Cerebrospinal Fluid Patterns and the Risk of Future Dementia in Early, Incident Parkinson Disease

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IMPORTANCE Alterations in cerebrospinal fluid (CSF) have been found in Parkinson disease (PD) and in PD dementia (PDD), but the prognostic importance of such changes is not well known. In vivo biomarkers for disease processes in PD are important for future development of disease-modifying therapies.

OBJECTIVE To assess the diagnostic and prognostic value of a panel of CSF biomarkers in patients with early PD and related disorders.

DESIGN, SETTING, AND PARTICIPANTS Regional population-based, prospective cohort study of idiopathic parkinsonism that included patients diagnosed between January 1, 2004, and April 30, 2009, by a movement disorder team at a university hospital that represented the only neurology clinic in the region. Participants were 128 nondemented patients with new-onset parkinsonism (104 with PD, 11 with multiple system atrophy, and 13 with progressive supranuclear palsy) who were followed up for 5 to 9 years. At baseline, CSF from 30 healthy control participants was obtained for comparison.

MAIN OUTCOMES AND MEASURES Cerebrospinal fluid concentrations of neurofilament light chain protein, Aβ1-42, total tau, phosphorylated tau, α-synuclein, and heart fatty acid–binding protein were quantified by 2 blinded measurements (at baseline and after 1 year). Follow-up included an extensive neuropsychological assessment. As PD outcome variables, mild cognitive impairment and incident PDD were diagnosed based on published criteria.

RESULTS Among the 128 study participants, the 104 patients with early PD had a different CSF pattern compared with the 13 patients with progressive supranuclear palsy (baseline area under the receiver operating characteristic curve, 0.87; \( P < .0001 \)) and the 30 control participants (baseline area under the receiver operating characteristic curve, 0.69; \( P = .0021 \)). A CSF biomarker pattern associated with the development of PDD was observed. In PD, high neurofilament light chain protein, low Aβ1-42, and high heart fatty acid–binding protein at baseline were related to future PDD as analyzed by Cox proportional hazards regression models. Combined, these early biomarkers predicted PDD with high accuracy (hazard ratio, 11.8; 95% CI, 3.3-42.1; \( P = .0001 \)) after adjusting for possible confounders.

CONCLUSIONS AND RELEVANCE The analyzed CSF biomarkers have potential usefulness as a diagnostic tool in patients with parkinsonism. In PD, high neurofilament light chain protein, low Aβ1-42, and high heart fatty acid–binding protein were related to future PDD, providing new insights into the etiology of PDD.

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The diagnosis of Parkinson disease (PD) is based on purely clinical criteria, which makes differentiation of PD from related movement disorders, such as multiple system atrophy (MSA) and progressive supranuclear palsy (PSP), challenging. There is a risk of misdiagnosis even in specialized movement disorder clinics, particularly during the early stages of disease. In addition to motor dysfunction, PD may entail long-term cognitive decline. Prospective data indicate that PD dementia (PDD) occurs in more than 75% of patients over the entire disease course but with interindividual variation. Hence, identification of biomarkers for PDD risk has become a key research priority.

Cerebrospinal fluid (CSF) biomarkers have shown potential usefulness in aiding parkinsonism diagnostics and in elucidating the pathogenesis of PDD. While studies have reported slight reductions in CSF Aβ1-42 concentrations in PD compared with control subjects, markedly reduced levels are found in PDD. This finding suggests an overlap with Alzheimer disease, in which a marked reduction in CSF Aβ1-42 reflects cortical deposition of Aβ. Elevated CSF neurofilament light chain protein (NFL) has been found in frontotemporal dementia. Cerebrospinal fluid tau and heart fatty acid–binding protein (HFABP) elevations have been linked to Lewy body disease, in which a lack of CSF Aβ1-42 reflects cortical deposition of Aβ. Furthermore, few studies have investigated combined CSF patterns. In this prospective study of 128 patients with parkinsonism, we analyzed 6 CSF biomarkers and aimed (1) to neurochemically distinguish between early PD, MSA, and PSP and (2) to predict PDD within 5 to 9 years among patients with PD.

