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Predictive Value of Serum Neurofilament Light Chain Levels in Anti-NMDA Receptor Encephalitis

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Abstract

Background and objectives Determinants of disease activity and prognosis are limited in anti-NMDAR encephalitis. Neurofilament light chains (NfL) are markers of axonal damage and have been identified as valuable biomarkers for neurodegenerative and other neuroinflammatory disorders. We aimed to investigate serum NfL levels in patients with anti-NMDAR encephalitis as a biomarker for disease severity and outcome.

Methods In this retrospective study, NfL values were measured in all available pre-treatment serum and paired CSF samples of the nationwide anti-NMDAR encephalitis cohort. The values were analyzed in duplicate using Single Molecule Array (SIMOA) and compared to measurements in healthy references. Follow-up sera were tested to analyze longitudinal responsiveness, if at least available from two time points after diagnosis. Serum NfL levels were compared to data on disease activity (seizures, MRI and CSF findings), severity (mRS, admission days, ICU admission) and outcome (mRS and relapses), using regression analysis.

Results We have included 71 patients (75% female; mean age 31.4, range 0-85 years). Paired CSF samples were available of 33 patients, follow-up samples of 20 patients. Serum NfL levels at diagnosis were higher in patients (mean 19.5 pg/mL, 95%-CI 13.7-27.7) than in references (mean 6.4 pg/mL, 95%-CI 5.8-7.2, $p<0.0001$). We observed a good correlation between serum and CSF NfL values (R=0.84, $p<0.0001$). Serum NfL levels and age correlated in patients (Pearson's R=0.57, $p<0.0001$) and references (R=0.62, $p<0.0001$). Increased NfL values were detected in

patients post-HSV1 encephalitis (mean 248.8 vs 14.1 pg/mL, p <0.0001) and in patients with brain MRI lesions (mean 27.3 vs 11.1 pg/mL, p=0.019). NfL levels did relate to the long-term follow-up (mRS at 12 months; β_{NfL}=0.55, p=0.013), although largely explained by the effect of age on NfL levels and prognosis. In serial samples, NfL values did roughly follow clinical disease activity, albeit with delay.

Discussion/Conclusions Increased serum NfL levels reflect neuro-axonal damage in anti-NMDAR encephalitis. No relationship was identified with disease severity, while the association with outcome was confounded by age. The implied role of sampling timing on NfL levels also limits the applicability of NfL as a prognostic marker.

Introduction

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a complex immune-mediated disorder characterized by antibodies in the cerebrospinal fluid (CSF) against the ionotropic glutamate receptor type 1 subunit of the NMDA receptor. Clinical features include behavioral changes, cognitive impairment, seizures, language disorders, movement disorders, and autonomic dysfunctions. It can occur as a paraneoplastic phenomenon, most often associated with ovarian teratomas, post-infectious after Herpes Simplex Virus encephalitis or sporadically.[1] The disease is treatable by removing the trigger (if paraneoplastic) and administering immunotherapy. Still, patients might require admission to the intensive care unit (ICU) during the acute stage. Many patients experience persisting neurological deficits and 12% of cases relapse within 2 years.[2] The outcome of anti-NMDAR encephalitis has previously been related to clinical factors like the requirement of ICU admission, treatment delay, and a lack of response to first line immunotherapy.[2,3] CSF leukocyte count and antibody titers correlate with outcome and clinical relapses.[3,4] However, titers do not consistently reflect disease activity.[5] Treatment decisions are

currently based on clinical assessment since, despite several attempts, biomarkers for disease severity and prognosis are very limited.[6]

Neurofilaments, and in particular the light chain subunit, are released from axons after acute damage. Neurofilament light chain (NfL) levels have therewith been identified as a useful biomarker for disease activity and prognosis in different neuro-inflammatory and degenerative neurological disorders.[7] The strong correlation between CSF and serum NfL values and the high sensitivity of novel diagnostic techniques, allowing to quantify the lower levels detectable in serum, seem to expand the applicability of serum NfL as a biomarker.[8] The pre-analytical stability of NfL values (i.e. to delayed freezing and repeated thawing/freezing cycles) additionally raises the potential to investigate NfL as a biomarker.[9] In this study we investigate serum NfL levels at diagnosis and follow-up in patients with anti-NMDAR encephalitis to evaluate whether this biomarker of ongoing axonal damage correlates with disease severity and long-term outcome.

Methods

Study subjects and sample selection

As the national referral center for autoimmune encephalitis of the Netherlands, accredited as European Reference Network site (ERN-RITA), we take note of all nationwide diagnoses of anti-NMDAR encephalitis. We have targeted all Dutch patients complying with the criteria for a definite anti-NMDAR encephalitis[10], based on (1) the availability of a sufficient amount of serum from the time of diagnosis, (2) serum drawn before start of immunotherapy, and (3) relevant clinical data of at least four months after diagnosis (eFigure 1). All eligible patients had previously consented to be in the nationwide anti-NMDAR encephalitis cohort and have been phenotyped clinically well (eTable 1).[11] We compared the data to a healthy reference group (n=61; 70% female; mean age 41.9, range 25-67 years) and to previously suggested age-based cut-off values.[12-14] To correlate

serum to CSF, we tested all available pre-treatment CSF samples drawn within 48 hours from the serum sample. To investigate NfL longitudinally, we selected those patients of whom we had sufficient amounts of sera from at least two different time points after diagnosis.

