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CSF biomarkers correlate with cerebral blood flow on SPECT in healthy elderly

Running title: CSF and CBF in healthy elderly

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Abstract

Background: The preclinical patterns of biological markers for Alzheimer's disease (AD) in vivo needs further exploration. The aim of this study was therefore to investigate CSF biomarkers, regional cerebral blood flow (rCBF) and cognitive performance in cognitively healthy older individuals.

Method: Within a two weeks period, 32 cognitively healthy older individuals underwent CSF analysis, rCBF measurement, and cognitive testing. The CSF was analysed for β-amyloid$_{1-42}$ (Aβ42), total tau protein (T-tau), and hyperphosphorylated tau protein (P-tau). The rCBF results were analysed with SPM (statistical parametric mapping) to investigate rCBF covariance with the other measurements.

Results: High CSF P-tau and T-tau levels correlated with decreased rCBF in the right superior posterior medial frontal lobe whereas high CSF P-tau levels also correlated with increased rCBF in the left fronto-temporal border zone area. No significant covariance was seen between rCBF with CSF Aβ42. Neither CSF P-tau and T-tau levels nor rCBF in the current right frontal and left posterior locations were associated with cognitive performance.

Conclusions: Our findings suggest a possible correlation between tau pathology and blood flow abnormalities in individuals without any overt cognitive symptoms. An association with AD development is possible but other explanatory mechanisms cannot be excluded.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder with a long preclinical, asymptomatic phase [1]. There is increasing evidence proposing that biological markers such as CSF biomarkers with good accuracy predict incipient AD and hence several recent published guidelines and research criteria have upgraded the value of CSF biomarkers in AD prediction and discrimination [2-4].

β-Amyloid$_{1-42}$ (Aβ42), Aβ42/40 ratio, hyperphosphorylated tau protein (P-tau) and total tau protein (T-tau) are currently the biomarkers in CSF with the highest predictive ability for AD, which increase if they are used in combination [5-10]. CSF biomarkers may also precede cognitive decline however their preclinical predictive ability still needs further exploration [11-16].

Beside changes in CSF biomarkers, biological markers of cerebral function such as regional cerebral blood flow (rCBF) change in certain patterns during AD development. A proposed sequence of the changes in rCBF is an initial reduction in the entorhinal cortex, posterior cingulate, precuneus and hippocampus. As the disease progresses reductions are in general seen in the temporal and parietal lobes with final involvement of the frontal lobes [17-20]. The early reductions predict conversion to AD in MCI subjects [21-25], and both AD-associated neuropathology pattern (Braak stages) as well as cognitive deterioration have in some studies been temporally correlated to this sequence of reductions [17,22,26].

Changes in CSF biomarkers and rCBF reflect different mechanisms in AD development. Combining the two has been shown to increase the predictive ability for AD in MCI individuals compared to using each biomarker separately [23,27]. Furthermore, Habert and colleagues have reported an association between tau protein levels and left parietal cortex hypoperfusion in AD patients [28]. This association was however not seen in another study on AD patients by Tsolaki et al [29]. In non-demented older individuals the relationship between

these biological markers is not extensively investigated. Nevertheless, Petrie and colleagues have observed an association between CSF biomarkers and hypometabolism (cerebral glucose metabolism) in areas affected early in AD development in non-demented individuals [30]. In the current study we aimed to investigate whether deviances in CSF biomarkers, rCBF and cognitive performance correlate in a group of cognitively healthy older individuals.

2. Material and methods

2.1. Subjects and study design

The included subjects were cognitively healthy, older individuals from a clinical control group at the Memory Clinic at Malmö University Hospital, Sweden. The individuals had been followed for 4.5 years and at the recruitment they hade undergone thorough examinations including medical history, somatic and psychiatric examination, CT scan and cognitive testing. In association with a clinical cognitive follow-up the participants underwent lumbar puncture and within 2 weeks rCBF measurement was also performed. The cognitive assessment included the mini-mental state examination (MMSE) [31], the Alzheimer's disease assessment scale-cognitive subscale (ADAS-cog) [32], and A Quick Test of Cognitive Speed (AQT) [33]. In the latter the participants are asked to name 40 figures in three subsets according to their colour, form and colour-form respectively while the time is monitored. The participants were included in the present study if they completed all investigations (CSF collection, rCBF measurement, and cognitive testing). They were excluded if they fulfilled the criteria for AD [34], other dementia types, or MCI [35] as well as if they performed a MMSE total score of 26 points or lower.