**Methods**

**Study Population**
All patients participated in a prospective, population-based incidence study of unselected cases of new-onset idiopathic parkinsonism from a defined geographic catchment area in Sweden. Study inclusion was between January 1, 2004, and April 30, 2009. Patients with secondary parkinsonism (eg, drug-induced parkinsonism) or dementia at baseline were excluded. Of 182 patients enrolled in the study, 128 (70.3%) agreed to CSF collection by lumbar puncture at study entry or after 1 year. The patients who declined were older than the other patients (77.9 vs 72.0 years) but had comparable scores for mood, motor, and cognitive dysfunction. All 128 patients were followed up with standardized clinical examinations, including the Modified Hoehn and Yahr Scale, Mini-Mental State Examination, and motor assessments at least yearly for 5 to 9 years except for 7 nondemented patients who died during the first years. At the latest follow-up, the 128 patients were diagnosed as having PD (n = 104), MSA (n = 11), or PSP (n = 13) (Figure 1). A diagnosis of PD required agreement among the examiners (neurologists specializing in movement disorders [J.L. and L.F.]) that the clinical criteria for the diagnosis were fulfilled based on the UK Parkinson’s Disease Society Brain Bank criteria. All 104 patients with PD underwent N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-123I-iodophenyl)nortropane, 123I-iolumpane (FP-CIT) single-photon emission computed tomography, and all demonstrated pathologic uptake. Diagnoses of MSA and PSP were made based on international consensus criteria. As a baseline reference, CSF was obtained from 30 adult, demographically similar, neurologically healthy control subjects (HCs) with normal FP-CIT uptake on single-photon emission computed tomography.

**Patient Consent and Institutional Review Board Approval**
All participants provided written informed consent. The study was approved by the Regional Medical Ethics Board in Umeå, Sweden, and was performed in accord with the Declaration of Helsinki.

**CSF Analysis**
At study entry (baseline), CSF was obtained from 99 patients with PD, 11 patients with MSA, 12 patients with PSP, and 30 controls (in total, 152 individuals) before initiation of dopaminergic treatment. During treatment, collection was repeated after 1 year in 57 patients with PD, 6 patients with MSA, and 9 patients with PSP (in total, 72 patients). Among patients from...
whom CSF was obtained after 1 year, 1 patient with PSP and 5 patients with PD donated CSF at this time only.

Cerebrospinal fluid levels of NFL were measured with a sandwich enzyme-linked immunosorbent assay (ELISA) (NF-light; UmanDiagnostics AB), as described by the manufacturer. The lower limit of quantification for this assay was 50 ng/L, and the coefficient of variation was 14.0%. Cerebrospinal fluid total tau was determined using an additional sandwich ELISA (INNOTEST hTAU-Ag; Innogenetics) specifically constructed to measure all tau isoforms irrespective of phosphorylation status, as previously described.18 Cerebrospinal fluid Aβ1-42 levels were determined using an additional sandwich ELISA (INNOTEST β-AMYLOID(1-42); Innogenetics) specifically constructed to measure Aβ containing the first and 42nd amino acids, as previously described.20 In this assay, the monoclonal antibody 21F12 (which is specific to the C-terminus of Aβ42) was used for capture, and 3D6 (which is specific to the N-terminus) was used as the detector. Tau phosphorylated at threonine 181 was also measured using a sandwich ELISA (INNOTEST PHOSPHO-TAU(181P); Innogenetics), as described previously.20 Cerebrospinal fluid levels of α-synuclein were analyzed using a commercially available α-synuclein human ELISA (KHBO061; Invitrogen), according to instructions by the manufacturer. In addition, levels of HFABP were measured using an ELISA (HK402; Hycult Biotech), following instructions by the manufacturer. For HFABP, 9 CSF samples (obtained from patients with PD) had unmeasurable values below the lowest calibrator in the assay (102 ng/L). These values were used in analyses of dichotomized HFABP concentrations (eg, <500 ng/L) but were not used as continuous variables. Hemoglobin in CSF was analyzed using a human hemoglobin ELISA (E80-135; Bethyl Laboratories, Inc) according to the manufacturer’s protocol. The α-synuclein concentrations were mildly correlated with hemoglobin concentrations; therefore, α-synuclein concentration was adjusted for CSF hemoglobin concentration. All CSF analyses were performed by experienced, board-certified laboratory technicians using procedures approved by the Swedish Board for Accreditation and Conformity Assessment. Analyses were performed blinded to clinical data, including clinical diagnoses.

