Molecular Neurobiology (2019) 56:3476–3483
https://doi.org/10.1007/s12035-018-1313-4

Cerebrospinal Fluid Total and Phosphorylated α-Synuclein in Patients with Creutzfeldt–Jakob Disease and Synucleinopathy

Matthias Schmitz[1] · Anna Villar-Piqué[1] · Franc Llorens[1,2,3] · Karin Gmitterová[4,5] · Peter Hermann[1] · Daniela Varges[1] · Saima Zafar[1] · Paul Lingor[1] · Hugo Vanderstichele[6] · Leentje Demeyer[6] · Erik Stoops[6] · John Q Trojanowski[7] · Virginia M-Y Lee[7] · Inga Zerr[1]

Received: 15 May 2018 / Accepted: 9 August 2018 / Published online: 22 August 2018

Abstract

High levels of total α-synuclein (t-α-synuclein) in the cerebrospinal fluid (CSF) were reported in sporadic Creutzfeldt–Jakob disease (sCJD). The potential use of t-α-synuclein in the discrimination of Lewy body dementias (i.e., Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB)) is still under investigation. In addition, phospho-serine-129 α-synuclein (p-α-synuclein) has been described to be slightly increased in the CSF of synucleinopathies. Here, we analyzed t-α-synuclein and p-α-synuclein concentrations and their ratio in the context of differential diagnosis of neurodegenerative diseases. We quantified the levels of CSF t-α-synuclein and p-α-synuclein in a cohort of samples composed of neurological controls (NC), sCJD, PDD, and DLB by means of newly developed specific enzyme-linked immunosorbent assays. T-α-synuclein and p-α-synuclein were specifically elevated in sCJD compared to other disease groups. The area under the curve (AUC) values for t-α-synuclein were higher for the discrimination of sCJD from dementias associated to Lewy bodies as compared to the use of p-α-synuclein. A combination of both markers even increased the diagnostic accuracy. An inverse correlation was observed in CSF between t-α-synuclein and p-α-synuclein, especially in the DLB group, indicating a disease-relevant association between both markers. In conclusion, our data confirm t-α-synuclein and p-α-synuclein as robust biomarkers for sCJD and indicate the potential use of colorimetric t-α-synuclein ELISAs for differential diagnosis of dementia types.

Keywords T-α-synuclein · Cerebrospinal fluid · Creutzfeldt–Jakob disease · Dementia with Lewy bodies · Parkinson's disease · Phospho-serine-129 α-synuclein

Matthias Schmitz, Anna Villar-Piqué and Franc Llorens contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12035-018-1313-4) contains supplementary material, which is available to authorized users.

✉ Matthias Schmitz
matthias.schmitz@med.uni-goettingen.de

✉ Anna Villar-Piqué
annavillarpique@gmail.com

✉ Franc Llorens
franc.llorens@gmail.com

[1] Department of Neurology, University Medical Center Goettingen, Goettingen, Germany

[2] Network Center for Biomedical Research in Neurodegenerative Diseases (CIBERNED), Barcelona, Spain

[3] Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain

[4] Second Department of Neurology, Comenius University, Bratislava, Slovakia

[5] Department of Neurology, University Medical Center Goettingen, Goettingen, Germany

[6] ADx NeuroSciences, Technologiepark 4, Ghent, Belgium

[7] Center for Neurodegenerative Research and Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, USA

Background

Intraneuronal inclusions named Lewy bodies (LBs), mainly consisting of aggregated α-synuclein, can be commonly found in patients with Parkinson's disease (PD), characterized by motor disturbances known as Parkinsonism or non-motor symptoms, including cognitive impairment as well as dementia (PD with dementia, PDD) and dementia with LBs (DLB) [1–3]. DLB patients are clinically characterized by almost simultaneous occurrence of Parkinsonism, cognitive impairment, and progressive dementia [4]. Levels of disease-relevant α-synuclein proteins in the cerebrospinal fluid (CSF) are modified not only in α-synucleinopathies but also in sporadic Creutzfeldt–Jakob disease (sCJD) patients [5, 6]. The current criteria for clinical diagnosis of prion diseases include a combination of CSF biomarkers, magnetic resonance imaging, electroencephalography, and clinical symptoms [3, 7, 8]. However, it must be taken into consideration that in the context of differential diagnosis of sCJD from DLB patients, there might be an overlap in clinical symptoms. Approximately 40% of DLB subjects meet the clinical neurological signs/symptoms of sCJD, such as extrapyramidal signs, myoclonus, and a common fluctuation of the cognitive symptoms which may mimic a rapid progression [1–3].

