

Journal Club: Neurofilament Light Chain Levels in Anti-NMDAR Encephalitis and Primary Psychiatric Psychosis

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Anti-NMDA receptor encephalitis (NMDARe) is an autoimmune brain disorder characterized by antibodies specific for the NMDA receptor in CSF.¹ A subset of patients with NMDARe present with isolated psychosis,² making it challenging to distinguish NMDARe from a primary psychiatric disorder without CSF antibody testing. Although CSF antibody testing is necessary for diagnosing NMDARe, complementary serum tests are needed, particularly in clinical settings where lumbar puncture is difficult to obtain.

In a recent study, Guasp et al.³ investigated the use of serum neurofilament light chain (NfL) in differentiating NMDARe from first-episode psychosis caused by a psychiatric disease (pFEP). Young patients with first-episode psychosis and elevated serum NfL had a higher likelihood of being subsequently diagnosed with NMDARe compared with pFEP. In this single-center study, testing serum NfL at disease onset—using NfL ≥ 15 pg/mL as a cutoff—had a sensitivity of 85% and a specificity of 96% for distinguishing NMDARe from pFEP. In summary, serum NfL testing in young adults presenting with psychosis may ensure that patients with a high likelihood of NMDARe receive comprehensive investigation with CSF anti-NMDA receptor antibody testing, MRI, and EEG. Learning objectives of the following Journal Club article are to (1) discuss the findings and significance of the study by Guasp et al.³ and (2) highlight relevant methods, including statistical methods for assessing new diagnostic tests and NfL detection by single molecule array (SiMoA).

Hypothesis and Design

The aims of this study were (1) to evaluate the use of serum NfL for distinguishing NMDARe from pFEP and (2) to determine whether serum NfL can serve as a biomarker for NMDARe severity and long-term outcome.

The study by Guasp et al.³ is an observational study in which NfL levels were compared between serum obtained from 118 patients with NMDARe, 45 patients with pFEP, 36 patients with herpes simplex encephalitis (HSE), and 36 individuals serving as healthy controls.

Methods

Serum samples from young adult patients (median age 23 [interquartile range (IQR) 17–30]) with a subsequent diagnosis of NMDARe⁴ were collected a median of 26 days after disease onset. 33 of 118 patients with NMDARe presented with isolated psychosis. The modified Rankin scale score (mRS)⁵ at 1 year was used to assess clinical outcomes. Serum samples from patients with a subsequent diagnosis of pFEP (diagnosed at least 1 year after presentation) were collected at their first hospital admission. Serum samples from patients with HSE were obtained at the time of initial diagnosis and used as a positive control for

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elevated NfL.⁶ Notably, HSE is a possible risk factor for NMDARe,⁷ although the authors do not indicate any study participants had HSE preceding NMDARe.

NfL levels were obtained using the SiMoA, an ultrasensitive ELISA technique using paramagnetic bead-based technology. Samples were analyzed in duplicate by blinded personnel, and all resulted NfL values were within the linear ranges of the assays. Intra-assay and interassay coefficients of variation were 4.8% and 10.7%, respectively.

Pairwise comparisons of NfL levels between patient groups were performed using nonparametric testing, and analysis of covariance was used to control for age and sex. Receiver operating characteristic (ROC) analysis and area under the curve (AUC) were used alongside the Youden index to identify a cut-off point with maximum sensitivity and specificity to distinguish NMDARe and pFEP. Using the threshold of serum NfL ≥ 15 pg/mL, the odds ratio for risk estimation was calculated comparing NMDARe and pFEP. Definitions of

diagnostic, methodologic, and statistical terms used may be found in Table.

