Cerebrospinal fluid biomarkers in dementia with Lewy bodies

van Steenoven, I.

2020

document version
Publisher's PDF, also known as Version of record

PART 1

Existing CSF biomarkers in dementia with Lewy bodies

CHAPTER 2

α-Synuclein species as potential cerebrospinal fluid biomarkers for Dementia with Lewy bodies

Inger van Steenoven, Nour K. Majbour, Nishant N. Vaikath, Henk W. Berendse, Wiesje M. van der Flier, Wilma D.J. van de Berg, Charlotte E. Teunissen, Afina W. Lemstra* and Omar M.A. El-Agnaf*
* shared senior authorship

Movement Disorders, 2018

ABSTRACT

Objective: To investigate the discriminating value of a range of CSF α-synuclein species for dementia with Lewy bodies in comparison to Alzheimer's disease, Parkinson's disease and cognitively normal controls.

Methods: We applied our recently published ELISA assays to measure the CSF levels of total α-synuclein, oligomeric α-synuclein and phosphorylated α-synuclein at Ser129 in dementia with Lewy bodies (n=42), Alzheimer's disease (n=39), PD (n=46) and controls (n=78). General Linear Models corrected for age and gender were performed to assess differences in α-synuclein levels between groups. We used backward-elimination logistic regression analysis to investigate the combined discriminating value of the different CSF α-synuclein species and Alzheimer's disease biomarkers.

Results: CSF levels of total α-synuclein were lower in dementia with Lewy bodies and PD compared to Alzheimer's disease as well as controls (p<0.001). In contrast, CSF levels of oligomeric α-synuclein were higher in dementia with Lewy bodies and PD compared to Alzheimer's disease (p<0.05) and controls (p<0.001). No group differences were found for phosphorylated α-synuclein. In dementia with Lewy bodies and PD, CSF total α-synuclein levels positively correlated with tau and phosphorylated tau (both r>0.40, p<0.01), but not with amyloid-β 1-42. The optimal combination to differentiate dementia with Lewy bodies from controls consisted of amyloid-β 1-42, total Tau, total α-synuclein, oligomeric α-synuclein, age and sex (AUC of 0.90). To differentiate dementia with Lewy bodies from Alzheimer's disease, the combination of tau and oligomeric α-synuclein resulted in an AUC of 0.83. CSF α-synuclein species do not contribute to the differentiation of dementia with Lewy bodies from PD.

Conclusions: CSF α-synuclein species could be useful as part of a biomarker panel for dementia with Lewy bodies. Evaluating of both oligomeric α-synuclein and total α-synuclein in CSF helps in the diagnosis of dementia with Lewy bodies.

INTRODUCTION

Dementia with Lewy bodies (DLB) is the second most common form of dementia in people above 65 years old. It accounts for 10-20% of dementia cases.[1] DLB is characterized by cognitive decline in combination with visual hallucinations, fluctuating cognition and parkinsonism as well as rapid eye movement (REM) sleep behavior disorder (RBD) and autonomic dysfunction.[2] Due to heterogeneity in clinical presentation and clinical and pathological overlap between DLB, Parkinson's disease (PD) and Alzheimer's disease (AD) accurate diagnosis of DLB is often challenging, especially at early stages of the disease.[3] Currently, the diagnosis of DLB is based on clinical diagnostic consensus criteria.[2, 4] These diagnostic criteria have a high specificity (80-100%), but a low sensitivity (20-60%). As a consequence, over 80% of DLB cases are initially diagnosed with other disorders, mainly AD or PD.[5] Post-mortem pathological confirmation of the presence of cortical Lewy bodies and Lewy neurites – intraneuronal inclusion composed primarily of α-synuclein aggregates[6] – constitutes the diagnostic gold standard. However, accurate diagnosis antemortem is important for adequate clinical management and patient care. There is an urgent need to discover biomarkers that can aid in an accurate and early diagnosis of DLB.

Analysis of cerebrospinal fluid (CSF) biomarkers is increasingly applied in the diagnostic work-up of neurodegenerative diseases. CSF amyloid-β 1-42 (Aβ42), total Tau protein (t-tau) and phosphorylated Tau at threonine 181 (p-tau) mirror the main neuropathological hallmarks of AD and are well established to aid in the diagnosis of AD.[7] AD-like pathology, i.e. neurofibrillary tangles and amyloid plaques – is also found in almost half of patients with DLB.[8, 9] The CSF AD biomarkers, therefore, have an added value to distinguish DLB from healthy subjects and to some extent from PD. To distinguish DLB from AD, however, additional biomarkers are necessary.

The discovery of α-synuclein as a major component of Lewy bodies[10] and the detection of α-synuclein in CSF[11, 12] has encouraged research into α-synuclein as a potential CSF biomarker for both DLB and PD. The discriminating value of CSF total α-synuclein (t-α-syn) has been addressed in multiple studies, with conflicting results. While some studies have shown that CSF levels of t-α-syn are decreased in patients with PD, PD dementia (PDD) or DLB compared to controls or patients with AD, other studies demonstrated increased levels or no group differences at all (see [13-15] for review). These mixed results could be due to a number of methodological factors, such as use of different antibodies and standard proteins used in the immunoassays, patient selection, variation in pre-analytical processing, and blood contamination due to traumatic lumbar puncture.[13]

Moreover, former studies all used immunoassays that detect CSF t-α-syn not taking into account its conformation or aggregation state and thus CSF t-α-syn might lack disease specificity.

Soluble α-synuclein oligomers could be more useful, because (i) early aggregates or "soluble oligomers" of α-synuclein (o-α-syn) might play a more essential role in the pathogenesis of synucleinopathies rather than the late aggregates, (ii) oligomeric forms of α-synuclein seem to be neurotoxic/ more pathogenic in vitro and in vivo[6, 16, 17] and (iii) soluble α-synuclein oligomers have been linked to synaptic and neuronal degeneration in an α-synuclein E57K transgenic mouse model.[18] Postmortem studies have shown high levels of soluble o-α-syn in the brain of patients with PD and DLB compared to AD and controls.[19, 20] Another α-synuclein specie of interest is α-synuclein phosphorylated at Serine 129 (pSer129-α-syn). pSer129-α-syn is specifically associated with Lewy body pathology, since approximately 90% of accumulated α-syn in Lewy bodies consists of pSer129-α-syn.[21] To investigate the use of CSF α-synuclein species as biomarkers for the diagnosis of DLB, we recently developed robust and specific enzyme-linked immunosorbent assays [ELISA] to quantify a wide range of α-synuclein species (t-α-syn, o-α-syn and pSer129-α-syn) in CSF.[20] We and others reported increased levels of soluble o-α-syn in PD(D) patients compared to other neurological disorders[22-25] and healthy controls.[20, 26] Only one study has shown that soluble o-α-syn was increased in DLB patients compared to patients with AD, but not compared to controls.[22] Two recent CSF studies reported elevated pSer129-α-syn levels in CSF of PD patients compared to controls.[20, 27] No studies investigating levels of CSF pSer129-α-syn in DLB have been published yet.

The aim of this study was to assess the diagnostic value of measuring CSF levels of a wide range of different CSF α-synuclein species (t-α-syn, o-α-syn and pSer129-α-syn) for the diagnosis of DLB in a well-established cohort of DLB patients, PD patients, AD patients and cognitively normal controls using our recently developed assays. In addition, we investigated whether these CSF α-synuclein species add discriminatory value to the CSF AD biomarkers.

METHODS

Participants

We included 106 participants with available CSF from the Amsterdam Dementia Cohort who had visited the VUmc Alzheimer center between 2002 and 2015 (41 DLB, 35 AD and 30 controls with subjective cognitive decline (SCD)). AD and SCD were matched for

age and gender with DLB patients. In addition, data and CSF-samples of 46 PD and 48 volunteers without neurological symptoms collected for a previous study[20] at the VUmc outpatient clinic for movement disorders were also included in our analyses.

The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines and approved by the local ethics committee of the VU University Medical Center. All study participants gave written informed consent for use of their clinical data and biomaterial for research purposes.

