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Cerebrospinal Fluid Markers in Differential Diagnosis of Alzheimer's Disease and Vascular Dementia

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A B S T R A C T

Alzheimer's disease (AD) and vascular dementia (VaD) are the two most common causes of dementia in old people. They remain difficult to differentiate in practice because of lack of sensitivity and specificity of current clinical diagnostic criteria. Recent molecular and cellular advancements indicate that the use of cerebrospinal fluid markers may improve early detection and differential diagnosis of AD. Our objective in this study was to determine diagnostic accuracy of three cerebrospinal (CSF) markers: total tau protein (t-tau), tau protein phosphorylated on threonine 181 (p-tau181) and tau protein phosphorylated on serine 199 (p-tau199). Using commercially available ELISA kits concentrations of t-tau, p--tau181 and p-tau199 were analyzed in 12 patients with probable AD, 9 patients with VaD and 12 NC subjects. The median levels of all three markers were significantly higher in AD group versus VaD and NC groups. However, when the sensitivity levels were set to 85% or higher, only t-tau and p-tau199 satisfied consensus recommendations (specificity more than 75%) when differentiating AD from VaD. In conclusion, our preliminary data on a small group of selected subjects suggest that the CSF t-tau and p-tau199 levels are useful markers for differentiating AD from VaD.

***Key words**: Alzheimer's disease, cerebrospinal fluid, ELISA, phoshorylated epitopes, tau proteins, vascular dementia*

Introduction

Development of novel therapeutic options as well as unsatisfactory sensitivity and specificity of current clinical diagnostic criteria for Alzheimer's disease (AD) require additional markers to help in early and accurate differentiation of AD from other dementias[1]. Besides amyloid plaques (AP), another important pathological hallmark of AD includes neurofibrillary tangles (NFT) which are mainly composed of hyperphosphorylated tau. Formation of both AP and NFT begins at least 20–30 years before the clinical onset of the disease[2]. During the years, progressive accumulation of the neuropathological changes eventually leads to appearance of first symptoms. Interestingly, the development of NFT, but not of AP, typically follows pattern of progression in topographical terms[3,4]. In contrast to AD, vascular dementia (VaD) is not characterized with formation of NFT. Due to the fact that biochemical changes in the brain are reflected in the CSF, in this context tau proteins may represent a promising biological marker for differentiating AD from VaD.

AD and VaD are the two most common dementia types in old people. Although they are clearly separate diseases, to differentiate them is often a serious problem in clinical practice, especially because of »mixed dementia« cases where characteristics of AD and VaD may persist concurrently. The best clinical discriminative factors

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for VaD versus AD include step-by-step progression, prominent impairment of executive functions, Hachinski ischemic score >4, and focal neurological signs implying extensive cortical and subcortical lesions[5,6].

In this study we analyzed CSF levels of total tau protein (t-tau), tau protein phosphorylated on threonine 181 (p-tau181) and tau protein phosphorylated on serine 199 (p-tau199) in patients with AD and VaD, as well as in control, cognitively normal subjects (NC). We wanted to test the diagnostic accuracy of these markers in the CSF in differentiation of AD from VaD and NC.

Materials and Methods

Subjects

A total of 21 patients were included in this pilot study. All patients fulfilled DSM-IV criteria for dementia[7]. Among the patients who fulfilled NINCDS-ADRDA criteria for probable AD[1], those with significant white matter changes on neuroimaging and/or Hachinski ischemic score >4 were excluded from the study. Finally, twelve patients with probable AD were recruited. Nine patients fulfilled NINCDS-AIREN criteria for VaD[8]. To exclude secondary causes of dementia, during the initial work-up all patients underwent general and neurological examination, Mini Mental State Examination (MMSE), complete blood tests including electrolytes, thyroid function, albumin, levels of vitamin B_{12}, VDRL, ECG and neuroimaging (CT or MRI scan of the brain). Patients without secondary causes of dementia finally underwent lumbar puncture for CSF analysis. None of the patients was under therapy with cholinesterase inhibitors or memantine. Additionally, 12 control subjects (NC) with no evidence of the cognitive decline, who were otherwise phy- sically and mentally healthy, were included in the study. More detailed data on subjects analyzed are given in Table 1. Study participants were recruited from the Department of Neurology, Ludwig-Maximilian University, Munich, Germany, and Department of Neurology, University Hospital Centre, Zagreb, Croatia. The study was approved by both local ethical committees and the Central ethical committee of the Zagreb Medical School. All patients consented that their CSF can be used for scientific studies.

