Serum neurofilament light chain in progressive supranuclear palsy

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Abstract (max 150 words)

Neurofilament light chain (NfL), a neuronal cytoskeletal protein, is a promising biomarker in neurodegenerative diseases. Elevated NfL levels (in CSF and/or plasma) have been observed in a growing number of neurodegenerative disorders, including frontotemporal dementia, Alzheimer's disease and amyotrophic lateral sclerosis. In the current study we have investigated serum NfL levels in a retrospective cohort of 131 patients with progressive supranulcear palsy (PSP) and 95 healthy controls. We found that serum NfL levels in PSP patients were twice as high compared to controls (p<0.001). Furthermore, higher NfL levels were significantly correlated with worse functional, motor and cognitive functioning and shorter survival. This study provides clear evidence that blood derived NfL is a relevant and promising biomarker in PSP, and may be used as a prognostic tool in clinical practice.

Max 1700 words

Introduction

There is a need for the development of sensitive, easily accessible biomarkers in neurodegenerative disorders to monitor disease progression and future treatment effects. Elevated levels of Neurofilament light chain (NfL), a neuronal cytoskeletal protein, reflect neuronal injury and have been found in the cerebral spinal fluid (CSF) of several neurodegenerative disorders.[1] An important recent finding is that NfL levels can be reliably detected in serum or plasma, which correlate very well with NfL levels in CSF. Results from studies in mouse models of neurodegenerative diseases, suggest that NfL is a dynamic biomarker, suitable to monitor treatment response in future trials. [2]

Progressive supranuclear palsy (PSP) is a neurodegenerative disorder, characterized by parkinsonism, frequent falls, supranuclear gaze palsy and widespread tau positive inclusions at brain autopsy. Elevated NfL levels have been found in the CSF of PSP patients.[3-8] More recently, plasma NfL in PSP patients has shown to be a useful biomarker for assessing disease severity and predicting disease progression.[9] In addition, blood derived NfL has a good diagnostic performance in the discrimination of atypical parkinsonism (including PSP) from Parkinson's disease and healthy controls.[10] NfL levels have also been associated with survival in FTD, AD and ALS patients,[11-13] but aren't investigated in PSP so far, except for a small study of 12 PSP patients where increased CSF NfL levels were found among patients who died at short follow up time.[3]

In the present retrospective case-control study, we have investigated blood based NfL levels in a large and well characterized cohort of PSP patients and demonstrate its relationship with disease severity and survival.

Methods

Subjects

PSP patients were ascertained in a nationwide genetic epidemiological study on PSP in the Netherlands, described elsewhere.[14] This study was approved by the Medical Ethical committee of the Erasmus Medical Center and all participants or legal representatives signed informed consent. All patients fulfilled the National Institute for Neurological Disorders and Society for PSP (NINDS-SPSP) criteria for possible or probable PSP during life.[15] Inclusion in the study took place at the out-patients clinic, or by visiting patients at home or in nursing homes. At study entry, blood collection and detailed clinical assessment was performed, including an interview with the care-giver, neurological examination and the quantitative assessment of functional, motor and cognitive disability by using the following rating scales: Hoehn and Yahr staging, Schwab and England Activities of Daily Living (SEADL), PSP-rating scale (PSP-RS), Mini-Mental State Examination (MMSE) and Frontal Assessment Battery (FAB). During follow-up, the diagnosis PSP was confirmed at autopsy in 23 patients. Survival time was calculated from the moment of serum collection till death or from serum collection till censoring date (July 1st 2017). In twelve cases who were lost to follow-up, we calculated survival time from serum collection till last contact alive.

Controls were healthy spouses or caregivers from stroke patients and free from neurodegenerative diseases. They were randomly selected from a large database and matched for age and sex.