Clinical parameters

Extensive clinical data had been collected as part of our nationwide study.[11] Age of onset, preceding Herpes Simplex Virus encephalitis, concomitant tumors, the presence of seizures or movement disorders, cerebral MRI abnormalities and antibody titers were considered potentially relevant covariates for NfL levels. Maximum modified Rankin Scores (mRS), duration of hospital admission and the need for ICU admission were used as measures for disease severity. Short and long-term outcomes were quantified as the mRS score at 4 and 12 months after diagnosis, respectively. A relapse was defined as the (re-)emergence or worsening of clinical symptoms fitting the diagnostic criteria for anti-NMDAR encephalitis, after a period of at least two months of improvement or stabilization, combined with the confirmation of anti-NMDAR antibodies in CSF.[2,11]

Procedures for NMDAR antibody and NfL measurements

Anti-NMDAR antibodies were detected using cell-based assays (Euroimmun, AG, Lübeck, Germany) in CSF, and confirmed by immunohistochemistry, as described before.[11] All patients had antibodies in CSF. NfL concentration in serum and CSF was measured in duplicate using SIMOA NfL-light kit with SR-X immunoassay analyzer (Quanterix Corporation, Billerica, Massachusetts) as previously described,[15] by investigators blinded to clinical data. Comparison was made with sera from 61 healthy controls. The Mean intra-assay coefficient of variation (CV) of duplicates and inter-assay CV were 6.7% and 6.4%, respectively. Samples with CV above 20% were reanalyzed.

Standard Protocol Approvals, Registrations, and Patient Consents

This retrospective study was waived and declared non-WMO complicit by the Institutional Review Board of Erasmus MC. Written informed consent was obtained from all patients.

Statistics

The data on NfL values in serum and CSF were logarithmically transformed to adjust for skewness of the distribution. The descriptive statistics provided in this paper are centered around the geometric means. The correlation between NfL levels in serum and CSF was investigated by calculating Pearson's correlation coefficient. A good correlation allowed serum NfL to be used as a surrogate biomarker. The serum NfL levels of the patients were compared to healthy adult references, as well as to age-based cut-off values from literature, also including pediatric references.[12-14] The known influence of age on NfL levels was confirmed by fitting a linear regression model. The rest of the analyses were corrected for this effect by the addition of age as a covariate. As the less-extensively-investigated effect of age on NfL in children does not seem strictly linear in the lowest age range, and the included healthy references were adults, we also performed all analyses in the subgroup of adult patients.

The relationship between the independent variables tumor, preceding HSV1 infections and visible MRI abnormalities and the dependent variable serum NfL, and the relationship between serum NfL levels (independent variable) and duration of hospital admission were tested with variants of linear regression models, univariable and multivariable with age as a covariate. Because of the reported effect of a HSV1 encephalitis on both NfL levels and prognosis of anti-NMDAR encephalitis[16,17], we have left these patients out of the analyses to determine prognostic value of serum NfL in anti-NMDAR encephalitis (eFigure 1). Logistic regression analysis was applied to investigate the relationship between serum NfL at diagnosis and the need for ICU admission, as measures of

disease severity. The predictive value of early NfL levels for maximum disease severity (maximum mRS), outcome (mRS score at 4 and 12 months after disease onset) and time to recovery (improving to an mRS score ≤2) was explored with ordinal regression analysis. Patients with an mRS > 2 *before* disease onset were excluded from the latter analyses as we would not be able to determine the outcome specifically related to the anti-NMDAR encephalitis (eFigure 1).

Data Availability

Any data not published within this article are available at the Erasmus University Medical Centre. Patient-related data will be shared on reasonable request from any qualified investigator, maintaining anonymity of the individual patients.

Results

We included 71 anti-NMDAR encephalitis patients (75% female; mean age 31.4, range 0-85 years; Table 1), representative of the complete national cohort (eTable 1).

NfL levels and associated clinical factors

The serum NfL concentration at diagnosis was higher in anti-NMDAR encephalitis patients (mean 19.5 pg/mL, 95%-confidence interval (CI) 13.7-27.7) than in healthy controls (mean 6.4 pg/mL, 95%-CI 5.8-7.2, $p<0.0001$). A positive association was observed between serum NfL values and age at sampling, in both patients (Pearson's R=0.57, $p<0.0001$) and healthy controls (R=0.62, $p<0.0001$; Figure 1A). Serum and CSF NfL levels (n=33) showed a good correlation (Pearson's R=0.84, $p<0.0001$; Figure 1B). Patients with a post-HSV1 anti-NMDAR encephalitis had higher serum NfL values than those without a preceding infection (mean 248.8 vs 14.1 pg/mL, $p <0.0001$; Figure 2). Serum NfL levels were significantly higher in patients with cerebral MRI lesions

compared to patients without (mean 27.3 vs 11.1 pg/mL, p=0.019, patients with post-HSV1 encephalitis were not included in this analysis; Figure 2). These effects were similar when age was added to the analysis as a co-variable (β_{HSV}=2.7, p<0.0001, β_{MRI}=0.70, p=0.012; Table 2). Analyzing these results in a slightly different way, using dichotomous age-based cut-off values, confirmed these results: patients with increased serum NfL levels (n=39 [55%]) more frequently had a preceding HSV1 encephalitis (21% vs 0%, p=0.019) and more frequently had MRI abnormalities (54% vs 16%, p=0.002), compared to patients with serum NfL levels below the cut-off (eTable 2).

The presence of concomitant tumors, seizures and movement disorders, the delay between symptom onset and sample drawing, and serum and CSF antibody titers did not significantly relate to NfL levels, with or without age as co-variable (Table 2 & eFigures 2–4).

A subgroup analysis of the adult patients (n=59) to account for different behavior of NfL as serum biomarker in children did not provide different results (eTable 3).

The prognostic value of NfL for disease severity and outcome

NfL levels at diagnosis did not significantly differ between patients who needed ICU admission or not and did not relate to the maximum mRS score over the course of the disease (eFigure 5) nor the duration of hospital admission (eFigure 6), as markers for disease severity. Similarly, no relation was noted between NfL levels at diagnosis and disability (mRS) four months after disease onset (eFigure 7).