Results

Guasp et al.³ demonstrated that serum NfL levels were significantly elevated across young adult patients with NMDARe (median 27.5 pg/mL, IQR 16.1–63.80) compared with patients with pFEP (median 7.1 pg/mL, IQR 5.6–10.5, $p < 0.001$) and healthy controls (median 5.1 pg/mL, IQR 3.7–6.2, $p < 0.001$), as well as in the subgroup of NMDARe patients with isolated psychosis (median 25.4 pg/mL, IQR 16.4–65.3) compared with patients with pFEP ($p < 0.001$). ROC analysis of NMDARe patients with isolated psychosis ($n = 33$) and patients with pFEP ($n = 45$) resulted in an AUC of 0.93 (95% CI 0.87–0.99). A serum NfL cutoff of ≥ 15 pg/mL discriminated between the 2 conditions with a specificity of 96% and sensitivity of 85%. Although elevated serum and CSF NfL levels were both associated with severe features of NMDARe at presentation (seizures, intensive care unit admission, CSF

Table Brief Definitions of Diagnostic, Methodologic, and Statistical Terms in the Reviewed Article

Term	Definition
Diagnostic terms	
Anti-NMDA receptor encephalitis	An autoimmune neurologic disease characterized by CSF antibodies targeting the NMDA receptor. Commonly presents with fever, psychosis (hallucinations, delusions), and confusion and can progress to seizures and dysautonomia. Can improve with immunotherapy (steroids, IV immunoglobulin, plasmapheresis, rituximab).
First-episode psychosis caused by a psychiatric disease (pFEP)	The initial presentation of psychosis in a patient with no history of psychosis symptoms (FEP). If the etiology is later determined to be a psychiatric condition (such as schizophrenia or bipolar disorder), then the term pFEP is used.
Herpes simplex encephalitis	An infection of the brain caused by herpes simplex virus characterized by fever, encephalopathy, and seizures and known to cause elevated serum neurofilament light chain (NfL) levels.
Methodologic terms	
Serum NfL	A serum biomarker of neurologic injury under investigation as a diagnostic or prognostic tool for several neurologic diseases. NfL is an intermediate filament protein in neurons which can be detected in serum or CSF in the setting of axonal damage.
Single molecule array	An ultrasensitive paramagnetic bead-based ELISA with capability of measuring protein down to fg/mL concentrations.
Modified Rankin score	A tool for measuring neurologic disability, originally developed to assess patients after stroke but commonly applied as a tool to measure disability due to other neurologic disorders.
Statistical terms	
Observational study	A study which does not involve direct intervention that is used to assess potential association between exposure and outcome, or the accuracy of diagnostic measures, often retrospectively.
Nonparametric tests	Statistical tests that do not rely on known distribution of data. Often used when the distribution of a measure is not normally distributed.
Analysis of covariance	A statistical method to test the effects of categorical variables (i.e., diagnosis) on a continuous variable (i.e., NfL level) while controlling for additional variables. Distinct from analysis of variance in its ability to account for additional covariates.
Receiver operating characteristic (ROC) analysis	A tool commonly used for evaluating the diagnostic performance of tests, which involves plotting the true positive rate (sensitivity) on the y-axis against the false positive rate (1-specificity) on the x-axis. The closer the ROC curve is to the upper left of the plot, the better the diagnostic performance.
Youden index (Youden J statistic)	A summary statistic of the ROC curve used to identify the optimal cut-off for a diagnostic test in which the measure is a continuous variable. The estimated maximum value of the Youden index ($J = \text{sensitivity} + \text{specificity} - 1$) is used to identify a cut-off value to maximize the sensitivity and specificity of a diagnostic test.

pleocytosis, absence of immunotherapy within 4 weeks of disease onset), no significant correlation was found between serum or CSF NfL at disease onset and mRS at 1 year.

Discussion

NfL is under investigation as a biomarker for neuroaxonal damage, with expected elevations in the CSF and blood in patients with neurodegeneration, traumatic brain injury, and immune-mediated diseases.⁸ Previous studies have observed elevated NfL in autoimmune encephalitis^{9,10}; however, NfL levels have not been previously studied in NMDARe with isolated psychosis. Based on findings by Guasp et al.,³ NfL has potential to serve as a serum biomarker to distinguish isolated new-onset psychosis in young adults due to NMDARe or pFEP. While serum NfL alone is not sufficient to diagnose NMDARe, it may serve as a complementary serum biomarker to identify high-risk patients for further MRI, EEG, and CSF antibody testing in clinical settings where lumbar puncture is difficult to obtain.