In this regard, CSF biomarkers might be useful for differential diagnosis of neurodegenerative dementias [5, 7, 9–11]. The use of new technologies, such as chemiluminescent-based platforms or mass spectrometry, allowed the discrimination of sCJD from other neurodegenerative diseases with a high diagnostic accuracy [6, 12, 13]. In particular, CSF α-synuclein may become especially useful in the differentiation of sCJD from DLB cases because its concentration is highly increased in sCJD patients [6, 13, 14]. In contrast, meta-analysis studies suggested only a slight to moderate decrease of CSF α-synuclein in the spectrum of α-synuclein aggregation disorders [15–17]. However, consensus for the latter is missing, resulting in an unclear role for total α-synuclein or non-aggregated synuclein. Recently, increased CSF phosphoserine-129 α-synuclein (p-α-synuclein) levels were reported in PD cases [18, 19], but its potential value in the differential diagnostic context has not been investigated yet. To our knowledge, only limited information is available on the concentration of CSF p-α-synuclein in synucleinopathies (i.e., PDD and DLB).

In order to assess whether CSF total α-synuclein (t-α-synuclein) and p-α-synuclein levels or their ratio might improve the diagnostic accuracy to detect sCJD, we have measured isoforms in a cohort of neurological controls (NC), sCJD, PDD, and DLB cases. To reach this goal, two new colorimetric enzyme-linked immunosorbent assays (ELISAs) were qualified for this purpose.

Methods

Patients

The present study includes sCJD samples ($n = 42$) that fulfilled the WHO criteria for diagnosis of probable ($n = 28$) or definite sCJD ($n = 14$) (confirmed by autopsy) [8, 20]. Patients with α-synucleinopathies were classified as PDD ($n = 16$) (criteria based on [21]) and DLB ($n = 27$) (criteria based on the *McKeith* criteria [4]; autopsies were not available). A neurological control group (NC) ($n = 42$) was composed of neurological diseases and neurodegenerative conditions (such as vascular dementia, Alzheimer's disease, vasculitis, hypoxia) in which prion disease and synucleinopathy diagnosis were excluded.

Collection and Pre-Analytical Sample Treatment

Lumbar puncture was performed for diagnostic purposes as previously described [11]. Samples were routinely analyzed for cell counts, total protein, hemoglobin, and immunoglobulins to rule out ongoing inflammatory processes or contamination of CSF with red blood cells. Routine investigation of the CSF did not reveal any abnormalities on the basis of these parameters.

Determination and Characterization of T-α-Synuclein by ELISA

The test protocol for the t-α-synuclein ELISA, using antibodies ADx301 and ADx302, was already described before [22]. Analytical performance data were extended with info on monoclonal antibodies (mAb) characteristics, specificity of the assay for detection of α-, ß-, or γ- isoform of the protein, and parallelism.

Determination and Characterization of P-α-Synuclein by ELISA

The p-α-synuclein prototype assay is designed using a combination of a p-α-synuclein specific mAb as capture mAb (Clone 81A, provided under license from University of Pennsylvania) and a pan-synuclein polyclonal antibody (FL140; Santa Cruz) as detection Ab. The test procedure is done at room temperature (18–30 °C) under static conditions. The assay procedure is initiated by adding 80 μL of undiluted CSF to 20 μL of the biotinylated capture Ab, followed by incubation for 3 h. Afterwards, wells are washed 5 times with 300 μL of wash solution. Next, 100 μL of peroxidase-labeled streptavidin (SV) is added and incubated for 30 min. For the colorimetric reaction, 100 μL of substrate solution was added into each well (after a second wash step) and incubated for

30 min (protected from light). The reaction was stopped by pipetting 100 μL stop solution (0.9 N sulfuric acid) to each well. After gently mixing, microplates were measured in a microplate reader for photometric measurement of the absorbance at a wavelength of 450 nm and a reference wavelength of 630 nm.

The phospho-dependency was assessed only for mAb ADx301 (Epitope, amino acid 110–120) where potential phosphorylation sites have been identified. No phosphorylation sites have been described in the region of the epitope of mAb ADx302 (amino acids 90–100) [23]. For this purpose, plates were coated with 100 μL of SV at a concentration of 5 μg/mL. The SV-coated plates were incubated with biotinylated peptides (non-modified, phosphorylated on tyrosine at position 125, 133, and 136 or on serine at position 129) (more details on the selected peptides are provided in Suppl. Fig. 2A). Biotinylated full-length synuclein, containing no phosphorylated amino acids (α-synuclein (1–140); Anaspec Cat#AS-55581), was used as a positive control. After a wash step, ADx301 and FL-140 antibody (Santa Cruz Cat#FL-140; positive control for the assay) were incubated for 1 h at room temperature. Excess of antibody was removed by wash steps. Finally, a peroxidase-labeled detector antibody was added for another 30 min at room temperature. After the wash step, substrate solution was added for 30 min. At the end of the test procedure, 0.9 N sulfuric acid is added to stop the reaction. Measurement was done in a microplate reader (wavelengths 450 nm, 630 nm).

Statistical Methods

All statistical evaluation of the data was performed by using the statistical software GraphPad Prism for Windows (version 6.01). Multiple comparisons were conducted with Kruskal–Wallis test followed for correction by Dunn's post-test. Area under the curve (AUC) values were obtained from receiver operating characteristic (ROC) curves. Spearman's rank 2-tailed coefficients were calculated to measure correlations between t-α-synuclein and p-α-synuclein. Best-fit lines in scatter plots were adjusted using linear regression. Statistical significance was considered at 5%.

