RESEARCH ARTICLE

Association of CSF α-Synuclein Seeding Amplification Assay Results With Clinical Features of Possible and Probable Dementia With Lewy Bodies

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Abstract

Background and Objectives

The clinical diagnosis of dementia with Lewy bodies (DLB) depends on identifying significant cognitive decline accompanied by core features of parkinsonism, visual hallucinations, cognitive fluctuations, and REM sleep behavior disorder (RBD). Hyposmia is one of the several supportive features. α-Synuclein seeding amplification assays (αSyn-SAAs) may enhance diagnostic accuracy by detecting pathologic αSyn seeds in CSF. In this study, we examine how different clinical features associate with CSF αSyn-SAA positivity in a large group of clinically diagnosed participants with DLB.

Methods

Cross-sectional and longitudinal CSF samples from the multicentered observational cohort study of the DLB Consortium and similar studies within the Parkinson's Disease Biomarker Program, contributed by academic medical centers in the United States, underwent αSyn-SAA testing. Participants included those clinically diagnosed with DLB and 2 control cohorts. Associations between core DLB features and olfaction with αSyn-SAA positivity were evaluated using logistic regression.

Results

CSF samples from 191 participants diagnosed with DLB (mean age 69.9 ± 6.8, 15% female), 50 age-matched and sex-matched clinical control participants, and 49 younger analytical control participants were analyzed. Seventy-two percent (137/191) of participants with DLB had positive αSyn-SAAs vs 4% of the control groups. Among participants with DLB, those who were αSyn-SAA–positive had lower Montreal Cognitive Assessment scores (18.8 ± 5.7 vs 21.2 ± 5.2, $p = 0.01$), had worse parkinsonism on the Movement Disorders Society Unified Parkinson's Disease Rating Scale part III (33.8 ± 15.1 vs 25.6 ± 16.4, $p = 0.001$), were more likely to report RBD (114/133 [86%] vs 33/53 [62%], $p < 0.0001$), and had worse hyposmia on the University of Pennsylvania Smell Identification Test (UPSIT) (94/105 [90%] below 15th percentile vs 14/44 [32%], $p < 0.0001$). UPSIT percentile had the highest area under the curve (0.87, 95% CI 0.81–0.94) in predicting αSyn-SAA positivity and participants scoring at or below the 15th percentile of age and sex normative values had 18.3 times higher odds (95% CI 7.52–44.6) of having a positive αSyn-SAA test. Among 82 participants with longitudinal CSF samples, 81 (99%) had the same αSyn-SAA result for initial and follow-up specimens.

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Class of Evidence
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Glossary

α-Syn = α→synuclein; **αSyn-SAA** = α-Syn seed amplification assays; **AD** = Alzheimer disease; **AT-DLB** = Assessment Toolkit for DLB; **AUC** = area under the curve; **CLIA** = Clinical Laboratory Improvements Amendments; **DLB** = dementia with Lewy bodies; **DLBC** = DLB Consortium; **LBCRS** = Lewy Body Composite Risk Score; **LR** = likelihood ratio; **MCI** = mild cognitive impairment; **MDS-UPDRS** = Movement Disorder Society Unified Parkinson's Disease Rating Scale; **MoCA** = Montreal Cognitive Assessment; **NPI** = Neuropsychiatric Inventory; **OR** = odds ratio; **PD** = Parkinson disease; **PDBP** = Parkinson's Disease Biomarker Program; **RBD** = REM sleep behavior disorder; **UPSIT** = University of Pennsylvania Smell Identification Test.

Discussion

A substantial proportion of clinically diagnosed participants with DLB had negative αSyn-SAA results. Hyposmia was the strongest clinical predictor of αSyn-SAA positivity. Hyposmia and αSyn-SAA may have utility in improving the diagnostic assessment of individuals with potential DLB.

Classification of Evidence

This study provided Class III evidence that CSF αSyn-SAA distinguishes patients with clinically diagnosed DLB from normal controls.

Introduction

In dementia with Lewy bodies (DLB), there are widespread abnormal deposits of α→synuclein (α-Syn) (Lewy bodies and Lewy neurites) in the brain.[1,2] Variable copathology, especially Alzheimer disease (AD), may accompany DLB in up to 70% of autopsy proven cases and can affect the phenotypic expression in these patients.[3-7] DLB is diagnosed clinically, and definitive diagnosis is only possible at autopsy. Studies show that the accuracy of this clinical diagnosis to predict the presence of Lewy pathology at autopsy is variable.[8-11] Dementia accompanied by 2 or more of the core clinical features of recurrent hallucinations (typically visual and well-formed), cognitive fluctuations, motor parkinsonism, and REM sleep behavior disorder (RBD) may strongly predict autopsy findings of Lewy body synucleinopathy in studies by experts.[1] The use of indirect biomarkers, including FP-CIT, MRI, cardiac MIBG, and polysomnogram, to definitively diagnose REM sleep without atonia can assist in the diagnosis.[1] However, underdiagnosis of DLB may occur in academic and community settings because core features may be subtle or not always present, and access to advanced testing modalities is not uniformly available. Conversely, DLB may be overdiagnosed or misdiagnosed due to overlapping symptoms that may occur in different dementias. Rating systems have been developed to standardize and improve the clinical diagnosis of DLB among patients with dementia, notably the Lewy Body Composite Risk Score (LBCRS)[12] and the Assessment Toolkit for DLB (AT-DLB)[13]; however, in a large-scale national study in Italy, both of these ratings overdiagnosed the prevalence of DLB in comparison with rigorous application of the consensus criteria.[14]