Diagnostic Criteria for Mild Cognitive Impairment, PDD, and Disease Progression

Full neuropsychological testing for PD-mild cognitive impairment (PD-MCI) and PDD diagnostics was performed at 0, 1, 3, 5, and 8 years. The MCI diagnoses were made based on Movement Disorder Society criteria.21 Because the protocol included 2 tests of all cognitive domains except language, MCI was diagnosed on modified level 2 criteria, consistent with previous research.22 Tests used for MCI classification and criteria for PDD diagnoses between 0, 1, 3, 5, and 8 years are described in the eAppendix in the Supplement. Structural magnetic resonance imaging and routine laboratory tests were performed in all patients to exclude non-PDD causes of dementia. Diagnoses of PDD were determined by neurologists experienced in neurodegenerative disorders (J.L. and L.F.), blinded to CSF data. No patients with PD had onset of cognitive symptoms 1 year or earlier after motor onset (eg, as in Lewy body dementia). All patients with dementia had PDD based on published criteria.23 Disease progression was defined as a sustained increase of at least 0.5 point on the Modified Hoehn and Yahr Scale at follow-up.

Statistical Analysis

Baseline correlations were tested by Spearman ρ and diagnostic group differences in biomarker concentrations by 1-way analysis of covariance. To allow adjustment for potential confounders (age, sex, and disease duration), biomarker concentrations were log transformed to obtain normal distributions before comparisons. Receiver operating characteristic curve and area under the receiver operating characteristic curve (AUROUC) were used to characterize biomarker sensitivity and specificity for specific diagnoses. An AUROC of 0.5 indicates no discrimination, while an AUROC of 1.0 indicates perfect discrimination. Optimal cutoff concentrations for CSF biomarkers were chosen at the highest Youden Index (sensitivity plus specificity minus 1).24 Hazard ratios for outcome in PD, with varying baseline biomarker levels, were estimated by Cox proportional hazards regression models, which were run with and without adjustment for age, sex, disease duration, and baseline MCI status. To improve diagnostic accuracy, 2 CSF biomarker ratios were calculated (ratio of NFL to Aβ1-42 and ratio of NFL + HFABP to Aβ1-42) by dividing biomarkers with positive correlation to the outcome by biomarkers with negative correlation to the outcome. Kaplan-Meier plots were used to show the effect of baseline CSF concentrations. Seven nondemented patients with PD who died less than 5 years from study inclusion (6.7% of 104 patients with PD) were excluded when sensitivity and specificity for PDD prediction were calculated because the long-term cognitive status of these patients was unknown. P < .05 was considered significant. However, if corrections for multiple comparisons were considered, a more conservative significance level based on 6 biomarkers was P ≤ .008 (at which the major findings were also significant). All statistical analyses were performed using a software program (SPSS 21.0; SPSS Inc).

Results

Baseline Characteristics

At baseline, there were no large differences in demographic characteristics across diagnostic groups (Table 1). Baseline concentrations of CSF biomarkers were moderately but significantly positively correlated with patient age (r range, 0.20–0.55) except for Aβ1-42, which was negatively correlated.

PD vs HCs

Patients with PD had significantly higher NFL, slightly lower Aβ (measured by Aβ1-42), and comparable α-synuclein concentrations in CSF compared with the neurologically HC at baseline after adjusting for age and sex (Table 1). Together with Aβ1-42, NFL had moderate accuracy for discrimination of patients with PD from HCs (Figure 2A).

