J Neural Transm (2006) 113: 1771–1778
DOI 10.1007/s00702-006-0537-z

Journal of Neural Transmission
Printed in Austria

CSF diagnosis of Alzheimer's disease and dementia with Lewy bodies

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Received: February 9, 2006 / Accepted: May 29, 2006 / Published online: August 17, 2006

Summary Differential diagnosis of Alzheimer's disease (AD) and dementia with Lewy bodies (DLB) is often crucial. CSF Tau protein and Amyloid-beta (Aβ) peptides have shown diagnostic value for the diagnosis of AD, but discrimination from DLB was poor.

Herein, we investigate CSF of 18 patients with probable AD, 25 with probable DLB and 14 non-demented disease controls (NDC) by Aβ-SDS-PAGE/immunoblot and commercially available ELISAs for Aβ1-42 and tau. CSF Aβ peptide patterns and tau exhibited disease specific alterations among AD and DLB. The ratio of Aβ1-42 to Aβ1-38 and Aβ1-42 to Aβ1-37, respectively, in combination with absolute tau, yielded a sensitivity and specificity of 100 and 92%, respectively. We conclude that CSF Aβ peptide patterns and tau levels reflect disease-specific pathophysiological pathways of these dementias as distinct neurochemical phenotypes. Combined evaluation of these biomarkers provides a reasonable accuracy for differential diagnosis of AD and DLB.

Keywords: Alzheimer's dementia, Lewy-body dementia, cerebrospinal fluid, amyloid-β peptides, tau protein, biomarker

Abbrevations: *Aβ peptides* amyloid-beta peptides, *Aβ-SDS-PAGE/immunoblot* amyloid-beta-sodium-dodecyl-sulphate-polyacrylamide-gel-electrophoresis with western immunoblot, *AD* Alzheimer's disease, *ApoE ε4* apolipoprotein E allele ε4, *APP* beta-amyloid precursor protein, *bicine* N,N′-bis-[2-hydroxyethyl]glycine, *CCD-camera* charge coupled device camera, *CSF* cerebrospinal fluid, *Ct-elongated* carboxyterminally elongated, *Ct-truncated* carboxyterminally truncated, *DLB* dementia with Lewy bodies, *ECL* enhanced chemiluminescence, *ELISA* Enzyme Linked Immunosorbent Assay, *MMSE* Mini-Mental-Status Examination, *NINCDS-ADRDA* National Institute Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association, *ND* non-demented, *PDD* Parkinson's disease dementia, *PVDF* polyvinylidene difluoride, *SDS* sodium dodecyl sulphate

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Introduction

Extracellular amyloid-plaques and intracellular neurofibrillary tangles (NFT's) are the two neuropathological hallmarks of Alzheimer's disease (AD). Amyloid-plaques deposited in the brain of patients suffering from AD (Glenner and Wong, 1984) and dementia with Lewy bodies (DLB) (Jendroska et al., 1997) mainly consist of carboxyterminally elongated forms of Amyloid-beta (Aβ) peptides, such as Aβ1-42.

Cleavage of the transmembrane amyloid precursor-protein (APP) by two enzymes, β- and γ-secretase (Haas and Selkoe, 1993), results in either carboxyterminally truncated (Ct-truncated) or elongated (Ct-elongated) Aβ peptides as referenced to Aβ1-40. Distinct γ-secretase activities are hypothesized to be responsible for this phenomenon (Citron et al., 1996).

One of the major constituents of the NFT's is the tau protein, a 68-kDa microtubule-associated phosphoprotein, which aggregates into paired helical filaments in AD (Lee et al., 1991).

The differential diagnosis of dementias based on established clinical criteria is often difficult during lifetime and the selective reduction of Aβ1-42 combined with an increase of tau protein in the cerebrospinal fluid (CSF) of AD patients has recently been reviewed as applicable to AD diagnostic testing in addition to clinical criteria (Andreasen et al., 2003). However, decreased Aβ1-42 levels have also been reported for DLB (Andreasen et al., 2001; Mollenhauer et al., 2005). Tau levels are often within the range of non-demented controls, but also higher levels with

a considerable overlap of value with AD have been reported (Molina et al., 1999; Kanemaru et al., 2000; Andreasen et al., 2001; Tschampa et al., 2001; Mollenhauer et al., 2005). Accordingly, the differential diagnostic value of the combined measurement of tau protein and Aβ1-42 to distinguish between AD and DLB in a definite case is assumed to be low (Verbeek et al., 2003).

A quantitative urea-based Aβ-sodium-dodecylsulphate-polyacrylamide-gel-electrophoresis with western immunoblot (Aβ-SDS-PAGE/immunoblot) recently revealed the regular abundance of the Ct-truncated Aβ peptides 1-37, 1-38, 1-39 in addition to 1-40 and 1-42 in CSF (Wiltfang et al., 2002). This Aβ peptide pattern displayed disease-specific variations in its absolute and relative quantities in the CSF of patients with AD, Creutzfeld-Jakob disease (CJD), chronic inflammatory diseases and other neuropsychiatric diseases (Wiltfang et al., 2002, 2003). Moreover, AD patients displayed a tendency to increased Aβ1-38 levels, and the introduction of Aβ peptide ratios (e.g. Aβ1-38/Aβ1-42) increased the diagnostic accuracy as compared to the measurement of absolute Aβ1-42 levels by either ELISA or Aβ-SDS-PAGE/immunoblot (Wiltfang et al., 2003; Bibl et al., 2004).

