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Inflammatory Biomarkers in Newly Diagnosed Patients With Parkinson Disease and Related Neurodegenerative Disorders

Camilla Christina Pedersen, MSc, Anastasia Ushakova, PhD, Ragnhild Eide Skogseth, MD, PhD, Guido Alves, MD, PhD, Ole-Bjørn Tysnes, MD, PhD, Dag Aarsland, MD, PhD, Johannes Lange, PhD, and Jodi Maple-Grødem, PhD

Correspondence
Dr. Maple-Grødem
jodi.maple@uis.no

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Abstract

Background and Objectives

Neuroinflammation contributes to Parkinson disease (PD) pathology, and inflammatory biomarkers may aid in PD diagnosis. Proximity extension assay (PEA) technology is a promising method for multiplex analysis of inflammatory markers. Neuroinflammation also plays a role in related neurodegenerative diseases, such as dementia with Lewy bodies (DLB) and Alzheimer disease (AD). The aim of this work was to assess the value of inflammatory biomarkers in newly diagnosed patients with PD and in patients with DLB and AD.

Methods

Patients from the Norwegian ParkWest and Dementia Study of Western Norway longitudinal cohorts (PD, n = 120; DLB, n = 15; AD, n = 27) and 44 normal controls were included in this study. A PEA inflammation panel of 92 biomarkers was measured in the CSF. Disease-associated biomarkers were identified using elastic net (EN) analysis. We assessed the discriminatory power of disease-associated biomarkers using receiver operating characteristic (ROC) curve analysis and estimated the optimism-adjusted area under the curve (AUC) using the bootstrapping method.

Results

EN analysis identified 9 PEA inflammatory biomarkers (ADA, CCL23, CD5, CD8A, CDCP1, FGF-19, IL-18R1, IL-6, and MCP-2) associated with PD. Seven of the 9 biomarkers were included in a diagnostic panel, which was able to discriminate between those with PD and controls (optimism-adjusted AUC 0.82). Our 7-biomarker PD panel was also able to distinguish PD from DLB and from AD. In addition, 4 inflammatory biomarkers were associated with AD and included in a panel, which could distinguish those with AD from controls (optimism-adjusted AUC 0.87). Our 4-biomarker AD panel was also able to distinguish AD from DLB and from PD.

Discussion

In our exploratory study, we identified a 7-biomarker panel for PD and a 4-biomarker panel for AD. Our findings indicate potential inflammation-related biomarker candidates that could contribute toward PD-specific and AD-specific diagnostic panels, which should be further explored in other larger cohorts.

From the The Norwegian Centre for Movement Disorders (C.C.P., G.A., J.L., J.M.-G.), Stavanger University Hospital; Department of Chemistry, Bioscience and Environmental Engineering (C.C.P., G.A., J.L., J.M.-G.), University of Stavanger; Section of Biostatistics (A.U.), Department of Research, Stavanger University Hospital; Department of Geriatric Medicine (R.E.S.), Haraldsplass Deaconess Hospital, Bergen; Department of Clinical Medicine (R.E.S., O.-B.T.), University of Bergen; Department of Neurology (G.A.), Stavanger University Hospital; Department of Neurology (O.-B.T.), Haukeland University Hospital, Bergen; Centre for Age-Related Medicine (D.A.), Stavanger University Hospital, Norway; and Department of Old Age Psychiatry (D.A.), Institute of Psychiatry, Psychology, and Neuroscience, King's College London, United Kingdom.

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Glossary

AD = Alzheimer disease; **APS** = atypical parkinsonian syndromes; **AUC** = area under the curve; **DLB** = dementia with Lewy bodies; **EN** = elastic net; **LLOD** = lower limit of detection; **MMSE** = Mini-Mental State Examination; **NPX** = normalized protein expression; **PD** = Parkinson disease; **PEA** = proximity extension assays; **ROC** = receiver operating characteristic; **UPDRS** = Unified Parkinson Disease Rating Scale; **VIFs** = variance inflation factors.

Parkinson disease (PD) is an age-related neurodegenerative disorder characterized by motor and nonmotor symptoms. Peripheral immune activation and neuroinflammation in the brain are believed to contribute to neuropathology and ultimately neurodegeneration.[1] Candidate inflammatory biomarkers have been associated with PD, severity of symptoms, and disease progression.[2-5] However, inflammation is not unique to PD and plays a role in neurodegenerative diseases with overlapping pathologies, such as dementia with Lewy bodies (DLB) and Alzheimer disease (AD).[6-8] Thus, further work is needed to characterize the profile and extent of inflammatory biomarker changes in PD.

Research using highly sensitive multiplex methodologies, such as proximity extension assays (PEAs), has enabled the identification and validation of panels of inflammation-related biomarkers that are altered in diseases involving inflammatory pathways, including AD and multiple sclerosis.[6,7,9,10] The same technology has also identified candidate inflammatory biomarkers for the diagnosis of PD, and the differential diagnosis of PD from atypical parkinsonian syndromes (APS), but these studies are limited by relatively small size and require validation.[11,12]

In this study, we used PEA technology to search for a panel of CSF inflammation-related biomarkers associated with PD in newly diagnosed patients with PD from the Norwegian ParkWest study and normal controls. We further analyzed the association of the same inflammatory biomarkers with AD or DLB to explore disease-specific changes in inflammatory biomarker profiles.

