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NfL as a biomarker for neurodegeneration and survival in Parkinson disease

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Abstract

Objective: To determine if Neurofilament Light chain protein in cerebrospinal fluid (cNfL); a sensitive biomarker of neuroaxonal damage, reflects disease severity or can predict survival in Parkinson's disease (PD).

Methods: We investigated if disease severity, phenotype or survival in patients with new-onset PD correlates with cNfL concentrations around the time of diagnosis in the population-based NYPUM study cohort (n = 99). A second, larger new-onset PD cohort (n = 194) was used for independent validation. Association of brain pathology with the cNfL concentration was examined using striatal dopamine transporter imaging and repeated diffusion tensor imaging, at baseline, 1 and 3 years.

Results: Higher cNfL in the early phase of PD was associated with greater severity of all cardinal motor symptoms except tremor, in both cohorts, and with shorter survival and impaired olfaction. cNfL concentrations above the median of 903 ng/L conferred an overall 5.8 times increased hazard of death, during follow-up. After adjustment for age and sex, higher cNfL correlated with striatal dopamine transporter uptake deficits and lower fractional anisotropy in diffusion tensor imaging of several axonal tracts.

Conclusions: cNfL shows usefulness as a biomarker of disease severity and to predict survival in PD. The present results indicate that the cNfL concentration reflects the intensity of the neurodegenerative process, which could be of importance in future clinical trials.

Classification of Evidence: This study provides Class II evidence that in patients with PD, cNFL concentrations are associated with more severe disease and shorter survival.

Introduction

The prognosis, life expectancy and clinical presentation in Parkinson's disease (PD) are highly variable.[1-4] For example, patients with PD that present with resting tremor have milder disease and longer survival compared to patients that present with gait disturbance.[4-6] Insufficient understanding of the mechanisms driving neurodegeneration, and the lack of biomarkers to dynamically measure their rate, hamper the development of disease-modifying therapies for PD.

A promising biomarker in neurodegenerative diseases is the neurofilament light chain protein (NfL), which provides a sensitive measurement of neuroaxonal damage, irrespective of cause.[7] NfL in cerebrospinal fluid (cNfL) was higher in PD compared with healthy controls in some, but not all studies.[8,9] While many patients with PD have normal levels,[10] we previously showed that high cNfL in early PD predicts later progression to PD dementia.[8] In animal models of PD, experimental induction of alpha-synuclein deposition in neurons is associated with marked increases of cNfL.[11] Higher cNfL levels might be explained by a more aggressive and widespread neurodegeneration in a subset of patients with PD.

Among idiopathic parkinsonian disorders, increased cNfL is particularly evident in multiple system atrophy, progressive supranuclear palsy and corticobasal syndrome,[9,12,13] *i.e.*, diseases with more extensive neurodegeneration than in typical PD. Technological advancement has now allowed sensitive quantification of NfL in blood, which may allow accessible monitoring.[7] However, NfL is not well studied as a biomarker of neurodegeneration in PD. The primary aim of this study was to investigate if the severity of PD is related to cNfL levels and if cNfL levels predict survival. To further investigate possible corrrelation with

neurodegeneration, striatal dopamine transporter (DAT) imaging, and specific symptoms such as hyposmia were also studied in relation to cNfL, as secondary endpoints. Finally, since MR diffusion tensor imaging (DTI) is a sensitive marker for axonal tract damage in early-stage PD,[14] and may expose regional patterns of disease pathology in relation to cNfL increases, we performed repeated DTI in the population-based PD cohort.

Methods

Study populations

All patients in the original cohort participated in a population-based incidence study of unselected cases of new-onset idiopathic parkinsonism from a geographic catchment area of ~142,000 inhabitants in northern Sweden (the NYPUM study). The inclusion period was between January 1, 2004, and April 30, 2009. A population screening procedure was performed to avoid selection bias and to make PD case identification as complete as possible.[15] Patients with atypical parkinsonism (*e.g.*, multiple system atrophy or progressive supranuclear palsy, n = 31), secondary parkinsonism (*e.g.*, drug-induced parkinsonism or stroke) or dementia at baseline, defined by an Mini Mental State Examination (MMSE) score <24 and clinical symptoms, were excluded. These patients are described in previous publications.[4,8] Patients with incident PD (n = 143) were included and followed up prospectively with standardized clinical examinations, including the modified Hoehn and Yahr (HY) and Unified Parkinson's disease Rating Scales (UPDRS), at least yearly. Medication was documented by Levodopa equivalent daily dose (LEDD). MRI and presynaptic imaging of dopamine transporter status with ^{123}I-FP-CIT single-photon emission computed tomography (SPECT) were done repeatedly. Of the 143 enrolled patients with PD, 99 agreed to CSF collection by lumbar puncture at study entry (baseline). The patients who

declined collection of CSF (n = 44) were older than the patients who participated (74.3 vs 69.8 years) but had comparable scores for mood, motor, and cognitive dysfunction.

For validation, a second cohort consisting of all patients with new-onset, idiopathic parkinsonism referred from primary care to the neurological department at Umeå university hospital (which is the only neurological department in the area), from May 2009 through September 2018 (the validation cohort) was investigated. All patients with PD were offered a lumbar puncture for CSF analysis around the time of diagnosis. 194 patients with new-onset PD agreed to perform CSF collection and were included. In agreement with the exclusion criteria in the original cohort (the NYPUM cohort), patients with secondary parkinsonism, atypical parkinsonism or dementia at baseline were excluded. All patients were investigated by neurological examination and motor assessments at baseline. In both cohorts, a diagnosis of PD required agreement among the examiners (neurologists specialized in movement disorders) that the clinical criteria for the diagnosis were fulfilled based on the UK PD Society Brain Bank criteria.[16] The diagnosis of PD was neuropathologically verified at autopsy in six cases in the population-based cohort.