In a univariable analysis, NfL serum levels at diagnosis were related to the outcome after 12 months (β_{NfL}=0.55, p=0.013) and the time until recovery (to an mRS≤2; β_{NfL}=0.31, p=0.050), although this seemed largely attributed to the effect of age at disease onset (β_{NfL}=0.38, p=0.14 and β_{Age}=0.018, p=0.26 for outcome after 12 months, Figure 3A; β_{NfL}=0.18, p=0.31 and β_{Age}=0.020, p=0.15 for

recovery time, Figure 3B; Table 2). These findings were confirmed when applying dichotomous age-based cut-off values (p=0.069 for outcome after 12 months, p=0.14 for recovery time; eTable 2), and a subgroup analysis of the adult patients showed no different results either (eTable 3).

NfL in longitudinal follow-up sera

We included a total of 58 follow-up samples of 20 patients, of whom 10 had had at least one relapse of the encephalitis (Figure 4A) and 10 had a monophasic course.

When monitoring NfL levels over time, we noted that NfL values often increased considerably in the weeks after onset, especially while on ICU, and had a subsequent decrease over time, more pronounced in patients discharged from ICU (Figure 4, B and C, eFigures 8 and 9). Interestingly, in an illustrative patient with a relapse, the main increase of NfL was seen only after the onset of symptoms (both in the initial episode and at relapse; Figure 4B). The suggestion of increase at the moment of onset of the relapse was similar to another patient who did not experience a relapse (Figure 4C). When focusing on the repeated serum measurements within the first months after disease onset, we see an increase of NfL levels up to 4-6 weeks (Figure 5A). This is in line with the observation that the majority of serum NfL measurements within the first weeks fall within the range of the healthy references, as opposed to the measurements after 2-4 weeks (Figure 5B).

Discussion

In this study, we have investigated serum NfL as a biomarker in a large cohort of well characterized patients with anti-NMDAR encephalitis. We demonstrate several important aspects: 1) although serum NfL levels are increased in patients with an anti-NMDAR encephalitis, these do not provide independent prognostic value at diagnosis, neither for maximum severity nor for long-term outcome; and 2) serum NfL can be used to monitor activity of disease in the chronic phase.

However, timing of serum NfL sampling has an influence on the values found, complicating the use as biomarker to identify relapses early.

We have first established that serum NfL levels are increased in patients with anti-NMDAR encephalitis compared to the general population. Identified associations between NfL levels and age, a preceding HSV1-encephalitis, and radiological signs of tissue damage are all in line with what we would expect, NfL being a marker of tissue injury associated with neuro-axonal damage.[8,17]

We identified no association between NfL levels at diagnosis and measures of maximum disease severity. In serial samples of patients admitted to the ICU, NfL levels increased within the first weeks, however the initial values at diagnosis had no predictive value for ICU admission. Using univariable analysis, an association between serum NfL values and outcome after a year seemed to be present. As we and others have identified age as a factor associated both with higher NfL levels and with longer time to recovery, correction for age at onset was warranted.[11] This explained at least the larger part of the difference in NfL levels, and no independent relationship between NfL and outcome at 12 months was identified.

These findings correspond partly with literature. Whereas other studies also negate the association between initial NfL levels, albeit in CSF, and disease severity[15,18], two studies do associate NfL levels with disease severity (i.e. ICU admission)[19,20]. The referred samples in one were of the moment of determining severity and did not *precede* or *predict* disease severity (i.e. at diagnosis)[19]. Two of the mentioned studies, in homogeneous cohorts of anti-NMDAR encephalitis patients, also described no applicability of NfL levels in CSF or serum as a biomarker for outcome.[18,20] Two other studies found a correlation between NfL levels in diagnostic CSF samples and long-term outcome, even after (partial) correction for age, albeit in heterogeneous cohorts of patients with

autoimmune encephalitis or paraneoplastic syndromes with diverse pathophysiological mechanisms (not limited to anti-NMDAR encephalitis).[21,22]

The observed NfL increase in the weeks *after* symptom onset, was previously observed in a cohort of anti-NMDAR encephalitis patients.[19] This might suggest that axonal damage is not a hyperacute initial feature of the disease *causing* clinical symptoms, rather serum NfL levels likely reflect an integral measure of antecedent and ongoing neuronal damage. This additionally discourages the deployment of NfL as a biomarker, as the timing of sampling largely affects the values found. Although the longitudinal data is limited, we provide some data to suggest that the same delay in increase hampers the use of serum NfL as a marker to predict relapses. As serum levels do often increase, a delayed NfL measurement may be used as a marker to differentiate between a relapse, pseudorelapse (i.e. due to infection) or persisting neurological symptoms. As serum NMDAR antibodies are not very reliable,[4] and CSF NMDAR antibody titers at remission are often not available, this could still be very valuable to decide upon escalation of treatment or instalment of maintenance immunotherapy.

Our study has limitations, mainly related to the sample size and retrospective design. Although we have included all available pre-treatment samples of our nationwide cohort, anti-NMDAR encephalitis is a rare disease and the consequentially moderate sample size limits the power of our analyses. The retrospective study design did not allow to monitor NfL values at regulated time points and the longitudinal analysis is based on a limited subgroup only. In addition, follow-up was relatively short and we did not perform regular imaging at consistent intervals, so we were unable to correlate NfL levels with lesion load and brain volume loss over time. Last, we used the mRS to quantify disability and outcome, which, despite being the most commonly used scale, is crude and not specific for this condition. More sensitive (cognitive) measures might yield different results

correlating NfL values and disability. Prospective, structured follow-up could solve the majority of these limitations in the future.

