Title: Diagnostic and prognostic value of serum NfL and p-Tau 181 in frontotemporal lobar degeneration

Alberto Benussi,[1] Thomas Karikari, Nicholas Ashton, Stefano Gazzina,[2] Juan Lantero Rodrigues, Joel Simrén, Enrico Premi,[3] Luisa Benussi,[4] Roberta Ghidoni,[4] Marcello Giunta,[1] Roberto Gasparotti,[5] Henrik Zetterberg, Kaj Blennow Barbara Borroni[1*]

Abstract

Objective To assess the diagnostic and prognostic value of serum neurofilament light (NfL) and serum phospho-Tau181 (p-Tau181) in a large cohort of patients with frontotemporal lobar degeneration (FTLD).

Methods In this retrospective study, performed on 417 participants, we analysed serum NfL and p-Tau181 concentrations with an ultrasensitive single molecule array (Simoa) approach. We assessed the diagnostic values of serum biomarkers in the differential diagnosis between FTLD, Alzheimer's disease (AD) and healthy ageing; their role as markers of disease severity assessing the correlation with clinical variables, cross-sectional brain imaging and neurophysiological data; their role as prognostic markers, considering their ability to predict survival probability in FTLD.

Results We observed significantly higher levels of serum NfL in patients with FTLD syndromes, compared with healthy controls, and lower levels of p-Tau181 compared with patients with AD. Serum NfL concentrations showed a high accuracy in discriminating between FTLD and healthy controls (area under the curve (AUC): 0.86, $p<0.001$), while serum p-Tau181 showed high accuracy in differentiating FTLD from patients with AD (AUC: 0.93, $p<0.001$). In FTLD, serum NfL levels

correlated with measures of cognitive function, disease severity and behavioural disturbances and were associated with frontotemporal atrophy and indirect measures of GABAergic deficit. Moreover, serum NfL concentrations were identified as the best predictors of survival probability.

Conclusions The assessment of serum NfL and p-Tau181 may provide a comprehensive view of FTLD, aiding in the differential diagnosis, in staging disease severity and in defining survival probability.

Introduction

Frontotemporal lobar degeneration (FTLD) encompasses a series of early onset progressive neurodegenerative conditions for which, in the last decade, the diagnostic workup has substantially changed with the publication of revised clinical criteria (Gorno-Tempini *et al.*, 2011; Rascovsky *et al.*, 2011). The careful characterization of clinical features of the behavioural variant frontotemporal dementia (bvFTD), the agrammatic or the semantic variant of primary progressive aphasia (avPPA and svPPA), and the spectrum of FTLD with extrapyramidal symptoms, such as corticobasal syndrome (CBS) and progressive supranuclear palsy (PSP), has enabled a better understanding of the heterogeneity of FTLD phenotypes (Bang *et al.*, 2015; Van Mossevelde *et al.*, 2018).

The pattern of brain atrophy and hypometabolism (Rosen *et al.*, 2002; Le Ber *et al.*, 2006), and the results of new positron emission tomography tracers (Makaretz *et al.*, 2017; Passamonti *et al.*, 2017; Tsai *et al.*, 2019), have assisted in increasing the diagnostic accuracy of FTD, while $A\beta_{1\text{-}42}$ or tau measurements in cerebrospinal fluid (CSF) have been proven to be key in ruling out Alzheimer's disease (AD) (Olsson *et al.*, 2016). Furthermore, the identification of monogenic FTLD, due to pathogenetic mutations within the *granulin (GRN)*, *chromosome 9 open reading frame 72 (C9orf72)* or *microtubule-associated protein tau (MAPT)*, has undoubtedly contributed to the diagnostic work-up (Borroni and Padovani, 2013).

Considering the possible drawbacks of these supportive biomarkers due to invasiveness, availability or expensiveness, there is an urgent need to identify robust and accessible screening tests to be used even in the earliest disease stages (Borroni *et al.*, 2015), in a disorder that is much more frequent than previously thought (Logroscino *et al.*, 2019).

Along with recently proposed neurophysiological markers, measuring FTLD-related neurotransmitter deficits by Transcranial Magnetic Stimulation (TMS) (Benussi *et al.*, 2017, 2020*c*), a giant step forward towards potentially useful biomarkers for AD-related pathologies has been made with the new ultrasensitive Single molecule array (Simoa) approach (Rissin *et al.*, 2010). It has been reported that concentrations of neurofilament light chain (NfL), a marker of axonal

damage which is measurable in CSF, plasma or serum, are increased in FTLD and may be related to parameters of disease severity and prognosis (Pijnenburg *et al.*, 2015; Meeter *et al.*, 2016; Rohrer *et al.*, 2016; Wilke *et al.*, 2016; Foiani *et al.*, 2018; Steinacker *et al.*, 2018; Heller *et al.*, 2020; Katisko *et al.*, 2020). Furthermore, a Meso-Scale Discovery (MSD) assay for plasma phospho-Tau$_{181}$ developed by Lilly Research Laboratories was found to differentiate AD from healthy controls, suggesting its ability to identify mixed 3R/4R tau pathology (Mielke *et al.*, 2018). Two recent studies have further highlighted the usefulness of this biomarker assay in the differential diagnosis between FTLD and AD, and in monitoring disease progression (Janelidze *et al.*, 2020; Thijssen *et al.*, 2020). A paper employing a Simoa assay developed at University of Gothenburg, also found a marked increase in plasma p-Tau$_{181}$ in AD, correlating with tau PET ligand retention, while levels were normal in other tauopathies including FTLD and progressive supranuclear palsy (Karikari). This retrospective study aimed at confirming and extending previous literature data, comprehensively assessing the clinical value of serum NfL and serum phospho-Tau$_{181}$ in a large cohort of FTLD patients. We discuss when either serum NfL or serum phospho-Tau$_{181}$ should be considered on clinical grounds on the basis of specific clinical questions and defined outcomes. We analysed three main aims: *a*) the role of serum NfL and serum phospho-Tau$_{181}$ as diagnostic markers, evaluating the accuracy in the differential diagnosis between FTLD and both AD and healthy ageing, and, most importantly, their usefulness in the earliest disease stages; *b*) their role as markers of disease severity, assessing the correlation with clinical variables, cross-sectional brain imaging and neurophysiological data; *c*) their role as prognostic markers, considering their ability to predict survival probability in FTLD.

