Cerebrospinal fluid α-synuclein does not discriminate between dementia disorders

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Abstract

α-Synuclein is the major constituent of Lewy bodies found in neurons in dementia with Lewy bodies (DLB) and might be of diagnostic value as a biomarker for DLB. We hypothesized that, as a consequence of increased accumulation of α-synuclein intraneuronally in DLB, the levels of α-synuclein in cerebrospinal fluid (CSF) of DLB patients would be lower than in other dementias. Our objective was to investigate the CSF levels of α-synuclein in several dementia disorders compared to control levels and to investigate the diagnostic value of CSF α-synuclein as a marker to discriminate between DLB and other types of dementia. We analysed the levels of α-synuclein in CSF of 40 DLB patients, 131 patients with Alzheimer's disease, 28 patients with vascular dementia, and 39 patients with frontotemporal dementia. We did not find any significant differences in CSF α-synuclein levels between DLB patients and patients with AD, VaD or FTD. We conclude that in clinically diagnosed patients, α-synuclein does not appear to be a useful biomarker for the differentiation between DLB and other types of dementia.

Introduction

Dementia with Lewy Bodies (DLB) is the second most common type of dementia [1,2,3], accounting for 15-20% of cases at autopsy [4]. It is difficult to differentiate between DLB and other dementias such as Alzheimer's disease (AD) using clinical examination [5]. Although specificity has improved with the introduction of the consensus guidelines for DLB in 1996 [1,6,7], sensitivity is low [1,3], resulting in many misclassified cases. Differentiation from other dementia syndromes is difficult since DLB patients can present with symptoms that resemble those of other dementias. Extrapyramidal signs and hallucinations, which are characteristic for DLB patients, may also occur in advanced AD. On neuropsychological assessment, most DLB patients present with language and memory deficits similar to those with AD [8]. In one study, most patients misdiagnosed with DLB had AD pathology at autopsy [5]. Accurately diagnosing DLB is important for its pharmacological management. Patient with DLB respond well to cholinesterase inhibitors, but are extremely sensitive to the side effects of neuroleptic drugs [3,4]. Furthermore, prognosis of patients with DLB may be different, with faster deterioration and shorter time between onset of cognitive symptoms and death compared to patients with AD [1,5,9].

Analysis of the cerebrospinal fluid (CSF) has been increasingly applied to differentiate the various dementia disorders. Especially in AD, a typical CSF pattern is often observed, with increased concentrations of total tau protein (t-tau) and phosphorylated tau protein (p-tau) and decreased concentrations of the amyloid β_{42} (Aβ_{42}) protein [10,11]. In contrast, there are no generally accepted biomarkers to distinguish DLB from other types of dementia. Some studies found equally high CSF t-tau concentrations in AD and DLB [12,13]. In most studies, however, CSF t-tau concentrations were normal to moderately elevated in DLB [2,8,12,14,15,16,17], with normal concentrations of p-tau [18,19]. In contrast, Aβ_{42} concentrations were decreased to a similar degree as in AD [2,8,14,16], resulting in a low discriminative value of Aβ_{42} / t-tau analysis to differentiate AD from DLB.

Little research has been done with respect to CSF α-synuclein as a diagnostic marker. α-Synuclein is the major constituent of Lewy bodies, which are characteristic cytoplasmic inclusions in

cortical neurons of DLB patients. Initially α-synuclein was thought to be an intracellular protein, but recently it was found in CSF and plasma [20]. Tokuda et al found that patients with Parkinson's disease (PD) had significantly lower α-synuclein levels in their CSF compared to controls [21]. This might be explained by accumulation of α-synuclein within affected neurons [21]. We hypothesized that levels of α-synuclein in synucleinopathies such as DLB would be decreased in comparison with dementias not characterized by α-synuclein inclusions, such as AD, VaD and FTD and with control levels. Therefore, we investigated the CSF levels of α-synuclein in these dementia disorders compared to control levels, and studied if CSF α-synuclein may serve as a biomarker for the differentiation of DLB from other dementias.

Materials and Methods

Patients

We screened 526 patients of the CSF database of the Alzheimer Centre of the Radboud University Nijmegen Medical Centre for inclusion in this study (figure 1). This database consists of clinical data and biobank materials of consecutive patients, in whom informed consent for lumbar puncture was obtained. We excluded 69 patients because CSF was not available for α-synuclein analysis. Patients whose diagnosis was uncertain or with a diagnosis other than DLB, AD, VaD or FTD (e.g. mild cognitive impairment) (n=98) were not included either, as were patients with mixed dementia (n=9). In total, we included 40 DLB, 131 AD, 39 FTD and 28 VaD patients whose diagnosis was established by a multidisciplinary panel, which consisted of a geriatrician, neurologist, neuropsychologist, and – if needed – an old-age psychiatrist. The panel used the accepted clinical diagnostic criteria, i.e. the 1996 consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies, the NINCDS-ADRDA criteria for AD, the 1998 consensus on clinical diagnostic criteria for frontotemporal lobar degeneration, and the NINDS-AIREN criteria for VaD [7,22,23,24]. If possible, information on MMSE was obtained and $A\beta_{42}$, p-tau and t-tau were analysed. The panel was aware of the CSF results for $A\beta_{42}$, p-tau and t-tau, but not for α-synuclein, when establishing the clinical diagnosis.

