Neurofilament light chain levels in anti-NMDAR encephalitis and primary psychiatric psychosis

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ABSTRACT

Introduction: An important challenge in diagnosing anti-NMDAR encephalitis (NMDARe) is differentiating it from a first episode of psychosis (FEP) caused by a psychiatric disease (pFEP). CSF antibody testing distinguishes these diseases, but spinal taps are difficult to obtain in psychiatric facilities. A separate problem is the lack of biomarkers of NMDARe severity and outcome. Here we assessed the performance of neurofilament light chain (NfL) testing in these settings.

Methods: NfL were determined with Single molecule array (SiMoA) in patients with NMDARe, pFEP, herpes simplex encephalitis (HSE), and healthy subjects (HC), the latter two groups used as controls. Receiver operating characteristic (ROC) analyses were performed to assess the prediction accuracy of NfL levels for NMDARe and pFEP, and to obtain cutoffs of clinical utility.

Results: 118 patients with NMDARe (33 with isolated psychosis), 45 pFEP, 36 HSE, and 36 HC were studied. NMDARe patients with seizures/status epilepticus, ICU admission, CSF pleocytosis (>20 WBC/μL), and without early immunotherapy were more likely to have higher NfL than NMDARe without these features. NfL levels at diagnosis of NMDARe did not correlate with outcome at 1 year follow-up assessed with the modified Ranking Scale (mRS). NMDARe patients had significantly higher NfL than pFEP and HC, and lower than HSE patients. ROC analysis of NfL levels between NMDARe with isolated psychosis and pFEP provided an AUC of 0.93 (95% CI 0.87-0.99) and a NfL cutoff ≥15 pg/mL to distinguish these disorders (sensitivity 85%, specificity 96%, positive likelihood ratio 19.3). Forty-three/45 (96%) pFEP had NfL<15pg/mL whereas 5/33 (15%) NMDARe with isolated psychosis were below this cutoff (risk estimation NMDARe vs pFEP: odds

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ratio 120.4 [95% CI 21.8-664], p<0.001). None of the HSE and 35/36 (97%) HC had NfL<15pg/mL.

Discussion: NfL measured at diagnosis of NMDARe associated with several features of disease severity but not with outcome at 1 year follow-up. Patients with FEP and serum NfL≥15pg/mL had 120 times higher chance of having NMDARe than pFEP. This cutoff correctly classified 96% of pFEP and 85% of NMDARe with isolated psychosis. Patients with FEP of unclear etiology and serum NfL≥15pg/mL should undergo CSF NMDAR-antibody testing.

INTRODUCTION

Anti-NMDAR encephalitis (NMDARe) is an autoimmune disease with a highly specific biological test (CSF antibody testing) and a clinically challenging differential diagnosis.[1] This is particularly important when psychosis is the initial or predominant manifestation, since current criteria of NMDARe and autoimmune psychosis depend on a combination of neurological and paraclinical features that are inadequate in this clinical setting.[2, 3] Most patients with first episode of psychosis (FEP) need antipsychotic drugs, which in turn may trigger neurologic adverse events undistinguishable from NMDARe.[4] If this occurs, the differential diagnosis becomes even more difficult, in a way that new deficits such as dyskinesias, hyperthermia, catatonia or coma may be attributed to a neuroleptic-induced complication in a psychiatric patient who, in fact, has an evolving NMDARe.[5-8]

CSF antibody testing is the gold standard to differentiate NMDARe from FEP caused by a primary psychiatric disease (pFEP).[2, 9] However, spinal taps are difficult to obtain in many psychiatric facilities often resulting in serum antibody testing, which has the limitation of false negative results (~12% NMDARe)[9] or positive results of unclear

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significance (~3-15% psychiatric diseases).[10-14] Moreover, delaying therapeutic interventions until antibody results are available or neurological symptoms develop, are not valid options, as early treatment is an important prognostic factor in those patients.

A separate problem is that there are no serological biomarkers of disease outcome in NMDARe patients. Determination of light chain neurofilaments (NfL) is becoming widely available for assessing neuroinflammatory and degenerative diseases.[15] Previous studies on autoimmune encephalitides have focused on the role of NfL as biomarkers of disease severity or prognosis, resulting in findings of unclear practical implications.[16-22] Here, we first assessed in a larger series of patients with NMDARe whether serum and CSF NfL levels at diagnosis of the disease associated with distinct symptoms or predicted long-term outcome. Then we explored the utility of serum NfL levels in differentiating pFEP from NMDARe in general, and focused in a subgroup of NMDARe patients with isolated psychosis at time of NfL testing, which is the phenotype where serological biomarkers are needed mostly.

METHODS

Patients and controls

Patients with NMDARe were randomly selected among patients whose serum or CSF were examined at Hospital Clínic-IDIBAPS, University of Barcelona, between January 2007 and December 2019, and fulfilled clinical-immunological criteria of definite NMDARe (antibodies present in CSF).[3] For all cases, extensive clinical information including the modified Rankin scale score (mRS)[23] as outcome measure at 1 year follow-up was available. Samples of serum and CSF were obtained within a median (IQR) of 26 (13-48) days from disease onset.

