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Comparative Performances of 4 Serum NfL Assays, pTau181, and GFAP in Patients With Amyotrophic Lateral Sclerosis

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Abstract

Background and Objectives

Selecting the most appropriate blood tests is crucial for the management of patients with amyotrophic lateral sclerosis (ALS). This study evaluates the diagnostic and prognostic performance of neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), and phosphorylated tau 181 (pTau181) biomarkers in ALS to establish their clinical relevance and cutoff values.

Methods

In a cohort of patients from the ALS center in Montpellier, we conducted a head-to-head comparison of 4 different technologies and 3 distinct serum analytes: NfL was tested using the ultrasensitive Simoa and the microfluidic Ella platforms, along with 2 assays recently set up on clinical-grade platforms: Lumipulse and Elecsys. We also used Elecsys to assess serum GFAP and pTau181.

Results

Our cohort included 139 patients with ALS and 70 non-ALS patients, with a mean age of 66.1 ± 11.4 years and 47.4% of women. The mean follow-up was 42 ± 26.3 months for patients with ALS and 141.6 ± 106.3 months for non-ALS patients, with a mortality rate of 85.5% vs 7.7%. There was a high correlation between all methods tested for serum NfL quantification (R^2 = 0.939 to 0.963). The area under the curve (AUC) for ALS diagnosis was 0.889 (0.827–0.932) for NfL Simoa, 0.906 (0.847–0.944) for Ella, 0.912 (0.853–0.948) for Lumipulse, and 0.910 (0.851–0.946) for Elecsys. Serum pTau181 and GFAP showed poor diagnostic performance with AUCs of 0.565 (0.472–0.649) and 0.546 (0.461–0.636), respectively. Kaplan-Meier survival analysis revealed significant hazard ratios (4.4–5.4) for blood NfL. Patients with ALS had a 40%–50% chance of surviving 50 weeks below the prognostic cutoff values while survival rates dropped to near zero above. NfL and GFAP levels were associated with age and body mass index, considered confounding factors. pTau181 levels varied significantly in patients with ALS depending on the site of onset.

Discussion

This study demonstrates the consistent performance of 4 immunoassays for serum NfL quantification in ALS. NfL showed high diagnostic and prognostic accuracy, making it suitable for individual assessment, unlike GFAP or pTau181. We propose diagnostic and prognostic cutoff values for serum NfL, providing a basis for wider implementation, especially with the clinically accredited Lumipulse and Elecsys platforms, which are becoming standard practice.

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Glossary

ALS = amyotrophic lateral sclerosis; **ALSFRS** = ALS Functional Rating Scale; **AUC** = area under the curve; **BMI** = body mass index; **CVs** = coefficients of variation; **FVC** = forced vital capacity; **GFAP** = glial fibrillary acidic protein; **IVD** = in vitro diagnostic; **NfL** = neurofilament light chain; **pNfH** = phosphorylated neurofilament heavy chain; **pTau181** = phosphorylated tau 181; **ROC** = receiver operating curve.

Classification of Evidence

This study provides Class II evidence that serum NfL levels are useful in identifying over 80% of patients with ALS and predicting survival in patients with ALS compared with pTau181 and GFAP levels.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons, leading to rapidly progressing muscle atrophy and severely affecting life expectancy. Diagnosis can be challenging because of the variability in clinical presentations and the criteria for progression. It is not uncommon for patients to be misdiagnosed with ALS, only to have their diagnosis reconsidered as a slowly progressing motor neuron disease; therefore, accurate diagnosis is essential.

Neurologic biomarkers are highly valuable for clinicians, serving various purposes such as aiding in clinical diagnosis, predicting prognosis, evaluating disease stage, and tracking progression or treatment response. This realm of neurology has greatly advanced in recent years, thanks to improvements in analytical assays that now enable neurologic biomarker detection in the blood. This is especially important for neurodegeneration, where a lumbar puncture is not always part of the diagnostic process, such as in cases of ALS.

In recent years, blood neurofilament light chain (NfL) protein has emerged as a promising biomarker for diagnosing ALS and assessing its prognosis. NfL is found in the cytoskeleton, mainly in myelinated axons.[1] NfL levels rise in various clinical contexts, such as neurodegenerative disorders and inflammatory, vascular, or traumatic conditions.[2] Thus, elevated NfL is a biomarker of neuronal damage in various neurologic conditions, including ALS.[3-5] Furthermore, the diagnostic performance of serum NfL for ALS is superior to that of other biomarkers of axonal degeneration, such as S100B and progranulin,[3] and is better than that of biomarkers of neuroinflammation.[6]

Although the clinical significance of serum NfL has been confirmed in diverse cohorts, there are still several unresolved matters requiring attention for its broad and routine clinical application. These include evaluating the growing array of analytical methods and establishing optimal cutoff values tailored to each specific quantification technique.

We previously compared 2 highly sensitive research-grade assays: Simoa from Quanterix and Ella from ProteineSimple/Bio-Techne. Using these technologies to measure blood NfL, we found that both techniques performed equally well in diagnosing and prognosing ALS.[7] The Simoa technique has been the gold standard for pilot research studies, but it is not appropriate for routine diagnostic testing.

