Plasma NfL, clinical subtypes and motor progression in Parkinson's disease

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Abstract

Introduction: neurofilament light chain (NfL) levels have been proposed as reliable biomarkers of neurodegeneration in Parkinson's disease (PD) but the relationship between plasma NfL, clinical subtypes of PD and motor progression is still debated.

Methods: plasma NfL concentration was measured in 45 healthy controls and consecutive 92 PD patients who underwent an extensive motor and non-motor assessment at baseline and after 2 years of follow-up. PD malignant phenotype was defined as the combination of at least two out of cognitive impairment, orthostatic hypotension and REM sleep behavior disorder. PD patients were divided according to the age-adjusted cut-offs of plasma NfL levels into high and normal NfL (H-NfL and N-NfL, respectively). A multivariable linear regression model was used to assess the value of plasma NfL as predictor of 2-years progression in PD.

Results: NfL was higher in PD patients than in controls ($p = 0.037$). H-NfL ($n = 16$) group exhibited more severe motor and non-motor symptoms, higher prevalence of malignant phenotype and worse motor progression (MDS-UPDRS-III 11.3 vs 0.7 points, $p = 0.003$) compared to N-NfL group ($n = 76$). In linear regression analyses plasma NfL emerged as the best predictor of 2-year motor progression compared to age, sex, disease duration, baseline motor/non-motor variables.

Conclusion: increased plasma NfL concentration is associated with malignant PD phenotype and faster motor progression. These findings support the role of NfL assessment as a useful measure for stratifying patients with different baseline slopes of decline in future clinical trials of putative disease-modifying treatments.

INTRODUCTION

Parkinson's disease (PD) is a complex disorders that encompasses various clinical, epidemiological and genetic subtypes characterized by different response to treatments and disease course[1–4].

Advanced age, longer disease duration, male sex, the presence of mild cognitive impairment (MCI), orthostatic hypotension (OH) and REM sleep behavior disorder (RBD) have been independently associated with worse outcomes in PD[4–6].

In spite of the recent advances in the field, there are currently no reliable biological markers for predicting disease progression in PD at single-subject level[7,8]. An early identification of different subgroups of patients with different disease trajectories is a pivotal step for a better planning of management strategies and symptomatic treatments[9].

Neurofilament light chain (NfL), a highly expressed protein in large caliber myelinated axons, has been recently proposed as marker of neuronal damage in different neurological disorders[10,11]. Indeed, there is evidence that cerebrospinal fluid (CSF) and plasma levels of NfL increases proportionally to the degree of axonal damage in several neurological disorders including inflammatory, neurodegenerative, traumatic and cerebrovascular diseases[10].

In parkinsonian disorders, CSF and plasma NfL levels discriminate between atypical parkinsonism and idiopathic PD, in which most subjects have normal levels[12–15]. Increased NfL levels in PD have been associated with more severe disease, longer disease duration and the presence of dementia[11,16].

A recent study by Lin and coauthors also suggest plasma NfL as possible predictor of motor and cognitive progression, despite the work had several methodological limitations, as it did not consider age-adjusted NfL cut-off levels, it includes PD patients with high NfL but also dementia at baseline, and it did not test the biomarker against the established clinical subtypes and factors associated with worse progression[16].

In this longitudinal study, we aimed to investigate the relationships between plasma NfL, motor and non-motor symptoms in PD and to evaluate the accuracy of plasma NfL as a predictor of motor progression compared to clinical subtypes.

METHODS

Patients selection

Consecutive patients with a clinical diagnosis of PD[17] were evaluated at the outpatient Movement disorder Clinic, Neurology Unit at the University of Brescia, Italy from October 2016 to March 2020 2016. This study was approved by the local ethics committee and was in conformity with the Helsinki Declaration. Informed consent was obtained from all participants. Levodopa equivalent daily dose (LEDD) was calculated according to standard conversion[18] and the diagnosis was supported by levodopa/dopaminergic response and at least two years of clinical follow-up. Only clinically established PD[17] were included in the final analyses.

All patients underwent routine blood analyses, magnetic resonance imaging (in our or other external centres) to exclude prominent cortical or subcortical infarcts or brain/iron accumulation or atypical parkinsonian disorders. The following exclusion criteria were applied: (1) atypical parkinsonism, at baseline or during follow-up; (2) prominent cortical or subcortical infarcts in structural imaging; (3) other neurologic disorders or medical conditions potentially associated with cognitive deficits; (4) bipolar disorder, schizophrenia, history of drug or alcohol abuse or impulse control disorder; (5) negative nigrostriatal imaging (6) recent traumatic events or acute fever/inflammation (potentially influencing NfL levels), (7) patients with dementia[19].

