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FVC-DiP correlates with neurofilament light chain levels in serum and cerebrospinal fluid in patients with ALS

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ABSTRACT

Background We previously reported a scale to assess the disease progression rate in patients with amyotrophic lateral sclerosis (ALS), the forced vital capacity decline pattern scale (FVC-DiP). In this study, we investigated the association between FVC-DiP scores and neurofilament light chain (NfL) in the serum and cerebrospinal fluid (CSF) in patients with ALS.

Methods We performed a retrospective study to examine the association between NfL levels and the rate of disease progression (N=41). The disease progression rate was assessed using three methods: the FVC-DiP score determined using the percentage of predicted FVC (%FVC) and disease duration at the %FVC measurement, the rate of decline in the ALS Functional Rating Scale Revised (ALSFRS-R) score (ΔFS) and the rate of decline in the %FVC (Δ%FVC).

Results The FVC-DiP scores were significantly correlated with NfL levels in both the serum and CSF (serum, R^2=0.274, p<0.001; CSF, R^2=0.274, p=0.001). Patients assessed as rapidly progressing by the FVC-DiP had high NfL levels, and patients assessed as slowly progressing had low NfL levels. In the group with a low ΔFS and/or Δ%FVC, although the disease progression rate assessed by the FVC-DiP may have differed from the assessments obtained using the ALSFRS-R and/or %FVC, the correlation between FVC-DiP scores and serum NfL levels remained consistent.

Conclusions The FVC-DiP was significantly associated with NfL levels in the serum and CSF, suggesting that the FVC-DiP is a reasonable scale to assess the rate of ALS progression.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The forced vital capacity decline pattern scale (FVC-DiP) is a novel and simple scale scoring the rate of disease progression in patients with amyotrophic lateral sclerosis (ALS).
⇒ Neurofilament light chain (NfL) levels in serum and cerebrospinal fluid (CSF) are associated with survival and the rate of decline in the ALS Functional Rating Scale Revised (ALSFRS-R) score.

WHAT THIS STUDY ADDS

⇒ FVC-DiP scores were significantly correlated with NfL levels in the serum and CSF. In the group with a low decline rate in ALSFRS-R score (ΔFS) and/or a low decline rate in percentage of FVC (Δ%FVC), although the disease progression rate assessed by the FVC-DiP may have differed from the assessments obtained using the ALSFRS-R and/or %FVC, the correlation between FVC-DiP scores and serum NfL levels remained consistent.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The FVC-DiP is a reasonable scale to assess the rate of disease progression in ALS and evaluates patient condition differently from current functional measures such as the ALSFRS-R and %FVC.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that progressively affects both upper and lower motor neurons. ALS exhibits substantial variability in progression rates,[1] and numerous prognostic models based on multiple prognostic factors have been developed to determine the rate of disease progression in individual patients.[2–10] We also recently reported a novel scale for the disease progression rate, the forced vital capacity (FVC) decline pattern scale (FVC-DiP).[11] The FVC-DiP consists of a score table with the percentage of predicted FVC (%FVC) on the vertical axis and duration from symptom onset on the horizontal axis. Each patient's FVC-DiP score is determined from two parameters at a single time point: %FVC value and duration from symptom onset at the %FVC measurement. A higher FVC-DiP score indicates slow progression, and a low score indicates rapid progression. The FVC-DiP is a simpler method than other prognostic models, but it correlates well with survival, even in the slowly progressing group, where the rate of %FVC decline is less likely to reflect survival.[11]

To develop objective methods to assess the prognosis, many studies have been conducted on fluid biomarkers in serum,

urine and cerebrospinal fluid (CSF).[12] In recent years, evidence has accumulated regarding the usefulness of measuring neurofilaments, particularly neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH), in serum and CSF.[13] High NfL and/or pNfH levels are reported to be associated with shorter survival[14–21] and a faster decline in the ALS Functional Rating Scale Revised (ALSFRS-R) score.[22–24] Although the usefulness of measuring neurofilaments to assess treatment efficacy is still controversial, NfL and pNfH have begun to be adapted as efficacy endpoints in clinical trials for the development of new therapies.[25 26] Single-molecule array (Simoa) can measure NfL with high accuracy, even in serum, where the NfL concentration is much lower than that in CSF,[14 27] but the invasiveness of blood sampling and CSF collection and the cost of measurement using Simoa are likely to be obstacles for repeat measurements in clinical practice.

