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ORIGINAL ARTICLE

Serum neurofilament light chain cut-off definition for clinical diagnosis and prognosis of amyotrophic lateral sclerosis

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Abstract

Background: The neurofilament light chain (NfL) assay is gradually becoming an essential diagnostic tool for the diagnosis of many neurological diseases including amyotrophic lateral sclerosis (ALS). Different methods for the determination of this biomarker in serum have been developed in recent years.

Methods: We measured blood NfL in 429 patients referred to the tertiary ALS center of Montpellier, France using two different ultrasensitive methods (Ella™ and Simoa™) and we compared the clinical performances of these two approaches. We also converted NfL values into age and body mass index-adjusted Z-scores to assess cut-off values of this biomarker in this clinical context.

Results: We show comparable diagnostic and prognostic performance of Ella™ and Simoa™ technologies in ALS, with specificities and sensitivities exceeding 80% for both. We propose cut-off values for serum NfL in this clinical context, thus enabling the routine clinical use of this biomarker.

Conclusion: The use of NfL in routine clinical practice will help predict survival and improve diagnostic accuracy by distinguishing ALS from other neurological diseases and motor neuron disease mimics.

KEYWORDS

ALS, diagnosis, neurofilament, prognosis

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a severely disabling neurodegenerative disease. It is caused by neurodegeneration of the upper and lower motor neurons and results in muscle weakness and paralysis. In Europe, the incidence of the disease is 2–3 per 100,000 individuals [1]. The clinical presentation and course of the disease are highly variable, but progression is inexorable and more than 50%

of patients usually die within 3 years [1]. Of the remaining patients, up to 10% survive for more than 8 years [2]. The diagnosis of ALS can be difficult to make [3] and relies on the use of comprehensive investigations including electroneuromyography. Recent research has highlighted the clinical relevance of a new blood biomarker in this context: neurofilament light chain (NfL). Neurofilaments are cytoskeletal proteins [4], predominantly found in the myelinated axons of neurons, whose level is increased in neurodegenerative,

Mehdi Brousse and Constance Delaby contributed equally to the work.

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inflammatory, vascular, or traumatic diseases, not only in cerebrospinal fluid (CSF) [5] but also in serum [6]. NfL represents a biomarker of neuronal injury, whose elevated levels have been proposed to reflect neurodegeneration in several neurological disorders including ALS [7–9]. Previous retrospective studies have shown that serum and CSF NfL concentrations are correlated [10] and that this biomarker can predict survival and improve diagnostic accuracy by distinguishing ALS from other neurological diseases and motor neuron disease mimics [10–12].

Analytically, the immunoassay methods available to quantify NfL have evolved considerably in recent years. Enzyme-linked immunosorbent assay (ELISA) allows the quantification of soluble NfL in CSF, but its limited sensitivity impairs its quantification in serum [13]. Tests based on electrochemiluminescence (ECL), which offer greater sensitivity, were then developed: the quantification of NfL in serum was thus possible [14], allowing sporadic ALS patients to be distinguished from healthy controls [15]. Several ultrasensitive immunoassay technologies are now available for the quantification of serum NfL (sNfL) at physiological levels, such as the single-molecule array (Simoa™, Quanterix) for digital immunoassays [16, 17], which has progressively become the benchmark technique for the quantification of this biomarker [18]. Another ultrasensitive immunoassay platform based on microfluidic cartridge assays (Simple Plex Ella™) has recently been developed by ProteineSimple/Bio-Techne for the quantification of sNfL [19]. The opportunity offered by these ultrasensitive technologies to measure sNfL is very promising for prompt and accurate diagnosis, potentially facilitating treatment trials in ALS [20, 21].

Although pre-analytical and analytical studies have been performed and the clinical value of sNfL has been demonstrated in different cohorts [10, 22, 23], several issues still need to be addressed to enable its routine use. These include: (1) accurately defining its context of use (COU), (2) selecting and comparing different analytical solutions, and (3) defining optimal cut-offs. In this study we defined the COU in the context of the tertiary ALS center of Montpellier, which has an important role in the diagnosis and follow-up of patients. We compared the two ultrasensitive reference approaches for sNfL measurement, Simoa™ and Ella™, which were found to be very similar and showed very good clinical performance for both the diagnosis and prognosis of ALS. Finally, we were able to define Ella™ and Simoa™ diagnostic and prognostic cut-offs that may be used as references in the future.

MATERIALS AND METHODS

Patients

A total of 429 patients were recruited (261 men and 168 women), of whom 377 had ALS and 52 did not. All patients were referred to the tertiary ALS center for suspicion of ALS and were recruited between June 2014 and September 2020. The ALS group was composed of patients fulfilling the criteria of either probable or definite

ALS according to the international ALS criteria of El Escorial and Airlie House [24]. Diagnoses were ascertained by at least two neurologists from the referral center. The non-ALS group was composed of patients whose final diagnosis was not ALS (Table S1). Presymptomatic C9 carriers were considered as asymptomatic C9 carriers, or C9-carriers without objectifiable abnormalities on neurological examination, as their complaints are rather psychosomatic and not caused by a C9-related disease. All subjects were prospectively recruited, most of them at their first diagnostic visit, in the Montpellier ALS referral center. Blood was collected at the recruitment visit. All patients were prospectively followed until either final diagnosis other than ALS or until March 1, 2021 for ALS patients. Demographic and clinical data (including age at first visit, age at first symptoms of motor loss, gender, site of onset [bulbar or spinal]) were collected in ALS patients. All subjects gave informed written consent to participate in the study. Ethics approval was granted by the Institutional Review Board, CHU Montpellier (Reference: IRB-MTP_2021_04_202100783) and the study conformed with the World Medical Association Declaration of Helsinki.

