $p < 0.01$) and SNAP-25 ($\rho = -0.54$; $p = 0.01$), and higher $\log_{10}(\text{YKL-40/SNAP-25})$ ($\rho = 0.48$; $p = 0.04$) associated with greater disease severity (higher mRS score) in prospectively followed patients. Higher YKL-40 ($\rho = 0.60$; $p = 0.02$) and neurogranin ($\rho = 0.55$; $p = 0.03$) at presentation were associated with higher mRS scores 12 months following hospital discharge.

Conclusions

CSF biomarkers suggest that neuronal integrity is acutely maintained in AME, despite neuroaxonal compromise. Low levels of biomarkers of synaptic function may reflect antibody-mediated internalization of cell surface receptors and may represent an acute correlate of antibody-mediated synaptic dysfunction, with the potential to inform disease severity and outcomes.

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Glossary

AD = Alzheimer disease; **AME** = antibody-mediated encephalitis; **ANCOVA** = analysis of covariance; **CASPR2** = contactin-associated protein-like 2; **CI** = confidence interval; **CN** = cognitively normal; **ICU** = intensive care unit; **LGII** = leucine-rich glioma-inactivated 1; **mRS** = modified Rankin Scale; **NfL** = neurofilament light chain; **NMDAR** = NMDA receptor; **ROC** = receiver operating characteristic; **SNAP-25** = synaptosomal-associated protein-25; **VILIP-1** = visinin-like protein-1; **YKL-40** = chitinase-3-like protein.

Although the majority of patients with autoimmune encephalitis associated with antibodies against cell-surface receptors (antibody-mediated encephalitis [AME]) return to independent living within 2 years of treatment with immunomodulatory therapies,¹⁻³ persistent deficits in memory and executive function are recognized in patients recovering from NMDA receptor (NMDAR), leucine-rich glioma-inactivated 1 (LGII), and contactin-associated protein-like 2 (CASPR2) antibody encephalitis.^{1,4-7} There is a clear need to develop objective measures that inform the causes and contributors to long-term impairment in patients recovering from AME. CSF biomarkers have been robustly adapted to this purpose in individuals with neurodegenerative dementing illnesses. Increases in CSF levels of total tau and visinin-like protein-1 (VILIP-1) and neurofilament light chain (NfL)—nonspecific markers of neuronal and neuroaxonal injury, respectively—predict accelerated rates of cognitive decline in individuals with early-symptomatic Alzheimer disease (AD)⁸⁻¹¹; chitinase-3-like protein (YKL-40)—a marker of astroglial activation—identifies patients with symptomatic AD,¹¹ HIV-associated dementia,¹² and refractory epilepsy.¹³ Elevated levels of presynaptic (synaptosomal-associated protein-25 [SNAP-25]) and postsynaptic proteins (neurogranin) are also reported in the CSF of patients with neurodegenerative diseases, implicating compromised synaptic integrity and synaptic failure in disease pathogenesis.¹⁴⁻¹⁶

To determine whether these biomarkers inform AME pathogenesis, we compared CSF biomarkers of neuronal and neuroaxonal injury, neuroinflammation, and synaptic dysfunction in patients with NMDAR and LGII/CASPR2 antibody encephalitis and age- and sex-similar cognitively normal (CN) individuals. In the subset of patients with AME for whom longitudinal data were available, we further considered whether CSF biomarkers associated with clinical measures of disease severity and outcomes.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Patients were enrolled and followed within prospective studies at Washington University School of Medicine (St. Louis, MO), University of Toronto (Canada), Charité Hospital (Berlin, Germany), or University Hospital Magdeburg (Germany) between April 2013 and December 2019.

Written informed consent was obtained from prospectively recruited individuals and study protocols were approved by the respective institutions' review boards. All protocols included provisions for retrospective review of medical records; prospective evaluation and documentation of clinical symptoms, signs, and results of clinically indicated investigations; and biofluid banking. Remnant CSF was obtained from a subset of individuals who tested positive for NMDAR or LGII autoantibodies at Mitogen Diagnostics (Calgary, Canada) between January 2013 and May 2015. Clinical information was limited to age at the time of testing and sex in these patients; a waiver of consent was granted for the use of nonidentifying clinical information. CN individuals were enrolled from the St. Louis community through research protocols at Washington University School of Medicine, permitting collection and banking of CSF for research purposes. Participants denied cognitive complaints or other active health issues. The Washington University School of Medicine Institutional Review Board approved all study procedures.

Patient Selection, Evaluation, and Follow-up

Patients were admitted to study hospitals and evaluated by experienced clinicians. Information about past history and presenting complaints was obtained through interview of a reliable collateral source. At the time of study enrollment, all patients met criteria for probable autoimmune encephalitis.¹⁷ Testing for disease-associated antibodies was requested by assessing physicians as part of standard of care and performed at the Mayo Clinic Neuroimmunology Laboratory (Rochester, MN), the Institute for Molecular and Clinical Immunology (Magdeburg, Germany), German Center for Neurodegenerative Diseases (Berlin, Germany), or Mitogen Diagnostics, using indirect immunofluorescence, Western blot, radioimmune, and cell binding assays. NMDAR and LGII autoantibody testing was performed using Euroimmun¹⁸ (Lübeck, Germany) cell-based assays in accordance with manufacturer's specifications. NMDAR autoantibodies were identified in the CSF of 34 patients; LGII or CASPR2 autoantibodies were identified in the serum or CSF of 11 patients.