We compared CSF α-synuclein levels of the dementia patients with those of 57 control persons aged >50 years (control group A). Data on $A\beta_{42}$, p-tau and t-tau was not available for this group. In order to have reference levels of $A\beta_{42}$, p-tau and t-tau levels to allow comparison of these variables, we included another 55 control persons (control group B). Both control groups underwent lumbar puncture for various reasons. However, for inclusion, intensive diagnostic investigations was not allowed to reveal any neurological disorder. Moreover, the CSF findings of both control groups for cell count, glucose, lactate, haemoglobin, bilirubin, total protein and oligoclonal IgG bands had to be normal.

A total of 238 patients with dementia and 112 controls were included (figure 1). Patient characteristics are listed in table 1. Characteristics of controls are listed in table 2. Mean age (± SD) of patients and controls together was 68 (± 10) years and 51% was male. AD patients were more often female. FTD patients were significantly younger than patients in the other three dementia groups.

CSF

CSF samples were collected in polypropylene tubes by lumbar puncture and transported to the laboratory within 30 minutes after withdrawal. The CSF was centrifuged and aliquots of each sample were immediately frozen at - 80°C until analysis. α-Synuclein in CSF was measured using a sandwich Elisa system based on a previously described procedure [25]. A disposable flat-bottom microtiterplate (Nunc Maxisorp F96, Roskilde, Denmark) was coated with 100 μl antibody 211 (0.2 μg/ml in 0.20 M carbonate buffer, pH 9.6) overnight at 4°C. A plate washer (BioTek, Beun de Ronde, Abcoude, The Netherlands) was used to wash the plate five times with 250 μl phosphate buffered saline containing 0.05% Tween-20 (PBS washing buffer). All further incubations were performed at 37°C, unless stated otherwise, and all measurements were performed in duplicate. 250 μl of blocking buffer (2.5% gelatin in PBS washing buffer) was added and incubated for 2 hours. The plate was subsequently washed five times with PBS washing buffer. Next, 100 μl α-synuclein solution (from 0 to 500 ng/ml diluted in PBS) or CSF was added to each well and incubated for 2.5 hours. After washing the plate five times with PBS washing buffer, 100 μl of antibody FL-140, diluted 1:1,000 in blocking buffer, was added for 1.5 hours. The plate was washed five times and 100 μl of the peroxidase labeled goat-anti rabbit antibody (dilution 1:5,000 in blocking buffer) was added and incubated for 1 hour. After a final washing step 100 μl of a freshly prepared solution of tetramethyl benzidine (TMB) was applied and incubated for 15 minutes in the dark at room temperature. The reaction was stopped with 50 μl 2 N H_2SO4 and the absorbance was measured at 450 nm in an ELISA plate reader (Tecan Sunrise, Salzburg, Austria). The technicians performing the analyses did not have access to the clinical data.

Statistical analysis

Differences in α-synuclein, age, MMSE score, $A\beta_{42}$, p-tau, and t-tau between dementia groups were analysed by ANOVA. Levels of α-synuclein, $A\beta_{42}$, p-tau, and t-tau did not follow a normal distribution. We used log transformation to analyse these variables. Differences in gender distribution among the dementia groups were determined by the chi square test. The associations between α-synuclein and age, MMSE score, $A\beta_{42}$, p-tau and t-tau were examined by linear regression. Statistical analyses were carried out using SAS, version 8.2.

Results

The mean CSF level of α-synuclein was not significantly different in the four dementia groups; nor did we find a significant difference between the dementia groups and the control group A. We found no association of CSF α-synuclein with MMSE score, sex, $A\beta_{42}$ or p-tau among the dementia patients. The level of α-synuclein decreased with increasing t-tau (B -0.17, p 0.030) and age (B -0.02, p 0.001) among the dementia cases (figure 2). Taking these variables into account did not change our initial findings about the absence of an association between dementia type and α-synuclein.

In comparison with the univariable associations with α-synuclein, the association with age remained stable and significant (B -0.02, p 0.002), while the association with t-tau diminished and lost significance (B -0.14, p 0.113) in a linear regression model that included both variables as well as dementia type.

