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# Comparative diagnosis interest of NfL and pNfH in CSF and plasma in a context of FTD–ALS spectrum

Jean Escal<sup>1,2</sup> · Anthony Fourier<sup>1,2</sup>  · Maité Formaglio<sup>3,4</sup> · Luc Zimmer<sup>2</sup> · Emilien Bernard<sup>5,6</sup> · H  l  ne Mollion<sup>3,4</sup> · Muriel Bost<sup>1</sup> · Mathieu Herrmann<sup>7</sup> · Elisabeth Ollagnon-Roman<sup>8</sup> · Isabelle Quadrio<sup>1,2,4</sup> · Jean-Michel Dorey<sup>7,9</sup>

## Abstract

**Objective** The ‘Frontotemporal dementia–Amyotrophic lateral sclerosis Spectrum’ (FAS) encompasses different phenotypes, including cognitive disorders (frontotemporal dementia, FTD) and/or motor impairments (amyotrophic lateral sclerosis, ALS). The aim of this study was to apprehend the specific uses of neurofilaments light chain (NfL) and phosphorylated neurofilaments heavy chain (pNfH) in a context of FAS.

**Methods** First, NfL and pNfH were measured in 39 paired cerebrospinal fluid (CSF) and plasma samples of FAS and primary psychiatric disorders (PPD) patients, considered as controls. Secondly, additional plasma samples were included to examine a larger cohort of 81 samples composed of symptomatic FAS and PPD patients, presymptomatic and non-carrier relatives individuals. The measures were performed using Simoa technology.

**Results** There was a positive correlation between CSF and plasma values for NfL ( $p < 0.0001$ ) and for pNfH ( $p = 0.0036$ ). NfL values were higher for all phenotypes of symptomatic FAS patients compared to PPD patients ( $p = 0.0016$  in CSF;  $p = 0.0003$  in plasma). On the contrary, pNfH values were solely increased in FAS patients exhibiting motor impairment. Unlike symptomatic FAS patients, presymptomatic cases had comparable concentrations with non-carrier individuals.

**Conclusion** NfL, but not pNfH, appeared to be useful in a context of differential diagnosis between FTD and psychiatric patients. Nevertheless, pNfH seem more specific for the diagnosis and follow-up of motor impairments. In each specific indication, measures in CSF and plasma will provide identical interpretations.

**Keywords** Frontotemporal lobar degeneration · Amyotrophic lateral sclerosis · NfL · pNfH · Blood biomarker

## Introduction

Frontotemporal lobar degeneration (FTLD) is an umbrella term encompassing various neurodegenerative diseases involving frontal and/or temporal lobes of the brain. The

✉ Anthony Fourier  
anthony.fourier@chu-lyon.fr

<sup>1</sup> Laboratory of Neurobiology and Neurogenetics, Department of Biochemistry and Molecular Biology, Lyon University Hospital, Bron, France

<sup>2</sup> BIORAN Team, Lyon Neuroscience Research Center, CNRS UMR 5292, INSERM U1028, Lyon 1 University, Bron, France

<sup>3</sup> Neurocognition and Neuro-Ophthalmology Department, Lyon University Hospital, Bron, France

<sup>4</sup> Center for Memory Resources and Research, Lyon University Hospital, Lyon 1 University, Villeurbanne, France

<sup>5</sup> Reference Center of ALS of Lyon, Lyon University Hospital, Lyon 1 University, Bron, France

<sup>6</sup> NeuroMyoG  ne Institute, CNRS UMR 5310, INSERM U1217, Lyon 1 University, Lyon, France

<sup>7</sup> Department of Aging Psychiatry, Hospital Le Vinatier, Bron, France

<sup>8</sup> Department of Predictive Medicine of Neurological and Neurodegenerative Diseases, Lyon University Hospital, Lyon, France

