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CSF Amyloid-beta 38 as a novel diagnostic marker for dementia with Lewy bodies.

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Abstract

Background

The clinical distinction between Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) is sometimes difficult, particularly in mild cases. Although cerebrospinal fluid (CSF) markers such as A β 42 and P-tau can distinguish between AD and normal controls, their ability to distinguish between AD and DLB is not adequate.

Objective

This study aims at investigating whether CSF markers, in particular the level of A β 38, can differentiate between mild AD and DLB.

Methods

In total 85 individuals were included after standardized diagnostic procedures: 30 diagnosed as **probable AD, 23 probable DLB, 20 with probable** Parkinson's disease dementia (PDD), and 12 non-demented controls subjects. CSF levels of A β 38, A β 40 and A β 42 were determined using commercially available Ultra-Sensitive multi-array kit assay (MSD) for human A β peptides. Total-tau (T-tau) and Phosphorylated tau (P-Tau) were analysed using ELISA (Innotest). In addition, combinations (A β 42/A β 38, A β 42/A β 40, A β 42/P-tau, and A β 42/A β 38/P-tau) were assessed.

Results

Significant between-group differences were found for all CSF measures, and all except A β 40, A β 42, and A β 42/P-tau differed between AD and DLB. A β 42/A β 38 ratio was the measure that best discriminated between AD and DLB (AUC 0.781; $p < 0.005$), with sensitivity 74% and specificity 77%.

Conclusion

This study suggests that the level of A β 38 can potentially contribute in the diagnostic distinction between AD and DLB when combined with A β 42. Single measures had low diagnostic accuracy, suggesting that developing a panel of markers is the most promising strategy. Studies with independent and larger samples and a priori cut-offs are needed to test this hypothesis.

Introduction

Dementia with Lewy Bodies (DLB) is a common dementia with a complex clinical presentation, reduced quality of life and higher costs [1, 2], higher mortality [3], poorer drug response [4], and increased risk for nursing home admission [5] compared to Alzheimer's disease (AD). Identifying people with DLB is therefore crucial, but can be difficult, in particular in early cases where the clinical features are less characteristic.[6] There is therefore a need for biomarkers to aid in the distinction between DLB and AD. However, with the exception of dopamine transporter SPECT, which is expensive and not available at all centres, there are yet no such established biomarkers.[7]

Potential biomarkers should be based on the underlying pathology of the disease. In AD, accumulation of amyloid peptides (A β -peptides) in plaques as well as species of microtubule-associated axonal protein tau, which is deposited in tangles, are key pathological features. The A β -peptides are derived from amyloid precursor protein (APP) by sequential cleavage involving proteolytic enzymes β - and γ secretases.[8] The A β 42 peptide variant is prone to aggregation, is predominantly deposited in senile plaques and is shown to be neuro-toxic.[9]

Hyper phosphorylated tau (P-tau) is one of the major components of neurofibrillary tangles.[10] Lewy bodies, consisting mainly of alpha-synuclein deposits, are the characteristic feature of DLB, although AD type changes are also common.[11]

Cerebrospinal fluid (CSF) A β 42 together with hyper-phosphorylated tau (P-tau) have been shown to identify incipient AD with good accuracy.[12, 13] However, these peptides discriminate less well between AD and other dementia subtypes, including DLB.[12, 13] In a study using Western blot and quantitative analyses, Bibl and co-workers showed that by expanding the number of amyloid species by including carboxy-terminally truncated peptides such as A β 37, A β 38, and A β 39, such markers might provide useful biomarker to distinguish AD from other dementias.[14, 15] In subsequent studies using ELISA, the same group reported that A β 38 and the A β 42/A β 38 ratio, have better discriminative power between AD and other dementias than A β 42 alone.[15, 16] However, in the latter study, only five patients had DLB. The objective of this paper was therefore to explore whether A β 38 and combinations including

A β 38 could distinguish between AD and DLB, using a larger sample size with DLB, and including normal controls and Parkinson's disease dementia (PDD) as comparison groups.

Methods

Subjects

Patients were drawn from two cohorts: The Norwegian DemWest study (n=42) recruited referrals to psychiatry, neurology, and geriatric medicine clinics in Rogaland and Hordaland counties who were diagnosed with mild dementia (i.e Mini-Mental State Examination (MMSE) ≥ 20) between 2005-2007.[17] For this study, only patients diagnosed as **probable AD (n=30), probable DLB (n=9) or PDD (n=3)** who consented to lumbar puncture were included. In addition, patients with **PDD (n=17) and probable DLB (n=14)** who were screened between 2006-2008 to participate in a clinical trial of memantine [17] and who consented to lumbar puncture were recruited from the Department of Clinical Sciences, Malmö, University of Lund, Sweden. Subjects without known brain disease who underwent lumbar puncture during orthopaedic surgery or neurologic outpatient assessment with a minimum MMSE score of >24 at the Stavanger University Hospital were recruited as non-demented controls.