Materials and Methods

Subjects

This retrospective study included 417 participants from two independent cohorts, 307 from the Centre for Neurodegenerative Disorders, University of Brescia, Italy and 110 from the IRCCS Istituto San Giovanni di Dio Fatebenefratelli, Brescia, Italy.

The cohort consisted of 291 patients meeting probable clinical criteria for a syndrome in the FTLD spectrum, namely 134 bvFTD, 48 avPPA, 27 svPPA, 51 CBS and 31 PSP (Gorno-Tempini *et al.*, 2011; Rascovsky *et al.*, 2011; Armstrong *et al.*, 2013; Höglinger *et al.*, 2017). Moreover, 63 AD patients fulfilling current clinical criteria (Jack *et al.*, 2018) and 63 healthy controls (HC), recruited among spouses or caregivers, were included as well.

Each FTLD patient underwent a neurological evaluation, routine laboratory examination and a neuropsychological and behavioural assessment (Borroni *et al.*, 2015). In all cases, the diagnosis was supported by brain structural imaging, while cerebrospinal fluid (CSF) dosage of tau, phospho-tau_{181} and $A\beta_{1-42}$ was performed in a subset of cases (45.7%), to rule out AD, as previously reported (Borroni *et al.*, 2014). Furthermore, in familial cases (based on the presence of at least one dementia case among the first-degree relatives) and early onset sporadic cases, genetic screening for *GRN*, *C9orf72* and *MAPT* P301L mutations was performed (given the low frequency of *MAPT* mutations in Italy (Binetti *et al.*, 2003), we considered only the P301L mutation; we sequenced the entire *MAPT* gene only in selected cases).

Each participant underwent blood collection for measurements of serum NfL and phospho-Tau_{181} biomarkers, and a subset of FTLD patients underwent standardized brain Magnetic Resonance Imaging (MRI) at baseline (n=132) to evaluate the correlation between serum biomarkers and imaging data. Moreover, a subgroup of patients underwent TMS protocols (n=113) to assess the correlation between serum biomarkers and neurophysiological data. For the purpose of the present study, we considered TMS measures that partially and indirectly reflect the activity of several neurotransmitters, including $GABA_A$ by short interval intracortical inhibition (SICI), glutamate by

intracortical facilitation (ICF), $GABA_B$ by long interval intracortical inhibition (LICI), and acetylcholine by short latency afferent inhibition (SAI) (Rossini *et al.*, 2015; Ziemann *et al.*, 2015). Full written informed consent was obtained from all subjects according to the Declaration of Helsinki. The Brescia Ethics Committee approved the study protocol.

Clinical evaluation

At baseline patients underwent a standardized neuropsychological battery which included the Mini-Mental State Examination (MMSE) (Magni *et al.*, 1996), the Short Story Recall test (Novelli *et al.*, 1970), the Rey Complex Figure (copy and recall) (Caffarra *et al.*, 2002), phonemic and semantic fluencies (Novelli *et al.*, 1986), the Token test (De Renzi and Vignolo, 1962), the Clock-Drawing Test (Sunderland *et al.*, 1989), and Trail Making Test (part A and part B) (Giovagnoli *et al.*, 1996). Disease severity was assessed with the FTLD modified Clinical Dementia Rating (FTLD-modified CDR) sum of boxes scale (Knopman *et al.*, 2008), while the level of functional independence was assessed with the Basic Activities of Daily Living (BADL) (Katz *et al.*, 1963) and the Instrumental Activities of Daily Living (IADL) (Lawton and Brody, 1969) questionnaires. Furthermore, neuropsychiatric and behavioural disturbances were evaluated with the Frontal Behaviour Inventory (FBI) (Alberici *et al.*, 2007; Cosseddu *et al.*, 2020).

HC underwent a brief standardized neuropsychological assessment (Mini-Mental State Examination ≥27/30); psychiatric or other neurological illnesses were considered exclusion criteria.

Serum biomarkers

Serum was collected by venipuncture, processed and stored in aliquots at -80°C according to standardised procedures. Serum NfL and serum phospho-Tau_{181} were measured using the multiplex Neurology 4-Plex A kit (Quanterix Corporation, Lexington, USA) and the Human Total Tau kit (Quanterix, Boston Massachusetts, USA), respectively, on the Simoa HD-1 Analyzer (Quanterix, Boston Massachusetts, USA) following manufacturer's instructions as previously described

(Gisslén *et al.*, 2016; Foiani *et al.*, 2018). The lower limits of detection of the assay for serum NfL and phospho-Tau$_{181}$ were 0.104 pg/mL and 0.019 pg/mL, respectively. Measurements were carried out at the same study site on consecutive days, using the same batch of reagents, and the operator was blinded to all clinical information. Quality control samples had a mean intra-assay and inter-assay coefficient of variation of less than 10%

MRI acquisition, processing and analysis

Brain images were collected using 1.5 Tesla (Siemens Symphony and Avanto, Erlangen, Germany) or 3 Tesla scanner (Siemens Skyra, Erlangen, Germany) equipped with a circularly polarized transmit-receive coil to obtain 3D magnetization-prepared rapid gradient echo (MPRAGE) T1-weighted scans. At 1.5T, sequences were acquired with the following parameters: repetition time 2100-2050 ms, echo time 2.95-2.56 ms, inversion time 1100 ms, slice thickness 1 mm, voxel size 1×1×1 mm, in-plane field of view 256 mm, flip angle = 15°. At 3T, sequences were acquired with the following parameters: repetition time 2000 ms, echo time 2.92 ms, inversion time 850 ms, slice thickness 1.1 mm, voxel size 1.1 × 1.1 × 1.1, field of view 282 mm, flip angle 8°.

T1 scans were visually inspected and excluded from subsequent analyses if excessive motion blurring or artifacts were present. Then, images were processed and analyzed with the fully automated surface-based morphometry pipeline in the Computational Anatomy Toolbox (CAT12.6) (http://www.neuro.uni-jena.de/cat/) for Statistical Parametric Mapping (SPM12 v. 7771) (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/), running on MATLAB 9.2 (The MathWorks, Inc, Natick, MA USA). Cortical meshes were resampled to the Human Connectome Project mesh and smoothed with a 15 mm filter.

Smoothed cortical thickness meshes were included in a multiple regression model, in which serum NfL and serum phospho-Tau$_{181}$ values represented the independent variables. Age, gender, clinical phenotype and MRI scanner type were considered as confounding factors. The statistical threshold

was set at 0.05 and corrected for multiple comparisons using false discovery rate (FDR) at whole-brain level.