Collection and analysis of serum samples

Serum was collected in 7 mL clot activator tubes (BD Vacutainer®) at inclusion and centrifuged at 1500g for 10 min at room temperature. Aliquots (from 0.5 to 1 mL) were stored at –80°C until use [25]. sNfL concentration was determined in parallel using a commercial Simoa™ NF-light™ Advantage Kit (Quanterix™) based on ultrasensitive Simoa™ HDX technology [16] and a commercial simplex NF-L assay Kit (ProteinSimple™) on an Ella™ analyser [19]. Ella™ was calibrated using the in-cartridge factory standard curve and Simoa™ using the providing standards. Internal quality controls (IQCs) represented by serum pool aliquots were used to monitor accuracy for both Simoa™ and Ella™. All samples were measured after a single thaw, with a four-fold dilution for Simoa™ and a two-fold dilution for Ella™. Dilutions were made using the provided dilution buffer.

Statistical analysis

Quantitative variables (age at onset, symptoms duration, sNfL concentration) were expressed as mean ± standard deviation. Spearman correlation coefficient with 95% confidence interval was calculated to assess the correlation between concentrations obtained with each platform. The Bland–Altman method [26] was used to investigate the agreement between the two assays. Biomarker levels in ALS and non-ALS patients were compared for continuous variables using the Wilcoxon–Mann–Whitney test if normality was rejected using the Shapiro–Wilk test or with t-test otherwise. The χ^2 test was used for dichotomous variables. Receiver operator characteristic (ROC) analysis was used to assess the sensitivity and specificity of each approach in differentiating ALS and non-ALS patients. The optimal diagnostic cut-offs were defined based on the whole population using the Youden

index which maximizes sensitivity and specificity based on the ROC curve. Survival was visualized by Kaplan–Meier analysis stratified by low and high levels of sNfL. The optimal survival cut-off based on Cox proportional hazards models was determined by identifying the cut-off value that divides the population (\leq cut-off and $>$ cut-off) with the most significant difference in survival (Fisher test) between the two groups thus generated [27]. Hazard ratios (HRs) including 95% confidence intervals are reported. To compare the classification of patients using a single cut-off or two cut-offs based on the median of age, we used the net reclassification index (NRI) [28]. The NRI is based on reclassification constructed separately for participants with and without the event of interest (i.e., ALS or non-ALS diagnosis) and quantifies the correct movement into classes, upwards for events and downwards for non-events. As a sensitivity analysis, serum sNfL values measured on Simoa™ were converted into Z-score values based on a large cohort of control individuals taking into account the confounding effect of age and body mass index (BMI) on the biomarker level [29]. sNfL Z-scores are an age- and BMI-corrected measure of how strongly a specific sNfL measure deviates from normal (Z-score=0). Age-adjusted Cox models using raw sNfL and sNfL Z-score were built to understand the independent effects of age and increased sNfL on survival and the model performances were compared using Harrell's C-index. All analyses were performed using MedCalc (20.111) software program and R software (R Core Team, 2019, R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria; <https://www.R-project.org/>).

RESULTS

Population characteristics

Distribution of gender as well as mean BMI between the ALS group ($n=349$) and the non-ALS group ($n=80$) were comparable (Table 1). At sample collection, the ALS population was significantly older (66.5 ± 13.4 years) than the non-ALS patients (62.7 ± 13.4 years). The median age of the overall population was 67 years. The diagnoses and

characteristics of non-ALS patients are described in detail in Table S1. Mean age at ALS onset was 64.0 ± 14.7 years and mean duration of the disease at sampling was 21.3 ± 23.9 months. There is a great variability in the duration because the center receives patients both for their initial diagnosis and also for confirmation of their diagnosis after a more or less long clinical phase. The onset of ALS was bulbar in 31.2% of cases (Table 2). A diagnosis of definite ALS was the final diagnosis in 66.5% of the patients, and familial ALS cases accounted for 14.6% of the whole group. Survival time of patients was analyzed until March 1, 2021 (database lock). Of the 349 ALS patients, 34 were lost to follow-up and thus the survival analysis could only be performed on 315 ALS patients. These latter patients were prospectively followed up for a maximum of 318 months, with a median of 30.8 months. At the time of analysis, 203 patients (64.4%) had died, and the mean ALS duration at death was 36.9 ± 22.5 months.

Analytical comparison of Simoa™ and Ella™ approaches

To confirm the accuracy of the assay methods, three quality control samples were measured in triplicate on three test runs. The intra-assay CVs were 3.5% and 2.0% for the Simoa™ and Ella™ platforms, respectively. Inter-assay CVs were 3.9% for both platforms. In the whole cohort, sNfL values were significantly higher when measured with the Ella™ platform compared with Simoa™ (112.0 ± 8.9 pg/mL vs. 79.3 ± 6.7 pg/mL respectively, $p < 0.001$). However, sNfL concentrations measured with both platforms were strongly correlated throughout the entire cohort ($r=0.974$ [95% CI 0.969–0.979], $Ella^{\text{TM}} = 22.442 + 1.13 \times Simoa^{\text{TM}}$) (Figure 1a). Moreover, Bland–Altman analysis showed a mean bias of 32.7 pg/mL (43.3%) for sNfL concentrations between both technologies (Figure 1b). However, since the line of equality is included in the 95% confidence interval, we can conclude that there is no significant systematic difference between the two methods [30]. Bias seemed inversely proportional to sNfL concentration, thus decreasing to 25.0% for high values (>100 ng/mL, $n=99$).

TABLE 1 Demographic data and serum neurofilament light chain levels in non-amyotrophic lateral sclerosis (non-ALS) and ALS patients.