Results

Part A: Analytical Performance

In the present study, we have partially qualified the analytical and clinical performance of two new colorimetric ELISAs for measurement of α-synuclein isoforms in CSF.

The α-synuclein immunoassay is specific for detection of the α-isoform (no immunoreactivity was obtained with the β- or γ-isoform of the protein) (Suppl. Fig. 1). The assay quantifies the monomeric, non-aggregated form of the protein. We confirmed the phospho-independency of the capture antibody of the t-α-synuclein ELISA (ADx301) by using a set of biotinylated peptides (Suppl. Fig. 2a). Experiments indicated that (hyper)-phosphorylation of synuclein in the CSF will not affect the t-α-synuclein concentration in CSF as measured with the t-α-synuclein ELISA (Suppl. Fig. 2b, c), although phosphorylation of S125 lowered the signal, as this is closer to the epitope of the detector antibody. Total variability (%CV) was below 10%. Good parallelism was shown for a set of CSF samples ($n = 5$; see Suppl. Fig. 3).

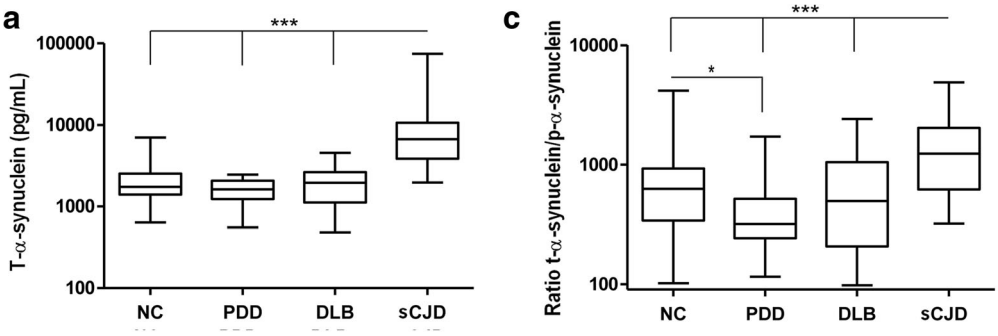
Part B: Diagnostic Performance

To evaluate the clinical performance of the ELISA methods, we measured the concentration of t-α-synuclein and p-α-synuclein in CSF of sCJD, PDD, DLB, and NC patients. T-α-synuclein concentrations were elevated in sCJD, as compared to NC ($p < 0.001$), PDD ($p < 0.001$), and DLB ($p < 0.001$) (Fig. 1a–d; Table 1). The levels of t-α-synuclein in PDD and DLB cases were not significantly different to those detected in NC ($p > 0.05$) (Fig. 1a, d). CSF concentrations of p-α-synuclein were higher in the sCJD group, compared to those in NC ($p < 0.001$), PDD ($p < 0.05$), and DLB ($p < 0.01$) (Fig. 1b, d). Mean p-α-synuclein values were higher in PDD and DLB compared to that in NC, but these differences did not reach statistical significance (Table 1 and Fig. 1b). The ratio of t-α-synuclein/p-α-synuclein was significantly increased in the sCJD group (Fig. 1c). In addition, we observed a significant decreased ratio in the PDD group in comparison to NC ($p = 0.02$), but not between PDD and DLB (Fig. 1c, d).

In order to assess the diagnostic accuracy for each α-synuclein isoform, the AUC values were calculated, and ROC curves had been created (Suppl. Fig. 4a–c). Significances were obtained for the differentiation of sCJD from PDD (AUC = 0.99; $p < 0.001$), DLB (AUC = 0.95; $p < 0.001$), and NC (AUC = 0.94; $p < 0.001$) (Fig. 1d). For these comparisons, AUC values were not statistically different between each other, indicating that t-α-synuclein was not a better discriminator of sCJD from NC, as for LB dementias.

T-α-synuclein was better than p-α-synuclein for the discrimination of sCJD patients with the other diagnostic groups (Fig. 1d). Indeed, for the discrimination of sCJD from NC, p-α-synuclein rendered an AUC of 0.77 ($p < 0.001$); while for t-α-synuclein, it amounted to 0.94. In this context, the sensitivity of t-α-synuclein for sCJD diagnosis was 85.7%, and the specificity varied between 90.5–97.5%. In contrast, p-α-synuclein merely exhibited a sensitivity of 57.1% and specificities between 75.0 and 85.7% (Table 2). A combination of t-α-synuclein and p-α-synuclein increased the sensitivity up to 90.5% (Table 2).