Information concerning the disease course and long-term outcomes was available from prospectively evaluated patients at Washington University School of Medicine, Charité Hospital, University Hospital Magdeburg, and University of Toronto hospitals. The modified Rankin Scale (mRS) was the most frequently reported measure of disability.¹⁹

Table 1 Demographics and CSF Biomarker Measures in Patients With Antibody-Mediated Encephalitis (AME) and Cognitively Normal (CN) Individuals

| | Patients with AME (n = 45) | CN individuals (n = 39) | p Value |
|--|----------------------------|-------------------------|---------|
| Demographics | | | |
| Age, y | 30.5 (4.0–83.2) | 34.0 (11.0–80.0) | 0.32 |
| Female sex | 34 (76) | 28 (72) | 0.81 |
| CSF biomarkers, pg/mL, mean (SD) | | | |
| Total tau | 25.4 (21.6) | 22.4 (15.7) | 0.40 |
| VILIP-1 | 40.9 (33.2) | 121.9 (54.4) | <0.001 |
| NfL ^a | 2467.2 (2100.6) | 1319.7 (2055.3) | 0.046 |
| YKL-40, mean ($\times 10^3 \pm SD$) ^b | 303.3 (212.4) | 169.9 (103.3) | <0.001 |
| Neurogranin | 567.2 (642.2) | 1501.5 (1015.0) | <0.001 |
| SNAP-25 | 1.5 (1.4) | 3.3 (1.3) | <0.001 |

Abbreviations: CASPR2 = contactin-associated protein-like 2; LGI1 = leucine-rich glioma-inactivated 1; NfL = neurofilament light chain; NMDAR = NMDA receptor; SNAP-25 = synaptosomal-associated protein-25; VILIP-1 = visinin-like protein-1; YKL-40 = chitinase-3-like protein.

Biomarker comparisons performed using analysis of covariance, controlling for age. Values are median (range), mean (SD), or n (%).

^a NfL measures available from 11 patients with NMDAR encephalitis, 10 patients with LGI1/CASPR2 antibody encephalitis, and 28 CN individuals.

^b YKL-40 measures available from 33 patients with NMDAR encephalitis, 11 patients with LGI1/CASPR2 antibody encephalitis, and 39 CN individuals.

CSF Processing and Biomarker Measures

Diagnostic lumbar punctures were performed in patients with AME using standard clinical techniques. An aliquot of CSF (minimum volume, 1 mL) was frozen at -80°C and maintained at study sites before being transported to the Knight Alzheimer Disease Research Center Biomarker Core (Washington University School of Medicine). CSF was obtained from fasted CN community-dwelling volunteers in accordance with established research protocols.²⁰

Measures of total tau (INNOTEST; Innogenetics, Ghent, Belgium),²¹ NfL (Uman Diagnostics, Umeå, Sweden),²² and YKL-40 (MicroVue; Quidel, San Diego, CA)¹⁶ were performed using commercially available ELISA and standard techniques. VILIP-1, neurogranin, and SNAP-25 were measured using microparticle-based immunoassays using the Singulex Erenna system (now part of EMD Millipore, Alameda, CA), incorporating antibodies developed in the laboratory of Dr. Jack Ladenson at Washington University School of Medicine.¹⁶ Samples were run in triplicate for VILIP-1, SNAP-25, and neurogranin, and in duplicate for total tau, NfL, and YKL-40. For concentrations below the limit of quantification, the lowest level of quantification was reported. To minimize variability, samples were tested in batch using assays from the same lot, with consistent freeze–thaw cycles. Specimens with known amounts of target biomarkers were included as positive controls.

Statistical Analyses

Data were analyzed using SPSS Statistics (version 25.0; IBM, Armonk, NY). Continuous and categorical measures were compared using the Mann-Whitney *U* test and Fisher exact test, respectively. Biomarker levels were compared between

patients with AME and CN controls and between patients with NMDAR and LGI1/CASPR2 antibody encephalitis using a univariate analysis of covariance (ANCOVA), with age added as an independent variable. Ratios of distinguishing biomarkers were generated and log-transformed to optimize discrimination between patients with AME and CN individuals. The ability of biomarkers (or biomarker ratios) to distinguish between patients with AME and CN individuals were evaluated using receiver operating characteristic (ROC) analyses, determined using logistic regression. Cutoff values were derived to maximize sensitivity and specificity using the Youden index (Youden *J* statistic). Correlations between biomarkers at presentation and mRS at disease nadir (worst mRS), discharge from hospital, and 12-month follow-up were evaluated using the nonparametric Spearman rho. Statistical significance was defined as $p < 0.05$.