Variable	Non-ALS patients ($n=80$)	ALS patients ($n=349$)		
	Mean	Mean	P value	P value ^a
Age (years)	62.7 ± 13.4	66.5 ± 11.1	0.0083	–
Sex (M %)	59.6	61.0	0.8472	0.2219
BMI	24.6 [21.9–29.3]	24.0 [21.8–26.7]	0.5115	0.5820
Simoa™ NfL (pg/mL)	15.5 [9.9–31.9]	67.4 [42.3–111.5]	<0.0001	<0.0001
Simoa™ NfL Z-score	0.755 ± 1.580	2.760 ± 0.950	<0.0001	<0.0001
Ella™ NfL (pg/mL)	27.9 [17.1–50.7]	106.0 [66.3–158.5]	<0.0001	<0.0001

Note: Values are indicated as mean \pm SD or median [25th–75th percentile] (if the normality of the data is rejected using the the Shapiro–Wilk test) and percentage (%) for sex. p : comparison between the two groups with t -test (Mann–Whitney test for non-normal distribution) or χ^2 . p^a : comparison between the two groups with linear regression adjusted for age (ranked values were used for non-normal distribution).

Abbreviations: ALS, amyotrophic lateral sclerosis; BMI, body mass index; M, male; NfL, neurofilament light chain.

TABLE 2 Characteristics of amyotrophic lateral sclerosis patients.

Characteristic	ALS patients (n = 349)
Age at onset (years)	64.0 ± 14.7
Age at diagnosis (years)	65.2 ± 14.8
ALS duration to death (months) ^a	36.9 ± 22.5
Site of onset	
Bulbar	109 (31.2)
Spinal	240 (68.8)
Inheritance	
Sporadic	298 (85.4)
Familial	51 (14.6)
Diagnostic criteria	
Definite ALS	232 (66.5)
Probable ALS	117 (33.5)
ALS duration at sampling (months)	21.3 ± 23.9
ALSFRS-R score at sampling	39.4 ± 5.8

Note: Values are means ± SD or n (%).

Abbreviations: ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised.

^aCalculated for the 208 patients who were deceased at the time of statistical analysis.

Comparison of sNfL in ALS and non-ALS patients

sNfL was significantly higher in the ALS group compared with the non-ALS patients using either the Simoa™ or Ella™ platforms ($p < 0.0001$) and with or without adjustment by age (Table 1 and Figure 1c,d). The computing of ROC curves (Figure 2a,b) resulted in an area under the curve (AUC) of 0.881 (95% CI 0.847–0.910) and 0.888 (95% CI 0.855–0.916) for Simoa™ and Ella™ sNfL, respectively. The difference between the two AUCs was statistically not significant as evaluated by the DeLong method [31]. The optimal cut-offs as defined by the best Youden index were 37 pg/mL and 57 pg/mL for Simoa™ or Ella™ values, respectively. The sensitivity and specificity for ALS detection reached with these cut-offs were close to 80% (Figure 2).

Impact of age in the determination of sNfL diagnostic cut-offs

Because age is a major confounding factor for sNfL [29], and because there was a difference in the age population between ALS and non-ALS patients (Table 1), we separated the population based on the age median that was rounded to 67 years old. In the younger population, the AUC was higher for both Simoa™ or Ella™ values: 0.916 (95% CI 0.869–0.950) and 0.910 (95% CI 0.862–0.945) when compared to the older population 0.837 (95% CI 0.782–0.883) and 0.859 (95% CI 0.806–0.902) (see Figure S1,A,B). These differences in AUC were however not significant ($p = 0.0677$ and

$p = 0.2166$ for Simoa™ or Ella™). The optimal cut-offs as defined by the best Youden index were 18.6 pg/mL and 45.3 pg/mL (Simoa™ sNfL) for the youngest and oldest population, respectively. When two cut-offs were used depending on the age (Table 3), the overall diagnostic performance values were higher than when using one single cut-off for the whole population. To evaluate this increase in performance we used the NRI approach and observed a 5.7% improvement in ALS classification (11 ALS patients were reclassified into the ALS diagnosis and 2 non-ALS patients were reclassified into the non-ALS diagnosis).

Performance of Simoa™ sNfL Z-score

After conversion of Simoa™ NfL values into age and BMI-adjusted Z-scores, a significant difference was observed between the ALS and non-ALS populations (Table 1). The Z-scores AUC (0.880 [95% CI 0.846–0.909]) was comparable to the raw Simoa™ NfL values (Figure 2c). With the cut-off value set at 2.20 we observed a sensitivity of 82.8% and a specificity of 85.0%, slightly higher therefore than with raw Simoa™ or Ella™ NfL values. This was associated with an improvement in the classification of the patients with a NRI of 5.4% (10 ALS patients were reclassified into the ALS diagnosis and 2 non-ALS patients were reclassified into the non-ALS diagnosis).

Prognosis value of sNfL

The optimal prognostic cut-offs of the ALS population determined as described in the Methods section were 81 pg/mL and 131 pg/mL for the Simoa™ and Ella™ assays, respectively, and 2.67 for sNfL Z-score using age as a covariate (Figure 3). The corresponding HRs were comparable at 4.56 (95% CI 3.51–6.16), 4.50 (95% CI 3.42–5.99), and 4.56 (95% CI 3.44–6.04) for Simoa™, Ella™, and NfL Z-score, respectively (Figure 3a–c). The prognosis in relation to the site of ALS clinical onset is illustrated in Figure 3d. The relationship between sNfL levels and prognosis was also assessed by the significant negative correlation between sNfL levels and ALS duration at sampling time as illustrated in Figure S1C,D. The age-adjusted Cox proportional hazards regressions (Table S2) revealed that age was not a significant covariate with raw sNfL for both Simoa™ and Ella™ but appeared as a significant factor with sNfL Z-score, representing an age- and BMI-independent measure of deviation of sNfL from normal. However, in ALS, the magnitude of this age effect appeared to be weak compared to the biomarker effect: whereas an increase by 1 Z-score unit was associated with a 3.2-fold increase in hazard, the hazard only increased by 2.9% per year of age (Model 3 in Table S2). In ALS, the separation of the effects of age and sNfL abnormality enabled the use of Z-scores; however, this did not result in a better prognostic model as indicated by the very similar concordance (Harrell's C-index) of the three adjusted models and legitimates the use of fixed NfL cut-offs in this disease leading to strongly increased sNfL levels (Table S2).