The relationship between the FVC-DiP score and prognostic biomarkers of ALS has not been reported. In this study, we investigated the association between the FVC-DiP score and NfL levels in serum and CSF in patients with ALS. The FVC-DiP is suitable for repeated evaluations because the disease progression rate can be evaluated using only spirometry, a non-invasive test that is routinely performed in clinical practice for ALS. Confirmation of an association between the FVC-DiP score and NfL level will increase the reliability of the FVC-DiP as a method to assess the rate of disease progression, and the FVC-DiP can be proposed as a useful scale for patients with ALS.

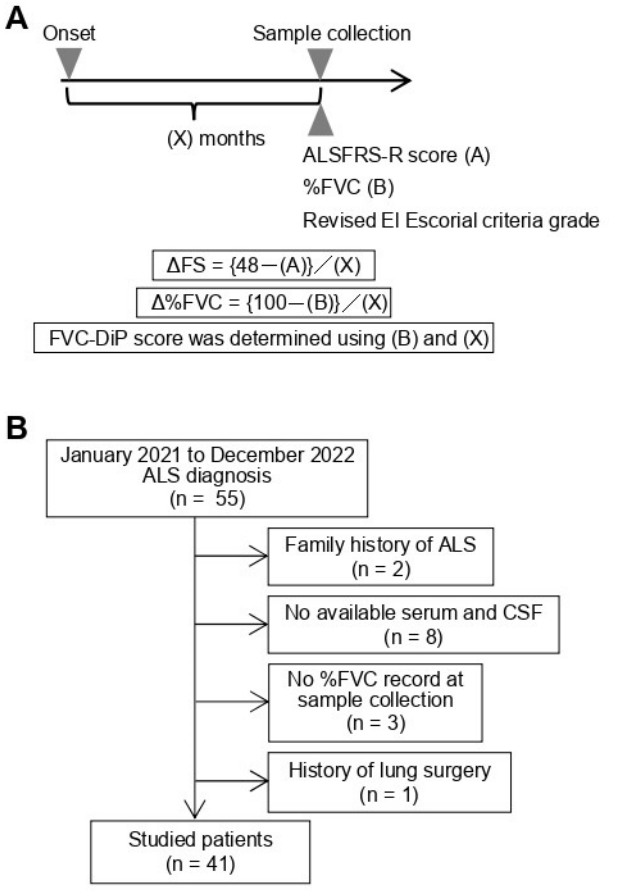

Figure 1 The three methods used to evaluate the disease progression rate (A) and patient selection flow (B). ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale Revised; CSF, cerebrospinal fluid; %FVC, percentage of predicted forced vital capacity; FVC-DiP, FVC decline pattern scale.

METHODS

Participants

We retrospectively analysed patients who fulfilled the following criteria: (1) Patients who were diagnosed with ALS according to the revised El Escorial criteria[28] by board-certified neurologists at Kyushu University Hospital from January 2021 to December 2022; (2) Patients with no family history of ALS; (3) Patients with available serum and/or CSF samples; (4) Patients with a record of %FVC measurement at serum and/or CSF collection and (5) Patients with no previous lung surgery. Data regarding %FVC, age at onset and sample collection, site and time of onset, revised El Escorial criteria grade, sex, body mass index (BMI) and ALSFRS-R score were collected from medical records. The disease progression rate for each patient was assessed using three methods (figure 1A). The FVC-DiP score was determined using the score table described in a previous study.[11] The FVC-DiP score table was created based on longitudinal %FVC data from the Pooled Resource Open-Access ALS Clinical Trials Cohort (PRO-ACT) database to quantify diverse patterns of %FVC decline. To determine the FVC-DiP score for individual patients, a range corresponding to the measured %FVC value was selected on the vertical axis, and a range corresponding to the disease duration was selected on the horizontal axis. The value at their intersection represents the patient's FVC-DiP score at the time of respiratory function measurement. The rate of decline in the ALSFRS-R score (ΔFS) was calculated by dividing (48–ALSFRS-R score at sample collection) by the duration in months between symptom onset and the assessment visit.[29] The rate of decline in the %FVC (Δ%FVC) was calculated by dividing (100–%FVC at sample collection) by the duration in months between symptom onset and the assessment visit. Data regarding the %FVC and ALSFRS-R score within 2 months of the date of serum and CSF collection were included.