In this study, we include 2 additional highly sensitive methods of serum NfL quantification on clinical-grade analyzers: Lumipulse from Fujirebio and Elecsys from Roche Diagnostics. Each of these immunoassays allows for the quantification of biomarkers at physiologic levels. However, their analytical approaches differ relying on either single-molecule array or digital immunoassay for Simoa,[8] microfluidic cartridge assays for Ella, chemiluminescent enzyme immunoassay for Lumipulse, and electrochemiluminescence immunoassay for Elecsys.

We also tested the clinical performance of 2 new Elecsys assays for glial fibrillary acidic protein (GFAP) and phosphorylated tau 181 (pTau181) quantification. GFAP is a type III intermediate filament almost exclusively expressed in astrocytes that has garnered interest as a marker for monitoring astrogliosis. Its release into biofluids during astrogliosis events makes it a promising candidate for noninvasive diagnosis and tracking of neurodegenerative diseases and possibly ALS.[9,10] pTau181 is a biomarker linked to amyloidosis and to the neurodegenerative process of Alzheimer disease, whose blood concentration is of diagnostic and prognostic interest.[11] pTau181 was found to differentiate ALS from the control population and from the Alzheimer disease population, and it was indicative of lower motor neuron impairment.[12,13]

The primary research question of this study is to determine the diagnostic and prognostic performances of NfL, GFAP, and pTau181 biomarkers in ALS and identify the most appropriate cutoff values for clinical use.

Methods

Study Population

This study is ancillary to a previous one,[7] in which patients were consecutively included based on the following criteria:

age older than 18 years, first visit to the ALS outpatient clinic for suspected ALS, blood sampling performed as part of routine care, and agreement (through informed consent) to use a portion of their sample for biomarker research. For this study, we used only a subset of the initial cohort of sporadic cases, based on the availability of remaining blood samples in the biobank. Baseline medical information collected at the first visit included age, sex, body mass index (BMI), forced vital capacity (FVC), site of symptom onset, and ALS Functional Rating Scale (ALSFRS) score. Patients were divided into 2 groups (ALS and non-ALS) according to their diagnosis, based on clinical international guidelines (Gold Coast criteria[14]) and electroneuromyography. Two neurologists from the ALS center confirmed the diagnoses in an independent manner. Patient follow-up was assured until the final diagnosis for the non-ALS group or until June 2021 for the ALS group. The characteristics of the study participants are presented in Table 1.

Serum Sampling and Analysis

Blood samples were collected in SST II tubes (BD vacutainer) with a clot activator and then centrifuged at 2,000*g* for 10 minutes. Afterward, serum samples were divided into 0.5–1 mL aliquots and conserved at −80°C until analysis in a storage facility with continuous temperature surveillance. Aliquots were thawed at +4°C and gently homogenized before analysis. One aliquot was used for each of the following NfL immunoassays: Simoa HD-X, Ella, Lumipulse G1200, and Elecsys. Analytical characteristics of the different NfL immunoassays are presented in eTable 1. pTau181 and GFAP were measured with NeuroToolKit Elecsys assays on a Cobas e402 analyzer.[15] All samples were analyzed by staff blinded to the clinical data.

Table 1 Clinical and Biological Data in ALS and Non-ALS Groups

	Non ALS	ALS	*p* Value	*p* Value*
Age (y)	63.8 (13.4)	67.3 (10.1)	0.0334	/
Men/women	41/29	69/70	0.2234	0.2254
BMI (kg/m^2)	24.9 (5.3)	24.4 (4.1)	0.6228	0.6757
NfL Elecsys (pg/mL)	4.32 (6.97)	13.97 (8.33)	<0.0001	<0.0001
NfL Ella (pg/mL)	44.7 (60.2)	127.2 (68.3)	<0.0001	<0.0001
NfL Lumipulse (pg/mL)	31.1 (46.2)	98.4 (63)	<0.0001	<0.0001
NfL Simoa (pg/mL)	29.6 (50.1)	86.1 (57.1)	<0.0001	<0.0001
pTau181 (pg/mL)	1.23 (1.11)	1.25 (0.78)	0.8527	0.8081
GFAP (pg/mL)	102 (57)	91 (45)	0.1551	0.005

Abbreviations: ALS = amyotrophic lateral sclerosis; BMI = body mass index; GFAP = glial fibrillary acidic protein; NfL: neurofilament light chain; pTau181 = phosphorylated tau 181.
Values in patients with ALS and control participants, with the Student t test or χ^2 (sex) and *linear regression adjusted for age; numbers were used to describe categorical variables (sex) and mean ± SD for continuous variables. There were no missing data.