Clinical and neuropsychological assessment

At baseline, standardized neurological examination was performed, including the Movement Disorder Society- Unified Parkinson Disease Rating Scale (MDS-UPDRS)[20].

Global non-motor function was evaluated with the Non-Motor Symptoms Scale for Parkinson's Disease (NMSS)[21]; hyposmia was assessed in all patients by Sniffin'Sticks Test[22,23]. The presence of REM-sleep behaviour disorder by administration of RBD-screening questionnaire (RBDSQ)[24,25].

Blood pressure (BP) and heart rate were evaluated in the sitting, supine (after at least 5 minutes of rest) and standing positions. OH was defined as a BP fall ≥ 20 mm/Hg systolic or 10 mm/Hg diastolic within 3 minutes of standing[26] and rated as severe OH if the BP fall was ≥ 30 mm/Hg systolic or 15 mm/Hg diastolic BP[27].

We selected four items to cover for different autonomic symptoms known to be associated with worse progression PD[28,29]: orthostatic symptoms, urinary, sexual and bowel dysfunction function were evaluated according to the Unified Multiple System Atrophy Rating Scale (UMSARS) part I[29]. Briefly, the severity of each symptom was scored on a 5-point scale, namely no presence of symptoms (score = 0), rare occurrence/minor impairment (score = 1), weekly occurrence/moderate impairment (score = 2), frequent occurrence/severe impairment (score = 3) and permanent medical therapy needed (score = 4). The cumulative score of dysautonomic symptoms was calculated and defined as Cumulative Dysautonomia Symptoms Scale (CDSS, range: 0-16 points).

A comprehensive, standardized cognitive and behavioural assessment was applied, including Mini-Mental State Examination (MMSE), ten test covering five cognitive domains as previously described [30] and the Neuropsychiatric Inventory (NPI) for the assessment of global cognitive and psychiatric functions[31]. Depressive symptoms were assessed by Beck Depression Inventory – II (BDI-II)[32].

PD-MCI was defined according to level II PD-MCI criteria[33] as an impairment on at least two neuropsychological tests (score below cut-off) with no impact on activities of daily living.

PD malignant subtype was defined as suggested by Fereshtehnejad and collaborators[4] by the presence of at least two out of MCI, OH and RBD.

All patients included in the analyses underwent a clinical and cognitive follow-up at 2 years.

Biochemical analyses

NfL blood measurement were performed at the Maurice Wohl Clinical Neuroscience Institute, London, UK. At the time of assessment, approximately 10 mL venous blood was collected in glass tubes containing sodium ethylenediaminetetraacetic acid (EDTA) from each subject. Participants were required to fast for at least 2 h prior to collection. The blood samples were centrifuged at 2000 x g at 4 °C for 8 min within 2 h of collection. Plasma supernatant was collected, divided into aliquots, and frozen at − 80 °C until further use. Plasma NfL concentration was measured using the Simoa platform (NF-light; Quanterix, Billerica, MA). Samples were randomized, blinded and measured in duplicate using a batch of reagents from the same lot. The intra-assay and inter-assay coefficients of variation were 8.1 and 11.2%, respectively. The limit of detection (LOD) was 0.52 pg/mL and the lower limit of quantification (LLOQ) was 3.26 pg/mL when compensated for a 4-fold sample dilution. Outliers with plasma NfL value above more than 5 standard deviations of the mean were excluded from the study analysis[34].

Statistical analyses.

Group differences were assessed with Mann-Whitney test or chi square for continuous or dichotomic variables, respectively. Partial correlation analyses adjusted for the effect of age, sex and disease duration was applied in order to test significant correlations between NfL values and clinical variables. The association between UPDRS-III, NMSS and MMSE and plasma NfL levels was additionally evaluated using a Linear regression model adjusted for the effect of age, sex, disease duration. Each PD patient was stratified according to the cut-off value established as 80th percentile of healthy individuals in the age-specific category[35], into subject with high and low NfL levels (PD-H-NfL and PD-L-NfL, respectively). Comparisons of clinical features, progression of the two subgroups were performed using Mann-Whitney tests or chi-square, as appropriate. SPSS 24 (IBM, Armonk, NY) was used for statistical analysis.