Transcranial Magnetic Stimulation

A TMS figure-of-eight coil (each loop diameter 70 mm – D70^2 coil) connected to a monophasic Magstim Bistim^2 system (Magstim Company, Oxford, UK) was employed for all TMS paradigms, as previously reported (Benussi *et al.*, 2019*b*). Electromyographic (EMG) recordings were performed from the first dorsal interosseous (FDI) muscles using 9 mm diameter, Ag-AgCl surface-cup electrodes. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint of the index finger. Responses were amplified and filtered at 20 Hz and 2 kHz with a sampling rate of 5 kHz.

Resting motor threshold (RMT) was determined on the left motor cortex as the minimum intensity of the stimulator required to elicit motor evoked potentials (MEPs) with a 50 μV amplitude in 50% of 10 consecutive trails, recorded form the right first dorsal interosseous muscle during full muscle relaxation.

SICI-ICF, LICI and SAI were studied using a paired-pulse technique, employing a conditioning-test design. For all paradigms, the test stimulus (TS) was adjusted to evoke a MEP of approximately 1 mv amplitude in the right first dorsal interosseous muscle.

For SICI and ICF, the conditioning stimulus (CS) was adjusted at 70% of the RMT, employing multiple interstimulus intervals (ISIs), including 1, 2, 3 ms for SICI and 7, 10, 15 ms for ICF (Kujirai *et al.*, 1993; Ziemann *et al.*, 1996). LICI was investigated by implementing two supra-threshold stimuli, with the CS adjusted at 130% of the RMT, employing ISIs of 50, 100 and 150 ms (Valls-Solé *et al.*, 1992). SAI was evaluated employing a CS of single pulses (200 μs) of electrical stimulation delivered to right median nerve at the wrist, using a bipolar electrode with the cathode positioned proximally, at an intensity sufficient to evoke a visible twitch of the thenar muscles

(Tokimura *et al.*, 2000). Different ISIs were implemented (0, +4), which were fixed relative to the N20 component latency of the somatosensory evoked potential of the median nerve.

For each ISI and for each protocol, ten different paired CS-TS stimuli and fourteen control TS stimuli were delivered in all participants in a pseudo-randomized sequence, with an inter trial interval of 5 secs (±10%).

The conditioned MEP amplitude, evoked after delivering a paired CS-TS stimulus, was expressed as percentage of the average control MEP amplitude. Average values for SICI (1, 2, 3 ms ISI), ICF (7, 10, 15 ms ISI), LICI (50, 100, 150 ms ISI) and SAI (0, +4 ms ISI) were used for analysis.

Stimulation protocols were conducted in a randomized order. Audio-visual feedback was provided to ensure muscle relaxation during the entire experiment and trials were discarded if EMG activity exceeded 100 μV in the 250 ms prior to TMS stimulus delivery. Less than 5% of trials were discarded for each protocol. All of the participants were capable of following instructions and reaching complete muscle relaxation; if, however the data was corrupted by patient movement, the protocol was restarted and the initial recording was rejected.

Statistical analysis

Linear regression and stepwise multiple regression analysis (including all variables with a $p<0.001$ at univariate analysis) were used to characterize the relationship between serum biomarkers and demographic characteristics (age, age at onset, sex and mutational status).

Differences in clinical variables and biomarker concentrations were assessed with one-way analysis of covariance (ANCOVA), corrected for age, sex and mutational status, with Bonferroni multiple comparisons correction. Pearson's correlations were used to assess associations between serum biomarkers, age and education corrected clinical variables and TMS measures.

Receiver operating characteristics (ROC) curve analyses were used to determine the ability of serum NfL and phospho-Tau_{181} to differentiate between diagnostic groups. The area under the curve

(AUC) including 95% confidence interval (CI) values are reported, with cut-off points set to achieve highest levels of sensitivity and specificity (Youden's index).

Survival was calculated as time from symptom onset to time of death from any cause (outcome=0) or censoring date (outcome=1). Survival analysis was carried out by the Kaplan-Meier method with log rank *post hoc* testing and by means of univariate and multivariate stepwise Cox proportional-hazard regression analysis; hazard ratios (HR) are provided with their respective 95% confidence intervals (CIs).

A two-sided p-value<0.05 was considered significant and corrected for multiple comparisons using false discovery rate (FDR) when appropriate (Pike, 2011). Statistical analyses were performed using SPSS (v.24; SPSS, IBM).

Data availability

All study data, including raw and analysed data, and materials will be available from the corresponding author, B.B., upon reasonable request.

Results

Participant characteristics

Baseline demographics, clinical variables and fluid biomarker levels are reported in **Table 1**.

In the FTLD group, serum NfL concentrations did not correlate with age (β=-0.07, p=0.272), age at onset (β=-0.03, p=0.614), or gender (β=-0.08, p=0.193), but correlated with the presence of a pathogenic mutation at both the linear regression (β=0.48, p<0.001) and at the stepwise multiple regression model (β=0.48, p<0.001). Serum NfL concentrations were higher in patients with a pathogenic mutation (mean±SE, *GRN* mutations n=30, 86.2±5.0; *MAPT* mutations n=3, 43.0±15.9) compared with patients without a pathogenic mutation (no mutation/unknown n=258, 36.0±1.7 pg/L). Serum phospho-Tau_{181} concentrations also did not correlate with age (β=0.07, p=0.209), age at onset (β=0.08, p=0.159), or gender (β=-0.08, p=0.200), but correlated inversely with the presence of a pathogenic mutation in both the linear regression (β=-0.16, p=0.006) and in the stepwise multiple regression model (β=-0.13, p<0.021). Serum phospho-Tau_{181} concentrations were lower in patients without a pathogenic mutation (no mutation/unknown, 3.9±0.4) compared with patients with a pathogenic mutation (*GRN* mutations, 0.6±1.1; *MAPT* mutations, 2.3±3.6).

Serum NfL and serum phospho-Tau_{181} concentrations in FTLD subgroups

Serum NfL concentrations were significantly increased in most FTLD subgroups (age- and sex-corrected ANCOVA, $F(6,408)=11.97$, p<0.001, η^2=0.15). In Bonferroni-corrected *post hoc* tests, we observed a significant increase in serum NfL levels in bvFTD, avPPA and CBS, and in AD patients compared with HC. Patients with avPPA had significantly higher levels of serum NfL compared with svPPA, CBS, PSP and AD (see **Table 1**).