Sample collection and measurement

Collected serum samples were centrifuged at 1740 g for 10 min at room temperature, and CSF samples were centrifuged at 120 g for 5 min at 4°C. Supernatants were immediately frozen and stored at −80°C until assayed. The concentrations of NfL in serum (sNfL) and CSF (cNfL) were measured with a Simoa HD-X Analyzer using the NfL Advantage Kit V.2 (Quanterix, Lexington, Massachusetts, USA) following the manufacturer's instructions. Samples were diluted to the range of the standard curve

(serum 1:4 dilution, CSF 1:100 dilution). In our measurements, the mean intraassay coefficients of variation for duplicate determinations of concentration were 2.8% in both serum and CSF. All sample measurements were performed while blinded to the clinical information.

Statistical analyses

The sNfL and cNfL concentrations showed a left-skewed, non-normal distribution (Shapiro-Wilk test for normality, p<0.0001 for sNfL and p=0.007 for cNfL). After common logarithm transformation, the data appeared to be normally distributed (Shapiro-Wilk test for normality, p=0.800 for sNfL and p=0.691 for cNfL), and we used the log-transformed NfL concentration ($\log_{10}$ (sNfL) and $\log_{10}$ (cNfL)) for subsequent analyses and graphical representation. The correlation between sNfL and cNfL levels was assessed using a linear regression model. Associations of sex, site of onset and El Escorial criteria grade with sNfL and cNfL levels were assessed using t-tests. The effects of the age and BMI at sample collection, ΔFS, Δ%FVC and FVC-DiP score on sNfL and cNfL levels were assessed using linear regression models. For the multivariate analysis, to assess the associations with sNfL and cNfL levels, linear regression models were performed including the following variables: the El Escorial criteria grade, age, site of onset and disease progression rate. For the disease progression rate, three different variables were used for each model: model 1, ΔFS; model 2, Δ%FVC and model 3, the FVC-DiP score.

RESULTS

Patient characteristics and NfL concentration in serum and CSF

55 patients were diagnosed with ALS during the study period. Of these, 41 patients who met the eligibility criteria were included in this study (figure 1B). Their characteristics are summarised in table 1. CSF samples at diagnosis were available for 36 patients. Serum samples at diagnosis were available for 41 patients, one of whom also had serum available 6 months after diagnosis. Thus, 36 CSF samples and 42 serum samples were included for the Simoa NfL assay. The cNfL concentration was much higher than the sNfL concentration, and these values were significantly correlated with each other (R^2=0.645, p<0.0001) (figure 2A).

The sNfL and cNfL levels did not differ by sex or onset site (bulbar or limb). Additionally, the sNfL and cNfL levels were not associated with age or BMI at sample collection (online supplemental figure 1). The sNfL level was significantly higher in the group with definite or probable ALS according to the revised El Escorial diagnostic criteria than that in the possible or probable laboratory-supported group ($\log_{10}$ (sNfL) pg/mL, mean±SD, 2.15±0.25 vs 1.88±0.22; p=0.002) (figure 2B), but the cNfL level was not different between the two groups ($\log_{10}$ (cNfL) pg/mL, mean±SD, 3.90±0.26 vs 3.77±0.27; p=0.157) (figure 2C).

Table 1 Patient characteristics

Variables	Patients (n=41)
Age at onset, years, mean (SD)	66.9 (12.7)
Men, n (%)	20 (49)
Bulbar onset, n (%)	7 (17)
Age at diagnosis, years, mean (SD)	68.3 (12.5)
Duration from symptom onset to diagnosis, months, mean (SD)	16.6 (12.3)
Revised El Escorial criteria at diagnosis, definite or probable, n (%)	25 (61)
%FVC at diagnosis, %, mean (SD)	74.8 (29.2)
ALSFRS-R at diagnosis, mean (SD)	35.3 (8.2)
BMI, kg/m^2, mean (SD)	20.6 (3.6)
sNfL, pg/mL, median (IQR)	113 (72.9–155)*
cNfL, pg/mL, median (IQR)	7503 (3981–10,949)†

*Calculated for 42 samples.
†Calculated for 36 samples.
ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale Revised; BMI, body mass index; cNfL, cerebrospinal fluid neurofilament light chain; %FVC, percentage of predicted forced vital capacity; sNfL, serum neurofilament light chain.