<sup>9</sup> Brain Dynamics and Cognition Team, Lyon Neuroscience Research Center, CNRS UMR 5292, INSERM U1028, Lyon, France

most common phenotype of FTLD, called behavioral variant of frontotemporal dementia (FTD) is characterized by progressive behavioral changes and executive dysfunctions [1]. A subset of patients additionally shows concomitant motor symptoms due to amyotrophic lateral sclerosis (ALS) that co-occurs, affecting motor neurons in the motor cortex and/or spinal cord [2]. Besides, since ALS and FTD share pathomechanisms, including common protein aggregates (e.g., TDP-43 and FUS) and genetic pathogenic mutations (e.g., *C9ORF72*, *TARDBP*, and *FUS*), these diseases can be considered as a continuum. In that respect, the ‘FTD–ALS spectrum’ (FAS) groups phenotypes of FTD and/or ALS [3]. The detection of known genetic pathogenic variants permits to classify FTD patients in the group of individuals with definite FTLD pathology [4]. Concerning ALS, when a family history of pathogenic mutation is observed, the diagnosis may be upgraded to clinically definite familial ALS [5]. Genetic analysis also provides a means of defining asymptomatic FAS relatives carrying pathogenic mutations as presymptomatic FAS (pre-FAS) and consequently offers a window to study the earliest FAS phases [6, 7]. Though, only approximately 30% of FTD patients have an autosomal dominant pattern of inheritance and 90–95% of ALS cases occur in people with no prior family history [8, 9]. Combined with a lack of specificity of clinical symptoms, it results in a challenging *ante mortem* diagnosis of FAS. Indeed, studies report that FTD is half of the time misdiagnosed as a primary psychiatric disorder (PPD) [10]. Conversely, PPD can be confused with FAS [11]. Moreover, about 10% of ALS ultimately turn out to be other diseases like multifocal motor neuropathy [12].

In this context, complementary markers to genetics are dramatically needed to facilitate the diagnosis and monitor the progression of FAS diseases. Among several promising candidates, neurofilaments seem particularly interesting. These intermediate filaments are selectively expressed by neurons and are localized in axons, where they may undergo post-translational modifications, including phosphorylation that is important for the interaction with each other and with other cytoskeletal proteins [13]. Neurofilaments are an essential component of neuronal cytoarchitecture in addition to participating in vital cellular functions like axonal transport or myelination [14]. Neuroaxonal damages result in the release of neurofilaments into cerebrospinal fluid (CSF) and then into blood, where they can be measured [15].

Within this family of proteins, neurofilaments light chain (NfL) and phosphorylated neurofilaments heavy chain (pNfH) are the two most studied markers in FAS. According to the literature, NfL are consistently elevated in CSF and blood of FAS patients relative to controls [16, 17]. Results are more ambiguous for pNfH, especially when individuals do not present motor impairment [18, 19]. It also appears that NfL and pNfH could increase just before the

phenoconversion of pre-FAS both in CSF and blood [20, 21].

Despite numerous publications available, further research is required to clarify practical indications for NfL and pNfH. Therefore, comparing both NfL and pNfH in CSF and blood in a single study, this work aimed at suggesting specific uses of these promising biomarkers in a context of FTD–ALS spectrum.

## Materials and methods

### Study participants

This retrospective study included symptomatic patients and asymptomatic cases. Symptomatic patients with cognitive disorders in the foreground were diagnosed with FTD or PPD at the Center for Memory Resources and Research of Lyon. They were assessed using a neurological clinical examination and a neuropsychological testing battery. Patients also underwent medical imaging and biological analyses. Thus, lumbar puncture and/or blood collection were performed as part of the diagnosis. Consensus diagnoses were made in multidisciplinary consultations according to the current international diagnostic criteria [4, 22, 23]. Patients with motor symptoms were diagnosed at the Reference Center of ALS of the Hospital of Lyon, where they were provided with clinical examination, medical imaging and biological tests, according to the revised El Escorial criteria [5].

Asymptomatic relatives to mutation carriers followed a multi-step protocol for genetic counseling, based on international recommendations established for the predictive genetic test for Huntington’s disease [24]. Finally, some were themselves carriers of a pathogenic mutation (pre-FAS) and others were not carriers (nc-FASR).

All subjects included in this study gave a written consent to save and use their biological fluids samples for research purposes. This study followed the Helsinki Declaration act of 1975 and was approved by the Lyon University Hospital ethics committee (No 19-42).

### Samples collection and neurofilaments’ analysis

Blood samples were collected in tubes with EDTA, rapidly centrifuged at 2000g for 15 min. CSF samples were obtained by lumbar puncture via standard procedures, collected in low binding polypropylene tubes (Sarstedt, Nümbrecht, Germany) and rapidly centrifuged at 2000g for 10 min. After centrifugation, CSF and plasma samples were divided into aliquots and stored until analyses in deep freeze (– 80 °C) in a biobank with authorization from the French Ministry of Health (Declaration number DC-2008-304).

