Journal of Alzheimer's Disease Reports 8 (2024) 1361–1371
DOI 10.3233/ADR-240110
IOS Press

Research Report

Biomarker Assessment in Parkinson's Disease Dementia and Dementia with Lewy Bodies by the Immunomagnetic Reduction Assay and Clinical Measures

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Received 20 June 2024
Accepted 14 August 2024
Published 25 October 2024

Abstract.
Background: Plasma biomarker assays provide an opportunity to reassess whether Alzheimer's disease, Parkinson's disease dementia (PDD), and dementia with Lewy bodies (DLB) plasma biomarkers are diagnostically useful.
Objective: We hypothesized that immunomagnetic reduction (IMR) of plasma biomarkers could differentiate between patients with PDD and DLB and healthy patients when combined with established clinical testing measures.
Methods: Plasma samples from 61 participants (12 PDD, 12 DLB, 37 controls) were analyzed using IMR to quantify amyloid-β 42 ($A\beta_{42}$), total tau (t-tau), phosphorylated tau at threonine 181 (p-tau181), and α-synuclein (α-syn). Receiver operating characteristic curve (ROC) analysis was used to obtain sensitivity, specificity, and area under the ROC curve. Biomarker results were combined with clinical measures from the Unified Parkinson's Disease Rating Scale (UPDRS), Montreal Cognitive Assessment, and Hoehn-Yahr stage to optimize diagnostic test performance.
Results: Participants with PDD had higher α-syn than those with DLB and healthy participants and were distinguishable by their biomarker products $A\beta_{42}\times$p-tau181 and $A\beta_{42}\times$α-syn. Patients with DLB had higher p-tau181 than those with PDD and healthy participants and were distinguishable by their concentrations of α-syn×p-tau181. Plasma α-syn plus UPDRS versus either test alone increased sensitivity, specificity, and AUC when healthy patients were compared with those with PDD and DLB. Combined clinical examination scores and plasma biomarker products demonstrated utility in differentiating PDD from DLB when p-tau181 was combined with UPDRS, α-syn was combined with UPDRS, and α-syn×p-tau181 was combined with UPDRS.

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Conclusions: In this pilot study, IMR plasma p-tau181 and α-syn may discriminate between PDD and DLB when used in conjunction with clinical testing.

Keywords: Alzheimer's disease, α-synuclein, dementia with Lewy bodies, immunomagnetic reduction assay, Parkinson's disease dementia, p-tau181, Unified Parkinson's Disease Rating Scale

INTRODUCTION

Whether Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB) represent distinct subtypes of dementia, or whether they exist along a continuous spectrum of α-synuclein (α-syn)–associated disorders, is an area of debate by medical professionals. Despite their wide clinical and morphologic overlap, PDD and DLB are generally regarded as separate neurocognitive disorders.[1] Diagnosis of both PDD and DLB is almost exclusively clinical and is based on a combination of a history of reported evolution of symptoms, characteristic examination findings, and the arbitrarily defined and strictly operational 1-year-rule that dementia preceding or emerging within 1 year of the onset of parkinsonian motor symptoms is characteristic of DLB rather than PDD, which features the insidious development of dementia over several years after motor symptoms emerge.[2,3] The differential diagnosis is difficult in presenting patients, particularly in those in the early stages of disease because wide clinical and neuropathologic overlap exists among PDD, DLB, and other neurodegenerative diseases such as Alzheimer's disease. Currently, only limited diagnostic and confirmatory testing is available to assist in the clinical characterization and differentiation between PDD and DLB. Imaging modalities such as dopamine transporter single-photon emission computed tomography (SPECT) and positron emission tomography (PET), metaiodobenzylguanidine scintigraphy, and magnetic resonance imaging volumetric analyses are neither routinely available nor able to exhibit sufficient specificity and sensitivity in discriminating among these disease subtypes.[4–6] Cardiac, skin, and gastrointestinal biopsies for α-syn deposition may provide diagnostic insight into DLB as a diagnosis in general, but they are limited in differentiating PDD from DLB, and they are not routinely accessible.[7] Although ongoing research actively aims to establish cerebrospinal fluid (CSF) amyloid-β 42 ($A\beta_{42}$), total tau (t-tau), and phosphorylated tau at threonine 181 (p-tau181) as robust prognostic biomarkers for identifying and differentiating Alzheimer's disease in its many stages and presentations, the utility of these markers in differentiating synucleinopathies like PDD and DLB is limited.[8–11] CSF analyses have demonstrated strikingly similar CSF profiles in patients with Alzheimer's disease and DLB, with reduced $A\beta_{42}$ in patients with DLB. The results of studies evaluating CSF α-syn measurements in the diagnosis of DLB preclude establishing a strong correlation because of their small sample sizes and low collection yields. In plasma biomarker analyses, p-tau181 has been shown to reliably distinguish mild cognitive impairment plus Alzheimer's disease from other pathologies such as frontotemporal dementia and progressive supranuclear palsy but not from DLB. Furthermore, concentrations of these plasma biomarkers have not been found to correlate well with PET-Aβ–positive or PET-Aβ–negative DLB. Enzyme-linked immunosorbent assay (ELISA) and single-molecule assay testing of p-tau181, α-syn, p-tau217, and glial fibrillary acidic protein have shown mixed clinical utility in distinguishing DLB from other neurodegenerative diseases, and plasma neurofilament light chain has demonstrated some introductory utility in differentiating Parkinson-plus syndromes but has not been shown to be reliable for diagnostic differentiation.[12,13]

