

# Cerebrospinal Fluid Neurochemical Phenotypes in Vascular Dementias: Original Data and Mini-Review

Mirko Bibl<sup>a</sup> Brit Mollenhauer<sup>b</sup> Hermann Esselmann<sup>c</sup> Michael Schneider<sup>d</sup>  
Piotr Lewczuk<sup>c</sup> Volker Welge<sup>e</sup> Martin Gross<sup>f</sup> Peter Falkai<sup>e</sup>  
Johannes Kornhuber<sup>c</sup> Jens Wiltfang<sup>a</sup>

<sup>a</sup>Klinik für Psychiatrie und Psychotherapie der Rheinischen Kliniken Essen, Universität Duisburg-Essen, Essen, <sup>b</sup>University of Göttingen, Paracelsus-Elena Klinik, Kassel, <sup>c</sup>Department of Psychiatry and Psychotherapy, University of Erlangen-Nuremberg, Erlangen, <sup>d</sup>Zentralinstitut für Seelische Gesundheit, Department of Biostatistics, Mannheim, <sup>e</sup>Department of Psychiatry, University of Göttingen, Göttingen, and <sup>f</sup>Neurologische Klinik Bad Aibling, Bad Aibling, Germany

## Key Words

Alzheimer's dementia · Vascular dementia · Cerebrospinal fluid · Amyloid- $\beta$  peptides · Biomarker

## Abstract

**Background/Aims:** The study evaluated the patterns of cerebrospinal fluid (CSF), amyloid- $\beta$  (A $\beta$ ) peptides, total tau and phospho-tau among Alzheimer's disease (AD) and vascular dementias (VAD). **Methods:** A $\beta$ -SDS-PAGE immunoblot and commercially available ELISAs were applied to the CSF analysis of 52 patients with probable (n = 21) and possible (n = 16) VAD, AD with cerebrovascular disease (n = 15), 30 patients with probable AD and 30 nondemented disease controls. **Results:** AD and AD with cerebrovascular disease displayed a similar neurochemical phenotype in contrast to nondemented disease controls and probable VAD with regard to tau, p-tau, A $\beta$ 1–40<sup>ox</sup> and A $\beta$ 1–42%. Possible VAD displayed AD-like changes only for A $\beta$ 1–40<sup>ox</sup> and A $\beta$ 1–42%. **Conclusion:** CSF neurochemical phenotypes sufficiently discriminate probable AD and VAD from each other, but their diagnostic value is limited in case of no clear-cut clinical ap-

pearance, such as possible VAD. Conversely, CSF A $\beta$  peptides and p-tau levels may help estimate the involvement of AD-like pathophysiological pathways in VAD subgroups.

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## Introduction

Besides Alzheimer's disease (AD) and dementia with Lewy bodies, vascular dementias (VAD) are one of the most common forms of dementia in the elderly. VAD represents a syndrome of a large etiological heterogeneity and a broad spectrum of clinical appearance. The clinical discrimination between VAD and AD is a routine diagnostic challenge and often has a profound impact on the therapeutic strategy applied. Although clinical criteria have been established for VAD [1, 2] and AD [3] to aid this crucial question, there is still a large overlap of symptoms and courses of disease. Likewise, overlapping vascular lesions and AD pathology are frequent neuropathologic findings [4] and both factors may contribute to a progressive loss of cognitive functions. However, in case of ad-

vanced AD, the effects of AD pathology on the cognitive status seem to increasingly overcome the impact of vascular factors [5].

The 2 neuropathological hallmarks of AD are amyloid plaques and neurofibrillary tangles. Amyloid plaques mainly consist of carboxyterminally elongated forms of amyloid- $\beta$  ( $A\beta$ ) peptides [6]. These peptides are cleaved from the transmembrane amyloid precursor protein by 2 enzymes,  $\beta$ - and  $\gamma$ -secretase [7]. Distinct  $\gamma$ -secretase activities are hypothesized to be responsible for the generation of either carboxyterminally truncated or elongated  $A\beta$  peptides as referenced to  $A\beta_{1-40}$  [8]. The major constituent of neurofibrillary tangles is tau, a 68-kDa microtubule-associated phosphoprotein that undergoes enhanced phosphorylation and aggregates into paired helical filaments in AD [9].

Biomarkers like  $A\beta_{1-42}$ , tau and phosphorylated tau have proved to be of debatable diagnostic value for the detection of AD among VAD [10].  $A\beta$  peptides other than  $A\beta_{1-42}$  have shown differential diagnostic value for AD among other neurodegenerative disorders [11–14]. Here, we address the question whether AD and VAD of different clinical phenotypes may be characterized by distinct neurochemical phenotypes in cerebrospinal fluid (CSF).

## Patients and Methods

A total of 112 consecutive CSF samples that had been referred to our laboratory between 2000 and 2004 from wards and the dementia outpatient clinic of the University of Göttingen and the University of Erlangen were prospectively investigated. The handling of CSF followed a standardized protocol according to previously published data [15]. The diagnosis was established by a neurologist and a psychiatrist, both very experienced in the clinical differential diagnosis of dementias. Both investigators were blinded to the neurochemical outcome measures. If possible, neuropsychological assessment, Mini-Mental State Examination (MMSE) according to Folstein et al. [16], was performed on patients suffering from cognitive impairments at the time of lumbar puncture.