Statistical Analyses

Quantitative data are expressed as mean ± SD. The Kolmogorov-Smirnov test was used to assess data normality. Means of NfL levels were compared by the Student *t* test. Proportions were compared by χ^2. Statistical significance was set at 5%, and a *p* value less than 0.05 was considered statistically significant. ALS diagnosis performances were assessed with determination of sensitivity (se), specificity (sp), and area under the curve (AUC) expressed with 95% bootstrap CI (1,000 iterations; random number seed: 978). Logistic regression was used to transform biomarker values into predicted probabilities, with ALS as the binary outcome variable. Calibration was evaluated using the Brier score, which measures the mean squared error between predicted probabilities and observed outcomes, and the Spiegelhalter Z-test, which compares observed and expected outcomes to test calibration.[16] Biomarker levels yielding the maximal Youden index value were considered as optimal cut-points for ALS diagnosis. AUCs were compared by the DeLong and DeLong test. Survival in the ALS group was analyzed using Kaplan-Meier curves and a right censoring for missing follow-up data. Optimal biomarker cutoff values for survival in ALS were selected for having the best *p* value in a Cox regression model, ensuring that the 2 distinct populations (below and above the cutoff value) represented at least 30% of the population. We used also a bootstrap procedure involving 500 iterations, recalculating the *p* values for each resampled data set. Confidence intervals for the *p* values were derived using the percentile method. Hazard ratios were also provided with a 95% CI. Analyses were performed in R version [R-4.4.2], with logistic regression using the glm function and AUC computed with pROC.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained for all patients, and the study was approved by the local institutional review board (declaration number: IRB-MTP_2021_04_202100783).

Data Availability

Anonymized data will be shared on request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with EU legislation on the General Data Protection Regulation and decisions by the Ethical and Scientific Board of CHU of Montpellier, which will be regulated in a material transfer agreement.

Results

Participants

Clinical characteristics of the 209 included patients are presented in Table 1. There were statistical differences between the 139 patients with ALS and 70 non-ALS patients in mean follow-up time (42 ± 26.3 vs 141.6 ± 106.3 months) and mortality rate (85.5 vs 7.7%), with a median time to death of 29.7 months. The mean age of patients with ALS was higher (67.3 ± 10.1 vs 63.8 ± 13.4 years) while

neither BMI nor gender distribution was statistically different between the groups. Regarding patients with ALS diagnosis, bulbar onset was recorded in 32.4% of cases, upper limbs in 30.9%, and lower limbs in 36.8%. The mean ALSFRS-R score was 40.5 ± 4.9, and the mean FVC was 87.6 L ± 25.1. Survival analysis was conducted on 138 patients with ALS, of whom 20 patients (14.5%) were lost to follow-up. The main diagnoses in the non-ALS group (summarized in eTable 2) were lower motor neuron disease (15.7%), primary lateral sclerosis (11.4%), and neuropathies (10%). Based on a power analysis that includes the determination of the effect size (Cohen d) using the means and standard deviations of Lumipulse NfL in different groups, followed by a 2-sample t test power calculation with a significance level of $\alpha = 0.05$, the result shows that the study has a power of 1.0 (100%), indicating that it is highly capable of detecting a true difference between the groups.

Analytical Comparison of NfL Assays

Values of the 4 NfL measurements in the population followed normal distributions and were above the limit of detection of the assays (eTable 1). The intra-assay and interassay coefficients of variation (CVs) were all less than 8%. A strong correlation as illustrated in Figure 1 was observed between the 4 NfL immunoassays (R^2 = 0.939 to 0.963, with p value <0.0001 in all cases). Using Simoa as reference, systematic differences in %, corresponding to [Variable - Reference]/Reference, were 67.2% (SD 51.6) for Ella, 20% (SD 42.4) for Lumipulse, and −83.4% (SD 3.8) for Elecsys. Elecsys values were thus the lowest, and the regression formula (Figure 1) indicated that NfL Elecsys values were 6–8 times lower than those of the other assays.