Correlation between the disease progression rate and NfL

We examined the association between NfL levels and the disease progression rate assessed by three different methods: ΔFS, Δ%FVC and the FVC-DiP score at sample collection. The disease progression rate, when assessed by any of the three methods, was correlated with both the sNfL and cNfL levels (figure 3). The ΔFS was positively correlated with both the sNfL and cNfL levels (sNfL p<0.001, cNfL p=0.003). Similarly, the Δ%FVC was positively correlated with the sNfL and cNfL levels (sNfL p<0.001, cNfL, p=0.003). In contrast, the FVC-DiP score was negatively correlated with the sNfL and cNfL levels (sNfL p<0.001, cNfL p=0.001). All of these findings indicate that patients identified as having faster disease progression by each method have higher NfL levels. Additionally, it is worth noting that the FVC-DiP scores were well distributed compared with the ΔFS and Δ%FVC (figure 3).

The multivariate analysis results for three models using different variables are shown in table 2. In all models, the disease progression rate was associated with the sNfL level (model 1, p=0.002; model 2, p=0.001; model 3, p=0.004) and the cNfL level (model 1, p=0.012; model 2, p=0.010; model 3, p=0.004). The revised El Escorial criteria grade was associated with the sNfL level (model 1, p=0.007; model 2, p=0.005; model 3, p=0.008) but not associated with the cNfL level.

Difference between assessment using the FVC-DiP and that using the ALSFRS-R or %FVC

The relationships between the FVC-DiP score, ALSFRS-R score and ΔFS are shown in figure 4A. In patients with

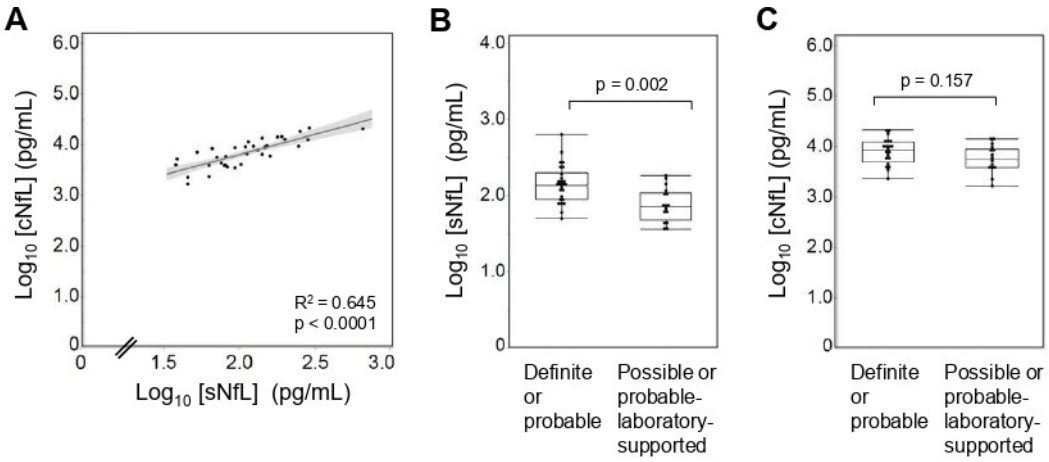

Figure 2 The serum neurofilament light chain (sNfL) and cerebrospinal fluid NfL (cNfL) levels and associations between NfL levels and the revised El Escorial criteria grade. The sNfL and cNfL levels were highly correlated (A). The sNfL level was significantly higher in patients with definite or probable amyotrophic lateral sclerosis (ALS) than in patients with possible or probable laboratory-supported ALS according to the revised El Escorial criteria (B). The cNfL level was not different between the two groups (C). Translucent bands indicate 95% CIs.