α-Syn seed amplification assays (αSyn-SAAs) are qualitative tests that detect aggregates of misfolded α-Syn by a protein amplification procedure. Sensitivity and specificity of tests that detect these aggregates (or seeds) in CSF samples obtained from autopsy confirmed patients with Parkinson disease (PD) and DLB are both >90%, even in the setting of significant AD copathology and when DLB phenotypic features are mild or absent.[15-22] Thus, CSF αSyn-SAA can reliably detect α-Syn aggregates in patients across different neurodegenerative diseases with dementia if limbic or neocortical stage Lewy body pathology is present, regardless of copathology or clinical phenotype. Studies of PD have shown that hyposmia is a strong predictor of αSyn-SAA positivity,[23] and in people with AD or mild cognitive impairment (MCI), mild parkinsonian symptoms and olfactory deficits associated with αSyn-SAA positivity.[24]

We now evaluate αSyn-SAA in CSF samples in relation to clinical features and a measure of olfaction in research participants diagnosed with DLB followed through a multicenter DLB Consortium (DLBC) (National Institute of Neurological Disorders and Stroke U01NS100610) and related research projects that fall under the National Institute of Neurological Disorders and Stroke Parkinson's Disease Biomarker Program (PDBP) who received standardized and detailed clinical evaluations. The primary research questions were whether αSyn-SAA in CSF differed in people with clinically diagnosed DLB compared with controls and whether people with DLB would be more likely to have positive αSyn-SAA in CSF in relation to hyposmia and a greater number of core clinical features.

Methods

Participants

CSF samples were selected from the PDBP biorepository that had been collected under various NIH-funded biomarker

projects from 2017 to 2021 under Institutional Review Board–approved research protocols. The samples were mostly from participants enrolled in studies selecting for DLB, although there were a small number of participants characterized as DLB-MCI (n = 5).[1,25] We also selected CSF samples available in the PDBP repository to represent 2 control groups: analytical controls who were younger individuals (mean age <50 years who had no evidence to support a neurologic diagnosis) to represent a group highly unlikely to have incidental Lewy body pathology and clinical controls (individuals with a mean age and sex distribution comparable with the DLB cohort) who were assessed as being cognitively and neurologically within normal limits.

Clinical assessments of the participants with DLB were standardized and included history and use of several rating scales to assess cognitive, neurobehavioral, and motor symptoms; general physical examination; and structured neurologic examination, including assessment of parkinsonian features. The PDBP protocol includes the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III,[26] Neuropsychiatric Inventory (NPI),[27] Mayo Sleep Questionnaire,[28] University of Pennsylvania Smell Identification Test (UPSIT),[29] the Montreal Cognitive Assessment (MoCA), and a battery of neuropsychological tests. Many participants were also assessed with the National Alzheimer Coordinating Center (NACC) DLB module.[30] DaT SPECT scans were performed, but the results were not used to assign clinical diagnoses. At each contributing center, local site PIs reviewed clinical data and made overall diagnoses based on the clinical data.

CSF α-Syn-SAA

CSF samples were collected at individual sites using similar standard operating procedures. All CSF αSyn-SAA tests were analyzed at a single central laboratory of Amprion. CSF samples were analyzed blind to a diagnostic group by a qualitative version of the αSyn-SAA that has been validated for clinical use under Clinical Laboratory Improvements Amendments (CLIA)/College of American Pathologists certifications (clinical assay, SYNTap). Each sample was analyzed in triplicate (40 μL CSF per well) in a 96-well plate (COSTAR, cat# 3603), with a final reaction volume of 200 μL. To each well, 0.3 mg/mL rec-αSyn (Amprion, cat# S2020) in 100 mM PIPES pH 6.50, 500 mM NaCl, 10 μM ThT, and a 2.5-mm borosilicate glass bead was added. Plates were sealed using an optical adhesive film placed on an orbital shaker and shaken at 800 rpm for 1 minute, followed by 29 minutes of resting. A TIMIX 5 shaker (Edmund Buehler) was used and kept in an incubator set to 37°C. Bottom fluorescence readings were obtained using a BMG Labtech FLUOstar Omega microplate reader set at 440 excitation/490 nm emission. This SYNTap assay was performed according to standard operational procedures meeting CLIA regulations. A preestablished threshold for the median maximum fluorescence of the triplicate wells was used to provide a readout of "Detected" or "Not Detected" for each CSF sample. Samples with quantity of CSF not sufficient for analysis or those assessed as "no call" (indeterminate) were excluded, yielding 191 participants with clinically diagnosed DLB with CSF ≤1 year of baseline visit, 50 clinical control participants, and 49 analytical control participants in addition to 126 follow-up samples from 82 of 191 of the participants with clinically diagnosed DLB.