Serum sampling

Between 2003 and 2014, serum samples were collected from PSP patients and healthy controls. After blood extraction, serum samples were centrifuged the same day and stored at -80°C. A

recently developed, ultrasensitive Simoa assay was used to measure NfL levels in serum from PSP patients and controls.[16] The measurements were performed in one round of experiments using one batch of reagents by board-certified technicians who were blinded to clinical data. For a quality control sample with a concentration of 16.8 pg/ml, repeatability was 7.36% and intermediate precision was 7.43%. For a quality control sample with a concentration of 127 pg/ml, repeatability was 6.69% and intermediate precision was 8.24%.

Statistical analysis

SPSS statistics version 21.0 was used and significance treshold was set on ≤ 0.05 for all tests (two-tailed). Difference between cases and controls were analyzed using Mann-Whitney U test and the area under the receiver operating characteristic (ROC) curve was calculated. Six samples (2 controls and 4 cases) showed extremely high NfL values (>3 SD above the mean, see figure 1). These values were replaced by values corresponding to the upper 2 SD cut-off. Since NfL levels were not normally distributed, we performed log transformation of the data (logNfL). LogNfL data were subsequently used in all of the following analyses. Analysis of covariance (ANCOVA) was used to study the differences in NfL levels between cases and controls adjusted for age and sex. Correlations between clinical variables and NfL within the PSP cohort was studied by using linear regression analyses with age and gender entered as covariates. Survival in patients was compared between NfL tertiles by Kaplan-Meier curves with according log-rank tests and Cox regressions adjusted for age and gender.

Results

Case control study

Serum NfL levels were determined in 131 PSP patients and 95 controls and table 1 summarizes the demographic and clinical data. There was a significant correlation between age and NfL in both cases (r=0.36, p<0.001) and controls (r=0.52, p<0.001). PSP patients showed higher NfL levels compared to controls (Figure 1; Median 64.2 versus 30.6 pg/ml, Mann Whitney U test p<0.001). This difference remained significant after correction for age and sex on log transformed NfL (logNfL) data (p<0.001). Serum NfL distinguished PSP patients from controls with high accuracy (area under the curve of 0.87, 95% confidence interval 0.82-0.92).

Relationship between NfL levels and disease severity within PSP cohort

Linear regression analyses (adjusted for age and sex) showed significant correlations between NfL levels and PSP-RS sum scores (β= 0.37, p<0.001), SEADL scores (β= -0.32, p=0.001), Hoehn and Yahr stages (β=0.30, p=0.001), FAB scores (β= -0.29, p=0.004) and MMSE scores (β= -0.18, p=0.05), indicating that higher motor, functional and cognitive disability are associated with higher NfL levels. No correlations were found between NfL levels and age at symptom onset or disease duration from onset till serum collection.

Survival analysis

On July 1st 2017, 119 PSP patients had deceased after a mean disease duration of 2.8 ± 2.1 years after serum collection. Twelve patients were lost to follow-up with a mean follow-up duration of 2.4 ± 1.5 years after serum collection. PSP-patients with high NfL levels showed worse survival

compared to patients with low NfL levels (Log Rank test p=0.001, Figure 2). The two years survival

probability was 73% in the lowest NfL tertiles, while this was 44% in the highest NfL tertiles. After

adjustment for age and sex, NfL levels remained significantly associated with worse survival (Hazard

Ratio 1.5 [1.1-1.9], p=0.003).

Discussion

The present study confirms that serum NfL levels are elevated in PSP patients and are associated with disease severity. We demonstrate for the first time that serum NfL levels can predict survival in PSP. This study provides strong evidence that blood derived NfL is a relevant and promising biomarker in PSP, and may be used as a prognostic tool in clinical practice. The use of blood based NfL instead of CSF is of great value, as the collection of blood samples is minimally invasive and would enable repeated measurements over time.

