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Serum NFL discriminates Parkinson disease from atypical parkinsonisms

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Abstract

Objective

To investigate the diagnostic value of serum neurofilament light chain (NFL) in patients with clear signs of parkinsonism but whose specific diagnosis was yet uncertain.

Methods

Serum samples were collected from patients with clear signs of parkinsonism but with uncertain diagnosis at the inclusion. Clinical diagnoses of Parkinson disease (PD) and atypical parkinsonism disorders (APDs) were established after 3 years of follow-up and updated again after a maximum of 12 years in case longer follow-up data were available. Serum NFL was quantified by single molecule array in patients with PD ($n = 55$) and APD ($n = 29$, multiple system atrophy = 22, progressive supranuclear palsy = 7) and 53 nonneurologic controls.

Results

Serum NFL levels were elevated and differentiated the APD group (mean 23.8 ± 10.3 ng/L) from PD (mean 10.4 ± 4.9 ng/L) and controls (mean 11.5 ± 6.5 ng/L, $p < 0.0001$) with accuracy levels up to 91% (sensitivity = 86% and specificity = 85%). Serum NFL strongly correlated with CSF NFL levels ($r = 0.72$, $p < 0.0001$) in all groups and with age in PD ($r = 0.78$, $p < 0.0001$) and controls ($r = 0.66$, $p < 0.0001$). In our cohort, the probability of having APD was 76% (positive predictive value) and of having PD 92% (negative predictive value).

Conclusion

Serum NFL levels are markedly elevated in APD compared to PD and discriminate APDs from PD with high accuracy. Serum NFL may be a useful clinical biomarker to identify APD, even at stages when clinical symptoms are not yet conclusive.

Classification of evidence

This study provides Class II evidence that serum NFL levels accurately discriminate APDs from PD.

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Glossary

$A\beta_{42}$ = β -amyloid 42; α -syn = α -synuclein; APD = atypical parkinsonism disorder; AUC = area under the curve; CBS = cortical basal syndrome; CI = confidence interval; H&Y = Hoehn and Yahr; MMSE = Mini-Mental State Examination; MSA = multiple system atrophy; NFL = neurofilament light chain; PD = Parkinson disease; PSP = progressive supranuclear palsy; ROC = receiver operating characteristic; Simoa = single molecule array.

Parkinson disease (PD) is difficult to discriminate from the various forms of atypical parkinsonism disorders (APDs), such as multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and cortical basal syndrome (CBS), especially at early disease stages. The rate of misdiagnosis based on clinical investigations alone can be as high as 15%, even in the hands of experienced movement disorder specialists.¹⁻³ Therefore, reliable biomarkers are needed for an accurate and early differentiation between PD and APD.

Several proteins directly related to PD, such as α -synuclein (α -syn) and DJ-1, or associated with neurodegeneration, such as neurofilament light chain (NFL), tau, and β -amyloid 42 ($A\beta_{42}$) have been investigated as potential biomarkers for parkinsonism in CSF (reviewed in reference 4). Quantification in serum of NFL, a protein involved in axonal growth and regeneration,⁵ has recently been reported to have a high diagnostic value to differentiate APD from PD. Clearly, analysis in serum is preferable to that in CSF.⁶

Our aim was to study the diagnostic value of serum NFL in a unique cohort of patients with clear signs of parkinsonism, such as bradykinesia with resting tremor or rigidity, but whose specific diagnosis was uncertain at the time of collection of serum and CSF, and who were followed for a maximum of 12 years.⁷ This cohort is representative of the daily situation when clinicians are confronted with a patient with an uncertain diagnosis, and where biomarkers are needed most for an accurate diagnosis.

Methods

Classification of evidence

Our goal was to determine whether NFL levels in serum could discriminate patients with APD (MSA and PSP) from patients with PD, at a disease stage when the clinical diagnosis was still uncertain. This study provides Class II evidence for the diagnostic value of serum NFL to discriminate APD from PD and controls.

