Cerebrospinal fluid neurofilament light concentration in motor neuron disease and frontotemporal dementia predicts survival

Skillbäck T; Mattsson N; Blennow K; Zetterberg H

[1]Institute of Neuroscience and Physiology, Department of Neurochemistry, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

[2]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

[3]Clinical Memory Research Unit, Department of Clinical Sciences Malmö, Lund University, Simrisbanvägen 14, Malmö 212 24, Sweden.

[4]Memory Clinic, Skåne University Hospital, Simrisbanvägen 14, Malmö 212 24, Sweden.

[5]Department of Neurology, Skåne University Hospital, Getingevägen 4, Lund 222 41, Sweden.

[6]UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK

Corresponding author: Tobias Skillbäck, Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital/Mölndal, S-431 80 Mölndal, Sweden. Telephone: 0707-909478. E-mail: tobias.skillback@neuro.gu.se

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Abstract

Background: Cerebrospinal fluid (CSF) neurofilament light protein (NFL) concentrations are increased in the related diseases frontotemporal dementia (FTD) and motor neuron disease (MND). Here, we compare CSF NFL concentrations in FTD and MND and examine if the biomarker predicts survival.

Methods: We cross-referenced the Swedish mortality registry with the laboratory database at the Sahlgrenska University Hospital to produce a dataset of CSF NFL concentrations and mortality information from 715 MND patients, 87 FTD patients, 3 patients with combined FTD and MND, and 107 healthy controls.

Results: MND patients had significantly higher CSF NFL concentrations than FTD patients. Both groups had significantly higher concentrations than the healthy controls. Higher concentrations of CSF NFL were associated with shorter survival in both MND and FTD.

Conclusion: The results of this study strengthen the notion of CSF NFL as a useful tool for determining disease intensity in MND and FTD patients. Further studies in patient cohorts with clinically subtyped and genetically classified MND and FTD diagnoses are needed.

Introduction

Motor neuron diseases (MND) are a group of related neurodegenerative disorders (primarily amyotrophic lateral sclerosis, progressive muscular atrophy and progressive bulbar), of which amyotrophic lateral sclerosis (ALS) is the most common, accounting for roughly 80% of the MND cases. ALS is a progressive and fatal neurodegenerative disease, primarily affecting upper and lower motor neurons, that has been shown to bear histopathologic and genetic resemblance to frontotemporal dementia (FTD), another neurodegenerative and fatal disease, characterized by frontotemporal brain atrophy with mainly cognitive and behavioral symptoms (Andersen, 2013, DeJesus-Hernandez et al., 2011, Gijselinck et al., 2012). There are at present no disease-modifying treatments for FTD, and only one, riluzole, for ALS, reported to have a modest life prolonging effect (Miller et al., 2003). To aid diagnostics, patient stratification and studies seeking to find further treatments for these diseases, there is a need to establish a way to reliably and objectively assess disease severity and the amount of ongoing neurodegeneration. Previous studies have suggested that cerebrospinal fluid (CSF) neurofilament light (NFL) may serve this purpose (Tortelli et al., 2012, Menke et al., 2015, Steinacker et al., 2016, Lu et al., 2015, Weydt et al., 2016). NFL is part of a family of proteins with all members acting as important parts of the scaffolding of the neuronal and axonal cytoskeleton. NFL is primarily expressed in myelinated large-caliber axons, and leak into CSF in conditions characterized by degeneration in, or injury to, areas of the brain dominated by white matter, such as multiple sclerosis (MS) (Madeddu et al., 2013), stroke, head trauma (Zetterberg et al., 2006), vascular dementia (Skillback et al., 2014), Alzheimer's disease (Zetterberg et al., 2016), idiopathic normal pressure hydrocephalus (Jeppsson et al., 2013), multiple system atrophy (Petzold et al., 2009), and various CNS infections (Hagberg et al., 2000, Mattsson et al., 2010, Grahn et al., 2013).