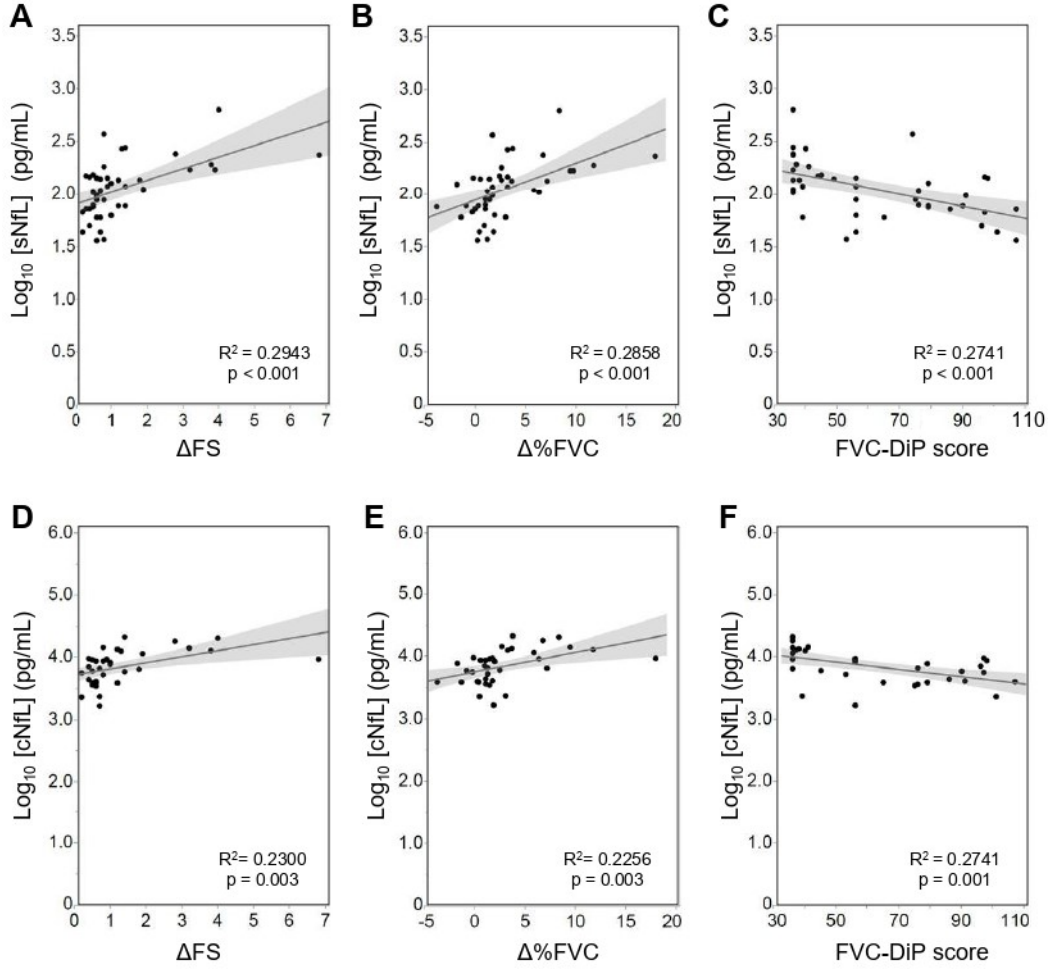

Figure 3 Correlation between the disease progression rate and NfL levels. The ΔFS (A), Δ%FVC (B) and FVC-DiP scores (C) were significantly correlated with sNfL levels. Additionally, the ΔFS (D), Δ%FVC (E) and FVC-DiP scores (F) were significantly correlated with cNfL levels. Translucent bands indicate 95% CIs. ΔFS, rate of decline in the Amyotrophic Lateral Sclerosis Functional Rating Scale Revised score; Δ%FVC, rate of decline in the percentage of predicted forced vital capacity; FVC-DiP, FVC decline pattern scale; sNfL, serum NfL; cNfL, cerebrospinal fluid NfL.

Table 2 Multivariate models examining the associations between NfL levels and clinical variables

		sNfL			cNfL		
Variables		Estimate*	95% CI	P value	Estimate*	95% CI	P value
Model 1							
Age at diagnosis		0.02	−0.04 to 0.08	0.510	0.01	−0.06 to 0.09	0.717
Onset site	Bulbar	−0.04	−0.13 to 0.05	0.380	−0.01	−0.13 to 0.11	0.811
Revised El Escorial criteria	Definite or probable	0.10	0.03 to 0.17	0.007	0.03	−0.06 to 0.12	0.448
Disease progression rate	ΔFS	0.09	0.04 to 0.15	0.002	0.09	0.02 to 0.16	0.012
Model 2							
Age at diagnosis		0.02	−0.04 to 0.07	0.572	0.01	−0.06 to 0.08	0.771
Onset site	Bulbar	−0.06	−0.15 to 0.04	0.221	−0.03	−0.16 to 0.09	0.568
Revised El Escorial criteria	Definite or probable	0.10	0.03 to 0.17	0.005	0.04	−0.05 to 0.13	0.346
Disease progression rate	Δ%FVC	0.03	0.01 to 0.05	0.001	0.03	0.01 to 0.05	0.010
Model 3							
Age at diagnosis		0.01	−0.05 to 0.07	0.681	0.01	−0.06 to 0.08	0.817
Onset site	Bulbar	−0.04	−0.13 to 0.06	0.460	−0.01	−0.12 to 0.11	0.914
Revised El Escorial criteria	Definite or probable	0.10	0.03 to 0.18	0.008	0.04	−0.05 to 0.13	0.351
Disease progression rate	FVC-DiP	−0.05	−0.08 to −0.02	0.004	−0.06	−0.09 to −0.02	0.004

