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Using Cerebrospinal Fluid Marker Profiles in Clinical Diagnosis of Dementia with Lewy Bodies, Parkinson's Disease, and Alzheimer's Disease

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Abstract.
Background: Dementia with Lewy bodies (DLB) is difficult to differentiate from other neuro-degenerative diseases. Patients are often mistaken to suffer from Parkinson's disease (PD) or Alzheimer's disease (AD) because of the overlapping clinical appearances concerning cognition and movement.
Objective: We investigated the possibility for a valid differential diagnosis using cerebrospinal fluid (CSF) biomarkers.
Methods: In the context of a large retrospective study, we analyzed data of patients suffering from degenerative, ischemic, or inflammatory CNS (central nervous system) diseases and identified those with DLB ($n = 34$), PD ($n = 37$), and AD ($n = 47$) for further analyses.
Results: We detected abnormalities in the CSF profiles of those patients with DLB while using a combination of decreased amyloid-β $(Aβ)_{42}$ and increased tau levels. By stratification of data by disease severity, we observed a high sensitivity of this combination especially in the subgroup of patients with advanced stages, while the sensitivity in early forms was lower. In addition, with clinical deterioration, the abnormalities in the CSF profile became more pronounced.
Conclusion: We conclude that DLB can be distinguished from PD, in spite of both being synucleinopathies, by CSF profiles using neurodegenerative marker analysis. The pathophysiology of increased tau and decreased Aβ levels in those conditions has to be elucidated further, since both proteins are known to be involved in the pathogenesis of AD, but no clear explanation has been postulated for DLB yet.

Keywords: Alzheimer's disease, amyloid-β, cerebrospinal fluid, clinical diagnosis, dementia, dementia with Lewy bodies, Parkinson's disease, tau

INTRODUCTION

Dementia with Lewy bodies (DLB) has emerged with particular importance since prevalence studies suggest it the second most common form of degenerative dementia following Alzheimer's disease (AD) [1, 2], accounting for up to 20% of cases in the elderly. The estimated numbers fluctuate because of several

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difficulties attaining the correct diagnosis, mostly due to a clinical appearance similar to Parkinson's disease (PD). In DLB, the diagnosis is based on clinical criteria, but even core features, like visual hallucinations and parkinsonism, can be absent in some patients [3]. To differentiate between PD with dementia (PDD) and DLB, a "one-year-rule" was defined [4] considering wide analogies in cognitive profiles, visuospatial dysfunction, and the presence of parkinsonism. It defines DLB when dementia occurs before or at the same time with motor symptoms, at least within 1 year. PDD develops in the context of an already well-established case of PD. So to differentiate between DLB and PDD, clinical follow-up is required.

In addition to physical examination and neuroimaging, an easy-to-determine biomarker in cerebrospinal fluid (CSF) or blood, which can be widely used in the clinical setting, could help and potentially identify those patients with PD that might develop dementia at later stages.

MATERIAL AND METHODS

Patient data collection and analysis

A clinical cohort suffering from different forms of neurological diseases who underwent lumbar puncture for diagnostic purposes, which included CSF dementia marker profile, was analyzed. We selected data from those with a clinical diagnosis of DLB ($n = 34$), PD ($n = 37$), and AD ($n = 47$), reaching a total number of 118 CSF samples for further analysis. Although more patients were evaluated for the respective diagnoses in clinical and outpatient settings, we decided to use only data from patients who fulfilled our strict inclusion criteria in the diagnostic workup. All tests were performed in the Neurochemistry laboratory at the Department of Neurology, University Medical Center, Göttingen, immediately after lumbar puncture. CSF was examined for tau, phosphorylated tau, and amyloid-β $(Aβ)_{1–42}$ according to established protocols. CSF tau protein was quantitatively analyzed using a commercially available ELISA kit according to manufacturer's instructions (INNOTEST® hTAU Ag, Innogenetics). Human tau, phosphorylated at Thr181 (phosphorylated tau) was measured quantitatively with a commercially available ELISA kit [INNOTEST® PHOSPHO-TAU (181P), Innogenetics]. $Aβ_{1–42}$ was detected with a commercially available ELISA kit [INNOTEST® β-AMYLOID (1–42) Innogenetics] for quantitative analysis. $Aβ_{1–40}$ was detected with a commercially available ELISA kit (Genetics Company (Schlieren/Schweiz). We intended to compare the levels of various proteins across three diagnostic groups. Since sensitivity, specificity, and predictive values were not the topic of the analysis, we decided not to use cut-offs for single markers, especially in view that they might be different for single disease entities. The study was approved by the local ethics committee (16/7/10).