Low AUC values were detected for p-α-synuclein in the discrimination of PDD and DLB cases (AUC = 0.62 for both)



d

	AUC	SE	95% CI	p-value
T-α-Synuclein				
NC vs PDD	0.62	0.08	0.46 to 0.78	0.16
NC vs DLB	0.51	0.07	0.37 to 0.66	0.85
NC vs *s*CJD	0.94	0.02	0.90 to 99	< 0.0001
PDD vs DLB	0.59	0.09	0.42 to 0.76	0.33
PDD vs *s*CJD	0.99	0.01	0.97 to 1.01	< 0.0001
DLB vs *s*CJD	0.95	0.02	0.90 to 1.00	< 0.0001
P-α-Synuclein				
NC vs PDD	0.62	0.09	0.45 to 0.79	0.17
NC vs DLB	0.62	0.07	0.48 to 0.76	0.09
NC vs *s*CJD	0.77	0.05	0.67 to 0.87	< 0.0001
PDD vs DLB	0.52	0.09	0.33 to 0.70	0.85
PDD vs *s*CJD	0.66	0.08	0.50 to 0.83	< 0.05
DLB vs *s*CJD	0.68	0.06	0.55 to 0.81	< 0.01
Ratio				
NC vs PDD	0.70	0.08	0.54 to 0.86	0.02
NC vs DLB	0.59	0.07	0.45 to 0.73	0.21
NC vs *s*CJD	0.73	0.06	0.62 to 0.84	< 0.001
PDD vs DLB	0.60	0.09	0.42 to 0.78	0.29
PDD vs *s*CJD	0.89	0.05	0.79 to 1.00	< 0.0001
DLB vs *s*CJD	0.79	0.06	0.68 to 0.90	< 0.0001

Fig. 1 CSF total t-α-synuclein, p-α-synuclein CSF levels, its ratio in the diagnostic groups, and its clinical value (ROC curve analysis). **a**–**b** CSF levels of t-α-synuclein and p-α-synuclein were measured a colorimetric ELISAs. **c** Ratio of t-α-synuclein/p-α-synuclein in the diagnostic groups. **d** Diagnostic accuracy (ROC analysis) for the discrimination between each disease group. *** $p < 0.001$; * $p < 0.02$. Four cases with p-α-synuclein cases close to 0 were excluded. SE, standard error; 95% CI, confidence interval at 95%; sCJD, sporadic Creutzfeldt–Jakob Disease; DLB, dementia with Lewy bodies; NC, neurological control; PDD, Parkinson's disease with dementia

from NC (Fig. 1d). Similar findings were obtained for the t-α-synuclein/p-α-synuclein ratio (Fig. 1d). Age or gender was excluded as a confounding factor for the CSF analysis of t-α-synuclein and p-α-synuclein (Suppl. Fig. 5a, b).

Finally, correlations between t-α-synuclein and p-α-synuclein were computed for the different disease groups. While no correlation between both synuclein isoforms was found in the groups of NC ($p = 0.490$), PDD ($p = 0.628$), or sCJD ($p = 0.581$), an inverse correlation ($p = -0.463$, $p = 0.015$) was observed in DLB (Fig. 2a–e).

Discussion

In the present work, the analytical and clinical performance of colorimetric ELISAs for detection of t-α-synuclein or p-α-

Table 1 Demographic features of the analyzed cohort and data corresponding to CSF biomarkers. Results are expressed as the mean ± standard deviation. *A.U.*, arbitrary units

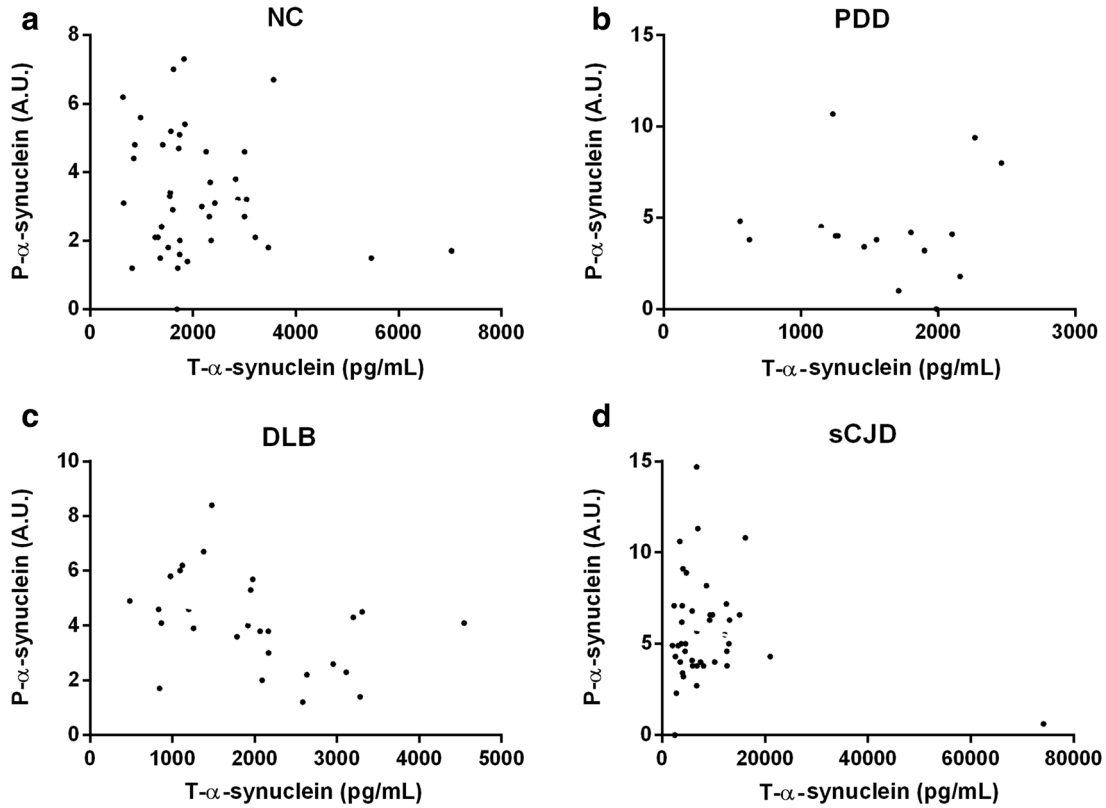
	n	Sex (f/m)	Age (years)	T-α-synuclein (pg/mL)	P-α-synuclein (A.U.)
Controls	42	22/20	67 ± 12	2100 ± 343	36.6 ± 0.55
synucleinopathies					
Parkinson's disease dementia	16	10/6	71 ± 9	1593 ± 298	4.42 ± 1.50
Dementai with Lewy bodies	27	16/11	70 ± 9	1973 ± 423	4.10 ± 0.69
Creutzfeldt–Jakob disease	42	21/21	67 ± 9	8929 ± 3645***	5.66 ± 0.88***