Here we analyzed results from measurements of NFL in CSF from 715 MND patients, 87 FTD patients and 3 patients with combined FTD and MND (FTD/MND) taken from a large database of clinical routine samples in combination with information on dates and causes of death from the Swedish mortality registry. We hypothesized that MND and FTD patients would display increased NFL concentrations in CSF, and that higher concentrations would correlate with shorter survival. We also hypothesized that the correlations of CSF NFL concentration and clinical progression rate to death would be similar in MND and FTD.

Methods

Three sources of information were combined to prepare the dataset used in this study. The first was a complete set of archived data on all clinical routine CSF NFL measurements made at the Mölndal site of the Sahlgrenska University Hospital, Sweden from January 1, 2002 to June 1, 2012 extracted from the lab software database. The laboratory at Mölndal handles CSF biomarker measurements for all of Sweden, and carries out about 15,000 – 20,000 such analyses yearly. The inclusion criteria were that patients were above 30 years of age at sampling, and that a dementia CSF screen including NFL had been requested by the clinician.

The second source of information was the Swedish mortality registry, a national registry maintained by the Swedish National Board of Health and Welfare, a Swedish government agency. The Swedish mortality registry keeps complete records on all deaths in Sweden, including causes of death as established by the medical doctor issuing the death certificate. This database was queried for underlying cause and date of death for the patients in our dataset. Patients that were coded as having a diagnosis from the G12.2 ICD10 category listed as a cause of death was extracted and labeled as MND patients in our data. For FTD the G31.0 ICD10 code was used in the same manner.

Finally, an independent dataset of healthy controls was included, consisting of 107 CSF samples from clinically ascertained neurologically healthy individuals (median age 70 years, range 41–99), as previously described (reference).

Information from the data sources was cross referenced using the Swedish personal identity number. For 14 FTD patients, 69 MND patients and 1 FTD/MND patient multiple CSF analyses spread out in time was registered. The repeated measurements were stripped out and put in a separate dataset for longitudinal analysis, and only the first measurement made was left in the main dataset.

Biochemical measurements

During the study period three methods were used for measurement of NFL concentration in CSF. The first two were based on an in house ELISA that had an original limit of detection of 250 ng/L which was improved to 125 ng/L in the second method version (Rosengren et al., 1996, Zetterberg et al., 2006). The third method was developed in collaboration with Uman Diagnostics (NF-light ELISA kit, UmanDiagnostics AB, Umeå, Sweden) and resulted in an even lower detection limit of 50 ng/L. Measurements from the methods are highly correlated but the third version gave a change in the absolute concentration of NFL reported. To correct for this systematic change, mean concentrations of all NFL measurements before and after the method change was calculated and the quota between them was used to regulate the measurements made after the change. Cases where a concentration below 250 ng/L was detected, were set to 250 ng/L to equalize results from different assays with different detection limits. Over time, the three NFL assays have had a coefficient of variation of 15.5%. For further details on these calculations see appendix A.

All CSF analyses were performed in clinical routine by board-certified laboratory technicians using procedures accredited by the Swedish Board for Accreditation and Conformity Assessment (SWEDAC).

Statistics

Age and NFL concentration distribution in subject groups were tested with ANOVA, and sex distribution by Chi^2 statistics. Differences in NFL concentrations across diagnosis groups and sex were tested by Kruskal-Wallis and Mann-Whitney U tests. Age corrected linear regression was used to analyze the relations between CSF NFL concentrations and time of survival in the patient groups. Log transformed CSF NFL concentrations were used for regression to correct for a significantly skewed data distribution. The Kaplan-Meier method was used to determine differentiation in survival between NFL quartiles within the patient groups. Kruskal-Wallis tests were used to analyze differences in survival between quartile groups, and Mann-Whitney U to test differences in groups with different longitudinal development.

Ethics

This study was approved by the regional ethical committee at the University of Gothenburg.