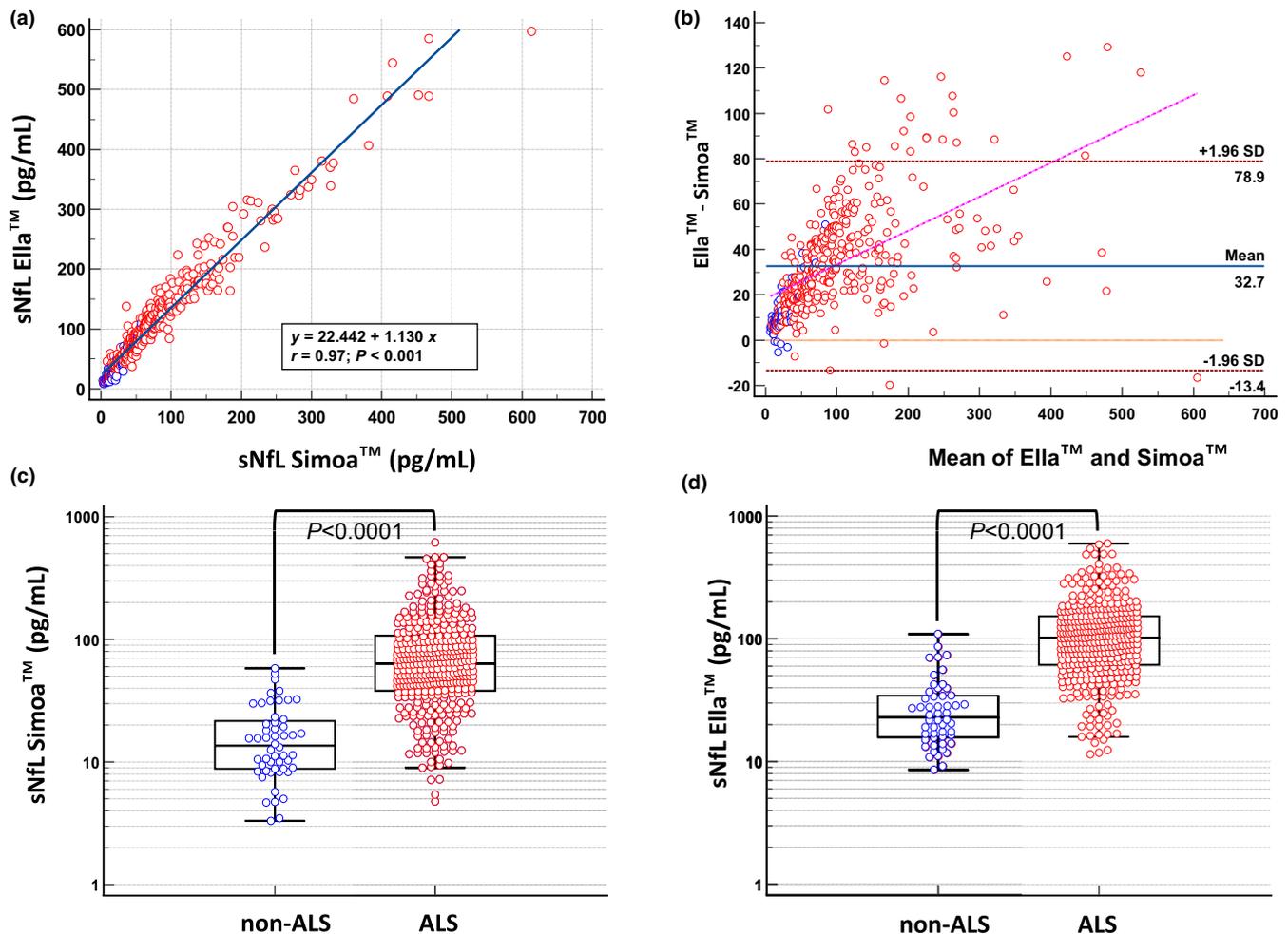


FIGURE 1 Simoa™ and Ella™ quantification of serum neurofilament light chain (sNfL) in the cohort. (a) Spearman correlation ($r=0.97$ [$0.97-0.98$, $p < 0.0001$]) between sNfL quantified by Simoa™ and Ella™ technologies. (b) Bland-Altman analysis assessing the agreement between sNfL concentration determined using the Simoa™ and Ella™ platforms. The central line represents the mean value of bias between assays (32.7 pg/mL) and the outer lines represent 95% limits of agreement (-13.4 to 78.9). (c, d): Boxplots of sNfL quantification using Simoa™ (c) or Ella™ (d) in non-amyotrophic lateral sclerosis (non-ALS) and ALS populations. The difference ($p < 0.0001$) was statistically assessed by Wilcoxon-Mann-Whitney test.

DISCUSSION

In this study we quantified sNfL in a cohort of ALS patients with two commercially available ultrasensitive technologies, Simoa™ and Ella™. These two approaches are those used in most clinical studies of sNfL to date [11, 32–34]. Both methods are very sensitive and can accurately measure picogram levels of analytes in different biological matrices (plasma, serum, or CSF). Ella™ has a lower analytical sensitivity for sNfL than Simoa™ (2.70 pg/mL vs. 0.241 pg/mL, suppliers' information), but for our study this had no impact since all samples were measurable with both technologies. The absolute values of sNfL were higher with Ella™ than with Simoa™ while being highly correlated. The use of different calibrators sets (recombinant human NFL for Simoa™ vs. bovine NFL for Ella™) may explain this difference. Despite some biases that are probably related to different analytical approaches (single-molecule array for Simoa™ vs. microfluidic array for Ella™), the two technologies seem commutable (Figure 1).