For comparison, 30 healthy control participants that were age- and sex-matched to the first 50 patients included in the NYPUM cohort were recruited. Controls were recruited by advertisements in the local newspaper, and in a few cases among friends and family of the PD participants. Requirements for controls were that they had no neurological disorders, had a normal neurological exam, and normal ^{123}I-FP-CIT SPECT brain imaging.

Standard protocol approvals, registrations, and patient consents

All participants provided informed consent. The study was approved by the Regional Medical Ethics Board in Umeå, Sweden (Um dnr 03-387, dnr 2014-163-31M), and was performed in accord with the Declaration of Helsinki.

Clinical evaluation

In both cohorts, motor function was assessed in the early phase of PD; prior to the start of dopaminergic medication at baseline, using the HYscale and the UPDRS. In the NYPUM cohort, the UPDRS scores were divided into subscores for tremor (sum of items 20 and 21) and postural imbalance and gait difficulty (PIGD; the sum of items 13-15, 29, and 30).[17] Bradykinesia (measured by the sum of UPDRS items 23-26 and 31) and axial symptoms (measured by the sum of UPDRS items 18, 27-29 and the neck rigidity of item 22) was also investigated. Data on UPDRS-subscales was lacking in the validation cohort. Instead, the most predominating symptom that first lead the patient to contact a health care service was recorded, and classified as (1) resting tremor, (2) bradykinesia (including, *e.g.*, micrographia and ”clumsiness”), (3) balance impairment or gait difficulty (*e.g.*, shuffling gait, freezing of gait or postural imbalance) or (4) other symptom. In the NYPUM cohort, olfactory function was tested at baseline and after 1 year by the 12-item Brief Smell Identification Test.[18] Mobility was measured by the Timed-Up-and-Go (TUG) test, which is the time it takes to rise up from a chair, walk 3 meters, and sit down again.[19]

Survival

After inclusion, all surviving patients in the population-based cohort (NYPUM) were followed yearly for approximately 8.5 to 13.5 years, until August 31, 2017. The surviving

patients in the validation cohort were followed until October 31, 2018. The survival data was complete for both cohorts. All-cause mortality was studied as the relevant outcome.

Imaging with SPECT and cerebrospinal fluid analysis

All 99 patients with PD from whom CSF was collected in the NYPUM cohort, all 194 patients in the validation cohort, and 30 age-matched healthy controls, underwent presynaptic DAT imaging by ^{123}I-FP-CIT (DaTSCAN; GE Healthcare BV, Eindhoven, the Netherlands) SPECT at baseline. All patients had a pathological scan. The DAT imaging protocol in the population-based study (NYPUM) was performed within a non-profit trial (EU no. 2009-011748-20) and constituted a sub-study within the research project. Semiquantitative analysis (based on regions of interest) and visual evaluation of the DAT SPECT were done unbiased by clinical information. Normal reference values were derived from the healthy control participants,[20,21] and reduction of DAT uptake in the putamen and caudate of the patients with PD was measured in standard deviations of the normal values. The imaging protocol, equipment, and semiquantitative evaluation methods were described earlier.[20] Two different SPECT cameras were used during the course of the population-based study; one brain-dedicated SPECT camera (the Neurocam) was later substituted by a multipurpose hybrid SPECT/CT (both General Electric, Milwaukee, WI). Twenty-two patients performed DAT imaging in the Neurocam and 77 in the hybrid SPECT/CT.

Standard procedures for lumbar puncture for collection of CSF were used, with the patient lying in the decubitus position. In the validation cohort, the lumbar puncture was generally performed somewhat later than in the NYPUM study, within 2-3 months from the first (baseline/diagnostic) visit. While on dopaminergic treatment, CSF collection was repeated in the NYPUM cohort after 1 year and 3 years in 53 and 35 patients, respectively. The patients

who participated in repeated CSF collection had a lower HY stage than the other PD patients (1.9 vs 2.3 at 1 year) but comparable ages and UPDRS scores. CSF levels of NfL were measured with a sandwich enzyme-linked immunosorbent assay (NF-Light; UmanDiagnostics AB, Umeå, Sweden), as described by the manufacturer.[22,23] The coefficient of variation was 14.0%. All analyses were performed by experienced, board-certified laboratory technicians, using procedures approved by the Swedish Board for Accreditation and Conformity Assessment. The CSF analyses were performed blinded to all clinical data.

Diffusion tensor imaging

In the NYPUM cohort, 45 of the 99 PD patients from whom CSF was collected at baseline conducted a 3.0 Tesla MRI scan at baseline. Twenty and 14 patients conducted both the MRI scan and CSF collection at the 1 and 3-year follow-up, respectively. MR DTI was performed using single-shot spin echo EPI sequences with the following parameters: TR = shortest, TE = 77 ms, flip angle = 90°, FOV = 224*224 mm, acquisition matrix = 112*112, reconstruction matrix = 256*256, b-value = 1100 s/mm2, # slices = 70 (continuous), 16 gradients and 2 mm slice thickness. One non-gradient (B0) volume was also sampled. The extracted B0- image was used in the procedure of realignment and motion and eddy current correction,[24] applying linear/quadratic terms for the spatial model of the eddy current induced field and linear terms for the model of the diffusion gradients. Postprocessing of the images included conversion from DICOM to NIFTI format, eddy current correction, and brain extraction. The images were processed using the diffusion toolbox FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT). All images were visually checked after correction and no sample was disregarded based on improper correction. The DTI map of fractional anisotropy (FA) was generated by fitting diffusion tensors to the corrected data. The postprocessing followed the Tract-Based Spatial

Statistics method, part of the FSL package.[25] The most typical subject was used for normalization. Randomize (part of the FSL package) was finally used to reveal areas in the brain where FA correlated negatively with cNfL levels (i.e., lower FA correlated with higher NfL).