Data Analysis

Clinical features were compared between DLB, clinical controls, and analytic controls, as well as across DLB αSyn-SAA+ or αSyn-SAA– using the analysis of variance, *t* test, χ^2, or Fisher exact test, as appropriate. Total motor scores and subscale scores for tremor, bradykinesia, and rigidity were derived from the MDS-UPDRS part III where higher scores indicate more severe motor impairment. The MoCA was analyzed—lower scores reflect worse cognitive impairment. Hallucinations were rated as present or absent based on questions in the NPI. REM Sleep Behavior symptoms were rated using the Mayo Sleep Questionnaire. The Mayo Fluctuation Scale[31] was used to rate fluctuations, with a score of 2 or higher (out of 4) assessed as positive. The MDS-UPDRS part III score of 6 or higher was used to determine the presence of parkinsonism based on a previous study,[32] where this value would have constituted 95% of MDS-UPDRS part III scores in a DLB cohort of comparable age. Olfaction scores for correct identification of odors on the UPSIT were rated according to percentiles for age-adjusted and sex-adjusted normal values from a recently published study.[29] To examine the relationships of clinical features with the likelihood of a positive αSyn-SAA test, we applied logistic regression and calculated area under the curves (AUCs). Logistic regression models controlling for the number of core features and examining the interaction between hyposmia and number of core features were also used. Because not all participants diagnosed as DLB had 2 or more core clinical features determined from rating scales, we also analyzed data using designations of probable (2 or more core features) and possible (1 core feature) DLB.

Standard Protocol Approvals, Registrations, and Patient Consents

All studies were conducted after approval by local institutional review boards, and written informed consent was obtained from all participants enrolled. All information analyzed was deidentified.

Data Availability

αSyn-SAA data and clinical data are available in the PDBP database to qualified researchers. Qualified researchers may request the data set used for these analyses from the corresponding author; requests will need to be approved by a review committee comprising DLBC primary investigators.

Results

A total of 193 samples were available from participants with a DLB diagnosis collected ≤1 year of their baseline study visit.

The CSF sample from 1 subject yielded an indeterminate αSyn-SAA result and was excluded. Two samples had insufficient CSF at baseline to run a αSyn-SAA test; one of these had a 12-month follow-up sample, which was analyzed. Thus, the total number of participants with DLB with initial CSF samples and interpretable αSyn-SAA results was 191 (185 samples obtained at the baseline visit, 2 participants with initial samples obtained at the 6-month visit, and 4 participants with initial samples at the 12-month visit). These originated from the DLBC (110 participants from 9 participating academic medical centers) and other projects (81 participants from 5 academic medical centers) that had enrolled participants who were clinically diagnosed with DLB using consensus criteria[1] (see eAppendix 1). DLB cohort characteristics and the characteristics of the clinical controls (n = 50) and analytical controls (n = 49, 50 samples analyzed, 1 sample indeterminant) are presented in Table 1. The analytical controls were younger than the clinical controls or participants with DLB, and there were more female participants in the analytical control group. Overall, 71.7% (137/191) of participants in the DLB cohort were αSyn-SAA–positive, whereas 4.1% (2/49) analytical controls and 4.0% (2/50) of the healthy controls were αSyn-SAA–positive; 5 participants were originally designated as DLB-MCI and 60% (3/5) had positive αSyn-SAA results. Overall, 44.8% (13/29) of subjects with 1 core feature present were αSyn-SAA–positive and 77.6% (121/156) of participants with 2 or more core features (i.e., "probable DLB" by clinical consensus criteria) was αSyn-SAA–positive. A greater number of core features was associated with a higher likelihood of a positive αSyn-SAA (likelihood ratio [LR] $\chi^2 = 17.6$, $p = 0.0005$; 1 core feature: odds ratio [OR] 0.65, 95% CI 0.19–2.12 $p = 0.4$; 2 core features: OR 2.72, 95% CI 0.91–8.12, $p = 0.07$; 3 core features: OR 4.00, 95% CI 1.13–12.22). Four core features could not be calculated due to collinearity). Rates of αSyn-SAA positivity did not show differences between sites that assessed participants and contributed CSF ($p = 0.7$, eAppendix 1). Within clinical DLB αSyn-SAA–positive and αSyn-SAA–negative participant groups (Table 2), age and sex did not differ. Participants with αSyn-SAA–positive DLB had lower MoCA scores (18.8 ± 5.7 vs 21.2 ± 5.2, $t = 2.6$, $p = 0.01$) and higher MDS-UPDRS part III scores than αSyn-SAA–negative participants (33.8 ± 15.1 vs 25.6 ± 16.4, $t = 3.2$, $p = 0.001$), driven by higher scores for signs of bradykinesia