Methods

Study Participants

One hundred twenty patients with incident PD were included from the population-based longitudinal ParkWest study.[13] PD was diagnosed according to the United Kingdom Brain Bank criteria.[14] Patients were assessed using a standardized examination program at baseline including the Unified Parkinson Disease Rating Scale (UPDRS),[15] Hoehn and Yahr (H&Y) staging,[16] and the Mini-Mental State Examination (MMSE).[17] During CSF sampling, all but 1 patient with PD were drug naïve.

Fifteen patients with DLB and 27 patients with AD were included from the longitudinal DemWest study, which was conducted at the same time and in the same region as the ParkWest study.[18] All patients experienced mild dementia during inclusion. DLB was diagnosed according to the revised consensus guidelines for clinical and pathologic diagnoses of DLB.[19] AD was diagnosed according to The National Institute of Neurological and Communicative Disorders and Stroke—AD and Related Disorders Association.[20] DemWest assessments at baseline included UPDRS part III and the MMSE.

The normal control group (n = 45) included people without suspected neurodegenerative disease and who underwent an elective neurologic examination or orthopedic surgery at Stavanger University Hospital, which is a site for both the ParkWest and DemWest studies. Basic demographic data and MMSE were obtained.

Standard Protocol Approvals, Registrations, and Patient Consents

The Regional Committee for Medical and Health Research Ethics in Western Norway approved both the ParkWest and DemWest studies. All participants signed written informed consent.

CSF Sampling and Storage

CSF samples were obtained from participants using lumbar puncture in accordance with standardized procedures.[21] On collection, samples were centrifuged and stored in polypropylene tubes at −80°C. All samples were subjected to 2 freeze-thaw events for aliquotation purposes.

Proximity Extension Assay Testing

Multiplex PEA technology was used to analyze 92 inflammatory markers (Olink Target 96 Inflammation panel; eTable 1, links.lww.com/NXI/A868) and was performed by Olink Bioscience, Uppsala, Sweden. Olink panel validation data are available.[22] Data for each biomarker were provided as normalized protein expression (NPX) values, an arbitrary unit on a Log2 scale after internal intensity normalization. NPX values are proportional to the concentration of the protein.[23]

Statistical Analysis

Statistical analyses for demographic and clinical data were performed using IBM SPSS Statistics, version 26.0 (Armonk, NY). Normally distributed continuous variables were summarized using the mean and SD, and differences between groups were assessed using the Student *t* test. Variables with skewed distribution were presented using the median with the 25th and 75th percentiles, and differences were assessed using the Mann–Whitney *U* test. Cases with missing data were omitted from the calculations performed to obtain summarizations. For categorical variables, differences were assessed using the χ^2 test.

Assays in the inflammation panel with more than 10% of values below the lower limit of detection (LLOD) were

Table 1 Demographic and Clinical Characteristics of Patients and Controls Included in the Study

Clinical variables	Controls	PD	DLB	AD
N Total	44	120	15	27
Male, N (%)	21 (47.7)	77 (64.2)	9 (60.0)	7 (25.9)[b,c]
Age at baseline, y, median (Q1–Q3)	65.1 (56.5–74.1)	67.2 (60.8–74.5)	73.7 (70.7–78.6)[a,b]	75.2 (68.9–80.1)[a,b]
Education, y, median (Q1–Q3)	10.0 (8.0–14.0)	11.0 (9.0–13.0)[c,d]	8.00 (7.0–11.0)	8.00 (7.0–13.0)
UPDRS III, mean (SD)	—	20.0 (14.0)	9.0 (16.0)	0.0 (2.0)[b,c]
H&Y, mean (SD)	—	2.0 (0.5)	—	—
MMSE score, median (Q1–Q3)	29.0 (28.0–30.0)[b,c,d,e]	28.5 (27.0–29.0)[c,d]	24.0 (22.0–26.0)	24.0 (23.0–26.0)
***APOE-ε4* carriers, N (%)**	—	37 (30.8)[c,d]	11 (73.3)[e]	20 (74.1)

Abbreviations: AD = Alzheimer disease; DLB = dementia with Lewy bodies; H&Y = Hoehn and Yahr stage; MMSE = Mini-Mental State Examination; NC = normal controls; PD = Parkinson disease; Q1 = 25th percentile; Q3 = 75th percentile; UPDRS III = Unified Parkinson Disease Rating Scale Part III.
[a] $p < 0.05$ vs controls.
[b] $p < 0.05$ vs PD.
[c] $p < 0.05$ vs DLB.
[d] $p < 0.05$ vs AD.
[e] MMSE scores for N = 43 controls and *APOE-ε4* carriers for N = 13 DLB.

excluded (n = 52; eTable 1, links.lww.com/NXI/A868). For the remaining assays (n = 40), values below the LLOD were included in the analyses.

Regularized logistic regression with elastic net (EN) penalization was performed to identify PEA biomarkers associated with each disease group, using R Project for Statistical Computing version 4.2.1 and the *glmnet*-package version 4.1–3.[24] EN penalizes predictors that lack prediction power, so it is an effective method for model selection of high-dimensional data.[25,26] EN has 2 parameters: λ, which is the overall strength of the penalty, and α (between 0 and 1), which is the weight of the l1 penalty. The minimum λ resulted in a prediction error within 1 standard error from the lowest value, as estimated by leave-one-out cross-validation, was selected as the level of the regularization parameter. EN combines regularization used in both Lasso (l1) and Ridge (l2) regressions. In the *glmnet*, the parameter α decides the balance between l1 and l2. In our analyses, EN was repeated for all values of α between 0 and 1 using 0.01 increments. Predictors with nonzero estimates for the range of α provided evidence for the association of a given PEA biomarker and disease status.