Data Availability

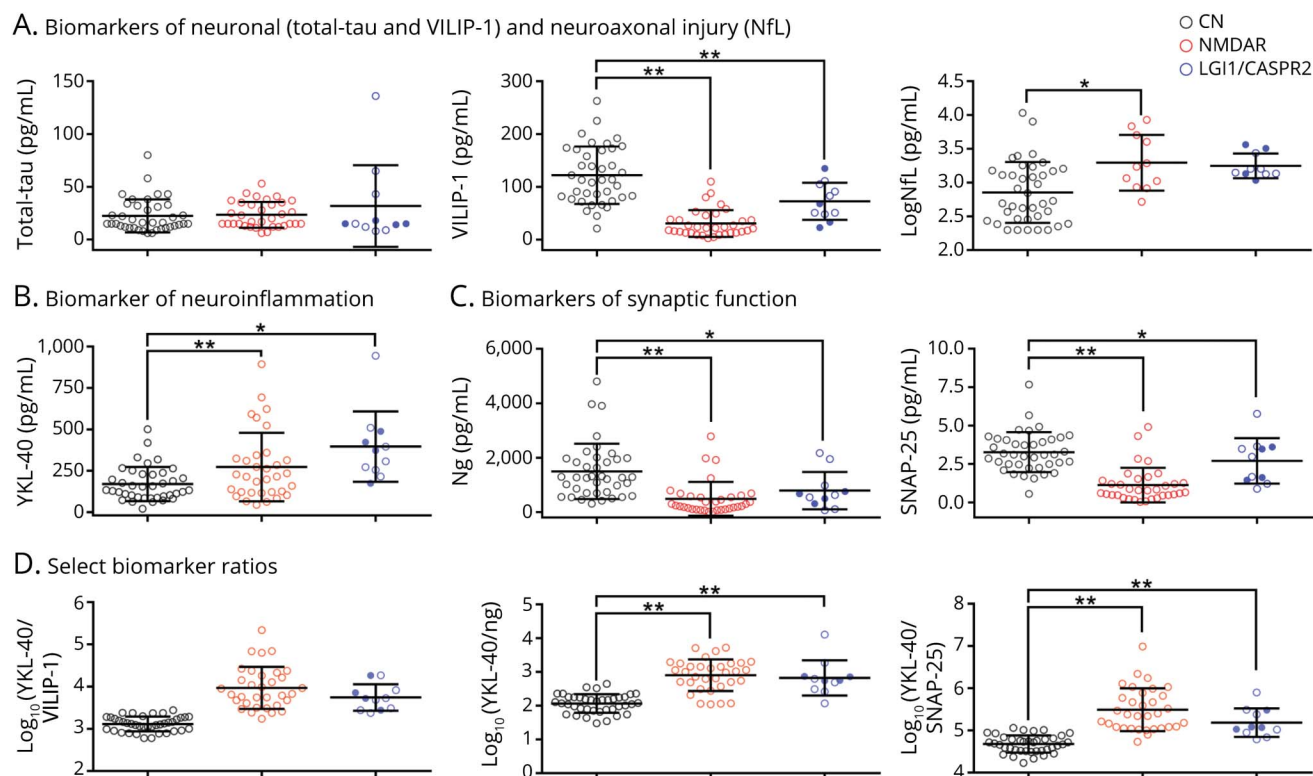
Anonymized study data will be shared with qualified researchers upon request. Supplemental data are available in tables e-1 through e-4 (orcid.org/0000-0001-5133-5538).

Results

Participants

CSF was obtained from 45 patients with AME, including 34 patients (76%) with NMDAR, 7 with LGI1 (16%), and 4 (11%) with CASPR2 antibody encephalitis, and 39 CN individuals. CSF was sampled at the time of AME diagnosis in 42 patients (93%) and at the time of diagnosis with relapsing/resurgent LGI1 antibody encephalitis in the remaining 3 patients (7%). As expected, patients with NMDAR encephalitis (median 27.3 years; range 4.0–41.8) were younger than those

Figure 1 Biomarkers at Presentation



Biomarkers of neuronal and neuroaxonal injury (A), neuroinflammation (B), and synaptic function (C) and selected biomarker ratios (D) in patients with antibody-mediated encephalitis (AME) and cognitively normal (CN) individuals. Biomarker measures are displayed in black for CN individuals, red for NMDA receptor (NMDAR) encephalitis, and blue for leucine-rich glioma-inactivated 1 (LGI1) (open circles)/contactin-associated protein-like 2 (CASPR2) (closed circles) antibody encephalitis. * $p < 0.05$; ** $p < 0.01$. NfL = neurofilament light chain; Ng = neurogranin; SNAP-25 = synaptosomal-associated protein-25; VILIP-1 = visinin-like protein-1; YKL-40 = chitinase-3-like protein.

with LGI1/CASPR2 antibody encephalitis (median 70.4 years; range 60.6–83.2; $p < 0.001$), and were more likely to be female (NMDAR = 85% [29/34], LGI1/CASPR2 = 36% [4/11]; $p = 0.004$).