Plasma and CSF values of NfL and pNfH were determined using the Simoa HD-1 analyzer (Quanterix, Lexington, USA) in a same batch, according to the manufacturer's protocols. The Simoa Neurology 4-plex and the PNF-HEAVY kits were used to measure NfL and pNfH, respectively.

## Study design

The study was structured in two steps. In a first step, only patients with paired plasma/CSF samples were included, to evaluate the correlation between the two biological fluids. Thus, NfL and pNfH values were measured in 39 symptomatic patients as follow: 29 FAS (12 familial FTD, 14 sporadic FTD, 1 ALS and 2 FTD-ALS) and 10 PPD patients (5 depressions and 5 bipolar disorders) considered

as controls (Table 1). The selection of 12 familial FTD patients included 9 *C9ORF72* expansions, 2 *GRN* variants and 1 *MAPT* variant.

In a second step, blood samples from patients who did not have lumbar puncture were added to the first cohort, to evaluate the diagnostic performances of both markers in plasma. In total, 42 additional plasma samples were added, leading to a second set of 81 samples as follow: 52 FAS (26 familial FTD, 16 sporadic FTD, 7 ALS and 3 FTD-ALS), 8 pre-FAS, 9 nc-FASR and 12 PPD patients (6 depressions, 5 bipolar disorders and 1 schizophrenia) (Table 2). Among the 26 familial FTD patients, there were 18 *C9ORF72* expansions, 6 *GRN* variants, 1 *MAPT* variant and 1 *TARDBP* variant. The selection of pre-FAS individuals included 6 *C9ORF72* expansions and 2 *GRN* variants.

**Table 1** Demographic characteristics of the paired CSF/plasma samples cohort, NfL and pNfH concentrations in CSF and plasma

	FTD-ALS spectrum ( <i>n</i> =29)	PPD ( <i>n</i> =10)	<i>p</i> value
Gender (male/female)	11/18	5/5	<i>p</i> =0.7311
Median age (years)	66.0	66.5	<i>p</i> =0.5208
CSF NfL (pg/mL)	2990.0 (1101.0–4360.0)	756.0 (606.0–1000.0)	<i>p</i> =0.0016
Plasma NfL (pg/mL)	46.0 (21.5–86.2)	14.1 (11.8–16.0)	<i>p</i> =0.0003
CSF pNfH (pg/mL)	246.5 (200.5–463.3)	270.0 <sup>a</sup> (227.8–360.0)	<i>p</i> =0.8908
Plasma pNfH (pg/mL)	56.0 <sup>a</sup> (20.1–104.5)	40.4 <sup>a</sup> (18.8–81.6)	<i>p</i> =0.8595

Concentrations are presented as median and interquartile range

ALS amyotrophic lateral sclerosis; CSF cerebrospinal fluid; FTD frontotemporal dementia; NfL neurofilaments light chain; pNfH phosphorylated neurofilaments heavy chain; PPD primary psychiatric disorders

<sup>a</sup>Data obtained with one fewer patient

**Table 2** Demographic characteristics of the complete cohort of patients, NfL and pNfH concentrations in plasma

	FTD-ALS spectrum ( <i>n</i> =52)			Pre-FAS ( <i>n</i> =8)	nc-FASR ( <i>n</i> =9)	PPD ( <i>n</i> =12)	<i>p</i> value
	ALS ( <i>n</i> =7)	FTD-ALS ( <i>n</i> =3)	FTD ( <i>n</i> =42)				
Gender (male/female)	1/6	1/2	19/23	3/5	4/5	6/6	0.7178
Median age (years)	59	72	62	40.5	52	66	0.0042
Median symptom duration at sampling (years)	0.5 (0.5–2.6)	1 (n/a)	3 (2–3.8)	–	–	4 (2–8.5)	0.0052
Plasma NfL (pg/mL)	84.4 (75.7–168.8)	108 (94.2–176.4)	46.8 (22.3–80)	10.1 (7.4–12.4)	9 (6.3–12.3)	13.6 (11.7–15.7)	<0.000001
Plasma pNfH (pg/mL)	579 (188–790.8)	82.2 (29.6–101.6)	30.5 <sup>a</sup> (11.6–87.4)	9.8 <sup>b</sup> (1.9–58.9)	5.5 <sup>b</sup> (2.4–39.6)	31.9 <sup>b</sup> (7.2–74.1)	0.0011