In conclusion, axonal damage is a feature of active anti-NMDAR encephalitis and measuring serum NfL might prove helpful in clinical practice to identify active disease, and monitor recovery. NfL levels are no independent predictors for disease severity or outcome. As timing of sampling seems to have a large impact on NfL values, the use of single values in prediction of disease severity, outcome or relapses is complicated.

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References

1. Dalmau J, Armangué T, Planagumà J, et al. An update on anti-NMDA receptor encephalitis for neurologists and psychiatrists: mechanisms and models. Lancet Neurol. 2019 Nov;18(11):1045-1057. doi: 10.1016/S1474-4422(19)30244-3. PMID: 31326280.

2. Titulaer MJ, McCracken L, Gabilondo I, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. Lancet Neurol. 2013 Feb;12(2):157-65. doi: 10.1016/S1474-4422(12)70310-1. PMID: 23290630; PMCID: PMC3563251.

3. Balu R, McCracken L, Lancaster E, Graus F, Dalmau J, Titulaer MJ. A score that predicts 1-year functional status in patients with anti-NMDA receptor encephalitis. Neurology. 2019 Jan 15;92(3):e244-e252. doi: 10.1212/WNL.0000000000006783. PMID: 30578370; PMCID: PMC6340387.

4. Gresa-Arribas N, Titulaer MJ, Torrents A, et al. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. Lancet Neurol. 2014 Feb;13(2):167-77. doi: 10.1016/S1474-4422(13)70282-5. PMID: 24360484; PMCID: PMC4006368.

5. Hansen HC, Klingbeil C, Dalmau J, Li W, Weissbrich B, Wandinger KP. Persistent intrathecal antibody synthesis 15 years after recovering from anti-N-methyl-D-aspartate receptor encephalitis. JAMA Neurol. 2013 Jan;70(1):117-9. doi: 10.1001/jamaneurol.2013.585. PMID: 23318518; PMCID: PMC3707142.

6. Leypoldt F, Höftberger R, Titulaer MJ, et al. Investigations on CXCL13 in anti-N-methyl-D-aspartate receptor encephalitis: a potential biomarker of treatment response. JAMA

Neurol. 2015 Feb;72(2):180-6. doi: 10.1001/jamaneurol.2014.2956. PMID: 25436993; PMCID: PMC4836910.

7. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol. 2018 Oct;14(10):577-589. doi: 10.1038/s41582-018-0058-z. PMID: 30171200.

8. Mariotto S, Sechi E, Ferrari S. Serum neurofilament light chain studies in neurological disorders, hints for interpretation. J Neurol Sci. 2020 Sep 15;416:116986. doi: 10.1016/j.jns.2020.116986. PMID: 32563076.

9. Altmann P, Leutmezer F, Zach H, et al. Serum neurofilament light chain withstands delayed freezing and repeated thawing. Sci Rep. 2020 Nov 17;10(1):19982. doi: 10.1038/s41598-020-77098-8. PMID: 33203974; PMCID: PMC7672085.

10. Graus F, Titulaer MJ, Balu R, et al. A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol. 2016 Apr;15(4):391-404. doi: 10.1016/S1474-4422(15)00401-9. Epub 2016 Feb 20. PMID: 26906964; PMCID: PMC5066574.

11. Bastiaansen AEM, de Bruijn MAAM, Schuller SL, et al. Anti-NMDAR Encephalitis in the Netherlands, Focusing on Late-Onset Patients and Antibody Test Accuracy. Neurol Neuroimmunol Neuroinflamm. 2021 Dec 22;9(2):e1127. doi: 10.1212/NXI.0000000000001127. PMID: 34937737; PMCID: PMC8696553.

12. Valentino P, Marnetto F, Martire S, et al. Serum neurofilament light chain levels in healthy individuals: A proposal of cut-off values for use in multiple sclerosis clinical practice. Mult Scler Relat Disord. 2021 June; 54: doi: 10.1016/j.msard.2021.103090. PMID: 34182224.

13. Hviid CVB, Knudsen CS, Parkner T. Reference interval and preanalytical properties of serum neurofilament light chain in Scandinavian adults. Scand J Clin Lab Invest. 2020

Jul;80(4):291-295. doi: 10.1080/00365513.2020.1730434. Epub 2020 Feb 20. PMID: 32077769.

14. Jin J, Cui Y, Hong Y, et al. Reference values for plasma neurofilament light chain in healthy Chinese children. Clin Chem Lab Med. 2021 Aug 27;60(1):e10-e12. doi: 10.1515/cclm-2021-0804. PMID: 34449172.

15. Mariotto S, Gajofatto A, Zuliani L, et al. Serum and CSF neurofilament light chain levels in antibody-mediated encephalitis. J Neurol. 2019 Jul;266(7):1643-1648. doi: 10.1007/s00415-019-09306-z. PMID: 30944980.

16. Armangue T, Leypoldt F, Málaga I, et al. Herpes simplex virus encephalitis is a trigger of brain autoimmunity. Ann Neurol. 2014 Feb;75(2):317-23. doi: 10.1002/ana.24083. Epub 2014 Feb 25. PMID: 24318406; PMCID: PMC3961499.

17. Westman G, Aurelius E, Ahlm C, et al. Cerebrospinal fluid biomarkers of brain injury, inflammation and synaptic autoimmunity predict long-term neurocognitive outcome in herpes simplex encephalitis. Clin Microbiol Infect. 2021 Aug;27(8):1131-1136. doi: 10.1016/j.cmi.2020.09.031. Epub 2020 Sep 23. PMID: 32979577.

18. Day GS, Yarbrough MY, Körtvelyessy PM, et al. Prospective Quantification of CSF Biomarkers in Antibody-Mediated Encephalitis. Neurology. 2021 May 18;96(20):e2546-e2557. doi: 10.1212/WNL.0000000000011937. Epub 2021 Apr 1. Erratum in: Neurology. 2021 Oct 19;97(16):795. PMID: 33795390.