CSF Biomarkers Distinguish Patients With AME From CN Individuals

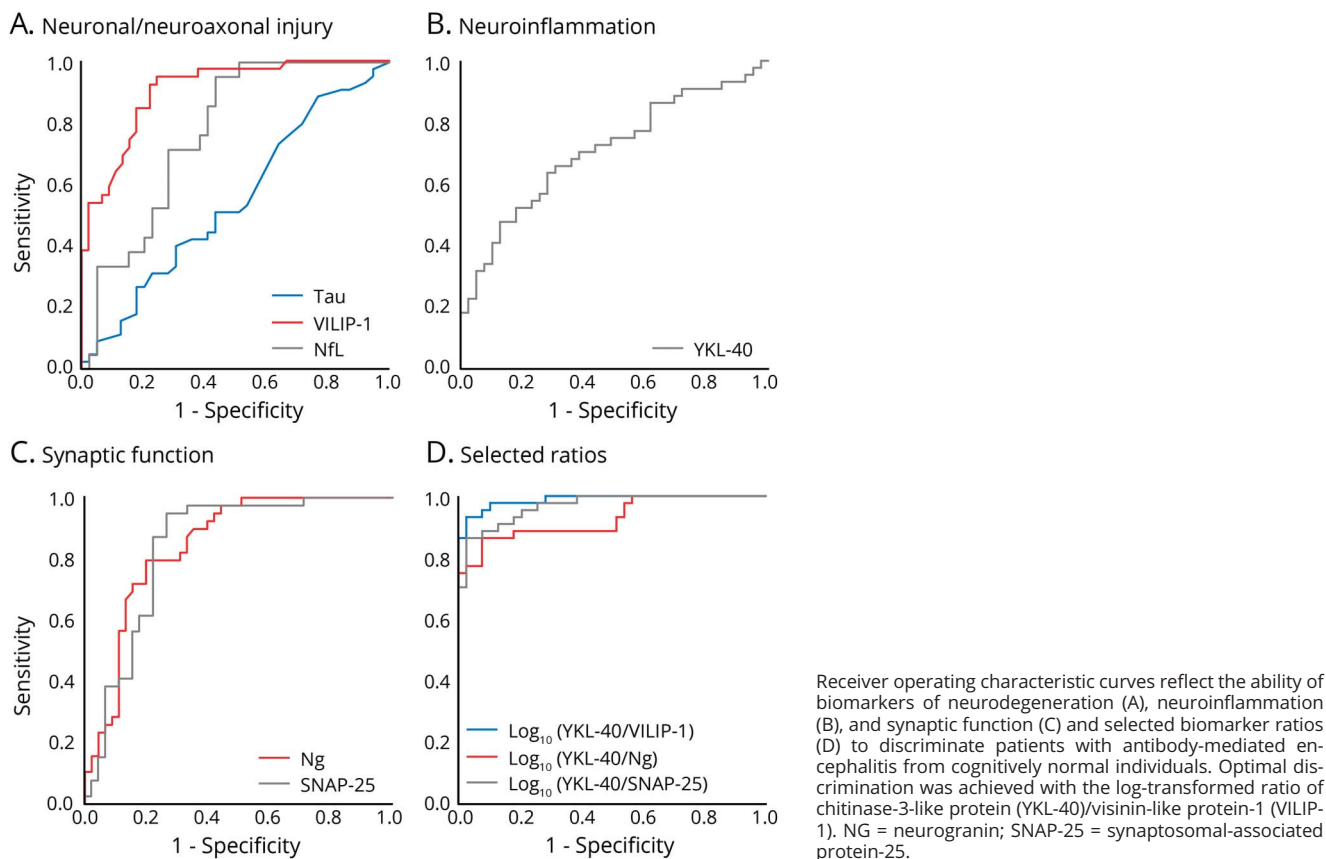
Biomarkers of neuronal (total tau, VILIP-1) and neuroaxonal injury (NfL), neuroinflammation (YKL-40), and synaptic function (SNAP-25, neurogranin) were measured in patients with AME and age- and sex-similar CN individuals (table 1). Increasing age was associated with increasing levels of VILIP-1 ($p < 0.001$), YKL-40 ($p = 0.001$), SNAP-25 ($p = 0.001$), and neurogranin ($p = 0.041$), but not total tau ($p = 0.08$) or NfL ($p = 0.36$). After controlling for age, markers of neuronal injury were similar (total tau) or decreased (VILIP-1) in patients with AME vs CN individuals (figure 1). The neuroinflammatory biomarker YKL-40 was elevated in patients with AME, while markers of synaptic function were markedly decreased in patients with AME. The overall pattern of biomarker changes was similar in patients with NMDAR and LGI1/CASPR2 AME vs CN individuals, when controlling for differences in age. One exception was NfL, which was elevated in NMDAR ($p = 0.046$) but not LGI1/CASPR2 antibody

encephalitis ($p = 0.56$) compared to CN individuals (figure 1A).

The ability of biomarkers and selected biomarker ratios to discriminate between patients with AME and CN individuals was further considered using ROC (figure 2 and table 2). VILIP-1 levels below 53.5 pg/mL identified patients with AME with excellent sensitivity (95%; 95% confidence interval [CI] 89, >99%) and reasonable specificity (76%; 95% CI 64, 89). Optimal discrimination was achieved when comparing the log-transformed ratio of the neuroinflammatory biomarker YKL-40 and marker of neuronal injury VILIP-1 (area under the ROC curve 0.99; 95% CI 0.97, >0.99): a cut point >3.4 discriminated between patients with AME and CN individuals with a sensitivity of 93% (95% CI 84, >99) and specificity of 97% (95% CI 92, >99).

Post hoc comparison of biomarker levels in patients with NMDAR and LGI1/CASPR2 AME confirmed that VILIP-1 levels differed between patients with NMDAR (mean 30.6 pg/mL, SD 25.4) and LGI1/CASPR2 antibody encephalitis (mean 72.6 pg/mL, SD 35.3; $p = 0.02$). In addition, the log-transformed ratio of YKL-40/VILIP-1 was higher in patients with NMDAR (mean 0.97, SD 0.50) than LGI1/CASPR2

Figure 2 Biomarker Performance



antibody encephalitis (mean 0.74, SD 0.31; $p = 0.02$; table 3). Although median time to diagnosis (and CSF sampling) tended to be longer in LGI1/CASPR2 (median 18.5 weeks; range 0.7–136.4) vs NMDAR encephalitis (median 6.6; range 1.3–12.1; $p = 0.06$), no association was observed between time from symptom onset and CSF biomarkers in patients with AME with CSF sampled at the time of diagnosis and available clinical information (table e-1, orcid.org/0000-0001-5133-5538). No trend was observed with visual inspection of biomarker data (data not shown). The relationships did not change substantially when controlling for age (ANCOVA; data not shown).

CSF Biomarkers at Presentation and Outcomes

Longitudinal clinical information was available from 10 patients with NMDAR and 10 LGI1/CASPR2 antibody encephalitis who were prospectively enrolled and followed at study hospitals (table 4). Wide variability in disease severity was noted across patients (table e-2, orcid.org/0000-0001-5133-5538). In general, patients with NMDAR encephalitis were more likely to require ICU admission (7/10 vs 2/10; $p = 0.07$) than patients with LGI1/CASPR2 antibody encephalitis, had higher median mRS at their illness nadir (4 vs 3; $p < 0.01$), and had longer hospital stays (median 4.3 vs 1.0 weeks; $p < 0.01$). Despite these differences, outcomes were similar. A

good outcome (mRS ≤ 2) was reported in 4/9 patients with NMDAR and 6/9 patients with LGI1/CASPR2 antibody encephalitis at hospital discharge ($p = 0.64$); 8/8 patients with NMDAR and 5/7 patients with LGI1/CASPR2 exhibited a good outcome at 12 months follow-up ($p = 0.20$). One patient died suddenly of a presumed acute thrombotic event (myocardial infarction vs pulmonary embolus) within 72 hours of receiving 2 g/kg of IV immunoglobulin for a presumed relapse of LGI1 antibody encephalitis. The patient's family declined postmortem examination.