Results

Dataset description

Detailed demographics of the study population can be found in table 1. There were no significant differences in age distribution between the subject groups. There were significant differences in the sex distribution ($p = .042$) between subject groups, however there were no significant differences in NFL levels between males and females in either diagnosis group.

NFL in the diagnosis groups

CSF NFL concentrations across subject groups can be found in figure 1. CSF NFL concentrations in the healthy controls group were significantly lower than in the FTD ($p < .001$), MND ($p < .001$) and the group of patients with combined FTD/MND ($p = .008$). The MND group had higher concentrations than the FTD group ($p < .001$). There were no significant differences between patients with combined FTD/MND and patients with isolated FTD or MND.

NFL and survival

Raw CSF NFL concentrations and days of survival in FTD and MND are plotted in figure 2. Higher concentrations of CSF NFL were found at later stages of disease in both the MND and FTD groups. In an age corrected analysis higher CSF NFL concentrations was associated with shorter survival in both the FTD ($R^2 = .175$, $\beta = -.392$, $p < .001$) and the MND ($R^2 = .297$, $\beta = -.489$, $p < .001$) groups. Age contributed significantly to the model in the MND group ($\beta = -.243$, $p < .001$), but not in the FTD group. The low subject count in the FTD/MND group precluded analysis here.

The FTD and MND patient groups were stratified into quartiles according to CSF NFL concentration. In both MND and FTD higher CSF NFL concentrations were associated with poorer survival (figure 3A-B). In MND, the survival distributions were shown to differ significantly ($p < .001$) between all quartiles except between the third and fourth one ($p = 1$). However, in FTD only the first and fourth quartile differed significantly ($p = .009$).

Longitudinal data

To further explore how CSF NFL concentrations relate to disease progression, the subset of patients with more than one recorded CSF NFL result was analyzed. In our dataset 14 FTD patients, 69 MND patients and 1 FTD/MND patient fulfilled this requirement. In 80% of the FTD and 67% of the MND NFL measurements a higher concentration of CSF NFL was detected at sampling at a later stage in the disease (figure 4A-B). The MND measurements that did not conform to the pattern of rising concentrations in a later stage of the disease differed from the others in that they measured higher CSF NFL levels at the index case (Mdn = 1570 ng/L vs Mdn = 760 ng/L, $p = .001$), but they did not differ significantly in age of the patient (Mdn = 63 vs Mdn = 62, $p = .57$) or survival (Mdn = 818 days vs Mdn = 1048 days, $p = .20$). The FTD measurements that did not conform did not differ significantly in neither measured NFL concentration (Mdn = 1570 ng/L vs Mdn = 920 ng/L, $p = .79$), age of patient (Mdn = 53 vs Mdn = 64, $p = .09$) or survival from sample date (Mdn = 1592 days vs Mdn = 2395 days, $p = .25$); However, this might stem from the very low sample count in this group (n=2).

One measurement in the FTD group and one in the MND groups did not show any change in NFL concentration from the previous measurement. This was in both cases due to the NFL concentrations being below the level of detection.

The FTD/MND patient with longitudinal data registered showed rising concentrations of NFL as the disease progressed (figure 4C).

Discussion

In this study, we explored the implication of CSF NFL concentrations in MND and FTD in a large cohort of patients drawn from the Swedish Mortality registry cross-referenced with a data set of CSF biomarker measurements from clinical routine testing in Sweden. We hypothesized that MND and FTD patients would exhibit high concentrations of CSF NFL, and that higher concentrations of CSF NFL would indicate shorter survival times. We found evidence for both these assertions in our data as MND and FTD patients had significantly higher CSF NFL concentrations than healthy controls, and higher concentrations of CSF NFL correlated with poor survival in both disease groups.