After correcting also for mutation status, considering the unbalanced distribution of pathogenic mutations across FTLD subgroups (see **Table 1**) and the increased NfL concentrations in mutation carriers, we observed a significant increase in NfL levels in all the FTD variants (bvFTD, avPPA

and svPPA) compared with HC (age, sex and mutation corrected ANCOVA, $F(6,408)=7.00$, $p<0.001$, $\eta^2=0.09$), without significant differences between avPPA and the other subgroups (see **Figure 1, panel A**).

Serum phospho-Tau_{181} concentrations were significantly reduced in all FTLD subgroups compared with AD (age and gender corrected ANCOVA, $F(6,408)=21.35$, $p<0.001$, $\eta^2=0.24$) (see **Table 1** and **Figure 1, panel B**). No significant differences between FTLD subgroups were found except for higher values in CBS compared with bvFTD (see **Figure 1, panel B**). Serum phospho-Tau_{181} was also significantly increased in AD patients compared with HC (see **Figure 1, panel B**). Comparable results were observed also after adjusting for age, gender and mutation status (ANCOVA, $F(6,408)=20.21$, $p<0.001$, $\eta^2=0.23$).

Diagnostic accuracy of serum NfL and serum phospho-Tau_{181}

To differentiate FTLD patients from HC, we applied a ROC curve analysis on serum NfL concentrations, observing an AUC of 0.862 ($p<0.001$, 95% CI 0.818-0.906); the serum NfL cut-off of 22.5 pg/mL differentiated FTLD from HC with a sensitivity of 71.5% and a specificity of 92.1% (see **Figure 2, panel A**). In patients with a mild disease stage (FTLD-modified CDR ≤ 5), a serum NfL cut-off of 19.1 pg/mL differentiated mild FTLD from HC with a sensitivity of 74.8% and specificity of 74.6%, with an AUC of 0.813 ($p<0.001$, 95% CI 0.753-0.874) (see **Figure 2, panel B**).

To differentiate FTLD from AD patients, we applied a ROC curve analysis on serum phopsho-Tau_{181} concentrations, observing an AUC of 0.930 ($p<0.001$, 95% CI 0.903-0.956); a serum phospho-Tau_{181} cut-off of 5.88 pg/mL differentiated FTLD from AD with a sensitivity of 81.4% and a specificity of 93.5% (see **Figure 2, panel C**).

In patients with a mild disease stage (FTLD with an FTLD-modified CDR ≤ 5 and AD with a MMSE ≥ 19), the serum phospho-Tau_{181} cut-off of 6.11 pg/mL differentiated mild FTLD from mild

AD with a sensitivity of 80.0% and specificity of 91.7%, with an AUC of 0.907 (p<0.001, 95% CI 0.862-0.951) (see **Figure 2, panel D**).

Serum NfL and serum phospho-Tau$_{181}$ associations with cognitive function and disease severity in FTLD

Cognitive and behavioural assessment. Serum NfL concentrations showed significant associations with baseline BADL (r=0.23, p<0.001), IADL (r=0.23, p<0.001) and FTLD-modified CDR sum of boxes (r=0.28, p<0.001), the higher the serum NfL levels, the greater impairment in functional activities and disease severity. Significant correlations were observed between serum NfL concentrations and MMSE scores (r=-0.30, p<0.001), phonemic (r=-0.24, p=0.001) and semantic fluencies (r=-0.24, p=0.001), clock-drawing (r=-0.24, p=0.001), short story (r=-0.25, p=0.002), trail-making part B (r=-0.22, p=0.011), digit symbol (r=-0.16, p=0.027), and token test (r=-0.17, p=0.035), with higher levels of serum NfL correlating with poorer scores. No significant correlations were observed for the Rey figure copy (r=-0.10, p=0.155) and recall (r=-0.09, p=0.222), and Trail-making test part A (r=0.11, p=0.117). Neuropsychiatric and behavioural disturbances, evaluated with the FBI, significantly correlated with serum NfL levels (r=0.18, p=0.007). All tests were age- and education-corrected; FDR-adjusted p-values for multiple comparisons are reported for each test.

No significant correlations were observed between serum phospho-Tau$_{181}$ concentration and FTLD-CDR sum of boxes score or other neuropsychological, behavioural or functional measures.

Brain imaging. As reported in **Figure 3**, serum NfL concentration correlated with cortical thinning of the frontotemporal and parietal regions, mainly on the left side (p<0.05 whole-brain FDR-corrected, cluster threshold = 200). There was no statistically significant association between serum phospho-Tau$_{181}$ and cortical thickness in patients with FTLD .

TMS measures. TMS measures were performed to evaluate average SICI, ICF, LICI and SAI. In the FTLD group (n=89), serum NfL levels were significantly associated with SICI (r=0.464, p<0.001) and LICI (r=0.545, p<0.001), but not with ICF or SAI (see **Figure 4, panel A and B**). No associations were observed between serum phospho-Tau_{181} and TMS measures.

Interestingly, in the AD group (n=12), we observed a significant association between serum phospho-Tau_{181} and average SAI (r=0.720, p=0.048) (see **Figure 4, panel C**). We did not observe any significant associations between serum NfL and TMS measures.

Reported p-values are FDR-adjusted for multiple comparisons.

Serum NfL and serum phospho-Tau_{181} associations with prognosis in FTLD

Serum NfL concentration significantly predicted the survival rate in FTLD patients.

The univariate and stepwise multivariate Cox regression analysis showed a significant association between survival and serum NfL levels (HR 1.01 95%CI 1.00-1.02, p=0.005), but not with phospho-Tau_{181}, age, age at onset or mutation status (see **Figure 5, panel A**). Patients with high serum NfL levels (upper half of median values) had significantly shorter survival than those with low serum NfL levels (lower than median value) at the Kaplan-Meier survival curves (p=0.034) (see **Figure 5, panel B**).

Discussion

In this work, we confirmed and extended previous literature claiming a different usefulness of serum NfL and serum phospho-Tau_{181} measurements in clinical practice, depending on specific clinical questions. Serum NfL concentrations showed high accuracy in identifying FTLD from cognitively unimpaired elderly, as well as in assessing FTLD severity and prognosis, while serum phospho-Tau_{181} concentrations showed high accuracy in discriminating FTLD from AD. Importantly, in this study we also further demonstrate high accuracy of these biomarkers even in the earliest disease stages.

The non-invasiveness and reliability of serum NfL and phospho-Tau_{181} measurements make these markers extremely useful in clinical practice for the diagnosis of FTLD, even in the early disease stages, compared to CSF biomarkers or more expensive brain imaging modalities.