Diagnostic procedures

Diagnostic procedures are described in detail elsewhere.[17, 18] In brief, patients were diagnosed as DLB if they fulfilled clinical consensus criteria for **probable DLB** [19], **probable AD** [20] or probable PDD [21] after a detailed clinical assessment by a registered specialist in psychiatry, neurology or geriatric medicine, using standardised assessments for parkinsonism[22] psychiatric symptoms including visual hallucinations (Neuropsychiatric Inventory, NPI)[23], and fluctuating cognition.[24, 25] Blood samples and brain imaging (CT or MRI) were taken from all patients, and a subset of DLB patients underwent dopamine transporter SPECT scans.

Pre-analytical treatment of CSF

Lumbar puncture (LP) was performed in the L3-L4 or L4-L5 interspace and CSF sampling was performed in all cases **between 7-10 am in order to minimize diurnal variation** of the level of

CSF A β [26]. The first 3-4mLs of the CSF were dedicated for routine analyses for assessment of relevant CSF abnormalities. Thus, samples were immediately sent on ice to the routine laboratory where routine assay for cell counts, levels of glucose and protein were performed. Study samples were collected in separate polypropylene tubes, and centrifuged at 2000xg, 4°C for 10 min to get rid of cell debris and other insoluble materials. Following centrifugation, samples were aliquoted and immediately frozen at -80°C until analyses were performed. Samples from the DemWest study, Stavanger, were originally stored in larger volumes, thus the portions of samples used in this study were aliquots derived from samples frozen and thawed (on ice) once.

Tri-plex human CSF A β 38, A β 40 and A β 42 Assay

All CSF analyses were performed randomized and in duplicates the same day by one of the authors (EM), blinded to clinical information. CSF levels of A β 42, A β 40, and A β 38 were determined using the A β triplex assay (Human A β peptide Ultra-Sensitive Kits) developed by Meso Scale Discovery, Gaithersburg, Maryland, USA. **This assay uses C-terminus specific antibodies to capture the different A β peptides and a SULFO-TAG™-labeled anti-A β antibody (4G8) for detection with electrochemiluminescence.**

The tri-plex assay was performed on CSF samples from patients and control subjects as well as standards of specific markers in duplicate and according to the manufacturer's instructions. Briefly, the assay technology is based on MULTI-ARRAY® technology combining electrochemiluminescence detection and patterned arrays offering combination of sensitivity and dynamic range. The triplex assay mentioned here utilizes peptide specific antibodies to capture A β 38, A β 40 and A β 42 peptides present in CSF. The CSF content of each peptide was then detected by SULFO-TAG-labeled 4G8 detection antibodies. **The standard ranges for A β 38 and A β 42 were 4-3000pg/mL respectively, and for A β 40 27.4-20000pg/mL. The lower limit of detection and limit of quantitation (LLOD/LOQ) for all three analytes were A β 38 (8.69/<25pg/mL), A β 40 (1.28/~50pg/ml) and A β 42 (12.37/~35pg/mL) pg/mL respectively. To determine inter and intra assay variations we included different CSF samples (1= low level, 2=medium level) in replicates as run controls. Within-assay precision for replicated samples on same plate (intra-assay) variation for the “low level” sample and for individual**

analytes; A β 38, A β 40 and A β 42 were between 5-6%. The inter-assay variability, (same sample analysed on different plates) for the “low level” sample and each of the analytes A β 38, A β 40 and A β 42 was 9, 13 and 8% respectively. Similarly the intra-assay variability for the “medium level sample” and each of the analytes mentioned above were between 4-7% and the inter-assay variability for “medium level sample” and each of the analytes were 15, 14 and 12% respectively.