***$p < 0.001$ (compared to controls)

Table 2 Diagnostic accuracy of t-α-synuclein and p-α-synuclein. Sensitivity and specificity values were calculated for t-α-synuclein and p-α-synuclein from the numbers of classified samples. A combination of t-α-synuclein and p-α-synuclein increased the diagnostic accuracy for sCJD to 90.5% sensitivity and 97.6% specificity. *A.U.*, arbitrary units; *PDD*, Parkinson's disease with dementia; *DLB*, dementia with Lewy bodies

	T-α-synuclein (> 3300 pg/mL)	P-α-synuclein (> 4.85 A.U.)	Ratio t-α-synuclein (> 1000)	Combination of t-α-synuclein and p-α-synuclein
sCJD	36/42 85.7% sensitivity	24/42 57.1% sensitivity	26/42 61.9% sensitivity	38/42 90.5% sensitivity
PDD + DLB	1/40 97.5% specificity	10/40 75.0% specificity	10/40 75.0% specificity	0/40 100% specificity
Controls	4/42 90.5% specificity	6/42 85.7% specificity	10/42 76.2% specificity	1/42 97.6% specificity

synuclein in CSF were qualified. We evaluated differences in CSF concentrations of t-α-synuclein and p-α-synuclein in sCJD as compared to NC and to the spectrum of some LB pathologies [6, 13]. T-α-synuclein levels in PDD and DLB were comparable to those detected in NC, as described previously [12, 13, 24–26]. Concentration of CSF t-α-synuclein in synucleinopathies has been extensively analyzed using a variety of methodological approaches, assays, and patient cohorts. Many publications reported a modest reduction compared to healthy or NC. However, conclusive data have not yet been obtained (see Eusebi et al. for a recent meta-analysis [15]). Altogether, it suggests that quantification of



e

	NC	*PDD*	*DLB*	*sCJD*
Spearman coefficient	-0.11	-0.13	-0.46	0.09
95% confidence interval	-0.41 to 0.21	-0.60 to 0.40	-0.72 to -0.09	-0.23 to 0.39
p-values	0.49	0.62	0.02*	0.58

Fig. 2 Correlation of CSF t-α-synuclein and p-α-synuclein in function of the disease group. **a**–**d** Non-parametric Spearman correlation study was performed for NC, DLB, PDD, and sCJD patients. **e** Spearman's correlation coefficients between t-α-synuclein and p-α-synuclein levels for each diagnostic group revealed a significant correlation only in the DLB group

changes in levels of t-α-synuclein levels in synucleinopathies (if any) has (i) a low to moderate clinical significance and/or (ii) is highly dependent on the patient cohorts, most probably linked to the type of controls that are integrated in the study design. In addition, the presence of confounding factors was not always taken into account during sample analysis [27]. Nevertheless, our methodology is able to discriminate NC from sCJD cases with a higher accuracy (sensitivity of 85.7% and a specificity > 90%) than other colorimetric assays for t-α-synuclein detection [14, 28].

In comparison to our previous electrochemiluminescence-based assay for t-α-synuclein detection (more costly and technical requirements), which showed a sensitivity of 94% and a specificity of 96% [6], the diagnostic assay accuracy of the colorimetric system was lower. Nevertheless, the diagnostic accuracy of the colorimetric t-α-synuclein detection is comparable to other biomarkers, such as 14-3-3 or tau detection [5, 9], being a good diagnostic alternative.