Clinical Diagnosis

All patients received a standardized and multi-disciplinary work-up, including medical history, physical, neurological and neuropsychological examination, MRI and laboratory tests. Diagnoses were made in multidisciplinary consensus meetings without knowledge of CSF AD biomarker results.[28, 29]

DLB patients were diagnosed according to the 2005 consensus criteria for probable DLB[4] and also fulfill novel consensus criteria.[2] The diagnosis of DLB was supported by [123I] FP-CIT SPECT findings showing presynaptic dopaminergic deficits (n=32) or slow-wave activity on EEG (n=8), or was confirmed at autopsy (n=1). AD patients were diagnosed using the criteria of the National Institute for Neurological and Communicative Diseases AD and Related Disorders Association (NIA-AA) criteria for probable AD.[30] PD patients were diagnosed according to the United Kingdom PD Society Brain Bank (UK-PDSBB) clinical diagnostic criteria by movement disorders specialists.[31] The diagnosis of PD was supported by abnormal [123I]FP-CIT SPECT scans (n=21). Severity of parkinsonism in the 'on' state was evaluated using the UPDRS-III. PD patients were only included if the MMSE and/or neuropsychological assessments did not indicate dementia. Subjects were labeled as SCD when the cognitive complaints could not be confirmed by cognitive testing and criteria for mild cognitive impairment, dementia or any other neurological or psychiatric disorder known to cause cognitive complaints were not met. To be included as controls in the present study, SCD subjects had to remain cognitively stable for at least 2 years. Cognition at baseline and yearly follow-up was evaluated with extensive neuropsychological assessment. Healthy volunteer group underwent a standardized clinical assessment that included medical history and neurological examination. Cognitive impairment in the healthy volunteers was excluded using the Cambridge Cognitive Examination (CAMCOG) scale. SCD subjects and healthy volunteers were analyzed as a single cognitively normal group (Supplementary table 1).

CSF collection

CSF was obtained by lumbar puncture between the L3/L4 or L4/L5 or L5/S1 intervertebral space, using a 25-gauge needle and syringe, collected in polypropylene tubes, centrifuged at 1800g at 4 °C for 10min, aliquoted in polypropylene tubes of 0.5mL and stored at -80 °C until further analysis, in line with international guidelines.[32] A small amount of CSF was used for routine analysis, including total cells, total protein, glucose and erythrocytes. Only samples containing <500 erythrocytes per microliter were included in the analysis, as excessive erythrocytes may influence α-synuclein levels.[33]

CSF Assays

CSF levels of Aβ42, t-tau and p-tau were determined with sandwich enzyme-linked immunosorbent assays [ELISA] (Innotest®, Fujirebio, Gent, Belgium) as described previously.[34]

CSF t-α-syn, pSer129-α-syn and o-α-syn levels were measured using our recently published ELISA assays.[20] More details on the α-synuclein assays are described in the supporting information. All biomarker analyses were carried out blinded to the clinical diagnosis.

Statistical analysis

Demographical and clinical characteristics were compared between groups using chi-square tests, ANOVA with post-hoc Bonferroni tests or Kruskal-Wallis tests followed by Mann-Whitney U tests, where appropriate.

CSF α-synuclein levels below the first quartile minus 3x interquartile range (IQR), or above the third quartile plus 3xIQR, were considered as outliers and excluded from subsequent analyses (more details about outliers is presented in Supplementary table 2). CSF t-tau and p-tau levels were log-transformed to meet assumptions of normally distributed data. Other biomarkers had a normal distribution.

For all CSF biomarkers, differences between diagnostic groups were assessed using general linear models (GLM) corrected for age and gender with post-hoc Bonferroni tests. We examined correlations using bivariate Pearson correlation coefficient within diagnostic groups. We used the Benjamini-Hochberg procedure to correct for multiple testing. Due to collinearity between t-tau and p-tau, only the strongest predictor (t-tau) was included in the following analyses.

Subsequently, we used stepwise linear discriminant function analysis to assess the accuracy of the combined CSF biomarkers in classification of the four groups. Stepwise linear discriminant function analysis identifies canonical discriminant functions based on

combinations of biomarkers which contribute maximally to group separation and evaluate how well these canonical discriminant functions discriminate the diagnostic groups.

Finally, to assess which subsets of CSF biomarkers performed best in distinguishing DLB from AD, PD and controls, respectively, we performed multivariate logistic regression analyses with backward stepwise selection (separate analyses for each comparison). DLB was entered as reference category and Aβ42, t-tau, t-α-syn, o-α-syn, pSer129-α-syn, age and sex as predictors. CSF data were Z-transformed. The resulting OR's therefore provide the increased odds per standard deviation increase in biomarker value. For the resulting models, we report AUC, sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) as well as OR (95%CI) of the individual biomarkers. Sensitivity, Specificity, NPV and PPV were calculated using the classification table (probability threshold: 0.5). All statistical analyses were performed using IBM SPSS software for Mac, version 22.0. A *p*-value of <0.05 was considered significant.

RESULTS

Demographical, clinical characteristics and CSF biomarkers levels of the diagnostic groups are presented in Table 1. There was an age difference between groups, as PD patients were younger than AD patients ($p<0.05$). The gender distribution also varied between the diagnostic groups ($p<0.001$). Patients with dementia (AD and DLB) had lower MMSE scores compared to controls and PD ($p<0.001$). GLM showed differences between diagnostic groups for Aβ42, t-tau and p-tau ($p<0.05$, adjusted for age and gender) (Table 1). AD patients had a CSF profile with lower levels of Aβ42 and higher levels of t-tau and p-tau compared to PD and controls. DLB patients had levels in between AD and PD with higher levels of Aβ42 and lower levels of Tau compared to AD and lower levels Aβ42 and higher levels of Tau compared to PD and controls. There were no differences between PD and controls.

Age and gender-adjusted GLM revealed differences in levels of CSF t-α-syn and o-α-syn between groups (both $p<0.001$; Table 1 and Figure 1). Subsequent Bonferroni adjusted t-tests showed lower levels of t-α-syn in PD and DLB compared to AD and controls ($p<0.001$). In contrast, the levels of o-α-syn were higher in PD and DLB compared to controls ($p<0.001$). Moreover, o-α-syn was also higher in PD compared to AD ($p<0.001$). There were no group differences for pSer129-α-syn. Both the ratio of o-α-syn/t-α-syn and pSer129-α-syn/t-α-syn were higher in PD and DLB compared to the ratio's in AD and controls (all $p<0.01$; Supplementary figure 1). Analysis including all cases showed similar results (data not shown).

Table 1 | Demographics and CSF biomarkers by diagnostic group

	DLB	PD	AD	Controls
N	41	46	35	78
Demographics				
Age	66.5 ± 6.1	62.8 ± 10.1[d]	67.8 ± 6.3	64.4 ± 6.9
Sex (% male)	35 (85.4%)[b,e]	28 (60.9%)[c]	33 (94.3%)[b]	41 (52.6%)
Disease duration (years)*	3 [2-4]	4 [2-9]	4 [3-5]	NA
MMSE§	23 [19-26][b,e]	29 [28-30][c]	23 [18-25][b]	29 [28-30]
CSF AD biomarkers				
Aβ42 (pg/ml)	695 ± 275[a,d,f]	917 ± 211[c]	486 ± 194[a]	926 ± 266
t-tau (pg/ml)	325 [224-431][b,c,e]	189 [157-275][c]	588 [398-787][a]	247 [174-308]
p-tau (pg/ml)	53 [35-66][c]	38 [28-51][c]	75.0 [62-99][a]	45 [35-57]
CSF α-syn biomarkers				
t-α-syn (ng/ml)#	1.4 ± 0.4[a,c]	1.4 ± 0.3[a,c]	2.0 ± 0.5	1.8 ± 0.6
o-α-syn (pg/ml)†	108 ± 34[a]	120 ± 49[a,c]	89 ± 30	72 ± 37
pSer129-α-syn (pg/ml)$	232 ± 79	258 ± 52	220 ± 61	235 ± 54