The ability to predict cognitive and motor impairment was assessed using a general linear model – repeated measures analysis adjusted for age, sex, disease duration and LEDD variation between t0

and t1 ($\Delta LEDD = LEDD_{t1} – LEDD_{t0}$). Plasma NfL levels and established prognostic factors (age, gender, disease duration, UPDRS III score, presence of MCI, OH or RBD) were analyzed by a multivariate linear regression in order to evaluate the best predictors of motor progression at two years. Significance was set at $p < 0.05$ for all the analysis.

RESULTS

Recruitment, clinical and cognitive baseline features

One-hundred and six patients with parkinsonism were consecutively enrolled, and 92 patients with a confirmed diagnosis of PD after two years of follow-up were included in the final analyses.

Fourteen patients were excluded because of i) diagnosis of atypical parkinsonism at follow-up (n = 4 dementia with Lewy bodies, n=3 progressive supranuclear palsy and n=1 corticobasal syndrome) ii) severe vascular chronic encephalopathy at brain MRI (n=2) iii) diagnosis of dementia after cognitive assessment at baseline (n=2) iv) deceased during the follow-up (n= 2) (Supplementary Figure 1).

In PD patients, plasma NfL levels correlated with age ($r = 0.546$; $p = 0.000$) and age at onset ($r = 0.410$; $p = 0.000$) but there was no correlation with gender ($p = 0.19$) and disease duration ($p = 0.142$).

Correlation between plasma NfL and motor and non-motor symptoms

Plasma NfL levels exhibited a positive correlation with total UPDRS-III scores ($r = 0.232$; $p = 0.030$), NMSS total score ($r = 0.280$, $p = 0.025$) and CDSS ($r = 0.394$, $p = 0.002$) (fig. 1). NfL levels correlated also with total UPDRS-I ($r = 0.370$, $p = 0.002$) and UPDRS-II ($r = 0.336$; $p = 0.009$) but no with MMSE scores, BDI-II and NPI. Total NMSS was the clinical variable with the strongest association ($\beta = 0.309$, $p = 0.040$) with plasma NfL in the adjusted linear regression model.

Subtyping of PD patients according to NfL levels

At baseline, sixteen PD patients were classified as PD-H-NfL and 76 showed normal NfL levels.

PD-H-NfL patients were significantly older compared to PD-L-NfL, but comparable for sex and disease duration. PD-H-NfL exhibited significantly higher UPDRS-II, UPDRS-III, NMSS, CDSS and BDI-II scores when compared to PD-L-NfL (table 1 and supplementary table 1). At baseline, PD-H-NfL and PD-L-NfL did not significantly differ neither for MMSE scores nor for the presence of MCI, whereas malignant phenotype was more prevalent in PD-H-NfL patients (37.6 % vs 13.1 % p=0.019) (Fig. 2).

Plasma NfL and clinical variables on progression

Compared to PD-L-NfL, PD-H-NfL patients showed a faster UPDRS-III progression over a follow-up period of 2 years (11.3 vs 0.7 points, p = 0.003) by a linear model repeated measures ANOVA analysis adjusted for the effect of age, sex, disease duration, baseline UPDRS-III and ΔLEDD (Fig. 3). Conversely, no difference in cognitive progression measured by the MMSE was found when adjusted for confounding variables in the model.

In univariate analyses, also OH (p=0.005), RBD (p=0.02), baseline MMSE (p=0.02) and disease duration (p=0.01) were associated with worse UPDRS-III progression at two years of follow-up. Plasma NfL levels and established prognostic factors (age, gender, disease duration, UPDRS III score, presence of MCI, OH or RBD) were analyzed by a multivariate linear regression in order to evaluate the best predictors of motor progression at two years. In analyses without NfL, OH was the best significant predictor of UPDRS-III progression (β = 0.32; p = 0.03). The inclusion of NfL increased the prediction of the linear model and NfL was the only significant predictor of motor progression (β = 0.569; p = 0.005) in the adjusted model (Table 2).

DISCUSSION

This study shows that plasma NfL is associated with measures of disease severity and progression thus supporting the claim that it might represent an accurate and powerful predictor of motor progression beyond phenotype classification in non-demented PD patients.