*Per 10-year increase for age, per 1-point increase for the ΔFS and Δ%FVC and per 10-point increase for the FVC-DiP score.
cNfL, cerebrospinal fluid neurofilament light chain; %FVC, percentage of predicted forced vital capacity; FVC-DiP, FVC decline pattern scale; sNfL, serum NfL; ΔFS, rate of decline in the Amyotrophic Lateral Sclerosis Functional Rating Scale Revised; Δ%FVC, rate of decline in the %FVC.

a low ΔFS, FVC-DiP scores exhibited a wide distribution, even at equivalent ALSFRS-R scores. In patients with a ΔFS of 1 or less, the NfL levels varied, even for those with a similar ΔFS; those with higher FVC-DiP scores tended to have lower sNfL levels, and those with lower FVC-DiP scores tended to have higher sNfL levels, although this trend was weaker for cNfL (figure 4B, C). Similarly, in patients with a lower Δ%FVC, FVC-DiP scores exhibited a wide distribution even at equivalent %FVC values (figure 4D). In patients with a Δ%FVC of 2 or less, the NfL levels varied, even in those with similar Δ%FVC; those with higher FVC-DiP scores tended to have lower sNfL levels, and those with lower FVC-DiP scores tended to have higher sNfL levels, although this trend was weaker for cNfL (figure 4E, F). These findings indicate that in patients with a low ΔFS and/or Δ%FVC, the assessment of the disease progression rate using the FVC-DiP was different from that obtained using the ALSFRS-R and/or %FVC, but the correlation between FVC-DiP scores and sNfL levels remained consistent.

DISCUSSION

In this study, the FVC-DiP score was significantly associated with the sNfL and cNfL levels: patients assessed as fast progressors by the FVC-DiP had high NfL levels, while those assessed as slow progressors by the FVC-DiP had low NfL levels. In the group with a low ΔFS and/or Δ%FVC, although the disease progression rate assessed by the FVC-DiP may have differed from the assessments obtained using the ALSFRS-R and/or %FVC, the correlation between FVC-DiP scores and sNfL remained consistent.

Many reports have examined NfL in patients with ALS, and NfL is a prime candidate biomarker for the diagnosis and prognosis of ALS. NfL is released from damaged or degenerated axons into the interstitial fluid, spreading into the CSF and eventually into the blood.[30] Thus, NfL is elevated in other neurological disorders and is not a specific biomarker for ALS. However, the NfL levels in serum and CSF in patients with ALS are much higher than those in patients with other neurological diseases, including diseases that require differentiation from ALS, and the usefulness of NfL as a diagnostic marker is proposed.[16 17 24 31–38] With regard to a prognostic biomarker, associations between NfL and the disease progression rate defined by survival and the rate of decline in the ALSFRS-R score have been reported.[14–17 19–21 24] In these reports, high sNfL and cNfL levels indicated a poor prognosis. Our results also showed that the ΔFS was correlated with sNfL and cNfL levels, with a higher ΔFS indicating higher levels of both sNfL and cNfL. This consistency between previous reports and our results supports the accuracy of NfL measurement in this study. Under these conditions, we were able to confirm the high correlation between FVC-DiP scores and NfL levels, suggesting