Investigations were carried out with the informed consent of the patients or their next of kin. The study was conducted under the guidelines of the Declaration of Helsinki [17] and approved by the ethics committees of the Universities of Göttingen and Erlangen-Nuremberg.

### Patients

We investigated 52 patients with VAD (20 men and 32 women) according to DSM IV and the NINDS-AIREN criteria [2]. The patients were further characterized by Hachinski's ischemic scale in its modified version according to Fischer et al. [18] and patients with a score less than 6 were excluded. All included patients exhibited typical signs of relevant cerebrovascular disease (CVD) in neuroimaging (computerized tomography, magnetic resonance

imaging). Subtypes of VAD were determined in accordance with the NINDS-AIREN criteria [2] and with the classification of sporadic vascular impairment [19]. Twenty-one and 16 patients displayed the clinical course of probable and possible VAD, respectively. Patients with a CVD that fulfilled the criteria of NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association) for possible AD were classified as AD with CVD ( $n = 15$ ).

Thirty patients (11 men and 19 women) with probable AD according to DSM IV and the NINCDS-ADRDA criteria [3] were investigated for comparison. AD patients displayed no signs of relevant CVD in either computerized tomography or magnetic resonance imaging, except for mild white matter lesions.

The 30 nondemented disease controls (NDC) (14 men and 16 women) comprised 15 patients suffering from depression, and 8 patients with peripheral and 7 with central neurological diseases. In detail, peripheral neurological patients were diagnosed as having polyneuropathy ( $n = 3$ ) and peripheral facial nerve palsy ( $n = 3$ ), facial hemispasm ( $n = 1$ ) and borreliosis without central nervous affection. Central neurological diseases included normal pressure hydrocephalus ( $n = 4$ ), Korsakow's syndrome ( $n = 1$ ), Wilson's disease ( $n = 1$ ), glioblastoma ( $n = 1$ ) and paraneoplastic cerebellar inflammation ( $n = 1$ ). All patients with cognitive complaints ( $n = 14$ ) underwent MMSE, none displayed a score below 26. The cognitive impairments of all depressive patients improved after antidepressant medication, except for 1 patient showing persistent visuospatial deficits.

### Preanalytical Treatment of CSF for $A\beta$ -SDS-PAGE Immunoblot, $A\beta_{1-42}$ , Tau and p-Tau ELISA

The preanalytical handling of all included CSF samples followed a standardized protocol according to previously published data [15].

Briefly, CSF drawn from patients by lumbar puncture was sampled in polypropylene vials and centrifuged (1,000 g, 10 min, 4°C). Aliquots of 200  $\mu$ l were kept at room temperature for a maximum of 24 h before storage at -80°C for subsequent  $A\beta$ -SDS-PAGE/immunoblot (amyloid beta sodium dodecyl sulphate polyacrylamide gel electrophoresis with Western immunoblot) analysis and p-tau ELISA, respectively. Freezing of samples was conducted by directly cooling 200  $\mu$ l of CSF in polypropylene cups down to -80°C. Neither an intermediate temperature stage nor shock freezing was performed. There was no additional freeze and thaw cycle before analysis.

CSF for  $A\beta_{1-42}$  and tau ELISA analysis was stored at +4°C and analyzed within 2 days. There was no impact of storage time before freezing as controlled for up to 48 h on  $A\beta$  peptide or tau protein levels.

### $A\beta$ -SDS-PAGE/Immunoblot

For direct analysis of different  $A\beta$  peptide species, 10  $\mu$ l of unconcentrated CSF were boiled in a sample buffer for SDS-PAGE, and  $A\beta$ -SDS-PAGE/immunoblot was conducted as published elsewhere [15, 20].

CSF samples of each individual patient were run as triplicates at minimum and each gel carried a 4-step dilution series of the synthetic  $A\beta$  peptides  $A\beta_{1-37}$ ,  $A\beta_{1-38}$ ,  $A\beta_{1-39}$ ,  $A\beta_{1-40}$  and  $A\beta_{1-42}$ . Synthetic peptides  $A\beta_{1-38}$ ,  $A\beta_{1-40}$  and  $A\beta_{1-42}$  were obtained from Bachem (Bubendorf, Switzerland),  $A\beta_{1-37}$  and

**Table 1.** Absolute abundances of tau and p-tau181, absolute and relative abundances of A $\beta$  peptides in the CSF of the diagnostic groups