As the connection between high concentrations of NFL and poor survival in both FTD and MND seemed clear in our data, it was surprising to find that in the set of MND patients with repeated measurements only 67% of the measured CSF NFL were at a higher level at later stages of disease. One explanation might be that an accelerating level of neurodegeneration in far advanced disease, reflected by higher CSF NFL, might plateau and fade out as neurons die off and eventually deplete. This was supported by the fact that the MND patients with a decreasing NFL concentration trend had significantly higher NFL concentrations at the index case, which could corroborate the theory of an accelerating disease up until neuron cell depletion. Further, the MND and the FTD patients with decreasing NFL concentrations had shorter survival; however, these results were not statistically significant.

Previous studies have shown MND and FTD to bear several similarities, which was somewhat corroborated here as both groups had higher CSF NFL concentrations than the healthy controls and had a clear correlation between survival and CSF NFL concentrations. The MND group had significantly higher CSF NFL concentrations that the FTD group. This is in keeping with previous studies, although to our knowledge this has only been examined in small cohorts and cohorts with specific genetic predispositions (Pijnenburg et al., 2015).

Approximately 10-15% of patients with FTD also develop MND symptoms, and 30% of the patients that debut with MND show signs of frontal lobe decline at later stages of the disease. However, in our data less than 1% were reported to have both diagnoses as contributing causes of death. This points to a large under-reporting that poses a limitation to this study, and might be due to lack of reassessment and/or registration of additional diagnoses once a patient has received one diagnosis of these. The combined FTD/MND patients in this study had high concentrations of CSF NFL most closely reminiscent of the patients with isolated MND.

The main strength of this study was the large study population and the inclusion of mortality information on every subject. The main limitation of this study was that detailed information on the clinical and pathological subtypes of the diseases was lacking in our data, and that we did not have any data on the genetic classification of the patients. This is unfortunate as MND mimics often display relatively low concentrations of CSF NFL (Steinacker et al., 2016). Also in FTD there are subtypes, such as progressive non-fluent aphasia, that do not have as markedly elevated CSF concentrations (Landqvist Waldö et al., 2013), as the common FTD variants. However, the main hypotheses of this study were clearly confirmed even though the influence of these subgroups might have hampered it. This might be due to the low incidence of the FTD subtypes and MND mimics which probably rendered them a minority in the study population, thus lessening their impact on the results. Another limitation of the study was the

use of three different NFL assays, which may introduce variability of the measurements, although the assays were highly correlated, supporting the normalization approach used here.

Conclusions

The results of this study strengthen the notion of CSF NFL as a biomarker for determining disease intensity and survival in MND and FTD patients. Further studies in patient cohorts with clinically subtyped and genetically classified MND and FTD diagnoses are needed.

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Figure 1. CSF NFL concentrations per subject group

The MND patients have the higher concentrations than the FTD patients. The FTD/MND subjects have high concentrations but a low subject count. The number of cases stacked up at 250 ng/L are due to limitations in the biomarker assay, where concentrations below that number could not be reliably detected. Note that the Y-axis is logarithmic.