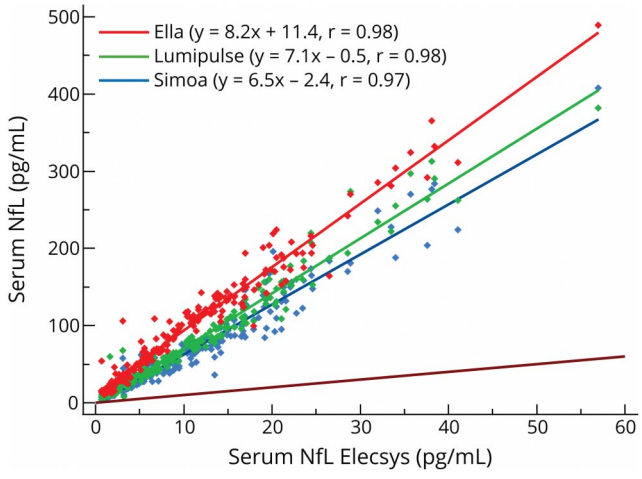
Diagnostic Performance of Blood Assays

NfL values measured with the 4 assays were significantly different between patients with ALS and non-ALS patients (Table 1, eFigure 1, eFigure 2). There was approximately 3 times more NfL in ALS than in non-ALS. Receiver operating curve analysis (Figure 2) revealed good performances for all techniques used for NfL: the AUC was 0.889 (0.827–0.932) for Simoa, 0.906 (0.847–0.944) for Ella, 0.912 (0.853–0.948) for Lumipulse, and 0.910 (0.851–0.946) for Elecsys (Table 2). The calibration of the AUCs, evaluated using the Brier score and Spiegelhalter Z-test (Table 2), confirmed that our observed outcomes align well with the predictions. Serum pTau181 and GFAP showed poor diagnostic performance with AUCs of 0.565 (0.472–0.649) and 0.546 (0.461–0.636), respectively. DeLong statistical comparison showed that the Simoa NfL AUC (0.889 [0.827–0.932]) was significantly lower than for Ella (0.906 [0.847–0.944], $p = 0.0224$), Elecsys (0.910 [0.851–0.946], $p = 0.0081$), or Lumipulse (0.912 [0.853–0.948], $p = 0.0076$), whose AUCs were statistically indistinguishable from one another (DeLong test $p > 0.05$). All NfL assays had significantly higher AUC than those of pTau181 and GFAP (in AUC values before, $p < 0.0001$). NfL cut-points, defined by the highest Youden index, yielded sensitivity close to 80% and specificity over 80% in all cases (Table 2).

Prognostic Performance of Blood Assays

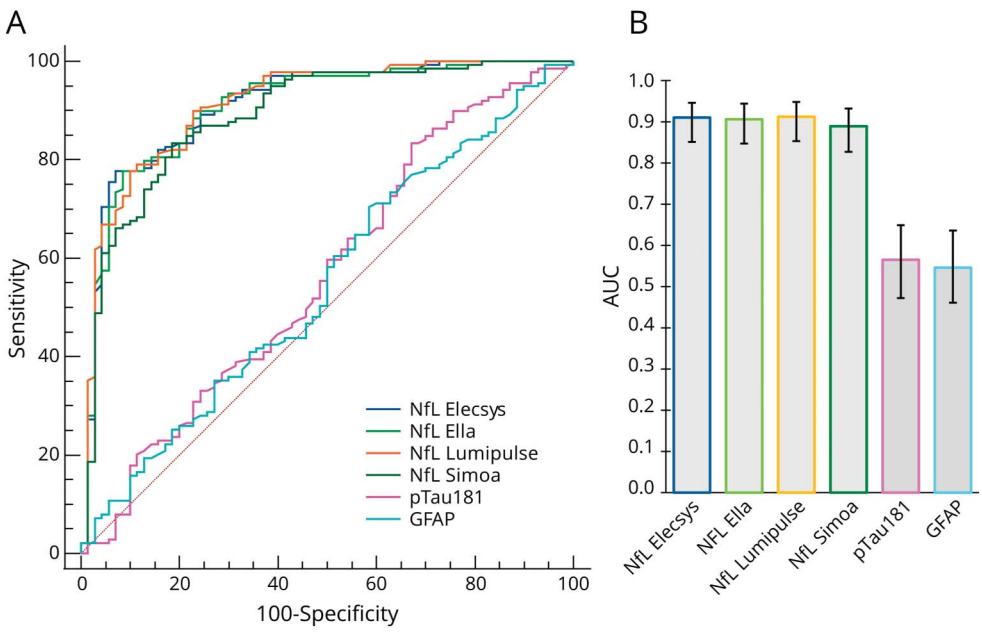
Selection of the best serum NfL cutoff values for survival analysis in the ALS population required values 2.2–2.4 times higher than the best cutoff values for diagnosis (Table 2). Kaplan-Meier survival analysis, presented in Figure 3, showed comparable HRs between NfL Simoa, Ella, Elecsys, and Lumipulse assays (HR = 4.4 to 5.4). One-year survival of patients with ALS was over 40% when values were below NfL thresholds and 0% when they were above. The prognostic performances of serum pTau181 and GFAP were at the limit of statistical significance, with Cox regression p values of 0.044 (7.44E-05-

Figure 1 Simoa, Ella, Elecsys, and Lumipulse NfL Blood Tests Are Highly Correlated



Serum NfL measured with Simoa, Ella, and Lumipulse plotted against Elecsys concentration. Lines of linear correlation with the formula are indicated on the graph along with correlation factor r. NfL = neurofilament light chain.

Figure 2 NfL Blood Tests Diagnose ALS With High Specificity and Sensitivity



(A) ROC and (B) AUC with 95% confidence intervals for ALS diagnosis of serum NfL measured with Simoa, Ella, Lumipulse, and Elecsys; GFAP; and pTau181. ALS = amyotrophic lateral sclerosis; AUC = area under the curve; GFAP = glial fibrillary acidic protein; NfL = neurofilament light chain; pTau181 = phosphorylated tau 181; ROC = receiver operating curve.

7.67E-01) and 0.065 (1.28E-04-8.62E-01), respectively (eTable 3), and HRs only slightly greater than 1 (Figure 2 with 95% CI). In a multivariable Cox regression analysis including age, sex, site of onset, and NfL values, pTau181 remained a significant independent prognostic factor (p = 0.047). The ALSFRS score between the 2 patient groups, stratified by prognostic cutoff values, was significantly different only for GFAP (eFigure 1).