In DLB patients, the concentration of $A\beta_{42}$ was significantly lower than in the control group B, although still above the cut-off value of 500 pg/ml for normal values. P-tau and t-tau concentrations were in the range of normal although significantly increased compared to these controls. For AD patients, $A\beta_{42}$ concentration was significantly decreased, while p-tau and t-tau concentrations were significantly higher than in the control group B.

Discussion

We investigated CSF α-synuclein in patients with different types of dementia. To our knowledge, this is one of the first studies comparing α-synuclein levels in DLB to other dementias. Since we did not observe differences in the CSF levels of α-synuclein between the various clinically diagnosed dementia disorders, α-synuclein is not a useful biomarker for the differentiation between DLB, AD, FTD or VaD.

What may have accounted for the lack of difference in α-synuclein levels that we observed between the different types of dementia, is that α-synucleinopathies, such as DLB, and tauopathies, such as AD and part of the FTD cases, may be disorders with considerable overlap, instead of distinct entities [26,27]. Neuropathological findings of the various disorders have been reported to be overlapping. The presence of Lewy bodies in brains of AD patients was higher than expected [26]. More than 60% of cases harboured α-synuclein positive Lewy bodies [28], whereas 87% of brains with a neuropathological diagnosis of DLB also met CERAD criteria for definite or probable AD [26]. When Iqbal et al divided AD patients into subgroups based on their CSF biomarker profile, a subgroup with low $A\beta_{42}$ and only a slight increase in t-tau levels consisted mainly of cases with AD of Lewy body type, their CSF biomarker profile comparable to our DLB group [29]. This supports the hypothesis of overlapping etiology of α-synucleinopathies and tauopathies. Interestingly, Lewy body pathology has also been found in brain tissue of elderly individuals without cognitive impairment [26,27].

Another explanation for the lack of difference in α-synuclein levels might be heterogeneity within the DLB group. Possibly, there are subgroups within the DLB group. For example, patients who display parkinsonism early in the disease could have lower levels of α-synuclein than patients whose parkinsonism develops later in the course of the disease. We are uncertain about such differences within our DLB group.

Studies addressing the association of α-synuclein in CSF with Parkinson's disease (PD) report contradictory findings. Tokuda et al found lower CSF α-synuclein in PD patients compared to controls

[21], which led to the assumption that α-synuclein could be used as a marker for α-synucleinopathies. However, others did not find significant differences between PD patients and controls [20,30]. In Lewy bodies, α-synuclein is aggregated and insoluble [3]. However, under normal conditions α-synuclein is a soluble synaptic protein, that can also be found in human CSF and blood plasma of healthy humans [3,16,20], and that may be secreted into the CSF by neurons [20,21,31]. Possibly, the CSF α-synuclein levels that we and others measured reflect a physiological level of soluble α-synuclein, independent of α-synuclein accumulation. The levels of α-synuclein that we measured were in the same order of magnitude as found in controls by Tokuda et al, using the same antibodies [21].

We found that age had a weak influence on the levels of α-synuclein, with α-synuclein decreasing with age. This is consistent with the study by Tokuda et al [21]. With aging, axonal transport of α-synuclein slows down and the amount of soluble α-synuclein decreases [27]. This may account for the decreasing levels of α-synuclein found in CSF. Our initial finding that the concentration of t-tau increased with decreasing α-synuclein lost significance after correcting for age and dementia type.

Our study population is representative for the population seen at a memory clinic and the CSF data indicate that our study group was very well comparable to the study groups used by other research centres. We observed the typical pattern of decreased $A\beta_{42}$ and increased t-tau /p-tau levels in AD patients [8,10]. In DLB we observed comparable decreased $A\beta_{42}$ concentrations, although not as low as in AD, and normal to slightly increased t-tau and p-tau concentrations, as has been reported before [2,12,14,15,16,17,18,19]. Also, our observations in the VaD and FTD groups are in accord with previous studies [8,17,32,33,34,35,36].

Although the clinical diagnoses in our study populations were made according to accepted clinical criteria, some misclassification of DLB patients as AD patients probably has occurred. The presence of AD pathology in DLB modifies its clinical presentation with a lower rate of visual hallucinations and parkinsonism [3]. This contributes to the complicated clinical differentiation between these two types of dementia and once more illustrates the urgent need for a discriminative biomarker. Our findings suggest that α-synuclein cannot be used as such a diagnostic marker. Future studies, e.g. with post-mortem verification of the clinical diagnosis, are warranted to confirm our

findings, but based on the findings of our study, α-synuclein cannot be advocated as a biomarker for DLB.