The associations between measures of disease severity, outcomes, and CSF biomarkers at presentation are summarized in table 5. Mean VILIP-1 ($p = 0.06$) and SNAP-25 ($p = 0.04$) were lower in patients with worst mRS ≥ 3 vs those with worst mRS ≤ 2 , a proxy for disease severity. Neurogranin ($p = 0.04$) and SNAP-25 ($p = 0.04$) were highest in the 2 patients with poorer outcomes (mRS ≥ 3) at 12-month follow-up. Similar findings were observed when considering the degree of correlation between CSF biomarkers and mRS at disease nadir, discharge, and 12-month follow-up (table e-3, orcid.org/0000-0001-5133-5538). Younger age ($\rho = -0.56$), lower VILIP-1 ($\rho = -0.60$) and SNAP-25 ($\rho = -0.54$), and higher $\log_{10}(\text{YKL-40/SNAP-25})$ values ($\rho = 0.48$) were associated with higher worst mRS. Higher YKL-40 ($\rho = 0.60$) and

Table 2 Ability of CSF Biomarkers (and Select Biomarker Ratios) to Discriminate Patients With Antibody-Mediated Encephalitis (AME) From Cognitively Normal (CN) Individuals

| CSF biomarkers | AME vs CN individuals | | | |
|-------------------------------|-----------------------|-------------------------------|----------------------|----------------------|
| | AUC (95% CI) | Cut point, pg/mL ^a | Sensitivity (95% CI) | Specificity (95% CI) |
| Neuronal/axonal injury | | | | |
| Total tau | 0.55 (0.42, 0.67) | 11.5 | 0.89 (0.80, 0.98) | 0.23 (0.10, 0.36) |
| VILIP-1 | 0.91 (0.84, 0.97) | 53.5 | 0.95 (0.89, >0.99) | 0.76 (0.64, 0.89) |
| NfL | 0.77 (0.65, 0.89) | 818.0 | 0.95 (0.86, >0.99) | 0.56 (0.41, 0.72) |
| Neuroinflammation | | | | |
| YKL-40 | 0.71 (0.60, 0.82) | 209.5 × 10 ³ | 0.68 (0.55, 0.82) | 0.82 (0.59, 0.86) |
| Synaptic function | | | | |
| Neurogranin | 0.84 (0.76, 0.93) | 695.5 | 0.80 (0.69, 0.91) | 0.80 (0.67, 0.92) |
| SNAP-25 | 0.84 (0.75, 0.93) | 1.68 | 0.95 (0.89, >0.99) | 0.73 (0.56, 0.85) |
| Ratios | | | | |
| log(YKL-40/VILIP-1) | 0.99 (0.97, >0.99) | 3.4 | 0.93 (0.84, >0.99) | 0.97 (0.92, >0.99) |
| log(YKL-40/neurogranin) | 0.93 (0.87, 0.98) | 2.4 | 0.86 (0.75, 0.96) | 0.92 (0.82, >0.99) |
| log(YKL-40/SNAP-25) | 0.97 (0.94, >0.99) | 5.0 | 0.86 (0.75, 0.96) | 0.97 (0.92, >0.99) |

Abbreviations: AUC = area under the receiver operating characteristic curve; CI = confidence interval; NfL = neurofilament light chain; SNAP-25 = synaptosomal-associated protein-25; VILIP-1 = visinin-like protein-1; YKL-40 = chitinase-3-like protein.

^a No units for ratios.

neurogranin ($\rho = 0.55$) at presentation were associated with higher mRS 12-month following hospital discharge. Differences were also observed when considering the association between CSF biomarkers at presentation and specific symptoms and signs (e.g., psychoses, seizures, requirement for ICU admission, and presence of disease-associated tumors; table e-4, [orcid.org/0000-0001-5133-5538](https://doi.org/10.1215/0000-0001-5133-5538)). Specifically, lower levels of VILIP-1 and SNAP-25 were observed in patients requiring ICU admission and those with disease-associated tumors—features that were most common in NMDAR encephalitis. NfL was also lower in patients with AME with disease-associated tumors. Owing to the exploratory nature of these analyses and modest cohort size, analyses were not adjusted for multiple comparisons.

Discussion

CSF biomarkers of neuronal and neuroaxonal injury, neuroinflammation, and synaptic function differentiated patients with NMDAR and LGI1/CASPR2 antibody encephalitis from CN individuals. Optimal discrimination was achieved using log-transformed ratios of YKL-40, a marker of astroglial activation/inflammation, and markers of neuronal injury (VILIP-1) or synaptic function (SNAP-25 or neurogranin), with sensitivity and specificity exceeding 90%. These candidate biomarkers may be adapted to improve early recognition of AME, warranting further evaluation in cohorts enrolling patients with disorders that may be mistaken for or overlap with AME,

including autoimmune encephalitis associated with antibodies against intracellular antigens,¹⁷ new-onset seizures,²³ rapidly progressive dementia,^{5,24} and primary psychiatric disorders.²⁵ In the subset of patients who were followed longitudinally, levels of VILIP-1 and SNAP-25 were associated with disease severity (lower levels at presentation associated with higher worst mRS), while markers of synaptic function (SNAP-25 and neurogranin) were associated with outcomes 12 months following discharge from hospital. Intriguingly, patients with worse outcomes at 12 months had higher levels of SNAP-25 and neurogranin at presentation, raising the possibility that acute elevations in these CSF biomarkers may reflect loss of synaptic integrity (i.e., synaptic damage), with implications for treatment responsiveness and recovery. These findings may provide insight into the relationships between markers of neuronal and neuroaxonal injury, neuroinflammation and synaptic function, and disease severity, informing the biologic processes that contribute to disability in AME and influence long-term outcomes in recovering patients.