The present study addressed the question of whether this finding might be relevant for the validation of the clinical differential diagnosis among AD and DLB, especially in combination with other AD biomarkers, like tau protein.

Here, we combine the results of the advanced Aβ-SDS-PAGE/immunoblot with the well established measurement of tau protein in CSF to produce a highly specific assay for the differential diagnosis of AD and DLB.

Patients and methods

Patients

We prospectively investigated 57 consecutive CSF samples that had been referred to our laboratory between 2000 and 2004 for the neurochemical evaluation of the differential diagnosis of AD and DLB, respectively.

The CSF was collected from hospitalized DLB patients of the Paracelsus-Elena Klinik, Kassel, which has specialized in the diagnosis and treatment of Parkinson's disease. CSF of AD patients and three patients with depression came from the memory clinic of the University of Goettingen. Non-demented disease controls came from wards of the university hospital of Goettingen.

The diagnosis was established by a psychiatrist and a neurologist due to thorough anamnesis, clinical examination, results of neuropsychological assessment, clinical records of the patients and the best clinical judgement. Both investigators are well experienced in the clinical differential diagnosis of dementias and were blinded to the neurochemical outcome measures. A clinical diagnosis of probable AD according to the DSM IV criteria for AD and the NINCDS-ADRDA criteria for clinical diagnosis of probable AD (McKhann et al., 1984) was made in 18 patients. The AD group comprised 5 men and 13 women. Age of this group was 69.7 ± 10.6 years (mean $\pm$ SD). Mini-Mental-Status-Examination (Folstein et al., 1975) (MMSE) was performed on all included patients. The mean MMSE score was 18.4 ± 4.5 (mean $\pm$ SD) in this group. 25 patients (21 men and 4 women) fulfilled the DSM IV criteria for dementia, the Mc Keith criteria for clinical diagnosis of probable DLB (McKeith et al., 1996) and presented with at least two core features required for the diagnosis of DLB. Enrolled patients were therefore hospitalized for at least several days to evaluate fluctuating cognition, extrapyramidal symptoms and visual hallucinations. Age of this group was 72.0 ± 7.5 years (mean $\pm$ SD). MMSE was available for 24 patients. One patient rejected the cognitive testing. He displayed moderate cognitive impairments at the time of lumbar puncture, and neuropathological postmortem analysis confirmed DLB. The mean MMSE score was 18.8 ± 4.9 (mean $\pm$ SD) in this group. Aβ1-42 ELISA was unavailable for 2 DLB patients.

The 14 (4 men and 10 women) non-demented disease controls (NDC) presented with no clinical features of neurodegenerative disease. Patients with anamnesis of persistent cognitive decline for more than six months were excluded from this group. Age of this group was 67.3 ± 6.6 years (mean $\pm$ SD). All patients with cognitive complaints ($n = 12$) were assessed by MMSE at minimum. The score was 27.5 ± 2.9 (mean $\pm$ SD). The group included patients suffering from depression ($n = 10$), normal pressure hydrocephalus (NPH) without dementia ($n = 2$), paraneoplastic cerebellar inflammation ($n = 1$), focal epilepsy ($n = 1$). The cognitive complaints of all depressive patients improved after antidepressant medication. One patient with cognitive complaints, who was suspected of suffering from NPH, improved after spinal tap.

The study was conducted under the guidelines of the Declaration of Helsinki (World Medical Organisation, 1996) and approved by the ethics committee of the University of Goettingen. All patients were enrolled after their informed consent.

Aβ-SDS-page/immunoblot, tau protein ELISA and Aβ1-42 ELISA

The preanalytical handling of all included CSF samples followed a standardized protocol according to previously

published data (Bibl et al., 2004): CSF was drawn from patients by lumbar puncture, sampled in polypropylene vials and centrifuged (1000 g, 10 min, 4°C). Aliquots of 200 µl were stored at −80°C within 24 hours for subsequent Aβ-SDS-PAGE/immunoblot analysis. Freezing of samples was conducted by directly cooling 200 µl of CSF in polypropylene cups down to −80°C without an intermediate temperature stage. The samples did not undergo additional freeze and thaw cycles. CSF for Aβ1-42 and tau ELISA analysis was stored at +4°C and analyzed within two days.

The commercially available assays Innotest hTAU Antigen and Innotest β-Amyloid$_{(1\text{-}42)}$, Innogenetics (Ghent, Belgium) were applied to the quantification of tau protein and Aβ1-42 levels in CSF, respectively. Tau and Aβ1-42 ELISA were performed according to previously published standard methods (Hulstaert et al., 1999).