Diagnosis	NDC (n = 30)	AD (n = 30)	AD with CVD (n = 15)	Possible VAD (n = 16)	Probable VAD (n = 21)
Age, years	64.27 $\pm$ 9.34	72.10 $\pm$ 9.38	68.87 $\pm$ 6.05	74.00 $\pm$ 6.42	70.10 $\pm$ 6.87
MMSE	28.76 $\pm$ 1.15	21.19 $\pm$ 4.94	19.15 $\pm$ 7.74	20.38 $\pm$ 4.93	18.64 $\pm$ 8.15
Tau, ng/ml	0.267 $\pm$ 0.220	0.591 $\pm$ 0.237	0.726 $\pm$ 0.565	0.393 $\pm$ 0.271	0.473 $\pm$ 0.356
p-tau, ng/ml	0.041 $\pm$ 0.020	0.091 $\pm$ 0.025	0.105 $\pm$ 0.062	0.049 $\pm$ 0.024	0.038 $\pm$ 0.014
A $\beta$ 1-42 ELISA, ng/ml	0.734 $\pm$ 0.324	0.437 $\pm$ 0.128	0.389 $\pm$ 0.179	0.368 $\pm$ 0.175	0.461 $\pm$ 0.252
A $\beta$ 1-37, ng/ml	0.997 $\pm$ 0.497	1.050 $\pm$ 0.496	1.236 $\pm$ 0.538	0.769 $\pm$ 0.317	0.747 $\pm$ 0.312
A $\beta$ 1-38, ng/ml	1.861 $\pm$ 0.685	2.087 $\pm$ 0.612	1.845 $\pm$ 0.581	1.494 $\pm$ 0.710	1.433 $\pm$ 0.640
A $\beta$ 1-39, ng/ml	1.140 $\pm$ 0.518	1.318 $\pm$ 0.391	1.329 $\pm$ 0.520	0.890 $\pm$ 0.401	0.922 $\pm$ 0.464
A $\beta$ 1-40, ng/ml	8.739 $\pm$ 3.101	8.988 $\pm$ 1.955	7.525 $\pm$ 1.819	6.533 $\pm$ 2.578	6.324 $\pm$ 2.210
A $\beta$ 1-40 <sup>ox</sup> , ng/ml	0.077 $\pm$ 0.039	0.154 $\pm$ 0.098	0.135 $\pm$ 0.099	0.126 $\pm$ 0.062	0.096 $\pm$ 0.036
A $\beta$ 1-42, ng/ml	1.373 $\pm$ 0.658	0.684 $\pm$ 0.349	0.840 $\pm$ 0.512	0.669 $\pm$ 0.340	1.026 $\pm$ 0.663
Sum of all analyzed A $\beta$ peptides	14.19 $\pm$ 5.070	14.28 $\pm$ 3.323	12.91 $\pm$ 3.562	10.48 $\pm$ 4.111	10.55 $\pm$ 4.207
A $\beta$ 1-37% <sup>b</sup>	6.916 $\pm$ 1.634	7.085 $\pm$ 2.275	9.235 $\pm$ 2.367	7.317 $\pm$ 1.196	7.047 $\pm$ 0.699
A $\beta$ 1-38% <sup>b</sup>	13.19 $\pm$ 2.261	14.52 $\pm$ 1.882	14.22 $\pm$ 1.840	13.94 $\pm$ 2.378	13.46 $\pm$ 1.209
A $\beta$ 1-39% <sup>b</sup>	7.941 $\pm$ 1.822	9.202 $\pm$ 1.541	10.1 $\pm$ 1.882	8.443 $\pm$ 1.818	8.533 $\pm$ 1.486
A $\beta$ 1-40% <sup>b</sup>	61.93 $\pm$ 4.502	63.36 $\pm$ 4.752	59.77 $\pm$ 5.159	62.57 $\pm$ 5.167	60.82 $\pm$ 3.344
A $\beta$ 1-40 <sup>ox</sup> % <sup>b</sup>	0.612 $\pm$ 0.371	1.118 $\pm$ 0.693	1.008 $\pm$ 0.528	1.226 $\pm$ 0.434	0.972 $\pm$ 0.378
A $\beta$ 1-42% <sup>b</sup>	9.414 $\pm$ 2.203	4.711 $\pm$ 1.493	6.341 $\pm$ 2.562	6.506 $\pm$ 2.416	9.163 $\pm$ 2.665

Values are means  $\pm$  SD. <sup>a</sup> Total A $\beta$  peptide concentration. <sup>b</sup> Percentage abundance of A $\beta$  peptides relative to the total A $\beta$  peptide concentration.

A $\beta$ 1-39 were synthesized automatically according to Janek et al. [21]. Standard preparations of synthetic A $\beta$  peptide mixture were created as described previously [15] and bands were quantified from individual blots of each patient relative to this dilution series using a charge-coupled device camera. The detection sensitivity for the 1E8 in this optimized immunoblot procedure was 0.6 pg (A $\beta$ 1-38, A $\beta$ 1-40) and 1 pg (A $\beta$ 1-37, A $\beta$ 1-39, A $\beta$ 1-42), respectively [15]. The inter- and intra-assay coefficients of variation for 80 as well as for 20 pg of synthetic A $\beta$  peptides were below 10% [15, 20].