Putative markers of neuronal injury (total tau, VILIP-1) were not increased in NMDAR and LGI1/CASPR2 antibody encephalitis at presentation. These findings are similar to those from a recent series, which reported normal total tau in 8/11 (73%) patients with NMDAR and LGI1 antibody encephalitis.²⁶ Interestingly, total tau was elevated in 3/11 patients with AME (NMDAR encephalitis, $n = 2$; LGI1 antibody encephalitis, $n = 1$), all of whom had T2–fluid-attenuated inversion recovery hyperintensities within the temporal lobes/

Table 3 Demographics and CSF Biomarker Measures in Patients With NMDA Receptor (NMDAR) vs Leucine-Rich Glioma-Inactivated 1 (LGI1)/Contactin-Associated Protein-Like 2 (CASPR2) Antibody Encephalitis

| | Patients with AME | | p Value |
|---|-------------------|----------------------|---------|
| | NMDAR (n = 34) | LGI1/CASPR2 (n = 11) | |
| Demographics | | | |
| Age, y, median (range) | 27.3 (4.0–41.8) | 70.4 (60.6–83.2) | <0.001 |
| Female sex, n (%) | 29 (85) | 4 (36) | 0.003 |
| Weeks from symptom onset, median (range) ^a | 6.6 (1.3–12.1) | 18.5 (0.7–136.4) | 0.06 |
| CSF biomarkers, pg/mL, mean (SD) | | | |
| Total tau | 23.4 (12.3) | 31.7 | 0.79 |
| VILIP-1 | 30.6 (25.4) | 72.6 (35.3) | 0.02 |
| NfL ^b | 2950.7 (2745.1) | 1935.3 (912.6) | 0.85 |
| YKL-40, mean ($\times 10^3 \pm SD$) ^c | 272.5 (206.5) | 396.0 (212.0) | 0.67 |
| Neurogranin | 493.9 (620.7) | 793.6 (684.8) | 0.75 |
| SNAP-25 | 1.1 (1.1) | 2.7 (1.5) | 0.34 |
| Biomarker ratios | | | |
| log(YKL-40/VILIP-1) | 0.97 (0.50) | 0.74 (0.31) | 0.02 |
| log(YKL-40/neurogranin) | −0.094 (0.47) | −0.18 (0.52) | 0.38 |
| log(YKL-40/SNAP-25) | 2.50 (0.51) | 2.18 (0.34) | 0.07 |

Abbreviations: AME = antibody-mediated encephalitis; CN = cognitively normal; NfL = neurofilament light chain; SNAP-25 = synaptosomal-associated protein-25; VILIP-1 = visinin-like protein-1; YKL-40 = chitinase-3-like protein.

Biomarker comparisons performed using analysis of covariance, controlling for age.

^a Weeks from symptom onset reported for patients with CSF sampled at the time of AME diagnosis, including 10 NMDAR and 7 LGI1/CASPR2 antibody encephalitis.

^b NfL measures available from 11 patients with NMDAR encephalitis, 10 patients with LGI1/CASPR2 antibody encephalitis, and 28 CN individuals.

^c YKL-40 measures available from 33 patients with NMDAR encephalitis, 11 patients with LGI1/CASPR2 antibody encephalitis, and 39 CN individuals.

limbic system at presentation, and findings consistent with hippocampal sclerosis at follow-up.²⁶ Thus, increased CSF total tau may identify patients with disease-associated neuronal loss. VILIP-1 is a brain-specific neuronal calcium-sensor protein found in greatest concentrations in the neuronal cell body.^{11,27} Although no prior studies have considered VILIP-1 in patients with AME, increases are noted following stroke²⁷ and in diseases with progressive neuronal loss (e.g., AD^{9,11}). Low levels observed in the present cohort suggest that neuronal integrity was acutely maintained in patients with AME. Whether antibody-antigen effects induced reductions in VILIP-1 through effects on signal transduction and neurotransmission remains to be determined. The low level of biomarkers associated with neuronal loss in patients with AME is consistent with findings from cellular models,^{28–31} animal studies,^{31,32} and limited neuropathologic data,^{33,34} and with the high potential for meaningful recovery following appropriate treatment observed in patients in this series and others.^{1–3}

In contrast to VILIP-1, NfL is abundantly expressed in large-caliber myelinated axons within the central and peripheral nervous systems.³⁵ Disproportionate increases are reported in

patients with diseases that preferentially affect subcortical areas and axonal projections, including frontotemporal lobar degeneration,³⁶ motor neuron disease,³⁷ and demyelinating diseases of the CNS.³⁸ The divergence of markers of neuronal and neuroaxonal injury in patients with AME suggests that persistent deficits in recovering patients are more likely attributable to neuroaxonal disruption, which may contribute to white matter changes and disrupted measures of functional connectivity reported in recovering patients.^{4,6,39,40}