Several previous works have assessed whether CSF and blood NfL discriminate between PD and atypical parkinsonism [12,13,16,36,37], but only a single work explored the relationships between NfL levels and progression in PD[11,38].

In this study, we found that plasma NfL was associated with motor impairment, in line with previous monocentric reports and recently multicenter validation studies in drug-naïve patients in the larger DeNoPa and PPMI cohorts[11,16].

In addition to this, the findings demonstrated that non-motor symptoms were the clinical features with the strongest association with plasma NfL levels in linear regression analysis. This might indicate that plasma NfL levels reflects the widespread neurodegeneration usually associated with non-motor features, in line with the correlation recently established between plasma NfL levels and brain MRI atrophy in the PPMI cohort[39].

When divided according to age-specific cutoff values, PD patients with abnormal NfL were more severely impaired in motor and non-motor function and showed a higher prevalence of malignant phenotype defined on the basis of presence of RBD, cognitive impairment and orthostatic hypotension in sit-to-stand blood pressure assessment. This subtype of PD[4,5] was indeed recently associated with faster progression, likely related to a wider impairment in nigrostriatal dopaminergic innervation and extensive cortical atrophy[6].

At follow-up, compared to PD-L-NfL, PD-H-NfL subgroup has a more severe UPDRS-III progression after 2-years adjusting for motor and non-motor baseline features. These data expanded the work of Lin and coauthors reporting an association between higher baseline plasma NfL values and an increase of at least 2 points in UPDRS-III[16], as they were confirmed by a multivariate linear

regression model taking in consideration age, disease duration, sex, OH, RBD, cognitive impairment and LEDD variation as independent predictors of motor progression.

Furthermore, plasma NfL exhibited the highest predictivity of motor score progression in addition to the baseline clinical features and phenotype in multivariate linear regression analyses.

At variance with early monocentric reports[16,40], cognitive progression assessed by MMSE score was not significantly different in PD-H-NfL at 2-years of follow-up. It should be underlined, however, that our study excluded a priori patients with dementia, who are known to be associated with increased CSF and plasma NfL levels[14,16,40] and with worse short-term progression[19] and that we did not observed any conversion to dementia in the cohort.

The ability of plasma NfL levels to predict cognitive progression thus still need to be verified in studies with longer follow-up, as this is strongly supported by several studies on peripheral and CSF NfL levels [37,41,42].

Several limitations should be acknowledged. First, a 2-year follow-up period is short to take these results as definitive, so they need further validations in longer on-going larger longitudinal studies and in independent cohorts. Second, the relatively small sample size required the use of non-parametric statistical analysis thus potentially increasing the risk of effect underestimation. Third, the longitudinal changes of plasma NfL were not evaluated, despite recent reports questioned their value in longitudinal cohorts[11,42]. The major strengths of the study were the exclusion of patients with atypical parkinsonism and dementia, the extensive baseline motor and non-motor assessment with clinical subtyping enabling the validation of longitudinal results through multivariate linear regression model.

Despite the limitations associated with an observational single-center study, our results have deep implications for clinical practice and research. The use of plasma NfL should be encouraged as routine marker able to predict motor progression at single-subject level- in addition to clinical subtypes. On

one hand, this can help clinicians in identifying patients who need different pharmacological and non-pharmacological management strategies. On the other, plasma NfL should be implemented in clinical trials for stratifying patients with divergent pattern of progression[11,43].

Conclusions

We found plasma NfL to be strongly associated with disease severity and faster motor progression in PD. NfL could thus be considered a strong peripheral marker reflecting neuropathological progression in PD and should be assessed in clinical practice and trials to predict disease progression at a single-subject level.

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Table 1. Demographic and clinical features of PD patients at baseline and after 2-years follow-up.