Table 1 Demographic and Clinical Features and CSF αSyn-SAA Results

	DLB	Clinical controls	Analytical controls
n	191	50	49
Age, y, mean ± SD	69.9 ± 6.8	69.8 ± 8.7	49.7 ± 9.3[d]
Sex (male:female and % male)	163:28 (85%)	42:8 (96%)	21:28 (43%)[d]
Ethnicity[a]	Caucasian 188 (98%) African American 2 (1%) Amerindian/Alaska Native 1 (1%)	Caucasian 48 (96%) African American 2 (4%)	Caucasian 43 (88%) African American 4 (9%) Asian American 1 (2%) Multiple ethnicities 1 (2%)
Latino	4 (2%)	3 (6%)	4 (8%)
Education, y	N = 185 16.1 ± 3.4	16.0 ± 2.5	15.4 ± 3.0
MoCA	19.5 ± 5.7[a]	27.0 ± 2.1	27.0 ± 2.5
MDS-UPDRS III	31.5 ± 15.9[a]	7.0 ± 7.4[c]	1.2 ± 1.8
Hallucinations	93/190 (49%)[b]	0	Not assessed
Acts out dreams	147/187 (79%)[a]	5 (10%)	7 (14%)
Cognitive fluctuations	39/70 (56%)	Not assessed	Not assessed
UPSIT	n = 149		N = 46
Items correct	18.6 ± 8.5[a]	31.2 ± 5.0	33.9 ± 4.0
Percentile	15.4 ± 22[a]	51.2 ± 27	48.1 ± 31
αSyn-SAA+	137 (72%)[a]	2 (4%)	2 (4%)

Abbreviations: α-Syn-SAA = α-synuclein seed amplification assay; DLB = dementia with Lewy bodies; MDS-UPDRS part III = Movement Disorder Society Unified Parkinson's Disease Rating Scale part III; MoCA = Montreal Cognitive Assessment; UPSIT = University of Pennsylvania Smell Identification Test.
Unless specified, values are indicative of the entire cohort.
[a] $p < 0.01$ for DLB vs clinical controls and analytical controls.
[b] $p < 0.01$ for DLB vs clinical controls.
[c] $p < 0.01$ for clinical controls vs analytical controls.
[d] $p < 0.01$ for analytical controls vs DLB and clinical controls.

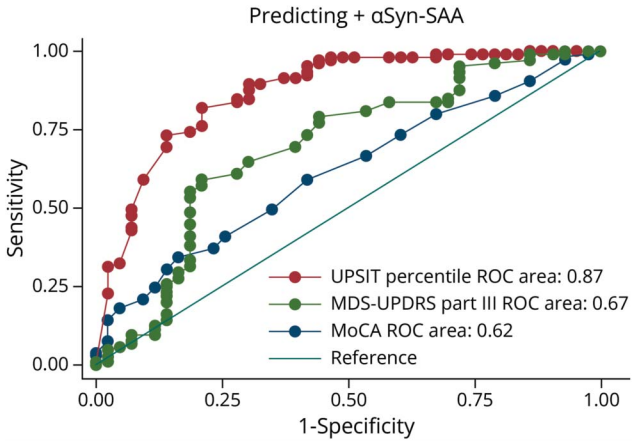
Table 2 αSyn-SAA Results in Participants With Clinically Diagnosed DLB

	αSyn-SAA–positive	αSyn-SAA–negative	*p* Value
n (%)	137 (72%)	54 (28%)	N/A
Age, y, mean ± SD	69.9 ± 6.7	70.1 ± 7.2	0.81
Sex (male:female and % male)	117:20 (87%)	46:8 (85%)	1.0
Ethnicity	Caucasian 135 (99%) African American: 2 (1%)	Caucasian: 53 (98%) Amerindian/Alaska Native: 1 (2%)	0.34
Latino	3 (2%)	1 (2%)	1.0
MoCA, mean ± SD	n = 135 18.8 ± 5.7	n = 53 21.2 ± 5.2	0.01
MDS-UPDRS III total, mean ± SD	33.8 ± 15.1	25.6 ± 16.4	0.001
Total >5	135 (99%)	50 (93%)	0.03
Total ≤5	2 (1%)	4 (7%)	
Rest tremor subscore	1.4 ± 2.6	1.0 ± 1.8	0.32
Bradykinesia subscore	13.9 ± 6.8	10.3 ± 7.7	0.002
Bradykinesia >5	121 (88%)	38 (70%)	0.003
Bradykinesia ≤5	16 (12%)	16 (30%)	
Rigidity subscore	4.8 ± 3.3	3.1 ± 2.5	0.001
Rigidity >2	99 (72%)	29 (54%)	0.01
Rigidity ≤2	38 (28%)	25 (46%)	
RBD history	114/133 (86%)	33/53 (62%)	<0.0001
Hallucinations	68/136 (50%)	25/53 (47%)	0.73
Fluctuation (Mayo Fluctuation scale >2/4)	27/45 (60%)	12/25 (48%)	0.33
Olfaction	N = 105	N = 44	
UPSIT total	15.1 ± 6.0	26.9 ± 8.0	<0.0001
UPSIT percentile	6.89 ± 9.1	35.7 ± 29.2	<0.0001
UPSIT ≤15th percentile:>15th percentile	94:11 (90%)	14:30 (32%)	<0.0001
No. of core features			
0	0 (0%)	1 (2%)	0.001
1	13 (9%)	16 (30%)	
2	52 (38%)	16 (30%)	
3	61 (44%)	13 (24%)	
4	11 (8%)	8 (15%)	

Abbreviations: α-Syn-SAA = α-synuclein seed amplification assay; DLB = dementia with Lewy bodies; MDS-UPDRS part III = Movement Disorder Society Unified Parkinson's Disease Rating Scale part III; MoCA = Montreal Cognitive Assessment; RBD = REM sleep behavior disorder; UPSIT = University of Pennsylvania Smell Identification Test.
p Values derived from the analysis of variance or χ^2 test, as appropriate.