19. Macher S, Zrzavy T, Höftberger R, et al. Longitudinal measurement of cerebrospinal fluid neurofilament light in anti-N-methyl-D-aspartate receptor encephalitis. Eur J Neurol. 2021 Apr;28(4):1401-1405. doi: 10.1111/ene.14631. PMID: 33145945; PMCID: PMC7984371.

20. Guasp M, Martín-Aguilar L, Sabater L, et al. Neurofilament Light Chain Levels in Anti-NMDAR Encephalitis and Primary Psychiatric Psychosis. Neurology. 2022 Apr 5;98(14):e1489-e1498. doi: 10.1212/WNL.0000000000200021. Epub 2022 Feb 10. PMID: 35145006.

21. Constantinescu R, Krýsl D, Andrén K, et al. Cerebrospinal fluid markers of neuronal and glial cell damage in patients with autoimmune neurologic syndromes with and without underlying malignancies. J Neuroimmunol. 2017 May 15;306:25-30. doi: 10.1016/j.jneuroim.2017.02.018. PMID: 28385184.

22. Nissen MS, Ryding M, Nilsson AC, et al. CSF-Neurofilament Light Chain Levels in NMDAR and LGI1 Encephalitis: A National Cohort Study. Front Immunol. 2021 Dec 16;12:719432. doi: 10.3389/fimmu.2021.719432. PMID: 34975832; PMCID: PMC8716734.

23. Kuhle J, Kropshofer H, Haering DA, et al. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology. 2019 Mar 5;92(10):e1007-e1015. doi: 10.1212/WNL.0000000000007032. Epub 2019 Feb 8. PMID: 30737333; PMCID: PMC6442011.

24. Dalmau J, Geis C, Graus F. Autoantibodies to Synaptic Receptors and Neuronal Cell Surface Proteins in Autoimmune Diseases of the Central Nervous System. Physiol Rev. 2017 Apr;97(2):839-887. doi: 10.1152/physrev.00010.2016. PMID: 28298428; PMCID: PMC5539405.

Tables

Table 1 Patient characteristics of the included anti-NMDAR encephalitis patients.

Variable	Included patients (n=71)
Sex (female, %)	53 (75%)
Age (mean, IQR, range)	32 (18-41; 0.7-86)
Tumor (n, %)	20/69 (29%)
Preceding HSV infection (n, %)	8 (11%)
MRI abnormalities (n, %)	26 (38%)
Mesiotemporal hyperintensity (n, % of patients with MRI abnormalities)	15 (58%)
Mesiotemporal atrophy (n, % of patients with MRI abnormalities)	2 (8%)
Thalamic lesions (n, % of patients with MRI abnormalities)	4 (15%)
Multifocal white matter lesions (n, % of patients with MRI abnormalities)	3 (12%)
Brainstem lesions(n, % of patients with MRI abnormalities)	2 (8%)
Baseline mRS (median, IQR, range)*	0 (0-0; 0-4)
mRS at onset (median, IQR, range)	3 (2-3; 2-5)
Maximum mRS (median, IQR, range)	4 (3-5; 3-5)
Hospital admission days (mean, IQR, range)	80 (28-93; 3-632)
ICU admission (n, %)	32 (45%)
mRS after 12 months (median, IQR, range)	2 (1-2; 0-6)
Time to mRS2 (in months; mean, IQR, range)	5.6 (2-10; 1-not achieved)
Last mRS (median, IQR, range)	2 (1-3; 0-6)
Follow-up time in months (mean, IQR, range)	35 (14-45; 3-180)

* Six patients had an mRS > 2

Table 2 Analyses with and without age correction.

Independent variables (NfL as a dependent variable)	Without age correction	With age as a covariate
Age[2]	β_{Age}= 0.037, ***p<0.0001***	Not applicable
Tumor (with vs without)[1]	Mean 14.7 vs 22.0 pg/mL, p=0.24	β_{Tumor} = -0.57, p=0.12
Preceding HSV infection (with vs without)[1,*]	Mean 248.8 vs 14.1 pg/mL, ***p<0.0001***	β_{HSV} = 2.7, ***p<0.0001***
Seizures (with vs without)[1]	Mean 11.5 vs 20.2 pg/mL, p=0.097	$\beta_{Seizures}$ = -0.25, p=0.36
Movement disorders (with vs without)[1]	Mean 13.1 vs 16.6 pg/mL, p=0.47	$\beta_{Movement}$ = -0.12, p=0.66
MRI abnormalities (mean with vs without)[1]	Mean 27.3 vs 11.1 pg/mL, ***p=0.019***	β_{MRI} = 0.70, ***p=0.012***
Time from onset to sample drawing[2]	β_{Delay}= -0.0006, p=0.59	β_{Delay} = -0.0006, p=0.64
Serum antibody titre[2]	β_{Titer} = -0.044, p=0.56	β_{Titer} = -0.014, p=0.82
CSF antibody titre[2]	β_{Titer} = 0.037, p=0.51	β_{Titer} = 0.006, p=0.90
mRS at onset[5]	β_{mRS} = -0.044, p=0.87	β_{mRS} = 0.22, p=0.34

Dependent variables (NfL as an independent variable)[**]

Maximum disease severity (mRS)[4]	β_{NfL}=0.18, p=0.38	β_{NfL} = 0.21, p=0.23
ICU admission (yes vs no)[3]	β_{NFL} = 0.10, p=0.65	β_{NFL} = 0.17, p=0.55
Duration of hospital admission (days)[5]	β_{NFL} = -0.086, p=0.44	β_{NFL}= -0.070, p=0.61
Disability (mRS) after 4 months[4]	β_{NfL}= 0.23, p=0.28	β_{NfL}= 0.10, p=0.69
Disability (mRS) after 12 months[4]	β_{NfL}= 0.55, ***p=0.013***	β_{NfL}= 0.38, p=0.14 β_{Age}= 0.018, p=0.26
Time to recovery (mRS2)[5]	β_{NfL}= 0.31, ***p=0.050***	β_{NfL}= 0.18, p=0.31 β_{Age}= 0.020, p=0.15

[1] Dichotomous independent variable, tested with a T-test.
[2] Continuous independent variable, tested with linear regression.
[3] Dichotomous dependent variable, tested by binomial logistic regression.
[4] Ordinal dependent variable, tested by ordinal logistic regression.
[5] Continuous dependent variable, tested by linear regression.
[*] Because of this known effect, we have excluded post-HSV encephalitis patients from the rest of the analyses.
[**] Patients with a premorbid mRS > 2 were excluded from these analyses.