CSF sampling and analysis

Lumbar puncture was performed after informed consent had been obtained. CSF was taken by a routine protocol in the L3/L4 or L4/L5 intervertebral space and was always performed between 9 a.m. and 11 a.m. CSF samples were collected in propylene tubes. Determination of routine biochemical parameters (leukocyte and erythrocyte cell count, glucose, lactate, total protein concentration, IgG index, TPHA) was performed using native CSF. The rest of the CSF sample was centrifuged for 10 minutes at 10,000 g, and aliquots of the remaining supernatant were immediately frozen at –80 °C for later t-tau, p-tau181, and p-tau199 protein determination. CSF analysis was performed in the Laboratory for Developmental Neuropathology of the Croatian Institute for Brain Research, Zagreb, by using enzyme-linked immunosorbent assay (ELISA). CSF t-tau and p-tau199 levels were determined by using Biosource International (Camarillo, CA, USA) ELISA kits, whereas the levels of t-tau were determined by using the ELISA kit Innotest hTau-Ag (Innogenetics NV, Ghent, Belgium). All three tests were carried-out according to the manufacturers' protocols.

TABLE 1
DEMOGRAPHIC CHARACTERISTICS OF GROUPS STUDIED WITH MMSE SCORES AND CSF T-TAU, P-TAU181 AND P-TAU199 LEVELS

	Age, Mean±SD (Range), y	Gender, W vs. M, No	MMSE, mean±SD (Range)	t-tau, median (25th–75th percentile), pg/mL	p-tau181, median (25th–75th percentile), pg/mL	p-tau199, median (25th–75th percentile), pg/mL
AD (n=12)	66.6 ± 8.1 (25.8)	6 vs. 6	18.3 ± 5.7 (17)	725 (489.5–1125)	77 (69.3–110.3)	58.5 (52.3–68)
NC (n=12)	60.4 ± 10 (40.2)	5 vs. 7	29.5 ± 0.8 (2.0)*	192 (94.5–215)*	50.5 (37.5–58.3)*	50 (46–54.8)#
VaD (n=9)	72.6 ± 5.9 (18.5)	4 vs. 5	17.35 ± 4.4 (13.0)	280 (197–310)* ¶	43 (25.5–68)*	49 (43.5–51.5)*

AD – Alzheimer's disease, VaD – vascular dementia, NC – normal controls, MMSE – Mini Mental Status Examination, T-tau – total tau, P-tau181 – tau phosphorylated at threonine 181, P-tau199 = tau phosphorylated at serine 199, * $p<0.001$ vs. AD, # $p<0.008$ vs. AD, ¶ $p<0.028$ vs. NC

TABLE 2
CORRELATION OF MMSE SCORES TO CSF T-TAU, P-TAU181 AND P-TAU 199 LEVELS IN ALL GROUPS OF SUBJECTS (AD, VAD AND NC)

Group	MMSE	R_s (t-tau)	R_s (p-tau181)	R_s (p-tau199)
AD (n=12)	18.3 ± 5.7	–0.204	0.018	0.025
NC (n=12)	29.5 ± 0.8	0.168	–0.565	–0.009
VaD (n=9)	17.35 ± 4.4	–0.252	–0.360	0.377

R_s=correlation coefficient, *$p<0.05$

Statistical analysis

Obtained data on CSF t-tau, p-tau181, and p-tau199 were presented as medians with 25th and 75th percentiles because the values were not normally distributed. Levels of total tau and phospho-tau markers were compared between groups using nonparametric Kruskal-Wallis ANOVA (as a test for the overall group differences), followed by the Mann-Whitney U test for pairwise comparisons. Cut-off levels of all of the markers used in the study were derived from the receiver operating characteristic (ROC) curve analysis, when the sum of sensitivity and specificity was maximized. Sensitivity and specificity were given with respective 95% CI. Additionally, areas under the curve (AUC) were calculated. The effect of MMSE score were tested separately in each diagnostic group by the Spearman's rank correlation coefficient (R_s). Statistical analysis was performed using SPSS v.12.0.1. P values less than 0.05 were considered statistically significant.