As expected, the inflammatory marker YKL-40 was increased in patients with AME relative to CN individuals. Elevations in YKL-40 along with additional inflammatory cytokines (tumor necrosis factor- α , interleukin-6, interleukin-10) were reported in a study enrolling 33 patients with NMDAR encephalitis, with decreases in CSF levels of YKL-40 correlated with improvement in mRS scores in the 15 patients who returned for 3-month follow-up and underwent repeat CSF sampling.⁴¹ Higher YKL-40 levels measured acutely associated with higher mRS at 12 months in our cohort (table e-2, [orcid.org/0000-0001-5133-5538](https://doi.org/10.1212/0000000151335538)). Taken together, these findings suggest that YKL-40 may represent a dynamic biomarker of CNS inflammation, with the potential for serial changes to identify

Table 4 Clinical Information From Prospectively Evaluated Patients With Antibody-Mediated Encephalitis

| | NMDAR (n = 10) | LG11/CASPR2 (n = 10) | p Value |
|---|---------------------|----------------------|---------|
| Demographics | | | |
| Age, y | 25.2 (18.0–35.2) | 70.1 (60.6–83.2) | <0.001 |
| Female sex | 8 (80) | 3 (30) | 0.07 |
| Presenting symptoms/signs | | | |
| Psychiatric features | 9 (90) | 3 (30) | 0.02 |
| Memory deficits | 4 (40) | 8 (80) | 0.17 |
| Altered mental status | 5 (50) | 2 (20) | 0.35 |
| Seizures | | | |
| Faciobrachial dystonic seizures | 0 | 3 (30) | 0.21 |
| Other | 5 (50) | 2 (20) | 0.35 |
| Focal neurologic signs | 2 (20) | 2 (20) | >0.99 |
| Disease-associated tumor | 5 (50) ^c | 0 | 0.03 |
| Clinical studies | | | |
| CSF pleocytosis ^a | 9 (90) | 3 (30) | 0.02 |
| Brain MRI consistent with autoimmune encephalitis | 4 (40) | 5 (50) | >0.99 |
| Clinical course | | | |
| Time to treatment, wk ^d | 5.3 (1.6–12.1) | 20.0 (0.9–78.1) | 0.37 |
| Duration of hospitalization, wk ^d | 4.3 (2.3–45.9) | 1.0 (0–5.6) | <0.01 |
| Requirement for ICU admission | 7 (70) | 2 (20) | 0.07 |
| Acute treatment | | | |
| IV solumedrol (high dose) | 10 (100) | 8 (80) | 0.47 |
| IV immunoglobulin (2 g/kg) | 9 (90) | 3 (30) | 0.02 |
| Plasma exchange (≥5 cycles) | 8 (80) | 3 (30) | 0.07 |
| Rituximab (375 mg/m ²) | 4 (40) | 6 (60) | 0.66 |
| Other ^b | 2 (20) | 2 (20) | >0.99 |
| mRS nadir ^d | 4 (3–5) | 3 (1–4) | <0.01 |
| mRS discharge ^d | 3 (2–4) | 2 (1–3) | 0.18 |
| mRS 12-mo follow-up ^d | 1 (0–2) | 2 (1–6) | 0.10 |

Abbreviations: CASPR2 = contactin-associated protein-like 2; CN = cognitively normal; ICU = intensive care unit; LG11 = leucine-rich glioma-inactivated 1; mRS = modified Rankin Scale score; NMDAR = NMDA receptor. Values are median (range) or n (%).

^a CSF pleocytosis defined as >5 white blood cells/mm³.

^b Other treatments including bortezomib (IV), cyclophosphamide (IV), and azathioprine (oral).

^c Ovarian teratoma; removed in all patients.

^d Data on initial hospitalization available from 9 patients with NMDAR encephalitis and 9 patients with LG11/CASPR2 antibody encephalitis (n = 18); 12-month follow-up data available from 8 patients with NMDAR encephalitis and 7 patients with LG11/CASPR2 antibody encephalitis (n = 15).

treatment-responsive patients who are more likely to experience better outcomes, or treatment-refractory patients who may benefit from rapid escalation of immunosuppressive therapies. Other markers of inflammation may also be adapted for this purpose (e.g., CXCL13⁴²), warranting further evaluation.

Ample cellular and animal data implicate antibody-mediated disruption of synaptic function in AME pathogenesis.^{32,43,44} Increasing in vivo evidence links neuroinflammation and synaptic dysfunction to impairments in functional networks, behavior, and cognition in recovering patients.^{4,6,39,40} Consistent with these observations, markers of synaptic function

Table 5 Relationship Between CSF Biomarkers and Good (Modified Rankin Scale Score [mRS] ≤ 2) vs Poor (mRS > 2) Outcomes in Prospectively Evaluated Patients With Antibody-Mediated Encephalitis (AME)