For each set of selected biomarkers, we applied a multiple logistic regression model that included all the selected biomarkers, age, and sex as covariates, with the disease status as the outcome. Logistic regression models, receiver operating characteristic (ROC) curve, and area under the curve (AUC) analyses were performed for each biomarker individually. Multicollinearity was simultaneously assessed based on variance inflation factors (VIFs), which were calculated using R package *car* version 3.0–11.[27] Of the biomarkers with high multicollinearity (VIF >5), only the biomarker with the highest AUC was included in the final multiple logistic regression model. The discriminative value of the final model was assessed by ROC curve analysis. ROC curves were constructed using R package *pROC car* version 1.18.0.[28] Optimism-adjusted AUCs were calculated using the bootstrapping method with 2,000 replications to compensate overfitting of the models using the R package *boot* version 1.3-28.[29,30] Sensitivity analysis was performed for analyses between groups where age was significantly different by restricting age at baseline to older than 65 years. Limiting the analyses to include only controls or patients with PD older than 65 years did not change these overall results (eTables 2–4, links.lww.com/NXI/A868).

Data Availability

Anonymized data are available on request to qualified investigators for the purposes of replicating procedures and results.

Results

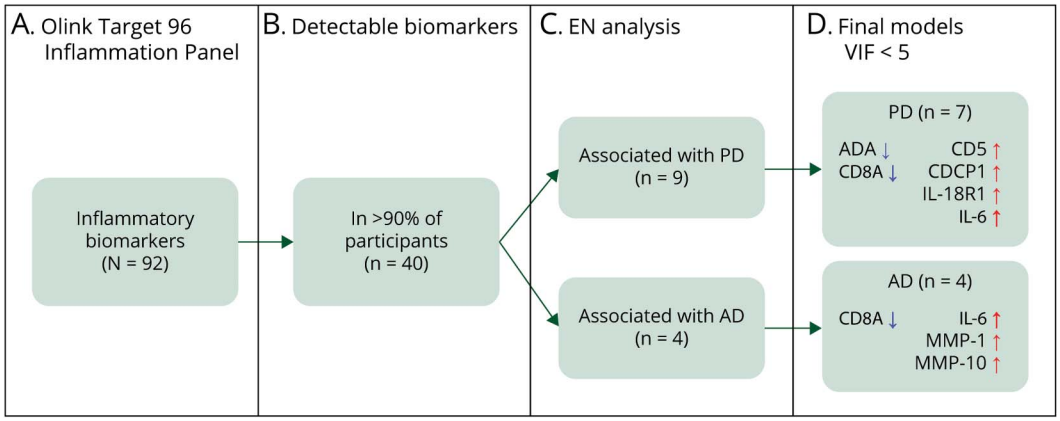
Demographic and Clinical Data

We analyzed the CSF from 207 individuals, including 120 patients with newly diagnosed PD, 15 patients with DLB, 27 patients with AD, and 45 normal controls. One control sample was excluded because of failing quality control, leaving 206 participants in the analysis (Table 1). Patients with DLB and AD were older than controls ($p = 0.014$ and $p = 0.001$, respectively) and patients with PD ($p = 0.02$ and $p < 0.001$, respectively). All patients had significantly lower MMSE scores than controls. Patients with DLB and AD had lower MMSE scores than patients with PD (both $p < 0.001$).

Inflammation-Related Biomarkers Associated With Parkinson Disease

Of the 92 PEA biomarker proteins included in the inflammation panel, 40 were detectable in the CSF of more than 90% of all participants (eTable 1, links.lww.com/NXI/A868) and were further analyzed. An overview of the workflow is

Figure 1 Workflow Diagram Tracking the Number of Biomarkers Included at Different Stages of the Study



(A) Of 92 biomarkers, (B) only the 40 that were detected in more than 90% of all participants were included in the study. (C) Through the EN analysis, the authors identified 9 and 4 biomarkers that were associated with PD and AD, respectively. (D) Finally, through the ROC curve analysis, the authors assessed the diagnostic performance of biomarkers associated with either PD or AD that had low multicollinearity (VIF <5). Arrows pointing up indicate biomarkers that were found to be increased in either PD or AD in the EN analysis, and arrows pointing down indicate biomarkers that were decreased with disease. Biomarkers in bold were identified as candidates in both the PD and AD EN analyses. VIFs = variance inflation factors.

presented in Figure 1. Biomarker candidates that differed between the group with PD and the controls were selected using EN, with sex and age at the point of testing forced to be included in the model as covariates. Nine PEA biomarkers were associated with PD across all levels of α in the EN analysis (Figure 2A). Specifically, increased levels of CD5, CDCP1, IL-18R1, and IL-6 and decreased levels of ADA, CCL23, CD8A, FGF-19, and MCP-2 were associated with PD (Table 2). Detailed descriptions and biological functions of the 9 candidate biomarkers are summarized in eTable 5.