Results

Study cohort

Demographic data of the subjects included in the study are presented in Table 1. When comparing mean age of participants no significant differences were found between groups. The relationships between MMSE scores and t-tau, p-tau181 and p-tau199 were analyzed. None of the parameters showed a significant correlation with MMSE score as shown in Table 2.

CSF biomarker levels

All CSF biomarkers analysed in the study (t-tau, p-tau181, p-tau199) are illustrated in detail in the box plots in Figures 1, 2 and 3, with values expressed as medians with 25th and 75th percentile (also listed in Table 1). As shown in Table 1, the median levels of all three biomarkers (t-tau, p-tau181, and p-tau199) were significantly higher in AD group versus VaD and NC groups, whereas comparison of VaD and NC revealed that only t-tau reached statistical significance.

Sensitivity and specificity of the CSF markers

Sensitivity and specificity levels, cut-off values, AUC and p values for paired comparisons (AD versus VaD and AD versus NC) of a single marker are shown in Table 3. Specificity levels for all three analyzed biomarkers, when sensitivity was set at 85% or higher, are shown in Figure 4. Additionally, ROC curves of all biomarkers are shown in Figure 4 (paired comparison between AD versus VaD) and Figure 5 (paired comparison between AD versus NC). ROC analysis in our study revealed that CSF t-tau differentiates between AD and VaD with sensitivity and specificity of 100% when the cut-off level was set at 350 pg/mL. P-tau181 differentiates between the same groups with a sensitivity of 77.8% and a specificity of 91.7% when the cut-off level was set at 64.5 pg/mL, whereas p-tau199 has a sensitivity of 88.9% and a specificity of 75% when the cut-off level was set at 52.5 pg/mL. When comparing AD and NC groups, t-tau shows a sensitivity of 100% and a specificity of 91.7% with a cut-off level set at 390 pg/mL; p-tau181 has a sensitivity of 83.3% and a

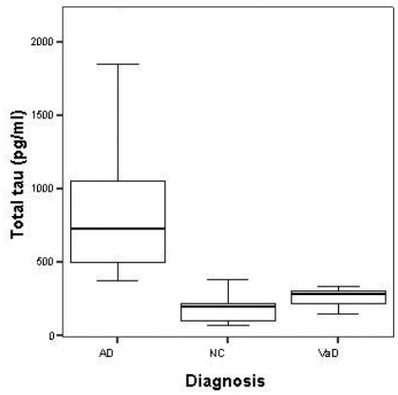

Fig. 1. Levels of CSF t-tau proteins in patients with AD, VaD and NC subjects. Data are presented as box plots. Boxes represent the 25th, 50th (median) and 75th percentiles.

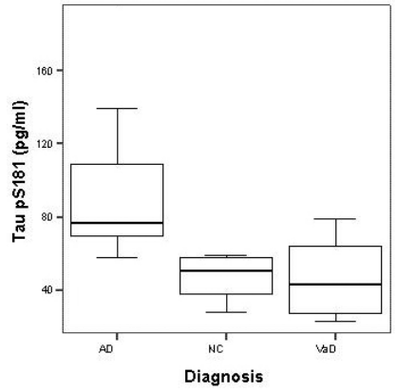

Fig. 2. Levels of CSF p-tau181 protein in patients with AD, VaD and NC subjects. Data are presented as box plots. Boxes represent the 25th, 50th (median) and 75th percentiles.