Impact of Onset Site

Kaplan-Meier survival analysis of the ALS population stratified by site of onset (Figure 4A) revealed that bulbar onset was associated with a worse outcome compared with upper limb onset (HR 1.53 [95% CI 0.94–2.49]) or lower limb onset (HR 1.65 [95% CI 1.04–2.62]). Blood biomarker concentrations between onset sites were differential only for pTau181, which showed a significant increase in the bulbar onset group compared with the upper and lower limb onset groups (Figure 4, B–D).

Influence of Confounding Factors on Blood Biomarkers

The association of blood biomarkers in the general population with age, sex, BMI, site of onset, ALSFRS score, and ALS diagnosis is illustrated by the forest diagram in Figure 5. Age, which also differs between patients with ALS and non-ALS patients (Table 1), is associated with increased blood biomarker concentrations. NfL and GFAP decrease with female sex and higher BMI. The site of disease onset has an impact on pTau181 concentration. Correlation, presented in eTable 4, confirmed the relationship between age, BMI, and biomarker concentrations.

Discussion

In a cohort of patients with amyotrophic lateral sclerosis, we found highly reproducible serum NfL quantification using 4 different commercially available technologies. The 4 techniques gave good clinical performances for diagnosis and prognosis. Two of the methods tested, Simoa and Ella, have so far been the most widely used in clinical research studies ascertaining the value of NfL for ALS.[4,5,7,17-27] Both are highly sensitive and can accurately measure analyte levels in serum. However, these technologies are hampered by their unsuitability for routine use regarding ISO15189 in vitro diagnostic (IVD) certification and their lack of random-access possibilities, integration into the clinical laboratory workflows, and IT systems. The 2 other technologies assessed on the contrary, Lumipulse and Elecsys, are fully certified IVD automated clinical-grade analyzers that are already routinely used in various clinical fields. Our study not only demonstrates the high performance of these new clinical tests for the diagnosis and prognosis of ALS but also proposes diagnostic and prognostic cutoff values that could serve as future references.

As NfL is proving increasingly useful in a number of clinical practice settings,[2,28] its use in routine laboratories is becoming a matter of course, and diagnostic laboratories must be prepared to assimilate a considerable increase in demand. The transition from research to clinical routine is already well underway. The data presented lay the foundation for the routine use of these tests in practice, with the understanding that the translation of our results into ordinary clinical practice must be approached cautiously because NfL is also indicative of many other diseases besides ALS.

Table 2 Diagnosis Performances and Cutoff Values for Serum NfL Assays

Biomarker	AUC (95% bootstrap CI)	Brier score	Spiegelhalter Z-score (*p* value)	Diagnostic cutoff (pg/mL)	Sensitivity	Specificity	Positive predictive power (PPV)	Negative predictive power (PPV)	Brier score	Prognosis cutoff (pg/mL)	Sensitivity (%)	Specificity (%)
NfL Simoa	0.889 (0.827–0.932)	0.131	$<10^{-11}$ ($p = 1$)	>37	83%	81%	89.9	71.2	0.131	>83	81	50
NfL Ella	0.906 (0.847–0.944)	0.114	$<10^{-11}$ ($p = 1$)	>57	86%	79%	88.9	74.3	0.114	>124	92	50
NfL Lumipulse	0.912 (0.853–0.948)	0.115	$<10^{-8}$ ($p = 1$)	>42	81%	84%	91.1	69.4	0.115	>95	93	50
NfL Elecsys	0.910 (0.851–0.946)	0.115	$<10^{-11}$ ($p = 1$)	>6.0	82%	84%	90.5	69.9	0.115	>14.4	95	45

Abbreviations: ALS = amyotrophic lateral sclerosis; AUC = area under the curve; NfL: neurofilament light chain.
Calibration, which indicates that observed outcomes align well with predictions (in the Methods section), was evaluated using the Brier score (ranging from 0 to 1, with scores less than 0.25 considered acceptable) and the Spiegelhalter Z-test (where a score close to 0 with a high *p* value (>0.05) indicates good calibration). Optimal cutoffs were determined using the highest Youden index value with sensitivity above 80%. Cutoff values for poor prognosis, selected based on the best Cox model, are also indicated.