ELISA of total and phosphorylated tau

CSF T-tau & P-tau₍₁₈₁₎

CSF T-tau was analyzed using a commercial sandwich ELISA (INNOTEST[®] hTAU-Ag, Innogenetics, Gent, Belgium) specifically constructed to measure all tau isoforms irrespective of phosphorylation status, as previously described [27]. In this assay, the monoclonal antibody AT120 was used for capture, while the biotinylated monoclonal antibodies HT7 and BT2 were used as detection antibodies. **AT120 and HT7 both react equally well with both normal and hyperphosphorylated, while BT2 preferentially recognizes normal tau protein. The standard range of the T-tau assay was 75-1200 pg/ml, the LOD was 59 pg/ml, and intra- and inter-assay CVs ranged from 1.2-5.9% and 1.7-6.0% respectively.**

CSF P-tau was measured using a commercial sandwich ELISA method (INNOTEST[®] PHOSPHO-TAU_(181P), Innogenetics, Ghent, Belgium), as described previously [28]. In this assay, the monoclonal antibody HT7 was used for capture and the biotinylated monoclonal antibody AT720 as detector antibody. **HT7 both react equally well with both normal and hyperphosphorylated, while AT270 specifically reacts with tau phosphorylated at threonine-181. The P-tau assay had a standard range of 15.6–500 pg/ml, a LOD of 15.6 pg/ml, and intra- and inter-assay CVs of <5% and <10% respectively.**

Statistics

Values of CSF markers were expressed as absolute (pg/ml). In addition to the single markers, pre-specified combinations were analysed: A β 42/ A β 40, A β 42/P-tau, A β 42/A β 38, and A β 42/A β 38/P-tau. Since measures were not normally distributed, median and interquartile range (IQR) were expressed, and Spearman correlations were performed. Between-group comparisons were

made using Kruskal-Wallis and chi square tests. Pre-planned post-hoc pair-wise comparisons (Mann-Whitney) between AD and DLB were performed subsequently. The global diagnostic accuracies were assessed by the received-operated characteristic curve (AUC). Since this was a hypothesis-generating study, a p value of < 0.05 was considered significant, and no attempts to adjust for multiple comparisons were made. Cut-off points and sensitivity and specificity were determined based on the coordinate points of the curves. Positive (sensitivity/1-specificity) and negative (specificity/1-sensitivity) likelihood ratios (LR) were calculated. Cut-off points and sensitivity and specificity were determined based on the coordinate points of the curves.

Results

Characteristics

In total, 85 subjects participated: AD (n=30), DLB (n=23), PDD (n=20) and normal controls (n=12). The characteristics of the subjects are shown in table 1. The groups did not differ in terms of age, and the dementia groups did not differ regarding MMSE score. As expected, there were more males in the DLB and PDD groups compared to the other groups, and the AD and DLB groups differed significantly (chi square 4.6, $p=.03$). The disease duration differed significantly, with a longer duration in DLB than AD ($p=.042$).

Table 1. Characteristics of the groups

	NC	AD	DLB	PDD	P
N	12	30	23	20	
Age	73.5(16.8)	75.5(11.3)	74(10.8)	73(11)	.89
MMSE	28.5(1.8)	23.5(4.3)	23(7.8)	23(9)	<.0005
Gender, M/F*	4/8	12/18	16/7	13/7	=.055
UPDRS motor	ND	0(2)	30.5(32.5)	34.5(17.3)	<.0005
Duration of disease	NA	2(2)	3.5(3)	8(4)	<.0005

Numbers represent median and inter-quartile range or *number of people and %

P values based on Kruskal-wallis test; ND: Not done NA: not applicable

NC=Normal control, AD=Alzheimer's disease, DLB=Dementia with Lewy bodies, PDD=Parkinson's disease dementia

CSF measures and associations with diagnosis and other characteristics

The CSF values are shown in table 2 and Figure 1. There were significant between-group differences on all markers. Compared to NC; the pattern in AD was as expected, with low A β 42 and high T-tau and P-tau. Significant differences between DLB and AD were observed for all measures except A β 40 and A β 42, and A β 42/P-tau. Whereas the single markers were changed in the same direction in AD and DLB, a different picture emerged for the combined markers involving A β 38, which were decreased in AD but increased in DLB and PDD compared to NC. **Mean CSF concentrations did not differ between the Norwegian and Swedish DLB/PDD patients.** The AUC analyses demonstrated that the A β 42/A β 38 ratio was the strongest marker for differentiation between AD and DLB, with an AUC of 0.732 (95% CI 0.587-0.876) (p=.007), with sensitivity 74% and specificity 67% at cut-off 0.50. Positive LR was 2.2 (95% CI 1.3 – 3.6 and negative LR 0.3 (0.25-0.8).

There was a trend towards association between A β 38 and gender (p=0.061). Since there were gender differences between AD and DLB, the analyses were therefore performed for each gender separately. The overall analyses were confirmed in males, whereas no significant differences were found in the smaller female group (data not shown). **When all subjects were included**, age correlated with total tau (rho 0.29, p=.007), A β 38 (0.24, p=.029), and A β 40 (0.26, p=.015), and all three A β species correlated significantly with MMSE score (rho 0.24-0.31, p 0.004 – 0.03).