Patients and samples

We have selected patients with PD and APD from a previously described longitudinal study performed at the Radboud University Medical Center (Nijmegen, the Netherlands).⁷ Patients were consecutively recruited from our movement disorders outpatient clinic between January 2003 and December 2006. All participants had clear signs of parkinsonism, but based on clinical grounds, their specific diagnosis was uncertain at the time of inclusion. Exclusion criteria were age younger than 18 years, history of brain surgery

or other neurodegenerative disease than parkinsonism, and unstable comorbidity. All patients underwent a structured interview, detailed and standardized neurologic examination, and, within 6 weeks after the initial visit, blood collection and lumbar puncture among other ancillary investigations (brain MRI, IBZM (iodobenzamide)-SPECT, anal sphincter EMG). The study design, methods, and the included patient populations have been extensively described.⁷ After 3 years, the clinical condition was reevaluated by a repeated structured interview and extensive neurologic examination. Using the clinical findings at baseline and the follow-up visit, a clinical diagnosis was established in consensus by 2 movement disorder specialists according to the existing clinical criteria by that time: the UK Brain Bank criteria for PD,³ Gilman criteria for MSA,⁸ National Institute of Neurological Disorders and Stroke and Society for Progressive Supranuclear Palsy criteria for PSP,⁹ Boeve criteria for CBS,¹⁰ and Zijlmans criteria for vascular parkinsonism. Twelve years after inclusion, 46 patients were reevaluated by movement disorder specialists, and all clinical diagnoses were evaluated again and updated according to most recent clinical criteria¹¹⁻¹⁴ and the most recent clinical information regarding survival, response to L-dopa, evolution of symptoms, and neuropathologic confirmation whenever available, were recorded. Diagnoses were determined by neurologists who were blinded to the NFL levels in both serum and CSF. Characteristics of patients are summarized in table 1.

Patients were selected for this study based on serum availability and classified according to the most recent diagnosis after 12 years of follow-up, consisting of 55 patients with PD (classified as possible $n = 1$, probable $n = 38$, clinically established $n = 15$, definite $n = 1$), 22 patients with MSA (classified as possible $n = 4$, probable $n = 15$, definite $n = 3$), and 7 patients with PSP (classified as possible $n = 3$, probable $n = 3$, definite $n = 1$). Patients with MSA and PSP were considered as one group (APD) because of the low number of patients with PSP in this study. An overview of patient inclusion and follow-up of clinical diagnoses is shown in figure 1.

Clinical assessments were performed at baseline and after 3 and 12 years of follow-up, including disease severity and cognitive function, by using the Hoehn and Yahr (H&Y) scores,¹⁵ Unified Parkinson's Disease Rating Scale,¹⁶ International Cooperative Ataxia Rating Scale,¹⁷ Mini-Mental State Examination (MMSE),¹⁸ and Scale for the Assessment and Rating of Ataxia.¹⁹ A summary of clinical parameters from all patients is available in table 2.

Table 1 Patient characteristics and biochemical measurements

	Control	PD	MSA	PSP	<i>p</i> Value ^a
No.	53	55	22	7	
Sex, M/F	29/24	38/17	15/7	4/3	0.4
Age at inclusion, y	57.5 ± 9.8	57 ± 10	60.7 ± 7.1	68.9 ± 4.1	0.01 ^{b,c}
Disease duration, mo	NA	34.2 ± 26.3	33.9 ± 26.4	35.7 ± 19.2	0.8
Follow-up, y	NA	10.3 ± 4.2 (n = 53)	3.9 ± 3.3 (n = 20)	4.7 ± 4.2 (n = 6)	NA
Dopaminergic medication at serum and CSF collection, no/yes	NA	44/11	15/7	4/3	NA
NFL in serum, ng/L	11.5 ± 6.5	10.4 ± 4.9	22.2 ± 11	25.6 ± 8.4	<0.0001 ^{b,c,d,e}
NFL in CSF, ng/L	1,265 ± 551 (n = 32)	1,249 ± 666 (n = 54)	65,487 ± 4,138 (n = 21)	4,809 ± 4,064 (n = 7)	<0.0001 ^{b,c,d,e}
Ratio CSF/serum NFL		122.5 ± 44.9	269.4 ± 143.0	176.1 ± 106.3	<0.0001 ^e
α-syn CSF, μg/L		26.4 ± 10 (n = 55)	28.4 ± 8.2 (n = 22)	33.7 ± 16.2 (n = 6)	0.4
Total tau, ng/L		213.2 ± 95.3 (n = 55)	275.2 ± 130.8 (n = 22)	266.6 ± 73.5 (n = 7)	0.03 ^e
Phosphorylated tau, ng/L		49.1 ± 18.4 (n = 55)	46.1 ± 15.6 (n = 22)	53 ± 16.8 (n = 7)	0.6
DJ-1, ng/L		534.7 ± 128.2 (n = 29)	721.5 ± 242.5 (n = 15)	ND	0.005 ^e
Aβ₄₂, ng/L		857.1 ± 206.6 (n = 55)	782.9 ± 199.6 (n = 22)	769 ± 156 (n = 7)	0.2
Ratio CSF/serum albumin		6.9 ± 3.0	7.0 ± 2.9	10.6 ± 7.0	0.3