Serum NfL concentrations, as already demonstrated in other neurodegenerative disorders (Bridel *et al.*, 2019; Forgrave *et al.*, 2019; Zhao *et al.*, 2019), were associated with measures of disease severity, and are helpful in assessing disease stage. In fact, higher serum NfL levels were significantly associated with more pronounced cognitive impairment and behavioural disturbances. We also observed an association with cortical thickness at brain imaging analysis. In particular, NfL concentrations were inversely correlated with cortical thickness values mainly in frontal, temporal and parietal regions, supporting the view that NfL is a neurodegeneration marker strongly related to FTLD (Ljubenkov *et al.*, 2018). These findings were also consistent with previous studies in FTLD that reported a correlation between brain structure and NfL concentrations, with a predominant involvement of the left frontotemporal area (Scherling *et al.*, 2014; Rohrer *et al.*, 2016; Falgàs *et al.*, 2020). To further corroborate the role of serum NfL as a marker of disease severity, we evaluated the association between serum NfL concentrations and indirect measures of GABAergic neurotransmission, which have been demonstrated to be impaired in FTLD (Burrell *et al.*, 2011; Benussi *et al.*, 2016, 2018, 2019*a*, 2020*a*, *b*; Murley and Rowe, 2018). We observed that the higher the serum NfL levels, the greater was the impairment in SICI and LICI, which are considered to

reflect short-lasting postsynaptic inhibition mediated through the $GABA_A$ and $GABA_B$ receptors at the level of local interneurons, respectively (Rossini *et al.*, 2015; Ziemann *et al.*, 2015).

Altogether, these findings strongly support the notion that serum NfL concentrations may be useful to stage disease severity, in a disorder where there is urgent need to find not only diagnostic but also prognostic markers, in light of the near onset of new pharmacological clinical trials. Compared with AD, FTLD is clinically heterogeneous, with patients presenting a combination of behavioural disturbances, impairment of executive functions or language deficits. Available standardised neuropsychological and clinical assessments may not be ideal in detecting the effects of future treatments, particularly in the early disease stages and across different FTLD subtypes. A non-invasive and easy to perform peripheral biomarker may represent a practical and valuable choice to assess disease severity, to monitor outcomes and to categorize patients into disease subgroups.

Most importantly, this study has demonstrated that serum NfL concentrations are able to predict survival rates. Indeed, several studies have now shown the prognostic value of NfL in patients with FTLD; however, concentrations were evaluated in CSF, or in small group of patients or in patients with monogenic disease (Skillbäck *et al.*, 2014; Donker Kaat *et al.*, 2018; Meeter *et al.*, 2019; van der Ende *et al.*, 2019). These confirmatory results observed using serum NfL concentrations in a large cohort of FTLD subjects are key to clearly prove that patients with higher NfL levels show decreased survival. These findings further prove that NfL, a major component of neuronal cytoskeleton involved in axonal and dendritic growth, signaling and transport (Yuan *et al.*, 2015), reflect the ongoing neuronal loss also in FTLD (Meeter *et al.*, 2019).

Conversely, serum phospho-Tau_{181} levels, besides being very accurate in discriminating AD from FTLD, were not helpful in monitoring disease severity or predicting prognosis in FTLD. Indeed, according to previous data, serum phospho-Tau_{181} may detect mixed 3R/4R neuropathology, *i.e.*, AD, but not other tauopathies, such as 4R tauopathy (*i.e.*, Pick's disease) or 3R tauopathy (i.e., PSP or CBS) (Mielke *et al.*, 2018) + Karikari. For these reasons, serum phospho-Tau_{181} was not able to identify FTLD subtypes. The modest increase in phospho-Tau_{181} concentrations observed in CBS

patients could be secondary to a concomitant AD neuropathology, which has been frequently observed in these patients (Schneider *et al.*, 1997; Boeve *et al.*, 1999). Accordingly, in patients carrying a *MAPT P301L* mutation, phospho-Tau$_{181}$ concentrations were not significantly higher than in other FTLD subtypes (data not shown), as they have a pure 4R tau pathology. It is however noteworthy that patients carrying *GRN* mutations, and consequently with FTLD-TDP43 pathology, showed decreased serum phospho-Tau$_{181}$ compared with patients without a pathogenetic mutation. The related pathological mechanism needs to be further explored. Finally, SAI, a TMS measure of cholinergic dysfunction widely associated with AD (Di Lazzaro *et al.*, 2002, 2006), correlated harmoniously with serum phospho-Tau$_{181}$ levels, further confirming the reliability of peripheral phospho-Tau$_{181}$ in detecting AD, as previously reported (Mattsson *et al.*, 2016).

Major strengths of our study are the large series of FTLD patients and the comprehensive approach in correlating clinical, imaging and neurophysiological data with fluid biomarkers, carried out at the same study site to minimize variability. A weakness of the study is the lack of autopsy confirmation, which prevented correlations between biomarkers and FTLD-related proteinopathies. Secondly, longitudinal serum NfL measurements were not available, and we were not able to draw conclusions on possible changes throughout disease progression.

In conclusion, our results show the usefulness of both peripheral NfL and phospho-Tau$_{181}$ assessment, with different and specific purposes in clinical practice. Assessing both blood-based biomarkers may provide a comprehensive view of FTLD, aiding in the differential diagnosis, in staging disease severity and in defining survival probability.

Acknowledgements

We thank A. Alberici for patients' recruitment, and E. Bonomi and C. Brattini for excellent practical work. This study was funded by the Italian Ministry of Health (Ricerca Corrente). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), and the UK Dementia Research Institute at UCL. KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-

2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), and European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236).

Disclosures

HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper. KB has served as a consultant or at advisory boards for Abcam, Axon, Biogen, Lilly, MagQu, Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper.

References

Alberici A, Geroldi C, Cotelli M, Adorni A, Calabria M, Rossi G, et al. The Frontal Behavioural Inventory (Italian version) differentiates frontotemporal lobar degeneration variants from Alzheimer's disease. Neurol Sci 2007; 28: 80–86.

Armstrong MJ, Litvan I, Lang AE, Bak TH, Bhatia KP, Borroni B, et al. Criteria for the diagnosis of corticobasal degeneration. Neurology 2013; 80: 496–503.

Bang J, Spina S, Miller BL. Frontotemporal dementia. Lancet 2015; 386: 1672–1682.