The diagnoses of the patients were based on the following criteria, corresponding to current guidelines: 1) The diagnosis of AD was based on the ICD-10 definition for Alzheimer's disease (F.00 G.30); 2) The diagnosis of DLB was based on the criteria of McKeith [5]; and 3) The diagnosis of PD was based on the ICD-10 definition for Parkinson's disease (G20 F02.3).

The PD group comprises patients with PD with and without dementia. Because numbers become too low, further splitting into PD and PDD was not practical in this analysis.

In order to investigate the correlation between biomarker levels and clinical parameters, we stratified the data into three stages based on disease severity. For this we used a combination of clinical criteria including aspects of the Clinical Dementia Rating (CDR) [6] and the Mini-Mental Status Examination (MMSE). For PD, Hoehn and Yahr staging was used. Criteria for disease severity in the single groups were applied according to previous publications [7, 8].

Statistical evaluation

The ANOVA test (Bonferroni and Tamhane T2) and Kruskal-Wallis test were used to compare the values. Values of $p < 0.05$ were considered to be significant. BOX-Plot were used for graphical presentation. Statistical analyses were done using IBM SPSS Statistics 19. The parameter $Aβ_{40/42}$-ratio was calculated with $Aβ_{42}$ in combination with $Aβ_{40}$ from the data of one sample (Aβ-Ratio = $Aβ_{42}/Aβ_{40}$*10).

RESULTS

In this study we analyzed clinical and CSF data from patients with AD, DLB, and PD (Table 1). The median age was comparable in all three groups (AD: 69 years range, DLB: 71 years, PD: 72 years); the male gender was predominant in DLB and PD patients (m:f DLB 23:12, PD 26:11), while the female prevailed in patients with AD (m:f 15:37). Duration of illness varied between the groups: the patients with AD had suffered the longest, on average for 18 month, followed by the

Table 1
CSF biomarker in AD, DLB, and PD

CSF pg/ml	AD mean (SD) median (min-max) *n*	DLB mean (SD) median (min-max) *n*	PD mean (SD) median (min-max) *n*
t-tau	391 (232) 289 (75–910) 47	351 (302) 245 (75–1340) 35	176 (107) 142 (75–443) 37
p-tau	74 (44) 70 (20–243) 31	66 (39) 54 (25–182) 25	54 (28) 48 (16–118) 13
$A\beta_{42}$	580 (211) 538 (246–1026) 46	413 (253) 351 (75–1062) 32	682 (333) 618 (75–1578) 37
$A\beta_{42/40}$ ratio	0.99 (0.6) 0.9 (0.4–2.4) 26	1.1 (0.7) 0.9 (0.2–2) 7	1.05 (0.4) 1.1 (0.3–1.8) 12

groups of DLB and PD, both diseases existed 12 month on average before lumbar puncture was performed.

The autonomy of the patients concerning their everyday life differs between the single groups (Table 2). Disease progression was most accelerated in DLB, showing considerable aggravation of symptoms in a short period of time, with finally serious impairment of cognition and self-dependency.

Cerebrospinal fluid marker analysis

Overall data on CSF analyses are given in Table 1.

Tau

AD patients had the highest values concerning the arithmetic mean and median of tau (arithmetic mean 391 pg/ml; median 289 pg/ml), followed by DLB (arithmetic mean 351 pg/ml; median 245 pg/ml) and PD (arithmetic mean 176 pg/ml; median 142 pg/ml) (Fig. 1). DLB could be differentiated from PD via t-tau levels ($p = 0.002$). However, in general the variables are in a similar range between diagnostic categories.

Tau in different disease stages

In AD and DLB, tau levels increased with disease severity (Table 2 and Fig. 1). There was no correlation between disease severity and age, but with tau and lower MMSE performance in DLB.

With further disease progression and increasing clinical severity, a decline in tau was observed in AD and DLB, but not in PD. We found only a trend for an increase from mild to moderate stage. Tau determination as a single marker was not useful to distinguish between DLB, PD, and AD.

Table 2
CSF biomarkers in AD, DLB, and PD according to disease severity in stages (stage 1: mild, stage 2: medium, stage 3: severe)