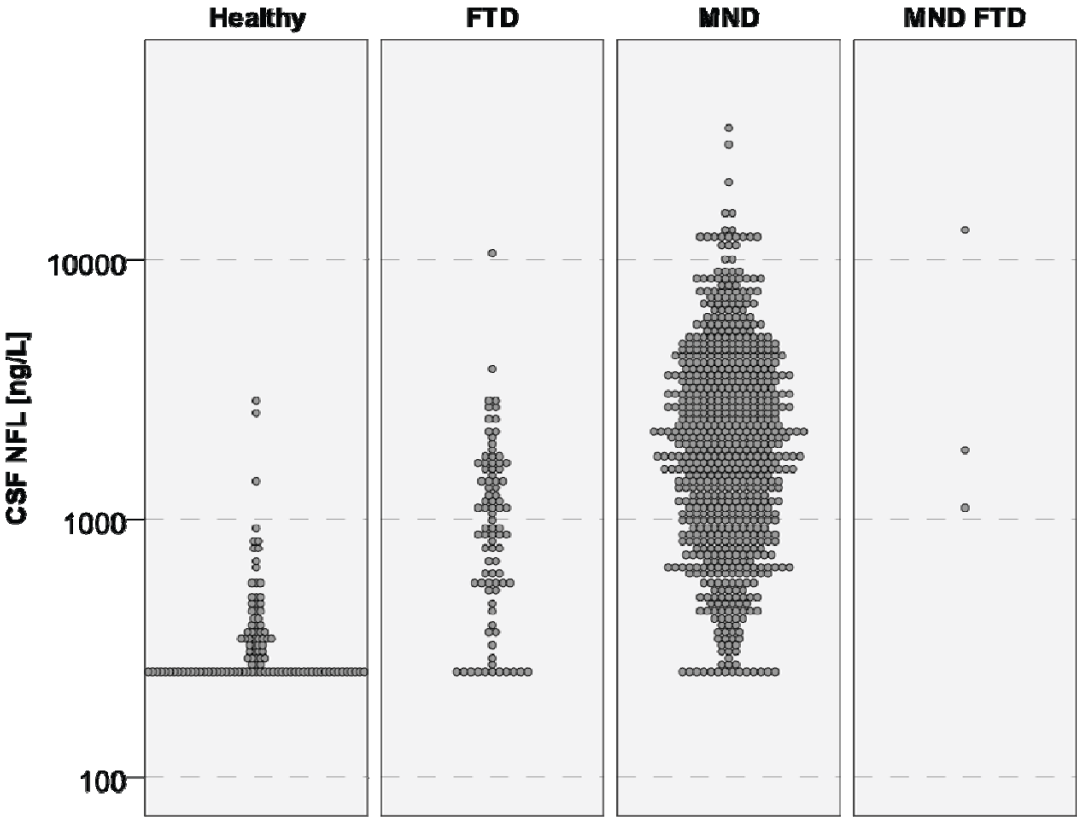


Figure 2. CSF NFL concentrations and survival in MND and FTD

The local regression slopes indicate increasing concentrations of CSF NFL at later stages of disease for both MND (red) and FTD (yellow). The patients' demise is at zero days of survival, i.e. at the far right of the x-axis. Note that the Y-axis is logarithmic.