Benussi A, Alberici A, Buratti E, Ghidoni R, Gardoni F, Di Luca M, et al. Toward a Glutamate Hypothesis of Frontotemporal Dementia. Front Neurosci 2019; 13: 304.

Benussi A, Cosseddu M, Filareto I, Dell'Era V, Archetti S, Sofia Cotelli M, et al. Impaired long-term potentiation-like cortical plasticity in presymptomatic genetic frontotemporal dementia. Ann Neurol 2016; 80: 472–6.

Benussi A, Dell'Era V, Cantoni V, Cotelli MS, Cosseddu M, Spallazzi M, et al. TMS for staging and predicting functional decline in frontotemporal dementia. Brain Stimul 2020; 13: 386–392.

Benussi A, Dell'Era V, Cantoni V, Cotelli MS, Cosseddu M, Spallazzi M, et al. Neurophysiological Correlates of Positive and Negative Symptoms in Frontotemporal Dementia. J Alzheimers Dis 2020; 73: 1133–1142.

Benussi A, Dell'Era V, Cantoni V, Ferrari C, Caratozzolo S, Rozzini L, et al. Discrimination of atypical parkinsonisms with transcranial magnetic stimulation. Brain Stimul 2018; 11: 366–373.

Benussi A, Gazzina S, Premi E, Cosseddu M, Archetti S, Dell'Era V, et al. Clinical and biomarker changes in presymptomatic genetic frontotemporal dementia. Neurobiol Aging 2019; 76: 133–140.

Benussi A, Grassi M, Palluzzi F, Koch G, Di Lazzaro V, Nardone R, et al. Classification Accuracy of Transcranial Magnetic Stimulation for the Diagnosis of Neurodegenerative Dementias. Ann Neurol 2020; 87: 394–404.

Benussi A, Di Lorenzo F, Dell'Era V, Cosseddu M, Alberici A, Caratozzolo S, et al. Transcranial magnetic stimulation distinguishes Alzheimer disease from frontotemporal dementia. Neurology 2017; 89: 665–672.

Le Ber I, Guedj E, Gabelle A, Verpillat P, Volteau M, Thomas-Anterion C, et al. Demographic, neurological and behavioural characteristics and brain perfusion SPECT in frontal variant of frontotemporal dementia. Brain 2006; 129: 3051–65.

Binetti G, Nicosia F, Benussi L, Ghidoni R, Feudatari E, Barbiero L, et al. Prevalence of TAU mutations in an Italian clinical series of familial frontotemporal patients. Neurosci Lett 2003; 338: 85–87.

Boeve BF, Maraganore DM, Parisi JE, Ahlskog JE, Graff-Radford N, Caselli RJ, et al. Pathologic heterogeneity in clinically diagnosed corticobasal degeneration. Neurology 1999; 53: 795–800.

Borroni B, Benussi A, Cosseddu M, Archetti S, Padovani A. Cerebrospinal fluid tau levels predict prognosis in non-inherited frontotemporal dementia. Neurodegener Dis 2014; 13: 224–9.

Borroni B, Cosseddu M, Pilotto A, Premi E, Archetti S, Gasparotti R, et al. Early stage of behavioral variant frontotemporal dementia: clinical and neuroimaging correlates. Neurobiol Aging 2015; 36: 3108–3115.

Borroni B, Padovani A. Dementia: A new algorithm for molecular diagnostics in FTLD. Nat Rev Neurol 2013; 9: 241–242.

Bridel C, Van Wieringen WN, Zetterberg H, Tijms BM, Teunissen CE, Alvarez-Cermeño JC, et al.

Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. JAMA Neurol 2019; 76: 1035–1048.

Burrell JR, Kiernan MC, Vucic S, Hodges JR. Motor Neuron dysfunction in frontotemporal dementia. Brain 2011; 134: 2582–2594.

Caffarra P, Vezzadini G, Dieci F, Zonato F, Venneri A. Rey-Osterrieth complex figure: normative values in an Italian population sample. Neurol Sci 2002; 22: 443–447.

Cosseddu M, Benussi A, Gazzina S, Alberici A, Dell'Era V, Manes M, et al. Progression of behavioural disturbances in frontotemporal dementia: a longitudinal observational study. Eur J Neurol 2020; 27: 265–272.

Donker Kaat L, Meeter LH, Chiu WZ, Melhem S, Boon AJW, Blennow K, et al. Serum neurofilament light chain in progressive supranuclear palsy. Park Relat Disord 2018; 56: 98–101.

van der Ende EL, Meeter LH, Poos JM, Panman JL, Jiskoot LC, Dopper EGP, et al. Serum neurofilament light chain in genetic frontotemporal dementia: a longitudinal, multicentre cohort study. Lancet Neurol 2019; 18: 1103–1111.

Falgàs N, Ruiz-Peris M, Pérez-Millan A, Sala-Llonch R, Antonell A, Balasa M, et al. Contribution of CSF biomarkers to early-onset Alzheimer's disease and frontotemporal dementia neuroimaging signatures. Hum Brain Mapp 2020: 1–10.

Foiani MS, Woollacott IOC, Heller C, Bocchetta M, Heslegrave A, Dick KM, et al. Plasma tau is increased in frontotemporal dementia. J Neurol Neurosurg Psychiatry 2018; 89: 804–807.

Forgrave LM, Ma M, Best JR, DeMarco ML. The diagnostic performance of neurofilament light chain in CSF and blood for Alzheimer's disease, frontotemporal dementia, and amyotrophic lateral sclerosis: A systematic review and meta-analysis. Alzheimer's Dement Diagnosis, Assess Dis Monit 2019; 11: 730–743.

Giovagnoli AR, Del Pesce M, Mascheroni S, Simoncelli M, Laiacona M, Capitani E. Trail making test: normative values from 287 normal adult controls. Ital J Neurol Sci 1996; 17: 305–309.

Gisslén M, Price RW, Andreasson U, Norgren N, Nilsson S, Hagberg L, et al. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. EBioMedicine 2016; 3: 135–140.

Gorno-Tempini ML, Hillis AE, Weintraub S, Kertesz A, Mendez M, Cappa SF, et al. Classification of primary progressive aphasia and its variants. Neurology 2011; 76: 1006–1014.

Heller C, Foiani MS, Moore K, Convery R, Bocchetta M, Neason M, et al. Plasma glial fibrillary acidic protein is raised in progranulin-associated frontotemporal dementia. J Neurol Neurosurg Psychiatry 2020: 263–270.