Diagnosis	CSF pg/ml	Stage 1 mean (SD) median (min-max) *n*	Stage 2 mean (SD) median (min-max) *n*	Stage 3 mean (SD) median (min-max) *n*
AD	t-tau	340 (201) 295 (75–910) 18	436 (251) 419 (75–910) 27	250 1
	p-tau	66 (28) 67 (20–104) 12	82 (53) 79 (22–243) 18	no data
	$A\beta_{42}$	574 (199) 543 (292–947) 17	599 (218) 590 (292–1026) 27	246 1
	$A\beta_{42}/_{40}$ ratio	1.01 (0.4) 0.9 (0.4–1.9) 10	0.99 (0.68) 0.75 (0.4–2.4) 14	no data
DLB	t-tau	275(158) 246 (110–556) 6	443(352) 340 (75–1340) 18	247(258) 161 (75–847) 8
	p-tau	58(14) 58 (42–73) 4	71(46) 61 (25–182) 15	48(26) 42 (27–82) 4
	$A\beta_{42}$	537(309) 510 (178–987) 6	326(196) 321 (75–703) 16	418(206) 369 (165–799) 8
	$A\beta_{42}/_{40}$ ratio	1.4 1	1.09(0.8) 0.9 (0.2–2) 5	0.9 1
PD	t-tau	171(103) 140 (75–421) 26	185(126) 159 (75–443) 10	233 1
	p-tau	55(30) 44 (21–118) 10	48(29) 56 (16–73) 3	no data
	$A\beta_{42}$	716(280) 645 (296–1260) 26	628(452) 456 (75–1578) 10	319 1
	$A\beta_{42}/_{40}$ ratio	1.1 (0.4) 1.1 (0.4–1.8) 10	0.8 (0.7) 0.8 (0.3–1.3) 2	no data

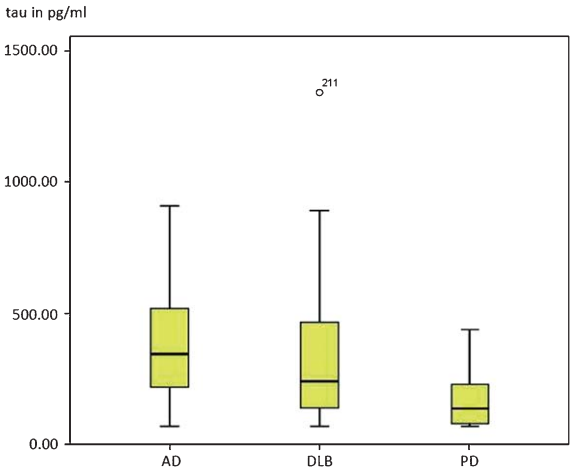

Fig. 1. Box plots showing median and confidence intervals of t-tau values.

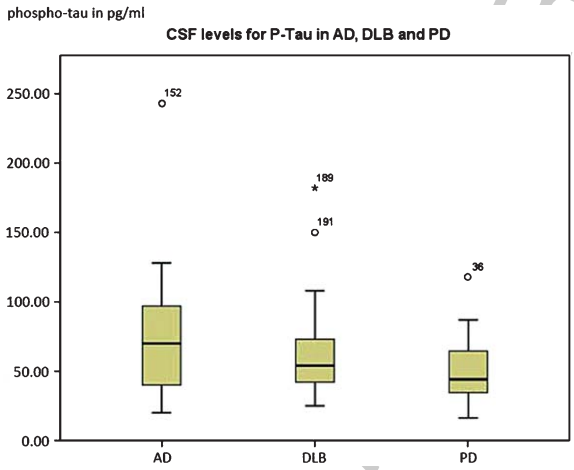

Fig. 2. Box plots showing median and confidence intervals of p-tau values.

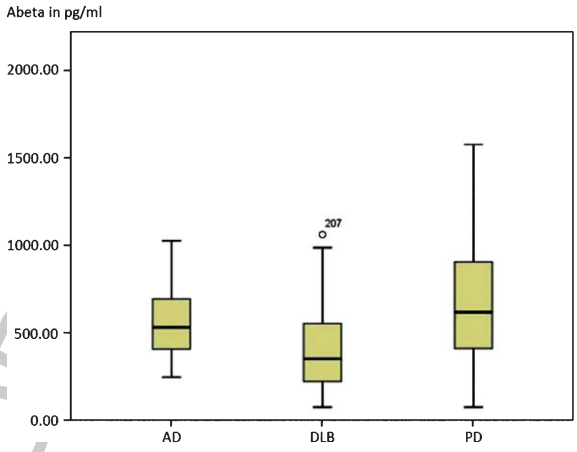

Fig. 3. Box plots showing median and confidence intervals of $A\beta_{42}$ values.

lar to t-tau, p-tau levels decline in advanced stages in DLB. Levels of p-tau in the PD group did not vary across stages (55 and 48 pg/ml).

P-Tau

In AD, p-tau levels in CSF were increased (arithmetic mean 74 pg/ml; median 70 pg/ml), followed by DLB (arithmetic mean 66 pg/ml; median 54 pg/ml) and PD (arithmetic mean 54 pg/ml; median 48 pg/ml) (Fig. 2). With regard to the single disease stages, p-tau increased from mild (66 pg/ml, $n = 12$) to moderate disease severity (82 pg/ml, $n = 18$) in AD. In DLB, only few data are available. In early disease stage, levels are low (arithmetic mean of p-tau of 58 pg/ml, $n = 4$), increased in moderate stage (71 pg/ml, $n = 15$) and decreased in severe stage (48 pg/ml, $n = 4$). Similar to t-tau, p-tau levels decline in advanced stages in DLB. Levels of p-tau in the PD group did not vary across stages (55 and 48 pg/ml).