Figure titles & legends

Figure 1: Serum NfL correlation with age and CSF.

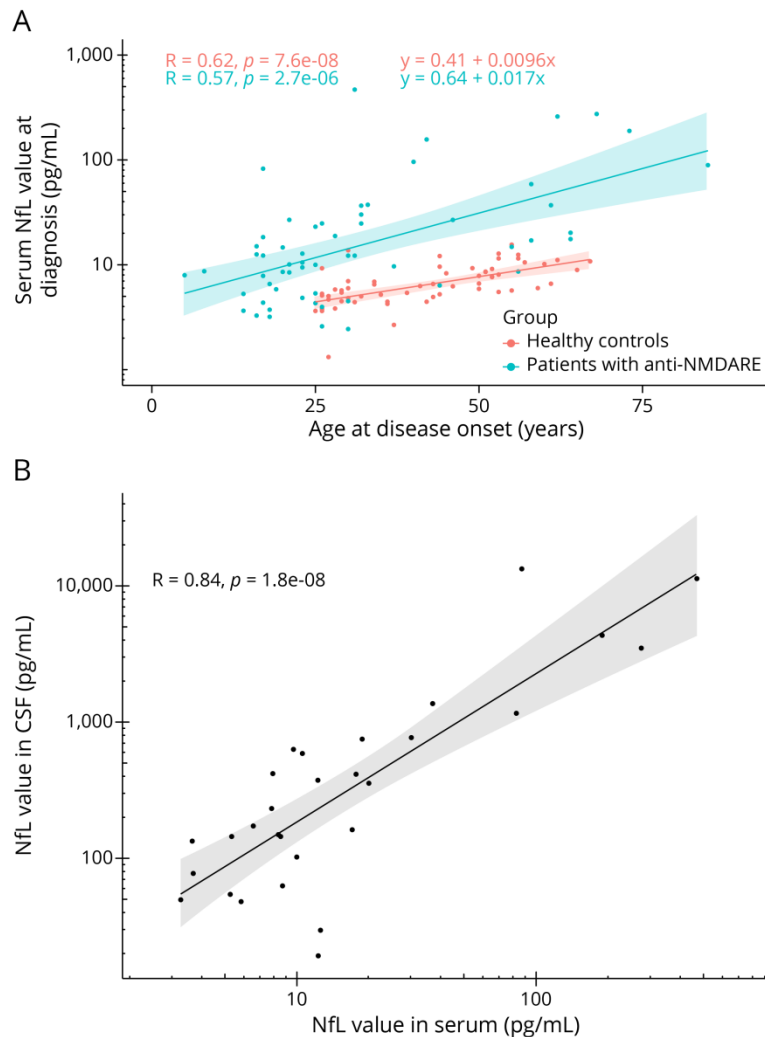NfL levels in serum correlate positively with age (A) and CSF (B).

A

B

Figure 2: Serum NfL related to radiological findings.

Anti-NMDAR encephalitis patients with MRI abnormalities had higher NfL levels in serum (p=0.019; geographic means of patients with and without MRI abnormalities are represented by the black horizontal lines). Patients with a preceding HSV1 encephalitis (depicted in blue; all with MRI abnormalities) had even higher NfL levels in serum compared to patients without preceding a preceding HSV1 encephalitis (p<0.0001; the geographic means of patients with and without a preceding HSV1 encephalitis are represented by the blue and red dotted horizontal lines, respectively).
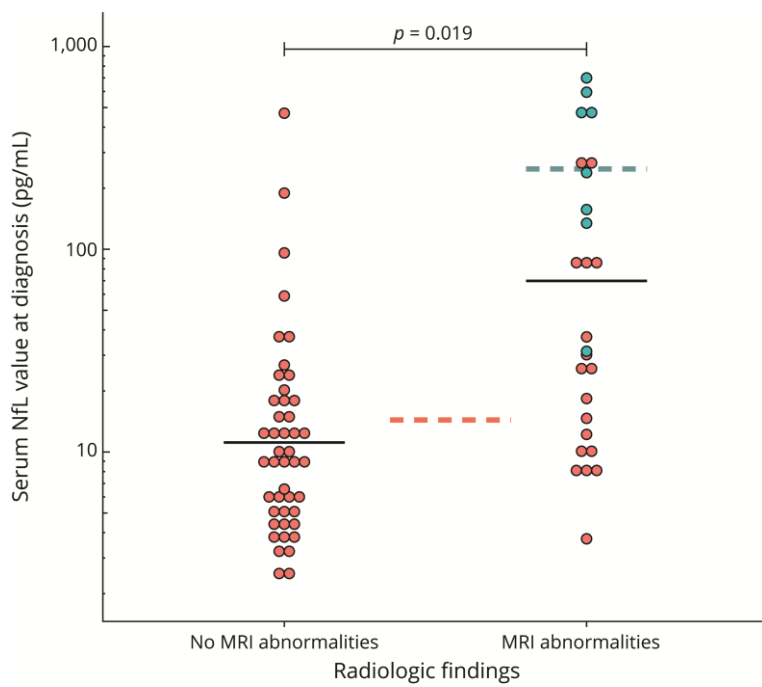
Figure 3: NfL, age and long-term outcome.