($t = 3.2$, $p = 0.002$) and rigidity ($t = 3.5$, $p = 0.001$). Participants with αSyn-SAA–positive DLB were also more likely to report RBD symptoms on the Mayo Sleep Questionnaire ($\chi^2 = 12.6$, $p < 0.001$). There were similar rates of reported visual hallucinations and cognitive fluctuations between αSyn-SAA–positive and αSyn-SAA–negative groups ($p > 0.3$), although there was a considerable amount of missing data for reported cognitive fluctuations. Smell performance on the UPSIT was markedly worse in the αSyn-SAA–positive group compared with that in the αSyn-SAA–negative group. Among participants who had completed an UPSIT, 90% (94/105) of those who tested positive for αSyn-SAA performed at <15th

Figure 1 Receiver Operator Curve Areas for Continuous Variables in Predicting αSyn-SAA Positivity in the Patients With Clinically Diagnosed DLB



MoCA, MDS-UPDRS part III scores, and age and sex normative hyposmia percentiles on UPSIT closest to CSF aSyn-SAA sampling were used in calculating receiver operator curves. α-Syn-SAA = α-synuclein seed amplification assay; DLB = dementia with Lewy bodies; MDS-UPDRS part III = Movement Disorder Society Unified Parkinson's Disease Rating Scale part III; MoCA = Montreal Cognitive Assessment; UPSIT = University of Pennsylvania Smell Identification Test.

percentile of age and sex normative values compared to 32% (14/44) of the αSyn-SAA negative group (χ^2 = 51.8, p < 0.0001). UPSIT percentile, adjusted for age and sex normative values, yielded a receiver operator curve AUC of 0.87 to predict a positive αSyn-SAA test, and being hyposmic (≤15th percentile) was associated with 18.3 times greater odds of having a positive αSyn-SAA test within the clinically diagnosed DLB cohort (95% CI 7.52–44.6, p < 0.0001). In the case of participants with DLB with 2 or more core features who had completed UPSIT, 74.8% (92/123) had positive αSyn-SAA tests. 89.4% (84/94) of hyposmic participants were αSyn-SAA-positive and 31.0% (9/29) normosmic participants were αSyn-SAA-positive. For participants with 1 core feature who had completed UPSIT, 47.6% (10/21) were αSyn-SAA–positive. This included 72.7% (8/11) of hyposmic participants and 20% (2/10) of normosmic participants.

An MDS-UPDRS part III score above 5 was associated with 6.9 times greater odds of having a positive αSyn-SAA test (95% CI 1.29–36.7, p = 0.002). Visual hallucinations and the presence of cognitive fluctuations did not associate with higher odds of having a positive αSyn-SAA test (OR 1.1 95%, CI 0.59–2.11; OR 1.6 95%, CI 0.61–4.35, respectively). UPSIT percentiles had higher AUC values than MoCA or MDS-UPDRS part III scores in predicting αSyn-SAA positivity within the DLB cohort (AUC 0.87, 0.62, and 0.67, respectively; Figure 1). Combinations of different numbers of core features and their association with αSyn-SAA positivity I the presence and absence of hyposmia from the DLB cohort exclusive of participants with DLB-MCI are listed in Table 3.

Table 3 Hyposmia, Normosmia, and Core Features

	αSyn-SAA positivity
Individual core features ± hyposmia	
Parkinsonism + visual hallucinations	7/12 (58.3%)
Parkinsonism + visual hallucinations + hyposmia	7/8 (87.5%)
Parkinsonism + visual hallucinations + normosmia	0/3 (0.0%)
Parkinsonism + fluctuations	1/1 (100%)
Parkinsonism + fluctuations + hyposmia	NA
Parkinsonism + fluctuations + normosmia	NA
Parkinsonism + RBD	43/52 (82.7%)
Parkinsonism + RBD + hyposmia	26/27 (96.3%)
Parkinsonism + RBD + normosmia	5/11 (45.5%)
Visual hallucinations + RBD	1/2 (50.0%)
Visual hallucinations + RBD + hyposmia	0/1 (0.0%)
Visual hallucinations + RBD + normosmia	NA
Parkinsonism + visual hallucinations + fluctuations	2/3 (66.7%)
Parkinsonism + visual hallucinations + fluctuations + hyposmia	1/1 (100%)
Parkinsonism + visual hallucinations + fluctuations + normosmia	1/2 (50%)
Parkinsonism + visual hallucinations + RBD	44/53 (83.0%)
Parkinsonism + visual hallucinations + RBD + hyposmia	29/33 (87.8%)
Parkinsonism + visual hallucinations + RBD + normosmia	2/6 (33.3%)
Parkinsonism + fluctuations + RBD	12/13 (92.3%)
Parkinsonism + fluctuations + RBD + hyposmia	11/11 (100%)
Parkinsonism + fluctuations + RBD + normosmia	0/1 (0.0%)
Visual hallucinations + fluctuations + RBD	1/2 (50.0%)
Visual hallucinations + fluctuations + RBD + hyposmia	1/2 (50.0%)
Visual hallucinations + fluctuations + RBD + normosmia	NA
No. of core features ± hyposmia	
One	13/29 (44.8%)
One and hyposmia	8/11 (72.7%)
One and normosmia	2/10 (20.0%)
Two	51/66 (77.3%)
Two and hyposmia	33/36 (91.7%)
Two and normosmia	5/14 (35.7%)
Three	60/72 (83.3%)
Three and hyposmia	43/48 (89.6%)
Three and normosmia	2/8 (25.0%)