PD vs PSP

The early CSF pattern differed in patients having PSP compared with patients having PD after adjusting for age, sex, and...
disease duration. At baseline, patients with PSP had higher NFL, lower Aβ-42, and lower α-synuclein levels. As shown in Figure 2B, NFL concentration was useful for discriminating PSP from PD. Baseline NFL exceeding 2020 ng/L in CSF had a sensitivity of 75% (95% CI, 43%-94%) and a specificity of 83% (95% CI, 74%-90%) for PSP against PD. Accuracy was improved using the ratio of NFL to Aβ-42 (AUROC, 0.87; P < .0001). At cutoff values exceeding 2.3, the ratio had baseline sensitivity of 100% (95% CI, 73%-100%) and specificity of 68% (95% CI, 58%-77%) for discriminating PSP from PD. At 1-year follow-up, the mean CSF NFL concentration had increased by 27.1% in patients with PSP who had provided 2 samples (P = .005), while it was unchanged in patients with PD. At 1 year, excellent discrimination of PSP from PD was obtained using NFL (AUROC, 0.93; P < .0001) (Figure 2C). This time, NFL exceeding 2916 ng/L (the highest Youden Index) had a sensitivity of 89% (95% CI, 52%-98%) and a specificity of 93% (95% CI, 83%-98%) for PSP against PD. In contrast, early MSA could not be clearly distinguished from PD by CSF at baseline or at 1 year. The other analyzed CSF biomarkers (total tau, phosphorylated tau [P-tau], and HFABP) showed similar levels in all diagnostic groups.

Clinical Outcome in PD

During longitudinal follow-up (5-9 years) of 99 patients with PD from whom baseline CSF samples were obtained, 35 (35.4%) developed PDD. The median time from study entry to dementia was 3.5 years. Baseline levels of 3 CSF biomarkers correlated with this decline. Higher NFL (P = .0005), lower Aβ-42 (P = .0053), and higher HFABP (P = .0037) conferred higher hazard ratios for PDD, as estimated by Cox proportional hazards regression models (Table 2). Using cutoff levels with the highest Youden Index and adjusting for differences in age, sex, and disease duration, patients having PD with baseline NFL exceeding 1100 ng/L, Aβ-42 less than 626 ng/L, and HFABP exceeding 500 ng/L had 2.6, 2.8, and 2.8 times, respectively, higher risk of future PDD compared with the other patients having PD. The relevance of NFL, Aβ-42, and HFABP concentrations at baseline was underscored by divergent curves for PDD incidence in Kaplan-Meier plots above and below cutoff levels (Figure 3A-C).

Prediction of PDD improved by combining CSF biomarkers. The baseline triad of high NFL, low Aβ-42, and high HFABP indicated high PDD risk. Expressed as the ratio of NFL + HFABP to Aβ-42, this pattern predicted PDD at follow-up with high accuracy (AUROC, 0.83; 95% CI, 0.74-0.92; P < .0001). Patients having PD with a baseline ratio exceeding 2.1 had an adjusted PDD hazard ratio of 11.8, yielding a sensitivity of 90% (95% CI, 74%-98%) and a specificity of 71% (95% CI, 56%-83%) for future PDD (Table 2 and Figure 3E). In contrast, patients with a baseline ratio of NFL to Aβ-42 of 1.0 or less (lower NFL than Aβ-42) had a low PDD incidence. In the PD subgroup with a ratio of 1.0 or less (similar to CSF findings in HCs), only 2 of 25 patients developed PDD during follow-up, while values exceeding 1.0 conferred a 6.7-times elevated, adjusted PDD hazard ratio (Table 2).
The other biomarkers (most notably α-synuclein, total tau, and P-tau) were not robust PDD risk markers. Furthermore, faster disease progression in PD was associated with low baseline αB4-42 (higher risk at αB4-42 <617 ng/L, P = .0006) (Table 2 and Figure 3F) but not with any other analyzed biomarkers.