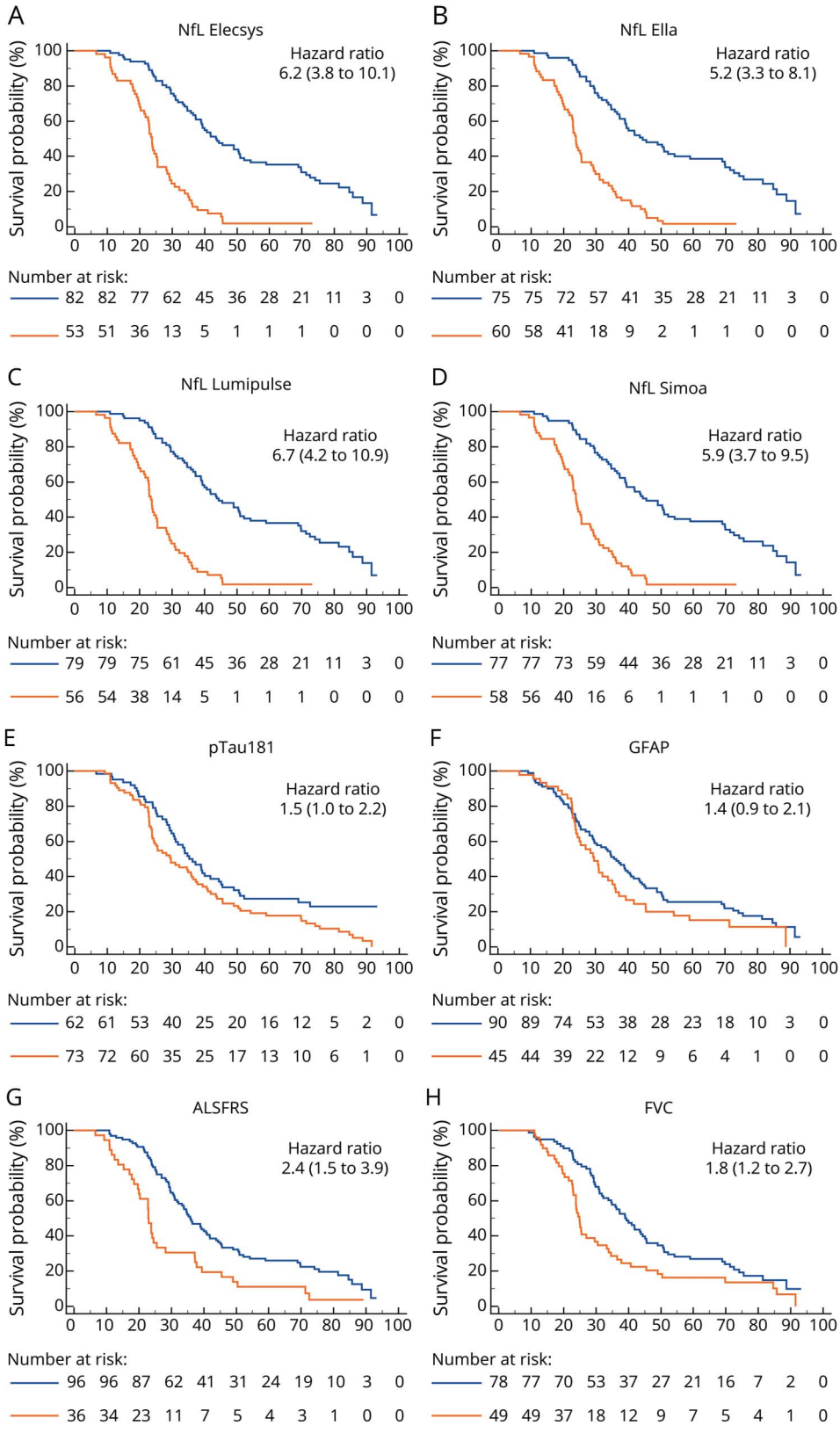
We have observed that the 4 NfL assays have similar precision (interassay and intra-assay CVs) and correlate exceptionally well with one another. The 4 technologies give different absolute values, with NfL Elecsys in particular giving values 6–8 times lower than the other techniques. The use of different calibrators may explain these differences, given that harmonizing NfL values between assays remains a major metrological challenge.[29] Efforts are underway to develop both a reference method and certified reference material, both of which will be needed. The 4 assays tested allow differentiation between patients with ALS and non-ALS patients in the context of a consultation in a motor neuron clinic. The specificities and sensitivities achieved exceed 80% with NfL optimal cutoff values of 37 pg/mL, 57 pg/mL, 42 pg/mL, and 6 pg/mL for Simoa, Ella, Lumipulse, and Elecsys, respectively.

The physiologic concentration of blood NfL is influenced by age and BMI, among other factors.[29-33] It is possible to derive NfL reference values corrected for age and BMI.[30] Even if the benefit of this correction seems limited in ALS,[7] which, unlike other pathologies such as multiple sclerosis, is characterized by a very sharp increase in NfL concentration, we nevertheless recommend using age-corrected and BMI-corrected NfL values from Lumipulse and Elecsys as soon as they become available.

Prognostic information is needed to improve management in ALS. In our cohort, we confirmed that the bulbar onset of the disease is associated with a more unfavorable evolution. ALSFRS-R score and FVC are also significant prognostic factors, as previously reported. However, serum NfL is a much more informative marker because its levels are associated with the extent and progression of neuronal loss. Our hazard ratios for survival of patients with ALS are comparable with those previously reported for NfL.[4,7,21,26,27] It is important to note that values above the prognostic cutoffs for the different assays are associated with a median survival that decreases by almost 50% from 46 to 25 weeks. The chance of surviving 50 weeks below the cutoff is 40%–50%, whereas above the cutoffs, survival rates among patients with ALS dropped to near zero.