Abbreviations: Aβ₄₂ = β-amyloid 42; α-syn = α-synuclein; MSA = multiple system atrophy; n = number of samples; NA = not applicable; ND = not determined; NFL = neurofilament light chain; PD = Parkinson disease; PSP = Progressive supranuclear palsy. Values are expressed as mean ± SD.

^a Parameters were analyzed with analysis of variance using Bonferroni as post hoc test in the case of gaussian distribution of data, Kruskal-Wallis with Dunn as post hoc test of nongaussian distribution for comparison of multiple groups, or Mann-Whitney *U* test for comparison of 2 groups. Sex was analyzed using χ² test.

^b Differences were found between control vs MSA.

^c Differences were found between control vs PSP.

^d Differences were found between PD vs PSP.

^e Differences were found between PD vs MSA.

To investigate potential correlations of serum NFL with CSF levels of NFL, α-syn, total tau, phosphorylated tau, DJ-1, Aβ₄₂, albumin, and serum albumin in patients with PD and APD, we used previously collected data published by our group.^{7,20–22} Results of the biochemical analyses are summarized in table 1.

The nonneurologic controls consisted of a group of 53 patients with suspicion of neurologic disorder who underwent serum and CSF collection in the diagnostic workup of cognitive symptoms, without evidence of dementia/Alzheimer disease (n = 16), diagnostic workup of neurologic symptoms without (somatic) neurologic explanation (n = 11), to exclude neuroinflammatory disease (n = 13) and subarachnoid hemorrhage (n = 10), or to explain disturbances of intracranial pressure (n = 3). In none of these control cases was a neurologic disease present, and leukocyte count, glucose, total protein, blood pigments, lactate, and (if assessed) oligoclonal immunoglobulin G bands were all normal in their CSF. Characteristics of nonneurologic controls are described in table 1.

Serum and CSF samples of PD, APD, and nonneurologic controls were collected in polypropylene tubes, centrifuged,

aliquoted, and stored in polypropylene tubes at –80°C until used for experiments.

Standard protocol approvals, registrations, and patient consents

This study was approved by the ethical committee review board Arnhem-Nijmegen (2002/188), and all participants provided written informed consent.