#### ELISA for Tau Protein, p-Tau and A $\beta$ 1-42

The commercially available assays Innostest hTAU Antigen and Innostest  $\beta$ -Amyloid<sub>(1-42)</sub>, Innogenetics (Ghent, Belgium) were applied for the quantification of tau protein and A $\beta$ 1-42 levels in CSF, respectively. Tau and A $\beta$ 1-42 ELISA were performed according to previously published standard methods [22]. The ELISA for tau phosphorylated at Thr181 (p-tau) was conducted as described by Vanmechelen et al. [23]. In brief, the HT7 monoclonal antibody directed against both normal tau and phospho-tau was used as the capturing antibody. The p-tau-specific biotinylated monoclonal antibody AT270 was applied for detection. Otherwise, the same reagents were used as for the Innostest hTAU Antigen.

#### Statistical Analysis

Patient groups were characterized by mean and standard deviation. The Mann-Whitney U test was employed for evaluation

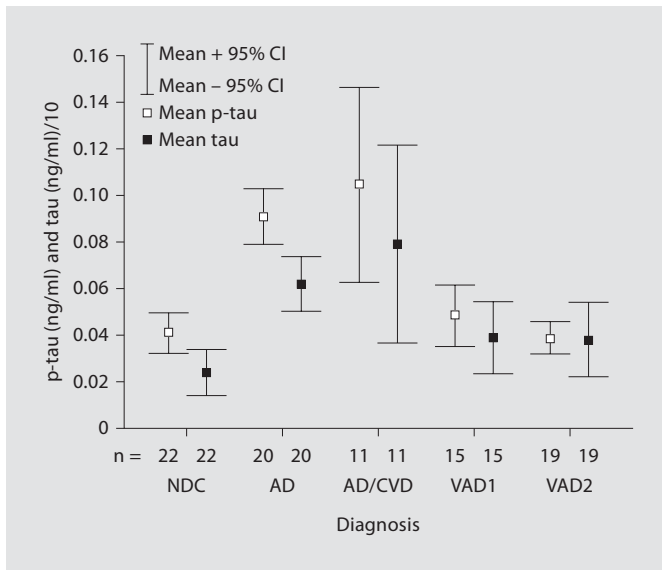
of significant differences of diagnostic groups (unpaired samples). Comparisons of multiple groups (i.e. age, MMSE) were evaluated by the Kruskal-Wallis test. The Wilcoxon signed rank test was applied for comparative evaluation of test accuracies. The 2-sided level of significance was taken as  $p < 0.05$ . The global diagnostic accuracies were assessed by the area under the receiver operating characteristic curve. Cutoff points were determined at the minimum sensitivity of 85%. The statistical software packages SPSS, version 10.0, and SAS, version 8.2, served for computations.

## Results

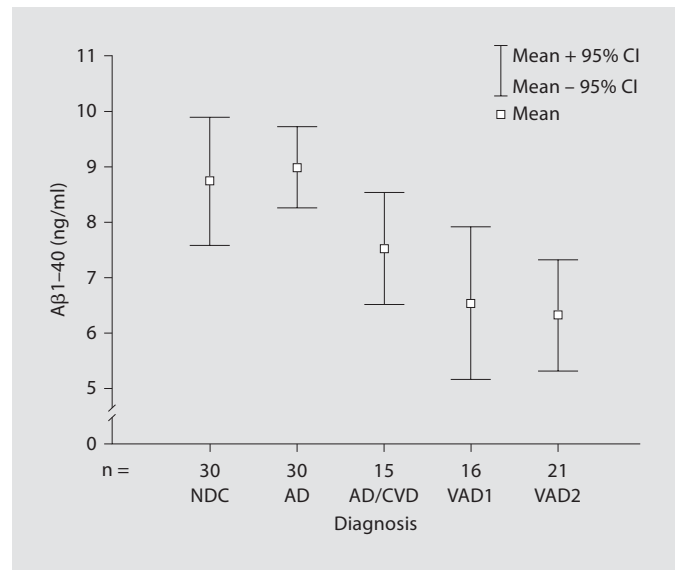
### Group Differences

The age did not significantly differ between the dementia diagnostic groups but was lower for the NDC group ( $p < 0.05$ ). The MMSE score did neither significantly differ between VAD and AD, nor among the subgroups of VAD ( $p > 0.05$ ).

Over all the groups, the regular abundance of 5 A $\beta$  peptide species aside A $\beta$ 1-42, all carboxyterminally shorter, could be detected. These were the A $\beta$  peptides A $\beta$ 1-37, A $\beta$ 1-38, A $\beta$ 1-39, A $\beta$ 1-40 and A $\beta$ 1-40<sup>ox</sup>. The latter has just recently been shown to represent an oxi-



**Fig. 1.** Mean and 95% confidence interval (CI) of p-tau181 and total tau for each diagnostic group. VAD1 = Possible VAD; VAD2 = probable VAD.



**Fig. 2.** Mean and 95% confidence interval (CI) of Aβ1-40 for each diagnostic group. VAD1 = Possible VAD; VAD2 = probable VAD.

dized and potentially  $\alpha$ -helical coiled form of Aβ1-40 [11].