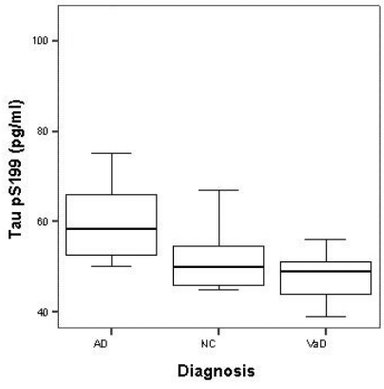

Fig. 3. Levels of CSF p-tau199 protein in patients with AD, VaD and NC subjects. Data are presented as box plots. Boxes represent the 25th, 50th (median) and 75th percentiles.

TABLE 3
DISCRIMINATIVE VALUES OF CSF MARKERS BETWEEN GROUPS

	Cut-off (pg/mL)	Sensitivity (%)	Specificity (%)	AUC	P-value
AD vs. VaD					
t-tau	350	100	100	1.0	<0.0001
p-tau181	64.5	77.8	91.7	0.89	0.003
p-tau199	52.5	88.9	75	0.91	0.002
AD vs. NC					
t-tau	390	100	91.7	0.99	<0.0001
p-tau181	62	83.3	91.7	0.89	0.001
p-tau199	55.5	83.3	66.7	0.92	0.009

AUC = area under curve

specificity of 100% with a cut-off level of 62 pg/mL; and p-tau199 has a sensitivity of 83.3% and a specificity of 66.7% with a cut-off level of 55.5pg/mL. P-values for all these comparisons and different tau markers were statistically significant ($p<0.05$).

Discussion

In the present study, we analyzed the diagnostic accuracy of three different CSF markers (t-tau, p-tau181, and p-tau199) in differentiation of patients with AD versus VaD and NC. We did not find any significant correlation between these biomarkers and MMSE scores. Previously, there were variable reports of correlation between MMSE score and various biomarkers in CSF, from strong[9,10] to insignificant correlations[11,12]. Although made on a limited sample of subjects, our results nevertheless showed significantly higher levels of all three markers analyzed in patients with AD compared to those with VaD and NC. Median levels of all three CSF markers in our study were comparable to those from previous studies. We also analyzed the discriminative power of each marker. For accurate interpretation of the results, we used recommendations of a consensus report for useful (ideal) biomarkers of AD, and therefore determined specificity levels after the sensitivity levels were set at 85% or higher[13]. According to the consensus report, ideal markers in these conditions should yield a specificity of at least 75% to 85%. In our study only t-tau satisfied these recommendations for both differentiating AD versus VaD (with specificity of 100%) and AD versus NC (with specificity of 91.7%). Additionally, p-tau199 satisfied these criteria for differenti-

TABLE 4
DISCRIMINATIVE POWER BETWEEN GROUPS WHEN SENSITIVITY WAS SET ON 85% OR HIGHER

	Specificity (%)
AD vs. VaD	
t-tau	100
p-tau181	58.3
p-tau199	75
AD vs. NC	
t-tau	91.7
p-tau181	41.7
p-tau199	50

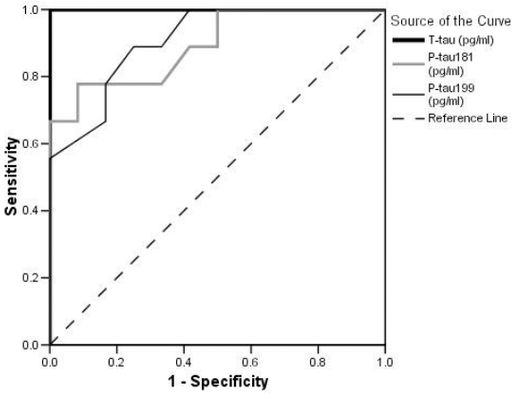

Fig. 4. ROC curves for CSF t-tau, p-tau181 and p-tau199 when patients with AD were compared to VaD patients. Diagonal line indicates an area of 50%, indicating no difference in marker levels between groups.