Höglinger GU, Respondek G, Stamelou M, Kurz C, Josephs KA, Lang AE, et al. Clinical diagnosis of progressive supranuclear palsy: The movement disorder society criteria. Mov Disord 2017; 32: 853–864.

Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018; 14: 535–562.

Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. Nat Med 2020: 1–8.

Katisko K, Cajanus A, Jääskeläinen O, Kontkanen A, Hartikainen P, Korhonen VE, et al. Serum neurofilament light chain is a discriminative biomarker between frontotemporal lobar degeneration

and primary psychiatric disorders. J Neurol 2020; 267: 162–167.

Katz S, Ford AB, Moskowitz RW, Jackson BA, Jaffe MW. Studies of Illness in the Aged The Index of ADL: A Standardized Measure of Biological and Psychosocial Function. JAMA 1963; 185: 914–919.

Knopman DS, Kramer JH, Boeve BF, Caselli RJ, Graff-Radford NR, Mendez MF, et al. Development of methodology for conducting clinical trials in frontotemporal lobar degeneration. Brain 2008; 131: 2957–2968.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. J Physiol 1993; 471: 501–519.

Lawton MP, Brody EM. Assessment of older people: Self-maintaining and instrumental activities of daily living. Gerontologist 1969; 9: 179–186.

Di Lazzaro V, Oliviero A, Tonali PA, Marra C, Daniele A, Profice P, et al. Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. Neurology 2002; 59: 392–397.

Di Lazzaro V, Pilato F, Dileone M, Saturno E, Oliviero A, Marra C, et al. In vivo cholinergic circuit evaluation in frontotemporal and Alzheimer dementias. Neurology 2006; 66: 1111–1113.

Ljubenkov PA, Staffaroni AM, Rojas JC, Allen IE, Wang P, Heuer H, et al. Cerebrospinal fluid biomarkers predict frontotemporal dementia trajectory. Ann Clin Transl Neurol 2018; 5: 1250–1263.

Logroscino G, Piccininni M, Binetti G, Zecca C, Turrone R, Capozzo R, et al. Incidence of frontotemporal lobar degeneration in Italy: The Salento-Brescia Registry study. Neurology 2019; 92: e2355–e2363.

Magni E, Binetti G, Bianchetti A, Rozzini R, Trabucchi M. Mini-Mental State Examination: a normative study in Italian elderly population. Eur J Neurol 1996; 3: 198–202.

Makaretz SJ, Quimby M, Collins J, Makris N, McGinnis S, Schultz A, et al. Flortaucipir tau PET imaging in semantic variant primary progressive aphasia. J Neurol Neurosurg Psychiatry 2017; 89: 1024–1031.

Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stomrud E, et al. Plasma tau in Alzheimer disease. Neurology 2016; 87: 1827–1835.

Meeter LH, Dopper EG, Jiskoot LC, Sanchez-Valle R, Graff C, Benussi L, et al. Neurofilament light chain: a biomarker for genetic frontotemporal dementia. Ann Clin Transl Neurol 2016; 3: 623–636.

Meeter LHH, Steketee RME, Salkovic D, Vos ME, Grossman M, McMillan CT, et al. Clinical value of cerebrospinal fluid neurofilament light chain in semantic dementia. J Neurol Neurosurg Psychiatry 2019; 90: 997–1004.

Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. Alzheimer's Dement 2018; 14: 989–997.

Van Mossevelde S, Engelborghs S, van der Zee J, Van Broeckhoven C. Genotype–phenotype links in frontotemporal lobar degeneration. Nat Rev Neurol 2018; 14: 363–378.

Murley AG, Rowe JB. Neurotransmitter deficits from fronto temporal lobar degeneration. Brain 2018; 141: 1263–1285.

Novelli G, Papagno C, Capitani E, Laiacona M. Tre test clinici di memoria verbale a lungo termine : Taratura su soggetti normali . / Three clinical tests for the assessment ... Arch Psicol

Neurol Psichiatr 1970; 47: 278–296.

Novelli G, Papagno C, Capitani E, Laiacona M, Vallar G, Cappa SF. Tre test clinici di ricerca e produzione lessicale. Taratura su sogetti normali. Arch Psicol Neurol Psichiatr 1986; 47: 477–506.

Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 2016; 15: 673–684.

Passamonti L, Vázquez Rodríguez P, Hong YT, Allinson KSJ, Williamson D, Borchert RJ, et al. 18F-AV-1451 positron emission tomography in Alzheimer's disease and progressive supranuclear palsy. Brain 2017; 140: 781–791.

Pijnenburg YAL, Verwey NA, van der Flier WM, Scheltens P, Teunissen CE. Discriminative and prognostic potential of cerebrospinal fluid phosphoTau/tau ratio and neurofilaments for frontotemporal dementia subtypes. Alzheimer's Dement Diagnosis, Assess Dis Monit 2015; 1: 505–512.

Pike N. Using false discovery rates for multiple comparisons in ecology and evolution. Methods Ecol Evol 2011; 2: 278–282.

Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 2011; 134: 2456–77.

De Renzi E, Vignolo LA. The token test: A sensitive test to detect receptive disturbances in aphasics. Brain 1962; 85: 665–678.

Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol 2010; 28: 595–599.

Rohrer JD, Woollacott IOC, Dick KM, Brotherhood E, Gordon E, Fellows A, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. Neurology 2016; 87: 1329–1336.

Rosen HJ, Gorno-Tempini ML, Goldman WP, Perry RJ, Schuff N, Weiner M, et al. Patterns of brain atrophy in frontotemporal dementia and semantic dementia. Neurology 2002; 58: 198–208.

Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol 2015; 126: 1071–1107.

Scherling CS, Hall T, Berisha F, Klepac K, Karydas A, Coppola G, et al. Cerebrospinal fluid neurofilament concentration reflects disease severity in frontotemporal degeneration. Ann Neurol 2014; 75: 116–126.

Schneider JA, Watts RL, Gearing M, Brewer RP, Mirra SS. Corticobasal degeneration: neuropathologic and clinical heterogeneity. Neurology 1997; 48: 959–69.

Skillbäck T, Farahmand B, Bartlett JW, Rosén C, Mattsson N, Nägga K, et al. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology 2014; 83: 1945–53.

Steinacker P, Anderl-Straub S, Diehl-Schmid J, Semler E, Uttner I, von Arnim CAF, et al. Serum neurofilament light chain in behavioral variant frontotemporal dementia. Neurology 2018; 91: e1390–e1401.