Among the control group, patients with central neurological diseases exhibited lower absolute levels of Aβ1-38, Aβ1-39 and Aβ1-42 but an increased percentage abundance of Aβ1-40<sup>ox</sup> as compared to peripheral neurological diseases and depressions ( $p < 0.05$ ).

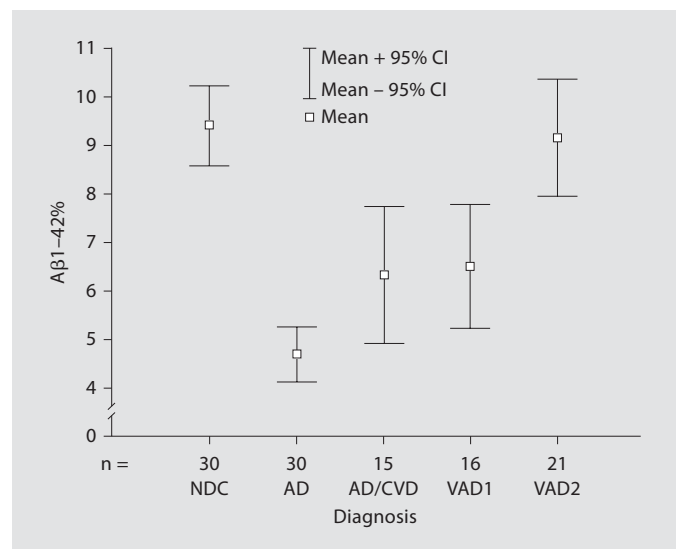
Table 1 summarizes absolute and relative protein levels. Figures 1 and 2 show the group-specific abundances of tau, p-tau and Aβ1-40, respectively. Figure 3 gives the Aβ1-42% values of each diagnostic group.

#### Neurochemical Phenotype in Probable AD

As expected, AD presented with elevated tau and p-tau levels ( $p < 0.01$ ) as compared to NDC. Aβ1-42 was decreased and Aβ1-40<sup>ox</sup> was elevated in absolute and percentage terms. In percentage terms, there were additional elevations of Aβ1-38% and Aβ1-39% ( $p < 0.01$ ).

#### Neurochemical Phenotype in Probable VAD

Probable VAD displayed lower overall Aβ peptide levels than NDC, except for Aβ1-40<sup>ox</sup>. Accordingly, the Aβ1-40<sup>ox</sup>% levels were increased in VAD ( $p = 2.6 \times 10^{-3}$ ). Additionally, the tau ( $p = 3.3 \times 10^{-2}$ ) levels and Aβ1-42 ( $p = 4.7 \times 10^{-3}$ ) as measured by ELISA were increased and diminished, respectively.



**Fig. 3.** Mean and 95% confidence interval (CI) of Aβ1-42% for each diagnostic group. VAD1 = Possible VAD; VAD2 = probable VAD.

Thus, in comparison to AD, there were common alterations in the absolute but not in the percentage Aβ1-42 levels, neither were the p-tau levels altered in VAD. It is noteworthy that the Aβ1-40 levels were reduced in probable VAD in absolute ( $p = 8.7 \times 10^{-5}$ ) and percent-

**Table 2.** The disease-specific peptide derangements of each diagnostic group in comparison to NDC

Diagnosis	AD (n = 30)	AD with CVD (n = 15)	Possible VAD (n = 16)	Probable VAD (n = 21)
Tau, ng/ml	▲▲**	▲▲**		▲*
p-tau, ng/ml	▲▲**	▲▲**		
Aβ1-42 ELISA, ng/ml	▽▽**	▽▽**	▽▽**	▽▽**
Aβ1-37, ng/ml				▽*
Aβ1-38, ng/ml				▽*
Aβ1-39, ng/ml				
Aβ1-40, ng/ml			▽▽**	▽▽**
Aβ1-40 <sup>ox</sup> , ng/ml	▲▲**	▲*	▲▲**	
Aβ1-42, ng/ml	▽▽**	▽▽*	▽▽**	▽*
Sum of all analyzed Aβ peptides			▽▽**	▽▽**
Aβ1-37% <sup>b</sup>		▲▲**		
Aβ1-38% <sup>b</sup>	▲▲**			
Aβ1-39% <sup>b</sup>	▲▲**	▲▲**		
Aβ1-40% <sup>b</sup>				
Aβ1-40 <sup>ox</sup> % <sup>b</sup>	▲▲**	▲▲**	▲▲**	▲▲**
Aβ1-42% <sup>b</sup>	▽▽**	▽▽**	▽▽**	

▲ = Increased peptide concentration as compared to NDC; ▽ = decreased peptide concentration as compared to NDC.

\* p = 0.05: significant difference; \*\* p = 0.01: significant difference.

age terms ( $p = 8.3 \times 10^{-3}$ ). Conversely, both dementias shared elevated levels of Aβ1-40<sup>ox</sup>% and total tau.

#### Neurochemical Phenotype in Possible VAD

In comparison to NDC, possible VAD exhibited lower Aβ1-42 levels in absolute and percentage terms. This was only paralleled by a drop in the absolute Aβ1-40 levels. Conversely, there were elevated levels of Aβ1-40<sup>ox</sup>% ( $p = 2.7 \times 10^{-5}$ ).