	PD (n = 92)	PD-H-NfL (n=16)	PD-L-NfL (n=76)	p - value[$]
Age, years	65.5 ± 10.9	75.8 ± 6.2	63.4 ± 0.5	**0.000**[a]
Sex M, % (n)	61.3 (57)	75 (12)	59.2 (45)	0.185[b]
Disease duration, years	5.5 ± 6.7	7.3 ± 6.0	4.6 ± 6.8	0.120[a]
MDS-UPDRS-III, total score	16.8 ± 9.5	22.3 ± 11.9	15.7 ± 8.5	**0.036**[a]
LEDD, mg/day	386 ± 355	426 ± 307	378 ± 366	0.334[a]
NMSS, total score	39.4 ± 29.8	69.9 ± 35.5	33.4 ± 24.8	**0.001**[a]
MMSE, total score	27.3 ± 3.4	25.8 ± 3.2	27.6±2.9	0.110[a]
OH, % (n)	31.5 (29)	31.3 (5)	22.4 (17)	0.238[b]
RBD, % (n)	29.3 (27)	18.8 (3)	26.3 (20)	0.490[b]
Malignant phenotype, % (n)	17.4 (16)	37.5 (6)	13.1 (10)	**0.019**[b]

NfL, pg/ml	31.3 ± 20.8	68.2 ± 19.4	23.6 ± 10.0	**0.000[a]**
Longitudinal follow-up at 2 years				
MDS-UPDRS-III total score	19.1±13.0	33.6 ± 18.8	16.3±9.5	**0.001[c]**
ΔUPDRS-III	2.3±8.3	11.3 ± 6.9	0.6±6.7	**0.006[c]**

Independent variables	**B**	**Standard error**	**Beta**	***t***	***p*-value**	
Constant	31.039	22.023		1.432	0.159	.521[a]
gender	-0.716	2.594	-0.037	-0.276	0.789	.355[a]
age	-1.360	1.701	-1.673	-0.799	0.428	.181[a]
Disease duration	1.366	1.739	1.072	0.786	0.436	.389[a]
UPDRS-III (t_0)	-0.287	0.185	-0.285	-1.554	0.127	
ΔLEDD	0.008	0.006	0.175	1.0289	0.204	
OH	5.111	2.819	0.246	1.813	0.07	
RBD	-3.466	2.737	-0.170	-1.266	0.212	
MMSE	-0.743	0.500	-0.256	-1.486	0.144	
NfL	**0.284**	**0.095**	**0.569**	**2.945**	**0.005**	

Abbreviations: LEDD, levodopa equivalent daily dose; NMSS, Non-Motor Symptoms Scale; PD, Parkinson's disease; PD-H-NfL PD patient subgroup with higher plasma NfL levels; PD-L-NfL, PD patients subgroup with lower plasma NfL levels; UPDRS, Movement Disorder Society Unified Parkinson's Disease Rating Scale (part III).
[$] Comparison between PD-H-NfL and PD-L-NfL subgroups have been performed by [a] Mann-Whitney *U* test or [b] Fisher test [c] repeated measures model adjusted for the effect of age, sex, disease duration, baseline MDS-UPDRS-III, MMSE

Table 2. Multivariate linear regression models for motor progression defined by UPDRS part III scores in PD cohort.

Abbreviations: LEDD, Levodopa Equivalent Daily Dose; ΔLEDD = ($LEDD_{t1} - LEDD_{t0}$); MMSE = Mini Mental State Examination score at the baseline; nfL, Neurofibrillary light chain; OH, Orthostatic Hypotension; RBD, REM sleep Behaviour disorder; UPDRS-III, Movement Disorder Society Unified Parkinson's Disease Rating Scale part III.

FIGURE CAPTIONS

Figure 1. Correlation between plasma NfL levels and MDS-UPDRS-III (a) and NMSS scores (b)

Abbreviations. MDS-UPDRS-III = Movement Disorder Society Unified Parkinson's Disease Rating Scale, part III. NMSS = Non Motor Symptoms Scale. r = Pearson's correlation coefficient.