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Patients with a pFEP were randomly selected from a prospective observational cohort study performed at Hospital Clínic-IDIBAPS, University of Barcelona (2019-present). All patients underwent neurologic and psychiatric assessment, paraclinical tests (MRI, EEG, NMDAR CSF studies), and at least one year of clinical follow-up; the clinical information of 22 pFEP patients has been previously reported.[2] In all cases NfL levels were examined in serum samples that had been obtained and kept frozen since their first hospital admission, when the primary psychiatric cause of FEP had not yet been established. In all cases the final diagnosis of pFEP was established after 1 year follow-up (35 patients developed schizophrenia, and 10 bipolar disorder as per DSM-5).[24-26]

Patients with herpes simplex virus encephalitis (HSE) were expected to have increased levels of serum NfL,[27] thus we included them as a potential high positive control group. Patients were randomly selected from a previously reported prospective observational cohort study developed at Hospital Clínic-IDIBAPS (2014-present).[28] In all cases, the diagnosis was confirmed by viral CSF PCR, and none had concomitant NMDAR antibodies by the time of the viral encephalitis, as reported.[28] Serum samples were obtained at HSE diagnosis.

For patients of the three cohorts, clinical information was obtained at diagnosis using a structured questionnaire; follow-up data was provided by their physicians using similar questionnaires. A fourth group of blood-donors without mental health problems (HC) and age-matched with NMDARe and pFEP patients were used as healthy controls.

Single molecule array (SiMoA) neurofilament light measurements

Serum and CSF samples from NMDARe patients and serum from patients of the other disease groups or healthy subjects were aliquoted and stored at -80ºC until needed.

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Measurement of serum and CSF NfL levels was performed as reported,[29] using the SiMoA Nf-light kit in SR-X immunoassay analyzer (Quanterix Corp, Boston, Massachusetts, USA), which runs ultrasensitive paramagnetic bead-based enzyme-linked immunosorbent assays. Samples were analyzed in duplicates by experienced personnel blinded to clinical diagnoses, following the manufacturer's instructions and standard procedures in Hospital de la Santa Creu i Sant Pau, Barcelona. All NfL values were within the linear ranges of the assays. The intra- and inter-assay coefficients of variation (ICV) at intermediate level (14.42 pg/mL) were 4.8% and 10.7%, respectively.

Statistical analyses

All data are described as median and IQR (25th, 75th percentiles), or absolute frequency and percentage for quantitative and qualitative variables, respectively. NfL levels were non-normally distributed as tested by the Shapiro-Wilk normality test. Baseline evaluations of NfL in relation to clinico-demographic variables within NMDARe were performed trough Generalized Linear Models (GLM) adjusted by age with a non-parametric approach consisting of mean-rank transformation of corresponding dependent variables. Pairwise comparisons between NMDARe, pFEP, HSE, and HC for age, sex and serum NfL were performed by means Mann-Whitney U test or Fisher's exact test. Group differences concerning serum NfL were also investigated by analysis of covariance controlling for age and sex. The ability of NfL to distinguish between NMDARe, pFEP and HSE and the accuracy for prediction of serum NfL levels among these groups were assessed by estimation of area under the curve (AUC) and their 95% confidence interval (CI) from receiver operating characteristic (ROC) analyses. Cutoff points for serum NfL with diagnostic value were determined by likelihood ratio positive (LR+) = sensitivity/(1-

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specificity) from described ROC curves and were derived to maximize sensitivity and specificity using the Youden index. Risk estimation through odds ratio between NMDARe and pFEP were calculated by binary logistic regression using the optimal cutoff of NfL concentrations. All analyses were addressed considering a two-sided type I error of 5% (p-value<0.05), using SPSS (version 26; IBM Corp, Armonk, NY), R-statistic (version 3.5.2) and Prism (version 7; GraphPad Software, La Jolla, CA).

Standard protocol approvals, registrations and patient consents

The Ethic Committee of the Hospital Clinic approved the study. All patients or proxies gave written informed consent for the storage and use of serum, CSF, and clinical information for research purposes.

Data availability

Anonymized data not published within this article will be made available by request from qualified investigators.

RESULTS

A total of 118 patients with NMDARe, 45 with pFEP, 36 with HSE, and 36 healthy controls were included in the study.

NfL and clinical features of NMDARe

To assess the correlation between clinical features and NfL levels within the group of NMDARe, only patients with available paired serum/CSF samples were investigated (n=103). The serum or CSF NfL levels obtained at disease diagnosis of NMDARe patients that showed significant associations with clinical features during the course of the disease are listed in Table 1. Patients who developed prodromal symptoms, seizures or status

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epilepticus, required ICU admission, had CSF pleocytosis (>20 WBC/µL), did not receive immunotherapy during the first 4 weeks of the disease or received second-line immunotherapy during the subsequent disease course were more likely to have higher CSF NfL levels than those who did not have these clinical features. Regarding serum, only ICU admission and not receiving immunotherapy during the first 4 weeks of disease associated with increased NfL.