The diagnostic ability of the panel of candidate markers for PD was estimated using ROC curves with AUC. When fitting logistic regression with all 9 PD-associated biomarkers, 3 candidates (CCL23, FGF-19, and MCP-2) had a VIF >5. Of the 3 biomarkers, MCP-2 had the highest AUC (optimism-adjusted AUC of 0.66) and was included in the final PD panel with the remaining 6 candidate biomarkers. The 7-biomarker PD panel yielded an estimated optimism-adjusted AUC of 0.82 (Table 3). In addition to being male, increased levels of CD5, CDCP1, IL-18R1, and IL-6 and decreased levels of ADA, CD8A, and MCP-2 were associated with PD (Table 3). Furthermore, we found that the 7-biomarker PD panel was also able to discriminate patients with PD from those with DLB (optimism-adjusted AUC of 0.73; eTable 6, links.lww.com/NXI/A868) and patients with PD from those with AD (optimism-adjusted AUC of 0.84; eTable 6).

Investigation of Inflammatory Biomarkers in Related Neurodegenerative Diseases

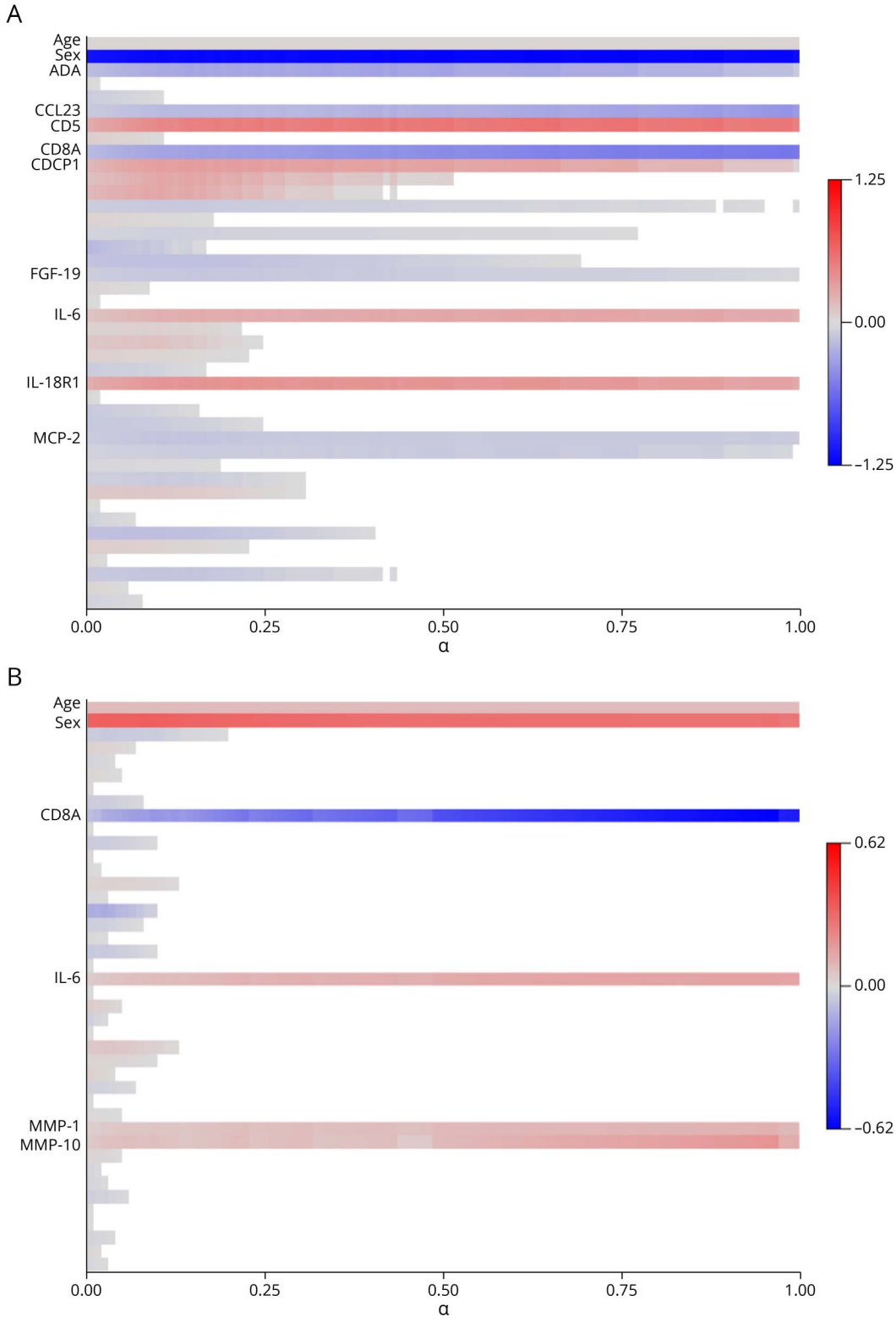
Our secondary aim was to investigate whether there are disease-specific changes in inflammatory biomarker profiles in related neurodegenerative diseases. EN analysis was next performed for either patients with AD, or with DLB, compared with controls. Of the 40 PEA biomarkers analyzed, 4 were associated with AD status across all levels of α (Figure 2B). Comparable with the direction of association with PD, increased levels of IL-6 and decreased levels of CD8A were associated with AD (Table 2). AD-specific biomarker candidates were also identified: increased levels of MMP-1 and MMP-10 were associated with AD (Table 2). EN analysis of patients with DLB compared with controls showed that increased levels of CD8A were associated with DLB, but this was not robust across all levels of α in the EN analysis (eFigure 1, links.lww.com/NXI/A868).