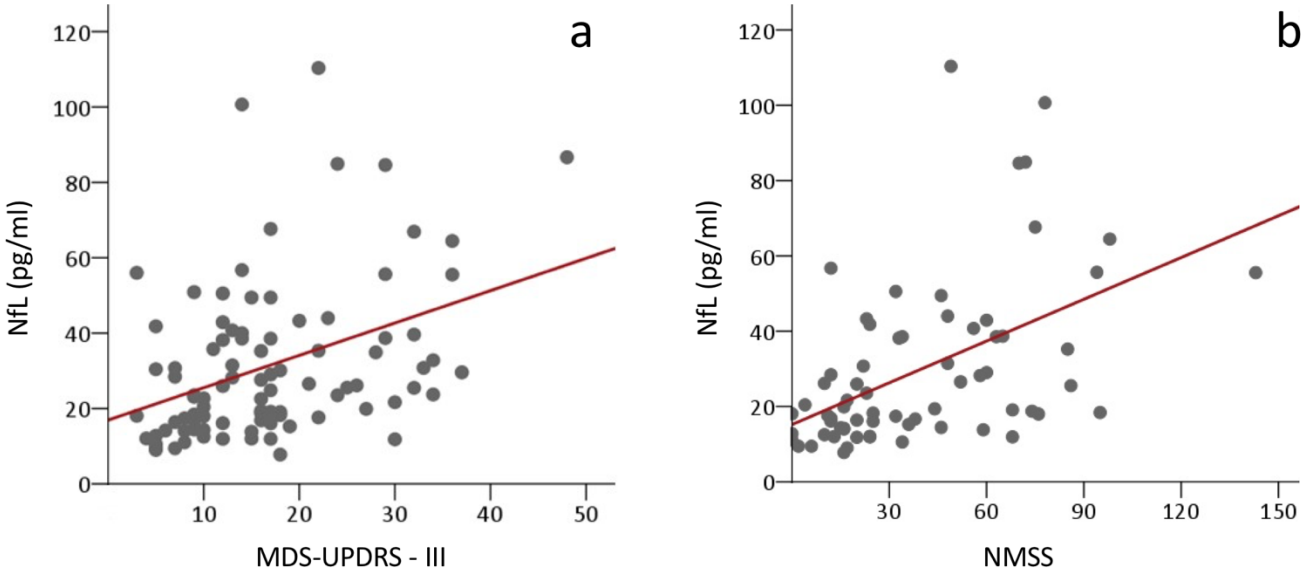


Figure 2. Proportions of PD patients with MCI, OH, RBD and malignant subtype between PD-L-NfL and PD-H-NfL subgroups. Significant p-value is shown above the columns. p-values are obtained with chi-square test. **Abbreviations.** MCI, mild cognitive impairment; RBD, REM – sleep behaviour disorder; OH, orthostatic hypotension.