CSF and serum NfL levels at disease onset did not significantly associate with sex, ethnicity, and presence or absence of any of the following clinical or paraclinical features during the course of the disease: psychiatric symptoms, cognitive alterations, sleep disturbances, movement disorders, impaired level of consciousness, autonomic instability, central hypoventilation, systemic tumor, brain MRI or EEG abnormalities, first line immunotherapy, clinical improvement within the first 4 weeks of treatment, and the development of relapses. No correlation was found between serum and CSF NfL levels at disease onset and the mRS score at 1 year follow-up (Table 1).

NfL in the differential diagnosis of NMDARe and pFEP

NMDARe patients (n=118) had significantly higher levels of serum NfL (median 27.5 pg/mL [IQR 16.1-63.80]) compared with pFEP patients (median 7.1 pg/mL [IQR 5.6-10.5], $p<0.001$) and HC (median 5.1 pg/mL [IQR 3.7-6.2], $p<0.001$), and significantly lower levels than patients with HSE (median 270.9 pg/mL [IQR 102.7-446.5], $p<0.001$) (Figure 1).

To further assess the potential assistance of serum NfL levels in the differential diagnosis between NMDARe and pFEP, we selected among all NMDARe patients, 33 who by the time of NfL testing had isolated psychosis, mainly characterized by disorganized or

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bizarre behavior in all 33 patients, delusional ideas in 31, hallucinations in 27, incoherent speech in 25, aggressiveness in 24, fluctuating agitation in 21, mood lability in 20, and disinhibition in 12. None of the 33 patients was in the ICU or had catatonia, and all had normal brain MRI, and no evidence of current or previous episodes of seizures or epileptic EEG activity. Comparing the NfL levels of these 33 patients (median serum NfL 25.4 pg/mL [IQR 16.4-65.3] with those of pFEP (shown above), the differences remained significant ($p<0.001$) (Figure 1). The main clinical features of pFEP patients are summarized in Table 2.

ROC analysis of serum NfL levels between the 33 NMDARe patients with isolated psychosis and pFEP patients provided an AUC of 0.93 (95% CI 0.87-0.99) (Figure 2). Based on this ROC curve we obtained a cutoff value of ≥15 pg/mL to discriminate between both disorders with a sensitivity of 85%, specificity of 96%, and a positive likelihood ratio of 19.3 (Figure 2). The risk estimation assessed between this subgroup of NMDARe and pFEP patients yielded an odds ratio of 120.4 (95% CI 21.8-664; $p<0.001$). These findings indicate that, despite an overlap of NfL levels between pFEP and NMDARe, patients with new onset psychosis of unclear etiology and serum NfL ≥15 pg/mL had 120 times higher chance of having NMDARe than pFEP.

Similar ROC analysis of serum NfL levels between all NMDARe patients and pFEP patients provided an AUC of 0.86 (95% CI 0.81-0.92), with a cutoff value similar to that of the NMDARe with isolated psychosis (≥15 pg/mL; sensitivity 76%, specificity 95%, and a positive likelihood ratio 11.2) (not shown).

When applying the indicated serum NfL cutoff, 35 of 36 (97%) healthy controls and 43 of 45 (96%) pFEP patients harbored <15pg/mL; the healthy control and the 2 pFEP patients

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with NfL above this cutoff had 33.5, 18.7, and 24.1 pg/mL respectively. In contrast, 5 of 33 (15%) of NMDARe with isolated psychosis, and 28 of all 118 (24%) NMDARe patients had serum NfL <15 pg/mL (Figure 3). Overall, no overlap of serum NfL levels was observed between HSE and pFEP or healthy controls. As shown in figure 1, most patients with HSE had substantially higher NfL levels than patients with NMDARe.

These findings show that the NfL cutoff of ≥15 pg/mL correctly classified 96% of patients with pFEP and 85% of patients with NMDARe and isolated psychosis, which in clinical practice are the two groups with more difficult differential diagnosis, particularly if NMDAR-antibody testing in CSF is not readily accessible.

DISCUSSION

In this study, we show that determination of NfL in the context of new onset psychosis of unclear etiology can assist in the diagnosis of pFEP vs NMDARe. The findings are important for two reasons; First, when both disorders are compared, the difference in NfL levels was significant, and remained significant for the subgroup of NMDARe patients with psychosis without neurological symptoms and with normal brain MRI and EEG without epileptic activity at time of evaluation. This is a challenging clinical scenario in which patients are often admitted to psychiatric facilities where spinal taps may be difficult to obtain upfront, and therefore, serological biomarkers are particularly needed.[2] Second, a serum NfL cutoff of ≥15 pg/mL was able to correctly classify 96% of patients who eventually had pFEP and 85% of patients with NMDARe and isolated psychosis.