Statistics

Baseline correlations between PD phenotype, severity scales, striatal DAT imaging uptake, and cNfL concentrations were tested by Spearman ρ or Pearson r, as appropriate. Differences in cNfL concentrations between the PD cohorts, PD subtype groups, and between patients and controls were tested by one-way ANCOVA, to allow adjustment for age, sex and disease duration. These tests were conducted after log transformation of cNfL values, to obtain normal distributions. Associations between PD phenotype, severity measures and cNfL in the larger, pooled cohort (n = 293) were also analyzed using multiple linear regression, and differences per unit of change of cNfL (measured by Beta values) were estimated with and without adjustments. In these analyses, the normality of data was assessed by inspection of residuals. Possible change of cNfL over time was investigated by Wilcoxon Signed Rank Test. Cox proportional hazards regression was used to investigate if the baseline cNfL level predicted mortality. Kaplan-Meier plots show effects on survival of a baseline cNfL concentration below/above the overall median in PD (903 ng/L) and in the highest and lowest quartile. The predictive value of baseline cNfL levels were described by Area Under the ROC curve (AUROC), and levels below/above cut-offs with the highest Youden Index (sensitivity+specificity -1) was investigated for all patients that were followed until death or 5 or 8 years. To investigate cNfL in relation to striatal dopaminergic denervation, DAT-uptake ratios were normalized by average standard deviations above or below the normal mean (*i.e.*, z score) in order to equate the numerical values derived from the two different scanners that

were used. Negative correlation between white matter integrity measured by fractional anisotropy and the cNfL concentration was analyzed. All correlations were adjusted for age and sex. In order to not exclude possible brain regions of interest a priori, the 10 largest contiguous "correlation clusters" (all being >30 voxels), for each occasion (baseline, 1 year, and 3 years), where all voxels exceeded the significance threshold $p < 0.05$ (uncorrected) are reported.

$P < 0.05$ was considered significant. However, in analyses with several tests (the comparisons shown in table 1, 2 and 3), Tukey HSD and Holm-Bonferroni correction were applied to correct for multiple comparisons. All statistical analyses were performed using SPSS 23.0; SPSS Inc.

Data availability

Anonymized data can be obtained by request from any qualified investigator for purposes of replicating procedures and results.

Results

Baseline characteristics of the cohorts

A total of 293 patients with incident, treatment-naïve PD (38.6% female and 61.4% male), and 30 neurologically healthy controls were included in this study. At baseline, there were no major demographic differences between the healthy controls and the patients with PD or between the PD patients in the population-based (NYPUM) and validation cohorts (table 1). However, the patients with PD in the population-based cohort were marginally older than the patients in the validation cohort (69.8 years v.s. 68.0 years, $p < 0.05$), and had higher HYand

UPDRS scores ($p < 0.001$), and higher cNfL concentrations ($p < 0.05$). Their cNfL concentrations were also higher compared with the healthy controls ($p < 0.05$), and this difference remained significant after adjusting for age and sex. In both PD cohorts, as well as in the controls, cNfL concentrations correlated positively with age (r range = 0.54 - 0.63). The variance of cNfL tended to be higher in PD than in the controls (figure 1A), and cNfL concentrations were similar in male and female patients (median 950 versus 798 ng/L, $p = 0.101$). In the six patients with an autopsy-verified diagnosis of PD (who had a mean age of 73.3 years at baseline), the baseline cNfL concentration ranged from 504 to 3490 ng/L and the baseline median was 1852 ng/L.

cNfL, disease severity and survival in Parkinson's disease

In both PD cohorts, the cNfL concentration correlated positively with the motor symptom severity as measured by clinical scales, with the exception of tremor (table 2), with and without adjustment for age, sex and disease duration and (at 1 year) medication. At baseline, after also adjusting for multiple comparisons, a higher cNfL concentration correlated positively with the total UPDRS score, the UPDRS III score, the bradykinesia and axial symptom subscores, the Timed-up-and-Go (TUG) test, and the severity of hyposmia (as measured by B-SIT) in the NYPUM cohort. The correlations between symptom severity and cNfL were in the range of $r = 0.28 – 0.48$ (p values between 0.005 and < 0.001, table 2). Tremor showed no correlation. At the 1-year follow-up in the NYPUM cohort, a positive correlation remained between the baseline cNfL concentration and the severity of all tested symptoms (the PIGD, bradykinesia, and axial subscores and with hyposmia) except tremor. In the validation cohort, a positive cNfL-correlation was confirmed for the UPDRS III and total UPDRS scores, and there was a significant correlation with the HY stage. In the NYPUM cohort, patients with tremor-dominant PD at the first visit had similar cNfL concentrations

compared with the healthy controls, while patients with Mixed- or PIGD subtypes had higher concentrations (median difference 238 ng/L, $p < 0.05$, and 440 ng/L, $p < 0.05$, respectively). However, after adjustment for age and sex, all PD phenotypes had significantly higher cNfL concentrations than the controls. The cNfL concentration also differed in relation to the major presenting symptom in the validation cohort (table 3), with patients presenting with balance impairment or gait difficulty (n = 30) having a higher cNfL concentration than patients presenting with bradykinesia ($p < 0.05$), or resting tremor ($p < 0.01$). These differences remained significant after age-adjustment. In patients with repeated measurements, cNfL did not change significantly between baseline and the 1-year follow-up but increased between the 1 and 3-year follow-ups (median increase: 98 ng/L, $p = 0.030$).