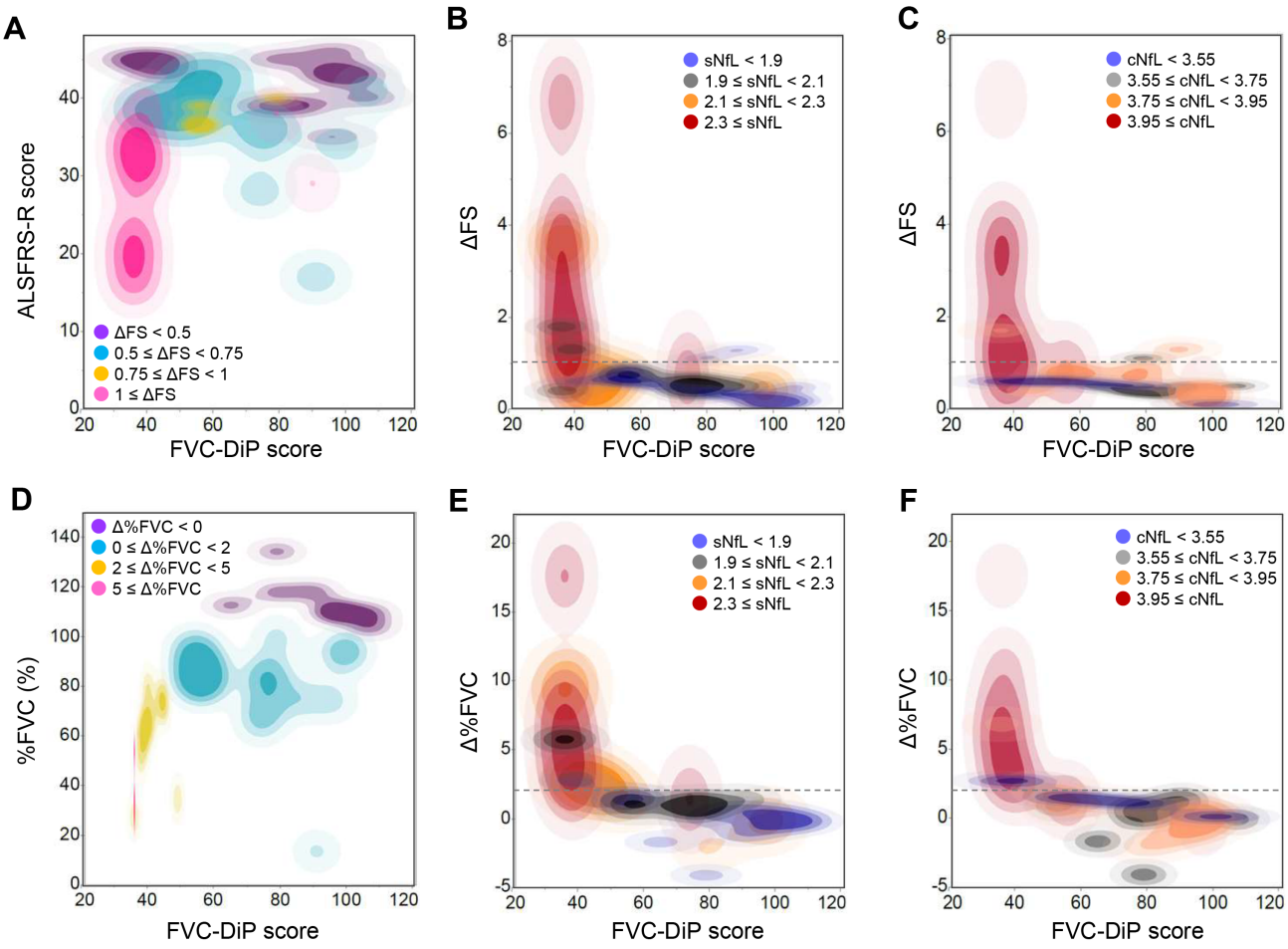

Figure 4 Difference in assessments using the ALSFRS-R, %FVC and FVC-DiP. A contour plot (A) illustrates the relationship between the FVC-DiP, ALSFRS-R score and ΔFS. Contour lines represent the distribution of patients by ΔFS levels, with higher densities indicated by darker zones. Contour plots illustrate the relationship between the FVC-DiP, ΔFS and sNfL (B) or cNfL (C). Contour lines represent the distribution of patients by NfL levels, with higher densities indicated by darker zones. The dashed line represents a ΔFS of 1. A contour plot (D) illustrates the relationship between the FVC-DiP, %FVC and Δ%FVC. Contour lines represent the distribution of patients by Δ%FVC levels, with higher densities indicated by darker zones. Contour plots illustrate the relationship between the FVC-DiP, Δ%FVC and sNfL (E) or cNfL (F). Contour lines represent the distribution of patients by NfL levels, with higher densities indicated by darker zones. The dashed line represents a Δ%FVC of 2. ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale Revised; ΔFS, rate of decline in the ALSFRS-R score, %FVC, percentage of predicted forced vital capacity; Δ%FVC, rate of decline in %FVC, FVC-DiP, FVC decline pattern scale; sNfL, serum NfL; cNfL, cerebrospinal fluid NfL.

that the FVC-DiP is a reasonable scale to assess the rate of disease progression in ALS. As shown by the results, the rate of disease progression assessed by the FVC-DiP had a much wider distribution, even in the group with comparable ΔFS and Δ%FVC. In patients with a low ΔFS and/or Δ%FVC, the FVC-DiP provides a different assessment compared with that of the ALSFRS-R and %FVC, while maintaining the correlation with sNfL. The FVC-DiP may more precisely detect disease progression than the ALSFRS-R or %FVC.