Consequently, AD and possible VAD shared decreased Aβ1-42% and elevated Aβ1-40<sup>ox</sup>% levels in contrast to elevated p-tau levels only in AD. Likewise in probable VAD, only the absolute but not the percentage levels of Aβ1-40 were decreased ( $p = 8.3 \times 10^{-4}$ ).

Lower Aβ1-42% and higher Aβ1-40<sup>ox</sup> levels in possible VAD were the sole differences to probable VAD.

#### Neurochemical Phenotype in AD with CVD

In comparison to NDC, AD with CVD displayed AD-like derangements of the peptides tau, p-tau, Aβ1-42 and Aβ1-40<sup>ox</sup>. Additionally, there were elevations of Aβ1-37% ( $p = 2.1 \times 10^{-3}$ ) and Aβ1-39% ( $p = 1.1 \times 10^{-3}$ ), paralleled by a drop in Aβ1-40% ( $p = 4.3 \times 10^{-2}$ ).

The differences between AD and AD with CVD included higher levels of Aβ1-37% ( $p = 1.1 \times 10^{-2}$ ) and

Aβ1-42% ( $p = 2.1 \times 10^{-3}$ ), respectively, in the latter group. Unlike in probable and possible VAD, the Aβ1-40 levels were unchanged.

AD with CVD shared increased levels of tau and Aβ1-40<sup>ox</sup>% with probable VAD, but diminished Aβ1-42% and elevated p-tau in AD with CVD marked the difference.

AD with CVD and possible VAD differed in tau and p-tau levels but exhibited similarities in elevated Aβ1-40<sup>ox</sup>% and reduced absolute and percentage Aβ1-42 levels.

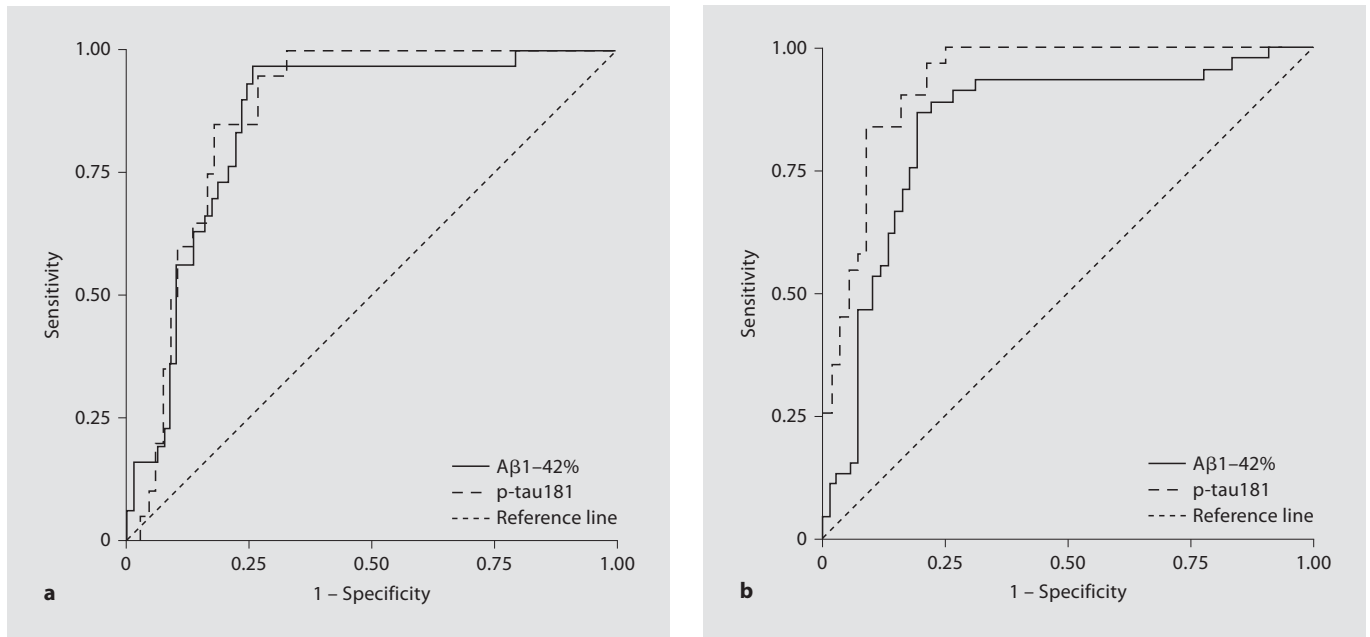
The disease-specific peptide derangements of each diagnostic group in comparison to NDC are given in table 2.

#### Detection of AD and AD with CVD

The discrimination of AD from NDC yielded contrasts of beyond 85% by the use of p-tau, Aβ1-42%, Aβ1-38% and Aβ1-40<sup>ox</sup> individually. p-tau exhibited the best accuracy for detection of AD among all other investigated patients with a sensitivity of 85% and a specificity of 82%.