In the NYPUM cohort, the follow-up was complete until 8 years. Of the 99 patients with CSF samples in this cohort, 13 (13.1%) had died at 5 years and 27 (27.3%) at 8 years. Nine patients died during follow-up in the validation cohort. The most common cause of death in all patients with PD was pneumonia, consistent with a previous study.[4] Other causes were described in the same study. A higher cNfL concentration at baseline predicted a shorter survival in the NYPUM cohort ($p < 0.001$) and this finding was confirmed in the validation cohort ($p < 0.001$). The hazard ratio for death during follow-up increased 1.36 times in the NYPUM cohort and 3.57 times in the validation cohort, per 1 ng/mL increase in cNfL (table 4). Considering all 293 patients in the pooled PD cohort, cNfL concentrations above the overall median of 903 ng/L around the time of diagnosis (as measured by the baseline sample) conferred a 5.8 times higher hazard for death during follow-up (95% CI: 2.82-11.85, $p < 0.001$; table 4, figure 1). Among patients with a low baseline cNfL concentration, below 965 ng/L and 925 ng/L (which were the cut-offs with the highest Youden index, and similar to the levels in the controls), 100% and 91% of the patients survived during the next 5 or 8 years,

respectively. The area under the ROC-curve (AUROC) for predicting survival by the cNfL concentration was slightly higher at the earlier (AUROC = 0.79, $p < 0.001$) compared to the later (AUROC = 0.75, $p < 0.001$) time point.

The relative impacts of age and cNfL on disease severity

Older age in PD correlated with higher cNfL concentration (increasing approximately 38 ng/L per year). However, all associations between cNfL and clinical features and survival remained significant after adjustment for age. An increase in one unit of cNfL at baseline (in ng/mL) in the pooled PD cohort was associated with an increase in the HY stage by 0.2 ($p < 0.001$), in the UPDRS III by 4.1 points ($p < 0.001$), and in the total UPDRS by 3.9 points ($p < 0.001$). The increases in clinical scores were somewhat lower after adjustment for age and sex (table 2). However, as shown by higher standardized ß values in the multivariable models, the cNfL concentration was a stronger predictor of UPDRS scores than age. The standardized ß for cNfL was 0.24 ($p < 0.001$) and for age 0.13 ($p = 0.012$) in prediction of the UPDRS III score, and 0.21 ($p = 0.002$) and 0.18 ($p = 0.010$), respectively, in prediction of the total UPDRS score (showing a stronger impact of cNfL than of age). Age contributed slightly more than cNfL to predict the HY stage, but cNfL also contributed significantly.

The association between high baseline cNfL and shorter survival in PD remained significant after adjustment for age and sex in both cohorts, as well as in the pooled cohort (table 4). In the validation cohort, high baseline cNfL was a stronger predictor for death during follow-up than was the age of the patient, rendering age at baseline a non-significant covariate ($p = 0.183$).

cNfL in relation to brain imaging

The severity of striatal ^{123}I-FP-CIT uptake deficits was positively correlated with the cNfL concentration at baseline (table 5). The strongest correlation was found in the right hemisphere and in the caudate nucleus. The anatomical locations where axonal fiber disintegrity, as measured by the FA value derived from DTI, correlated with a higher cNfL in clusters with at least > 30 voxels are shown in table 5. Associations between cNfL concentration and axonal lesions on DTI were found, *e.g.*, in the anterior and posterior limb of internal capsule unilaterally (at the right side) at the level of the thalamus at baseline. This finding was more pronounced and found bilaterally at a similar location after 1 year, together with several cNfL-associated lesions on DTI in frontal lobe axonal tracts in proximity to the cortex, and at 3 years in the pons and, among other locations, the limbic lobe (table 5, figure 2).

Discussion

In this population-based and clinical study of incident PD, we investigated if high CSF neurofilament light chain protein (cNfL) levels in the early disease phase reflect disease severity and risk of increased mortality at follow up. We show that higher cNfL was related to more severe PD symptoms, as measured by clinical scales, and shorter survival in the population-based NYPUM cohort, as well as independently, in the validation cohort. These relationships were evident although the absolute cNfL values, at the group level, were not particularly high in PD in comparison with many other neurodegenerative diseases, such as frontotemporal dementia or amyotrophic lateral sclerosis.[7] At the subgroup level, however, the cNfL values were higher in PD patients presenting with postural instability or gait difficulty, compared with PD patients with other phenotypes.

In the population-based NYPUM cohort, all patients with incident idiopathic parkinsonism in the studied area, rather than the referred cases, were included to avoid selection bias. This PD cohort should, therefore, provide information that is generalizable to the "real-life" experience of the population with idiopathic PD. Of note, some differences between the population-based- and the validation cohort (consisting of the referred, consecutive patients with new-onset PD) were found. The HY stage and the disease severity as measured by the UPDRS were higher in the population-based cohort. The cNfL levels in the population-based cohort was higher than in the validation cohort, and this was not explained by the minor age difference. Even so, the relationships between higher cNfL and increased PD severity and higher all-cause mortality were similar in both cohorts.

In the validation cohort, the follow-up period was shorter and there were fewer fatalities than in the NYPUM cohort, but the increase in the hazard ratio (HR) for death per unit of baseline cNfL increase (HR: 3.6) was higher. cNfL was a stronger predictor of death during follow up than age in this cohort. These characteristics may indicate that cNfL is a particularly sensitive marker of increased mortality in early PD.