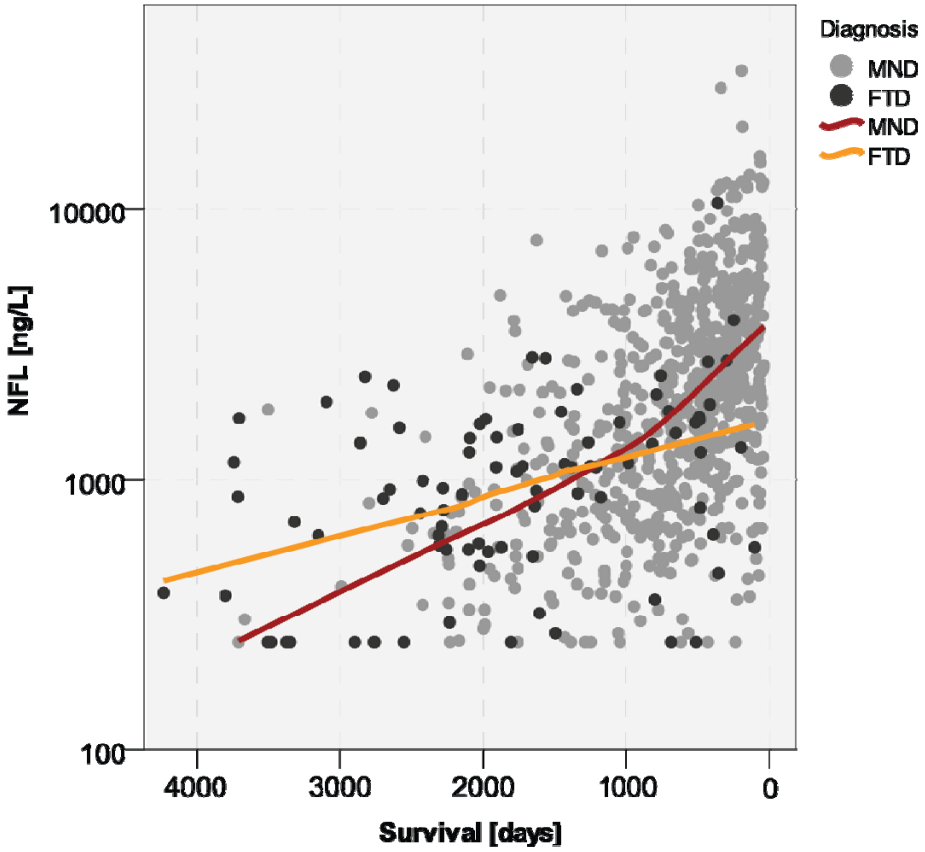
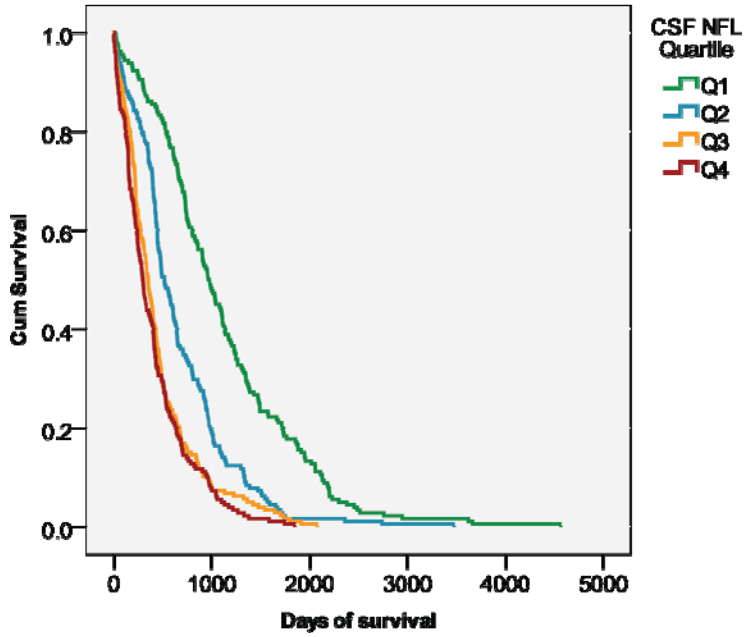


Figure 3. Survival functions

The MND and FTD groups were individually divided into quartile groups according to CSF NFL concentrations. Q1 had the lowest concentrations, Q2 the second lowest and so on.

A. MND. *The quartile groups with the higher CSF NFL concentrations have poorer survival, although Q3 and Q4 are undistinguishable.*