CSF testing requires advanced scheduling, is often invasive, features peri- and postprocedural risks, and may yield CSF samples of varying quantity and purity, which makes studies prone to poor reproducibility, depending on the individual researcher performing the procedure. Given the wide clinical overlap that exists in these synuclein-related dementia subtypes, the arbitrary clinical criteria currently used to differentiate them, and the overall paucity of reliable, objective diagnostic assays available to assist in differentiation, there would be tremendous value in developing reliable ancillary testing, such as biomarker screening in plasma, which is less invasive, more cost-effective, easily reproducible, and far more accessible than other modalities. The utility of plasma biomarkers for the diagnosis of PDD and DLB has long been hampered by issues of assay sensitivity and specificity, as well as low detec-

tion thresholds; concentrations of disease-associated components in plasma are precipitously lower than those in CSF, most notably in traditional ELISA measurements.[14–16]

Over the past several years, the continual development of more sophisticated detection assays has addressed classic limitations in plasma biomarker measurement. Antigen-targeting detection assays, such as immunomagnetic reduction (IMR), single-molecule array, multimer detection system, and immunoprecipitation/mass spectrometry, have increased sensitive quantification of biomarkers.[16–21] IMR is of particular interest in this study. IMR uses nanobead-conjugated antibodies that bind to specific target analytes of interest. A superconducting quantum interference device and an alternating current magnetic susceptometer record the resulting differentials in measured magnetic signal, which correlate to the amount of antigen binding to the antibodies (i.e., to minute changes in buffer viscosity), thereby allowing for the ultra-sensitive measurement of plasma concentrations of classic neurodegenerative biomarkers such as tau and $A\beta_{42}$ with exceptional accuracy.[14,22,23] IMR not only has been clinically validated and shown to have accuracy superior to that of other plasma detection platforms such as ELISA but also has demonstrated utility in distinguishing healthy patient controls from patients with Alzheimer's disease, and it has even distinguished between different stages of cognitive impairment, namely the prodromal and dementia phases of Alzheimer's disease.[24,25] With detection thresholds in the low pg/mL to fg/mL range, technologies like the IMR-superconducting quantum interference device immunoassay provide tremendous utility in optimizing the quantification of low-abundance plasma proteins.[14] This technology may therefore be used in developing plasma assays for common biomarkers implicated in PDD and DLB.

Our study used the IMR assay to assess the diagnostic utility of plasma biomarkers, including $A\beta_{42}$, t-tau, p-tau181, and α-syn, in identifying and differentiating among PDD, DLB, and healthy patient controls. Plasma concentrations of these biomarkers were then combined with scores on classic PDD and DLB functional assessments to further evaluate potential opportunities for optimizing the sensitivity and specificity of the biomarker measures, all with the intent of advancing clinical methodologies used in diagnosing and differentiating PDD and DLB. We hypothesize that these plasma assays could serve not only as reliable markers for disease but also as tools to distinguish healthy patients from PDD and DLB and PDD from DLB when used in conjunction with established clinical tests.

METHODS

Standard protocol approvals, registrations, and patient consents

All interested patients who were eligible to participate were informed about the study protocol and its aims, including their ability to withdraw from the study at any time. The patients who opted to participate were consented for participation according to the human subject study protocol approved by the institutional review board of Barrow Neurological Institute at St. Joseph's Hospital and Medical Center in Phoenix, Arizona. The recruitment and informed consent processes were conducted by qualified personnel within the Department of Neurology.

Classification of participants

Sixty-one participants were consecutively recruited from the movement disorder and memory disorder clinics. Because this study was a pilot study, the sample size was not prespecified, and the sample pool was 61 people. Of these 61 participants, 12 had PDD, 12 had DLB, and the rest ($n = 37$) composed the healthy control group with intact physical and cognitive functions and no demonstratable cognitive complaints. The inclusion criteria for the 12 participants with PDD were categorized on the basis of positive clinical diagnostic and supportive prospective criteria from the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria first outlined in 1992 by Hughes et al.[26] the inclusion criteria for the 12 participants with DLB were categorized on the basis of clinical diagnostic criteria delineated in 2017 by McKeith et al.[2] as part of the third DLB consortium. Participants were excluded if they did not meet the diagnostic categorical criteria. There were no exclusions on the basis of severity or medications.