2.2. Cerebrospinal fluid collection and assays

Lumbar puncture was performed in the sitting position. The CSF samples were obtained in the L3/L4 or L4/L5 interspaces. After disposal of the first 1 ml of CSF, the consecutive 10 ml were collected in plastic (polypropylene) tubes to avoid absorbance of β-amyloid by the tube wall. All CSF samples were mixed gently to avoid possible gradient effects. No CSF sample contained more than 500 erythrocytes/µl. The CSF samples were centrifuged at 2,000 *g* at 4˚ C for 10 min to eliminate cells and other insoluble material, and were then immediately frozen and stored at -80˚ C pending biochemical analyses, without being thawed or refrozen. At the same time serum samples were collected. The CSF samples were analysed for T-tau, tau protein phosphorylated at threonine 181 (P-tau) and Aβ42 with xMAP technology using the INNO-BIA AlzBio3 kit (Innogenetics, Ghent, Belgium) and the same batch of reagents [36]. The CSF/serum albumin ratio was calculated for evaluation of the blood-brain barrier function.

2.3. SPECT-CT acquisition and processing

^{99m}Tc-exametazime and SPECT-CT (Siemens Symbia® T2, Siemens Medical Solutions) were used to measure the rCBF. The individuals were given an intravenous injection of 900 MBq ^{99m}Tc-exametazime (Ceretec®, GE Health Care) in a well-lit setting apart from ambient noise, resting in a supine position, awake with eyes open. Image acquisition began 30 minutes later with a total acquisition time of about 40 min. The gamma camera was equipped with low energy high-resolution collimators, rotating in a non-circular orbit, 360 degrees and recording 128 projections per detector head. The recorded images were reconstructed into a 128 x 128 x 128 voxel matrix using 3D-OSEM (Flash 3D, Siemens Medical Solution) with DEW scatter correction and attenuation correction based on the sequentially performed low dose CT measurements. The spatial resolution of the images was about 10 mm FWHM (full-width half maximum) at the centre of rotation.

Statistical parametric mapping (SPM5) (The Wellcome Trust Centre for Neuroimaging, London, UK) together with MATLAB 7.1 (The Mathworks) were used for voxel-based analyses. The SPECT images were normalized into a standard stereotactic space [37,38]. The images were then smoothed by means of an isotropic Gaussian filter (16-mm FWHM). In the statistical analysis voxels were normalized to the global level of CBF.

2.4. Statistical analysis

Statistical analysis was performed using the SPSS software (version 17.0.1 for Windows, SPSS Inc., Chicago, I11, USA) and SPM5. Analysis of covariance between the SPECT measurements versus linear measurements as CSF biomarker levels, results on cognitive tests and age was performed by entering a regressor of respective covariate. Voxels were considered significant at a threshold of $p < 0.001$, uncorrected. Clusters were considered significant at a threshold of $p < 0.05$, corrected for multiple comparisons. For anatomical labelling, the coordinates obtained were converted from MNI to Talairach space using a developed script (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach). The anatomical localization was then determined using Talairach Client [39].

Spearman rank correlation coefficient (r_s) was used to test the degree of correlation between CSF biomarkers and cognitive test results as well as the influence of age. Mann-Whitney U test was used when one of the variables was dichotomized (gender). Kruskal-Wallis test was used to test the influence of *APOE*-ε4 allele. The level of significance was set to $p < 0.05$.

3. Results

3.1. Demographics

Forty-four individuals from the clinical control group underwent cognitive assessment. Of these nine individuals did not complete all investigations and an additional three individuals

were excluded after performing 26 points or lower on the MMSE. The demographics of the included 32 participants are described in table 1.

