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Negative results

CSF D-serine concentrations are similar in Alzheimer's disease, other dementias, and elderly controls

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ABSTRACT

Cerebrospinal fluid (CSF) levels of D-serine were recently reported as a potential new biomarker for Alzheimer's disease (AD), showing a perfect distinction between AD patients and healthy controls. In this study, we aimed to confirm these results and extend these previous findings to dementia with Lewy bodies and frontotemporal dementia. D-Serine levels in CSF of 29 AD patients, 8 dementia with Lewy bodies patients, 14 frontotemporal dementia patients, and 28 nondemented controls were measured using ultra-high-performance liquid chromatography-tandem mass spectrometry. In contrast to previous findings, in our study CSF D-serine levels were only slightly increased in AD patients compared with controls. CSF D-serine in AD did not differ from other dementias and was also not correlated to mini-mental state examination–scores. Owing to the large overlap of D-serine levels, we conclude that CSF D-serine is neither a suitable biomarker for AD nor for cognitive decline.

1. Introduction

Diagnosis of probable Alzheimer's disease (AD) is made using a combination of clinical and neurological investigations and neuropsychological testing. In addition, brain imaging results and cerebrospinal fluid (CSF) analyses can increase the certainty of the diagnosis (McKhann et al., 2011). Recently, a 5-fold increase in CSF D-serine, a coagonist of the N-methyl-D-aspartate receptor, was observed in AD patients compared with healthy controls (Madeira et al., 2015). Remarkably, a perfect distinction between AD and controls was observed and addition of D-serine to the established CSF biomarkers (amyloid-β 42 [Aβ42], total-tau [t-tau], and hyperphosphorylated tau [p-tau]) (Rosen et al., 2013) increased sensitivity (96.3%) and specificity (100%) for the diagnosis of probable AD.

In this study, we aimed to confirm these findings and extend them to other dementia syndromes.

2. Methods

2.1. Patients

CSF samples of 29 AD patients, 8 dementia with Lewy bodies (DLB) patients, 14 frontotemporal dementia (FTD) patients, and 28 nondemented controls were obtained from the Radboud University Medical Center, Nijmegen, The Netherlands. Based on the values obtained by Madeira et al. (2015), a sample size of $n = 6$ per group should be sufficient to repeat the previous findings ($\alpha = 0.05$, $\beta = 0.80$). Diagnosis was based on clinical and neuropsychological examination and supported by routine laboratory investigation. Mini-mental state examination (MMSE) was performed at the time of lumbar puncture to assess cognitive function. Clinical diagnosis for AD patients was confirmed by NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association) criteria because samples were collected before the introduction of recent diagnostic criteria (McKhann et al., 2011). DLB and FTD were diagnosed according to published consensus criteria (McKeith, 2006; Neary et al., 1998).

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Nondemented control patients were initially examined for a neurological disorder but were diagnosed either without neurological disorder or with a systemic disease without neurological manifestations. Routine CSF parameters were normal for these control subjects. All patients were informed that remaining CSF could be used for future research application and were given the option to object to this use, in which case their CSF and data were not used in this study. CSF samples were obtained by lumbar puncture, collected in polypropylene tubes and centrifuged, aliquoted and stored at −80 °C within 2 hours after collection. Patient characteristics are listed in Table 1.

2.2. Serine measurements in CSF

Levels of D- and L-serine in CSF were determined after derivatization with (*S*)- N-(4-nitrophenoxycarbonyl)-l-phenylalanine 2-methoxyethyl ester using an ultrahigh-performance liquid chromatography-tandem (HPLC) mass spectrometry approach which has been validated and described before (Luykx et al., 2013; Visser et al., 2011). This assay has an limit of quantification of 22 nM with an intra-assay variation of 2.4% and an interassay variation of 7%, accuracy was 98.2% (Visser et al., 2011).

2.3. Data analysis

Statistical analysis was performed using Graphpad Prism 5 for Windows (La Jolla, CA, USA) and IBM SSPS Statistics, version 20 (Armonk, NY, USA). Statistical outliers for CSF D-serine were determined by using Grubbs' test. Biomarker levels were analyzed using an analysis of variance or ANCOVA with Bonferroni's *post hoc* test for multiple comparisons. Alternatively, the Kruskal-Wallis test with Dunn's *post hoc* test for multiple comparisons was used when not all groups showed Gaussian distribution. *p* values less than 0.05 were considered statistically significant. Correlation between D-serine and MMSE scores was calculated using Pearson *r*. The diagnostic value of D-serine in addition to other biomarkers (Aβ42, t-tau, and p-tau) was determined by linear regression and receiver operating characteristic analysis.