Quantitative variables collected during the study included the participants' sex, age, educational level, scores on the motor portion of the Unified Parkinson's Disease Rating Scale (UPDRS) and the Montreal Cognitive Assessment (MoCA), and their Hoehn-Yahr (H-Y) stage, which is a clinical measure of functional disability in Parkinson's disease. Clinical

assessments were conducted by the same attending neurologist for each participant. Participants who tested within the normal range on the motor UPDRS, MoCA, and H-Y assessments (healthy controls) were compared directly to the participants with PDD and DLB.

Laboratory techniques

Nonfasting plasma samples were collected from each participant in K3-EDTA–coated vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA) for use in quantifying the plasma biomarkers $A\beta_{42}$, t-tau, p-tau181, and α-syn. These samples were subsequently centrifuged at $2000 \times g$ for 15 min at room temperature. The upper layer (plasma) was then transferred to new 15-mL standard tubes, aliquoted at 1.5 mL in standard tubes, and stored at –70°C. Sample aliquots were shipped on dry ice to MagQu Co, Ltd (Surprise, AZ, USA) for quantification via IMR assays. Before the assays were performed, all samples were deidentified to mask the participants' demographic features and clinical diagnoses. The frozen aliquoted samples were thawed on ice before sample preparation, and assays were performed at room temperature. For each sample, assays were performed in duplicate for $A\beta_{42}$, t-tau, p-tau181, and α-syn. The volumes of the reagent (MF-AB2-0060; MagQu) and plasma sample were 60 μL reagent and 60 μL plasma for $A\beta_{42}$; 80 μL reagent and 40 μL plasma for t-tau; 80 μL reagent and 40 μL plasma for p-tau181; and 80 μL reagent and 40 μL plasma for α-syn. Samples and reagents were mixed briefly in glass tubes and sealed. The tubes were then placed inside the sample channels of the IMR analyzer (XacPro-S; MagQu) for analysis. The final concentrations of $A\beta_{42}$, t-tau, p-tau181, and α-syn were calculated according to the standard curves of each marker. Duplicate measurements of each marker were obtained, and the mean (SD) was calculated for each marker and each sample and then tabulated. These values are expressed as a concentration in pg/mL.

Statistical analysis

Plasma biomarker concentrations were tabulated, as described in the preceding section on laboratory techniques. These concentrations and their computed products were regarded as continuous variables and were primarily normally distributed. The parametric comparison of means was performed by one-way analysis of variance. Sample means (SDs) were then compared using the Student-Newman-Keuls method or the Kruskal-Wallis test for pairwise analysis to further test the sample means for significance. Receiver operating characteristic curve (ROC) analysis was used to determine the ability of the biomarkers to discriminate among patients with PDD, DLB, and healthy control patients, and to survey optimal sensitivity and specificity between the biomarkers and clinical data, as well as to derive sensitivity, specificity, and area under the ROC curve (AUC) values. Multivariate logarithmic logistic regression was used to combine plasma biomarker products and clinical assessment scores into unified variates for ROC analysis. The Bonferroni correction was used for multiple comparisons. All statistical analyses were performed using MedCalc statistical software, version 17.4.4 (MedCalc Software, Ltd, Ostend, Belgium). Statistical significance was defined as $p < 0.05$. The same neurology resident physician performed all statistical calculations. All data that were collected, including demographic assessment data and quantified plasma concentrations, are expressed as absolute numbers and as means (SDs) for the purpose of descriptive statistical analysis. Analyses were performed to differentiate among patients with PDD, DLB, and healthy status.

RESULTS

This pilot study included a sample of 61 participants: 12 with a diagnosis of PDD, 12 with a diagnosis of DLB, and 37 healthy controls. The mean (SD) age of participants in the PDD group was 74.8 (8.5) years and 71.8 (6.4) in the DLB group; these results were higher than that of the control group 69.6 (8.5) years; both $p < 0.05$. No significant differences were found in the years of education among the groups. The DLB group demonstrated markedly worse performance on the MoCA than the healthy control group (17.8 [4.6] versus 27.5 [2.3]; $p < 0.001$). As expected, the mean UPDRS score of the PDD group was higher than that of the control group (31.8 [21.1] versus 2.8 [3.7]; $p < 0.001$) and even higher for the DLB group versus the healthy control group (51.6 [16.8] versus 2.8 [3.7]; $p < 0.001$). The PDD (1.7 [0.9]) and DLB 2.2 [1.0]) groups exhibited higher H-Y stages than the healthy control group (0.1 [0.3]); $p < 0.001$ (Table 1).