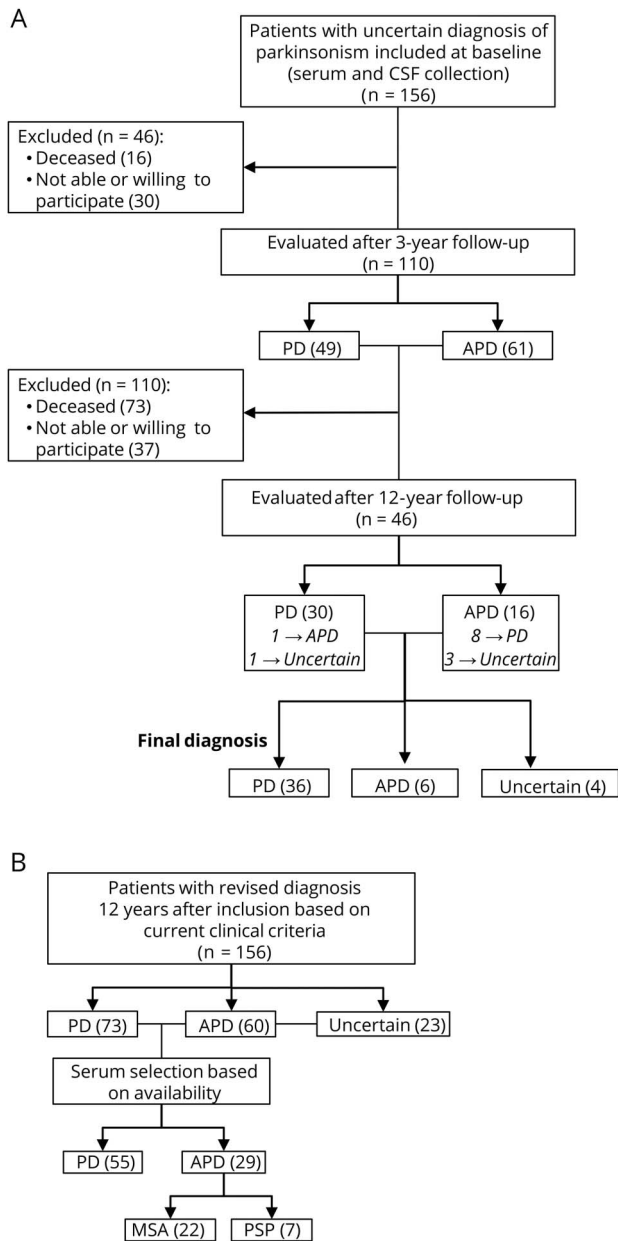
Single molecule array

Serum NFL was measured with the single molecule array (Simoa) NF-light Advantage Kit from Quanterix (Lexington, MA). Serum samples were diluted 1:4 for the measurements according to the manufacturer's recommendation. Serum quality-control samples with high- and low-endogenous NFL concentration were included in all measurements (coefficient of variation <5%). Serum NFL levels were determined by researchers who were blinded to the clinical diagnosis.

Data analysis

The data analysis of this study was done by using SPSS Statistics 22 (IBM Corp., Armonk, NY) and GraphPad Prism 5

Figure 1 Inclusion and follow-up of patients included in this study



Flowchart of follow-up of patients included in this study. (A) Patients with uncertain diagnosis were included at baseline and part of the cohort was evaluated again after 3 and 12 years for follow-up. Changes in the diagnosis between the 3- and 12-year follow-up are indicated in italics. (B) Revision of the diagnosis of all 156 cases after 12 years based on the current clinical criteria and with all clinical information available at that time (even for patients who were not available for follow-up after 3 or 12 years) and the selection of the sample set for this study. APD = atypical parkinsonism disorder; MSA = multiple system atrophy; PD = Parkinson disease; PSP = progressive supranuclear palsy.

(La Jolla, CA). Student *t* test, Mann-Whitney *U* test, analysis of variance, followed by Bonferroni post hoc test, and Kruskal-Wallis, followed by Dunn post hoc test, were used to assess group differences. Sex was analyzed by χ^2 test. Analysis of covariance was done using age and sample storage time as confounding factors. Correlations between 2 variables were

investigated by Spearman test, and partial correlation was done using age as a covariate. To determine the diagnostic accuracy, a receiver operating characteristic (ROC) curve was constructed to determine the area under the curve (AUC) and the values of sensitivity and specificity with 95% confidence interval (CI), and Youden index was determined (sensitivity + specificity – 1.0) to find the optimal cutoff value. Comparison between ROC curves was done by using MedCalc software trial version 18.2.1 (Ostend, Belgium). Risk estimation was calculated yielding positive and negative predictive values and by binary logistic regression to determine odds ratio by using the cutoff of serum NFL concentration determined by the optimal Youden index.

Data availability

Anonymized data will be shared on request from any qualified investigator.

Results

We observed significantly higher NFL levels in serum of patients with an APD compared to patients with PD and nonneurologic controls (figure 2A). Group differences remained highly significant after correction for age and sample storage time ($p < 0.0001$). Serum NFL levels were similar for patients with MSA and PSP ($p = 0.4$). Analysis of diagnostic accuracy by construction of ROC curve yielded an AUC of 0.91 for discrimination of APD from PD (95% CI 0.83–0.98, sensitivity = 86% and specificity = 85%, cutoff = 14.8 ng/L, Youden index = 0.7) and an AUC of 0.88 to discriminate patients with APD from controls (95% CI 0.80–0.96, sensitivity = 93% and specificity = 71%, cutoff = 13.6 ng/L, Youden index = 0.6) (figure 2B).