$A\beta_{42}$

$A\beta_{42}$ showed a wide range of values in each group investigated here (Table 2). The lowest levels were found in DLB (arithmetic mean 413 pg/ml; median 351 pg/ml), followed by AD (arithmetic mean 580 pg/ml; median 538 pg/ml) and PD (arithmetic mean 682 pg/ml; median 618 pg/ml) (Fig. 3). $A\beta_{42}$ levels were useful to differentiate DLB from PD ($p < 0.001$) and from AD as well ($p = 0.023$). With regard to disease progression, no clear differences were observed between mild and moderate stages. In DLB, we noticed a decrease from the early stage to moderate and to severe stages.

$A\beta_{42}/A\beta_{40}$ ratio

We observed an arithmetic mean of 0.9 in AD, of 1.11 in DLB, and of 1.05 in PD (Table 2, Fig. 4). Although we could not calculate the ratio for all patients, we observed a decline from mild ($n = 10$) to moderate ($n = 15$) stage (1.01 to 0.95) in AD. For DLB, data were very limited, but similar: one patient in the mild stage had the highest ratio (1.4) followed by moderate stage ($n = 5$) with 1.09 and one in severe stage with a ratio of 0.9. In PD, we obtained data for early disease stage only ($n = 10$) with an arithmetic mean of 1.1 and for the moderate ($n = 2$) a ratio of 0.8.

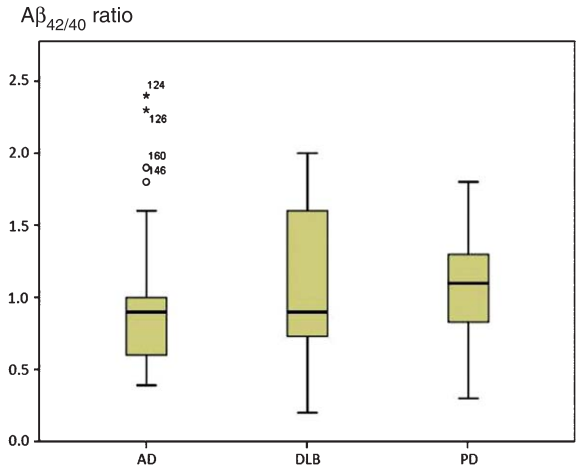

Fig. 4. Box plots showing median and confidence intervals of Aβ ratio values.

Other ratios

In addition to the commonly used ratio, we calculated other combinations to test if we might obtain a better ratio to differentiate between diseases (Table 3).

First we calculated the ratio $A\beta_{40/42}$ and achieved arithmetic means of 13 in AD, 17 in DLB, and 12 in PD. This ratio does not seem to be useful for distinction between the diseases, because of the similar scatter of the single values. Further, we combined $A\beta_{42}$/tau and achieved arithmetic means of 2.5 to 2.2 to 5.1 for AD:DLB:PD (Fig. 5a). It seems to be a valuable ratio to differentiate between PD and DLB or AD (both $p < 0.001$). With regard to patients suffering from PD, their data are arranged in the area of higher values and considerably above those of AD and DLB. The combination of tau/p-tau resulted in similar arithmetic means for AD:DLB:PD 5.3:5.5:3.8 and is not suitable for a valid distinction between the groups. A combination that yields more significance to distinguish DLB from PD is $A\beta_{42}$/p-tau (Fig. 5b). In this case, the majority of the data in the DLB group stays below a ratio of 10 and at the same time, the main data of the PD group ranges between a ratio of 10 up to 30 (AD:DLB:PD 12.5:7.4:20). It significantly separates PD from DLB ($p = 0.002$). The ratio p-tau/tau shows a wide range of data in the single groups and does not help to differentiate between them. The same problems show up considering the combination of tau/$A\beta_{42}$.

To summarize, in addition to the commonly used ratio, we found two new ones which might reach a certain utility in differential diagnosis of DLB versus PD and should be considered for further evaluation in a prospective setting. The best data for DLB and PD were obtained using the $A\beta_{42}$/tau ratio (mean 2.2 in DLB and 5 in PD) and the $A\beta_{42}$/p-tau ratio (DLB 7.4 versus PD 20). With regards to AD, the commonly used ratio $A\beta_{42}/A\beta_{40}$ allows the distinction between AD and DLB and PD (AD <1.0, DLB and PD >1.0).