Even though patients with FEP of unclear etiology and serum NfL levels ≥15 pg/mL had 120 higher chance of eventually having NMDARe than pFEP, there was an overlap of

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NfL levels between both diseases; for example, 5/33 (15%) patients with NMDARe and isolated psychosis had NfL levels below 15 pg/mL, that is within the range of most cases (96%) with pFEP. On the other hand, only 4% of patients who eventually had pFEP harbored NfL levels above 15 pg/mL. These findings indicate that serum NfL testing can be an important diagnostic aid in these clinical settings but it does not override the need of comprehensive neurological evaluation along with brain MRI, EEG, and serum NMDAR antibodies, as recently proposed.[2] If serum NfL levels are ≥15 pg/mL or any of the other tests is abnormal, patients with new onset psychosis without a clear etiology should undergo a neurological examination and work-up, including CSF analysis for NMDAR antibodies.

Serum NfL levels, reflecting neuroaxonal damage, are currently used as biomarkers of response to therapy and prognosis in several neurodegenerative diseases, traumatic brain injury, and immune-mediated peripheral neuropathies or multiple sclerosis.[15] In the field of autoimmune encephalitis, the limited number of studies on NfL have mainly focused on their role as biomarkers of disease severity and prognosis.[16-19, 21, 22] A study showed that patients with anti-LGI1 encephalitis had higher CSF NfL (measured by ELISA) than patients with depressive disorders associated with cognitive complaints.[20] In line with some of these studies, we found that NfL levels were significantly higher in patients who had seizures or status epilepticus, CSF pleocytosis (>20 WBC/μL), or were admitted to intensive care. However, NfL levels measured at disease diagnosis did not correlate with the outcome calculated with the mRS score at 1 year follow-up. A recent report with a small number of NMDARe patients, suggested that NfL levels measured 3-4 months after disease onset, were potentially a better biomarker of long-term outcome than NfL determined at earlier stages.[30]

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Several considerations and limitations should be noted in our study. The prospective cohort of pFEP patients is from a single center, indicating the need of confirmation (or adjustment) of the selected NfL cutoff values in other centers. For this, the observation that 96% of pFEP patients and 97% of healthy controls overlapped below a specific threshold (15 pg/mL), may help to establish a serum NfL cutoff above which patients with new onset psychosis may benefit from CSF NMDAR antibody investigations. We have not examined patients with psychosis as manifestation of other antibody-mediated encephalitis or systemic inflammatory diseases (e.g., lupus) because in most of these diseases the presence of accompanying neurological symptoms or previous history of the disease suggests the diagnosis.[3] This also applies to many patients with NMDARe who at the time of initial evaluation have already developed neurological symptoms or clear alterations in paraclinical tests, as occurred in 85/118 (72%) patients in the current study. It is likely that for other autoimmune encephalitis (e.g., anti-AMPAR), the very few cases who present with isolated or predominant psychosis have also increased serum NfL levels, but this needs to be assessed in the future. Our study was initially designed to assess the role of NfL in the differential diagnosis of NMDARe and pFEP, thus, it does not allow full assessment of the potential utility of NfL as predictors of long-term outcome in NMDARe. For this, a prospective assessment of NfL at different time points through the stages of the disease is needed.

Overall, the current findings have an important practical implication: in the setting of new onset psychosis of unclear etiology, determination of serum NfL levels is able to select patients who should undergo CSF testing for NMDAR antibodies. As far as NMDARe is concerned, future studies should focus on follow-up of NfL levels during the course of the disease. It is reasonable to postulate that determination of NfL by the time patients leave the

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ICU or are discharged from the hospital may have a better correlation with long-term outcome measured with mRS or scales optimized to provide better assessment of behavioral and cognitive deficits, such as the Clinical Assessment Scale in Autoimmune Encephalitis (CASE) scale.[31]

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Appendix 1. Authors:

Name	Location	Role	Contribution
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Table 1. Relationship between serum and CSF NfL levels at diagnosis of the disease, main clinical features in the acute phase of the disease, and outcome at 1 year follow-up.