Data are expressed as mean ± SD, median [IQR] or n (%). Demographical differences between groups were analyzed using ANOVA with post hoc Bonferroni tests (age), χ^2 tests (sex), and Kruskal Wallis with post hoc Mann-Whitney U tests (MMSE, Disease duration). Differences in CSF biomarker levels between groups were assessed with GLM, adjusted for age and gender. t-tau, p-tau were log-transformed, but are presented as raw data. Aβ42 = amyloid-β 1-42; AD = Alzheimer's disease; DLB = dementia with Lewy bodies; MMSE = Mini-Mental State Examination; NA = not applicable; PD= Parkinson's disease; pSer129-α-syn = phosphorylated α-synuclein protein at Serine 129; p-tau = Tau phosphorylated at threonine 181; o-α-syn = oligomeric α-synuclein; t-α-syn = total α-synuclein; t-tau = total Tau protein.* AD: n=35; DLB: n=40; PD: n=45, § Controls: n= 78; AD: n=34; DLB: n=40; PD: n=46, # Controls: n=77; AD: n=34; DLB: n=41; PD: n=46, † Controls: n= 78; AD: n= 35; DLB: n=41; PD: n=42, $ Controls: n=75; AD: n=33; DLB: n=38; PD: n=45. [a]$p<0.001$ compared to Controls, [b]$p<0.05$ compared to Controls, [c]$p<0.001$ compared to AD, [d]$p<0.05$ compared to AD, [e]$p<0.001$ compared to PD, [f]$p<0.05$ compared to PD.

Subsequently, by use of Pearson correlations we evaluated associations between different CSF biomarkers (Figure 2, Supplementary table 3). For DLB, but not for any of the other groups, we found a positive association between o-α-syn and pSer129-α-syn (r=0.45, $p<0.05$). Evaluating correlations between α-synuclein species and the AD biomarkers, we found a positive correlation between t-α-syn and (p)tau in all patient groups (both r>0.40, $p<0.05$), but not in controls. By contrast, levels of o-α-syn and pSer129-α-syn did not correlate with any of the AD biomarkers. Correlations of the α-synuclein species with clinical parameters (age, disease duration, MMSE and UPDRS-III) are shown in Supplementary table 4. Briefly, we observed a negative correlation between t-α-syn and MMSE within the PD group (r=-0.42, $p<0.01$), but not in any of the other groups. CSF o-α-syn did not correlate with any of the clinical parameters. In DLB, we found a positive correlation between pSer129-α-syn and age (r=0.39, $p<0.05$) and a negative correlation between pSer129-α-syn and MMSE scores (r=-0.45, $p<0.01$).

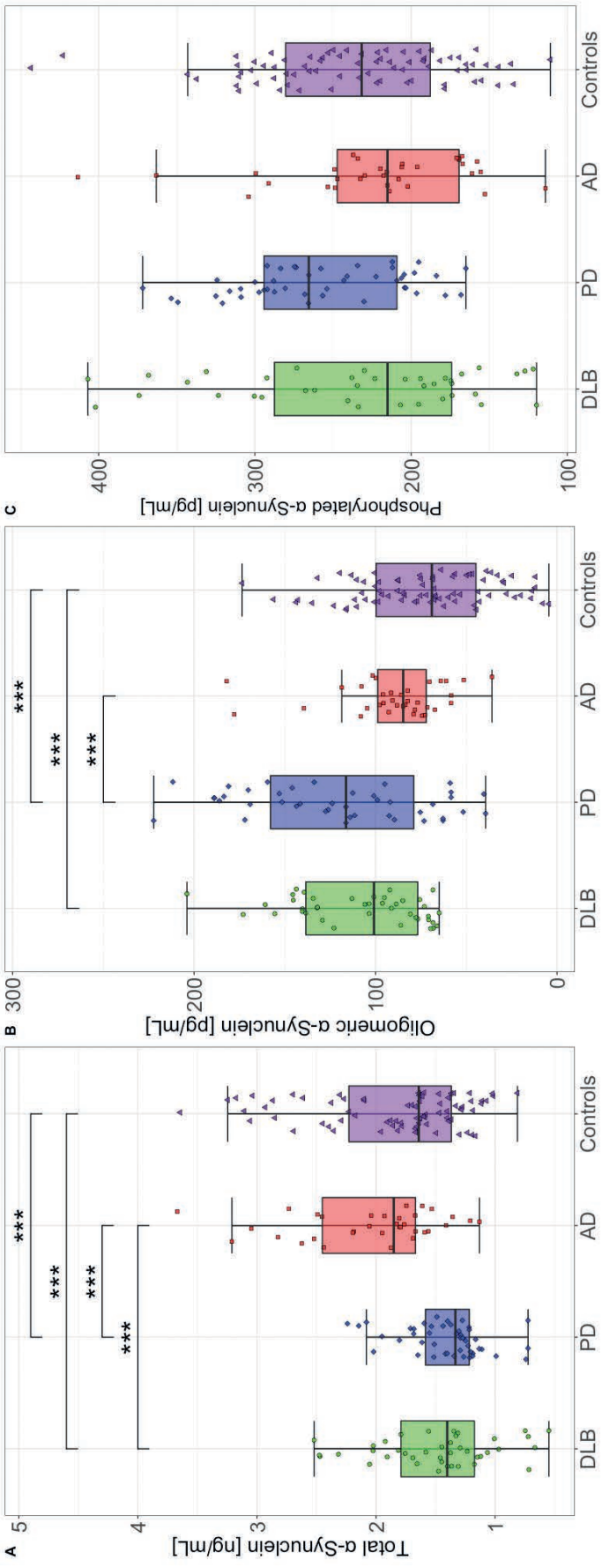
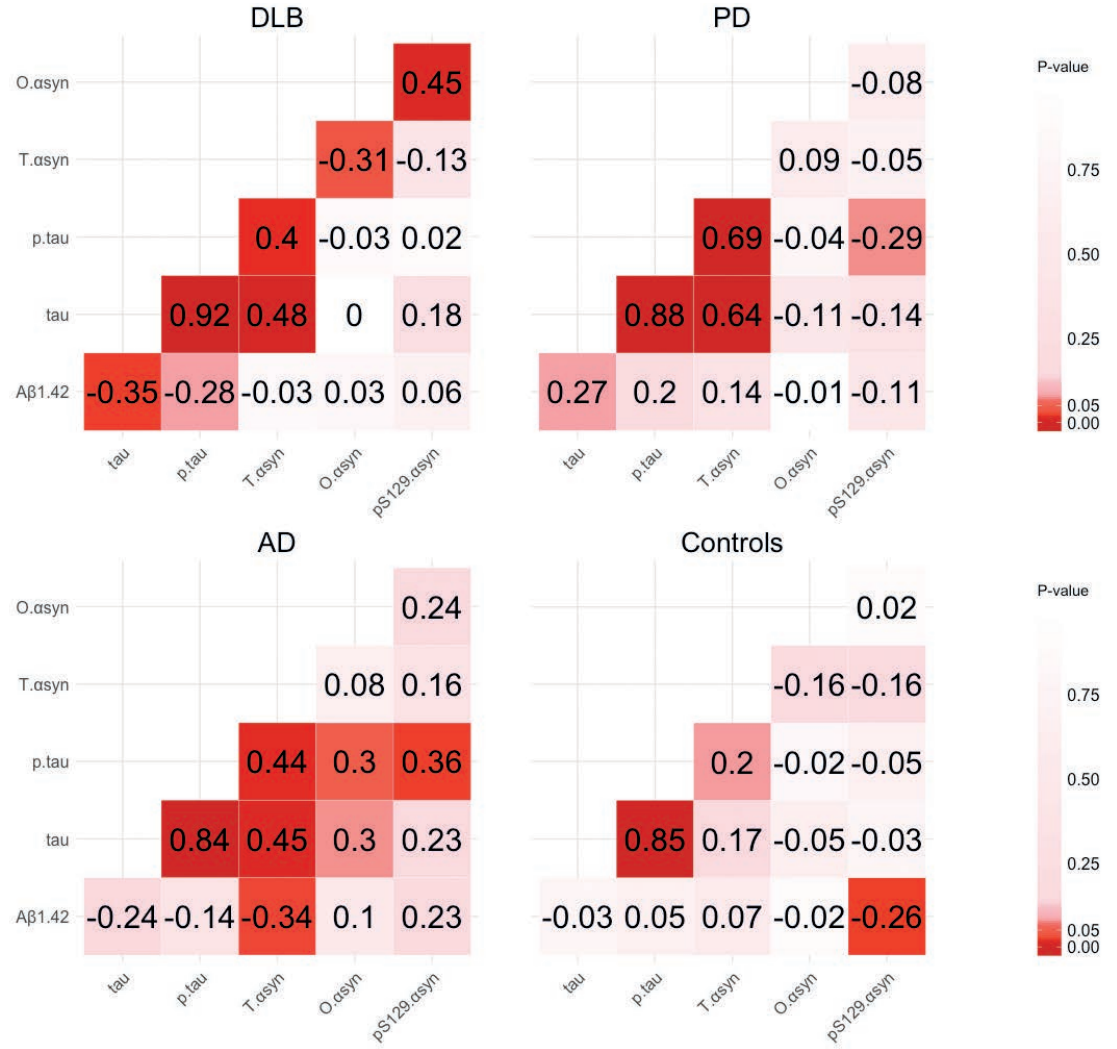