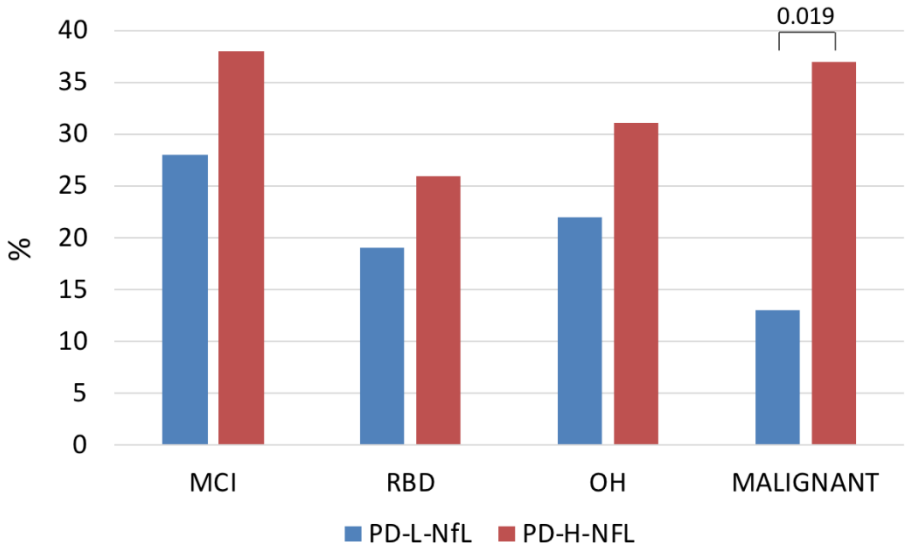
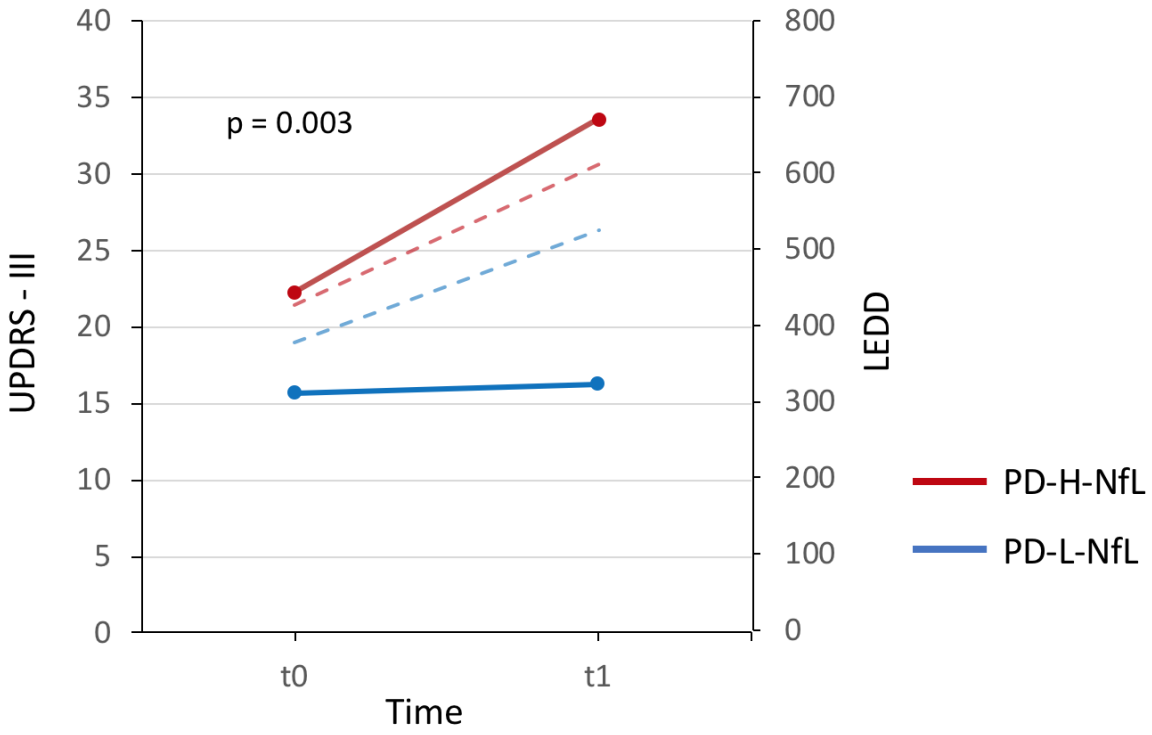
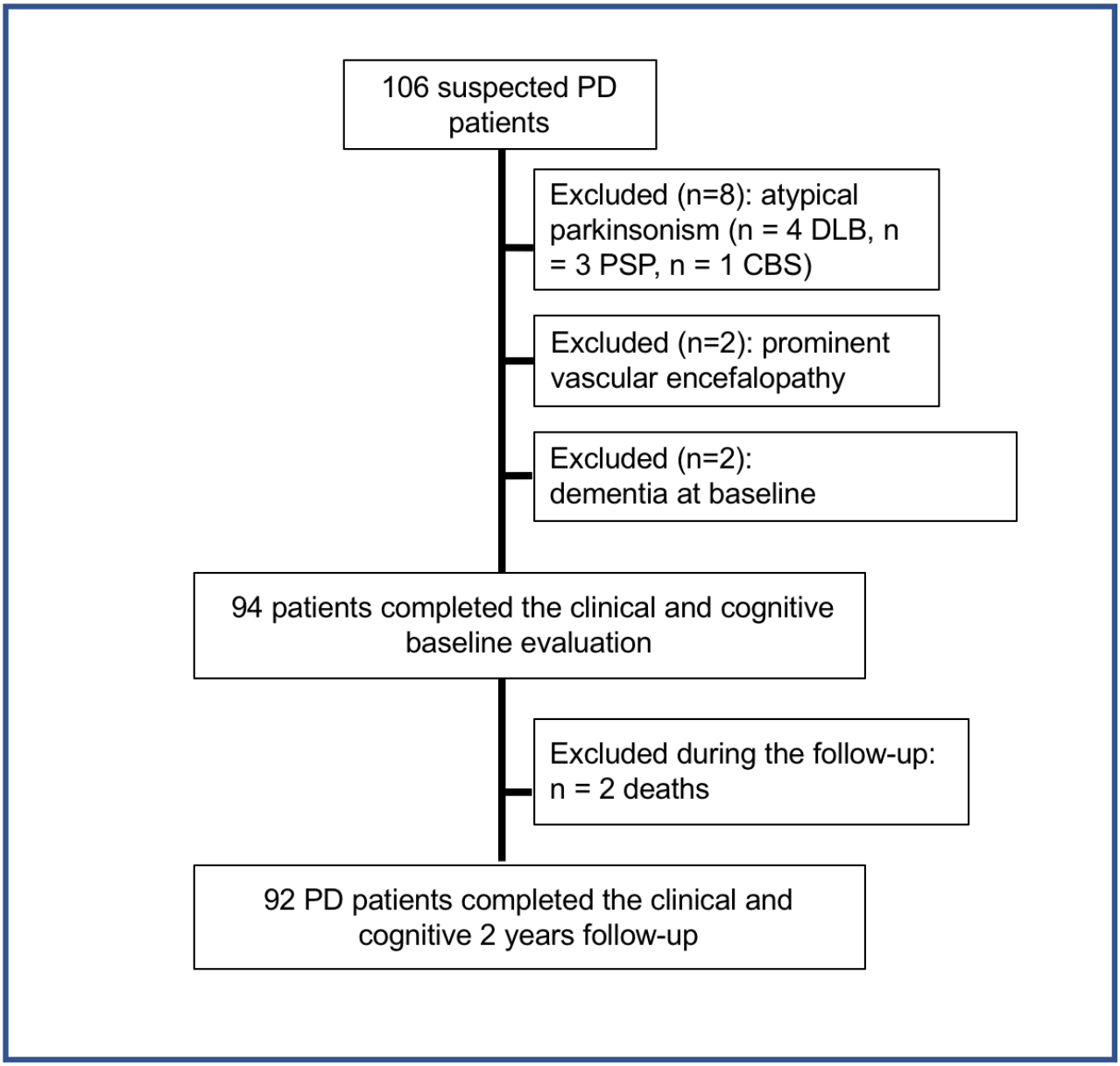