Clinical features (n=103)	**N (%)**	**Serum NfL** median (IQR)[a]	**p**[b]	**CSF NfL** median (IQR)[a]	**p**[b]
Female Male	86 (83) 17 (17)	26.3 (14.6-65.9) 40.6 (21.7-56.6)	0.257	195 (51-776.4) 140.5 (82.7-676.1)	0.669
Prodromal symptoms – Present – Absent	 70 (68) 33 (32)	 25.9 (14.3-81.7) 34.4 (17.4-60.9)	 0.398	 239.3 (116.2-911.6) 69.1 (41.4-301.1)	 **0.010**
Behavior alterations – Present – Absent	 91 (88) 12 (12)	 46.7 (14.7-63.1) 55.9 (17.1-73.2)	 0.232	 289.4 (54.7-714.3) 708.7 (118.4-3847.3)	 0.208
Cognitive dysfunction[d] – Present – Absent	 83 (81) 20 (19)	 26.5 (14.3-65.3) 34.5 (16.1-70.7)	 0.581	 219.6 (51.1-903.8) 128.4 (64.0-561.9)	 0.377
Seizures – Present – Absent Status epilepticus – Present – Absent	 71 (69) 32 (31) 12 (12) 91 (88)	 26.6 (17-60.5) 34.5 (8.2-73.7) 44.4 (26.3-124.1) 26.2 (14.4-60.9)	 0.583 0.052	 300.2 (78.1-1011.6) 175.1 (45.3-391) 876.1 (281.1-2375.3) 172.1 (53.1-666.4)	 **0.043** **0.004**
Movement disorders – Present – Absent	 73 (71) 30 (29)	 26.7 (15.3-63.1) 33.9 (13.1-81.7)	 0.847	 189.4 (50.4-780.6) 205.2 (119.7-776.4)	 0.746
Decreased consciousness level – Present – Absent	 66 (64) 37 (36)	 29.7 (16.1-75.5) 22.2 (13.1-48.3)	 0.430	 205.2 (48.1-1056.8) 180.2 (82.7-483.3)	 0.669
Autonomic instability – Present – Absent	 57 (55) 46 (45)	 29.2 (18.8-65.9) 24.1 (9.4-60.2)	 0.335	 238.4 (63.5-899.1) 136.7 (53.1-640.5)	 0.544
Central hypoventilation – Present – Absent	 43 (42) 60 (58)	 30.2 (19.7-75.3) 24.3 (11.4-62.9)	 0.373	 205.2 (94.9-1332.0) 157.8 (54.7-648.5)	 0.515
ICU admission No ICU admission	84 (82) 19 (18)	31.3 (17.4-82.6) 13.1 (4.1-35.7)	**0.001**	239.3 (61.2-984.2) 106 (47-230.3)	**0.033**
CSF ≥20 WBC/µL CSF <20 WBC/µL	54 (58) 39 (42)	29.7 (17.3-72.2) 19.1 (9.5-59.9)	0.168	432.3 (150.5-1501.4) 108.9 (44.2-190.6)	**<0.001**
Normal brain MRI Abnormal brain MRI	71 (69) 32 (31)	25.9 (15.7-51.3) 30.2 (14.8-72.2)	0.400	175.2 (53.1-776.4) 274.6 (73.1-789.9)	0.580
Normal EEG Abnormal EEG[e]	8 (8) 95 (92)	26.7 (46.1-60.9) 48.3 (17.5-72.2)	0.286	137.1 (26.4-676.1) 198.6 (68.1-908.7)	0.149
Tumor[f] – No	 56 (54)	 34.5 (14.3-75.3)	 0.208	 138.8 (48.1-808.7)	 0.576

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– Yes	47 (46)	25.7 (16.1-54.1)		253.6 (120.0-676.2)	
First line immunotherapy – No – Yes	 99 (96) 4 (4)	 34.5 (13.1-36.7) 26.6 (15.7-65.9)	 0.881	 272.6 (51.0-573.6) 190.6 (58.9-776.4)	 0.760
Second line immunotherapy – No – Yes	 65 (63) 38 (37)	 26.2 (13.1-56.7) 31.5 (18.9-83.5)	 0.107	 137.1 (49.7-483.3) 281.1 (94.9-1132.0)	 **0.049**
Treated within first 4 weeks – No – Yes	 43 (42) 59 (58)	 40.6 (16.1-92.3) 23.9 (14.6-47.7)	 **0.022**	 301 (82.7-2406.7) 143.4 (46.9-375.5)	 **0.002**
Improved within first 4 weeks – No – Yes	 51 (49) 52 (51)	 32.8 (17.3-75.3) 26.3 (12.1-63.1)	 0.333	 240.2 (58.9-903.8) 149.4 (51.4-653.5)	 0.365
Relapses – No – Yes	 92 (89) 11 (11)	 27.5 (15.3-65.6) 26.8 (9.9-72.3)	 0.722	 194.9 (57.6-714.2) 137.8 (51.1-1056.8)	 0.831
Functional outcome (at 1 year) – mRS 0-2 – mRS 3-6	 77 (75) 26 (25)	 25.9 (16.6-56.6) 29.2 (14.6-65.9)	 0.652	 172.1 (58.9-483.3) 531.7 (51.2-1591.4)	 0.146

(a) IQR, interquartile range in pg/mL. (b) Age-adjusted p-values. (c) Includes all patients who at the time of diagnosis had neurological symptoms of NMDARe with or without psychiatric symptoms. (d) Memory or speech disorder. (e) Focal or diffuse slow and/or epileptic activity. (f) Ovarian teratoma in all cases. (^) Reference category of the variable for age-adjusted post-hoc pairwise comparisons. CSF: cerebrospinal fluid; ICU: intensive care unit; MRI: magnetic resonance imaging; mRS: modified Rankin scale; WBC: white blood cells count.

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Table 2: Main clinical features of 45 patients with pFEP.