3.2. CSF biomarkers and resting cerebral blood flow

In the SPM5 evaluation a cluster with a significant negative covariance between levels of CSF P-tau and resting CBF was found in the superior posterior right medial frontal lobe. When an automated Talairach Atlas labels data software program (Talairach Deamon) [39] was used the covariance could be more precisely located to the dorsal medial and superior frontal gyruses. The Brodmann areas most likely located at this location would be Brodmann area 6 and 8 ($p < 0.001$) (figure 1) (Table 2). A similarly located cluster with negative covariance was found for CSF T-tau levels and resting CBF in the medial frontal gyrus ($p < 0.01$), however compared with CSF P-tau it had a slightly lesser degree of significance at cluster levels. In addition a cluster with a significant positive covariance between CSF P-tau and resting CBF was found in the left fronto-temporal border zone and according to Talairach coordinates located close to the transverse and superior temporal gyrus ($p < 0.001$) (figure 1) (Table 3). In contrast to CSF P-tau and T-tau, no significant clusters with covariance were found between CSF Aβ42 levels and resting CBF. No additional effect was seen if the combination of CSF biomarkers in ratios (P-tau/Aβ42 and T-tau/Aβ42) were used.

Recently a multicenter study published in JAMA® on CSF biomarkers in MCI proposed the optimal cut-off levels for predicting incipient AD (P-tau > 52 ng/l and Aβ42 < 482 ng/l) [5]. These optimized P-tau cut-off levels were used in order to dichotomize the individuals into groups with high versus low CSF P-tau levels. If this was performed, rCBF differences between the groups were seen with the same pattern and in the same brain areas as was seen when continuous CSF P-tau levels were used. Dichotomization with combined P-tau and Aβ42 cut-off levels did not result in any rCBF differences between the groups.

3.3. Association of biomarkers with cognition and demographic factors

Neither CSF P-tau nor T-tau was related to any of the cognitive measurements (MMSE, ADAS-cog 85 total score, ADAS-cog delayed word recall, and AQT subtests) in this study. Instead, CSF Aβ42 was associated with cognitive performance in this study population, as previously published [40]. No clusters with significant covariance were found with the SPM analysis between any of the cognitive measurements and resting CBF in the locations with CSF P-tau covariance. Similarly, age did not have any significant covariance with resting rCBF in any of the locations with CSF P-tau covariance. Neither age nor gender correlated with any CSF biomarker or with cognitive performance. Furthermore, occurrence of *APOE*-ε4 allele was associated with a higher CSF T-tau level ($\chi^2 = 9.022$, $p < 0.05$) (as previously published [40]) but not with CSF P-tau, CSF Aβ42 or cognitive performance.

4. Discussion

In the current study high CSF P-tau and T-tau levels were associated with decreased rCBF in the right posterior medial frontal cortex (MFC) in a group of cognitively healthy older individuals and high CSF P-tau levels also correlated with increased rCBF in the left fronto-temporal area. Individuals with AD-indicative CSF P-tau levels presented the same rCBF changes in the same areas when compared to those without AD-indicative levels. CSF P-tau levels had a slightly stronger covariance than CSF T-tau with rCBF in the right posterior MFC.

Tau is a protein binding to microtubule in the axons. When hyperphosporylated it is detached from the microtubule leading to a destabilization and compromised axonal transport. Hyperphosphorylated tau is also the main component of the neurofibrillary tangles seen in

AD. The T-tau levels in CSF appears to represent a general damage to cortical axons whereas the P-tau levels are related to the hyperphosphorylation and hence more specific to AD than T-tau [41]. In the current study both CSF T-tau and P-tau show similar covariance with rCBF suggesting a possible relationship with AD development. The stronger covariance with P-tau in specific further strengthens this possibility. In contrast, CSF Aβ42 did not show covariance with rCBF in the current study, which could contradict such a relationship. However, previously only CSF tau, and not Aβ42, has correlated with hypoperfusion or hypometabolism [28,30]. Increasing evidence proposes that Aβ biomarkers abnormalities precede biomarkers of neurodegeneration such as CSF tau, hypometabolism, blood flow abnormalities and brain atrophy in AD development [41,42]. This possible temporal difference between changes in CSF biomarker could perhaps explain why CSF Aβ42 did not show covariance with rCBF when CSF T-tau and P-tau did.