Table 3
Calculated biomarker ratios

Ratio	AD mean (SD) median (min-max) *n*	DLB mean (SD) median (min-max) *n*	PD mean (SD) median (min-max) *n*
$A\beta_{42}/A\beta_{40}$	0.99 (0.6) 0.9 (0.4–2.4) 26	1.1 (0.7) 0.9 (0.2–2.0) 7	1.05 (0.4) 1.1 (0.3–1.8) 12
$A\beta_{40}/A\beta_{42}$	12.9 (6.4) 11.2 (4.1–25.8) 24	17 (19.05) 10.85 (5.6–50.9) 5	12.2 (8.4) 8.9 (5.6–32.7) 12
$A\beta_{42}$/tau	2.5 (2.9) 1.5 (0.04–13) 46	2.2 (2.5) 1.6 (0.1–13.4) 32	5.1 (3.3) 4.4 (0.4–11.4) 37
$A\beta_{42}$/p-tau	12.5 (11.1) 8.3 (1.7–47.4) 30	7.4 (4.3) 8.1 (0.9–15.4) 23	20.1 (14.4) 13.2 (5.6–45.1) 13
tau/$A\beta_{42}$	0.8 (0.5) 0.7 (0.1–2.2) 46	1.2 (1.8) 0.6 (0.1–9.8) 32	0.4 (0.4) 0.2 (0.1–2.7) 37
tau/p-tau	5.3 (2) 4.6 (2.6–12.1) 31	5.5 (3.7) 4.3 (2.5–20.3) 25	3.8 (1.7) 3.7 (1–6.3) 13
p-tau/tau	0.2 (0.1) 0.2 (0.1–0.4) 31	0.2 (0.1) 0.2 (0.1–0.4) 25	0.3 (0.2) 0.3 (0.2–1.0) 13

DISCUSSION

The lack of commonly accepted biomarkers to distinguish DLB from other dementias [9] encouraged us to put DLB, PD, and AD into the focus for further examination. We analyzed the levels of tau, p-tau, $A\beta_{42}$, and calculated ratios. We found slightly increased tau in AD and DLB but not in PD. P-tau was considerably increased in AD, marginally increased in DLB, and normal in PD (Table 1). Furthermore, there is a distinct decrease of $A\beta_{42}$ in DLB, which was surprisingly much more pronounced than in AD. The commonly used ratio $A\beta_{42/40}$ was found to be the lowest in AD, followed by PD and DLB in our study.

In previous studies (Table 4), p-tau had already been suggested to display a high discriminative value

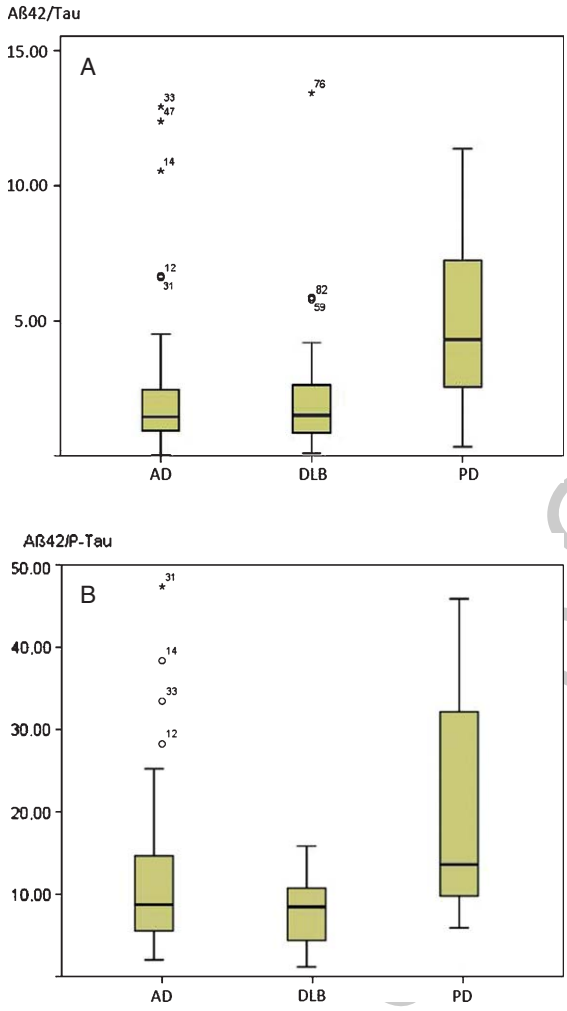

Fig. 5. A) Box plots showing median and confidence intervals of the new calculated ratio $A\beta_{42}$/total tau. B) Box plots showing median and confidence intervals of the new calculated ratio $A\beta_{42}$/p-tau.

differentiating between AD and DLB [10]. In addition to its diagnostic potential, it might be also a marker for disease progression as has been suggested by Henneman et al. [11]. In their study, high values of p-tau were associated with progression of AD as defined by the worsening of memory functions. Vanderstichle et al. [12] determined that p-tau is statistically significant in distinguishing between AD and DLB, results which are comparable to ours.