In the model to assess the discriminative ability of the 4-biomarker AD panel for AD compared with controls, no multicollinearity was detected. The 4-biomarker AD panel yielded an optimism-adjusted AUC of 0.87 (Table 3). Increased levels of MMP-10 and decreased levels of CD8A were associated with AD (Table 3). The 4-biomarker AD panel was also assessed for its ability to distinguish patients with AD from the other patient groups: the analysis of AD compared with DLB yielded an optimism-adjusted AUC of 0.71, and the analysis of AD compared with PD yielded an optimism-adjusted AUC of 0.92 (eTable 7, links.lww.com/NXI/A868).

Discussion

In our study, we identified 9 CSF inflammatory biomarker candidates that were differentially expressed in patients with PD, at the time of diagnosis, compared with controls, using the highly specific and sensitive PEA technology along with regularized logistic regression with EN penalization. Seven of the 9 biomarker candidates were included in a panel that was found to be able to distinguish PD from controls. In addition, we found a panel of 4 inflammatory biomarkers associated with AD, of which 2 (CD8A and IL-6) were common in both the AD and PD panels.

Figure 2 Regularized Regression With Elastic Net Penalization for α Values Between 0 and 1 to Identify Biomarkers Associated With PD or AD Compared With Controls



Variables are highlighted in red when increased values are associated with disease and they are highlighted in blue when decreased values are associated with disease. The intensity of color reflects the strength of association. (A) Nine PEA biomarkers were associated with PD across all levels of α, and (B) 4 PEA biomarkers were associated with AD across all levels of α. The names of biomarkers associated with disease status across all levels of α are indicated. For abbreviations, see eTable 5 (links.lww.com/NXI/A868). PEA = proximity extension assay.

Table 2 Mean NPX Values of Inflammatory Biomarkers and Association With Disease Status According to Elastic Net Analysis

CSF biomarker	NC, NPX, mean (SD)	PD, NPX, mean (SD)	DLB, NPX, mean (SD)	AD, NPX, mean (SD)	PD EN[a]	AD EN[a]
ADA	4.76 (0.64)	4.47 (0.79)	4.47 (1.00)	4.52 (0.78)	↓	
CCL23	1.96 (1.04)	1.53 (0.96)	2.19 (0.88)	2.58 (1.24)	↓	
CD5	1.05 (0.43)	1.18 (0.63)	1.17 (0.37)	1.16 (0.41)	↑	
CD8A	4.17 (0.80)	3.55 (0.90)	3.76 (0.85)	3.63 (0.74)	↓	↓
CDCP1	2.98 (0.56)	3.01 (0.55)	3.07 (0.37)	3.10 (0.47)	↑	
FGF-19	3.91 (1.30)	3.26 (1.17)	3.84 (0.95)	4.32 (1.13)	↓	
IL-18R1	2.90 (0.59)	3.00 (0.52)	3.00 (0.40)	3.20 (0.53)	↑	
IL-6	2.13 (0.83)	2.70 (1.69)	2.26 (0.79)	3.04 (1.50)	↑	↑
MCP-2	3.68 (1.26)	3.09 (1.09)	3.78 (0.95)	3.94 (1.22)	↓	
MMP-1	6.43 (1.25)	6.10 (1.09)	6.30 (1.42)	7.60 (1.58)		↑
MMP-10	3.66 (0.78)	3.51 (0.74)	3.74 (0.58)	4.28 (0.82)		↑

Abbreviations: AD = Alzheimer disease; DLB = dementia with Lewy bodies; EN = elastic net; NC = normal controls; PD = Parkinson disease.
[a] "↑" indicates that increased values are associated with disease status, and "↓" indicates that decreased values are associated with disease status. No PEA biomarkers were robustly associated with DLB status

Multiplex biomarker studies are a valuable tool to identify inflammatory biomarkers. The same inflammation PEA multiplex panel has been applied to the CSF in 2 previous studies that each aimed to distinguish patients with APS from patients with PD and healthy controls.[11,12] One study found no differences between patients with PD and controls in the biomarkers assessed.[11] However, this study was notably smaller (including 37 patients with PD and 34 controls), and the patients had substantially longer disease duration during lumbar puncture (mean 11.6 ± 6.1 years), which makes comparison with our study of newly diagnosed patients with PD challenging. The second study, with 44 patients with PD (average disease duration of 3.5 ± 2.9 years) and 25 controls, found significant increases of CCL23 and MCP-2 in patients

Table 3 Receiver Operating Characteristic Models for Normal Controls vs Patients With Parkinson Disease or Alzheimer Disease