Figure 1 | Box and Whisker plots of CSF levels of α-synuclein species in DLB, PD, AD and controls

(A) CSF levels of t-α-syn, (B) CSF levels of o-α-syn, (C) CSF levels of pSer129-α-syn. The line through the middle of the boxes corresponds to the median and the lower and the upper lines to the 25th and 75th percentile, respectively. The whiskers extend from the 5th percentile on the bottom to the 95th percentile on top. Differences between groups were assessed with GLM, adjusted for age and gender. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Figure 2 | Correlations (Pearson) between CSF biomarkers in the diagnostic groups

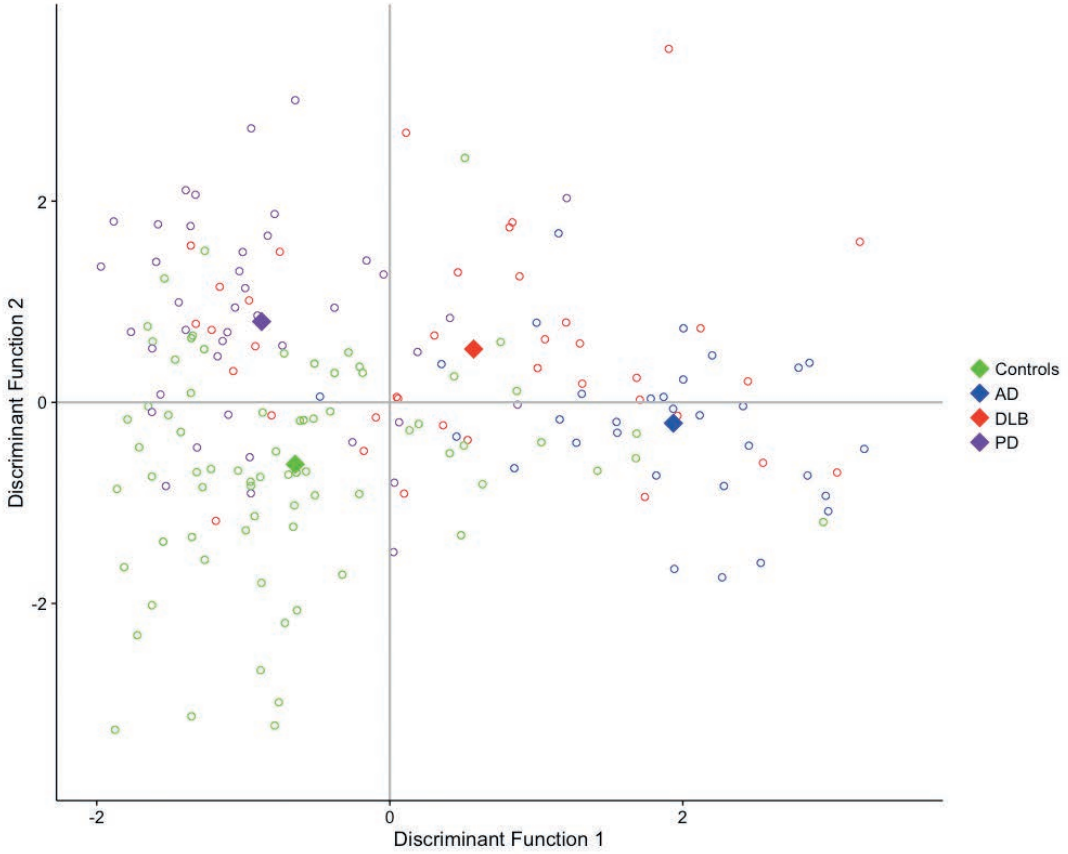


Pearson correlation coefficients are depicted by the number within the plots. The colors represent the *p*-value of the association. Darker colors represent lower p-values and lighter colors represent higher p-values. Aβ42 = amyloid-β 1-42; AD = Alzheimer's disease; DLB = dementia with Lewy bodies; PD = Parkinson's disease; pS129-α-syn = phosphorylated α-synuclein protein at Serine 129; p-tau = Tau phosphorylated at threonine 181; o-αsyn = oligomeric α-synuclein; t-α-syn = total α-synuclein; t-tau = total Tau protein.

Subsequently, we conducted a discriminant analysis to identify the best combination of biomarkers to classify the four groups. A panel of Aβ42, t-tau, t-α-syn and o-α-syn together classified 64.5% of all cases correctly in DLB, AD, PD or control groups (lambda=0.351, $p<0.001$). Figure 3 shows the discrimination plot of the two canonical discriminant functions for discrimination of the four groups. The loadings of individual predictors on each discriminant function are shown in Supplementary table 5. Canonical discriminant function 1 strongly correlates with the AD biomarkers (Aβ42: r=0.647, t-tau: r=-0.789, p-tau: r=-0.596) and discriminated AD patients and DLB patients from PD

patients and controls. We will refer to this function as the dementia function. Canonical discriminant function 2 strongly correlates with the α-synuclein species (t-α-syn: r=-0.620, o-α-syn: r=0.829, pSer129-α-syn: r=0.205) and adds by discriminating PD patients and DLB patients from AD patients and controls. We will refer to this function as the movement disorders function. DLB is located at the intersection of both the dementia-axis and the movement disorders-axis.

Figure 3 | Discriminant function plot of canonical discriminant functions for discrimination of DLB, PD, AD and controls



Red circles indicate individual data of DLB patients, purple circles indicate individual data of PD patients, blue circles indicate individual data of AD patients and green circles indicate individual data of controls. The diamonds represent the group centroids.

Finally, we used backward-elimination multiple logistic regression analyses to identify optimal biomarker panels for bilateral comparisons between (i) DLB and controls, (ii) DLB and AD and (iii) DLB and PD. Aβ42, t-tau, t-α-syn, o-α-syn, pSer129-α-syn, age and sex were entered as predictors. DLB was used as the reference group in each comparison. Table 2 shows a summary of the final models. The combination of Aβ42, t-tau, t-α-syn, o-α-syn, pSer129-α-syn, age and sex discriminated DLB from controls. Low levels

of Aβ42 (OR: 0.42; 95%CI:0.22-0.80), high levels of t-tau (OR: 3.62; 95%CI:1.58-8.27), low levels of t-α-syn (OR: 0.30; 95%CI: 0.13-0.74) and high levels of o-α-syn (OR: 4.55; 95%CI:1.78-11.66) give a higher risk for DLB compared to controls. For the discrimination between DLB and AD, we found that low levels of tau (OR: 0.22, 95%CI:0.10-0.50) and high levels of o-α-syn (OR: 2.67; 95%CI:1.03-6.94) give a higher risk for DLB compared to AD. Finally, low levels of Aβ42 (OR: 0.43; 95%CI:0.22-0.87) and high levels of tau (OR: 3.63; 95%CI:1.63-8.06) give a higher risk for DLB compared to PD. Receiver operating characteristic curves (ROC) are illustrated in Supplementary figure 2. All models had an AUC > 0.80.