The cohort of 33 patients with NMDARe and isolated psychosis presented in this study was sizable, considering that this presentation is uncommon.² However, this was a single-center study, and broad implementation would require validation in multiple centers. Most of the patients with NMDARe in this study were young adults and female. Older age is associated with higher and more variable NfL levels¹¹ in addition to worse prognosis, particularly in women, who are also diagnosed more often.¹² Given that elevated serum NfL levels are also seen in neurodegenerative disorders more common in older adults,⁸ the conclusions from this study should be limited to young adult patients, and a broader differential should be considered in the evaluation of older adult patients with psychosis. Further studies with older adult patients and sample collection at different time points may help determine the broader applicability of serum NfL as a screening test. A strength of this study was the high sensitivity (85%) and specificity (96%) for a serum NfL level of ≥ 15 pg/mL to differentiate NMDARe presenting with isolated psychosis from pFEP. However, to screen for NMDARe in first-episode psychosis when CSF studies are difficult to obtain, it would be preferable to have a test with a higher sensitivity as opposed to a higher specificity. Furthermore, although NfL is being investigated as a biomarker for several neuroimmune disorders, this test is not yet commonly used in most clinical settings.⁸

The study by Guasp et al. highlights the statistical methods used to determine thresholds for diagnostic tests. ROC curve analysis and AUC calculation evaluated test performance by assessing the relationship between the true positive rate (sensitivity) and the false positive rate (1-specificity) (Table). The Youden index was used to identify an optimum cut-off point for serum NfL levels. The Youden index (or Youden *J* statistic) is defined across the ROC curve as $J = \text{sensitivity} + \text{specificity} - 1$. Estimating the maximum value of the Youden index allows for selection of the optimum cut-off point for a dichotomous diagnostic test where the measurement is a continuous variable (such as NfL) to

maximize the sensitivity and specificity. Statistical methodology accounting for non-normally distributed values was also a strength of the study. Normality assumptions for NfL levels were appropriately tested using the Shapiro-Wilk test, which determined that the data were non-normally distributed. Because of this, nonparametric test methods were used for analysis including the Mann-Whitney *U* test and the Fisher exact test.

This study also highlights the SiMoA method, which is particularly equipped to accurately measure low concentrations of protein (i.e., NfL 5–10 pg/mL),^{12,13} and is preferred over traditional ELISA methods for precision.¹⁴ Although studies have shown NfL in the CSF and blood to be highly correlated, serum concentrations are much lower,^{13,14} requiring more sensitive and quantitative measurement than allowed by the traditional ELISA method. The SiMoA method used in this study has been shown to measure NfL down to fg/mL concentrations and is sensitive enough to measure a discreet binding event between a paramagnetic bead and single protein.¹⁵

In conclusion, this single-center observational study found that serum NfL ≥ 15 pg/mL measured at disease onset distinguished NMDARe with isolated psychosis from pFEP in young adult patients with a sensitivity of 85% and specificity of 96%. Although diagnosis of NMDARe cannot be based on serum NfL levels alone, Guasp et al. provide strong evidence for the utility of this serum test as a complementary approach to definitive evaluation with MRI, EEG, and CSF antibody testing. If validated across multiple centers, NfL could serve as a serum biomarker to aid in identifying which young adult patients with new-onset psychosis would benefit from CSF antibody testing for NMDARe, particularly in clinical settings where lumbar puncture is difficult to obtain.

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Disclosure

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Appendix (continued)

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James Giarraputo, MD	Duke University School of Medicine, Durham, NC	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Megha Gupta, MD	Duke University School of Medicine, Durham, NC	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Christopher Eckstein, MD	Duke University School of Medicine, Durham, NC	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data; additional contributions: supervision

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