Sunderland T, Hill JL, Mellow AM, Lawlor BA, Gundersheimer J, Newhouse PA, et al. Clock

drawing in Alzheimer's disease. A novel measure of dementia severity. J Am Geriatr Soc 1989; 37: 725–729.

Thijssen EH, La Joie R, Wolf A, Strom A, Wang P, Iaccarino L, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. [Internet]. Nat Med 2020Available from: http://www.ncbi.nlm.nih.gov/pubmed/32123386

Tokimura H, Di Lazzaro V, Tokimura Y, Oliviero A, Profice P, Insola A, et al. Short latency inhibition of human hand motor cortex by somatosensory input from the hand. J Physiol 2000; 523 Pt 2: 503–513.

Tsai RM, Bejanin A, Lesman-Segev O, LaJoie R, Visani A, Bourakova V, et al. 18F-flortaucipir (AV-1451) tau PET in frontotemporal dementia syndromes. Alzheimers Res Ther 2019; 11: 13.

Valls-Solé J, Pascual-Leone A, Wassermann EM, Hallett M. Human motor evoked responses to paired transcranial magnetic stimuli. Electroencephalogr Clin Neurophysiol 1992; 85: 355–364.

Wilke C, Preische O, Deuschle C, Roeben B, Apel A, Barro C, et al. Neurofilament light chain in FTD is elevated not only in cerebrospinal fluid, but also in serum. J Neurol Neurosurg Psychiatry 2016; 87: 1270–1272.

Yuan A, Sershen H, Veeranna, Basavarajappa BS, Kumar A, Hashim A, et al. Neurofilament subunits are integral components of synapses and modulate neurotransmission and behavior in vivo. Mol Psychiatry 2015; 20: 986–94.

Zhao Y, Xin Y, Meng S, He Z, Hu W. Neurofilament light chain protein in neurodegenerative dementia: A systematic review and network meta-analysis. Neurosci Biobehav Rev 2019; 102: 123–138.

Ziemann U, Reis J, Schwenkreis P, Rosanova M, Strafella A, Badawy R, et al. TMS and drugs revisited 2014. Clin Neurophysiol 2015; 126: 1847–68.

Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol 1996; 496: 873–881.

Table 1. Demographic and clinical characteristics of FTLD patients and controls

	FTLD					Controls	
Variable	**bvFTD**	**avPPA**	**svPPA**	**CBS**	**PSP**	**AD**	**HC**
Number	134	48	27	51	31	63	63
Age, years	64.5± 8.0	67.7± 8.8	64.0± 8.2	65.8± 7.6	72.9± 7.4	75.5± 8.1	65.4± 12.1
Sex, female %	58.2	43.8	59.3	52.9	51.6	31.7	20.6
Age at onset, years	61.5±7.8	64.9±8.6	60.5±8.0	63.2±7.5	68.8±7.3	74.0 ±8.3	-
Monogenic disease, %	14.9	25.0	0.0	2.3	0.0	0.0	-
Serum NfL (pg/mL)							
mean±SE	43±2.4	54.6±3.9	33.3±5.2	36.5±3.8	30.4±4.9	32.7±3.6	14.2±3.5
lower-upper bound	38.3-47.8	46.9-62.3	23.0-43.6	29.1-44.0	20.7-40.1	25.6-39.9	7.4-21.1
Serum phospho-Tau$_{181}$ (pg/mL)							
mean±SE	2.5±0.7	3.3±1.1	3.8±1.5	7.1±1.1	3.9±1.4	16.4±1.1	5.4±1.0
lower-upper bound	1.1-3.8	1.1-5.5	0.8-6.8	4.9-9.3	1.0-6.7	14.3-18.5	3.5-7.5

FTLD = Frontotemporal Lobar degeneration; bvFTD = behavioural variant frontotemporal dementia; avPPA = agrammatic variant of primary progressive aphasia; svPPA = semantic variant of primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy controls; NfL = Neurofilament Light Chain; SE = standard error. Results are expressed as mean ± standard deviations, unless otherwise specified. Monogenic disease: all *GRN* mutations, but 3 *MAPT* mutations (2 bvFTD and 1 CBS).

Legend to Figures

Figure 1. Serum biomarkers concentrations in participants by clinical diagnosis.

(A) Serum NfL and (B) serum phospho-Tau_{181} concentrations in participants by clinical diagnosis. bvFTD = behavioural variant frontotemporal dementia; avPPA = agrammatic variant of primary progressive aphasia; svPPA = semantic variant of primary progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy controls. Bar graphs represent mean values and error bars represent 95% confidence intervals. *p<0.050; **p<0.010; ***p<0.001 after Bonferroni corrected *post hoc* tests.

Figure 2. ROC curves for serum biomarkers in differentiating FTLD from HC and AD.

ROC curves for serum NfL in differentiating (A) FTLD and (B) mild FTLD patients from HC. Serum phospho-Tau_{181} in differentiating (C) FTLD from AD and (D) mild FTLD from mild AD patients. ROC = receiver operating characteristic; AUC = area under the curve; FTLD = frontotemporal lobar degeneration; mild FTLD = FTLD patients with FTD-CDR ≤ 5 and AD; FTD-CDR = frontotemporal dementia clinal dementia rating scale; HC = healthy controls; AD = Alzheimer's disease; mild AD = AD patients with MMSE>=19/30; MMSE = Mini-Mental State Examination.

Figure 3. Significant association between serum NfL and whole-brain cortical thickness.

The significant clusters (inverse relationship) from the multiple regression model where serum NfL values were considered as independent variable (age, gender, clinical phenotype and MRI scanner type included as confounding factors). The statistical threshold was set at p<0.05 and corrected for multiple comparisons using false discovery rate (FDR) at whole-brain level. The significant clusters were superimposed on a 3-dimensions T1 standardized template.

Figure 4. Significant associations between serum biomarkers and neurophysiological measures.

Association between serum NfL and (A) average SICI (ISI 1, 2, 3 ms ISI), (B) average LICI (ISI 50, 100, 150 ms ISI) and (C) average SAI (0, +4 ms ISI).

SICI = short-interval intracortical inhibition; LICI = long-interval intracortical inhibition; SAI = short latency afferent inhibition; ISI = interstimulus interval.

Figure 5. Survival curves

(A) Survival probability curves and (B) Kaplan-Meier survival curves in FTLD patients for serum NfL subgroups (upper half vs lower half of median values).