Several longitudinal studies on changes in NfL levels over time in patients with ALS indicated that sNfL and cNfL levels show little or no change during the natural course of ALS.[14–16] This characteristic of stability is similar to that of the FVC-DiP score, which does not show significant changes during the disease course.[11] However, the rate of decline in the ALSFRS-R score and %FVC is not uniform and not always constant during the disease course.[39 40] Although the usefulness of NfL as a monitoring marker for treatment response remains controversial,[25] indicators that remain unchanged in an untreated state would be more suitable for treatment effect monitoring than indicators that show diverse changes, especially in diseases with heterogeneous phenotypes such as ALS. The usefulness of the FVC-DiP score as an indicator to monitor the treatment response will be worthy of exploration.

Our study has some limitations. First, most of the data and samples were taken at the time of ALS diagnosis, and we did not follow the correlation between NfL levels and FVC-DiP scores longitudinally. Given all of the previously reported characteristics of the FVC-DiP and NfL level, it

is expected that they would remain correlated even in the progressive stage, but follow-up data are needed to confirm this consistent correlation. Second, the number of samples, especially CSF samples, is relatively small. Our results showed a significant association between the revised El Escorial criteria grade and the sNfL level, but not the cNfL level. Reports have indicated that the sNfL and cNfL levels are associated with the extent of the affected regions of upper and lower motor neurons.[21 31] Our lack of a statistically significant association between the cNfL level and the revised El Escorial criteria grade may have occurred because of the insufficient sample size. Third, we investigated only sporadic cases, and it remains unknown whether the correlation between the FVC-DiP score and NfL level is present in familial patients with ALS. Fourth, this study is a single-centre, retrospective study and further validation in a multicentre, prospective study is needed to validate the correlation between the FVC-DiP score and NfL level in more detail. In addition to these limitations, there are still aspects of the FVC-DiP that require further consideration. The FVC-DiP score table was created based on the PRO-ACT database, which consists of patients who meet the selection criteria for clinical trials, including age and/or severity limits. The participants in this study were significantly older than those in the PRO-ACT database. In a previous paper, we verified the reproducibility of FVC-DiP characteristics in an external cohort with older onset ages and a varying proportion of bulbar-onset cases. Therefore, the differences in patient characteristics between this study and the PRO-ACT database are unlikely to have a significant impact when applying the FVC-DiP to individual patients, but it is desirable to validate the FVC-DiP characteristics in various cohorts. Lastly, the FVC-DiP is not applicable to patients who are unable to perform pulmonary function tests (eg, those with severe bulbar involvement). In this study, most data were obtained from tests conducted at the time of diagnosis, and only patients who successfully performed pulmonary function tests were included. However, to make the FVC-DiP applicable to all patients with ALS, including those with advanced disease, it is necessary to explore alternative methods for situations where pulmonary function tests cannot be performed.

The Simoa is a much more sensitive method than conventional ELISA and can measure NfL levels with high accuracy, even in serum, where the concentration is much lower than that in CSF .[14 27] However, this assay has not been applied in clinical practice, and the high cost of the assay will affect its widespread use. Pulmonary function tests using spirometry are commonly performed on patients with ALS in clinical practice, and the FVC-DiP has an advantage in that it can assess the rate of disease progression without additional cost or invasiveness.

In conclusion, we showed that FVC-DiP scores correlated with serum and CSF NfL levels in patients with ALS, suggesting the FVC-DiP as a reasonable scale to assess the disease progression rate of ALS. Additionally, the FVC-DiP evaluates patient condition differently from current functional measures such as the ALSFRS-R and %FVC and may contribute to the detection of disease progression that cannot be captured by conventional tools such as the rate of decline in the ALSFRS-R score or %FVC.

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Contributors YK, SK, RY and NI conceived and designed the study. YK and SK performed data collection. YK, TT, GM and J-IK performed the measurements and analysed the results. RY and NI provided technical advice for the experiments. All authors interpreted the results. YK, J-JK and NI drafted the manuscript. All authors reviewed and approved the manuscript. NI is the guarantor.

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Competing interests No, there are no competing interests.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the institutional review board for clinical research of Kyushu University Hospital and Medical Institutions (approval number: 22319-00). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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