The IMR assay was used to measure plasma biomarker concentrations because of its demonstrated improved detection threshold for measuring

Table 1
Demographics of 61 patients who underwent biomarker assessment[a]

Variable	NC ($n = 37$)	PDD ($n = 12$)	DLB ($n = 12$)
Sex, *n* (%)			
Women	25 (68)	7 (58)	7 (58)
Men	12 (32)	5 (42)	5 (42)
Age, y	69.6 (8.5)	**74.8 (8.5)**[b]	71.8 (6.4)
Education, y	15.3 (2.7)	15.7 (3.0)	14.8 (3.7)
UPDRS score	2.8 (3.7)	**31.8 (21.1)**[c]	**51.6 (16.8)**[c]
MoCA score	27.5 (2.3)	25.3 (5.5)	**17.8 (4.6)**[c]
H-Y stage	0.1 (0.3)	**1.7 (0.9)**[c]	**2.2 (1.0)**[c]

DLB, dementia with Lewy bodies; H-Y, Hoehn-Yahr; MoCA, Montreal Cognitive Assessment; NC, normal controls; PDD, Parkinson's disease dementia; UPDRS, Unified Parkinson's Disease Rating Scale. [a]Values are mean (SD) unless otherwise indicated. [b]Bolded values indicate significance ($p < 0.05$ compared to NC). [c]Bolded values indicate significance ($p < 0.001$ compared to NC).

Table 2
Plasma biomarker concentrations and computed products in 61 patients[a]

Biomarker/Clinical Diagnostic Marker	NC ($n = 37$)	PDD ($n = 12$)	DLB ($n = 12$)
$A\beta_{42}$, pg/mL	16.91 (1.38)	16.30 (1.33)	16.25 (1.23)
t-tau, pg/mL	20.22 (4.55)	20.21 (5.04)	20.61 (4.38)
p-tau181, pg/mL	3.32 (0.80)	3.03 (0.65)	**3.83 (0.79)**
α-syn, pg/mL	0.09 (0.05)	**0.17 (0.13)**	0.11 (0.06)
$A\beta_{42}$ × p-tau181	56.54 (15.94)	**49.04 (10.06)**	75.41 (51.56)
$A\beta_{42}$ × α-syn	1.52 (0.90)	**2.81 (1.99)**	1.81 (0.98)
α-syn × t-tau	1.84 (1.27)	3.62 (2.86)	2.30 (1.28)
α-syn × p-tau181	0.29 (0.20)	0.51 (0.40)	**0.42 (0.21)**

$A\beta_{42}$, amyloid-β 42; α-syn, α-synuclein; DLB, dementia with Lewy bodies; NC, normal controls; PDD, Parkinson's disease dementia; p-tau181, phosphorylated tau at threonine 181; t-tau, total tau. [a]Values are mean (SD). Bolded values indicate significance ($p < 0.05$, as compared to NC).

biomarkers in plasma compared to that of other blood-based assays and because of its potential utility for the development of readily available testing of human samples. The mean (SD) of plasma biomarker concentrations was compared among the participants with PDD, DLB, and healthy controls (Table 2). No significant differences were found in $A\beta_{42}$ and t-tau among the groups. The DLB group had a higher plasma concentration of p-tau181 than the healthy group (3.83 [0.79] versus 3.32 [0.80]; $p < 0.05$). The PDD group had a higher plasma concentration of α-syn than the healthy group (0.17 [0.13] versus 0.09 [0.05]; $p < 0.05$).

Previous comparisons of assay analyses of neurodegenerative biomarkers have demonstrated that plasma biomarker measurements conducted by IMR, which has the propensity to measure plasma monomers and oligomers equally (unlike single-molecule array, which has the propensity to measure predominantly oligomers) can be optimized by mathematical manipulation, thereby obtaining a new biomarker.[19,22] Therefore, we measured biomarker products in the 3 groups of participants. Similar to their lack of significance when measured alone, the $A\beta_{42}$ × t-tau product showed no significant difference among groups. The mean [SD] for the $A\beta_{42}$ × p-tau181 product was lower in participants with PDD (49.04 [10.06]) than in healthy controls (56.54 [15.94]) and those with DLB (75.41 [51.56]); $p < 0.05$ (Table 2). The α-syn × p-tau181 product was higher in participants with PDD (0.51 [0.40]) than in healthy controls (0.29 [0.20]) and those with DLB (0.42 [0.21]); $p < 0.05$. The $A\beta_{42}$ × α-syn product was higher in participants with PDD (2.81 [1.99]) than in healthy controls (1.52 [0.90]) and those with DLB (1.81 [0.98]); $p < 0.05$.

ROC analyses were performed on the measured plasma biomarker concentrations, their computed products, participants' scores on established functional clinical assessments, and various combinations of these clinical modalities to assess test sensitivity, specificity, and AUC for the ability of each modality to differentiate healthy participants from those with PDD and DLB. Clinical assessments such as the UPDRS, MoCA, and H-Y stage performed alone are sufficiently sensitive and specific to discriminate

Table 3
Sensitivity and specificity of biomarkers and clinical diagnostic markers for the 61 study participants