Both allow differentiation between ALS and non-ALS patients in the context of a consultation in a motor neuron clinic. The specificities and sensitivities achieved exceed 80% with sNfL threshold values of 37 pg/mL and 57 pg/mL for Simoa™ and Ella™, respectively. These performances are comparable with previous retrospective studies with similar populations [35, 36]. In the study of Behzadi et al. [23] involving both CSF and blood, plasma NfL already showed a very strong diagnostic interest, and it allowed the differentiation of patients with long or short survival as in our study. Of note, our estimates of the HR for survival are indeed comparable to those reported previously [36, 37], which confirms the significance of our results and the interest of this biomarker for the management of patients. Finally, in the study by Halbgebauer et al. [38] using the Ella™ platform, a slightly higher AUC was observed, which may be related to a lower proportion of ALS patients in the studied population.

We know that physiological levels of sNfL are influenced by age and BMI, among other factors [39]. Furthermore, our two clinical

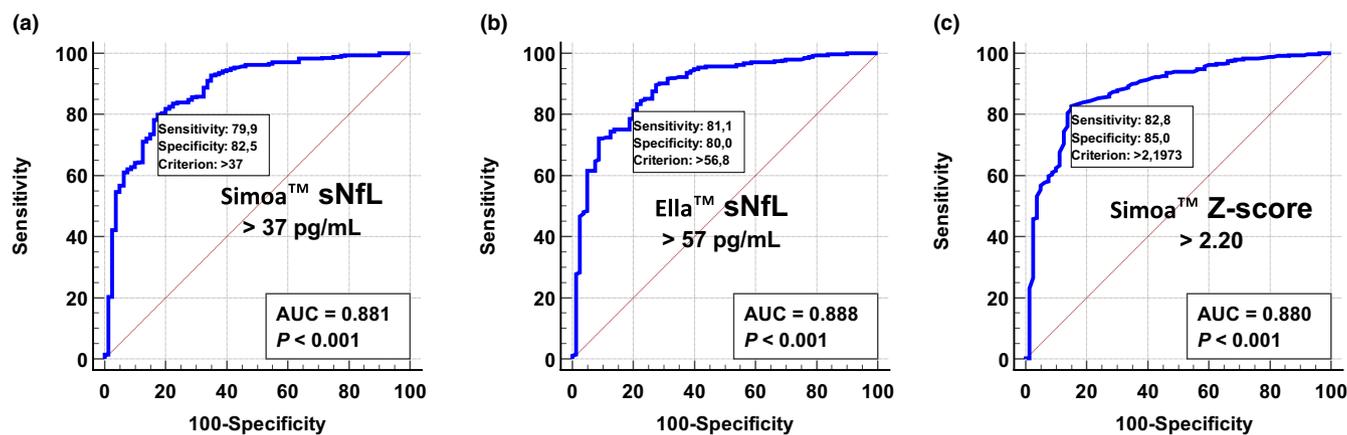


FIGURE 2 Receiver operator characteristic (ROC) analysis of serum neurofilament light chain (sNfL) for amyotrophic lateral sclerosis (ALS) detection in the cohort. sNfL was quantified using Simoa™ (a, c) and Ella™ (b) in the entire cohort. The raw data (a, b) or the sNfL data after Z-score computation (c) were used to plot the ROC curve using the ALS diagnosis as a dichotomous factor. The area under the curve (AUC) as well as the optimal cut-offs corresponding to the Youden index associated with the highest values of sensitivity and specificity are indicated.

TABLE 3 Comparison of diagnostic performance according to patients' age.

Simoa™	Total population	Age ≤ median	Age > median	Two age cut-offs combination	NfL Z-score
AUC	0.881 (0.847–0.910)	0.916 (0.86–0.950)	0.837 (0.782–0.883)		0.882 (0.846–0.909)
Cut-off (pg/mL)	>37.0	>18.6	>45.3	>18.6 or >45.3	>2.20
Sensitivity (%)	79.9	92.4	75.4	83.1	82.8
Specificity (%)	82.5	87.0	82.4	85.0	85.0

Note: Median age is 67 years old. AUC as well as the optimal cut-offs corresponding to the Youden index associated with the highest values of sensitivity and specificity are indicated. The two-age cut-off combination corresponds to the use of one cut-off when "Age ≤ median" and another one for "Age > median".

Abbreviations: AUC, area under the curve; NfL, neurofilament light chain.

groups have different mean ages. If we dichotomized our population by age, we observed very different optimal cut-offs in young and older groups (e.g., 18.6 pg/mL and 45.3 pg/mL for Simoa™) that when combined resulted in a slight improvement of the diagnostic performance (NRI +5.7%). The use of sNfL Z-scores resulted in a similar improvement and therefore qualifies as an optimized solution, especially in diseases other than ALS showing less pronounced increases in sNfL to optimize cut-offs to individual age and BMI [39].

Prognostic factors for ALS are well known and the most clinically relevant is the ALSFR-S score (Amyotrophic Lateral Sclerosis Functional Rating Scale) [11, 12]. In our cohort we confirmed that bulbar onset of disease is a significant prognostic factor (Figure 3d). The prognostic performance of sNfL is, however, much more significant with median survival of 25 weeks [18–34; 25th–75th percentile] above the 81 pg/mL cut-off for Simoa™ and 47 weeks [33–72; 25th–75th percentile] below that threshold. In addition, similar values were observed for Ella™ and sNfL Z-score in a multivariable Cox model (Figure 3a–c). It is notable that age was a covariate with sNfL Z-score but not for raw sNfL measured with Simoa™ and Ella™ (Table S2). The interpretation of this result is that raw sNfL reflects both increased, ongoing neuroaxonal injury as well as higher age, which

itself is affecting survival. The fact that age was a significant covariate in the model with sNfL Z-score (reflecting increased sNfL levels not confounded by age and BMI) indicates that survival also depends on age. However, the prognostic power of increased sNfL appeared to be much higher, and the separation of the raw sNfL effect in a component of increased sNfL compared to healthy controls (i.e., the Z-score) and age did not improve the prognostic performance and led to a limited improvement in the diagnostic performance.