Finally, A β 38 correlated with duration of disease (0.25, p=.04). **Including patients only, significant correlations with age were found for total tau (rho =0.25, p<.05), A β 42 correlated with UPDRS motor score (0.37, p=.04), and duration with A β 38 (rho=-0.26, p=.032), whereas a non-significant trend between MMSE and A β 38 was found (rho=0.22, p=.06).**

Table 2. CSF concentration of protein markers in the diagnostic groups

Markers	NC	AD	DLB	PDD	P1	P2	AUC
A β 38 (pg/mL)	635(712)	440(305)	385(244)	404(299)	.005	.013	0.70
A β 40 (pg/mL)	8286(6652)	5461(2632)	5507(2109)	5036(3633)	.048	.36	0.43
A β 42 (pg/mL)	337(378)	192(74)	223(163)	287(107)	.001	.84	0.52
T-tau (pg/mL)	250(166)	382(363)	303(116)	303(122)	.023	.009	0.68
P-tau (pg/mL)	58.3(35.4)	86.5(61)	60.2(52.8)	57.1(41.3)	.027	.011	0.69
A β 42/A β 38	0.62(0.14)	0.46(0.29)	0.66(0.34)	0.74(0.28)	.0005	.001	0.77
A β 42/A β 40	0.058(0.02)	0.032(0.02)	0.043(0.02)	0.055(0.1)	.002	.004	0.64
A β 42x1000/A β 38/P-tau	9.2(6.3)	4.8(9.5)	10.8(11.1)	11.4(15.2)	.0005	.015	0.74
A β 42/P-tau	9.0(2.9)	2.1(2.0)	3.9(5.0)	4.8(2.7)	.0005	.076	0.71

NC = non-demented control subjects, AD = Alzheimer's disease, DLB = Dementia with Lewy

Bodies and PDD = Parkinson's Disease Dementia

Numbers represent median and interquartile range (pg/ml) or ratio

P1=All groups, Kruskal-wallis test; p2=AD vs DLB, Mann-Whitney test

AUC: Area under the curve.

Discussion

We investigated whether CSF markers could distinguish between mild DLB and AD. The main finding was that the ratio between A β 42 and A β 38 was the CSF marker that best distinguished between AD and DLB. However, the accuracy is still well below the 85% level which is recommended by *Consensus Report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease"* for a diagnostic test.[29] Given the increasingly recognised need for an early dementia diagnosis [30], and the difficulties in making an accurate clinical diagnosis of DLB, in particular early in course, these findings are nevertheless encouraging. Our findings further suggest that the pattern from a panel of CSF markers is the best strategy forward, although continued research is needed to develop an adequate biomarker for the differentiation between AD and DLB.

There were associations between A β -peptide species with cognitive impairment, and A β 38 correlated with disease duration. This is consistent with a recent study demonstrating that the CSF level of alpha-synuclein was associated with dementia severity in DLB,[31] and suggests that CSF markers can be useful markers of the progression of disease pathology, and thus might serve as biological outcome measures in clinical trials of disease modifying therapies.

This study has limitations which need to be considered when interpreting the findings. First, the diagnoses were based on clinical assessment and may thus not be 100% accurate. However, the two research groups have long experience in diagnosing DLB, and standardised and recommended diagnostic procedures and consensus criteria were used. These have shown good clinico-pathologic correlation [32], suggesting that the diagnostic accuracy is relatively high. Of note, there is also pathological overlap between AD and DLB, and the pathological classification of the two diseases is still under discussion.[33]

There were gender differences in the expected direction. The overall findings were confirmed in the male patients, supporting the validity of the overall findings. The lack of significant differences in the female patients should be interpreted in light of the low statistical power due to the low number of female DLB patients. Difference in duration of disease might also have introduced a bias. **Lumbar puncture was not standardised in relation to meals at one study site (Malmö). This might have influenced the findings [26], although such an effect is most likely to be minor as no differences in mean values were observed in the DLB/PDD group between study centres.**

Finally, as most previous studies, we derived cut-offs from the population under study. This procedure has an inherent risk for overestimating diagnostic accuracy.[34] Further studies are therefore need to test the accuracy of a priori determined cut-offs rather than establishing a cut-off directly on the cohort under investigation.