Risk estimation was calculated by using the cutoff value (14.8 ng/L) yielded by the optimal Youden index in the ROC analysis (APD vs PD). Risk was estimated considering the most recent clinical diagnosis (after 12 years of follow-up) in our cohort. Among those patients who had serum NFL levels above the cutoff value, the probability of having an APD is 76% (positive predictive value), and patients who had serum NFL levels below the cutoff value have a 92% probability of having PD (negative predictive value). In addition, analysis by binary logistic regression yielded an odds ratio of 36 (95% CI 10.0–134.0), which means that patients with serum NFL levels above the cutoff value have a 36-times-higher chance of having APD over PD. Risk estimation values were retained after taking age as covariate.

Serum NFL concentration correlated with age in both the PD ($r = 0.78$, $p < 0.0001$) and control groups ($r = 0.66$, $p < 0.0001$) but not in the APD group. Therefore, age was used as a confounding factor for all further analyses described below. Serum NFL was highly correlated with CSF NFL levels in the 3 combined groups ($r = 0.72$, $p < 0.0001$) (figure 3), as well as in the individual groups: PD, $r = 0.34$,

Table 2 Clinical parameters of the parkinsonism cohort at baseline and 3- and 12-year follow-up

	PD	MSA	PSP	p Value ^a
Baseline				
H&Y score	2.0 ± 0.6 (n = 53)	2.4 ± 1.0 (n = 22)	3.3 ± 0.7 (n = 7)	<0.0001 ^{b,c}
UPDRS score	26.5 ± 11.9 (n = 52)	29 ± 13.9 (n = 21)	35.9 ± 15.6 (n = 7)	0.2
ICARS score	2.8 ± 3.0 (n = 50)	11.7 ± 11.7 (n = 18)	12 ± 7.4 (n = 5)	<0.0001 ^{b,c}
MMSE score	28.3 ± 2.1 (n = 54)	28 ± 2.2 (n = 21)	25.4 ± 2.8 (n = 7)	0.01 ^{c,d}
Tandem gait	0.1 ± 0.3 (n = 54)	1.6 ± 1 (n = 21)	2.5 ± 0.5 (n = 7)	<0.0001 ^{b,c}
3-y follow-up				
H&Y score	2.3 ± 0.7 (n = 52)	3.8 ± 1.4 (n = 17)	4.4 ± 0.5 (n = 5)	<0.0001 ^{b,c}
UPDRS score	28.8 ± 13.6 (n = 47)	32.5 ± 9.3 (n = 11)	41.6 ± 10.5 (n = 5)	0.03 ^c
ICARS score	2.5 ± 2.2 (n = 43)	23.5 ± 19 (n = 10)	18 ± 9 (n = 5)	<0.0001 ^{b,c}
MMSE score	28.2 ± 2.2 (n = 44)	25.8 ± 2.8 (n = 11)	24.8 ± 4.4 (n = 5)	<0.01 ^b
Tandem gait	0.2 ± 0.7 (n = 49)	2.4 ± 1.1 (n = 14)	2.7 ± 0.6 (n = 3)	<0.0001 ^{b,c}
12-y follow-up				
H&Y score	2.9 ± 0.8 (n = 29)	n = 1	5 (n = 1)	NA
UPDRS score	36 ± 14 (n = 22)	ND	91 (n = 1)	NA
SARA score	5.7 ± 2.2 (n = 29)	7.5 (n = 1)	20 (n = 1)	NA
MMSE score	27.1 ± 4.7 (n = 30)	26 (n = 1)	22 (n = 1)	NA
Tandem gait	1.4 ± 1.4 (n = 29)	ND	4 (n = 1)	NA

Abbreviations: H&Y = Hoehn and Yahr; ICARS = International Cooperative Ataxia Rating Scale; MMSE = Mini-Mental State Examination; MSA = Multiple System Atrophy; n = number of samples; NA = not applicable; ND = not determined; PD = Parkinson Disease; PSP = progressive supranuclear palsy; SARA = Scale for the Assessment and Rating of Ataxia; UPDRS = Unified Parkinson's Disease Rating Scale.

Values are expressed as mean ± SD.

^a Parameters were analyzed with analysis of variance using Bonferroni as post hoc test in the case of gaussian distribution of data, Kruskal-Wallis with Dunn as post hoc test of nongaussian distribution for comparison of multiple groups.