Figure 3. UPDRS-III score progression over 2-years follow-up in NfL High and NfL Low subgroup (continuous lines). LEDD progression for the two subgroups is showed as dashed lines.



Abbreviations. UPDRS-III = Unified Parkinson's Disease Rating Scale, part III; LEDD = Levodopa Equivalent Daily Dose. t0 = baseline; t1 = 2 years. p-value refers to repeated measures - GLM analysis of UPDRS – III progression of PD-H-NfL and PD-L-NfL subgroups.

Supplementary Figure 1. Flowchart of patient enrolment and final study sample


106 suspected PD patients
Excluded (n=8): atypical parkinsonism (n = 4 DLB, n = 3 PSP, n = 1 CBS)
Excluded (n=2): prominent vascular encefalopathy
Excluded (n=2): dementia at baseline
94 patients completed the clinical and cognitive baseline evaluation
Excluded during the follow-up: n = 2 deaths
92 PD patients completed the clinical and cognitive 2 years follow-up

Supplementary table 1. Additional clinical variables in PD-L-NfL and PD-H-NfL

	PD (n = 92)	PD-H-NfL (n=16)	PD-L-NfL (n=76)	p - value[$]
Age, years	65.5±10.9	75.8±6.2	63.4 ± 0.5	**0.000[a]**
UPDRS-I	8.2±5.4	12.2±7.8	7.5±4.6	0.075[a]
UPDRS-II	5.8±5.4	11.1±7.8	5.0±4.6	**0.025[a]**
UPDRS-III	16.8±9.5	22.3±11.9	15.7 ± 8.5	**0.036[a]**
UPDRS-IV	1.4±3.3	1.0±2.2	1.5±3.5	0.786[a]
LEDD, mg/day	386±355	426±307	378±366	0.334[a]
OH, % (n)	31.5 (29)	31.3 (5)	22.4 (17)	0.238[b]
RBD, %	29.3 (27)	18.8 (3)	26.3 (20)	0.490[b]
Malignant phenotype, % (n)	17.4 (16)	37.5 (6)	13.1 (10)	**0.019[b]**
Sniffing's sticks, score	5.6 ±3.1	4.4 ±3.3	6.3±2.7	**0.780**
NMSS, total score	39.4±29.8	69.9±35.5	33.4±24.8	**0.001[a]**
CDSS, total score	3.0±2.2	4.7±2.2	2.8±2.1	**0.029[a]**
MMSE, total score	27.3±3.4	25.8±4.7	27.6±2.9	0.110[a]
BDI-II, total score	7.3±6.4	13.8±8.2	6.6±5.8	**0.035[a]**
NPI, total score	8.1±7.0	12.8±14.0	7.2±4.8	0.747[a]