Both p-tau and Aβ1-42% yielded contrasts of beyond 85% for discriminating AD and AD with CVD (combined to 1 group) from NDC. p-tau gave the best discrimination with a sensitivity of 87% at a specificity of 84% for detection of AD and AD with CVD among all other investigated patients (table 3, fig. 4).





**Fig. 4. a** Receiver operator curves for detection of AD among all other patients based on A $\beta$ 1-42% and p-tau181. **b** Receiver operator curves for detection of combined group of AD and AD with CVD among all other patients based on A $\beta$ 1-42% and p-tau181.

**Table 3.** Cutoff points, sensitivities and specificities for the most relevant differential diagnostic testing

Differential diagnosis	Parameter	Cutoff	Sensitivity, %	Specificity, %	Youden	AUC
AD versus NDC	p-tau, ng/ml	0.066	85	91	0.76	0.94 (0.87-1.01)
	A $\beta$ 1-42%	5.85	90	97	0.87	0.96 (0.91-1.02)
AD versus all other	p-tau, ng/ml	0.069	85	82	0.67	0.87 (0.80-0.94)
	A $\beta$ 1-42%	5.85	90	77	0.67	0.86 (0.78-0.93)
AD and AD with CVD versus NDC	p-tau, ng/ml	0.056	90	86	0.76	0.94 (0.87-1.01)
	A $\beta$ 1-42%	7.15	91	87	0.78	0.92 (0.86-0.99)
AD and AD with CVD versus all other	p-tau, ng/ml	0.057	87	84	0.71	0.93 (0.88-0.98)
	A $\beta$ 1-42%	6.36	87	81	0.68	0.84 (0.76-0.92)

Figures in parentheses are 95% CI. AUC = Area under the receiver operating characteristic curve.

## Discussion and Mini-Review

Herein, we present first results on the detailed characterization of A $\beta$  species in CSF of VAD subgroups in comparison to AD and NDC.

The detailed characterization of carboxyterminally shorter A $\beta$  peptides as referenced to A $\beta$ 1–42 and oxidized A $\beta$  peptide species (i.e. oxidized A $\beta$ 1–40) in CSF (A $\beta$  peptide patterns) is a novel approach that has been enabled by a urea-based quantitative A $\beta$ -SDS-PAGE/immunoblot [15, 20]. The data published so far on this issue suggest A $\beta$  peptide patterns to reflect disease-specific interactions of neurodegenerative processes with the A $\beta$  peptide metabolism more adequately than the sole measurement of CSF A $\beta$ 1–42 [11, 14, 15, 24].

### *Biomarker Studies in VAD*

Previous CSF studies have revealed contradictory results about the concentration of the established biomarkers tau, A $\beta$ 1–42 and p-tau in VAD and their differential diagnostic value for discrimination from AD. This may be mainly due to the fact that especially VAD are of heterogeneous etiology. Some studies on tau postulate high levels in VAD [25–27], whereas others found tau to be in the normal range [28–30]. In comparison, data on the performance of CSF A $\beta$ 1–42 in discriminating between AD and VAD are limited. A mild to moderate reduction and considerable overlap with AD has been reported in most of the studies [22, 31, 32]. As reviewed by experts [33], the differential diagnostic power of tau and A $\beta$ 1–42 in a definite case was usually found to be limited [10]. The elevated concentration of phosphorylated tau protein in CSF is a relatively new biomarker that is claimed to be closely related to the pathogenic process of AD and specific to AD, even in contrast to other forms of dementia. Measurement has been performed using ELISA with antibodies capturing specifically distinct phosphorylation sites of the peptide (e.g. Thr181, Thr199, Thr231 and Thr396/404) [34, 35]. Thr181 phosphorylated tau was evaluated for the differential diagnosis of AD and VAD in a study that found a pronounced increase in AD as compared to VAD [32]. Due to some overlap of values, there was no sufficient diagnostic discrimination among AD and VAD. A large comparative CSF study revealed similar accuracies of the ELISAs specific to Thr181, Thr199 and Thr231 in discriminating AD among various other dementias, including VAD [34]. These results suggest the outcome of Thr181 p-tau ELISA in discriminating among AD and VAD to be representative of the Thr199 and Thr231 p-tau ELISAs, too. However, an ELI-

SA specific to the phosphorylation site of tau at Thr396/404 enabled contrasts of beyond 85% sensitivity and specificity for the discrimination of AD from VAD [35].

The presented data of our study reconfirm the majority of the results of others summarized above with regard to tau and absolute A $\beta$ 1–42 values. In particular, we found contrasts for tau and A $\beta$ 1–42 in discriminating AD from probable VAD comparable to those of one of the largest community-based studies made up for the establishment of reference values of these biomarkers [31]. Another study included solely subcortical arteriosclerotic encephalopathy following a modified version of the criteria of Bennett et al. [36] that were made up for the clinical diagnosis of Binswanger's disease, for comparison to AD [30]. Their results for elevated tau and p-tau levels in AD correspond to our contrasts between AD and VAD. With regard to p-tau, our results for the discrimination of AD among probable VAD by p-tau181 resemble those from another study that included 20 cases of VAD in comparison to AD and investigated p-tau231 [37]. Likewise, de Jong et al. [38] achieved accuracies of 85% or beyond when p-tau and A $\beta$ 1–42 were applied to the discrimination of AD and VAD.