NC ($n = 37$) versus PDD ($n = 12$) and DLB ($n = 12$)				
Biomarker or Biomarker+Clinical Diagnostic Marker	Threshold	Sensitivity (%)	Specificity (%)	AUC
p-tau181	3.23	0.62	0.58	0.57
α-syn	0.09	0.71	0.64	0.67[a]
$A\beta_{1\text{-}42}$ × p-tau181	50.99	0.62	0.53	0.52
$A\beta_{1\text{-}42}$ × α-syn	1.47	0.71	0.64	0.68[a]
α-syn × p-tau181	0.33	0.67	0.72	0.71[b]
UPDRS score	11	1.00	0.97	1.00[c]
MoCA score	23	0.58	0.97	0.80[c]
H-Y stage	0	0.92	0.97	0.94[c]
p-tau181 + UPDRS score	0.24	1.00	0.97	1.00[c]
α-syn+UPDRS score	0.22	1.00	0.97	1.00[c]
p-tau181 + MoCA score	0.54	0.58	0.97	0.81[c]
α-syn+MoCA score	0.61	0.71	1.00	0.87[c]
p-tau181 + H-Y stage	0.11	0.92	0.97	0.93[c]
α-syn+H-Y stage	0.22	0.95	0.97	0.98[c]

Aβ, amyloid-β; AUC, area under the receiver operating characteristic curve; α-syn, α-synuclein; DLB, dementia with Lewy bodies; H-Y, Hoehn-Yahr; MoCA, Montreal Cognitive Assessment; NC, normal controls; PDD, Parkinson's disease dementia; p-tau181, phosphorylated tau at threonine 181; t-tau, total tau; UPDRS, Unified Parkinson's Disease Rating Scale. [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

between persons with and without disease, and the addition of plasma biomarkers augmented the ability of these examinations to differentiate between diseases. For example, the UPDRS score and H-Y stage had AUCs of 1.00 and 0.94, respectively, when differentiating the healthy controls from the PDD or DLB groups (Table 3). The AUC values for UPDRS scores were 0.99 and 0.94 when differentiating the healthy controls from the PDD group; AUCs were 1.00 and 0.95 for H-Y stage when differentiating the healthy controls from the DLB group. When plasma biomarkers were combined with clinical scores, the α-syn+UPDRS combination improved test sensitivity to 1.00, specificity to 0.97, and AUC to 1.00 ($p < 0.001$). The α-syn+H-Y combination improved test sensitivity to 0.95, specificity to 0.97, and AUC to 0.98 ($p < 0.001$) (Table 3). In the clinical differentiation of PDD and DLB, the use of the UPDRS, MoCA, and H-Y alone yielded mixed sensitivity and specificity, with AUCs of 0.79, 0.85, and 0.66, respectively (Table 4, Fig. 1). Plasma biomarker concentrations and their combined products also demonstrated mixed sensitivity and specificity when used alone to distinguish PDD from DLB, with the $A\beta_{42}$ × p-tau181 product proving to be the most robust, with sensitivity of 0.83, specificity of 0.75, and an AUC of 0.79 ($p < 0.01$) (Table 4).

The utility of plasma biomarkers and their computed products in diagnostic differentiation became most apparent when these values were combined with functional clinical data. For example, combining the UPDRS score with α-syn and p-tau181 increased the sensitivity and specificity of both examinations to 0.83 and yielded AUCs of 0.85 and 0.88, respectively ($p < 0.001$) (Table 4, Fig. 1). The α-syn × p-tau181 product, when combined with the UPDRS score, also yielded a more robust sensitivity, specificity, and AUC (all 0.83; $p < 0.001$) (Table 4). The p-tau181 + MoCA combination yielded an AUC of 0.92 ($p < 0.001$), and the $A\beta_{42}$ × p-tau181 product combined with the MoCA score yielded an AUC of 0.90 ($p < 0.001$); both demonstrated markedly improved aggregate measures of test performance over each test individually (Table 4, Fig. 1).

DISCUSSION

Our pilot study included 3 groups of participants: healthy control patients and patients with an established clinical diagnosis of PDD or DLB. Plasma samples from these participants were tested by IMR, an assay that has demonstrated a measurably increased yield of biomarkers whose detection in plasma has been challenging with other testing modalities. ELISA, for example, demonstrates trace detection of plasma α-syn but has not been able to produce yields fit for clinical differentiation of Parkinson's disease from atypical Parkinson's disease or DLB.[27,28] IMR was able to measure biomarker concentrations in plasma samples and to detect statistically significant concentration differences among

Table 4
Sensitivity and specificity of biomarkers and clinical diagnostic markers for participants with known diagnoses of PDD and DLB