Our main findings are in line with two publications that have studied blood based NfL in PSP so far. [9, 10] Rojas *et al.* found that plasma NfL concentrations in PSP were twice as high as those in controls,[9] which is similar to what we have observed. The absolute values are higher in the current study and may be explained by older age and more severely affected patients in our cohort. Interlaboratory variation may add to the difference although both studies used the newly developed Simoa method, which has been proven to be highly sensitive.[16]

We found that NfL levels correlate with disease severity on motor, cognitive and functional domains. This correlation is in line with pervious observations, where serum NfL was correlated with Hoehn and Yahr stages and motor scores.[10] In addition, high baseline NfL levels in PSP patients were found to be associated with a more rapid neurological, functional and neuropsychological decline after 1 year compared to low baseline NfL levels.[9] In line with this observation, we have shown that high serum NfL levels can predict shorter survival in PSP patients. The association of high NfL levels with shorter survival, has been demonstrated in other neurodegenerative diseases, usually measured in CSF [11-13], while in plasma this association has only been demonstrated in ALS patients.[17, 18]

Extremely high NfL levels were observed in a few PSP patients. Whether other comorbidities (such as a minor stroke or brain trauma due to the severe postural instability) could have influenced NfL levels cannot be ruled out. These patients were severely disabled, but did not have signs of motor neuron disease at the time of serum collection. Clinical misdiagnosis with FTD is less likely as all patients fulfilled the clinical criteria for possible or probable PSP during life. In 23 individuals the diagnosis PSP was confirmed at autopsy and the percentages of autopsy proven subjects were equally distributed among the different NfL levels (see also figure 1), indicating that definite PSP patients can exhibit either low or high NfL levels. The presence of high NfL values in a few control subjects remains unsolved, as we do not have additional clinical information or neuroimaging in these subjects. The presence of (unknown) comorbidities or (presymptomatic) neurological disorders that increases NfL levels remains speculative.

The strengths of the current study are the large sample size with substantial pathological conformation, a clinically well characterized cohort with the assessment of several rating scales, and the long period of follow-up. We also acknowledge a few limitations. First, this study relies on cross-sectional acquired data with many patients in advanced disease stage. Longitudinal data would be very interesting in order to determine whether and how NfL concentrations in PSP patients change over time. Studies in ALS patients have shown stable NfL levels over time,[17, 18] while in patients with primary progressive aphasias, serum NfL increased in one year follow-up.[19] Secondly, many samples were stored at − 80°C for more than 10 years. Although the effect of long storage on serum NfL levels are unknown, NfL concentrations in CSF seem to be stable under pre-analytical variations and no negative effect have been observed by long-term storage or delayed processing.[20]

In summary, this study contributes to the growing evidence that blood derived NfL is an important,

easily accessible biomarker to monitor disease severity and progression in neurodegenerative

disorders, including PSP.

Variables	PSP patients	Controls	p-value
Gender (M/F)	72/59	44/51	0.20
Age at onset, y	66.4 ± 7.6	na	
Age at serum collection, y	71.3 ± 7.7	68.5 ± 6.3	0.003
Disease duration at serum collection, y	4.9 ± 2.9	na	
Median NfL level (pg/ml)	64.2	30.6	<0.001
NINDS-PSP criteria*			
Possible	49	na	
Probable	59	na	
Definite	23	na	
PSP-RS sum score (n=113)	47.2 ± 15.5	na	
Hoehn and Yahr stage (n=116)			
II	1	na	
III	23	na	
IV	30	na	
V	62	na	
MMSE (n=107)**	23.9 ± 4.6	na	
FAB (n=92)	10.0 ± 3.5	na	
Disease duration from onset till death, y (n=119)	7.8 ± 3.3	na	

Table 1. Demographics of PSP cohort.

Data are given as mean ± standard deviation or frequency (n).