B. FTD. *The quartile groups with the higher CSF NFL concentrations have poorer survival.*

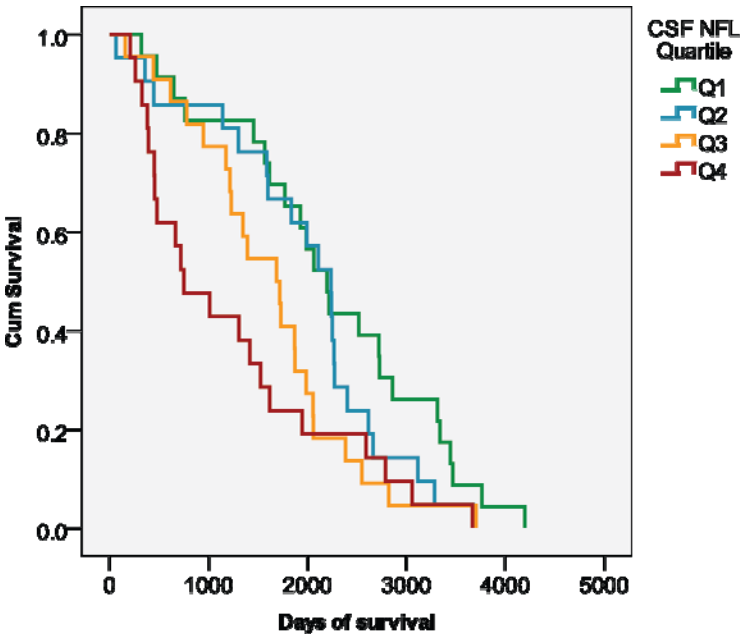
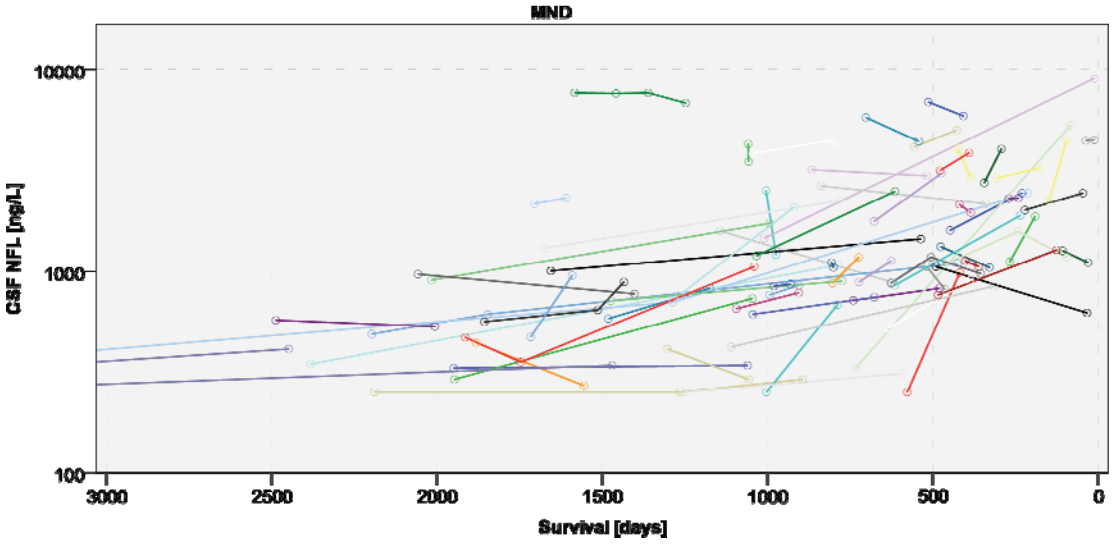