	PDD ($n = 12$) versus DLB ($n = 12$)			
Biomarker or Biomarker+Clinical Diagnostic Marker	Threshold	Sensitivity	Specificity	AUC
p-tau181	3.45	0.75	0.92	0.78[a]
α-syn	0.18	0.42	0.92	0.65
$A\beta_{42}\times$p-tau181	55.92	0.83	0.75	0.78[a]
$A\beta_{42}\times\alpha$-syn	3.50	41.67	1.00	0.65
α-syn×p-tau181	0.71	0.33	1.00	0.54
UPDRS score	32	0.75	0.92	0.79[a]
MoCA score	24	1.00	0.75	0.85[b]
H-Y stage	1	0.92	0.33	0.66
p-tau181 + UPDRS score	0.45	0.83	0.83	0.88[b]
α-syn+UPDRS score	0.49	0.83	0.83	0.85[b]
p-tau181 + MoCA score	0.17	1.00	0.67	0.92[b]
α-syn+MoCA score	0.31	1.00	0.75	0.86[b]
p-tau181 + H-Y stage	0.49	0.67	0.92	0.78[a]
α-syn+H-Y stage	0.28	1.00	0.33	0.69
[$A\beta_{42}\times$p-tau181]+UPDRS score	0.25	1.00	0.58	0.83[b]
[$A\beta_{42}\times\alpha$-syn]+UPDRS score	0.31	1.00	0.58	0.83[b]
[α-syn×p-tau181]+UPDRS score	0.49	0.83	0.83	0.83[b]
[$A\beta_{42}\times$p-tau181]+MoCA score	0.17	1.00	0.67	0.90[b]
[$A\beta_{42}\times\alpha$-syn]+MoCA score	0.29	1.00	0.75	0.86[b]
[α-syn×p-tau181]+MoCA score	0.26	1.00	0.75	0.86[b]
[$A\beta_{42}\times$p-tau181]+H-Y stage	0.48	0.67	0.92	0.76[c]
[$A\beta_{42}\times\alpha$-syn]+H-Y stage	0.50	0.75	0.58	0.70
[α-syn×p-tau181]+H-Y stage	0.54	0.58	0.75	0.67

$A\beta_{42}$, amyloid-β 42; AUC, area under the receiver operating characteristic curve; α-syn, α-synuclein; DLB, dementia with Lewy bodies; H-Y, Hoehn-Yahr; MoCA, Montreal Cognitive Assessment; NC, normal controls; PDD, Parkinson's disease dementia; p-tau181, phosphorylated tau at threonine 181; t-tau, total tau; UPDRS, Unified Parkinson's Disease Rating Scale. [a]$p < 0.01$; [b]$p < 0.001$; [c]$p < 0.05$.

the healthy control patients and those with PDD and DLB. Our results show that persons with an established clinical diagnosis of PDD have higher levels of plasma α-syn and that persons with DLB have higher levels of p-tau181. This report is among the first reports of plasma α-syn assays clinically applied.

Plasma biomarker concentrations had no obvious association with demographic features such as age and education or with the performance of clinical testing in the healthy control group. In participants with PDD, age was mildly associated with increased plasma α-syn. Unsurprisingly, participants with a known dementia diagnosis performed markedly worse on established functional cognitive and neurologic testing measured by the UPDRS, MoCA, and H-Y stage. Our results demonstrate that these clinical measures, particularly the UPDRS score and H-Y stage, have modest utility individually in differentiating patients with PDD and DLB from healthy control patients, but they are not especially useful in differentiating between these disease processes.

A key finding of this study is that IMR plasma biomarker concentrations demonstrate utility in differentiating PDD from DLB. This finding may be explained by existing clinical histopathologic and morphologic data that show different topographic spreading patterns for α-syn pathology and different degrees of corresponding Alzheimer's disease copathology in DLB and PDD, despite their otherwise wide overlap, hence, the synuclein spectrum model of disease.[29,30] Our data demonstrate that combining clinical cognitive and neurologic functional assessments with IMR plasma biomarker concentrations increases the sensitivity and specificity of these established clinical tests. In contrast to how we compared the healthy control participants to the PDD and DLB participants, we used more combinations of plasma concentration and clinical examination, as well as more combinations of plasma concentration product and clinical examination in our comparison of the PDD and DLB groups to assess combinations of potential higher sensitivity and specificity. Most notably, testing above a certain