### Adjusting Outcome in PD for Possible Confounders

Older age and preexisting cognitive impairment are considered risk factors for PDD. In the present cohort, age at baseline was related to PDD risk (hazard ratio, 1.1; 95% CI, 1.0-1.1 per year; P = .0027). Adjusting for age slightly changed PDD hazard ratios, varying with baseline CSF findings (Table 2). However, adjusting the effect of age for the ratio of NFL to αB4-42 demonstrated that the CSF measure was the stronger PDD predictor, rendering age a nonsignificant covariate (P = .2291). Essentially normal CSF profiles (as defined by a ratio of NFL to αB4-42 ≤1.0) were also found in some older patients (Figure 3D).

### Mild Cognitive Impairment

At baseline, CSF profiles of patients having PD-MCI (n = 40) did not differ from those of patients with PD having normal cognition (PD-NC) (n = 58) (eTable 1 in the Supplement). After adjusting for baseline MCI status, CSF risk biomarkers in Table 2 still showed significant associations with PDD (eTable 2 in the Supplement). In the PD-NC subgroup, 11 patients developed PDD during follow-up. When PDD risk was analyzed in this subgroup (among whom the median age was lower than that in the whole cohort), many of the same CSF risk biomarkers as in Table 2 were found, although they were associated...
Figure 3. Cerebrospinal Fluid (CSF) Biomarkers in Relation to Clinical Outcome in Parkinson Disease

A. Dementia risk stratified by CSF NFL

B. Dementia risk stratified by CSF Aβ1-42

C. Dementia risk stratified by CSF HFABP

D. Range of ages for 2 ratio groups

E. Dementia risk stratified by ratio of NFL + HFABP to Aβ1-42

F. Risk of progression stratified by CSF Aβ1-42

A-C, E, and F, Kaplan-Meier plots show outcome (dementia or disease progression) in 99 patients having Parkinson disease with baseline CSF biomarkers above and below cutoff levels. D, Age ranges are shown for patients having Parkinson disease with baseline ratios of NFL to Aβ1-42 above and below 1.0. P values are by log-rank test. HFABP indicates heart fatty acid–binding protein; H&Y, Modified Hoehn and Yahr Scale stage; and NFL, neurofilament light chain protein.

No. at risk
NFL <1100 ng/L 48 47 40 24 9
NFL >1100 ng/L 51 40 26 9 3

No. at risk
Aβ1-42 >626 ng/L 36 28 19 9 3
Aβ1-42 ≤626 ng/L 63 58 46 23 8

No. at risk
HFABP <500 ng/L 64 60 49 25 9
HFABP >500 ng/L 35 27 17 8 3

No. at risk
NFL + HFABP to Aβ1-42 ≤2.1 40 40 37 23 9
NFL + HFABP to Aβ1-42 >2.1 50 38 23 5 2

No. at risk
Aβ1-42 >617 ng/L 35 19 15 8 2
Aβ1-42 ≤617 ng/L 64 55 47 31 9
with higher hazard ratios (eTable 3 in the Supplement). After adjusting for all covariates, high NFL was associated with a rapid decline from PD-NC to PDD.

Discussion

In this population-based, prospective study, we found that CSF levels of NFL, Aβ1-42, and HFABP were important diagnostic and prognostic biomarkers in patients with early, drug-naive parkinsonism. First, we showed that high baseline NFL, together with low Aβ1-42, discriminated PSP from PD and demonstrated that this finding was stable when CSF was analyzed after 1 year (during treatment with dopaminergic medication). Cerebrospinal fluid levels of NFL alone separated PSP from PD with high diagnostic accuracy (AUROC, 0.93 at 1 year). This result is consistent with previous findings of elevated NFL in atypical parkinsonism (eg, moderate to advanced MSA and PSP) in cross-sectional studies. Although elevated NFL has not clearly been shown in early PSP and by repetitive measurements. Marked elevation in NFL might reflect a more aggressive, accumulating subcortical axonal degeneration in PSP compared with PD. If validated, this difference could serve as a supportive diagnostic criterion for PSP in early disease. In addition, NFL might serve as a biomarker for neurodegeneration in PSP.