Clinical history of interest, n (%)	
– Neurologic disease[a]	2 (4)
– Systemic autoimmune disease[b]	1 (2)
– Oncologic disease	0 (0)
First degree family history of psychiatric disease, n (%)	19 (42)
Prevalence of previous life stressful events , n (%)	26 (58)
Duration of untreated psychosis, in days: median (IQR)	23 (8-45)
PANSS: median (IQR), severity of psychotic symptoms	
– Positive symptoms score	26 (8)
– Negative symptoms score	18 (9)
– General score	39 (12)
– Total score	81 (24), moderate
YMRS: median (IQR), severity of manic symptoms	18 (20), hypomania
HAM-D: median (IQR), severity of depressive symptoms	16 (14), mild
Neurologic abnormalities during the episode[c] , n (%)	2 (4)
Abnormal cerebrospinal fluid studies[d], n (%)	0 (0)
Abnormal brain MRI, n (%)	0 (0)
Abnormal EEG	¿??
Treatment, , n (%)	
– Atypical antipsychotics	45 (100)
– Mood stabilizers	10 (22)
– Typical antipsychotics	5 (11)
– Electroconvulsive therapy	0 (0)
Duration of hospital admission, in days: median (IQR)	18 (9)
GAF: median (IQR), level of dysfunction	
– Six months prior to admission	78 (13), normal
– On admission	30 (12), severe
– Three months after discharge	61 (14), mild-moderate
Confirmed cause of pFEP after 1 year follow-up, n (%)	
– Schizophrenia	35 (78)
– Bipolar disorder	10 (22)

(a) One migraine, 1 learning difficulties. (b) One autoimmune hypothyroidism. (c) One postural tremor, 1 stereotype movements. (d) Defined by pleocytosis (>5 white blood cells/μL in CSF) and/or increased protein

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concentration (>60 mg/dL). GAF: Gobal Assessment of Functioning Scale; HAM-D: Hamilton Depression Rating Scale; IQR: Interquartile Range; MRI: magnetic resonance imaging; PANSS: Positive and Negative Syndrome Scale; YMRS: Young Mania Rating Scale.

LEGENDS TO FIGURES

Figure 1: Serum NfL levels in disease groups

Comparison of baseline serum NfL levels between 36 healthy controls (HC) (median age 25 years [IQR 21-32] 21 female), 45 patients with a first episode of psychosis (pFEP) (median age 20 years [IQR 17-26] 20 female), the whole group of 118 patients with anti-NMDAR encephalitis (NMDARe) (median age 23 years [IQR 17-30] 98 female), the subgroup of 33 NMDARe patients with a psychotic presentation (NMDARe psych) (median age 22 years [IQR 17-28] 27 female), and 36 patients with herpes simplex virus encephalitis (HSE) (median age 56 years [IQR 34-68] 16 female). All NMDARe or NMDARe with isolated psychotic patients vs pFEP, $p<0.001$; NMDARe vs HC or HSE, $p<0.001$.

Figure 2: ROC curve for serum NfL between the groups of patients with pFEP and NMDARe with isolated psychosis