Table 3 Hyposmia, Normosmia, and Core Features *(continued)*

	αSyn-SAA positivity
Four	10/18 (55.6%)
Four and hyposmia	7/10 (70.0%)
Four and normosmia	2/7 (28.6%)

Abbreviations: α-Syn-SAA = α-synuclein seed amplification assay; DLB = dementia with Lewy bodies; MCI = mild cognitive impairment; NA = not available; RBD = REM sleep behavior disorder.
participantsIndividual combinations of core features present when 2 or more were present in clinical DLB cohort exclusive of with DLB-MCI and associations with αSyn-SAA positivity and the presence or absence of hyposmia. Combinations not listed did not have any participants who had those particular sets of core features. The lower section shows the number of core features in the DLB cohort exclusive of with DLB-MCI and the associations with αSyn-SAA positivity and the presence or absence of hyposmia.

Full test characteristics for the entire cohort are listed in eAppendix 1. After adjustment for age, sex, and other clinical features available, MDS-UPDRS part III scores and UPSIT percentiles remained significant predictors of αSyn-SAA positivity in the DLB cohort in multivariable models (multivariable model pseudo R^2 = 0.53, LR χ^2 = 41.1, p < 0.001; Table 4). Nonsignificant factors were sequentially removed from the multivariable models until a model with the highest pseudo R^2 value was identified, which included age, MDS-UPDRS part III scores, presence of RBD, presence of cognitive fluctuations, presence of visual hallucinations, and UPSIT percentile (pseudo R^2 = 0.53, LR χ^2 = 41.8, p < 0.0001; Table 4). Removal of UPSIT percentile from the model resulted in a significant drop in variance accounted (pseudo R^2 = 0.13, LR χ^2 = 11.9, p = 0.03). When controlling for number of core features present, hyposmia was still associated with a significantly increased likelihood of αSyn-SAA positivity (OR 17.4, 95% CI 6.9–43.8, p < 0.001).

αSyn-SAA was performed on CSF samples from follow-up visits on 82 of 191 participants (126 additional follow-up samples available) in the DLB cohort; there was only 1 participant who had a single discordant result (negative results from samples at baseline, year 1, year 2, and year 5 but positive for year 4 sample). All other participants had fully concordant longitudinal results (i.e., all participants with negative αSyn-SAA results at baseline remained negative, and all participants with baseline positive αSyn-SAA results remained positive; Figure 2). Hemoglobin concentration in CSF was not associated with a greater likelihood of a positive or negative αSyn-SAA result (p = 0.8).

Classification of Evidence

This study provided Class III evidence that CSF αSyn-SAA distinguishes patients with clinically diagnosed DLB from normal controls.