^b Differences were found between PD and MSA.

^c Differences were found between PD and PSP.

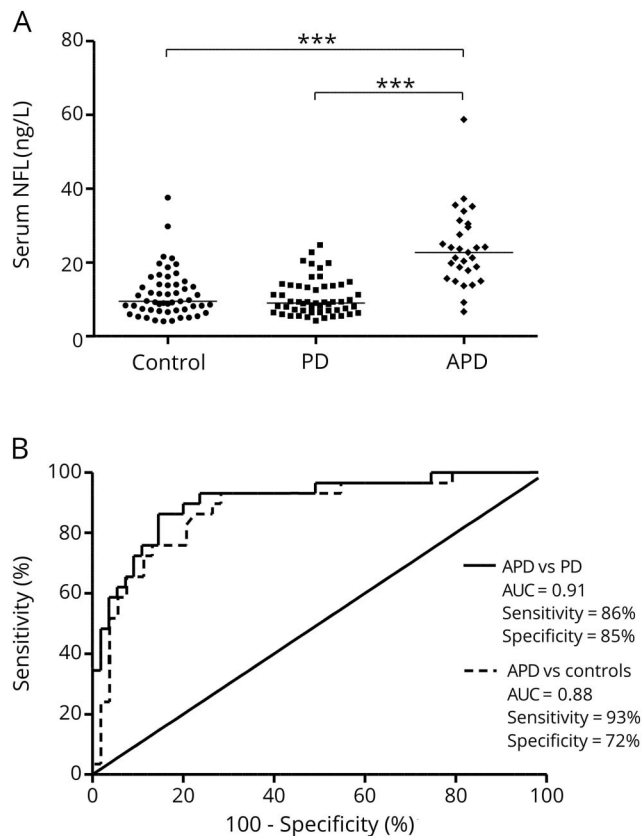
^d Differences were found between MSA and PSP.

$p = 0.012$; APD, $r = 0.60$, $p < 0.001$; controls, $r = 0.39$, $p = 0.029$. Serum NFL levels in the combined cohorts, but not in the individual groups, correlated with CSF DJ-1 ($r = 0.47$, $p < 0.001$). We observed a weak correlation between the albumin CSF/serum ratio (a measure of blood-CSF barrier integrity) and the NFL CSF/serum ratio ($r = 0.24$, $p = 0.04$). No correlations were found between serum NFL and CSF α -syn, $A\beta_{42}$, total tau, or phosphorylated tau. Serum NFL levels were associated with clinical parameters only in the APD group. At baseline, serum NFL was correlated with the International Cooperative Ataxia Rating Scale score ($r = 0.60$, $p = 0.003$) and tandem gait test ($r = 0.54$, $p = 0.004$). At 3-year follow-up, serum NFL was associated with tandem gait test ($r = 0.53$, $p = 0.03$), H&Y score ($r = 0.68$, $p < 0.001$), MMSE ($r = 0.60$, $p = 0.019$), and with the difference in H&Y scores measured at 3-year follow-up and baseline ($r = 0.69$, $p < 0.001$). No correlations between serum NFL and clinical parameters could be determined at 12-year follow-up since only 2 patients with APD were available for follow-up. Clinical parameters

were not determined in nonneurologic controls; therefore, it was not possible to investigate any correlations in this group.

Since our publication of CSF NFL concentrations in this cohort,²⁰ the diagnoses of the patients have been revised resulting in a change in clinical diagnosis for 21 patients. Therefore, we reanalyzed the previously published data of NFL levels in CSF. NFL levels in CSF were higher in APD ($n = 32$; $5,544 \pm 4,137$ ng/L) and discriminated APD from PD ($n = 65$; $1,239 \pm 638.1$ ng/L, $p < 0.0001$) and controls ($n = 65$; $1,348 \pm 569.1$ ng/L, $p < 0.0001$). Diagnostic accuracy was as follows: APD vs PD, AUC = 0.90, 95% CI 0.82–0.95, sensitivity = 75% and specificity = 98%; APD vs controls, AUC = 0.89, 95% CI 0.81–0.94, sensitivity = 75% and specificity = 100%. ROC curves for the discrimination of APD from PD or controls using either serum or CSF NFL were compared, but no significant differences were found: PD vs APD, $p = 0.94$; controls vs APD, $p = 0.85$.