Concentrations are presented as median and interquartile range

<sup>a</sup>Data obtained with three fewer patients

<sup>b</sup>Data obtained with one fewer patient

ALS amyotrophic lateral sclerosis; FTD-ALS FTD coupled to ALS; CSF cerebrospinal fluid; FTD frontotemporal dementia; nc-FASR non-carrier FTD-ALS spectrum relatives; NfL neurofilaments light chain; pNfH phosphorylated neurofilaments heavy chain; pre-FAS presymptomatic FTD-ALS spectrum; PPD primary psychiatric disorders

## Statistical analysis

Statistical analysis was performed using Medcalc software (v 15.11.4). Gender repartition was studied using the Chi-square test. Correlations between CSF and plasma values of NfL and pNfH were studied calculating the Spearman's correlation coefficient.

The non-parametric Mann–Whitney test was applied to test differences between two groups, while the Kruskal–Wallis test was applied for multiple group comparisons. ROC analyses were performed to establish the diagnostic performances of each marker. The optimal cutoff values of NfL in CSF and plasma in terms of sensitivity and specificity were determined by the Youden index.

## Results

In the first step, analyzing the paired CSF/plasma samples, patients showed no significant difference regarding gender and age at biological fluids collection time (Table 1). NfL and pNfH values were found in higher concentrations in CSF than in plasma. There was a positive correlation between CSF and plasma values for the two analytes. Though, the correlation was stronger for NfL ( $Rho = 0.880$ ;  $p < 0.0001$ ) than for pNfH ( $Rho = 0.467$ ;  $p = 0.0036$ ) (Fig. 1). For both markers, correlations were stronger in patients exhibiting high NfL or pNfH values (mostly FAS patients) and were maintained when excluding outliers (data not shown).

NfL values were significantly higher in the 29 FAS patients compared with the 10 PPD patients in CSF ( $p = 0.0016$ ) and in plasma ( $p = 0.0003$ ) (Table 1). This increase was found regardless of the phenotype and the genetic status of the FAS patients in both matrices. To discriminate FAS from PPD patients, the optimal cutoff values

determined by the Youden index, were 1000 pg/mL in CSF (sensitivity = 79%; specificity = 80%) and 16 pg/mL in plasma (sensitivity = 86%; specificity = 90%) (Supplemental Fig. 1). In this sample set, NfL were not statistically different between sporadic and familial FTD patients, neither in CSF ( $p = 0.41$ ) nor in plasma ( $p = 0.78$ ). Conversely to NfL, pNfH values were not significantly different in the cohort of FAS patients compared with the PPD patients both in CSF ( $p = 0.8908$ ) and plasma ( $p = 0.8595$ ) (Table 1). However, pNfH values in CSF were higher in patients with motor impairment (ALS and FTD–ALS) in comparison to FTD patients ( $p = 0.0122$ ). In plasma, this difference was not retrieved ( $p = 0.3160$ ). In this sample set, pNfH values were not statistically different between sporadic and genetic FTD cases, neither in CSF ( $p = 0.57$ ) nor in plasma ( $p = 0.41$ ).

In the second step, analyzing the extended plasma samples cohort, subjects showed no significant difference regarding gender (Table 2). Symptomatic FAS and PPD patients did not show a significant difference regarding age at biological fluids collection time.

Neither did the asymptomatic pre-FAS nor nc-FASR cases, but both were significantly younger than the symptomatic subjects ( $p = 0.0042$ ). The 52 FAS patients had significantly higher plasma NfL values than the 12 PPD patients ( $p < 0.0001$ ). As previously described in the first step, this increase was found regardless of the phenotype and the genetic status of the FAS patients. In addition, the optimal cutoff value of 16 pg/mL in plasma, determined by the Youden index, was retrieved, with a sensitivity of 92% and a specificity of 92% for this larger cohort (Supplemental Fig. 2). More in detail, the highest values were observed for FAS patients with motor impairment. FTD patients exhibited increased values compared to pre-FAS, nc-FASR and PPD patients. These last three groups did not show significant difference of NfL values (Fig. 2A). In FTD patients, NfL