	Full model, PD				Full model, AD			
Predictors	**OR (95% CI)**	***p*[a]**	**AUC**	**Optimism-adjusted AUC**	**OR (95% CI)**	***p*[a]**	**AUC**	**Optimism-adjusted AUC**
Age	1.04 (0.99–1.09)	0.137	0.86	0.82	1.09 (1.00–1.20)	0.051	0.91	0.87
Female	0.17 (0.06–0.46)	**0.001**			1.87 (0.34–12.04)	0.483		
ADA	0.40 (0.16–0.91)	**0.038**			—	—		
CD5	4.10 (1.37–15.08)	**0.019**			—	—		
CD8A	0.35 (0.14–0.83)	**0.021**			0.07 (0.01–0.25)	**0.001**		
CDCP1	4.06 (1.18–15.37)	**0.030**			—	—		
IL-18R1	3.00 (0.90–10.88)	0.082			—	—		
IL-6	1.84 (1.12–3.49)	**0.034**			1.38 (0.49–3.86)	0.532		
MCP-2	0.33 (0.17–0.62)	**0.001**			—	—		
MMP-1	—	—			1.39 (0.62–3.38)	0.427		
MMP-10	—	—			6.49 (1.76–32.75)	**0.011**		

Abbreviations: AD = Alzheimer's disease; AUC = area under the curve; OR = odds ratio; PD = Parkinson disease.
[a] *p* values in bold are statistically significant at $p < 0.05$.

with PD compared with controls.[12] Similarly, a larger study investigating inflammation-related biomarkers for PD and PD progression found a significantly higher level of CCL23 in patients with PD than in healthy controls and no differences in IL-6 levels.[31] However, this study investigated biomarkers in the plasma, while we used the CSF.

With a considerably higher number of patients, our study expands on the previous findings by demonstrating that a broader panel of inflammatory markers is associated with PD during diagnosis. Our analyses identified 2 biomarkers (CDCP1 and IL-18R1) that have not previously been associated with PD, although their link to the disease is supported by evidence from other fields. IL-18R1 is a receptor that specifically binds interleukin 18 (IL-18). Of interest, IL-18 has been found to be significantly higher in controls compared with patients with PD and MCI,[32] suggesting a role for IL18-mediated signal transduction in disease risk and the development of cognitive symptoms in PD. Second, CDCP1 has been associated with idiopathic normal pressure hydrocephalus, which can include parkinsonian-like features in addition to the classical triad of gait disturbances, urinary incontinence, and cognitive deterioration.[33] Of the other biomarkers identified in this study, ADA, CD5, and MCP-4 were also new candidates for the association with PD risk, and further studies are justified to validate these findings.

Because neuroinflammation is not exclusive to PD, we also analyzed the inflammatory biomarker profile in patients with DLB and AD. While no biomarker was robustly associated with DLB, likely attributed to the low sample size, 4 biomarkers were associated with AD. Two of these (MMP-1 and MMP-10) were unique to the AD panel in this study. In line with our findings, previous studies have also identified MMP-10 as a potential AD-specific biomarker candidate, demonstrating differences in MMP-10 levels in patients with AD and patients with other neurologic disorders or patients with mild cognitive impairment due to AD (AD-MCI) and cognitively normal controls.[6,9,34] Furthermore, 1 study found elevated MMP-1 levels in postmortem brain tissue samples from patients with AD compared with controls.[35]

IL-6 and CD8A were identified in both the AD and PD panels. Previous findings comparing CSF levels of IL-6 in patients with PD or AD to controls, measured using enzyme-linked immunosorbent style assays, are inconsistent. While a meta-analysis from 2018, including 6 studies from 1995 to 2017, found an overall increased level of IL-6 in PD,[36] more recent studies including larger numbers of patients found no association with PD.[3,37] IL-6 is associated with a broad range of diseases with links to inflammation and may be a more general biomarker for inflammation.[38,39] CD8A is a T-cell surface glycoprotein that is expressed by $CD8^+$ T cells.[40] Two previous studies using the same PEA panel did not report the levels of CD8A in the CSF of patients with PD.[11,12] Notably, a postmortem study of patients with PD found an association between the increase in $CD8^+$ T-cell infiltration and the increase in the presence of α-synuclein (α-syn) aggregation and subsequent dopaminergic neuronal death.[41]Because these are both hallmarks of PD pathology, this shows that CD8A plays a role in PD, and further investigation is needed to better define its potential as a biomarker for the disease. Together, the overlap of IL-6 and CD8A in the biomarker profile may indicate that these represent common pathways in PD and AD.

While the biomarkers identified in our study might be interesting candidates in themselves, their inclusion in a panel may be more powerful to support a diagnosis of PD. In addition to being able to distinguish those with PD from controls, our final 7-biomarker PD panel could also be used to distinguish PD from both DLB and AD. Previous PEA studies have shown the use of inflammatory biomarker panels in discriminating PD from either a group of APS, which included multiple system atrophy (MSA), or MSA alone.[11,12] We also found that our 4-biomarker AD panel could be used to discriminate patients with AD from patients with both DLB and PD. Future exploration of the PEA inflammatory biomarker in other cohorts is warranted to develop PD-specific and AD-specific inflammatory biomarker panels.