CSF P-tau and T-tau were related to decrease in right-sided superior posterior medial frontal lobe CBF. Although, the figure resolution and template compatibility in the SPM5 statistical evaluation prohibits very precise estimation of regions involved, the Talairach coordinates of the covariance indicate engagement of Brodmann area 6 and 8. Among other functions, the superior posterior MFC is partly responsible for executive function, i.e. direct executive control [43,44]. Executive deficits are generally associated with frontal degenerative disorders and vascular dementia [45], however a subtle but measurable executive deficit is also observed in early AD development [46,47].

The current study proposes an additional covariance in the left fronto-temporal region between increased CSF P-tau protein levels and increased rCBF. This covariance appears to be located close to the superior and transverse temporal gyrus cortex, which is a location that

previously has been reported to have rCBF changes early in AD development [19,48]. However, rCBF in this area has also been suggested to be relatively spared in AD [26].

Any interpretation of the findings in the current study should be made with caution. There are several aspects that could argue against a relationship with AD development. Hence, the covariance could reflect other underlying neuropathologic or aging mechanisms leading to hypo-/hyperperfusion and tau-hyperphosphorylation.

The first argument is that the early rCBF changes previously have been located to the posterior cingulate, the precuneus, the hippocampus, and the temporal and parietal cortex [17-20]. In addition, the associations between CSF tau and decreased rCBF in AD and between CSF tau and hypometabolism in non-demented individuals have also had a posterior orientation [28,30]. Hence, the findings in the current study differ in their locations. However, if CBF studies in early AD are studied in detail, it becomes evident that rCBF changes in many cases also have involved increases and decreases in frontal lobe cortex [21,24,26,29,48-50]. These areas have been reported as secondary findings and not highlighted as the more expected, concurrent posterior changes. In fact, several studies have reported rCBF decreases in the same frontal lobe location as in the current study, i.e. the superior posterior MFC [21,26,29,49,50]. In one of these studies Caffarra et al reported that all studied MCI subtypes had decreased rCBF in these regions, including individuals with dysexecutive MCI who also had the highest conversion rate to AD [50].

A second argument is the co-variance with an increase in rCBF observed. However, associations between neurodegenerative disease and relative increases in rCBF have been observed previously [21,24,51,52]. One plausible explanation, that has been put forward, could be that the increased rCBF might be an attempt to preserve neuronal function in a situation with neuronal stress and deterioration due to progressive neuropathology. Hence,

this compensatory mechanism would temporarily maintain cognitive functions until eventually the neuronal loss become insurmountable [21,51,52].

A third argument is that the covariance with rCBF is not located to the areas early affected with AD neuropathology. However, this is not exclusive for the current study but is rather the case in almost all rCBF studies investigating early stages of AD. It has therefore been suggested that rCBF changes in early AD are primarily a remote effect, which is further supported by the initial rCBF changes appearing to be reversible [18,19,53]. The loss of neuron cells in one region would lead to a break in neuronal connectivity with and/or deafferentation towards another region with rCBF decrease or increase as the result [17-20,53]. In animal studies for example, Aβ has had toxic properties for cholinergic neurons, which may lead to blocking of the neurogenic vascular control [54]. In addition, Aβ has 'vasotoxic' effects *in vitro* possibly further affecting cerebral autoregulation [54].

It should be emphasized that the exact mechanism behind hypoperfusion in AD development and whether it is a cause, an effect or both is still not fully established. Hence, despite unexpected findings the possibility of a relationship with AD cannot be excluded but at the same time not verified.

It needs to be stated that the covariance seen in this study does not necessarily imply that the participants' rCBF decreases or increases are pathologic, merely that the variability in CSF tau protein is significantly related to the variability in relative rCBF levels in certain regions. Moreover, a possible source of error is that the rCBF measurement could be modified by a partial volume effect due to cortical atrophy. However, the brain areas with covariance in the present study are located away from both the interhemispheric fissure and the fissure Sylvius and hence any cortical atrophy would not likely affect rCBF measurements in these areas.