Table 2 | Logistic Regression analysis of multiple CSF biomarkers

	DLB				
	Predictors	**OR for DLB (95% CI)**	***p*-value**	**Accuracy of model**	
Controls	Aβ42	0.42 (0.21-0.77)	<0.01	AUC: 0.90 (0.84-0.96)	
	t-tau	3.61 (1.67-8.89)	<0.01	Sens: 68%	PPV: 84%
	t-α-syn	0.30 (0.11-0.68)	<0.01	Spec: 93%	NPV: 85%
	o-α-syn	4.55 (1.91-12.87)	<0.01		
	Age	0.91 (0.81-1.00)	<0.05		
	Sex	0.19 (0.04-0.64)	<0.05		
AD	t-tau	0.21 (0.09-0.43)	<0.001	AUC: 0.84 (0.75-0.93)	
	o-α-syn	2.90 (1.24-7.97)	<0.05	Sens: 81%	PPV: 79%
				Spec: 74%	NPV: 77%
PD	Aβ42	0.43 (0.20-0.82)	<0.05	AUC: 0.84 (0.75-0.93)	
	t-tau	3.65 (1.76-8.86)	<0.01	Sens: 74%	PPV: 85%
	Sex	0.23 (0.05-0.85)	<0.05	Spec: 88%	NPV:79%

CSF biomarker predictors were Z transformed before analyses; therefore, odds ratio's (OR) represent higher odds for DLB per standard deviation (SD) decreased amyloid and t-α-syn or increased tau and o-α-syn.
Aβ42 = amyloid-β 1-42; AD = Alzheimer's disease; α-syn = α-synuclein; AUC = area under the curve; DLB = dementia with Lewy Bodies; NPV = negative predictive value; o-α-syn = oligomeric α-synuclein; OR = odds ratio; PD = Parkinson's disease; PPV = positive predictive value; Sens = sensitivity; Spec = specificity; t-α-syn = total α-synuclein; t-tau = total Tau protein.

DISCUSSION

The major findings of this study are that CSF levels of t-α-syn are lower in DLB and PD compared to both AD and cognitively normal controls, whereas CSF levels of o-α-syn are higher in DLB and PD. In addition, we observed that CSF t-α-syn was associated with t-tau and p-tau, while o-α-syn is not associated with any of the AD biomarkers.

Third, we demonstrated that CSF α-synuclein species in combination with the CSF AD biomarkers are promising biomarker candidates for DLB.

Most previous research on CSF α-synuclein in DLB focused on t-α-syn and generated conflicting results as compared to AD or controls, α-synuclein levels are reportedly increased, decreased or unchanged (see [13, 14] for review). These discrepancies are likely due to differences in the assay platform, antibodies' characteristics, CSF collection, storage and processing steps, blood contamination and heterogeneity of patients included in studies.[13] By using highly specific and sensitive ELISAs[20] in a well-characterized cohort of patients with DLB, PD, AD and non-demented controls we now report a decrease of t-α-syn in DLB and PD compared to AD and controls. Moreover, we observed elevated levels of o-α-syn in both DLB and PD, especially compared to the levels in AD and controls. These findings are in line with a previous CSF studies that showed increased o-α-syn levels in DLB compared to AD[22] and in PD compared to controls[23-25]. However, we did not observe differences in pSer129-α-syn levels between diagnostic groups. To date, no previous studies have evaluated CSF pSer129-α-syn in a DLB patient cohort. In a previous study in PD using a Luminex assay, however, CSF pSer129-α-syn levels were increased in PD compared to healthy controls, but not compared to AD.[27] In the present study, we observed a trend towards higher CSF levels of pSer129-α-syn in PD compared to controls as previously reported[20], but possibly as a result of the large dispersion of pSer129 within the groups, especially in the control group, this increase did not achieve statistical significance. We found a negative association between pSer129-α-syn and MMSE only in DLB. These findings together might suggest that pSer129-α-syn will not aid in differential diagnosis, but rather that pSer129-α-syn might play a role specific in DLB (and PD).

The reduction in t-α-syn in DLB and PD is likely due to α-syn aggregation and sequestration in Lewy bodies[6], similar to the reduction in CSF Aβ42 that is thought to mirror increased amyloid deposition in the AD brain. However, the regulation of t-α-syn in DLB seems to be more complex. We observed a positive association between tau proteins and t-α-syn in DLB, PD and AD, but not in controls. These results concur with previous studies.[35-39] Tau protein is considered as a biomarker of neurodegeneration.[7] Synapse loss and disruption could cause a release of tau and t-α-syn from damaged neurons into the brain's interstitial fluid and then into the CSF, resulting in higher CSF levels of both tau and t-α-syn. Hence, it could be hypothesized that DLB patients with more synaptic loss have elevated levels of t-α-syn, whereas, DLB patients with limited synaptic loss has decreased levels of t-α-syn. This hypothesis is supported by the findings that CSF levels of t-α-syn are elevated in AD, characterized by marked

neuron and synapse loss, compared to controls[39] and t-α-syn levels increased with disease progression in PD.[40, 41] In the present study, we found a negative correlation (r=-0.42) between t-α-syn and MMSE score in PD. This finding is in line with previous studies.[41, 42] Studies with longitudinal measurements of CSF biomarkers in PD indeed showed that t-α-syn and tau increased over 2 years in PD and were associated with worsening cognition.[40, 41] A possible explanations for the association might be that impaired synaptic function is linked to cognition in Parkinson's disease.[43-45] In line with our results, most studies performing correlation analysis between t-α-syn and AD biomarkers showed a positive correlation between t-α-syn and tau, and no correlation with Aβ42 in PD/DLB.[20, 25, 35, 37, 46-49] Other studies, however, have shown a positive correlation between t-α-syn and Aβ42 in patients with PD(D).[26, 41, 50-52] This discrepancy might be due to inclusion of more severely affected PD patients with lower levels of CSF Aβ42. In a previous study in early PD patients no correlation between t-α-syn and Aβ42 was found[53]. These results seem to suggest that t-α-syn and Aβ42 reflect unrelated disease processes. The elevated levels of o-α-syn might be associated with increased levels of soluble α-synuclein aggregates resulting from a clearance failure.[54, 55]

Although differences in t-α-syn and o-α-syn were found between diagnostic groups, there is substantial overlap of individual α-synuclein levels, which limits the diagnostic value of α-synuclein species for individual patients. A potential confounding factor is the overlap in histopathology in neurodegenerative diseases. Neuropathological studies reported the presence of α-synuclein pathology in 20-50% of AD patients.[36, 56-58] In addition, α-synuclein pathology was also present in approximately 25% of aged healthy controls.[59] Another possible explanation could be that these CSF α-synuclein species are not sensitive or disease specific enough to distinguish DLB and/or PD from AD and controls. Several authors have suggested that CSF α-synuclein species might be more informative when used in combination with other biomarkers, for example Aβ42, t-tau and p-tau.[25, 36, 37, 49] In the present study, we demonstrated that CSF α-synuclein species add discriminatory value to traditional CSF AD biomarkers. AD biomarkers can be used to discriminate both types of dementia (i.e. AD and DLB) from PD and controls, and α-synuclein species add by discriminating both types of synucleinopathies (i.e. PD and DLB) from AD and controls, illustrating that DLB is at the cross-roads of dementia disorders and synucleinopathies. This was further substantiated when we found that in a bilateral comparison, the combination of o-α-syn and tau optimally discriminates DLB from AD. Taken together with the results of previous studies[38, 46, 49, 60], our observations underline the potential of combining α-synuclein species with other biomarkers like Aβ42, t-tau and p-tau to improve the differential diagnosis of DLB. Other, yet to discover,

potential biomarker candidates or post-translationally modified α-synuclein species, may also be useful for this purpose.

One of the strengths of this study is that the diagnosis of PD and DLB was supported by [123I]FP-CIT SPECT findings showing presynaptic dopaminergic deficits and/or slow-wave activity on EEG. Furthermore, the cohort was relatively large for a CSF biomarker study. Third, our assays are sensitive, highly target specific and robust. Among the limitations is the lack of postmortem validation in most patients. Only one DLB patient underwent postmortem examination. Another limitation is the use of erythrocytes instead of hemoglobin to measure the contamination of red blood cells in CSF. The erythrocytes were measured in the first 2 mL of CSF during routine analysis and might not reflect the actual erythrocyte count in the CSF sample used to measure α-synuclein species. Using this procedure, we may have overestimated the actual erythrocyte count. To note, as we excluded all CSF samples with an erythrocyte count ≥500 cells/µL, it is unlikely that traces of blood may have influenced CSF α-synuclein levels in our study.