Total tau levels [13] can be used to differentiate between AD and healthy controls with a sensitivity of 92% and a specificity of 89%, as was demonstrated in various studies. T-tau levels were found to be highest in AD compared to DLB and other types of dementia [9]. In our data, tau levels correlated with disease stage rather than with the duration of illness. We assume tau to depend more on the disease severity and to reflect obvious pathological processes in the brain. $A\beta_{42}$ levels were reported to be decreased in AD compared to healthy controls and DLB [14]. At the same time, $A\beta_{42}$ is supposed to be very useful for differential diagnosis in combination with t-tau and p-tau with a sensitivity of 93% and a specificity of 95% when all three biomarkers were used [15]. We also conclude that a combined detection of increased tau and a pronounced decrease in $A\beta_{42}$ helps to distinguish DLB from other dementias, especially AD and PD, which was unfortunately not true for mild stages of DLB.

In contrast to the biomarkers as single variables, their ratios have not been examined systematically so far. In particular, studies that analyze various ratios in the same cohort are rarely available. Ratios are considered valuable for the differential diagnosis of degenerative dementias. Spies et al. postulated that the CSF $A\beta_{42}/A\beta_{40}$ ratio improves differentiation of AD patients from vascular dementia, DLB, and non-AD dementia patients, in comparison to $A\beta_{42}$ alone [14]. Jellinger describes the ratio total-tau/$A\beta_{42}$ as a potential marker of the severity of neurodegeneration in PD [34], just the same as Prikrylova et al. who found this combination significantly altered compared to controls and as a potential laboratory marker of the presence and severity of neurodegeneration [34] (Table 4).

With regard to α-synucleinopathies, CSF analysis is increasingly becoming an additional tool that is recommended for distinction between the different forms.

In particular for pharmacological interventions, the diagnosis has to be achieved early. Guidelines recommend the use of cholinesterase inhibitors in DLB and in PDD [17–19]. In opposite to that, there is only a partial response to levodopa in DLB, which is the first line therapy for PD in the elderly. A need for HI extreme care with the use of neuroleptics is given in DLB in the context of vivid visual hallucinations [20] causing physical and cognitive decline up to an akinetic-rigid syndrome, as well as increased mortality [21, 22].

Considering CSF biomarker analysis of patients with a diagnosis of PD, we realized that this group was quite heterogeneous. As a disadvantage of a retrospective analysis, we had to be content with the data available and had no influence on further examinations. Obviously patients with classical idiopathic PD did not undergo spinal tap in most of the situations, but patients with additional symptoms did. This situation might explain why many patients in this group displayed dementia (low MMSE, cognitively impaired, frequently tumbling, visual hallucination). Of special interest, 3 out of 6 patients related to probable PDD

Table 4
CSF studies on dementia biomarkers

Author	Disease	CSF Biomarker	Comment
Hertze et al. [29]	AD (94) MCI (166) healthy controls (38)	t-tau p-tau $A\beta_{42}$	MCI progresses to AD more likely with lower Aβ and high levels of tau
Snider et al. [30]	AD (49)	t-tau p-tau $A\beta_{42}$	progression of AD is enforced with low Aβ and increased p-/t-tau levels, they are proper markers for prognostic use
Mattsson et al. [31]	MCI (750) AD (529) healthy controls (304)	t-tau	271 MCI patients developed AD within 2 years and showed higher levels of tau
Shaw et al. [32]	mild AD (100) MCI (192) AD autopsy confirmed (56) healthy controls (114)	t-tau p-tau $A\beta_{42}$	Aβ sensitivity (96%) and specificity (77%) is above those of the tau biomarkers
Hennemann et al. [11]	AD (31) MCI (25) healthy controls (19)	p-tau	high p-tau is associated with worse memory function and progression of disease
Formichi et al. [33]	AD (2287) healthy controls (1384)	t-tau	tau distinguishes between AD and healthy controls with sensitivity (81%) and specificity (89%), but low specificity for dementia differential diagnosis
Sunderland et al. [34]	AD (131) healthy controls (72)	t-tau	AD > healthy controls sensitivity (92%), specificity (89%)
Mollenhauer et al. [35]	AD (82) DLB (44) healthy controls (71)	$A\beta_{42}$	AD < controls Aβ is decreased
Aerts et al. [15]	AD (45) DLB (23)	t-tau p-tau $A\beta_{42}$	all markers DLB < AD sensitivity of combination (92.9%), specificity (95%)
Spies et al. [14]	AD (69) DLB (16) VaD (26) FTD (27) controls (47)	t-tau p-tau $A\beta_{42/40}$	ratio $A\beta_{42/40}$ is significantly increased in AD, helps to differentiate AD from the other groups in addition to the biomarkers alone
Kasuga et al. [9]	DLB (34) AD (31) other dementias (21)	t-tau p-tau $A\beta_{40/42}$ tau/$A\beta_{42}$	CSF biomarker levels AD > DLB/OD –> tau and Aβ analysis are useful for differential diagnosis of AD-DLB/OD in addition to α-synuclein
Wada-Isoe et al. [36]	AD (34) DLB (22) controls (37)	p-tau $A\beta_{42}$/p-tau	p-tau levels in AD = DLB, but an increase of the ratio in AD in comparison to DLB
Simic et al. [37]	AD (11) DLB (2)	t-tau p-tau	p-tau distinguish between AD and DLB with sensitivity (91%) and specificity (95%)
Vanderstichele et al. [12]	AD (94) DLB (60) controls (12)	p-tau	AD > DLB p-tau is statistically significant to distinguish between AD and DLB
Parnetti et al. [38]	AD (23) DLB (19) PD (20) healthy controls (20)	t-tau p-tau $A\beta_{42}$	t-tau AD > DLB>PD, ∼MMSE p-tau increased only in AD, $A\beta_{42}$ the lowest in DLB
Bibl et al. [16]	AD (23) DLB (21) PDD (21) controls (23)	Aβ peptides	$A\beta_{40}$ DLB > PDD sensitivity (81%), specificity (71%), not useful as solid biomarker