NfL= neurofilament light chain. PSP-RS= PSP-rating scale; MMSE: MiniMental State Examination;

FAB= Frontal Assessment Battery. na= not applicable

*according to Litvan et al. 1996.[15]

** In 20 subject the maximal possible score was less than 30 due to severe motor disability
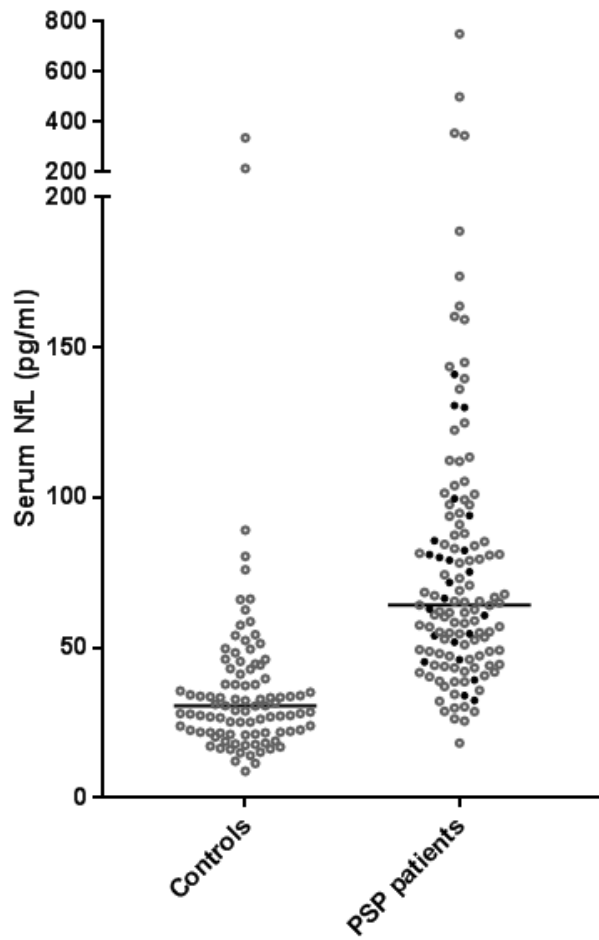
Figure 1.

Serum NfL levels (Y-axis) in 131 PSP-patients and 95 controls. Horizontal lines represent medians. Filled black circles indicate individuals with a definite PSP diagnosis.
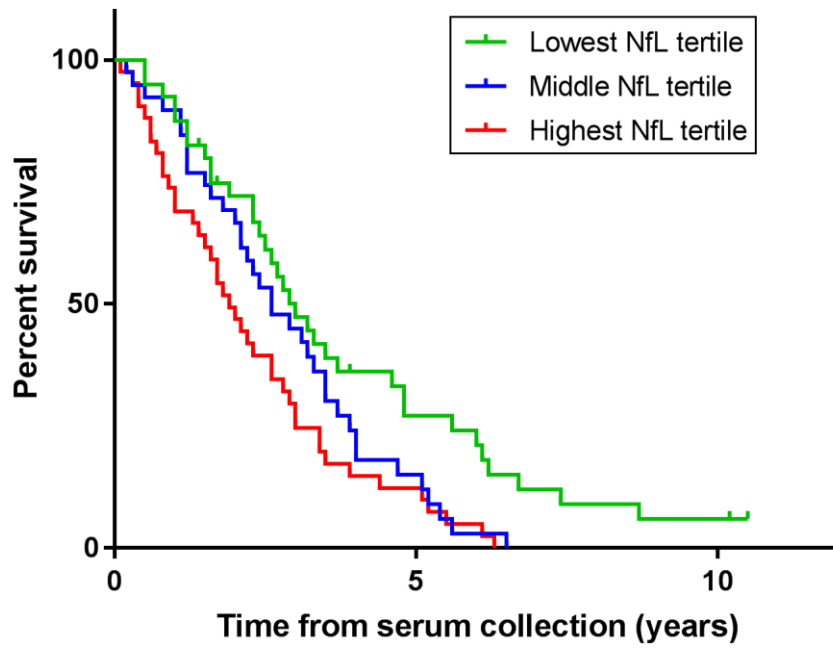
Figure 2. Survival curves for NfL levels (log transformed) in PSP patients. Log Rank test p=0.001.

(max 40)

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