In conclusion, DLB is a disease-entity that is located at the cross-roads of dementia disorders and movement disorders. We here showed that CSF α-synuclein species, especially t-α-syn and o-α-syn, in combination with the AD biomarkers could be useful as part of a biomarker panel to support DLB diagnosis. This approach would allow for better and timelier diagnosis, characterization of disease subtypes, patient selection for clinical trials that are designed to evaluate new disease-modifying treatments and treatment monitoring. An important next step is to prospectively validate CSF α-synuclein species in patients at an early disease stage or prodromal phase.

Acknowledgements

Research of the VUmc Alzheimer center is part of the neurodegeneration research program of Amsterdam Neuroscience (www.amsterdamresearch.org). The VUmc Alzheimer Center is supported by Stichting Alzheimer Nederland and Stichting VUmc fonds. The clinical database structure was developed with funding from Stichting Dioraphte. DLB specific research is further funded by the Scientific Excellence Program of Amsterdam Neuroscience, the Memorabel grant programme of the Netherlands Organisation for Health Research and Development (ZonMW grant: 733050509) and Stichting Alzheimer Nederland.

REFERENCES

1. Vann Jones SA, O'Brien JT. The prevalence and incidence of dementia with Lewy bodies: a systematic review of population and clinical studies. Psychol Med. 2014 Mar;44(4):673-83.
2. McKeith IG, Boeve BF, Dickson DW, et al. Diagnosis and management of dementia with Lewy bodies: Fourth consensus report of the DLB Consortium. Neurology. 2017 Jul 4;89(1):88-100.
3. Walker Z, Possin KL, Boeve BF, Aarsland D. Lewy body dementias. Lancet. 2015 Oct 24;386(10004):1683-97.
4. McKeith IG, Dickson DW, Lowe J, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology. 2005 Dec 27;65(12):1863-72.
5. Nelson PT, Jicha GA, Kryscio RJ, et al. Low sensitivity in clinical diagnoses of dementia with Lewy bodies. Journal of neurology. 2010 Mar;257(3):359-66.
6. Vekrellis K, Xilouri M, Emmanouilidou E, Rideout HJ, Stefanis L. Pathological roles of alpha-synuclein in neurological disorders. The Lancet Neurology. 2011 Nov;10(11):1015-25.
7. Blennow K, Dubois B, Fagan AM, Lewczuk P, de Leon MJ, Hampel H. Clinical utility of cerebrospinal fluid biomarkers in the diagnosis of early Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2015 Jan;11(1):58-69.
8. Howlett DR, Whitfield D, Johnson M, et al. Regional Multiple Pathology Scores Are Associated with Cognitive Decline in Lewy Body Dementias. Brain pathology (Zurich, Switzerland). 2015 Jul;25(4):401-8.
9. van Steenoven I, Aarsland D, Weintraub D, et al. Cerebrospinal Fluid Alzheimer's Disease Biomarkers Across the Spectrum of Lewy Body Diseases: Results from a Large Multicenter Cohort. J Alzheimers Dis. 2016 Aug 18;54(1):287-95.
10. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997 Aug 28;388(6645):839-40.
11. Borghi R, Marchese R, Negro A, et al. Full length alpha-synuclein is present in cerebrospinal fluid from Parkinson's disease and normal subjects. Neurosci Lett. 2000 Jun 16;287(1):65-7.
12. El-Agnaf OM, Salem SA, Paleologou KE, et al. Alpha-synuclein implicated in Parkinson's disease is present in extracellular biological fluids, including human plasma. FASEB J. 2003 Oct;17(13):1945-7.
13. Simonsen AH, Kuiperij B, El-Agnaf OM, et al. The utility of alpha-synuclein as biofluid marker in neurodegenerative diseases: a systematic review of the literature. Biomark Med. 2016;10(1):19-34.
14. Lim X, Yeo JM, Green A, Pal S. The diagnostic utility of cerebrospinal fluid alpha-synuclein analysis in dementia with Lewy bodies - a systematic review and meta-analysis. Parkinsonism Relat Disord. 2013 Oct;19(10):851-8.
15. Delgado-Alvarado M, Gago B, Navalpotro-Gomez I, Jimenez-Urbieta H, Rodriguez-Oroz MC. Biomarkers for dementia and mild cognitive impairment in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2016 Jun;31(6):861-81.

16. Sulzer D. Clues to how alpha-synuclein damages neurons in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2010;25 Suppl 1:S27-31.
17. Winner B, Jappelli R, Maji SK, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. Proceedings of the National Academy of Sciences of the United States of America. 2011 Mar 8;108(10):4194-9.
18. Rockenstein E, Nuber S, Overk CR, et al. Accumulation of oligomer-prone alpha-synuclein exacerbates synaptic and neuronal degeneration in vivo. Brain : a journal of neurology. 2014 May;137(Pt 5):1496-513.
19. Paleologou KE, Kragh CL, Mann DM, et al. Detection of elevated levels of soluble alpha-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies. Brain : a journal of neurology. 2009 Apr;132(Pt 4):1093-101.
20. Majbour NK, Vaikath NN, van Dijk KD, et al. Oligomeric and phosphorylated alpha-synuclein as potential CSF biomarkers for Parkinson's disease. Mol Neurodegener. 2016 Jan 19;11:7.
21. Anderson JP, Walker DE, Goldstein JM, et al. Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J Biol Chem. 2006 Oct 6;281(40):29739-52.
22. Hansson O, Hall S, Ohrfelt A, et al. Levels of cerebrospinal fluid alpha-synuclein oligomers are increased in Parkinson's disease with dementia and dementia with Lewy bodies compared to Alzheimer's disease. Alzheimers Res Ther. 2014;6(3):25.
23. Tokuda T, Qureshi MM, Ardah MT, et al. Detection of elevated levels of alpha-synuclein oligomers in CSF from patients with Parkinson disease. Neurology. 2010 Nov 16;75(20):1766-72.
24. Park MJ, Cheon SM, Bae HR, Kim SH, Kim JW. Elevated levels of alpha-synuclein oligomer in the cerebrospinal fluid of drug-naive patients with Parkinson's disease. J Clin Neurol. 2011 Dec;7(4):215-22.
25. Parnetti L, Farotti L, Eusebi P, et al. Differential role of CSF alpha-synuclein species, tau, and Abeta42 in Parkinson's Disease. Front Aging Neurosci. 2014;6:53.
26. Compta Y, Valente T, Saura J, et al. Correlates of cerebrospinal fluid levels of oligomeric- and total-alpha-synuclein in premotor, motor and dementia stages of Parkinson's disease. Journal of neurology. 2015 Feb;262(2):294-306.
27. Wang Y, Shi M, Chung KA, et al. Phosphorylated alpha-synuclein in Parkinson's disease. Sci Transl Med. 2012 Feb 15;4(121):121ra20.
28. van der Flier WM, Pijnenburg YA, Prins N, et al. Optimizing patient care and research: the Amsterdam Dementia Cohort. J Alzheimers Dis. 2014;41(1):313-27.
29. van Dijk KD, Bidinosti M, Weiss A, Raijmakers P, Berendse HW, van de Berg WD. Reduced alpha-synuclein levels in cerebrospinal fluid in Parkinson's disease are unrelated to clinical and imaging measures of disease severity. Eur J Neurol. 2014 Mar;21(3):388-94.

30. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2011 May;7(3):263-9.
31. 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 Mar;55(3):181-4.
32. Teunissen CE, Petzold A, Bennett JL, et al. A consensus protocol for the standardization of cerebrospinal fluid collection and biobanking. Neurology. 2009 Dec 1;73(22):1914-22.
33. Barbour R, Kling K, Anderson JP, et al. Red blood cells are the major source of alpha-synuclein in blood. Neurodegenerative diseases. 2008;5(2):55-9.
34. Duits FH, Teunissen CE, Bouwman FH, et al. The cerebrospinal fluid "Alzheimer profile": easily said, but what does it mean? Alzheimer's & dementia : the journal of the Alzheimer's Association. 2014 Nov;10(6):713-23 e2.
35. Wennstrom M, Surova Y, Hall S, et al. Low CSF levels of both alpha-synuclein and the alpha-synuclein cleaving enzyme neurosin in patients with synucleinopathy. PLoS One. 2013;8(1):e53250.
36. Toledo JB, Cairns NJ, Da X, et al. Clinical and multimodal biomarker correlates of ADNI neuropathological findings. Acta neuropathologica communications. 2013 Oct 9;1:65.
37. Chiasserini D, Biscetti L, Eusebi P, et al. Differential role of CSF fatty acid binding protein 3, alpha-synuclein, and Alzheimer's disease core biomarkers in Lewy body disorders and Alzheimer's dementia. Alzheimers Res Ther. 2017 Jul 28;9(1):52.
38. Slaets S, Vanmechelen E, Le Bastard N, et al. Increased CSF alpha-synuclein levels in Alzheimer's disease: correlation with tau levels. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2014 Oct;10(5 Suppl):S290-8.
39. Majbour NK, Chiasserini D, Vaikath NN, et al. Increased levels of CSF total but not oligomeric or phosphorylated forms of alpha-synuclein in patients diagnosed with probable Alzheimer's disease. Sci Rep. 2017 Jan 10;7:40263.
40. Majbour NK, Vaikath NN, Eusebi P, et al. Longitudinal changes in CSF alpha-synuclein species reflect Parkinson's disease progression. Movement disorders : official journal of the Movement Disorder Society. 2016 Oct;31(10):1535-42.
41. Hall S, Surova Y, Ohrfelt A, et al. Longitudinal Measurements of Cerebrospinal Fluid Biomarkers in Parkinson's Disease. Movement disorders : official journal of the Movement Disorder Society. 2016 Jun;31(6):898-905.
42. Stewart T, Sossi V, Aasly JO, et al. Phosphorylated alpha-synuclein in Parkinson's disease: correlation depends on disease severity. Acta neuropathologica communications. 2015 Jan 31;3:7.
43. Bereczki E, Branca RM, Francis PT, et al. Synaptic markers of cognitive decline in neurodegenerative diseases: a proteomic approach. Brain : a journal of neurology. 2018 Feb 1;141(2):582-95.
44. Selnes P, Stav AL, Johansen KK, et al. Impaired synaptic function is linked to cognition in Parkinson's disease. Ann Clin Transl Neurol. 2017 Oct;4(10):700-13.
45. Bereczki E, Bogstedt A, Hoglund K, et al. Synaptic proteins in CSF relate to Parkinson's disease stage markers. NPJ Parkinsons Dis. 2017;3:7.

46. Llorens F, Schmitz M, Varges D, et al. Cerebrospinal alpha-synuclein in alpha-synuclein aggregation disorders: tau/alpha-synuclein ratio as potential biomarker for dementia with Lewy bodies. Journal of neurology. 2016 Nov;263(11):2271-7.
47. Reesink FE, Lemstra AW, van Dijk KD, et al. CSF alpha-synuclein does not discriminate dementia with Lewy bodies from Alzheimer's disease. J Alzheimers Dis. 2010;22(1):87-95.
48. Parnetti L, Chiasserini D, Bellomo G, et al. Cerebrospinal fluid Tau/alpha-synuclein ratio in Parkinson's disease and degenerative dementias. Movement disorders : official journal of the Movement Disorder Society. 2011 Jul;26(8):1428-35.
49. Shi M, Tang L, Toledo JB, et al. Cerebrospinal fluid alpha-synuclein contributes to the differential diagnosis of Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2018 Aug;14(8):1052-62.
50. Buddhala C, Campbell MC, Perlmutter JS, Kotzbauer PT. Correlation between decreased CSF alpha-synuclein and Abeta(1)(-)(4)(2) in Parkinson disease. Neurobiol Aging. 2015 Jan;36(1):476-84.
51. Gao R, Zhang G, Chen X, et al. CSF Biomarkers and Its Associations with 18F-AV133 Cerebral VMAT2 Binding in Parkinson's Disease-A Preliminary Report. PLoS One. 2016;11(10):e0164762.
52. Goldman JG, Andrews H, Amara A, et al. Cerebrospinal fluid, plasma, and saliva in the BioFIND study: Relationships among biomarkers and Parkinson's disease Features. Movement disorders : official journal of the Movement Disorder Society. 2018 Feb;33(2):282-8.
53. Kang JH, Irwin DJ, Chen-Plotkin AS, et al. Association of cerebrospinal fluid beta-amyloid 1-42, T-tau, P-tau181, and alpha-synuclein levels with clinical features of drug-naive patients with early Parkinson disease. JAMA Neurol. 2013 Oct;70(10):1277-87.
54. Xin W, Emadi S, Williams S, et al. Toxic Oligomeric Alpha-Synuclein Variants Present in Human Parkinson's Disease Brains Are Differentially Generated in Mammalian Cell Models. Biomolecules. 2015 Jul 22;5(3):1634-51.
55. Ingelsson M. Alpha-Synuclein Oligomers-Neurotoxic Molecules in Parkinson's Disease and Other Lewy Body Disorders. Front Neurosci. 2016;10:408.
56. Mikolaenko I, Pletnikova O, Kawas CH, et al. Alpha-synuclein lesions in normal aging, Parkinson disease, and Alzheimer disease: evidence from the Baltimore Longitudinal Study of Aging (BLSA). J Neuropathol Exp Neurol. 2005 Feb;64(2):156-62.
57. Leverenz JB, Fishel MA, Peskind ER, et al. Lewy body pathology in familial Alzheimer disease: evidence for disease- and mutation-specific pathologic phenotype. Arch Neurol. 2006 Mar;63(3):370-6.
58. Hamilton RL. Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. Brain pathology (Zurich, Switzerland). 2000 Jul;10(3):378-84.
59. Markesbery WR, Jicha GA, Liu H, Schmitt FA. Lewy body pathology in normal elderly subjects. J Neuropathol Exp Neurol. 2009 Jul;68(7):816-22.
60. Hall S, Ohrfelt A, Constantinescu R, et al. Accuracy of a panel of 5 cerebrospinal fluid biomarkers in the differential diagnosis of patients with dementia and/or parkinsonian disorders. Arch Neurol. 2012 Nov;69(11):1445-52.

SUPPLEMENTAL DATA

Supplementary Table 1 | Characteristics of the control groups

	SCD	Healthy controls	*p*-value
N	30	48	
MMSE	29 [28-30]	29 [29-30]	0.611
t-α-syn (ng/ml)	1.8 ± 0.6	1.9 ± 0.8	0.854
o-α-syn (pg/ml)	82 ± 29	68 ± 41	0.169
pSer129-α-syn (pg/ml)	304 ± 184	227 ± 55	0.152

Data are expressed as mean ± SD or median [interquartile range]. Differences between groups were assessed with student t-tests for normally distributed continuous variables or with Mann-Whitney U tests for non-normally distributed continuous variables. MMSE = Mini-mental state examination; pSer129-α-syn = phosphorylated α-synuclein protein at Serine 129; o-α-syn= oligomeric α-synuclein; SCD = Subjective cognitive decline; t-α-syn = total α-synuclein

Supplementary Table 2 | Characteristics of outliers

Outliers ID	Diagnosis	Age	Sex	t-α-syn (ng/ml)	o-α-syn (pg/ml)	pSer129-α-syn (pg/ml)	Aβ42 (pg/ml)	t-tau (pg/ml)	p-tau (pg/ml)
ADC-5085	DLB	67.8	f	1.23	161	**798**	484	557	66
ADC-3709	DLB	76.5	m	1.58	138	**697**	912	218	34
ADC-4081	DLB	73.3	f	0.72	86	**627**	617	134	17
MOV-18	PD	84.0	m	1.32	**343**	197	431	116	51
MOV-94	PD	59.0	m	2.02	**299**	253	1234	393	73
ADC-223	AD	66.4	m	**5.55**	95	196	237	425	55
ADC-3416	AD	55.9	m	2.49	139	**639**	460	921	99
ADC-3418	AD	65.0	m	3.05	58	**600**	634	620	75
MOV-176	HC	79.0	m	**4.58**	56	164	1414	458	63
ADC-3490	SCD	62.2	m	2.70	97	**968**	1280	300	47
ADC-1157	SCD	66.2	m	1.60	106	**713**	848	188	71
ADC-158	SCD	70.0	f	1.28	100	**676**	192	251	44