Higher NfL levels in serum were correlated to a worse outcome (higher mRS) after 12 months (A) and a longer time to recovery (B). As can be seen by the colored dots, this was largely influenced by the age of onset. Correction for age at onset negated the significant association.
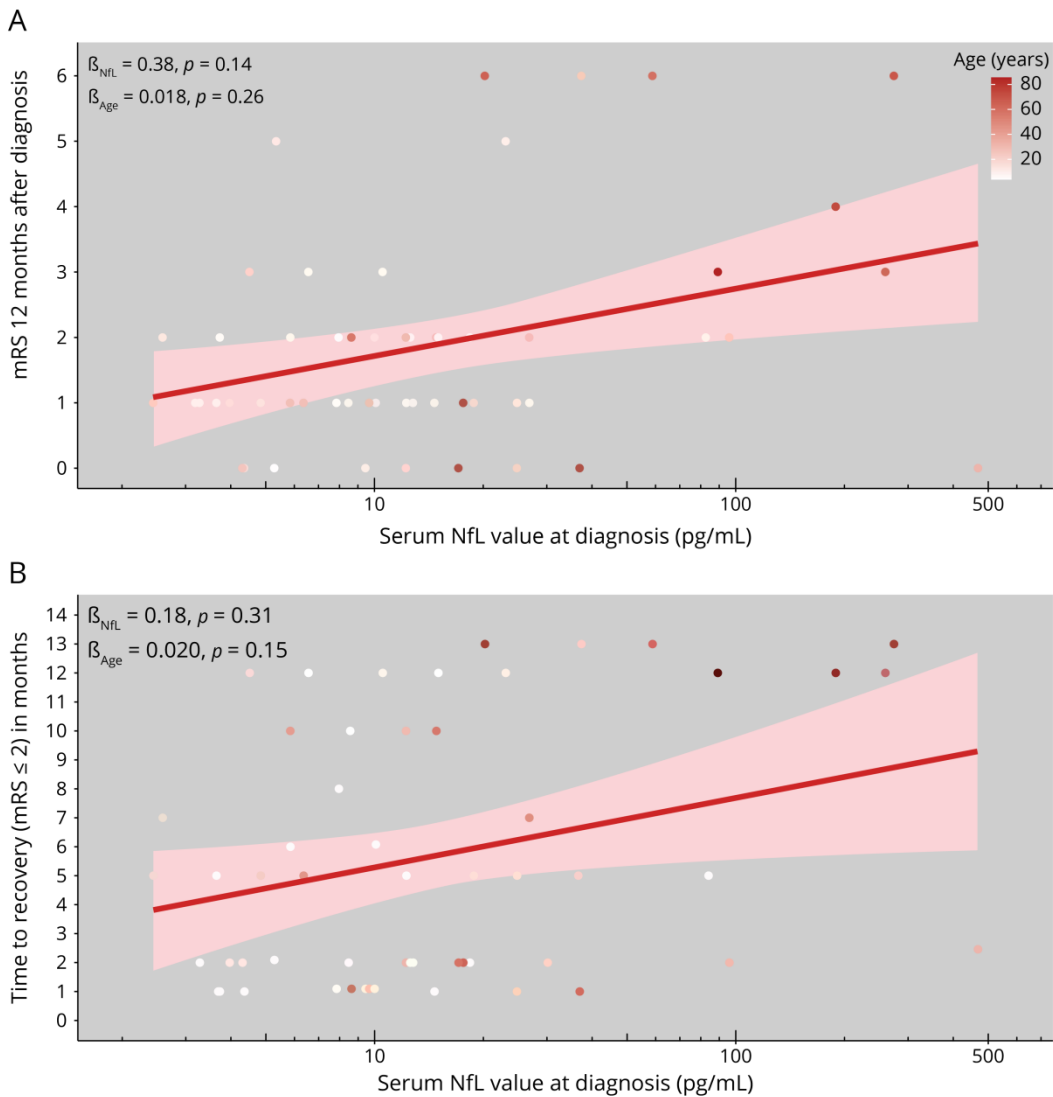
Figure 4: Longitudinal NfL levels in serum.