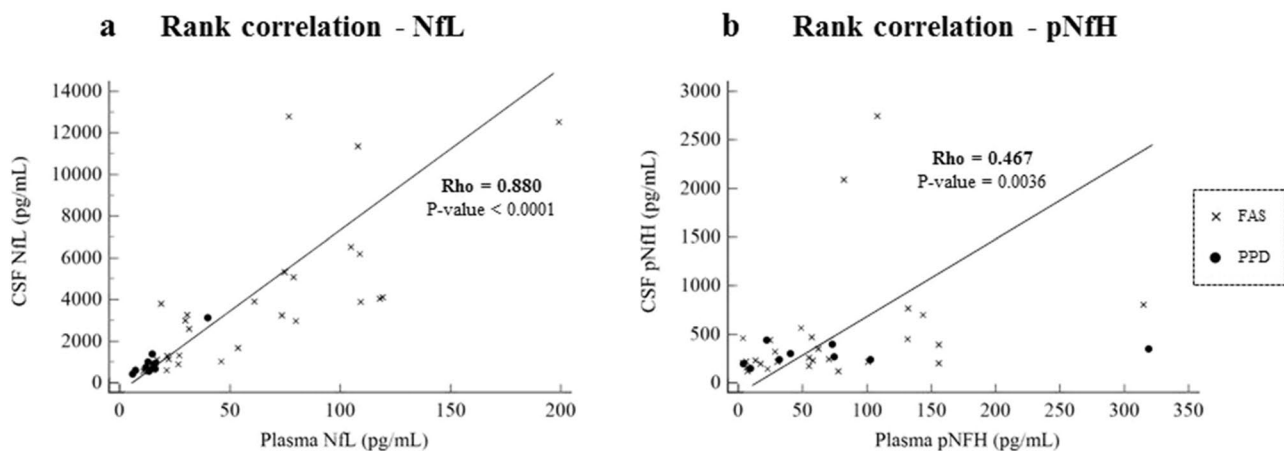
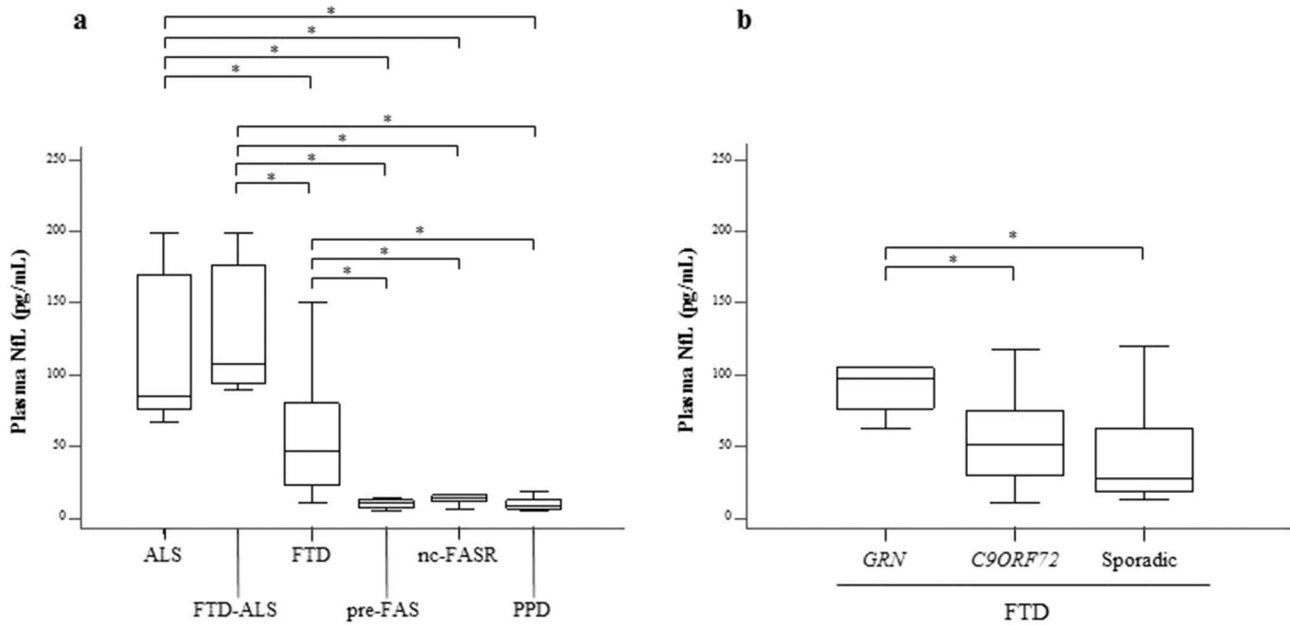
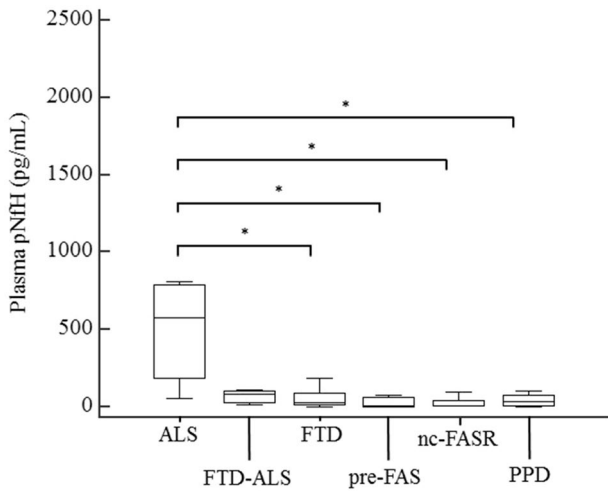