Hazard ratios comparing how quickly death occurs in both groups above and below the cut-offs are greater than 4.5, which is highly significant. This strong survival prediction of sNfL is likely related to the biomarker's ability to witness the extent of motor neuron degeneration, independent of the site of onset. The general correlation between sNfL and ALS duration reinforces this idea.

Regarding the comparison of Ella™ and Simoa™ technologies, we observed greater inter-assay variation for Simoa™, in contrast to another comparison performed on multiple sclerosis samples [40]. Both assays are not yet compliant with the United States Food and Drug Administration (FDA) or the European Union In Vitro Diagnostic Regulation (IVDR), and particular attention should be paid to the control of analytical variation, especially

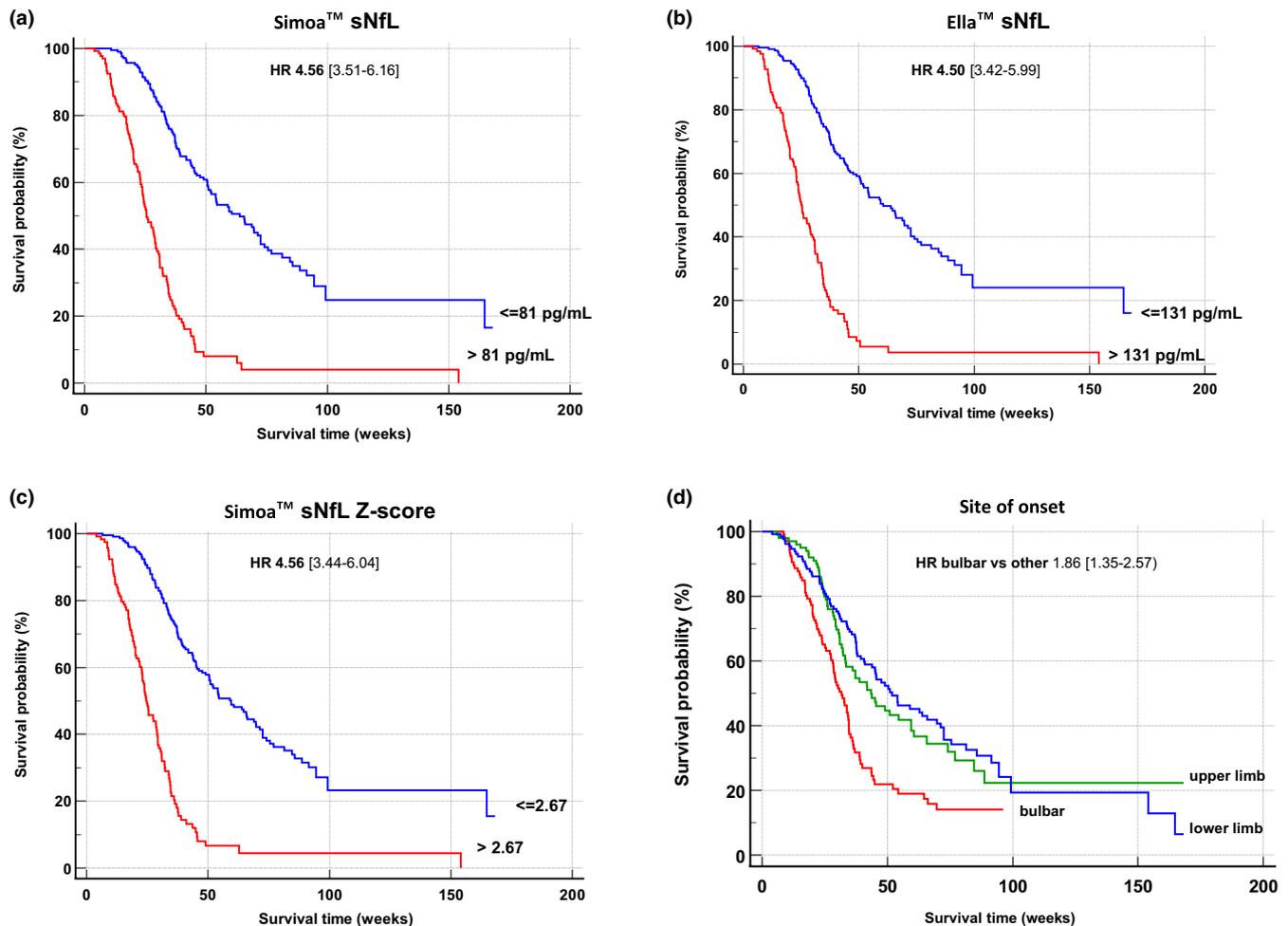


FIGURE 3 Survival analysis. Kaplan–Meier analysis and corresponding hazard ratios (HRs) from Cox proportional hazards models were plotted for Simoa™ (a), Ella™ (b), and serum neurofilament light chain (sNfL) Z-score (c) using age as a covariate. Cox models were plotted depending on the onset site of amyotrophic lateral sclerosis in patients (d).

between batches of reagents, rather than intra-assay performance which is more related to the technology itself. Both approaches are much more expensive than most routine blood tests, with the Ella™ analyzer, however, being less expensive than that for Simoa™ to purchase and maintain. Turnover is clearly in favor of Ella™, which performs parallel measurement of biomarkers with limited sample handling. Conversely, Simoa™ has the advantage of offering relevant multiplexed kits measuring sNfL in parallel with biomarkers such as glial fibrillary acidic protein (GFAP), C-terminal ubiquitin hydrolase-L1 (UCH-L1), or tau protein [41, 42]. In terms of clinical relevance in our context of use, the two technologies achieved very comparable performances. In conclusion, the choice between one or other technology is dependent on the analytical requirements of the individual laboratory.