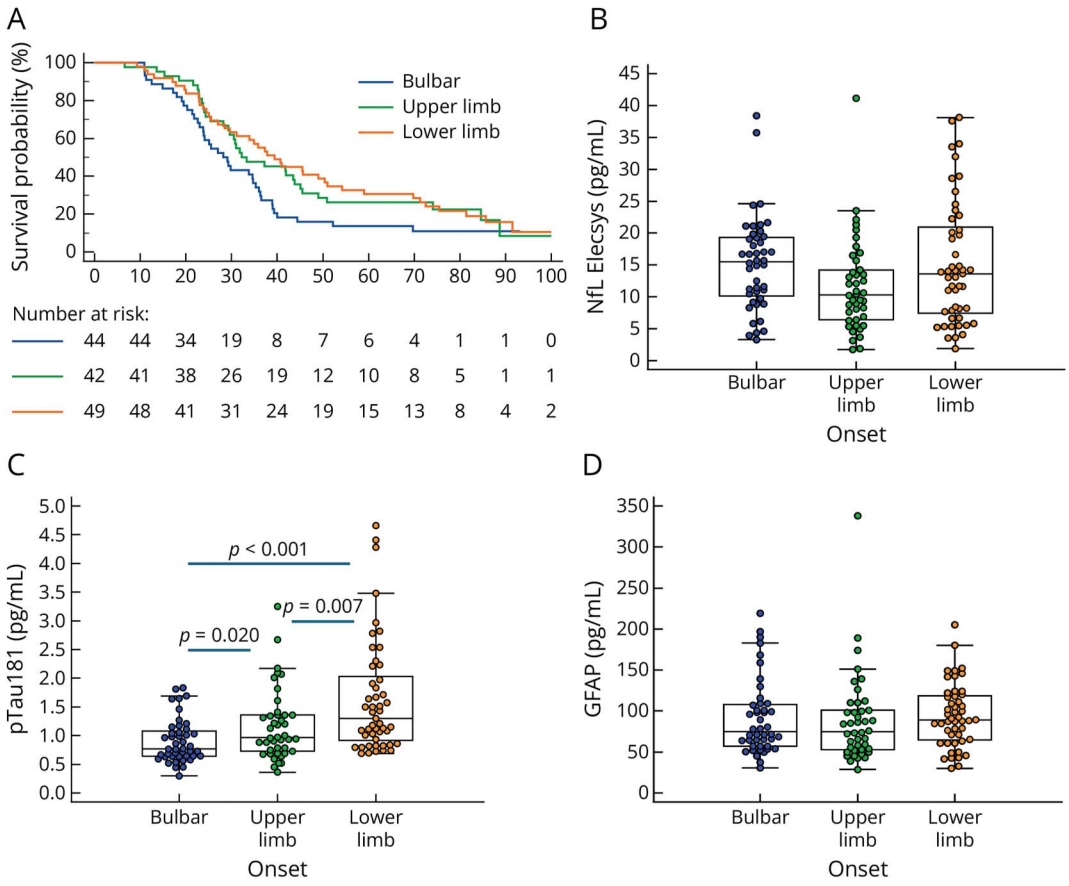
We also studied the variation in GFAP and pTau181 levels in our cohort. Blood GFAP has recently been shown to be of significant interest in Alzheimer research as an indicator of astrogliosis and of cerebral amyloidosis.[34,35] We did not observe a significant increase in ALS, unlike other studies[9,10] in which the difference was, however, with neurologic controls rather than, in our study, with relevant ALS mimic situations. This could well explain the discrepancies. In one study,[9] the authors observed an association between GFAP and concomitant beta-amyloid pathology, suggesting that GFAP may represent a biomarker of Alzheimer disease rather than ALS. When the analysis is restricted to the ALS groups, the results of our study concurred with previous studies showing that GFAP is also a weak prognostic marker of ALS. The hypothesis would be that astrogliosis is an additional element for assessing the severity of ALS, which corresponds well to the

Figure 3 NfL Blood Cutoff Values Predict Survival



Kaplan-Meier survival analysis in ALS using the cutoff values in Table 2 for (A) serum Elecsys NfL, (B) Ella NfL, (C) Lumipulse NfL, (D) Simoa NfL, (E) pTau181, (F) GFAP, (G) ALSFRS score, and (H) FVC. Log-rank proportional HR with 95% CI is indicated for each biomarker. ALS = amyotrophic lateral sclerosis; ALSFRS = ALS Functional Rating Scale; FVC = forced vital capacity; GFAP = glial fibrillary acidic protein; HR = hazard ratio; NfL = neurofilament light chain; pTau181 = phosphorylated tau 181.