Second, by following up patients in the longest prospective CSF study to date with an extensive neuropsychological test protocol in PD, we found that the early CSF pattern of NFL, Aβ1-42, and HFABP predicts the risk of PDD. Several mechanisms have been suggested in the pathogenesis of PDD. The results of some postmortem studies suggest limbic and neocortical Lewy body deposition as the main determinant, whereas other studies point to cortical Aβ deposition or reduced cortical cholinergic innervation. Pathologic Aβ in the brain is a common finding at autopsy in PDD. Our result of low CSF Aβ1-42 in patients subsequently developing PDD supports involvement of Aβ pathology in PDD, consistent with cross-sectional study findings in PDD and in 2 longitudinal PD studies. This finding also highlights the possibility of using CSF as a presymptomatic screening tool for dementia. Once interventions for halting cognitive decline are available, they are likely to be most beneficial in early phases of disease. Therefore, PDD risk biomarkers are needed. In Alzheimer disease, low CSF levels of Aβ1-42 have been demonstrated several years before onset of dementia. We found that low Aβ1-42 predicted cognitive decline to PDD up to 9 years after clinical presentation of PD. However, the low baseline CSF Aβ1-42 related to disease progression in PD in the present study (consistent with findings in a cross-sectional study) and the low CSF Aβ1-42 in PSP suggest that low Aβ1-42 is not specific to PDD (or Alzheimer disease).

Third, and most important, we extend previous results by showing a CSF pattern of high NFL, low Aβ1-42, and high HFABP in patients developing PDD. The associations were robust to age adjustment. The PDD hazard ratios were slightly lowered by adjusting for baseline MCI status, which could imply that NFL, Aβ1-42, and HFABP alterations in those who developed PDD were also related to PD-MCI development. In the younger subgroup of patients with PD-NC at baseline, high NFL predicted rapid progression to PDD. In frontotemporal dementia, CSF levels of NFL are higher than those in Alzheimer disease and correlate positively with disease severity and brain atrophy. Elevated NFL in patients developing PDD possibly reflects a more widespread and aggressive disease process than that in PD without dementia. A study of patients with Lewy body dementia suggested a trend of elevated CSF HFABP compared with controls. Because HFABP is a protein that facilitates the transport of fatty acids to the mitochondria for oxidation, our findings might also link PDD to mitochondrial dysfunction, which has repeatedly been reported as a component in the pathogenesis of PD.

Limitations of our study are that few patients had atypical parkinsonism and the fact that there was no independent validation cohort for confirmation of findings. Another limitation was that 7 of 104 nondemented patients with PD (6.7%) were followed up for less than 5 years. Data from these patients could not be used in some outcome analyses. Our study also has several strengths, including a population-based and longitudinal design, evaluation of incident cases, and a high proportion of patients who provided CSF samples. The risk of incorrect diagnosis was minimized by follow-up periods exceeding 5 years, the finding of pathologic uptake on FP-CIT examination in all 104 patients with PD, and the use of an extensive neuropsychological test battery.

Conclusions

Our results suggest that early CSF analysis of NFL, Aβ1-42, and HFABP can aid in the diagnosis of parkinsonian disorders and may enhance prediction of PDD. Although additional, confirmatory studies are needed, the diagnostic accuracy of high NFL, low Aβ1-42, and high HFABP (with 90% sensitivity and 71% specificity for PDD) is likely sufficiently high to be clinically useful. Changes in these biomarkers, even at the time of the diagnosis of PD, may alert physicians to a patient’s risk of developing dementia. In the future, CSF analysis could also enhance selection of patients at risk for PDD to participate in neuroprotective trials.
Conflict of Interest Disclosures: Dr Linder reported receiving honoraria for lectures from GSK, Lundbeck, Boehringer Ingelheim, Abbott, AbbVie, Solvay, Orion Pharma, UCB, Nordic InfuCare, Medtronic, and IPSEN and reported serving on advisory boards for Boehringer Ingelheim, Lundbeck, and GSK. No other disclosures were reported.

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