The application of the A $\beta$ -SDS-PAGE/immunoblot in addition to commercially available ELISAs for tau, p-tau and A $\beta$ 1–42 demonstrated that the drop in CSF A $\beta$ 1–42 in AD was selective and paralleled by a percentage increase of carboxyterminally shorter A $\beta$  peptides and the oxidized form of A $\beta$ 1–40. It is noteworthy that AD with CVD and single VAD patients exhibit a CSF neurochemical phenotype with regard to CSF tau, p-tau, A $\beta$ 1–42 and A $\beta$ 1–40<sup>ox</sup>. From these data, we conclude that CSF neurochemical phenotypes, especially p-tau and A $\beta$ 1–42 levels, sufficiently discriminate between probable AD and VAD. In case of no clear-cut clinical appearance (e.g. possible VAD), distinct CSF biomarker constellations may direct the diagnosis towards either AD or VAD. Moreover, the CSF neurochemical phenotype may indicate the involvement of AD-like pathophysiological pathways and the impact of oxidative stress mechanisms in VAD. In this respect, CSF biomarkers may even help predict an individual's benefit from an AD-specific therapeutic strategy, irrespective of whether it may be symptomatic (e.g. acetylcholinesterase inhibitors) or a hopefully forthcoming causative therapy.

### *Pathophysiological Implications*

It is still under debate whether a dementia syndrome that exhibits clinical features of both CVD (e.g. history of stroke or vascular lesions in magnetic resonance imag-

ing) and AD (e.g. gradual progressive dementia) may be caused by a self-containing disease, the so-called mixed (AD/VAD) dementia.

This hypothesis is underlined by the frequent finding of overlapping Alzheimer-typical lesions and vascular pathology in postmortem neuropathological studies [5, 10]. Rather than simple coincidence, a growing body of evidence points to a causative relationship between the vascular disease and AD. Atherosclerosis, as a major part of vascular disease, is considered to promote higher local concentrations and accumulation of A $\beta$  via an impaired clearance from the brain into the peripheral blood system [39, 40]. Conversely, AD may support vascular disease by disturbance of cholinergic innervations of cerebral blood vessels [41, 42], which are essentially involved in the autonomic regulation of brain blood flow [43]. In addition to deregulated cerebral blood flow, amyloid precursor protein overexpression [44] and amyloid deposition in cerebral blood vessels (cerebral amyloid angiopathy) [40] have been hypothesized to predispose the AD brain to ischemic injury. Moreover, inflammatory processes and oxidative stress, which are supposed to play a major pathogenic role in AD and VAD, may be initialized by both intracerebral A $\beta$  accumulation and repeated brain ischemia [44]. This fits in with our finding of elevated absolute A $\beta$ <sub>1–40</sub><sup>ox</sup> levels in AD, AD with CVD and possible VAD, but not in probable VAD, as this peptide is considered to represent an oxidized form of A $\beta$ <sub>1–40</sub> [11]. In our study, such a mixed (AD/VAD) dementia syndrome may be present in AD with CVD and partly in possible VAD, which is characterized by an AD-like CSF biomarker constellation.

#### *Limitations of the Study*

One major drawback of the presented data is the heterogeneity of the NDC group that includes 7 patients with organic brain disorders aside peripheral neurologic diseases and depressions. For example, patients with normal pressure hydrocephalus might additionally suffer from small vessel disease in deep parts of the brain and a pathogenic overlap with AD has been hypothesized [45]. This may have subsequently led to an underestimation of diagnostic accuracies for the discrimination of either VAD or AD among the control group.

A second weakness is the small sample size, especially for VAD subgroups representing a broad spectrum of clinical appearance accompanied by an inhomogeneous neurochemical phenotype, indicating that there may be different subtypes summarized under the diagnosis VAD. We cannot exclude pre-existing forms of dementia, for

example due to AD, or cognitive impairment of another kind in patients classified as VAD. Thus, especially the course of disease in patients with possible VAD could supposedly rely on any form of pre-existing dementia, impaired by stroke. Gamaldo et al. [46], for example, found an increased risk of developing dementia after clinically overt stroke in individuals who had mild cognitive impairment prior to the stroke.

The reliability on clinical diagnosis limits our results in consideration of potential misclassification. Pathogenic conclusions that may be drawn from CSF biomarker constellations are also limited due to the lack of neuropathological data. In the present study, none of the clinical diagnoses have been confirmed by autopsy. Therefore, we propose larger and neuropathologically controlled studies on CSF peptide patterns.

In addition, biomarker-controlled therapy studies will have to be arranged to validate the hypothesis that a CSF biomarker constellation may predict the successfulness of AD-specific therapeutic strategies in other dementias. In this context, it will be of particular importance to additionally assess the presence of apolipoprotein 4 alleles, which may influence the clinical course as well as the neurochemical CSF phenotype of dementia patients.

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