Regarding CSF p-α-synuclein levels, significant differences with less diagnostic impact were detected between sCJD and the other groups. In brain tissue, α-synuclein phosphorylation at position 129 occurs under normal physiological conditions [29], as well as in pathological conditions, not directly associated to an underlying synucleinopathy, as it has been shown in sCJD [28]. Thus, elevated p-α-synuclein in sCJD may reflect massive synaptic damage and, therefore, basal p-α-synuclein levels could be detected in the CSF of these patients. On the contrary, the higher mean p-α-synuclein values in PDD and DLB compared to that in NC were not statistically significant. Although our data are not in line with previous findings concluding that p-α-synuclein is slightly increased in PD compared to healthy controls [18, 19], it should be noted that none of these studies included DLB and PDD and that our control group was composed of neurological and neurodegenerative diseases cases instead of healthy individuals.

A limitation of our study is that our findings still need to be confirmed in a larger patient cohort. Also, the robustness of p-α-synuclein detection needs to be further documented. It is worthwhile to note that we did not observe any difference in CSF α-synuclein species between DLB and PDD, supporting the idea that these two disorders are strongly related with a very thin boundary defined by the temporal sequence of appearance of motor and cognitive symptoms [30, 31]. Taken together, we can assert from our analyses that quantification of p-α-synuclein alone or the t-α-synuclein/p-α-synuclein ratio does not provide additional value in the discrimination between these disease groups. In this regard, it is also worth noting that we found that the ratio of t-α-synuclein and p-α-synuclein decreased in PDD when compared to that in NC which still needs to be confirmed in a larger cohort.

A final interesting finding of our work is the inverse correlation in DLB between t-α-synuclein and p-α-synuclein. The fact that this correlation is not observed in the other diagnostic groups, nor in the NC group indicates an underlying disease-specific mechanism. Importantly, a very similar correlation was recently found between CSF t-α-synuclein and p-α-synuclein at early stages of PD [32]. It shold be noted that the number of PDD cases analysed might be too low to detect a significant correlation in this diagnostic group. In synucleinopathies, it is believed that t-α-synuclein concentration in the CSF results from the balance of synaptic damage (increased levels) and sequestration of α-synuclein in LBs (decreased levels). This, together with the fact that α-synuclein phosphorylation in the brain tissue is directly associated with its aggregation capacity, could explain the inverse correlation between both α-synuclein species seen in DLB.

Conclusion

Overall, our study qualified colorimetric ELISAs for two α-synuclein isoforms. We have detected elevated CSF t-α-synuclein levels in sCJD and reported for the first time increased p-α-synuclein levels in sCJD patients, which may be important for diagnostic purposes. However, our data do not support the use of α-synuclein forms for the discrimination of LB dementias.

Acknowledgements We thank Silja Köchy for indispensable technical assistance.

Author Contribution Matthias Schmitz: designed the study, analyzed data, and wrote the manuscript

Anna Villar-Piqué: performed experiments, analyzed data, and wrote the manuscript

Franc Llorens: performed experiments, analyzed data, and critically revised the manuscript

Karin Gmitterová, Daniela Varges, and Peter Hermann: provided samples

Saima Zafar: critically revised the manuscript

Paul Lingor: critically revised the manuscript

Leentje Demeyer: development and qualification of the assay

Erik Stoops: development and qualification of the assay, delivery of materials for the study, and critically reviewed the manuscript

Hugo Vanderstichele: data interpretation and critically reviewed of the manuscript

John Trojanowski: contributed antibody reagents and critically reviewed the manuscript

Virginia M-Y Lee: contributed antibody reagents and critically reviewed the manuscript

Inga Zerr: analyzed and interpreted data and wrote the manuscript

Funding AV-P was funded by Georg-August University of Göttingen (Dorothea Schlözer Programme). FL was funded by the Spanish Ministry of Health–Instituto Carlos III (Miguel Servet–CP16/00041). The assay development work has been supported by the Michael J. Fox Foundation (Grant No. 10147). JQT and VM-YL were supported by NIA Grant AG-10124.

Compliance with Ethical Standards

Ethics Approval The present study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines and has been approved by the local ethics committee in the University Medical Center, Göttingen (No. 9/6/08, 19/11/09, and 18/8/15). Informed consent was given by all study participants or their legal next of kin.

Consent for Publication Not applicable.

Availability of Data and Material The full databank of the data is available to each co-author on the paper.

Competing Interest Leentje Demeyer and Erik Stoops are employees of ADx NeuroSciences. Hugo Vanderstichele is a co-founder of ADx NeuroSciences and a founder of Biomarkable. All other authors declare there are no competing interests.