Acknowledgements

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Table 1 Clinical characteristics and results of CSF analysis of dementia patients

|	DLB n=40	AD n=131	FTD n=39	VaD n=28	p-value*
No. of males/females	27/13	54/77	24/15	18/10	0.005‡
Age (years)	74.0 ± 8.3	71.7 ± 8.8	66.4 ± 8.6	75.4 ± 8.4	<0.0001
MMSE score	18.9 ± 8.2 n=7	19.6 ± 3.9 n=71	20.3 ± 6.1 n=16	18.5 ± 2.9 n=6	0.843
Amyloid β$_{42}$ (pg/ml)	533.0 ± 231.0 n=33	451.3 ± 147.2 n=110	702.1 ± 257.8 n=33	650.1 ± 244.6 n=24	<0.0001
T-tau (pg/ml)	318.7 ± 161.6 n=33	716.4 ± 408.9 n=109	407.3 ± 285.9 n=33	400.1 ± 543.1 n=23	<0.0001
P-tau (pg/ml)	56.9 ± 22.2 n=31	108.7 ± 47.0 n=109	68.5 ± 34.6 n=33	52.9 ± 18.6 n=23	<0.0001
α-Synuclein (ng/ml)	38.0 ± 29.0	37.0 ± 36.1	49.1 ± 41.5	44.3 ± 40.3	0.187

Values are expressed as means ± SD. *, p-value by ANOVA; ‡, p-value by chi square test.
DLB, dementia with Lewy bodies; AD, Alzheimer disease; FTD, frontotemporal dementia; VaD, vascular dementia; MMSE, mini mental state examination (0-30, with higher scores indicating better cognition); t-tau, total tau; p-tau, phosphorylated tau.

Table 2 Clinical characteristics and results of CSF analysis of controls

	Control group A n=57	Control group B n=55
No. of males/females	30/27	26/29
Age (years)	61.3 ± 8.8	61.1 ± 8.9
Amyloid β₄₂ (pg/ml)	n.a.	822.9 ± 256.3
T-tau (pgm/l)	n.a.	212.4 ± 81.6
P-tau (pg/ml)	n.a.	47.5 ± 15.1
α-Synuclein (ng/ml)	30.4 ± 19.1	n.a.

Values are expressed as means ± SD.
T-tau, total tau; p-tau, phosphorylated tau; n.a., not available.

Figure 1 Overview of the patients included in this study
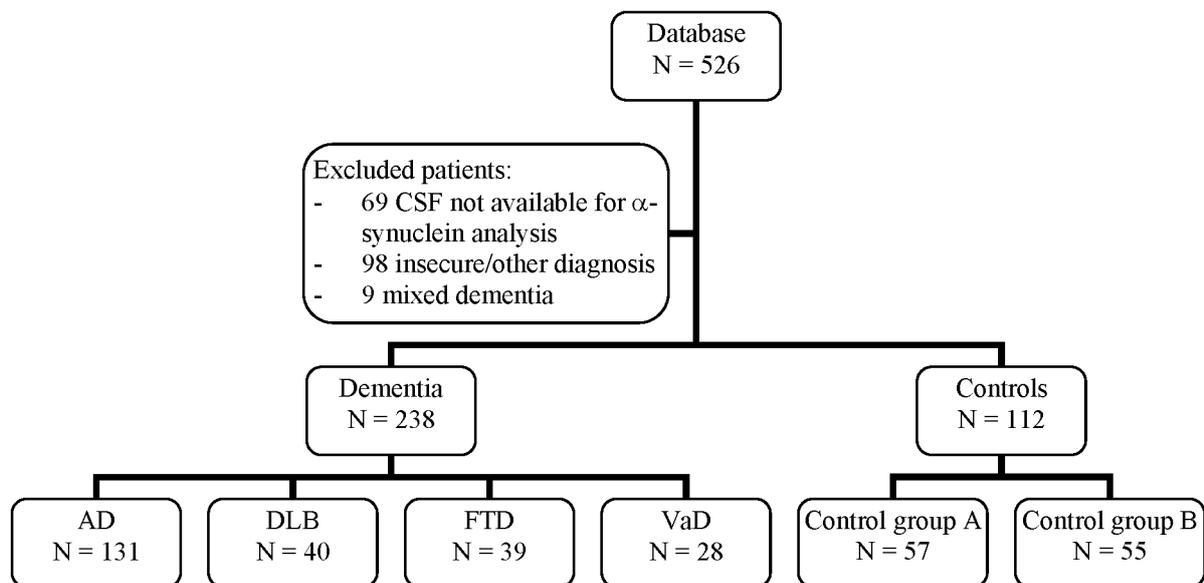
AD, Alzheimer disease; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; VaD, vascular dementia

Figure 2 Scatter plot of CSF α-synuclein versus age in the dementia groups