This study is robust, with a large sample size and population characteristics similar to those in the literature and tertiary ALS centers, but it also has its limitations. First, it is a monocentric study, and the geographical area may induce a bias, as all patients in our cohort were from southern France. Second, the limited number of non-ALS patients in the cohort compared to ALS patients should be

mentioned. Further multisite validation of our results might therefore be useful.

In conclusion, we compared the performance of highly sensitive approaches (Simoa™ and Ella™) for quantifying sNfL in a large cohort of ALS patients. We show that both platforms are highly accurate and can easily be used in routine practice as a confirmatory test for patients with an equivocal diagnosis and especially as a prognostic factor for ALS. One of the major interests of our study is to propose diagnostic thresholds for both technologies that can be used by other centers with a similar context of use as ours [10].

AUTHOR CONTRIBUTIONS

Study conception and design: S.L., W.C. Patient recruitment and consent: F.E., E.d.I.C., W.C. Serum banking and sampling: N.G., E.M. Acquisition of data: M.B., E.M., S.L. Statistical analysis and writing of the manuscript: W.C., E.d.I.C., C.D., S.L. Critical revision of the manuscript: all authors.

FUNDING INFORMATION

None.

CONFLICT OF INTEREST STATEMENT

None to be declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICAL APPROVAL

Ethics approval was granted by Institutional Review Board, CHU Montpellier (Reference: IRB-MTP_2021_04_202100783) and consent obtained from all participants in this study.

PATIENT CONSENT

Obtained (confirmation).

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REFERENCES

- Hardiman O, van den Berg LH, Kiernan MC. Clinical diagnosis and management of amyotrophic lateral sclerosis. *Nat Rev Neurol*. 2011;7:639-649. doi:10.1038/nrneurol.2011.153
- Kiernan MC, Vucic S, Cheah BC, et al. Amyotrophic lateral sclerosis. *Lancet* 2011;2011;377(9769):942-955. [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(10\)61156-7/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(10)61156-7/fulltext) (Accessed 18 May 2021)
- Richards D, Morren JA, Piro EP. Time to diagnosis and factors affecting diagnostic delay in amyotrophic lateral sclerosis. *J Neurol Sci*. 2020;417:117054. doi:10.1016/j.jns.2020.117054
- Herrmann H, Strelkov SV, Burkhard P, et al. Intermediate filaments: primary determinants of cell architecture and plasticity. *J Clin Invest*. 2009;119:1772-1783. doi:10.1172/JCI38214
- Deisenhammer F, Egg R, Giovannoni G, et al. EFNS guidelines on disease-specific CSF investigations. *Eur J Neurol*. 2009;16:760-770. doi:10.1111/j.1468-1331.2009.02595.x
- Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol*. 2018;14:577-589. doi:10.1038/s41582-018-0058-z
- Steinacker P, Feneberg E, Weishaupt J, et al. Neurofilaments in the diagnosis of motoneuron diseases: a prospective study on 455 patients. *J Neurol Neurosurg Psychiatry*. 2016;87:12-20. doi:10.1136/jnnp-2015-311,387
- Poesen K, de Schaepdryver M, Stubendorff B, et al. Neurofilament markers for ALS correlate with extent of upper and lower motor neuron disease. *Neurology*. 2017;88:2302-2309. doi:10.1212/WNL.0000000000004029
- Tortelli R, Copetti M, Ruggieri M, et al. Cerebrospinal fluid neurofilament light chain levels: marker of progression to generalized amyotrophic lateral sclerosis. *Eur J Neurol*. 2015;22:215-218. doi:10.1111/ene.12421
- Feneberg E, Oeckl P, Steinacker P, et al. Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. *Neurology*. 2018;90:e22-e30. doi:10.1212/WNL.0000000000004761
- Thouvenot E, Demattei C, Lehmann S, et al. Serum neurofilament light chain at time of diagnosis is an independent prognostic factor of survival in amyotrophic lateral sclerosis. *Eur J Neurol*. 2020;27:251-257. doi:10.1111/ene.14063
- Lu C-H, Macdonald-Wallis C, Gray E, et al. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. *Neurology*. 2015;84:2247-2257. doi:10.1212/WNL.0000000000001642
- Norgren N, Karlsson J-E, Rosengren L, et al. Monoclonal antibodies selective for low molecular weight neurofilaments. *Hybrid Hybridomics*. 2002;21:53-59. doi:10.1089/15368590252917647
- Gaiottino J, Norgren N, Dobson R, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. *PLoS One*. 2013;8:e75091. doi:10.1371/journal.pone.0075091
- Lu C-H, Petzold A, Topping J, et al. Plasma neurofilament heavy chain levels and disease progression in amyotrophic lateral sclerosis: insights from a longitudinal study. *J Neurol Neurosurg Psychiatry*. 2015;86:565-573. doi:10.1136/jnnp-2014-307,672
- Rissin DM, Kan CW, Campbell TG, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at sub-femtomolar concentrations. *Nat Biotechnol*. 2010;28:595-599. doi:10.1038/nbt.1641
- Wilson DH, Rissin DM, Kan CW, et al. The Simoa HD-1 analyzer: a novel fully automated digital immunoassay analyzer with single-molecule sensitivity and multiplexing. *J Lab Autom*. 2016;21:533-547. doi:10.1177/2211068215589580
- Kuhle J, Barro C, Andreasson U, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med*. 2016;54:1655-1661. doi:10.1515/cclm-2015-1195
- Dysinger M, Marusov G, Fraser S. Quantitative analysis of four protein biomarkers: an automated microfluidic cartridge-based method and its comparison to colorimetric ELISA. *J Immunol Methods*. 2017;451:1-10. doi:10.1016/j.jim.2017.08.009
- Thompson AG, Gray E, Verber N, et al. Multicentre appraisal of amyotrophic lateral sclerosis biofluid biomarkers shows primacy of blood neurofilament light chain. *Brain Commun*. 2022;4:fcac029. doi:10.1093/braincomms/fcac029
- Esselin F, de la Cruz E, Hirtz C, et al. Repeated neurofilament light chain measurements did not capture Riluzole therapeutic effect in amyotrophic lateral sclerosis patients. *CNS Neurosci Ther* 2022;28:1532-1538. doi:10.1111/cns.13894
- Benatar M, Zhang L, Wang L, et al. Validation of serum neurofilaments as prognostic and potential pharmacodynamic biomarkers for ALS. *Neurology*. 2020;95:e59-e69. doi:10.1212/WNL.0000000000009559
- Behzadi A, Pujol-Calderón F, Tjust AE, et al. Neurofilaments can differentiate ALS subgroups and ALS from common diagnostic mimics. *Sci Rep*. 2021;11:22128. doi:10.1038/s41598-021-01499-6
- Traynor BJ, Codd MB, Corr B, Forde C, Frost E, Hardiman OM. Clinical features of amyotrophic lateral sclerosis according to the El Escorial and Airlie House diagnostic criteria: a population-based study. *Arch Neurol*. 2000;57(8):1171-1176. <https://jamanetwork.com/journals/jamaneurology/article-abstract/777262> (Accessed 31 May 2021)
- Teunissen CE, Petzold A, Bennett JL, et al. Deisenhammer: a consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. *Neurology*. 2009;73:1914-1922.
- Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1:307-310.
- Budczies J, Klauschen F, Sinn BV, et al. Cutoff finder: a comprehensive and straightforward web application enabling rapid biomarker