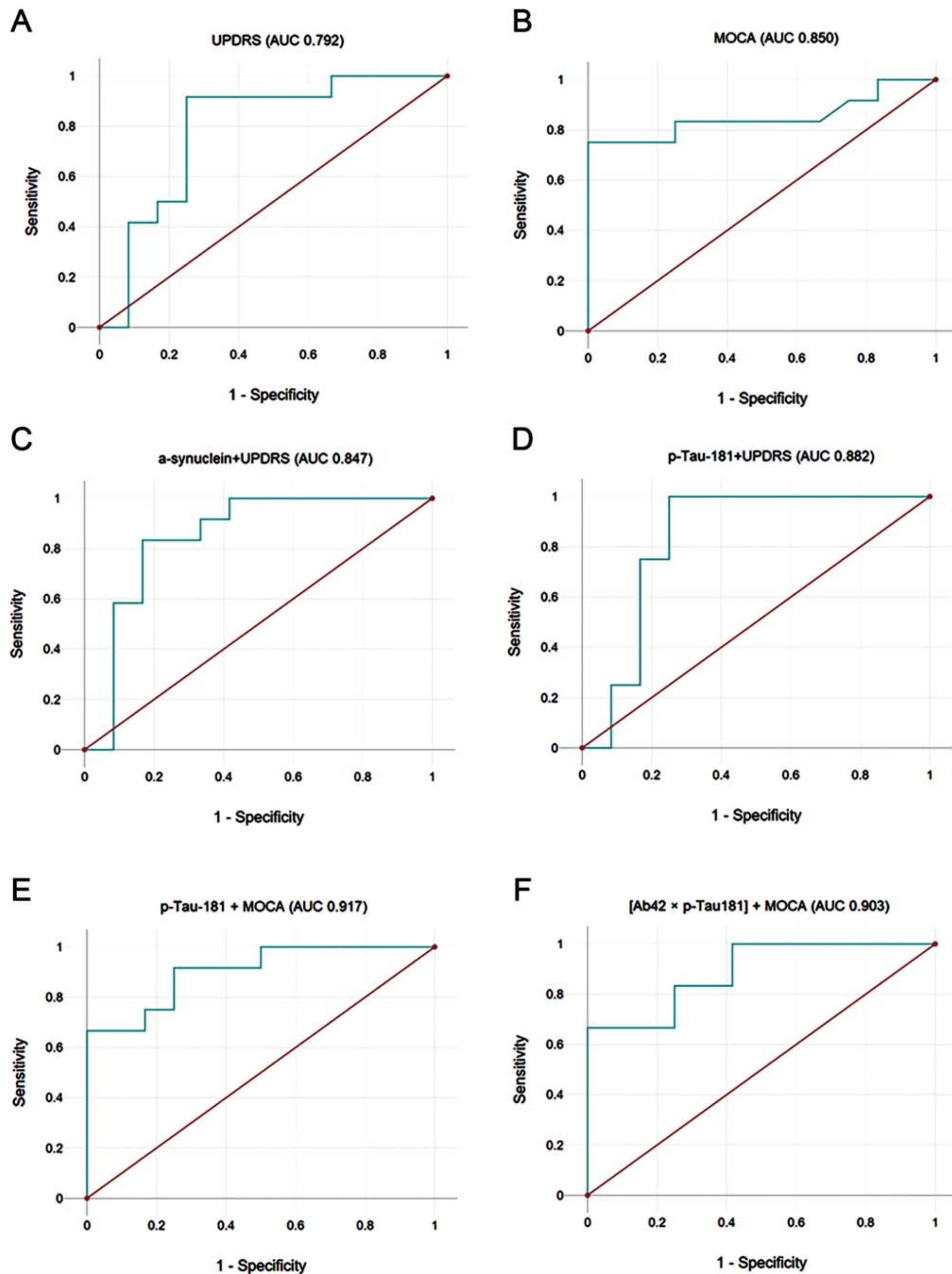

Fig. 1. Receiver operating characteristic curve (ROC) plots for the differentiation of Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB) using various clinical diagnostic measures with and without combined plasma biomarkers performed using the immunomagnetic reduction assay. The ROC yielded improved area under the ROC curve (AUC) when clinical measures were combined with plasma biomarkers. A) Unified Parkinson's Disease Rating Scale (UPDRS) had an AUC of 0.79. B) The Montreal Cognitive Assessment (MoCA) had an AUC of 0.85. C) The combination of α-synuclein (α-syn) and UPDRS had an AUC of 0.85. D) The combination of phosphorylated tau at threonine 181 (p-tau181) and UPDRS had an AUC of 0.88. E) The combination of p-tau181 and MoCA had an AUC of 0.92. F) The product of amyloid-β 42 ($A\beta_{42}$) and p-tau181 combined with MoCA had an AUC of 0.90. Used with permission from Barrow Neurological Institute, Phoenix, Arizona.

threshold in the combined p-tau181 + UPDRS and the combined α-syn+UPDRS categories was highly sensitive and specific for DLB compared to PDD. Plasma biomarkers therefore demonstrate utility in differentiating patients with PDD and DLB from healthy control patients, in that they augment a diagnostic

suspicion generated by clinical examination and help differentiate between these nuanced diagnoses.

Clinical differentiation between PDD and DLB has relied historically on subjective information about symptom duration and type, nuanced physical examination findings that must be carefully considered within the context of the patient's total clinical presentation, and postmortem histopathologic studies. The use of affordable, accessible, and reliable ancillary tests has the potential to mitigate diagnostic uncertainty and assist clinicians in making the correct diagnosis without relying solely on subjective history, clinical examination results, and watchful waiting to assess symptomatic progression. Earlier clinical discrimination can lead to earlier prognostication and management, thereby alleviating the disease burden on patients, their caregivers, and the overall health care system.