Figure 2 Serum NFL highly discriminates APD from PD and controls



Serum NFL concentration in patients with parkinsonism and diagnostic accuracy. (A) Serum NFL levels are elevated in APD compared to PD and nonneurologic controls. Data were analyzed using Kruskal-Wallis test and Dunn as a post hoc test; mean levels are shown with SD; *** $p < 0.0001$. (B) Receiver operating characteristic curves showed high accuracy levels for discrimination of APD from PD (solid line) and APD from controls (dashed line) by using NFL levels in serum. NFL levels were quantified by single molecule array. APD = atypical parkinsonism disorder; AUC = area under the curve; NFL = neurofilament light chain; PD = Parkinson disease.

Discussion

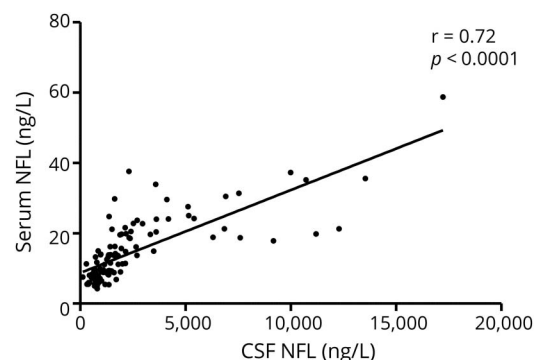
In this study, we aimed to analyze whether NFL levels in serum could be used as a biomarker for discrimination of APD from PD and controls at a disease stage when the clinical diagnosis is still uncertain. For this purpose, we measured NFL concentration in the serum of patients with clear signs of parkinsonism but with an uncertain diagnosis at the time of inclusion. Our findings indicated that serum levels of NFL are significantly increased in APDs vs PD or controls and discriminated APDs from PD and controls with high accuracy levels. Furthermore, high serum NFL levels were associated with a very high risk of having an APD and low NFL levels were associated with a high chance of having PD in our cohort.

NFL is one of the components of the axonal cytoskeleton, thus release of NFL likely occurs after injury to axons with subsequent release into interstitial fluid, which is drained toward the CSF, and later to peripheral blood.²³ Elevated NFL

levels in CSF have been described in various neurodegenerative disorders, such as Alzheimer disease,²⁴ multiple sclerosis,^{25,26} amyotrophic lateral sclerosis,^{24,27,28} frontotemporal dementia,^{29,30} Creutzfeldt-Jakob disease,³¹ Huntington disease,³² PSP,³³ CBS,⁴ and MSA.⁴ In parkinsonisms, CSF NFL has been previously suggested as a biomarker for discrimination of APD from PD (reviewed in reference 4).

Our findings of increased serum NFL in APDs are in line with previous studies^{6,34,35} in which serum or plasma NFL levels were studied in patients with PD and APD. The first study³⁴ reported elevated NFL levels in the plasma of patients with PSP compared to controls in 2 cohorts, and higher NFL plasma levels were correlated with impairment of PSP symptoms after a 1-year follow-up. We also observed positive correlations of NFL levels and parameters of disease severity in patients with APD with comparable age and cognitive function (MMSE). The second study⁶ assessed NFL levels in the serum of patients with PD and APD from 3 independent cohorts. Two of these cohorts were characterized by inclusion of patients with long disease duration (approximately 5 years) with well-established diagnoses. The third cohort consisted of patients with short disease duration up to 3 years. NFL levels were higher in APDs in all 3 cohorts, with accuracy up to 91% in the 2 cohorts with long-term follow-up and 81% in the cohort with relative short follow-up. In comparison with this latter cohort, we found even higher diagnostic accuracy (91%). Our patients with PD and MSA were younger but had similar disease severity compared to this published cohort (based on H&Y score). The higher accuracy in our cohort is probably attributable to a more accurate diagnosis after long-term follow-up of our patients with repetitive clinical reassessments of the diagnosis. The most recent study³⁵ showed higher serum NFL levels in patients with cerebellar-type MSA compared to patients with sporadic adult-onset ataxia and healthy controls. The NFL levels in those patients with MSA were almost 3 times higher compared to our study. Although

Figure 3 NFL levels in serum are highly correlated with NFL levels in CSF



Correlation analysis of NFL concentration measured in serum and in CSF. NFL levels in serum and CSF were highly correlated in the combined parkinsonism and nonneurologic control groups. Spearman ρ coefficient value and p value are indicated in the graph. NFL = neurofilament light chain.

these patients had an age comparable to our patients at serum withdrawal, their disease duration was longer, which may explain the higher NFL levels.