Fig. 1 Rank correlations between CSF and plasma concentrations for a NfL and b pNfH in the paired CSF/plasma samples' cohort



**Fig. 2** Box-whisker plots for plasma NfL concentrations **a** in ALS, FTD-ALS, FTD, pre-FAS, nc-FASR and PPD and **b** among the FTD sub-group. \*  $p$  values < 0.05



**Fig. 3** Box-whisker plots for plasma pNfH concentrations in ALS, FTD-ALS, FTD, pre-FAS, nc-FASR and PPD. \*  $p$  values < 0.05

plasma values were significantly higher for patients with *GRN* variants compared to patients with *C9ORF72* and sporadic cases (Fig. 2B). Concerning pNfH, there was no significant difference concerning plasma pNfH values of FAS and PPD patients ( $p = 0.2978$ ). In the FAS group, only ALS patients had increased plasma pNfH values. Indeed, FTD patients exhibited identical values than pre-FAS, nc-FASR and PPD patients (Fig. 3). In this extended cohort, pNfH values in patients with motor impairment (ALS and FTD-ALS) were increased in comparison to FTD patients ( $p = 0.0019$ ).

Among FTD group, there was no difference of plasma pNfH values between patients with *GRN* and *C9ORF72* mutations, or between familial and sporadic patients.

## Discussion

In this retrospective study, NfL and pNfH were measured in CSF and/or plasma of FAS and PPD patients, pre-FAS and nc-FASR individuals with a highly sensitive single-molecule array technology.

NfL were increased for all FAS in CSF and plasma, regardless of their clinical manifestations, compared with PPD patients. This observed increase for all FAS phenotypes could be explained by the fact that NfL are non-specific markers of neuronal damages. Despite a relatively modest cohort of psychiatric patients, our optimal plasma NfL cutoff value of 16 pg/mL determined by the Youden index was consistent with the literature [25]. Indeed, Al-Shweiki et al. found close plasma NfL cutoff values comparing a larger psychiatric cohort to FTD patients with the same SIMOA technology. This allow us to consider that NfL can be helpful to perform a differential diagnosis between FTD and PPD patients, responding to a need of neurologists and psychiatrists in current medical practice [10, 11]. Within FTD patients, those with *GRN* variants had higher plasma NfL values than others, which was again in agreement with the literature [26]. Combined with the fact that FTD patients with *GRN* mutation have also more white matter lesions and

a faster rate of neurodegeneration, it suggests that different processes, dependent in part on genetics, are involved in the FTLD pathophysiology [27, 28].