Figure 3: Distribution of disease groups according to serum NfL cutoff

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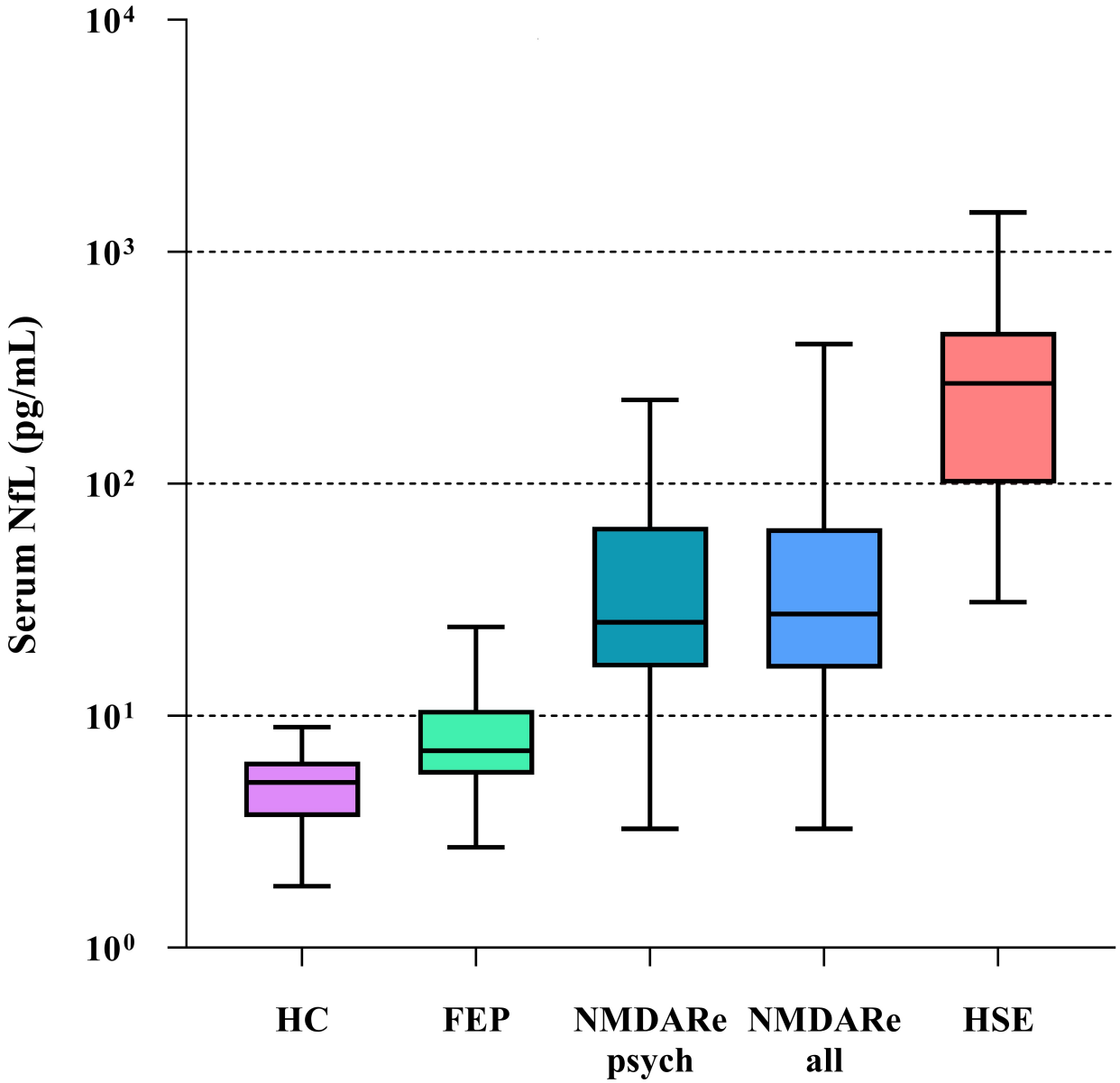
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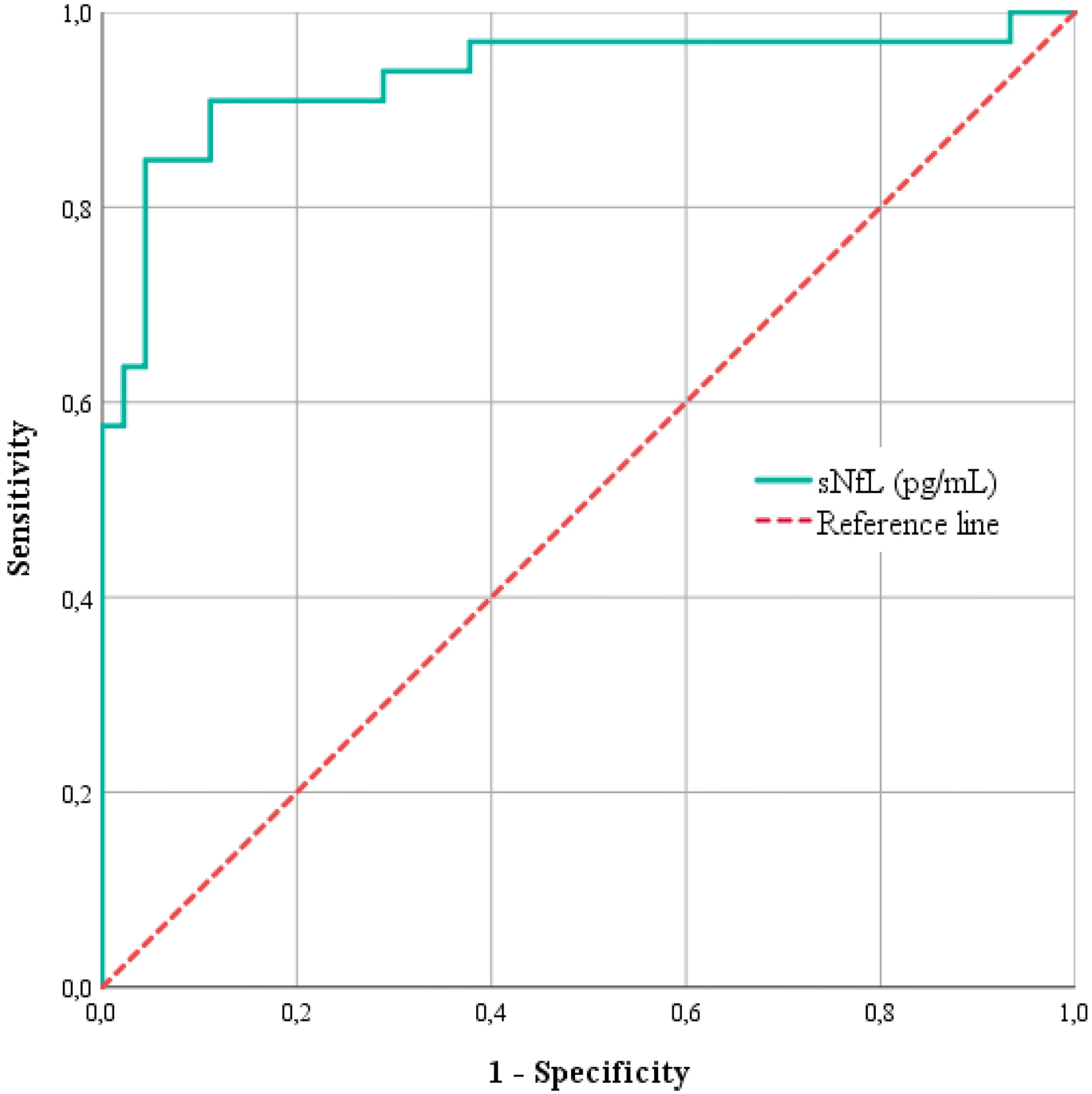
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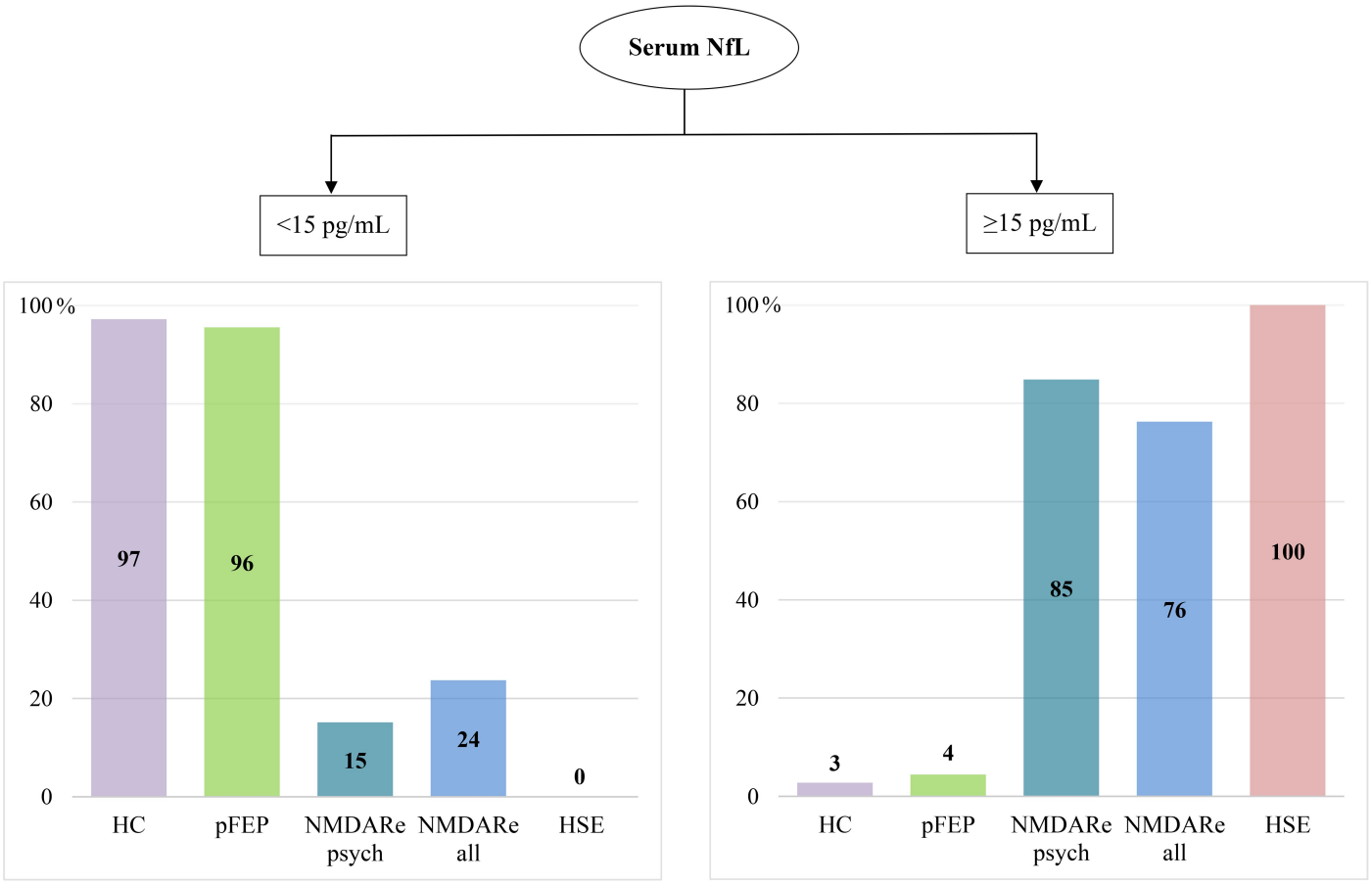
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Serum NfL (pg/mL)
10⁴
10³
10²
10¹
10⁰
HC
FEP
NMDARe psych
NMDARe all
HSE


1,0
0,8
0,6
0,4
0,2
0,0
Sensitivity
0,0
0,2
0,4
0,6
0,8
1,0
1 - Specificity
sNfL (pg/mL)
Reference line


Serum NfL
<15 pg/mL
≥15 pg/mL
100 %
80
60
40
20
0
97
96
15
24
0
HC
pFEP
NMDARe psych
NMDARe all
HSE
100 %
80
60
40
20
0
3
4
85
76
100
HC
pFEP
NMDARe psych
NMDARe all
HSE