Abbreviations PD, Parkinson's disease; ROC, receiver operating characteristic; AUC, area under the curve; sCJD, sporadic Creutzfeldt–Jakob disease; CSF, cerebrospinal fluid; DLB, dementia with LBs; ELISA, enzyme-linked immunosorbent assay; LBs, Lewy bodies; NC, neurological controls; p-α-synuclein, phospho-serine-129 α-synuclein; PDD, Parkinson's disease dementia; t-α-synuclein, total α-synuclein

References

1. Burkhardt CR, Filley CM, Kleinschmidt-DeMasters BK, de la Monte S, Norenberg MD, Schneck SA (1988) Diffuse Lewy body disease and progressive dementia. Neurology 38(10):1520–1528
2. Yamamoto T, Imai T (1988) A case of diffuse Lewy body and Alzheimer's diseases with periodic synchronous discharges. J Neuropathol Exp Neurol 47(5):536–548
3. Tartaglia MC, Johnson DY, Thai JN, Cattaruzza T, Wong K, Garcia P, Dearmond SJ, Miller BL et al (2012) Clinical overlap between Jakob-Creutzfeldt disease and Lewy body disease. Can J Neurol Sci 39(3):304–310
4. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, Aarsland D, Galvin J et al (2017) Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB consortium. Neurology 89(1):88–100. https://doi.org/10.1212/WNL.0000000000004058
5. Llorens F, Schmitz M, Karch A, Cramm M, Lange P, Gherib K, Varges D, Schmidt C et al (2016) Comparative analysis of cerebrospinal fluid biomarkers in the differential diagnosis of neurodegenerative dementia. Alzheimers Dement 12(5):577–589. https://doi.org/10.1016/j.jalz.2015.10.009
6. Llorens F, Kruse N, Schmitz M, Gotzmann N, Golanska E, Thune K, Zejneli O, Kanata E et al (2017) Evaluation of alpha-synuclein as a novel cerebrospinal fluid biomarker in different forms of prion diseases. Alzheimers Dement 13(6):710–719. https://doi.org/10.1016/j.jalz.2016.09.013
7. Zerr I, Bodemer M, Gefeller O, Otto M, Poser S, Wiltfang J, Windl O, Kretzschmar HA et al (1998) Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. Ann Neurol 43(1):32–40. https://doi.org/10.1002/ana.410430109
8. WHO (1998) Human transmissible spongiform encephalopathies. Wkly Epidemiol Rec 73(47):361–365
9. Cramm M, Schmitz M, Karch A, Mitrova E, Kuhn F, Schroeder B, Raeber A, Varges D et al (2016) Stability and reproducibility underscore utility of RT-QuIC for diagnosis of Creutzfeldt-Jakob disease. Mol Neurobiol 53(3):1896–1904. https://doi.org/10.1007/s12035-015-9133-2
10. Schmitz M, Cramm M, Llorens F, Muller-Cramm D, Collins S, Atarashi R, Satoh K, Orru CD et al (2016) The real-time quaking-induced conversion assay for detection of human prion disease and study of other protein misfolding diseases. Nat Protoc 11(11):2233–2242. https://doi.org/10.1038/nprot.2016.120
11. Schmitz M, Ebert E, Stoeck K, Karch A, Collins S, Calero M, Sklaviadis T, Laplanche JL et al (2016) Validation of 14-3-3 protein as a marker in sporadic Creutzfeldt-Jakob disease diagnostic. Mol Neurobiol 53(4):2189–2199. https://doi.org/10.1007/s12035-015-9167-5
12. Llorens F, Kruse N, Schmitz M, Shafiq M, da Cunha JE, Gotzman N, Zafar S, Thune K et al (2015) Quantification of CSF biomarkers using an electrochemiluminescence-based detection system in the differential diagnosis of AD and sCJD. J Neurol 262(10):2305–2311. https://doi.org/10.1007/s00415-015-7837-x
13. Oeckl P, Metzger F, Nagl M, von Arnim CA, Halbgebauer S, Steinacker P, Ludolph AC, Otto M (2016) Alpha-, beta-, and gamma-synuclein quantification in cerebrospinal fluid by multiple reaction monitoring reveals increased concentrations in Alzheimer's and Creutzfeldt-Jakob disease but no alteration in synucleinopathies. Mol Cell Proteomics 15(10):3126–3138. https://doi.org/10.1074/mcp.M116.059915
14. Kasai T, Tokuda T, Ishii R, Ishigami N, Tsuboi Y, Nakagawa M, Mizuno T, El-Agnaf OM (2014) Increased alpha-synuclein levels in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. J Neurol 261(6):1203–1209. https://doi.org/10.1007/s00415-014-7334-7
15. Eusebi P, Giannandrea D, Biscetti L, Abraha I, Chiasserini D, Orso M, Calabresi P, Parnetti L (2017) Diagnostic utility of cerebrospinal fluid alpha-synuclein in Parkinson's disease: a systematic review and meta-analysis. Mov Disord 32(10):1389–1400. https://doi.org/10.1002/mds.27110
16. Gao L, Tang H, Nie K, Wang L, Zhao J, Gan R, Huang J, Zhu R et al (2015) Cerebrospinal fluid alpha-synuclein as a biomarker for Parkinson's disease diagnosis: a systematic review and meta-analysis. Int J Neurosci 125(9):645–654. https://doi.org/10.3109/00207454.2014.961454
17. Zhou B, Wen M, Yu WF, Zhang CL, Jiao L (2015) The diagnostic and differential diagnosis utility of cerebrospinal fluid alpha -synuclein levels in Parkinson's disease: a meta-analysis. Parkinsons Dis 2015:567386–567311. https://doi.org/10.1155/2015/567386
18. Majbour NK, Vaikath NN, van Dijk KD, Ardah MT, Varghese S, Vesterager LB, Montezinho LP, Poole S et al (2016) Oligomeric and phosphorylated alpha-synuclein as potential CSF biomarkers for Parkinson's disease. Mol Neurodegener 11:7. https://doi.org/10.1186/s13024-016-0072-9
19. Wang Y, Shi M, Chung KA, Zabetian CP, Leverenz JB, Berg D, Srulijes K, Trojanowski JQ et al (2012) Phosphorylated alpha-synuclein in Parkinson's disease. Sci Transl Med 4(121):121ra120. https://doi.org/10.1126/scitranslmed.3002566
20. Zerr I, Kallenberg K, Summers DM, Romero C, Taratuto A, Heinemann U, Breithaupt M, Varges D et al (2009) Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain 132(Pt 10):2659–2668. https://doi.org/10.1093/brain/awp191
21. Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, Broe GA, Cummings J et al (2007) Clinical diagnostic criteria for dementia associated with Parkinson's disease. Mov Disord 22(12):1689–1707; quiz 1837. https://doi.org/10.1002/mds.21507
22. Chiasserini D, Biscetti L, Eusebi P, Salvadori N, Frattini G, Simoni S, De Roeck N, Tambasco N et al (2017) 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 9(1):52. https://doi.org/10.1186/s13195-017-0276-4