Another possible source of error is the relative small number of participants in relation to the expected small differences in the investigated biomarkers and the cognitive screening tests. The study could therefore risk type-II bias, i.e. not detecting an existing difference.
It would have been desirable to have cognitive follow-up data of the participants in order to more thoroughly investigate the relationship with dementia in general and AD in specific. It would also have been preferable to have specific cognitive assessments of executive function for the individuals, since the covariance with hypoperfusion was located to brain areas involved in this cognitive domain.

5. Conclusions

In the current study increase and hyperphosphorylation of CSF tau were associated with right-sided frontal hypoperfusion and a paradoxal left-sided posterior hyperperfusion in cognitively healthy older individuals. The findings suggest a possible correlation between tau pathology and blood flow abnormalities in individuals without any overt cognitive symptoms. An association with AD development is possible but other explanatory neurodegenerative mechanisms cannot be excluded. Future studies as well as future follow-ups of the current study sample will be needed to establish the relevance of the observed correlation.

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6.1. Disclosure statement

The authors declare no conflicts of interest. The study was approved by the regional ethics committee at Lund University, Lund, Sweden and the participants gave their written consent to participate.

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Tables

Table 1

Demographics, cognitive assessments, and CSF biomarker levels

	Baseline
Demographics	
Number	32
Age, mean (±SD)	76.6 (± 8.4)
Age, range	65 - 99
Gender (F/M)	22 / 10
APOE-ε4 heterozygote, N (homozygote)	8 (1)
Cognition	
MMSE score, mean (±SD)	28.7 (± 1.2)
MMSE score range	27 - 30
ADAS-cog85 score, mean (±SD)	7.5 (± 4.2)
ADAS-cog85 score range	1 – 19
AQT (colour-form-colour/form), mean (±SD)	24 (±4) – 33 (±6) – 65 (±14)
CSF biomarkers, median (25th - 75th pcntle)	
CSF T-tau, ng/l	363 (245 – 507)
CSF P-tau, ng/l	60 (43 – 80)
CSF β-amyloid1-42, ng/l	644 (504 – 781)

MMSE = Mini-Mental State Examination, ADAS-cog85 = Alzheimer's Disease Assessment Scale-cognitive subscale with total score of 85 points, AQT =A Quick Test of cognitive speed, CSF = Cerebrospinal Fluid

Table 2

Statistics from SPM analysis showing clusters with statistically negative covariance between rCBF and CSF P-tau levels. The anatomical localization was determined using Talairach Client [39]. $p < 0.05$ (cluster-level, corrected for multiple comparisons).

Cluster-level			Voxel-level			Coordinates			Region
$p_{corrected}$	Cluster size (k_E)	$p_{uncorrected}$	T	Z_E	$p_{uncorrected}$	x	y	z	
0.000	**1366**	**0.000**	**5.11**	**4.34**	**0.000**	**8**	**20**	**50**	R frontal lobe, superior gyrus, (close to BA 8)
			4.61	4.01	0.000	6	-8	59	R frontal lobe, medial gyrus, BA 6
			4.32	3.81	0.000	2	-13	50	R frontal lobe, medial gyrus, (close to BA 6)

R = right. BA = Brodmann area.

Table 3

Statistics from SPM analysis showing clusters with statistically positive covariance between rCBF and CSF P-tau levels. The anatomical localization was determined using Talairach Client [39]. $p < 0.05$ (cluster-level, corrected for multiple comparisons).

Cluster-level			Voxel-level			Coordinates			Region
$p_{corrected}$	Cluster size (k_E)	$p_{uncorrected}$	T	Z_E	$p_{uncorrected}$	x	y	z	
0.000	**1448**	**0.000**	**4.59**	**3.99**	**0.000**	**-36**	**-30**	**10**	L temporal lobe, sup temporal cortex, w-m (close to transverse temporal gyrus, BA 41)
			4.52	3.95	0.000	-42	-33	19	L sub-lobar, insula, w-m (close to sup temporal gyrus BA 41)
			4.52	3.94	0.000	-26	-8	29	L frontal lobe, sub-gyral, w-m

L = left. Sup = superior. w-m = white matter. BA = Brodmann area.