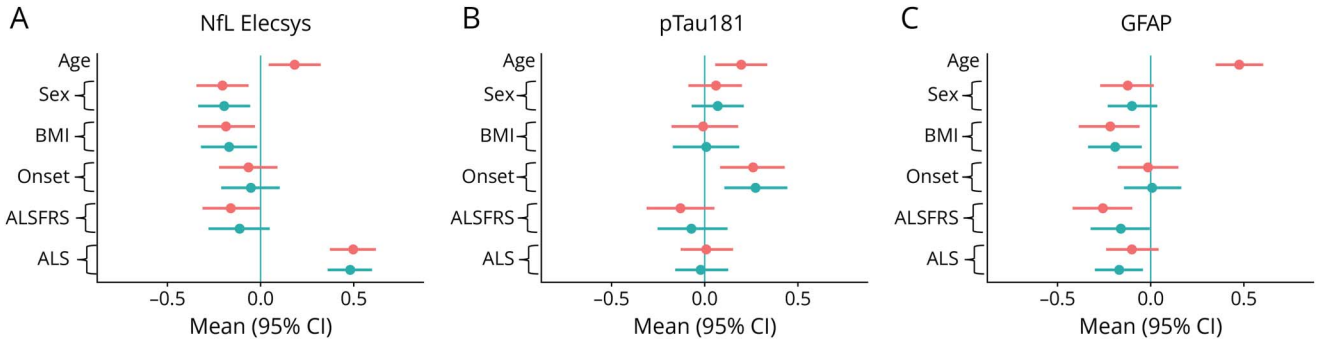
Figure 4 Bulbar-Onset ALS Has Poorer Prognosis and the Relation Between Onset Sites and Biomarkers



(A) Kaplan-Meier survival analysis in ALS for different sites of onset. Bulbar onset HR compared with upper limb onset is 1.53 (95% CI 0.94–2.49) and with lower limb onset is 1.65 (95% CI 1.04–2.62). Biomarker concentration (pg/mL) by site of onset for serum Elecsys NfL (B), pTau181 (C), and GFAP (D). Only pTau181 showed a significant difference between sites of onset (*p* values of the Student *t* test are indicated). ALS = amyotrophic lateral sclerosis; GFAP = glial acidic protein; HR = hazard ratio; NfL = neurofilament light chain; pTau181 = phosphorylated tau 181.

involvement of astrocytes in ALS.[36] Plasma pTau181 was previously shown to be higher in lower limb–predominant ALS than in the bulbar phenotype and an independent prognostic factor in multivariable Cox regression.[12,13] We have confirmed these results and shown that pTau181 has no diagnostic value. Several biomarkers other than those examined in our study have been proposed for the diagnosis and prognosis of ALS. These include phosphorylated neurofilament

Figure 5 Relationship Between Biomarkers and Variables in All Populations



Forest plots of associations using linear regression between serum Elecsys NfL (A), pTau181 (B), or GFAP (C) and age, sex, BMI, site of onset, ALFRS score, and ALS using linear regression. Unadjusted (red) and age-adjusted means and 95% CIs are provided. ALS = amyotrophic lateral sclerosis; ALSFRS = ALS Functional Rating Scale; BMI = body mass index; GFAP = glial fibrillary acidic protein; NfL = neurofilament light chain; pTau181 = phosphorylated tau 181.

heavy chain (pNfH), microRNA-206, superoxide dismutase 1, and chitotriosidase-1, among others.[37] Each of these markers is likely to provide information on the pathophysiologic processes underlying ALS. However, we chose to focus primarily on NfL because of its superior performance in ALS-related studies, particularly in comparison with pNfH, and the recent availability of automated clinical-grade techniques for its measurement. These clinical detection methods have also been developed in parallel for GFAP and pTau181, which have also been proposed as potential ALS biomarkers. Ultimately, the clinical application of these biomarkers using clinically validated platforms, rather than for research purposes only, was one of the main reasons we focused on these 3 markers.

This study has certain limitations, and our conclusions would benefit from external validation. However, the fact that our results and the cutoffs determined for NfL Simoa are similar to those obtained with larger studies reinforces the robustness of our conclusions. Prolonged follow-up could also strengthen the relevance of our study because it could reveal further conversions to ALS. Longitudinal NfL data could also be interesting in patients with ALS who are below the cutoffs because it may reveal increases in NfL values over time. Another limitation is represented by the fact that our study is monocentric, with participants coming from a single tertiary center, and that geographical area may induce bias, because all patients in our cohort originate from the south of France. For investigators, it may be wise to validate our selected cutoffs using their own populations.

In conclusion, the Simoa, Ella, Lumipulse, and Elecsys assays all gave highly accurate and reproducible ALS diagnosis and prognosis. Thus, these tests can be useful in confirming a diagnosis in rare cases where it is uncertain. Ultimately, the prognostic information they provide is certainly the most valuable aspect. Taking confounding factors such as age and BMI into account may even improve the performance of the assays. We also measured serum levels of pTau181 and GFAP, which show pathophysiologic variations that are not, however, sufficiently informative for individual clinical application.

Finally, we propose diagnostic and prognostic cutoffs for serum NfL measured on these 4 platforms. This information can serve as a basis for implementing these tests in other laboratories, particularly because Lumipulse and Elecsys, which are clinically validated, are already present in many routine laboratories and are becoming standard practice with reduced cost and turnaround time.

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Disclosure

The authors report no relevant disclosures. Go to Neurology.org/N for full disclosures.

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