Diagnostic accuracy is very similar for NFL in serum and in CSF, as previously reported.⁶ Therefore, serum analysis could replace CSF analysis for this goal, since blood collection is less invasive and offers less discomfort for patients. Since NFL concentrations are very low in serum, it was previously not possible to detect its levels in serum by conventional methods, such as ELISA. The recent development of Simoa technology allowed researchers to assess very low NFL levels in serum.³⁶ However, Simoa technology is an expensive technique, whereas NFL levels in CSF could be quantified with cheaper ELISAs. To reach even higher accuracy numbers, panels of biomarkers, including NFL, have been proposed for discrimination of APDs from PD and controls in CSF.^{37–39} In the near future, such studies could include serum NFL to further evaluate its diagnostic value for APD diagnosis.

Serum NFL levels correlated with age in PD and controls, but not in the APD group. We could speculate that because NFL levels are much higher in patients with APD this correlation with aging is lost, although it might have existed earlier in the disease process or even in a presymptomatic state. We did not find associations between serum NFL levels and age in either MSA or PSP group, which could be caused by the relative small numbers of patients.

Serum NFL concentrations may be related to disease severity. Although in a previous study no correlations between serum NFL and clinical parameters were observed in the cohort with relatively short follow-up,⁶ we observed several correlations with clinical assessments in our APD cohort. This difference is most likely explained by a more extensive clinical follow-up in our study. Associations between NFL and clinical assessments may suggest the use of NFL for monitoring or predicting disease severity in APDs.

Elevated concentrations of NFL in body fluids are not specific for one type of APD and may be a biomarker for general axonal degeneration. Although NFL highly discriminated APD from PD and controls, NFL levels cannot discriminate among APDs, such as MSA and PSP in our cohort, which could be caused by the small number of patients with PSP in our study. Additional biomarkers may be needed, which may aid clinicians to make a more detailed differentiation between the various forms of APDs, such as misfolded forms of α -syn, tau, and A β ₄₂.^{39,40}

A few limitations may apply to our study. First, our samples were stored for an extended period of time at -80°C . Previous studies assessed the effects of long-term storage of plasma and CSF at -80°C .^{41,42} No effect of long-term storage effect was found for several CSF proteins. However, for 18 plasma proteins, concentrations may decrease or increase after long-term storage, such as interleukin 13, interleukin 27, and

CD40. None of the studies reported on NFL. Therefore, we assume that NFL concentrations remained the same in CSF and serum after storage at -80°C . Moreover, sample storage time did not affect our data analysis when it was taken as a covariate. A second limitation of our study is that the final diagnosis, which was used to determine diagnostic accuracy, was only based on clinical evaluation and not yet confirmed by neuropathologic examination. Although a team of movement disorder specialists determined the clinical diagnoses according to international diagnostic criteria and after long-term follow-up, which increased the chance of correct classification, we cannot exclude that some patients might have been incorrectly diagnosed. Third, we assessed a high relative risk of having a diagnosis of APD when serum NFL levels are elevated. However, these risk estimates are only based on our population and thus require further confirmation.

This study confirms the high diagnostic value of serum NFL for discrimination of APD at early disease stages, offering a great opportunity for further confirmation studies in larger patient cohorts. Our findings suggest a possibility for differentiation between PD and APD by serum rather than CSF analysis. Serum NFL levels were associated with clinical parameters of disease severity and cognitive decline, suggesting that NFL might be used in the future for monitoring or prediction of disease severity.

Author contributions

Drs. Verbeek, Bloem, Otto, and Marques designed the study. Drs. van Rumund, Esselink, and Bloem were responsible for recruiting and collection of patient data. Dr. Oeckl performed the serum NFL analysis. Drs. Marques, Kuiperij, and Verbeek interpreted the data. Dr. Marques analyzed the data and wrote the first manuscript draft. All authors reviewed and contributed to the manuscript and approved the final version.

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Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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