The reason for the marked disease-heterogeneity in PD is largely unknown but could relate to differences in the pattern and/or extent of neurodegeneration.[26-28] Tremor-dominant PD is consistently associated with a more benign prognosis and less striatal dopamine depletion, compared with non-tremor dominant phenotypes.[29-31] The tremor-dominant phenotype is also associated with a lower risk of dementia, less cortical pathologies and a longer life expectancy.[4,5,32] In the present study, more severe bradykinetic-rigid and/or axial symptoms, and postural instability and gait impairment were related to higher cNfL, while tremor was unrelated. This may be consistent with less nigrostriatal degeneration as well as less cortical

pathology in tremor-dominant PD. Our results add to the hypothesis that resting tremor may reflect a relatively benign circuit-dysfunction in PD.[5]

Anatomical distribution of brain lesions

DTI is an MRI technique that measures water diffusivity in white matter axon tracts. FA represents the directionality of diffusion, which is particularly sensitive to microstructural integrity in axons.[33] Axonal tract degeneration is detectable early in PD, and there is evidence that diffusion tensor imaging changes precede changes observed on conventional, structural MRI, such as atrophy.[14] Brain areas that have shown altered diffusion in PD include substantia nigra and thalamic projections, the projection fibers from substantia nigra to the striatum, and subcortical-cortical, callosal and cortical pathways such as the superior longitudinal fasciculus. [33-39]

cNfL-correlated diffusion impairments in this study were found, among other locations, in internal capsule axon tracts unilaterally (at the right side) at the level of the thalamus at baseline, and after 1 year more markedly and bilaterally in a similar location. At 3 years, such cNfL-associated lesions were found in right-sided superior corona radiata (possibly in axon tracts extending from the internal capsule) in proximity to the frontal cortex, and in the cingulum and pons. This longitudinally developing pattern of cNfL associated axon fiber disintegrity on DTI may reflect a progressive, spreading pathology in PD. Although the resolution limitations call for some caution, the observed pattern could suggest the spread of alpha-synuclein disease pathology in connected axonal projecting systems of the brain, with a predominant caudo-rostral course. The early finding (at baseline and the 1-year follow-up) of several cNfL-associated lesions in association fiber tracts of the cerebral hemispheres, such as

the superior longitudinal fasciculus and axon tracts of the frontal lobe, is in agreement with previous findings in PD.[38,39]

SPECT and PET studies in PD consistently show more prominent denervation of dopaminergic neurons in the putamen than in the caudate nucleus. The caudate nucleus is affected later, as a result of disease progression.[21,40] Early denervation in the caudate nucleus, which was related to higher cNfL in the present study may, therefore, be a more sensitive marker of rapidly progressive neurodegeneration, compared with the putamen. Impaired function of the caudate nucleus is also related to a higher risk of developing dementia and shorter life expectancy in PD.[8,41] The reason for the possible right-sided disease predominance in our imaging data is unknown, but was also observed in a previous study.[41]

Our center has extensive experience in using DTI for visualizing neuronal fiber disintegrity.[37,42] Even so, it is not clear exactly what the cNfL-related white matter lesions on DTI represent. They may represent other pathological processes than the alpha-synuclein proteopathy of PD. NfL concentration is also, for instance, increased by ischemic small vessel disease[43] and Alzheimer disease.[44] Co-morbid brain pathology such as ischemic small vessel disease and/or Alzheimer disease pathology could contribute to increased mortality in PD and DTI findings, and may relate to balance and gait impairment in PD.[45,46] However, the finding that increased cNfL is associated with lower striatal ^{123}I-FP-CIT uptake, higher scores in validated PD severity scales, and with hyposmia, indicate that the increase of cNfL is at least partially specific for PD neurodegeneration. Furthermore, the cNfL related lesions on DTI were not found in areas typically affected by chronic, ischemic vascular injury, such as in the periventricular white matter.[47] Hence, the cNfL levels appear to reflect the intensity of the neurodegenerative process of PD. In parkinsonism, high cNfL could increase the suspicion of

either a more agressive form of PD or (if atypical features are present) atypical parkinsonism. Because of the close correlation between cNfL and NfL measured in blood, the biomarker qualities of cNfL are also likely to pertain to even more accessible blood NfL.[48,49]

The possibility of uncontrolled confounding factors and the limited number of neuropathologic diagnoses and healthy control participants are further limitations in this observational study. The risk of incorrect PD diagnosis was nonetheless minimized by long follow-up by specialized neurologists at a movement disorders unit, and the finding of pathologic uptake on DAT imaging in all patients. The population-based and prospective study designs are strengths. The possibility of unspecific findings on DTI, as discussed above, limits inferences in relation specifically to PD pathology. However, the repeated DTI in patients with PD enables a search for disease patterns that are likely to change over time.

In conclusion, no validated neurochemical biomarker exists in PD to measure disease activity and to predict prognosis. NfL is likely to have clinical utility in PD for several purposes, *e.g.* as shown by the ability to predict disease severity and long term survival in two different PD cohorts. NfL may enhance the selection of patients with PD in clinical trials by enrichment of patients with a faster disease progression. Further research is needed to determine if NfL in CSF and/or blood can be used to longitudinally monitor the progression of PD, or if NfL increases can be reversed by neuromodulatory therapy.