Figure legends

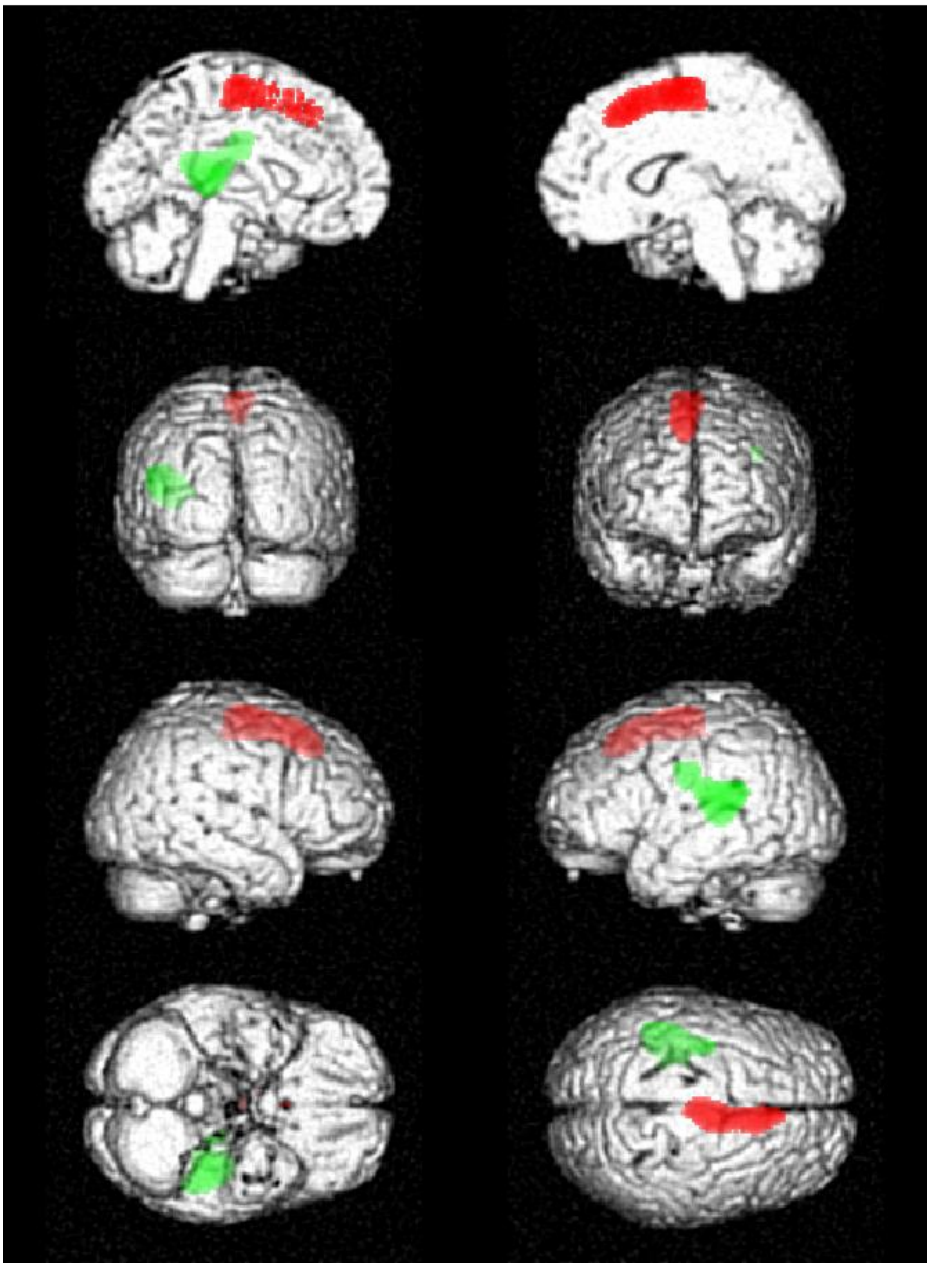

Figure 1

SPM analysis showing clusters with significant covariance between SPECT-assessed CBF measurements versus CSF P-tau levels in cognitively healthy elderly. Three-dimensional renderings of a brain including a sagittal interhemisphere slice are used with dual-colour display showing negative covariance (red) and positive covariance (green).