Discussion

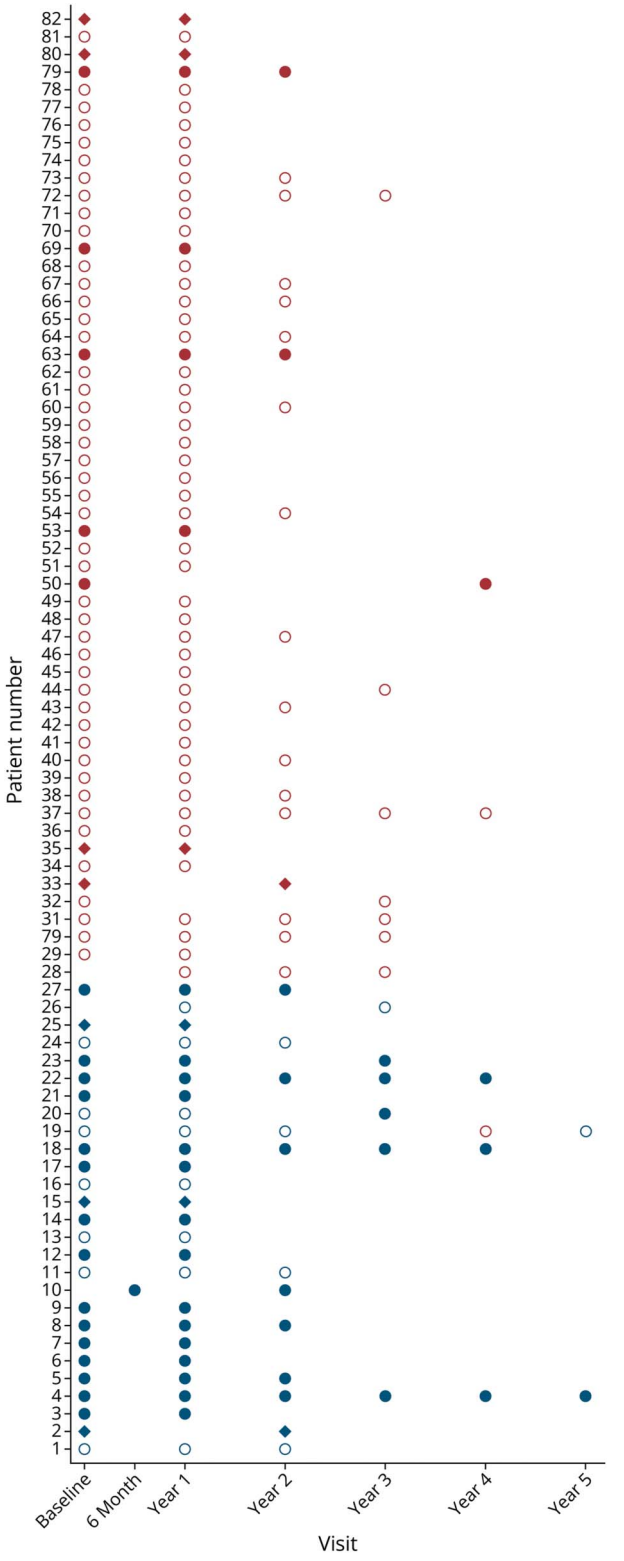
Previous clinicopathologic studies have shown varying accuracy in the ability of a clinical diagnosis of DLB to predict the presence of limbic or neocortical stage Lewy body pathology at autopsy. Revised clinical criteria and the application of new biomarkers have improved the accuracy of this diagnosis over time in tertiary care academic centers.[1] Structured assessments for DLB, such as the LBCRS and the AT-DLB, may improve the diagnostic approach but seem to over detect DLB in comparison with the application of consensus criteria.[14] Biomarkers for DLB have focused on indirect assessment of the effects of pathology, for example, patterns of regional

Table 4 Logistic Regression Models

Univariable model	OR	95% CI	*p* Value	AUC	Multivariable model	OR	95% CI	*p* Value	Optimal model	OR	95% CI	*p* Value
MDS-UPDRS part III	1.04	1.01–1.06	0.002	0.67		1.14	1.02–1.28	0.03		1.14	1.02–1.28	0.02
MoCA	0.92	0.87–0.98	0.01	0.62		1.06	0.86–1.30	0.6				
Hallucinations	1.12	0.59–2.11	0.7	0.51		0.54	0.07–3.87	0.5		0.44	0.07–2.76	0.38
RBD	3.63	1.73–7.60	0.001	0.62		3.98	0.22–70.1	0.3		4.03	0.24–67.6	0.33
Fluctuations	1.63	0.60–5.35	0.3	0.56		1.21	0.17–8.78	0.8		1.33	0.21–8.32	0.38
UPSIT %	0.90	0.87–0.94	<0.0001	0.87		0.84	0.75–0.94	0.002		0.85	0.76–0.94	0.002
Sex	1.00	0.41–2.43	1.00	0.50		0.68	0.05–9.50	0.77				
Age	0.99	0.95–1.04	0.81	0.49		1.06	0.86–1.27	0.5		1.05	0.89–1.23	0.58

Abbreviations: α-Syn-SAA = α-synuclein seed amplification assay; AUC = area under the curve; DLB = dementia with Lewy bodies; MDS-UPDRS part III = Movement Disorder Society Unified Parkinson's Disease Rating Scale part III; MoCA = Montreal Cognitive Assessment; RBD = REM sleep behavior disorder; UPSIT = University of Pennsylvania Smell Identification Test.
Univariable logistic regression models showing effects of individual features on likelihood of aSyn-SAA positivity in the DLB diagnosed cohort. Associated AUCs for individual measures are reported. In the multivariable model, all factors are considered simultaneously with UPDRS part III scores and UPSIT percentiles significantly predicted αSyn-SAA positivity when controlling for other factors. Factors were sequentially removed until an optimal multivariable model was created to predict αSyn-SAA positivity in this DLB cohort with a pseudo R^2 of 0.