Values in bold are above the third quartile plus 3xIQR. Aβ42 = amyloid-β 1-42; AD = Alzheimer's disease; DLB = dementia with Lewy bodies; f = female; m = male; PD = Parkinson's disease; pSer129-α-syn = phosphorylated α-synuclein protein at Serine 129; p-tau = Tau phosphorylated at threonine 181; o-α-syn = oligomeric α-synuclein; SCD = subjective cognitive decline; t-α-syn = total α-synuclein; t-tau = total Tau protein

Supplementary Table 3 | Associations between CSF biomarkers

	t-α-syn	o-α-syn	pS129-α-syn	Aβ42	t-tau	p-tau
t-α-syn	1					
DLB		- 0.31	- 0.13	- 0.03	0.48**	0.40*
PD		0.09	- 0.05	0.14	0.64***	0.69***
AD		0.08	0.16	- 0.34	0.45*	0.44*
Controls		- 0.16	- 0.16	0.07	0.17	0.20
o-α-syn		1				
DLB	- 0.31		0.45*	0.03	0.00	- 0.03
PD	0.09		- 0.08	- 0.01	- 0.11	- 0.04
AD	0.08		0.24	0.10	0.30	0.30
Controls	- 0.16		0.02	- 0.02	- 0.05	- 0.02
pSer129-α-syn			1			
DLB	- 0.13	0.45*		0.06	0.18	0.02
PD	- 0.05	- 0.08		- 0.11	- 0.14	- 0.29
AD	0.16	0.24		0.23	0.23	0.36
Controls	- 0.16	0.02		- 0.26	- 0.03	- 0.05

Associations between CSF biomarkers were assessed with Pearson Correlation Coefficients. The Benjamini-Hochberg procedure was used to correct for multiple testing. Data shown as r. * p<0.05, ** p<0.01, *** p<0.001. Aβ42 = amyloid-β 1-42; AD = Alzheimer's disease; DLB = dementia with Lewy bodies; PD = Parkinson's disease; pSer129-α-syn = phosphorylated α-synuclein protein at Serine 129; p-tau = Tau phosphorylated at threonine 181; o-α-syn = oligomeric α-synuclein; t-α-syn = total α-synuclein; t-tau = total Tau protein

Supplementary Table 4 | Associations between CSF α-synuclein species and clinical parameters

	Age	Disease duration	MMSE	UPDRS-III
t-α-syn				
DLB	0.11	0.18	0.30	NA
PD	0.17	0.22	- 0.42**	- 0.06
AD	- 0.07	0.10	0.09	NA
Controls	0.06	NA	0.10	NA
o-α-syn				
DLB	0.12	- 0.04	- 0.10	NA
PD	0.09	- 0.28	- 0.02	0.10
AD	0.16	0.04	0.33	NA
Controls	0.16	NA	- 0.11	NA
pSer129-α-syn				
DLB	0.39*	- 0.21	- 0.45**	NA
PD	- 0.15	- 0.10	0.16	- 0.24
AD	- 0.28	0.11	- 0.05	NA
Controls	0.04	NA	- 0.03	NA

Associations between CSF α-synuclein species and clinical variables were assessed with Pearson Correlation Coefficients. Data shown as r. * p<0.05, ** p<0.01, *** p<0.001. AD = Alzheimer's disease; DLB= dementia with Lewy bodies; MMSE= Mini-mental state examination; NA = not applicable; UPDRS-III = Unified Parkinson Disease Rating Scale motor score; PD= Parkinson's disease; pSer129-α-syn = phosphorylated α-synuclein protein at Serine 129; o-α-syn = oligomeric α-synuclein; t-α-syn = total α-synuclein.

Supplementary Table 5 | Discriminant loadings for each individual predictor

	Function	
	1	**2**
Aβ42	**- 0.64**	- 0.11
t-tau	**0.81**	- 0.19
t-α-syn	0.19	**- 0.62**
o-α-syn	0.01	**0.83**

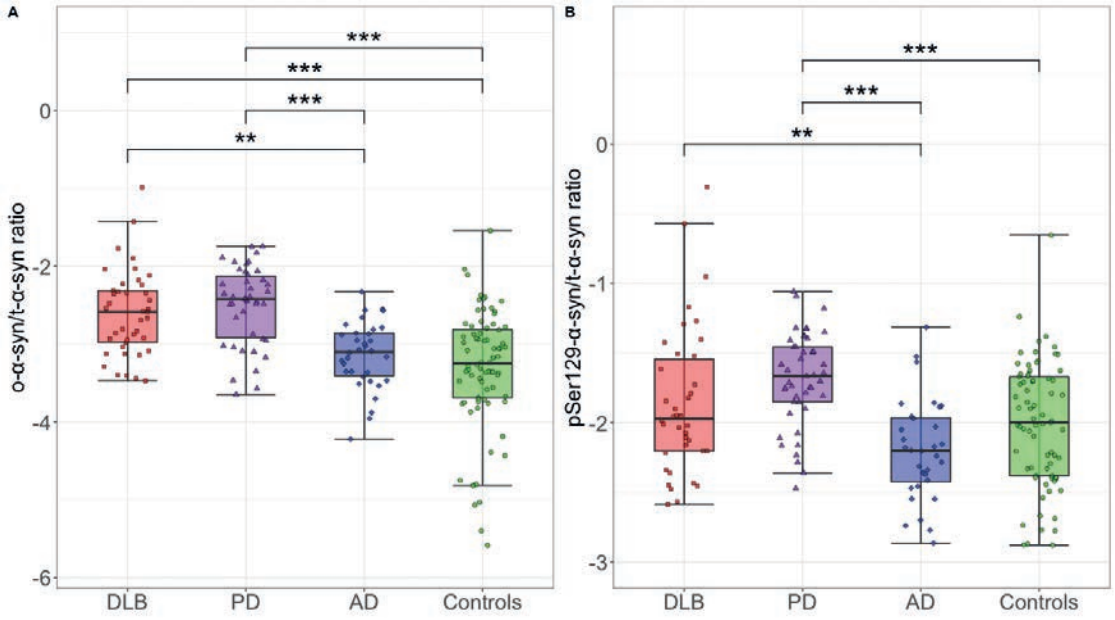
The correlation coefficient represents the relative contribution for each predictor to group separation. Aβ42 = amyloid-β 1-42; o-α-syn = oligomeric α-synuclein; t-α-syn = total α-synuclein; t-tau = total Tau protein.

Supplementary Table 6 | Logistic Regression analysis of multiple CSF biomarkers for discrimination between PD and controls

	PD				
	Predictors	**OR for PD (95% CI)**	***p*-value**	**Accuracy of model**	
Controls	t-α-syn	0.27 (0.11-0.57)	<0.01	AUC: 0.85 (0.77-0.92)	
				Sens: 67%	PPV: 74%
	o-α-syn	3.32 (1.97-6.09)	<0.001	Spec: 87%	NPV: 82%

CSF biomarker predictors were Z transformed before analyses; therefore, odds ratio's (OR) represent higher odds for PD per standard deviation (SD) decreased t-α-syn or increased o-α-syn. AUC = area under the curve; NPV = negative predictive value; o-α-syn = oligomeric α-synuclein; OR = odds ratio; PD = Parkinson's disease; PPV = positive predictive value; Sens = sensitivity; Spec = specificity; t-α-syn = total α-synuclein.

Supplementary Figure 1 | Box and Whisker plots of ratios of α-synuclein species in DLB, PD, AD and controls



(A) Ratio of o-α-syn/ t-α-syn, (B) Ratio of pSer129-α-syn/ t-α-syn. The line through the middle of the boxes corresponds to the median and the lower and the upper lines to the 25th and 75th percentile, respectively. The whiskers extend from the 5th percentile on the bottom to the 95th percentile on top. Differences between groups were assessed with GLM, adjusted for age and gender. * p<0.05, ** p<0.01, *** p<0.001.