| CSF biomarkers | Worst mRS ^a | | | mRS discharge ^b | | | mRS 12-month follow-up ^c | | |
|--------------------------------------|------------------------|-------------------|---------|----------------------------|-------------------|---------|-------------------------------------|------------------|---------|
| | ≤ 2 (n = 4) | ≥ 3 (n = 16) | P Value | ≤ 2 (n = 8) | ≥ 3 (n = 10) | P Value | ≤ 2 (n = 13) | ≥ 3 (n = 2) | P Value |
| Neuronal/axonal injury, pg/mL | | | | | | | | | |
| Total tau | 43.4 (61.8) | 22.1 (16.5) | 0.85 | 34.1 (39.9) | 18.1 (14.5) | 0.62 | 21.5 (18.2) | 74.0 (87.7) | 0.55 |
| VILIP-1 | 88.0 (48.3) | 43.4 (29.3) | 0.06 | 63.2 (42.5) | 43.4 (32.7) | 0.29 | 44.3 (32.3) | 94.0 (15.6) | 0.09 |
| NfL | 2393.1 (1217.5) | 2252.0 (1811.6) | 0.60 | 2700.3 (1933.9) | 2079.4 (1454.2) | 0.49 | 2250.7 (1945.8) | 1466.1 (184.0) | 0.84 |
| Neuroinflammation, pg/mL | | | | | | | | | |
| YKL-40 | 339.2 (737.6) | 334.0 (238.3) | 0.37 | 268.8 (112.2) | 458.9 (258.7) | 0.10 | 324.3 (239.2) | 381.9 (180.2) | 0.31 |
| Synaptic function, pg/mL | | | | | | | | | |
| Neurogranin | 1105.3 (739.6) | 623.1 (615.8) | 0.09 | 754.4 (616.4) | 814.4 (747.7) | 0.86 | 586.2 (552.0) | 2064.5 (154.9) | 0.04 |
| SNAP-25 | 3.42 (0.31) | 1.79 (1.48) | 0.04 | 2.36 (1.20) | 2.05 (1.93) | 0.40 | 1.71 (1.20) | 4.71 (1.51) | 0.04 |
| Selected ratios | | | | | | | | | |
| log(YKL-40/VILIP-1) | 3.66 (0.40) | 3.89 (0.41) | 0.27 | 3.66 (0.34) | 4.10 (0.39) | 0.03 | 3.90 (0.44) | 3.59 (0.14) | 0.31 |
| log(YKL-40/neurogranin) | 2.5 (0.35) | 2.9 (0.55) | 0.19 | 2.70 (0.44) | 2.89 (0.61) | 0.50 | 2.86 (0.54) | 2.24 (0.25) | 0.09 |
| log(YKL-40/SNAP-25) | 4.99 (0.10) | 5.29 (0.38) | 0.11 | 4.91 (0.21) | 5.44 (0.44) | 0.08 | 5.30 (0.40) | 4.90 (0.07) | 0.13 |

Abbreviations: CN = cognitively normal; NfL = neurofilament light chain; SNAP-25 = synaptosomal-associated protein-25; VILIP-1 = visinin-like protein-1; YKL-40 = chitinase-3-like protein.

Values are mean (SD).

^a NfL measures available from 18 patients with AME; YKL-40 measures available from 19 patients with AME.

^b NfL measures available from 16 patients with AME; YKL-40 measures available from 17 patients with AME.

^c NfL measures available from 13 patients with AME.

differentiated patients with AME from CN individuals, and served as reasonable proxies of disease severity in this cohort (lower levels at presentation identified patients with more severe disease). Marked decreases in SNAP-25 and neurogranin at presentation are presumed to reflect depressed neurotransmission and synaptic transmission (i.e., synaptic dysfunction), secondary to antibody-mediated internalization of cell-surface receptors, with maintained neuronal integrity.²⁸⁻³¹ These findings contrast those reported in patients with early symptomatic AD, in whom the accumulation of AD neuropathology results in neurodegeneration and compromised synaptic integrity (i.e., synaptic damage), leading to extracellular increases in SNAP-25 and neurogranin.^{9,14,16} With this in mind, it is interesting that higher SNAP-25 and neurogranin levels at presentation associated with worse 12-month outcomes in our cohort. Thus, while lower levels of biomarkers of synaptic function at presentation may associate with acute severity of AME, higher levels may mark those patients with compromised synaptic integrity who may be at higher risk of poorer long-term outcomes. These patients may be most likely to benefit from novel

therapeutic approaches to mitigate antibody-mediated synaptic damage.^{43,45}

Limitations

This study has several limitations, including those associated with cross-sectional sampling of CSF from patients assessed at academic medical centers in 3 different countries, and the limited access to clinical data from patients whose CSF was obtained from a reference laboratory following identification of NMDAR or LGII/CASPR2 autoantibodies. Prospective evaluation of greater numbers of patients with AME is required to validate findings, including the performance of thresholds for biomarkers/biomarker ratios proposed here. Ideally these studies will include CSF sampling at standardized intervals, informing the longitudinal relationship between putative biomarkers of neuronal and neuroaxonal injury, inflammation and synaptic function, and clinical features. Access to expanded cohorts is also required to explore differences in biomarkers across diseases—including those that may be mistaken for AME—with the goal of determining

the diagnostic utility of biomarkers and biomarker ratios in clinically representative samples (critical to establishing external generalizability of findings), and informing the relationships between candidate CSF biomarkers, mechanisms of disease, and outcomes. Future studies should also consider the influence of other clinically relevant features on CSF biomarker levels, including features commonly associated with AME (e.g., seizures, dyskinesia/dystonia) and medications commonly prescribed to manage AME and accompanying features (e.g., anticonvulsant, sedative/paralytic and immunosuppressant medications). The Clinical Assessment Scale for Autoimmune Encephalitis was recently proposed and validated as a means to prospectively quantify disease severity and grade clinical status in patients with autoimmune encephalitis⁴⁶; this measure was not applied to enrolled patients. Future studies should leverage comprehensive measures of disease severity in AME together with CSF biomarker measures to determine the influence of patient- and disease-specific factors on established and innovative measures of function in patients recovering from AME.^{47,48}

CSF biomarkers of neuronal injury suggest that neuronal integrity is acutely maintained in NMDAR and LGII/CASPR2 antibody encephalitis, despite increases in NfL, a marker of neuroaxonal injury. Low levels of biomarkers of synaptic function may reflect antibody-mediated internalization of cell-surface receptors and may present an acute correlate of antibody-mediated synaptic dysfunction. Biomarkers of neuronal and neuroaxonal injury, neuroinflammation, and synaptic function may predict disease severity, with the potential to influence decision-making regarding the selection and escalation of immunotherapy acutely and inform monitoring in patients recovering from AME.

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Continued

Appendix (continued)

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