Figure 2 Longitudinal αSyn-SAA Results in 82 of 191 Clinical Participants With DLB



Red: αSyn-SAA–positive, blue: αSyn-SAA–negative. Open circles: hyposmia ≤15th percentile of age and sex expected performance on the UPSIT at baseline. Closed circles: normosmic (>15th percentile of age and sex expected performance). Diamonds: UPSIT not completed at baseline. Subject 19 was the only subject with a discordant value. α-Syn-SAA = α-synuclein seed amplification assay; DLB = dementia with Lewy bodies; UPSIT = University of Pennsylvania Smell Identification Test.

hypometabolism on FDG-PET, dopaminergic transporter imaging to detect denervation using DaT-SPECT and others, autonomic cardiac denervation using MIBG scintigraphy, polysomnogram confirmation of REM sleep without atonia, and quantitative EEG assessment.[1]

The recent development of αSyn-SAA, which can detect endogenous α-Syn aggregation-competent seeds in CSF in vivo, offers the ability to further improve the accuracy of the clinical evaluation of suspected DLB. Prior studies have shown remarkably high sensitivity and specificity of these assays to determine that α-Syn seeds are present in CSF of individuals with PD, DLB, and prodromal synucleinopathy states compared with controls and other neurodegenerative disorders.[15-20,23] In this study, we explored the αSyn-SAA positivity in CSF samples from participants in the PDBP biorepository, evaluated by clinicians at tertiary care academic centers across the United States, with most samples from participants contributed by members of the DLBC. In this cohort, 72% of 191 participants with clinically diagnosed DLB and 78% of 156 participants with at least 2 core features had positive CSF αSyn-SAA tests, implying that a proportion of these enrollees do not harbor significant Lewy body pathology. Thus, assuming that αSyn-SAA has high sensitivity compared with previously published studies, it is likely that the clinical methods and ratings used by the DLBC and other PDBP projects may overcall DLB. We observed that αSyn-SAA–positive participants tended to have worse hyposmia, cognitive impairment, and higher MDS-UPDRS part III scores and were more likely to endorse symptoms of RBD. Reports of visual hallucinations and cognitive fluctuations were similar between the αSyn-SAA–positive and αSyn-SAA–negative groups. This differs from prior studies where visual hallucinations were noted as one of the more specific features to aid in the differentiation between DLB and AD at early stages.[33-36] Although missing data and ascertainment using the NPI might have contributed to diagnostic inaccuracy in participants with DLB, visual hallucinations have also been noted to occur occasionally in neurodegenerative diseases that include AD, multiple systems atrophy, and posterior cortical atrophy (some of which can have Lewy body pathology).[37] Hyposmia, as measured by the UPSIT and using a 15th percentile, the cutoff for age-adjusted and sex-adjusted scores was an especially strong predictor of αSyn-SAA positivity. Objective assessment of hyposmia contributed to this predictive value; self-reported hyposmia is known to be less reliable than objective testing.[38] A small number of control participants had hyposmia or reported symptoms of RBD, which could have qualified them as potentially prodromal participants; however, the analytical control group had a mean age where prodromal prevalence would likely be quite low and the αSyn-SAA positivity was similar to other control groups tested using the same αSyn-SAA platform.[19,23] Our findings align with a recent publication in PD where hyposmia was also a strong predictor of αSyn-SAA positivity in participants with sporadic and

LRRK2-related PD in the Parkinson's Progression Marker Initiative study.[23] In that study, 93% of patients with sporadic PD were SAA–positive, which increased to 98% in those with hyposmia. Only 67.5% of PD patients with LRRK2 mutations had positive αSyn-SAA tests (with 89.9% of patients with LRRK2 PD who were also hyposmic being positive). Although it is possible that patients with DLB who lack hyposmia may have a form of synuclein pathology less likely to produce detectable seeds, among the limited studies of hyposmia in DLB, it was identified in a majority of patients and was a significant predictor of Lewy body pathology postmortem.[39,40] Given these prior studies and this study linking hyposmia to αSyn-SAA positivity in DLB, we suggest that olfaction assessment should be considered in the DLB evaluation.

There are limitations to this study. The cohort may not generalize because it could only include participants who agreed to (and did not have a contraindication to) a lumbar puncture. Participants were overwhelmingly White and predominantly male, which is similar to other previously published reports but continues to limit generalizability. There was an incomplete data set from both the Mayo Fluctuation scale and reports of visual hallucinations, both core diagnostic features. The presence of RBD was determined using a questionnaire; polysomnogram confirmation was not available in the PDBP database. The diagnosis of DLB was made on a clinical basis at tertiary care centers, and autopsy confirmation was not available as a gold standard to assess brain tissue for Lewy body pathology. Furthermore, indicative biomarker testing (e.g., dopamine transporter scan, 123 iodine-MIBG scintigraphy, or polysomnography) was not uniformly used, although those tests might have further refined the diagnostic classification. However, assuming that the αSyn-SAA is a close proxy for the presence of significant Lewy body pathology, as demonstrated in several autopsy-confirmed series, our data imply that a proportion of participants were clinically misclassified. Autopsy confirmation would be needed to determine the definitive accuracy of DLB diagnosis in this study, and what neuropathologies are present in αSyn-SAA–negative participants. Besides SAA, histology for p-Ser-129-α-synuclein on skin biopsy has been shown to have strong diagnostic value for PD and synucleinopathy.[41] Our data suggest that integration of αSyn-SAA (which is now being studied in skin biopsies[42,43] and blood as less invasive alternatives to CSF) and evaluation of hyposmia would likely improve the accuracy of clinical diagnosis of people suspected of having DLB.

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Continued

Appendix *(continued)*

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