This study has some limitations. First, only 40 of the 92 biomarkers in the Olink Target 96 Inflammation Panel were detected in more than 90% of the patients. This is attributed to the panel being optimized for the measurement of serum and plasma samples rather than the CSF, and other studies have reported similar detection rates.[11,12] Second, our main focus was PD, and the smaller number of CSF samples available from patients with DLB or AD limits the power of the analyses to detect small effects for these disease groups. In addition, patients with PD were newly diagnosed at baseline, whereas all patients with DLB or AD had mild dementia at the time of inclusion. We did not account for other factors that might be related to inflammation, for example, we had insufficient information regarding the use of anti-inflammatory medication or *APOE* genotype to assess the potential effects on the biomarkers. Finally, validation of these results is important. However, we did not have available data to perform the same analysis in a validation cohort. This study also has notable strengths. First, we chose to use the highly specific and sensitive PEA technology, which avoids some challenges with quantifying biomarkers such as cross-reactivity. Our study is also the largest study using this technology to examine levels of inflammatory biomarkers in PD. In addition, our study includes well-defined prospective cohorts where patients were all recruited early in the disease stage using standardized diagnostic criteria for PD, DLB, and AD. Last, we used EN analysis and correlation analysis to reduce the risk of overfitting the model.

In conclusion, we report the analysis of 40 biomarkers involved in inflammation and their association with PD, DLB, and AD. We identified 9 CSF inflammation-related biomarkers that were associated with PD and 4 biomarkers that were associated with AD. When assessing the ability of these

biomarkers to distinguish patients from controls, a combination of 7 and 4 biomarkers showed high discriminatory power for PD and AD, respectively. The inflammation-related biomarkers identified in our study may be further combined with other biomarkers (for example, α-syn or tau) to improve diagnostic performance, which should be investigated in future studies in other large cohorts.

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Disclosure

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Appendix Authors

Name	Location	Contribution
Camilla Christina Pedersen, MSc	The Norwegian Centre for Movement Disorders, Stavanger University Hospital; Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger, Norway	Drafting/revision of the article for content, including medical writing for content; study concept or design; and analysis or interpretation of data
Anastasia Ushakova, PhD	Section of Biostatistics, Department of Research, Stavanger University Hospital, Norway	Analysis or interpretation of data
Ragnhild Eide Skogseth, MD, PhD	Department of Geriatric Medicine, Haraldsplass Deaconess Hospital; Department of Clinical Medicine, University of Bergen, Norway	Drafting/revision of the article for content, including medical writing for content
Guido Alves, MD, PhD	The Norwegian Centre for Movement Disorders, Stavanger University Hospital; Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger; Department of Neurology, Stavanger University Hospital, Norway	Drafting/revision of the article for content, including medical writing for content
Ole-Bjørn Tysnes, MD, PhD	Department of Clinical Medicine, University of Bergen; Department of Neurology, Haukeland University Hospital, Bergen, Norway	Drafting/revision of the article for content, including medical writing for content
Dag Aarsland, MD, PhD	Centre for Age-Related Medicine, Stavanger University Hospital, Norway; Department of Old Age Psychiatry, Institute of Psychiatry, Psychology, and Neuroscience, King's College London, United Kingdom	Drafting/revision of the article for content, including medical writing for content
Johannes Lange, PhD	The Norwegian Centre for Movement Disorders, Stavanger University Hospital; Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger, Norway	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Jodi Maple-Grødem, PhD	The Norwegian Centre for Movement Disorders, Stavanger University Hospital; Department of Chemistry, Bioscience and Environmental Engineering, University of Stavanger, Norway	Drafting/revision of the article for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

References

1. Tansey MG, Wallings RL, Houser MC, Herrick MK, Keating CE, Joers V. Inflammation and immune dysfunction in Parkinson disease. *Nat Rev Immunol.* 2022;22(11):657-673.
2. Lindqvist D, Hall S, Surova Y, et al. Cerebrospinal fluid inflammatory markers in Parkinson's disease–associations with depression, fatigue, and cognitive impairment. *Brain Behav Immun.* 2013;33:183-189.
3. Hall S, Janelidze S, Surova Y, Widner H, Zetterberg H, Hansson O. Cerebrospinal fluid concentrations of inflammatory markers in Parkinson's disease and atypical parkinsonian disorders. *Sci Rep.* 2018;8(1):13276.
4. Rathnayake D, Chang T, Udagama P. Selected serum cytokines and nitric oxide as potential multi-marker biosignature panels for Parkinson disease of varying durations: a case-control study. *BMC Neurol.* 2019;19(1):56.
5. Green HF, Khosousi S, Svenningsson P. Plasma IL-6 and IL-17A correlate with severity of motor and non-motor symptoms in Parkinson's disease. *J Parkinsons Dis.* 2019;9(4):705-709.
6. Whelan CD, Mattsson N, Nagle MW, et al. Multiplex proteomics identifies novel CSF and plasma biomarkers of early Alzheimer's disease. *Acta Neuropathologica Commun.* 2019;7(1):169.
7. Jiang Y, Zhou X, Ip FC, et al. Large-scale plasma proteomic profiling identifies a high-performance biomarker panel for Alzheimer's disease screening and staging. *Alzheimers Dement.* 2022;18(1):88-102.