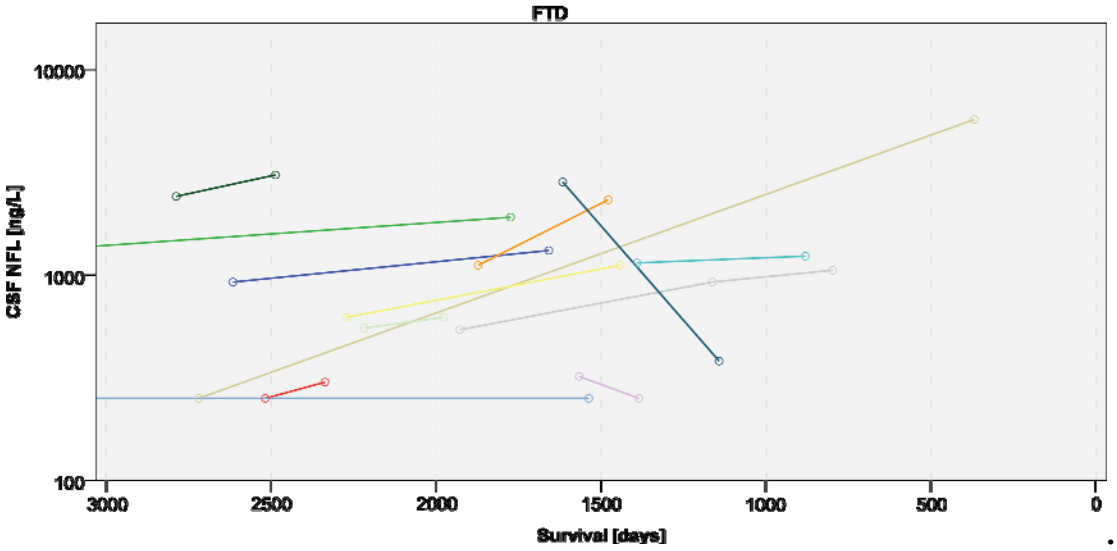
Figure 4. Longitudinal data

Each line represents one patient, and each ring a CSF NFL measurement. The patients' demise is at zero days of survival, i.e. at the far right of the x-axis. Note that the Y-axis is logarithmic.

A. *The MND patients with repeated measures of CSF NFL. Most cases show rising concentrations of CSF NFL at later stages of disease.*



B. *The FTD patients with repeated measures of CSF NFL. Most patients have a higher concentration of CSF NFL at sampling at a later stage of disease.*



C. *The FTD/MND patient with repeated measures presented rising CSF NFL concentrations as the disease progressed.*

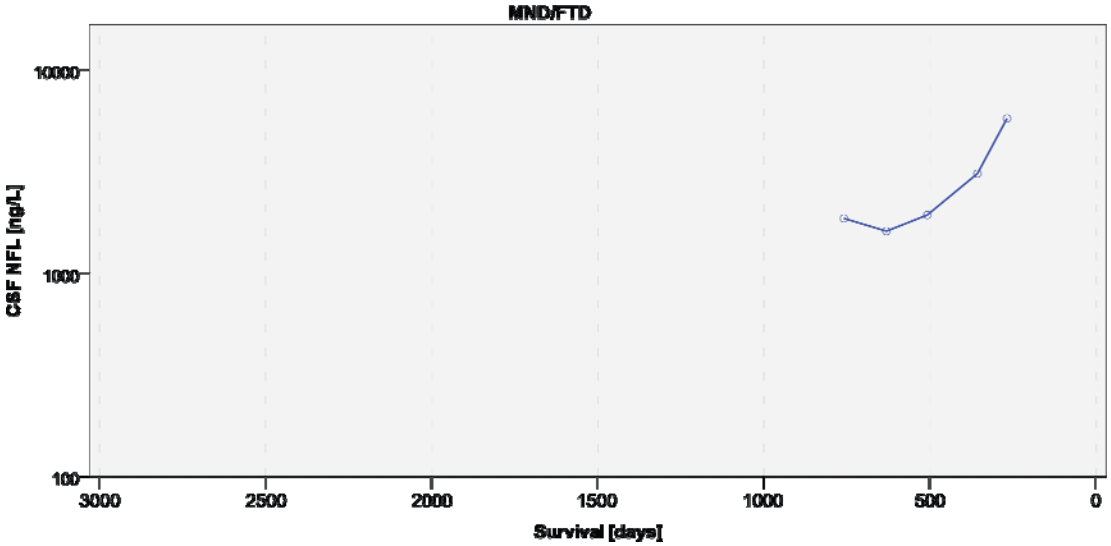


Table 1. Demographics of the study population

		FTD	Healthy	MND	MND/FTD
Total	**Count (% Female)**	87 (45%)	107 (61%)	715 (47%)	3 (33%)
Age at sampling (years)	**Mean (SD)**	66 (10)	69 (11)	66 (11)	63 (16)
	Median (IQR)	66 (59-74)	70 (63-76)	68 (60-74)	67 (46-77)
CSF NFL (ng/L)	**Mean (SD)**	1214 (1252)	395 (372)	2800 (2945)	5261 (6568)
	Median (IQR)	930 (550-1600)	275 (250-384)	1969 (970-3600)	1860 (1091-12832)