In the absence of definitive antemortem diagnostic modalities, a laboratory standard is needed that can assist with the clinical differentiation of PDD and DLB. Although CSF $A\beta_{42}$, t-tau, p-tau181 are subject to intensive ongoing study as biomarkers for identifying Alzheimer's disease and prodromal Alzheimer's disease, the utility of similar markers in the exploration of PDD and DLB has yielded variable results.[31] Invasive CSF testing is inconsistently available, especially in lower-income geographic areas of the US and in countries with low healthcare resources, and it can be difficult for older patients. Specialized neuroimaging modalities, such as dopamine transporter SPECT, PET, and metaiodobenzylguanidine scintigraphy, are expensive, less accessible, and demonstrate mixed diagnostic utility.[32]

The correlation between CSF and plasma α-syn remains to be explored. CSF dynamics in diseases like PDD and DLB continue to become better understood. For example, CSF α-syn decreases as the disease progresses compared to that of healthy controls (Parkinson's Progression Markers Initiative study).[33] α-Syn levels are low in the CSF of patients with PD but are higher in patients with dementia.[34] This finding might explain some of the differences observed in our study since we analyzed patients with PDD and not PD. Additionally, several reports indicate that plasma α-syn levels are higher than that in CSF.[35,36] Also, the antibodies used in the IMR platform to capture α-syn may bind to different forms of α-syn (i.e., monomeric versus oligomeric versus posttranslationally modified forms). Therefore, further studies are needed to correlate CSF and plasma α-syn to better understand their relationship.

Plasma biomarkers should continue to be explored because of their potential utility in neurodegenerative disease. The development of more sophisticated biomarker assays, such as IMR, has enabled higher-yield biomarker detection, which may facilitate sophisticated analyses that can aid in otherwise nuanced diagnoses. Our study demonstrated that using an IMR assay may enable the quantification of characteristic neurodegenerative biomarkers in plasma with good reliability and may help correlate these biomarkers in patients with established clinical diagnoses of PDD and DLB compared to those of healthy control patients. Furthermore, it demonstrated that using biomarkers in conjunction with established functional clinical measures may increase the diagnostic sensitivity and specificity of those tests over the individual use of the tests. The quantification of plasma biomarker concentrations by IMR identified objective differences in biomarker levels among the 3 groups, and it enabled the differentiation of PDD from DLB with a reasonable degree of certainty. These findings have immediate implications for maximizing the capabilities of diagnostic differentiation, which may lead to better prognoses, earlier patient and family counseling, and more optimal symptomatic management.

Limitations

This study has limitations, most notably, its small sample size of 61 participants. This limitation is multifactorial, given the pilot nature of the study, the disease groups selected, and the limited availability of this specific assay for processing plasma biomarker samples. The measured plasma concentrations of biomarkers also have few established comparative references in the medical literature, such that their measured concentrations alone are not indicative of disease severity compared to other commonly tested plasma levels with well-defined normal ranges. As these assays continue to be developed, other biomarkers such as glial fibrillary acidic protein and neurofilament light chain can also be tested, which may further establish relationships between these disease states. Another limitation is that the UPDRS scores seem worse for DLB than PDD, which might be related to duration of disease. Subsequent studies and analyses should consider these characteristics as covariates.

This pilot study creates many opportunities for future IMR research, namely, to compare plasma yield with CSF yield and yield in other fluids such as

saliva or biopsy and even with histopathologic analyses. This study should be repeated with a larger cohort to examine its reproducibility.

AUTHOR CONTRIBUTIONS

Giovanni Malaty (Writing – original draft); Boris Decourt (Conceptualization; Methodology; Validation; Writing – review & editing); Holly Shill (Project administration; Resources); Marwan Sabbagh (Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Visualization; Writing – original draft; Writing – review & editing).

ACKNOWLEDGMENTS

We thank Lih-Fen Lue, PhD, for her career-long contributions to the development of procedures for isolation of plasma biomarkers and modeling mechanisms for human neurodegenerative diseases. We thank the staff of Neuroscience Publications at Barrow Neurological Institute for assistance with manuscript preparation.

FUNDING

Research in this manuscript was supported by the National Institutes of Health under award numbers R01AG059008, P30 AG072980, and R01AG073212.

CONFLICT OF INTEREST

Dr. Malaty declares no conflict of interest at the time this publication was written. Dr. Decourt declares no conflict of interest at the time this publication was written. Dr. Shill received research support from NIH, the Michael J. Fox Foundation for Parkinson's Research, Transposon Therapeutics, Inc, Saccadous, Inc, the National Institute of Neurological Disorders and Stroke at NIH, Jazz Pharmaceuticals, Inc, Supernus Pharmaceuticals, Inc, The Parkinson's Foundation, and Barrow Neurological Foundation, and also received consulting honoraria from AbbVie, Inc, and the Tremor Research Group. Dr. Sabbagh discloses ownership interest (stock or stock options) in uMETHOD, Athira Pharma, Inc, CervoMed, Inc, and Lighthouse Pharmaceuticals, Inc; consulting for Alzheon, Inc, Genentech (Roche Group), Prothena Corp, plc, Eisai Co, Ltd, Eli Lilly and Co, Cognito Therapeutics, Inc, and Anavex Life Sciences Corp. Dr. Sabbagh is an Editorial Board member of this journal, but was not involved in the peer-review process of this article nor had access to any information regarding its peer review.

An overview of key findings from this study was previously presented in a poster titled "Assessment of Plasma Biomarkers by ImmunoMagnetic Reduction in Parkinson's Disease Dementia and Dementia with Lewy Bodies" (P10.008) at the American Academy of Neurology meeting held on April 25-26, 2023, in Boston, MA.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request and institutional review board approval, as applicable.

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