23. Schmid AW, Fauvet B, Moniatte M, Lashuel HA (2013) Alpha-synuclein post-translational modifications as potential biomarkers for Parkinson disease and other synucleinopathies. Mol Cell Proteomics 12(12):3543–3558. https://doi.org/10.1074/mcp.R113.032730
24. Aerts MB, Esselink RA, Abdo WF, Bloem BR, Verbeek MM (2012) CSF alpha-synuclein does not differentiate between parkinsonian disorders. Neurobiol Aging 33(2):430 e431–430 e433. https://doi.org/10.1016/j.neurobiolaging.2010.12.001
25. Hansson O, Hall S, Ohrfelt A, Zetterberg H, Blennow K, Minthon L, Nagga K, Londos E et al (2014) 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 6(3):25. https://doi.org/10.1186/alzrt255
26. Reesink FE, Lemstra AW, van Dijk KD, Berendse HW, van de Berg WD, Klein M, Blankenstein MA, Scheltens P et al (2010) CSF alpha-synuclein does not discriminate dementia with Lewy bodies from Alzheimer's disease. J Alzheimers Dis 22(1):87–95. https://doi.org/10.3233/JAD-2010-100186
27. Schade S, Mollenhauer B (2014) Biomarkers in biological fluids for dementia with Lewy bodies. Alzheimers Res Ther 6(5–8):72. https://doi.org/10.1186/s13195-014-0072-3
28. Llorens F, Lopez-Gonzalez I, Thune K, Carmona M, Zafar S, Andreoletti O, Zerr I, Ferrer I (2014) Subtype and regional-specific neuroinflammation in sporadic Creutzfeldt-Jakob disease. Front Aging Neurosci 6:198. https://doi.org/10.3389/fnagi.2014.00198
29. Muntane G, Ferrer I, Martinez-Vicente M (2012) Alpha-synuclein phosphorylation and truncation are normal events in the adult human brain. Neuroscience 200:106–119. https://doi.org/10.1016/j.neuroscience.2011.10.042
30. McKeith I (2007) Dementia with Lewy bodies and Parkinson's disease with dementia: where two worlds collide. Pract Neurol 7(6):374–382. https://doi.org/10.1136/jnnp.2007.134163
31. Irwin DJ, Grossman M, Weintraub D, Hurtig HI, Duda JE, Xie SX, Lee EB, Van Deerlin VM et al (2017) Neuropathological and genetic correlates of survival and dementia onset in synucleinopathies: a retrospective analysis. Lancet Neurol 16(1):55–65. https://doi.org/10.1016/S1474-4422(16)30291-5
32. Stewart T, Sossi V, Aasly JO, Wszolek ZK, Uitti RJ, Hasegawa K, Yokoyama T, Zabetian CP et al (2015) Phosphorylated alpha-synuclein in Parkinson's disease: correlation depends on disease severity. Acta Neuropathol Commun 3:7. https://doi.org/10.1186/s40478-015-0185-3
33. Lee JW, Devanarayan V, Barrett YC, Weiner R, Allinson J, Fountain S, Keller S, Weinryb I et al (2006) Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res 23(2):312–328. https://doi.org/10.1007/s11095-005-9045-3