The observation of a similar pattern of CSF markers in DLB and PDD, with largely shared underlying brain changes, adds biological validity to the observations. However, we found somewhat lower diagnostic accuracies for the A β species compared with a previous study.[16]. Furthermore, our findings of Tau and P-Tau, although significantly different between the groups,

show lower diagnostic accuracy compared to a recent study [35]. Possible explanations for these differences include that we specifically explored the differentiation between AD and DLB, whereas only 10% of the non-AD group in the previous study were DLB or PDD. Thus, since AD-type brain changes are typically found in DLB [36], this differentiation may be more challenging than distinguishing AD from frontotemporal and vascular dementias. Secondly, our sample included older patients (age 73-75) with mild dementia, with a mean MMSE score of 23-24, compared to younger patients (mean age below 70) with a lower mean MMSE score (below 20) in [16]. Whereas the previous study (35) was based on an autopsy-cohort, we used clinically diagnosed cases with presumed lower diagnostic accuracy. **In addition, there were differences in the pre-analytic handling and assay methods between the different studies, possibly leading to variation in CSF marker concentrations between different centres. This was shown in a recent multi-centre study [12], in which some of the Stavanger cases participated. Finally, although there are strong correlations between the different methods and commercially available kits used to determine level of CSF markers, there is considerable variability in the average values of results.**

In conclusion, our findings suggest that A β 38 should be included in future studies to identify CSF biomarkers to differentiate between AD and DLB. Future studies should also explore whether CSF markers can increase diagnostic accuracy over and above standard clinical assessment, and whether they can predict disease progression. Finally, whether establishing CSF panels combining specific A β -peptides and tau markers with alpha-synuclein species might improve this differentiation should also be investigated.

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Competing Interests

None declared.

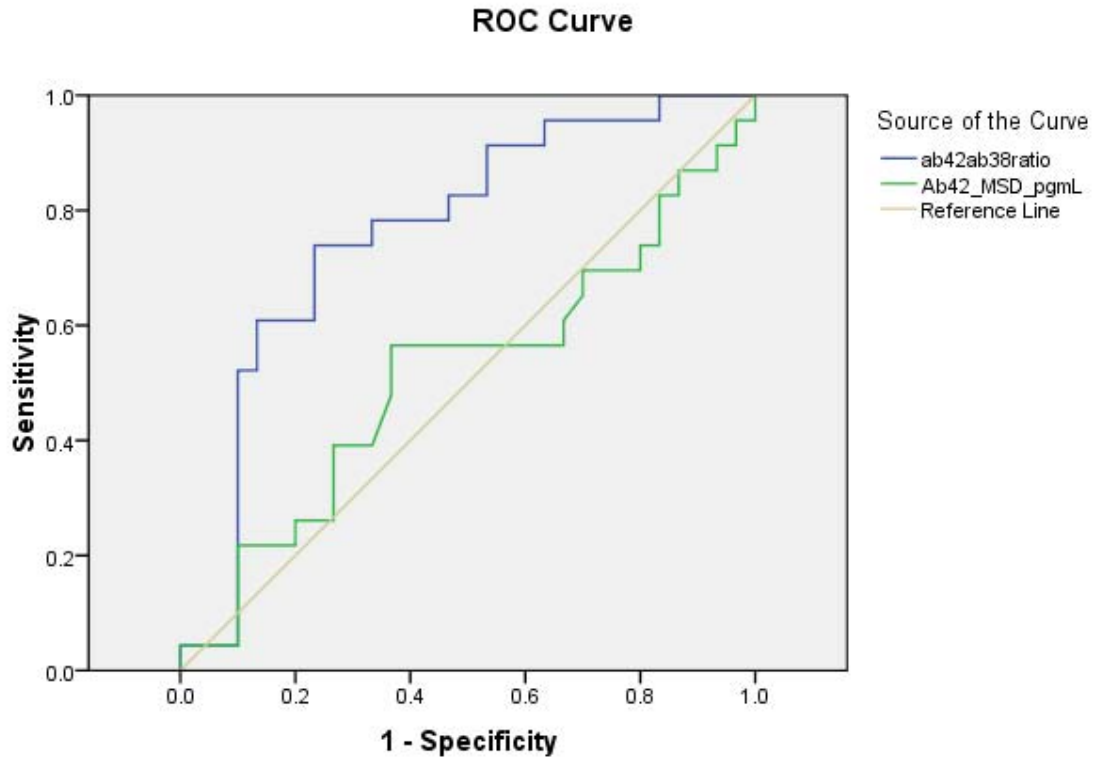
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Figure 1. Diagnostic accuracy for DLB versus AD for A β 42/A β 38 ratio and A β 42 as measured using the area under the curve



Diagonal segments are produced by ties.

Note. A β 42 A β 38ratio is the ratio between CSF A β 42 and A β 38.
A β 42_MSD_pgml is the CSF level of A β 42 in pg/ml.