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Appendix 1. Authors

Name	Location	Contribution
David Bäckström, MD, PhD	Umeå University, Sweden	Conception and design of the study, acquisition and analysis of data, drafted the manuscript for intellectual content.
Jan Linder, MD, PhD	Umeå University, Sweden	Acquisition and interpretation of data, revised the manuscript for intellectual content.
Susanna Jakobson Mo, MD, PhD	Umeå University, Sweden	Acquisition and interpretation of data, revised the manuscript for intellectual content.
Katrine Riklund, MD, PhD	Umeå University, Sweden	Interpretation of data, revised the manuscript for intellectual content.
Henrik Zetterberg, MD, PhD	Gothenburg University, Sweden and UCL Queen Square Institute of Neurology, London, UK	Acquisition and interpretation of data, revised the manuscript for intellectual content.
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Figure 1. Survival in Parkinson's disease in relation to baseline cNfL concentration

A. Boxplots of baseline cNfL levels

B. NYPUM cohort

C. Validation cohort

D. Pooled cohort

Figure legend: A: Boxplots of baseline cNfL levels in Parkinson's disease. Cumulative survival for patients with a baseline cNfL below the median concentration of 903 ng/L (blue line) compared with those with cNfL above 903 ng/L (red line) in the population-based NYPUM cohort (B) and the validation cohort (B). C: Cumulative survival in the combined, pooled cohort for patients (n = 293) with baseline cNfL levels in the lowest (< 660 ng/L; blue line) and highest (> 1255 ng/L; green line) quartile, and those with concentrations between these levels (red line). Abbreviations: cNfL = Neurofilament light chain protein in cerebrospinal fluid

B. Number at risk, year	0	4	8	12
NfL < 903 ng/L	38	38	36	6
NfL > 903 ng/L	61	53	36	1

C. Number at risk, year	0	4	8
NfL < 903 ng/L	109	51	3
NfL > 903 ng/L	85	32	0

D. Number at risk, year	0	4	8	12
NfL < 660 ng/L	76	46	19	3
NfL 660 to 1255 ng/L	143	78	32	4

NfL > 1255 ng/L	74	50	24	0

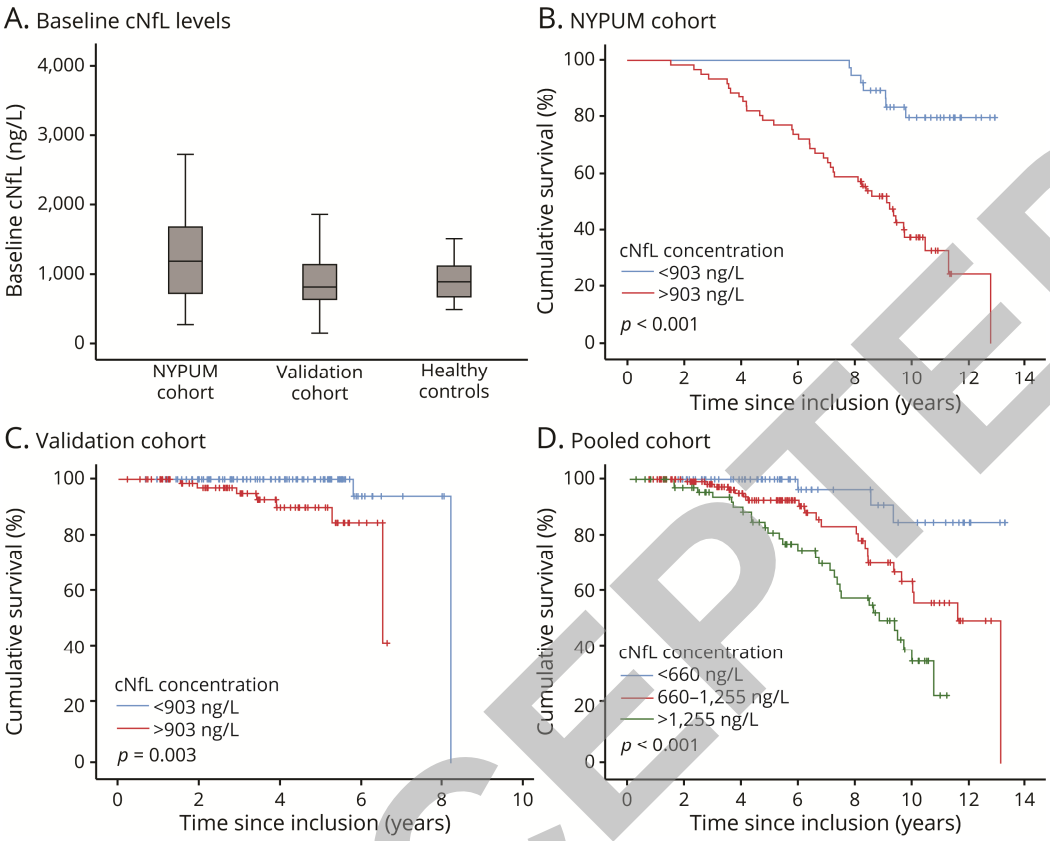

Figure 2. Imaging of impaired diffusion associated with cNfL elevations in Parkinson's disease

At Baseline At 1 year At 3 years

Figure legend: Brain diffusion tensor imaging of patients in the NYPUM cohort. Imaging was performed in 45, 20 and 14 patients with PD, respectively, at baseline (left image), the 1-year (middle image) and 3-year (right image) follow-ups. For all contiguous diffusion tensor imaging clusters, the FA value in all individual voxels significantly correlated with the cNfL level after adjustment for age and sex. A more yellow color denotes a larger cluster.

Abbreviations: cNfL = Neurofilament light chain protein in cerebrospinal fluid.