Table 4
(*Continued*)

Author	Disease	CSF Biomarker	Comment
Prikrylova et al. [39]	subgroups PD (48) AD (18) controls (19)	t-tau $A\beta_{42}$ tau/$A\beta_{42}$	AD and non-tremor dominant PD higher levels of tau & t/Aβ, tau potentially is a marker for presence and disease severity
Jellinger [40]	PD (12) AD (27) healthy controls (17)	t-tau $A\beta_{42}$ tau/$A\beta_{42}$	t-tau and tau/$A\beta_{42}$ levels are increased in AD and non-tremor dominant PD
Compta et al. [13]	PDD (20) non demented PD (20) controls (30)	t-tau p-tau $A\beta_{42}$	t-tau and p-tau levels are increased PDD, $A\beta_{42}$: controls < PD < PDD
Maetzler et al. [41]	DLB (9) PDD (12) non demented PD (14)	$A\beta_{42}$	DLB and PDD < ndPD

FTD, frontotemporal dementia; MCI, mild cognitive impairment; OD, other dementias; VaD, vascular dementia.

diagnosis (MMSE <27, cognitively affected) showed decreased values of $A\beta_{42}$ (<410 pg/ml), but those without dementia ($n = 11$) had a normal CSF profile.

Our study has some limitations, which are the sample size and the lack of neuropathological confirmation of the diagnoses. Although more patients were evaluated for the respective diagnoses in clinical and outpatient settings during the five-year period of time, we decided to use only data from patients who fulfilled our strict inclusion criteria for the diagnostic workup. This resulted in a reduction of the cohort size. Therefore, although the sample size might appear to be relatively small, this cohort is unique with respect to the inclusion criteria on one hand, but also because it represents a prospective cohort of patients and differential diagnostic challenges which clinicians face in every day clinical routine. It reflects the clinical practice rather than a study population, which is frequently artificial and hampered by unique problems. Another problem to address is the lack of neuropathological verification of our clinically-based diagnoses according to current guidelines. We excluded all cases with a high risk of possible misdiagnosis or overlapping diseases but we are aware that a neuropathological confirmation would have been superior concerning the possible coexistence of DLB and AD at the same time.

Lack of neuropathological confirmation is a serious problem of all type of CSF based studies in dementia research [23]. Actually the current clinical AD diagnostic methods show much variability among studies. Highest validity is given by multi-center studies. Beach et al. [23] collected clinical and neuropathological data from the National Alzheimer's Coordinating Center, comprising 919 patients, seeking to determine the accuracy of currently used clinical diagnostic methods. They found the sensitivity of clinical diagnosis ranging widely from 70.9% to 87.3%; the specificity ranged from 44.3% to 70.8%. Furthermore a mismatch in terms of clinical and neuropathological diagnosis was given depending on the exact clinical and neuropathological criteria used. More precisely, they were stressing the fact that neuropathological criteria for AD have changed several times over the past 30 years bringing up the question "how good is the present neuropathological gold standard?" and summarized: "When the minimum neuropathological threshold for diagnosis is defined as moderate or frequent neuritic plaques together with Braak stage III-VI, [. . .] 83% of subjects with that clinical diagnosis were confirmed neuropathologically to have AD lesions." [. . .] On the other hand, to put it into another perspective, the clinical diagnosis is confirmed by neuropathological examination in more than 80% in general and in patients with classical AD presentation almost always. Another support of clinical diagnostic criteria is given by Alladi et al. who described 20 patients with clinically typical AD, of whom 19 could be verified by pathology [24].