In all patients with a relapse (A), marked by the arrows. In two exemplary patients (B & C) we see an increase in NfL whilst admitted to the ICU (ICU admission annotated in red). The increase measured at the moment of relapse in patient B is similar to the one in the still improving patient (C), without a relapse. The considerable increase is only seen later during the relapse. The treatment regime is represented by the colored squares at the top of the figure; intravenous methylprednisolone courses in light blue, immunoglobulins in dark blue, Rituximab in light green and cyclophosphamide in dark green.
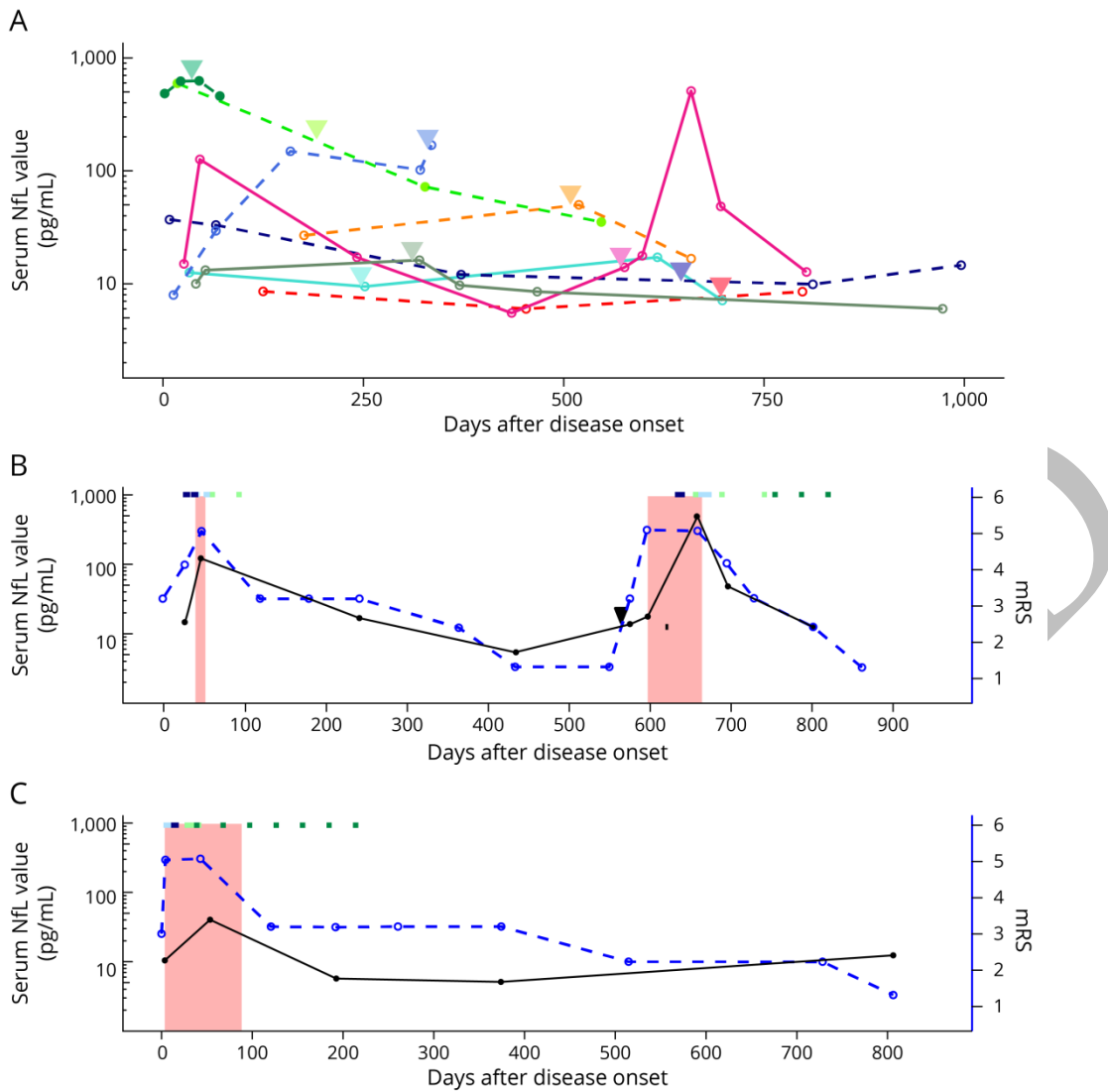
Figure 5: Details on timing of NfL measurements.

In all patients with multiple serum samples in the first two months after diagnosis, we see that the second measurements, starting at 28 days after diagnosis, exceed the normal range (A). The majority of all samples taken within the first two weeks after onset fall in the range of the healthy references (annotated with the green square; B).
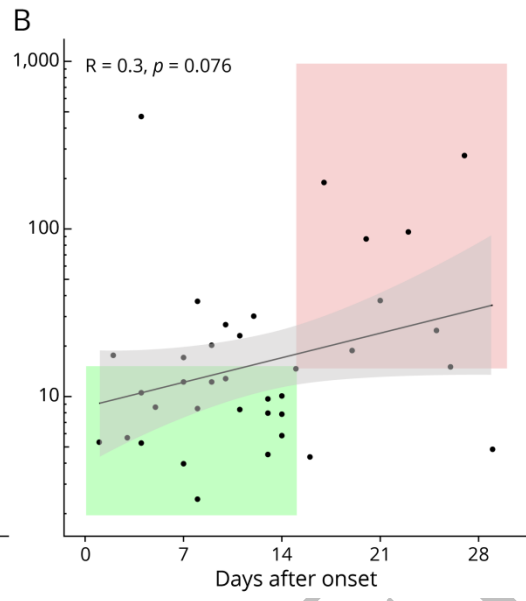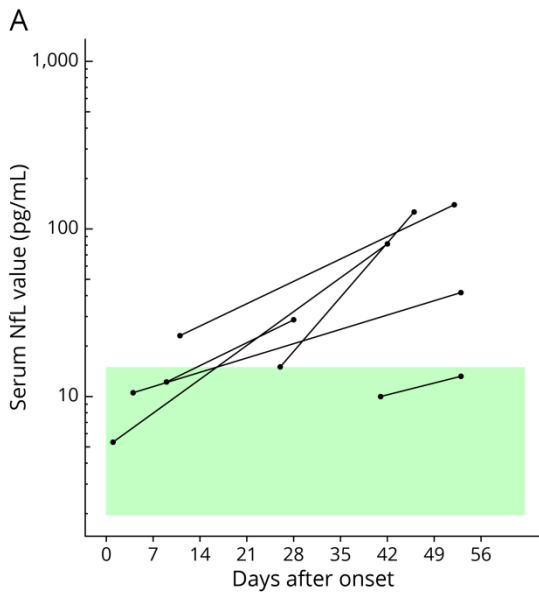
A

Serum NfL value (pg/mL)

Days after onset

B

R = 0.3, *p* = 0.076

Days after onset

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Predictive Value of Serum Neurofilament Light Chain Levels in Anti-NMDA Receptor Encephalitis

Juliette Brenner, Sara Mariotto, Anna E.M. Bastiaansen, et al.

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