8. King E, O'Brien JT, Donaghy P, et al. Peripheral inflammation in prodromal Alzheimer's and Lewy body dementias. *J Neurol Neurosurg Psychiatry*. 2018;89(4):339-345.
9. Gaetani L, Bellomo G, Parnetti L, Blennow K, Zetterberg H, Di Filippo M. Neuroinflammation and Alzheimer's disease: a machine learning approach to CSF proteomics. *Cells*. 2021;10(8):1930.
10. Huang J, Khademi M, Fugger L, et al. Inflammation-related plasma and CSF biomarkers for multiple sclerosis. *Proc Natl Acad Sci U S A*. 2020;117(23):12952-12960.
11. Jabbari E, Woodside J, Guo T, et al. Proximity extension assay testing reveals novel diagnostic biomarkers of atypical parkinsonian syndromes. *J Neurol Neurosurg Psychiatry*. 2019;90(7):768-773.
12. Santaella A, Kuiperij HB, van Rumund A, et al. Inflammation biomarker discovery in Parkinson's disease and atypical parkinsonisms. *BMC Neurol*. 2020;20(1):26.
13. Alves G, Muller B, Herlofson K, et al. Incidence of Parkinson's disease in Norway: the Norwegian ParkWest Study. *J Neurol Neurosurg Psychiatry*. 2009;80(8):851-857.
14. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. 1992;55(3):181-184.
15. Fahn S. Unified Parkinson's disease rating scale. *Recent Dev Parkinsons Dis*. 1987;2:153-163.
16. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. *Neurology*. 1967;17(5):427-442.
17. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12(3):189-198.
18. Breitve MH, Chwiszczuk LJ, Bronnick K, et al. A longitudinal study of neurocognition in dementia with Lewy bodies compared to Alzheimer's disease. *Front Neurol*. 2018;9:124.
19. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. *Neurology*. 2005;65(12):1863-1872.
20. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology*. 1984;34(7):939-944.
21. Alves G, Brønnick K, Aarsland D, et al. CSF amyloid-beta and tau proteins, and cognitive performance, in early and untreated Parkinson's disease: the Norwegian ParkWest Study. *J Neurol Neurosurg Psychiatry*. 2010;81(10):1080-1086.
22. Olink. Transparent validation for data you can trust [online]. Accessed October 21, 2022. olink.com/our-platform/assay-validation/.
23. Olink. What is NPX? [online]. Accessed November 2, 2022. olink.com/faq/what-is-npx/.
24. Friedman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models via coordinate descent. *J Stat Softw*. 2010;33:1-22.
25. Lange K, Papp JC, Sinsheimer JS, Sobel EM. Next generation statistical genetics: modeling, penalization, and optimization in high-dimensional data. *Annu Rev Stat its Appl*. 2014;1:279-300.
26. Wei Z, Wang W, Bradfield J, et al. Large sample size, wide variant spectrum, and advanced machine-learning technique boost risk prediction for inflammatory bowel disease. *Am J Hum Genet*. 2013;92(6):1008-1012.
27. Fox J, Weisberg S. *An R Companion to Applied Regression*, 3rd ed. Sage; 2019.
28. Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics*. 2011;12(1):77.
29. Davison AC, Hinkley DV. *Bootstrap Methods and Their Applications*. Cambridge University Press; 1997.
30. Canty A, Ripley BD. *Boot: Booststrap R (S-Plus) Functions*; 2021.
31. Bartl M, Dakna M, Schade S, et al. Blood markers of inflammation, neurodegeneration, and cardiovascular risk in early Parkinson's disease. *Mov Disord*. 2023;38(1):68-81.
32. Kıçık A, Tüzün E, Erdoğdu E, et al. Neuroinflammation Mediators are reduced in Sera of Parkinson's disease patients with mild cognitive impairment. *Noro Psikiyatr Ars*. 2020;57(1):15-17.
33. Lolansen SD, Rostgaard N, Andreassen SN, et al. Elevated CSF inflammatory markers in patients with idiopathic normal pressure hydrocephalus do not promote NKCC1 hyperactivity in rat choroid plexus. *Fluids Barriers CNS*. 2021;18(1):54.
34. Boström G, Freyhult E, Virhammar J, et al. Different inflammatory signatures in Alzheimer's disease and Frontotemporal dementia cerebrospinal fluid. *J Alzheimers Dis*. 2021;81(2):629-640.
35. Leake A, Morris CM, Whateley J. Brain matrix metalloproteinase 1 levels are elevated in Alzheimer's disease. *Neurosci Lett*. 2000;291(3):201-203.
36. Chen X, Hu Y, Cao Z, Liu Q, Cheng Y. Cerebrospinal fluid inflammatory cytokine aberrations in Alzheimer's Disease, Parkinson's disease and amyotrophic lateral sclerosis: a systematic review and meta-analysis. *Front Immunol*. 2018;9:2122.
37. Starhof C, Winge K, Heegaard NHH, Skogstrand K, Friis S, Hejl A. Cerebrospinal fluid pro-inflammatory cytokines differentiate parkinsonian syndromes. *J Neuroinflammation*. 2018;15(1):305.
38. Papadopoulos A, Palaiopanos K, Björkbacka H, et al. Circulating interleukin-6 levels and incident ischemic stroke: a systematic review and meta-analysis of prospective studies. *Neurology*. 2022;98(10):e1002-e1012.
39. Luo Y, Zheng SG. Hall of fame among pro-inflammatory cytokines: interleukin-6 gene and its transcriptional regulation mechanisms. *Front Immunol*. 2016;7:604.
40. Bevan MJ. Helping the CD8+ T-cell response. *Nat Rev Immunol*. 2004;4(8):595-602.
41. Galiano-Landeira J, Torra A, Vila M, Bové J. CD8 T cell nigral infiltration precedes synucleinopathy in early stages of Parkinson's disease. *Brain*. 2020;143(12):3717-3733.

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