Facing DLB diagnosis, the Third Consortium on DLB neuropathologic criteria scheme performed reasonably well, according to Fujishiro et al. [25]. They describe 43 clinically probable DLB patients, showing diffuse cortical Lewy bodies in more than 80%. In this study, the frequency of core clinical features and the accuracy of the clinical diagnosis of probable DLB were described as significantly greater in pathologic high-likelihood cases and it concludes that the DLB clinical syndrome is directly related to Lewy body pathology.

The potentially overlapping pathological changes found in all three disease entities are not addressed by clinical criteria, since no test (imaging, biomarker, neuropsychology) has been developed so far which helps

to overcome this problem. Another point to consider is the intrinsic problem that the pathological confirmation of clinical diagnoses of AD and PD made today requires a follow up of at least 10, maybe 15 years. This type of study will be available in the future and will allow the analysis of the differential diagnostic value of Aβ and tau tests performed at time of clinical diagnosis. Also, previous studies on clinicopathological correlations did not take into account the modern imaging methods such as MRI and PET/SPECT, which significantly contribute to the field in recent years and improve the clinical diagnosis. Modern CSF analyses help to exclude other treatable conditions such as autoimmune inflammatory disorders [26, 27]. To summarize, neuropathology does not always confirm the clinical diagnosis. On the other hand, it confirms the clinical diagnosis in the vast majority of cases and the chances are high that modern techniques will further help to minimize the gap.

Some of the pathological CSF changes observed need further attention. In AD, pathology development probably starts decades before the first symptoms occur. The pathology is defined by plaques consisting of Aβ peptides and neurofibrillary tangles containing tau proteins that lead to inflammatory damage and synaptic dysfunction [28]. Therefore, the decreased values of $A\beta_{42}$ in AD are not surprising, because of its affinity to aggregation and potential involvement in disease pathogenesis being a major constituent of the core of the senile plaques. The amount of senile plaques accumulates with the progression of AD. The number of neurofibrillary tangles is also a pathologic marker of the disease and correlates with its severity. These tangles consist mainly of abnormal hyperphosphorylated and aggregated tau, which inhibits the stability of microtubules and vesicle transport mechanism. In CSF, tau and, in particular, phosphorylated tau are increased in AD.

On the contrary, the finding of abnormal tau and $A\beta_{42}$ in DLB is not easily explained by the same assumptions as in AD and need to be studied further. One hypothesis to test is the involvement of tau and Aβ in neurodegenerative processes in general; the other needs to test the specific distribution of the pathological changes in the brain and the drainage of brain-derived proteins into the CSF.

CONCLUSIONS

In view of the rising world-wide prevalence of dementias, there is a need for improved and early detection of degenerative dementias in general and their differential diagnosis. Considering CSF analyses, it is neither very expensive or time consuming for investigation, compared to other tools such as neuroimaging.

The present study critically analyzed the value of the single CSF biomarkers and their various ratios in a large cohort from a single center. Our major conclusions are: 1) For AD, the best single CSF parameter is p-tau. The combination with $A\beta_{42}$ and the ratio is highly valid. T-tau levels might reflect advanced pathologies in later stages of disease progression; 2) As a rule, the distinction between DLB and AD was difficult. Markedly decreased $A\beta_{42}$ was detected in DLB; 3) PD patients had no distinct deviation in CSF profile and normal biomarkers were more frequently observed in PD than in DLB; 4) New ratios seem to be promising in differential diagnosis and should be investigated further. Most useful to differentiate DLB and PD were the $A\beta_{42}$/tau ratio (mean 2.2 in DLB and 5 in PD) and the $A\beta_{42}$/p-tau ratio (mean 7.4 in DLB and 20 in PD); 5) As a general rule, the typical biomarker profiles were: a) DLB: very low $A\beta_{42}$, normal $A\beta_{40/42}$ ratio, only moderate increase in total tau and p-tau; AD: $A\beta_{42}$ slightly decreased, $A\beta_{40/42}$ ratio <1.0, considerably increased total tau and p-tau; PD: normal $A\beta_{40/42}$ ratio, normal total tau and p-tau.

There is increasing evidence that CSF biomarkers are already of great importance in the differential diagnosis in neurodegenerative diseases. CSF analysis will become even more important in the future when specific biomarkers for distinct neurodegenerative conditions will become available.

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