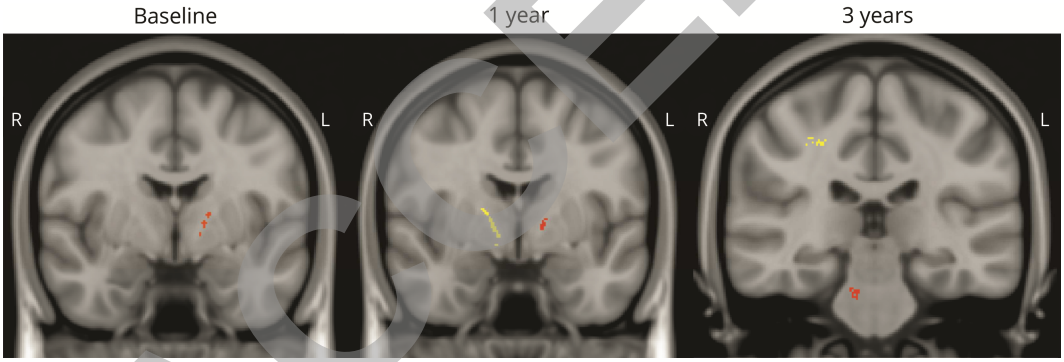

Table 1. Characteristics of the two Parkinson's disease cohorts and healthy controls

	HC	NYPUM	Validation cohort
	N = 30	N = 99	N = 194
Baseline characteristics			
Age, years	68.9 (5.6)	69.8 (9.2) [a]	68.0 (9.7)
Sex (M/F, Male%)	16/14 (53%)	60/39 (61%)	118/74 (61%)
MMSE score	29.1 (0.8)	28.6 (1.3)	28.0 (2.2)
Disease duration, years	-	1.7 (1.2)	1.2 (1.0)
Hoehn and Yahr stage	-	2.2 (0.5) [b]	1.7 (0.6)
Total UPDRS	-	37.2 (12.0) [b]	31.1 (11.2)
UPDRS III	-	26.9 (9.9) [b]	20.7 (8.5)
Olfactory function, B-SIT	9.5 (2.0)	6.5 (2.7) [c]	-
TUG, seconds (median, IQR)	-	8 (7-10)	-
Neurofilament light chain protein concentration and follow-up data			
cNfL, at baseline, ng/L	879 (667-1120) [d]	1189 (706-1706) [e]	805 (630-1140)
cNfL, at 1 year, ng/L	-	1194 (942-1758)	-
cNfL, at 3 years, ng/L	-	1266 (842-1870)	-
Median LEDD, mg, at 1 year	-	343 (232)	-
Median follow-up, years	-	9.2 (7.3-10.5)	3.6 (2.2-5.2)

Data are mean (SD) for the baseline variables and median (interquartile range) for cNfL values, unless otherwise specified. Disease duration is the time from onset of first motor symptom, as recalled by the patient, to baseline. LEDD at 1 year is given only for patients in

the NYPUM cohort, because motor functions were not analyzed in the validation cohort at 1 year. Post hoc comparisons was made using Tukey HSD, to correct for multiple comparisons.

[a] older in NYPUM than in the validation cohort, $p < 0.01$.

[b] higher in NYPUM than in the validation cohort, $p < 0.001$.

[c] lower scores in PD than in the healthy controls, $p < 0.001$.

[d] higher in NYPUM than in the healthy controls, $p < 0.05$.

[e] higher in NYPUM than in the validation cohort, $p < 0.05$.

Both differences in cNfL levels (d and e) were more strongly significant after adjustment for age and sex. Abbreviations: HC = neurologically healthy controls; MMSE = Mini Mental State Examination; UPDRS = Unified PD Rating Scale; IQR = Interquartile range; TUG = timed up and go; cNfL = Neurofilament light chain protein in cerebrospinal fluid; LEDD = Levodopa equivalent daily dose.

Table 2. Association of clinical features in Parkinson's disease with cNfL level

	NYPUM cohort		Validation cohort		Pooled cohort			
Variable	n = 99		n = 194		n = 293			
	Correlation		Correlation		Univariate		Multivariable	
At baseline:	coefficient, *r*		coefficient, *r*		*ß*		*ß*	
Hoehn &Yahr	0.144	$p = 0.158$	0.357	$p < 0.001$	0.2 [a]	$p < 0.001$	0.1 [a]	$p = 0.004$
UPDRS Tot.	0.320 [a]	$p = 0.002$	0.261	$p = 0.003$	3.9[a]	$p < 0.001$	2.8 [a]	$p = 0.002$
UPDRS III	0.355 [a]	$p < 0.001$	0.241	$p = 0.003$	4.1[a]	$p < 0.001$	2.7 [a]	$p < 0.001$
Hyposmia	-0.296[a]	$p < 0.001$						
TUG test	0.479 [a]	$p < 0.001$						
Tremor score	-0.031	$p = 0.764$						
PIGD score	0.199	$p = 0.051$						
Bradykinesia	0.281 [a]	$p = 0.005$						
Axial score	0.413 [a]	$p < 0.001$						
At 1 year:								
Hyposmia	-0.311[a]	$p = 0.003$						
Tremor score	-0.115	$p = 0.260$						
PIGD score	0.306 [a]	$p = 0.002$						
Bradykinesia	0.389 [a]	$p < 0.001$						
Axial score	0.399 [a]	$p < 0.001$						

Associations with baseline cNfL concentration were tested in the early, drug naïve phase of PD (baseline) and after 1 year (while receiving PD medications). Hyposmia was investigated by the Brief Smell Identification Test. Data are Spearmans rho coefficients for the two left

columns and *ß* values, which show units of change in clinical scales per 1 unit change in cNfL (ng/mL), for the two right columns. In the multivariable model, the cNfL-associations are adjusted for differences in age and sex. Disease duration or LEDD did not affect the cNfL-association with any symptoms.

[a] Association that was was significant after Holm-Bonferroni correction for multiple comparisons. Abbreviations: cNfL = Neurofilament light chain protein in cerebrospinal fluid; UPDRS = Unified PD Rating Scale; PIGD = Postural imbalance and gait difficulty; TUG = timed up and go; LEDD = Levodopa equivalent daily dose.