- cutoff optimization. *PLoS One*. 2012;7:e51862. doi:10.1371/journal.pone.0051862
28. Pencina MJ, D'Agostino RB, D'Agostino RB, et al. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med*. 2008;27:157-172; Discussion 207-212. doi:10.1002/sim.2929
 29. Benkert P, Meier S, Schaedelin S, et al. Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *Lancet Neurol*. 2022;21:246-257. doi:10.1016/S1474-4422(22)00009-6
 30. Stöckl D, Rodríguez Cabaleiro D, van Uytvanghe K, et al. Interpreting method comparison studies by use of the Bland-Altman plot: reflecting the importance of sample size by incorporating confidence limits and predefined error limits in the graphic. *Clin Chem*. 2004;50:2216-2218. doi:10.1373/clinchem.2004.036095
 31. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44:837-845.
 32. Delaby C, Julian A, Page G, et al. NFL strongly correlates with TNF-R1 in the plasma of AD patients, but not with cognitive decline. *Sci Rep*. 2021;11:10283. doi:10.1038/s41598-021-89749-5
 33. Alcolea D, Delaby C, Muñoz L, et al. Use of plasma biomarkers for AT(N) classification of neurodegenerative dementias. *J Neurol Neurosurg Psychiatry*. 2021;92(11):1206-1214. doi:10.1136/jnnp-2021-326603
 34. Delaby C, Alcolea D, Carmona-Iragui M, et al. Differential levels of neurofilament light protein in cerebrospinal fluid in patients with a wide range of neurodegenerative disorders. *Sci Rep*. 2020;10:9161. doi:10.1038/s41598-020-66090-x
 35. Gray E, Oeckl P, Amador MDM, et al. A multi-center study of neurofilament assay reliability and inter-laboratory variability. *Amyotroph Lateral Scler Frontotemporal Degener*. 2020;21:452-458. doi:10.1080/21678421.2020.1779300
 36. Poesen K, van Damme P. Diagnostic and prognostic performance of neurofilaments in ALS. *Front Neurol*. 2018;9:1167. doi:10.3389/fneur.2018.01167
 37. Gaiani A, Martinelli I, Bello L, et al. Diagnostic and prognostic biomarkers in amyotrophic lateral sclerosis: neurofilament light chain levels in definite subtypes of disease. *JAMA Neurol*. 2017;74:525-532. doi:10.1001/jamaneurol.2016.5398
 38. Halbgebauer S, Steinacker P, Verde F, et al. Comparison of CSF and serum neurofilament light and heavy chain as differential diagnostic biomarkers for ALS. *J Neurol Neurosurg Psychiatry*. 2022;93:68-74. doi:10.1136/jnnp-2021-327,129
 39. Manouchehrinia A, Piehl F, Hillert J, et al. Confounding effect of blood volume and body mass index on blood neurofilament light chain levels. *Ann Clin Transl Neurol*. 2020;7:139-143. doi:10.1002/acn3.50972
 40. Gauthier A, Viel S, Perret M, et al. Comparison of SimoaTM and EllaTM to assess serum neurofilament-light chain in multiple sclerosis. *Ann Clin Transl Neurol*. 2021;8:1141-1150. doi:10.1002/acn3.51355
 41. Darlix A, Hirtz C, Mollevi C, et al. Serum glial fibrillary acidic protein is a predictor of brain metastases in patients with metastatic breast cancer. *Int J Cancer*. 2021;149:1605-1618. doi:10.1002/ijc.33724
 42. Bailey DM, Bain AR, Hoiland RL, et al. Hypoxemia increases blood-brain barrier permeability during extreme apnea in humans. *J Cereb Blood Flow Metab*. 2022;42:1120-1135; 271678X221075967. doi:10.1177/0271678X221075967

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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