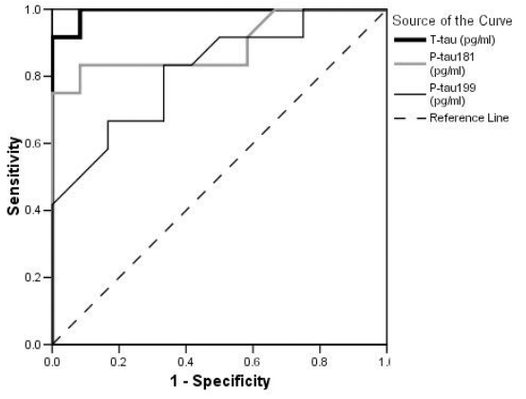

Fig. 5. ROC curves for CSF t-tau, p-tau181 and p-tau199 when patients with AD were compared to NC subjects. Diagonal line indicates an area of 50%, indicating no difference in marker levels between groups.

ating AD and VaD with specificity of 75% (as shown in Table 4).

So far, only one study showed high discriminatory power in distinguishing between AD and VaD when using the ratio of CSF amyloid β42 (the main component of amyloid plaques) and p-tau181[14]. A prior study which was done using CSF t-tau and amyloid β42 levels, yielded specificity of only 48% when comparing these two groups[12]. Levels of p-tau199 showed to be highly specific in differentiating AD patients from non-AD group, although differentiation of AD and VaD was not investigated[15].

A recent neuropathological review on assessment of the cognitive impact of AD and vascular burden in the aging brain stressed the relative weakness of clinicopathological correlations[16]. To bridge this gap from clinical criteria to neuropathological findings, many hopes are currently being directed towards CSF biomarkers. In this context, we report here that levels of t-tau and p-tau199 may represent robust markers for differentiating AD from VaD. Levels of p-tau181 did not yield adequate level of specificity to satisfy consensus recommendations. Since AD and VaD are the most common dementias, these results, although from a small series, provide an insight on how to diagnose these disorders in the early phase of their progression, which also facilitates the patients' therapeutic management. However, additional studies in larger series, including mixed cases, are needed to further explore the potential of these and other CSF markers in early detection and tracking of primary causes of cognitive impairment in the elderly.

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MARKERI IZ CEREBROSPINALNE TEKUĆINE U DIFERENCIJALNOJ DIJAGNOZI ALZHEIMEROVE BOLESTI I VASKULARNE DEMENCIJE

SAŽETAK

Alzheimerova bolest (AD) i vaskularna demencija (VaD) dva su najčešća uzroka demencije u starih ljudi. Uslijed nedostatka osjetljivosti i specifičnosti kliničkih dijagnostičkih kriterija ova se dva poremećaja još uvijek teško razlikuju u praksi. Novija saznanja iz područja molekularne i stanične biologije ukazuju da bi korištenje markera iz cerebrospinalne tekućine (CSF) moglo unaprijediti ranu detekciju i diferencijalnu dijagnozu AD. Naš je cilj u ovom istraživanju bio odrediti dijagnostičku preciznost triju markera iz CSF: ukupnog tau proteina (t-tau), tau proteina fosforiliranog na treoninu 181 (p-tau 181) i tau proteina fosforiliranog na serinu 199 (p-tau199). Upotrebom komercijalno dostupnih ELISA kitova, analizirali smo razine t-tau, p-tau181 i p-tau199 u 12 pacijenata s dijagnozom vjerojatne AD, 9 pacijenata s VaD i 12 normalnih kontrola (NC). Medijane vrijednosti razina svih triju markera bile su značajno više u skupini

pacijenata s vjerojatnom AD u odnosu na VaD i NC. Ipak, kada je prilikom diferenciranja AD od VaD razina osjetljivosti postavljena na 85% ili više, samo su t-tau i p-tau199 zadovoljili međunarodno usuglašene preporučene vrijednosti (specifičnost veću od 75%). Zaključno, naši preliminarni podaci na manjoj grupi odabranih ispitanika ukazuju da su t-tau i p-tau199 iz CSF korisni markeri za rano diferenciranje AD od VaD.