Table 3. cNfL concentrations in different Parkinson's disease subtypes

NYPUM cohort (n = 99)

Subtype	HC	Tremor dominant	Mixed	PIGD
	n = 30	n = 32	n = 12	n = 55
Age, mean (SD)	68.2 (6.7)	68.4 (9.8)	69.6 (9.3)	72.9 (9.8)
cNfL, ng/L, median	879	963 [b]	1117 [a, b]	1319 [a, b]
(IQR)	(667-1120)	(730-1270)	(883-1427)	(664-2155)

Validation cohort (n = 194)

Presenting symptom	Resting tremor	Bradykinesia	Balance/Gait
	n = 106	n = 57	n = 30
Age, mean (SD)	67.6 (9.7)	67.3 (11.0)	71.2 (6.7)
cNfL, ng/L, median	755	790	1205 [c,d]
(IQR)	(600-1040)	(630-1020)	(780-1530)

PD subtypes classified by UPDRS scores in the NYPUM cohort and by presenting symptom in the validation cohort. Balance/Gait denotes patients presenting with balance impairment or gait difficulty. Post hoc comparisons, using Tukey HSD to correct for multiple comparisons, showed the following differences:

[a] cNfL was higher in PIGD and Mixed PD phenotypes compared to the healthy controls ($p < 0.05$) in the NYPUM cohort.

[b] After adjusting for age and sex, cNfL was higher in all three PD phenotypes (Tremor, Mixed or PIGD) compared to the healthy controls ($p < 0.05$).

[c] and [d] In the validation cohort, patients presenting with balance impairment or gait difficulty had higher cNfL than patients presenting with ([c]) tremor ($p < 0.01$) or ([d]) bradykinesia ($p < 0.05$). These differences remained significant after adjusting for age and sex.

Abbreviations: cNfL = Neurofilament light chain protein in cerebrospinal fluid; HC = neurologically healthy controls; PIGD = Postural imbalance and gait difficulty; SD = standard deviation; IQR = interquartile range; UPDRS = Unified PD Rating Scale

Table 4. Mortality in Parkinson's disease in relation to baseline cNfL concentration

	Univariate		Multivariable	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
NYPUM cohort, n = 99				
cNfL, ng/mL	1.36 (1.18-1.56)	<0.001	1.13 (1.08-1.18)	0.025
Validation cohort, n = 194				
cNfL, ng/mL	3.57 (1.72-7.40)	<0.001	2.57 (1.08-6.07)	0.032
Pooled cohort, n = 293				
cNfL, ng/mL	1.39 (1.22-1.58)	<0.001	1.26 (1.07-1.48)	0.006
cNfL < vs > 903, ng/L	5.77 (2.82-11.85)	<0.001	2.56 (1.12-5.84)	0.026

Hazard ratios (HR) for mortality during follow up, in relation to one unit increase in cNfL (ng/mL) at baseline. In the multivariable analysis, adjustment was made for age and sex. Disease duration at baseline had no impact on survival. Abbreviations: cNfL = Neurofilament light chain protein in cerebrospinal fluid; CI = Confidence interval.

Table 5. Relation of brain imaging findings with cNfL concentration in Parkinson's disease

Striatal DAT uptake	Correlation coefficient		Multivariable, adjusted for age and sex	
	r	*p*-value	ß	*p*-value
Putamen, right	-0.226	0.024	-0.189	0.020
Putamen, left	-0.048	0.635	-0.027	0.778
Caudate, right	-0.354	<0.001	-0.277	0.003
Caudate, left	-0.202	0.045	-0.132	0.218

Diffusion tensor imaging

Cluster size	MNI coordinate			Anatomical designation
At baseline (n = 45):				
	X	Y	Z	
121	122	64	62	Posterior thalamic radiation (optic radiation)
119	124	108	96	Superior longitudinal fasciculus
106	114	56	100	Occipital lobe, sub-gyral white matter
93	126	88	94	Superior longitudinal fasciculus
83	61	147	49	Frontal lobe, inferior frontal gyrus
75	122	104	109	Superior longitudinal fasciculus
68	104	120	73	Anterior and posterior limb of internal capsule
68	58	111	114	Frontal lobe, sub-gyral white matter
55	71	81	92	Splenium of corpus callosum
53	119	135	36	Temporal lobe, superior temporal gyrus

At the 1 year follow up (n = 20):

	X	Y	Z	
126	78	123	63	Anterior and posterior limb of internal capsule
58	51	151	61	Frontal lobe, inferior frontal gyrus
55	126	159	67	Frontal lobe, inferior frontal gyrus
49	102	81	52	Cerebellum, anterior lobe
37	43	87	106	White matter in inferior parietal lobe
36	102	122	71	Posterior limb of internal capsule
36	44	133	84	Frontal lobe, sub-gyral white matter
34	68	150	105	Frontal lobe, cingulate gyrus
33	94	130	67	Caudate nucleus

At the 3 year follow up (n = 14):

	X	Y	Z	
210	58	96	111	Superior corona radiata
137	100	117	129	Frontal lobe, middle frontal gyrus
86	75	111	129	Frontal lobe, sub-gyral white matter
61	79	98	43	Pontine crossing tract
58	60	107	106	Superior corona radiata
57	62	130	35	Limbic lobe, uncus.
53	97	57	107	Occipital lobe, cuneus
49	50	73	106	White matter in inferior parietal lobe
46	137	73	96	Superior corona radiata

Brain imaging of PD patients in the NYPUM study. Striatal DAT uptake in standard deviation from normal values, and cNfL (ng/mL), was measured at baseline in all 99 patients. Diffusion tensor imaging was performed in 45, 20 and 14 patients with PD at baseline and the 1 and 3 year follow up, respectively. For all contiguous diffusion tensor imaging clusters, the FA value in all individual voxels significantly correlated with cNfL after adjustment for age and sex (see methods section for further description). The cluster size is the number of voxels in each cluster. Abbreviations: cNfL = Neurofilament light chain protein in cerebrospinal fluid; DAT = Dopamine active transporter.