No significant difference was observed for pNfH values in CSF and plasma between all FAS patients and PPD patients. In particular, FTD and PPD patients exhibited comparable pNfH values. Thus, contrary to NfL, pNfH could not discriminate between FTD and PPD patients, in accordance with other studies [29, 30]. Nevertheless, increased pNfH values were found in CSF of FAS with motor impairment compared to FTD. In plasma, the first sample set showed no difference between these two groups, contrary to the extended sample set. This discrepancy is probably due to the small size of the cohort of ALS patients in our first sample set, which was specifically designed for paired CSF and plasma samples. The results observed in the larger sample set are in line with other studies [31, 32]. On another note, it was also reported in the literature that the dosage of pNfH presents a better sensitivity and specificity for ALS than the dosage of NfL, and that there are greater changes in pNfH values than in NfL values throughout the ALS course [33, 34]. These observations combined with our results suggest that pNfH more than NfL might reliably support the diagnosis of ALS.

There was a strong correlation between CSF and plasma values for both NfL and pNfH markers. This finding suggests that plasma and not only CSF values are the reflection of pathological changes in the brain for NfL and pNfH, even if only a small fraction of brain proteins that cross the blood–brain barrier is finally measured in blood [15]. Such a correlation has already been reported in FTD–ALS spectrum, but was less described for PPD patients [29, 35]. The correlation between pNfH values in plasma and CSF was not as strong as that of NfL. One reason could be the sequestration of pNfH in hetero-aggregates in blood, but all the mechanisms responsible for the release and the degradation of neurofilaments are not yet fully understood [36, 37]. The correlations were probably strengthened by the use of a same assay kit, containing identical capture and detection antibodies, to assess CSF and plasma samples. Indeed, single-molecule array technology, offering the possibility to measure a wide range of concentrations, is suitable for both CSF and plasma.

Consequently, CSF and plasma can be considered as interchangeable matrices, their use depending on specific clinical indications. On the one hand, in a context of cognitive disorders, NfL could be measured in CSF if core Alzheimer's disease biomarkers are negative, to support the differential diagnosis between FTD and PPD. On the other hand, when patients exhibit strong behavioral disturbances rendering the lumbar puncture difficult to perform, measuring NfL from blood seems to be an interesting alternative. Moreover, plasma samples fit well with

longitudinal iterative studies of symptomatic patients, but also with the follow-up of presymptomatic individuals.

In our study, asymptomatic (pre-FAS and nc-FASR) individuals had lower NfL plasma values than FAS patients. Despite the higher median age of the latter, this difference in concentrations is only partly explained by the 2% per year increase in neurofilaments described in the general population [38]. Indeed, this slight physiological progression is largely surpassed by the disease effect. Moreover, NfL and pNfH plasma values were comparable between pre-FAS individuals and nc-FASR, considered as negative controls. In the literature, studies describing the kinetics of neurofilaments in CSF and/or plasma for presymptomatic FTD and/or ALS cases remain contradictory. Indeed, some authors reported no difference between presymptomatic carriers and controls for NfL in FTD [20] and for pNfH in ALS [39]. Nevertheless, Benatar et al. showed an increase of CSF and serum pNfH for converters compared to at-risk and control cases [21]. A possible reason for these different observations could be linked to the time of blood collection, in relation to the onset of symptomatology. At blood collection time, the neurological examination of the pre-FAS individuals included in our study did not show any clinical symptom. Pending more detailed international guidelines, it seems relevant to monitor disease onset in presymptomatic cases measuring both plasma NfL and pNfH [40].

To conclude, CSF and plasma appear to be interchangeable matrices for NfL and pNfH measurements. The use of one and other biological fluid would depend on specific clinical indications. Concerning neurofilaments' analysis, NfL appeared particularly useful for the differential diagnosis between FTD and behavioral impairment linked to psychiatric diseases. For their part, pNfH showed a weak sensitivity for cognitive symptoms but could be specifically used for the diagnosis and the follow-up of FAS patients with motor impairment. Thus, NfL and pNfH are non-commutable markers but both very promising for the diagnosis and the monitoring of the FTD–ALS spectrum.

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## Declarations

**Conflicts of interest** The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

**Ethics approval** This study followed the Helsinki Declaration act of 1975, and was approved by the Lyon University Hospital ethics committee (No 19-42).

**Consent to participate** All the individuals included in this study gave a written consent to participate.

**Consent for publication** All the individuals included in this study signed informed consent regarding publishing their data.

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