3. Results

CSF D-serine levels were slightly increased in AD patients (1.56 ± 0.29 μM) compared with controls (1.35 ± 0.29 μM; $p = 0.03$, analysis of variance with Bonferroni's *post hoc* test) but did not differ from other dementias. One AD patient showed an extremely high value of D-serine in CSF compared with the other AD patients, which could not be attributed to any other characteristic of this individual patient. According to the Grubbs' test, this value was a significant outlier and was therefore excluded from the analysis. No other outliers were detected using Grubbs' test in any of the groups. Because age was significantly different between diagnostic groups, additional analysis with age as a possible confounder was performed and abolished the statistical difference between the control and AD groups ($p = 0.25$, ANCOVA with Bonferroni's *post hoc* test). D-Serine levels did not differ between those who reported either to be present or former smokers or used alcohol vs. those who did not ($p = 0.70$ and $p = 0.86$ respectively). L-Serine levels in CSF were not significantly different between diagnostic groups ($p = 0.65$, Fig. 1B). In addition, the ratio between D- and L-serine was not significantly different between these groups ($p = 0.26$, Fig. 1C). Also, we observed no correlation between D-Serine CSF levels and MMSE scores in the entire cohort (Fig. 1D, r = 0.25, $p = 0.11$). Finally, linear regression analysis followed by receiver operating characteristic analysis showed that the addition of D-serine to the panel of biomarkers (Aβ42, t-tau, and p-tau protein levels in CSF) did not improve discrimination between control and AD subjects (area under the curve = 0.985 for both models).

4. Discussion

Recently, CSF levels of D-serine were found to be 5 times higher in AD patients compared with healthy controls, with a perfect separation between AD and controls. In addition, CSF D-serine levels were twice as high compared with patients with a depression or hydrocephalus (Madeira et al., 2015). Furthermore, addition of CSF D-serine levels to the Aβ42, t-tau, and p-tau biomarker combination to separate AD from non-AD cases increased both the sensitivity (to 96.3%) and the specificity (to 100%) for diagnosis of probable AD. In our current cohort, we could not confirm the previously reported

Table 1
Group characteristics

	Control	AD[a]	DLB	FTD	*p* value[b]
Number of patients	28	28	8	14	
Gender (M/F)	17/11	10/18	8/0	9/5	$p = 0.006$
Age (y)[c]	60.07 (5.34)	71.57 (8.35)	77.25 (9.66)	63.79 (7.31)	$p < 0.001$
Smoking (yes/no/unknown)	10/15/3	9/10/9	3/2/3	5/6/3	NS ($p = 0.50$)
Alcohol use (yes/no/unknown)	15/10/3	17/3/8	3/2/3	6/5/3	NS ($p = 0.23$)
Disease duration (mo)[d]	NA	19.04 (16.31)	21.5 (25.19)	18.92 (19.76)	NS ($p = 0.86$)
MMSE score[e]	NA	22.1 (3.3)	21.3 (4.6)	19.2 (6.2)	NS ($p = 0.39$)
Aβ42 (pg/ml)[f]	676 (307)	473 (176)	584 (227)	734 (183)	$p = 0.001$
t-Tau (pg/ml)[f]	302 (192)	822 (625)	299 (122)	496 (387)	$p < 0.0001$
p-Tau (pg/ml)[f]	48 (19)	121 (61)	54 (13)	75 (33)	$p < 0.001$
D-serine (μM)	1.35 (0.29)	1.56 (0.29)	1.45 (0.26)	1.34 (0.26)	$p = 0.03$
L-serine (μM)	23.5 (4.6)	25.2 (4.0)	26.9 (7.6)	24.9 (5.8)	NS ($p = 0.65$)
Ratio D/L-serine	0.058 (0.011)	0.063 (0.012)	0.056 (0.015)	0.055 (0.010)	NS ($p = 0.26$)

Values are expressed as mean (±standard deviation).
Key: AD, Alzheimer's disease; DBL, dementia with Lewy bodies; FTD, frontotemporal dementia; NA, not available; NS, not significant; MMSE, mini-mental state examination.
[a] One patient in the AD group was excluded from analysis after determination of statistical outliers using Grubbs' test.
[b] Gender, smoking, and alcohol use differences between groups were compared using the chi-square test. Mean age of groups and CSF D-serine concentration were compared using ANOVA with Bonferonni's *post hoc* analysis for multiple comparisons. All other parameters showed a non-Gaussian distribution and were compared using Kruskal-Wallis with Dunn's *post hoc* test for multiple comparisons.
[c] Age at the time of lumbar puncture.
[d] Disease duration was not available for 1 AD, 2 DLB, and 2 FTD patients.
[e] MMSE scores were not available for 2 DLB patients and 3 FTD patients.
[f] Biomarker levels were not available for 3 controls, 1 AD, and 1 FTD patient.