Aβ peptide patterns were analyzed by the recently established Aβ-SDS-PAGE/immunoblot. For separation of Aβ peptides and subsequent detection 10 µl of unconcentrated CSF were boiled in a sample buffer for SDS-PAGE, and Aβ-SDS-PAGE/immunoblot was conducted as published elsewhere (Wiltfang et al., 2002; Bibl et al., 2004).

Samples were run as triplicates and each gel carried a four step dilution series of the synthetic Aβ peptides Aβ1-37, Aβ1-38, Aβ1-39, Aβ1-40 and Aβ1-42. Synthetic peptides Aβ1-38, Aβ1-40, Aβ1-42 were obtained from Bachem (Bubendorf, Switzerland), Aβ1-37 and Aβ1-39 were synthesized automatically according to Janek et al. (2001). Standard preparations of synthetic Aβ peptide mixture were created as described previously (Bibl et al., 2004) and bands were quantified from individual blots of each patient relative to this dilution series using a charge coupled device camera (CCD-camera). Absolute concentrations of proteins in CSF are given in ng/ml.

The detection sensitivity for the 1E8 in this optimized immunoblot procedure was 0.6 pg (Aβ1-38, Aβ1-40) and 1 pg (Aβ1-37, Aβ1-39, Aβ1-42), respectively (Bibl et al., 2004). The inter- and intra-assay coefficients of variation for 80 as well as for 20 pg of synthetic Aβ peptides were below 10% (Wiltfang et al., 2002; Bibl et al., 2004).

All neurochemical measurements and quantifications were performed in the laboratory of neurobiology of the University of Goettingen by two experienced technical assistants, blinded to clinical diagnosis.

Statistical analysis

Aβ peptide and tau levels were expressed as absolute values (ng/ml). The data on peptide levels were obtained from individual blots of each individual patient. We have characterized patient groups by mean and standard deviation (SD).

The Mann-Whitney U-test was applied to evaluate significant group differences.

Receiver operating characteristic (ROC) curve analysis was used to determine cut-off points. Additionally, the optimal cut-off level for dichotomising values was selected as the situation maximizing the Youden index. Only the best discriminating cut-off values alone or in combination are presented.

The chi-square test was used to determine statistical significance of different sensitivities and specificities, respectively.

The two-sided level of significance was taken as $p < 0.05$. A p-value less than 0.01 was considered as highly significant.

Computations were performed using the statistical software package SPSS, version 10.0.

Results

Group differences and diagnostic accuracies

The mean age did not significantly differ between the diagnostic groups.

The mean MMSE score did not significantly differ between AD and DLB and was significantly lower than in the NDC group ($p = 1.6 \times 10^{-6}$ and 1.9×10^{-6}, respectively).

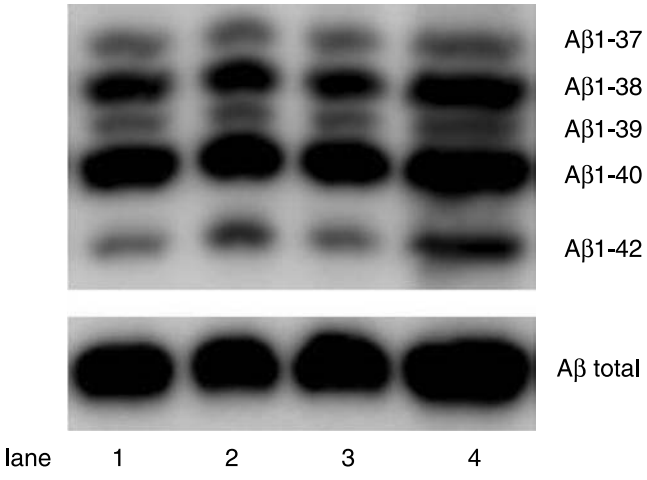

Fig. 1. Urea-based Aβ-SDS-PAGE/immunoblot (A) and conventional SDS-PAGE (B) of CSF (lane 1–3) and synthetic Aβ peptides 1-37, 1-38, 1-39, 1-40, 1-42 (lane 4). The figure shows a blot of pooled CSF of seven representative AD (lane 1), NDC (lane 2) and DLB (lane 3) patients, whereas all quantifications have been obtained from individual blots of each patient

The Aβ-SDS-PAGE/immunoblot revealed a highly conserved pattern of four Aβ peptides in addition to Aβ1-42 in the CSF of all investigated patients. All Aβ peptides migrate as a single band if urea is absent in otherwise unchanged separation gels (Fig. 1). Aβ peptides generally appeared in the following order of abundance: Aβ1-40, Aβ1-38, Aβ1-42, Aβ1-39 and Aβ1-37.

Both dementia groups, AD and DLB displayed decreased absolute levels of Aβ1-42 in comparison to the NDC group as measured by ELISA ($p = 1.9 \times 10^{-5}$ and 3×10^{-3}, respectively) (Fig. 2). The reduction of Aβ1-42 levels in absolute terms as measured by the Aβ-SDS-PAGE/immunoblot was significant for AD ($p = 2 \times 10^{-3}$), but failed the level of significance in DLB patients ($p > 0.05$). None of the carboxyterminally shorter Aβ peptides, in absolute terms, was significantly altered in AD or

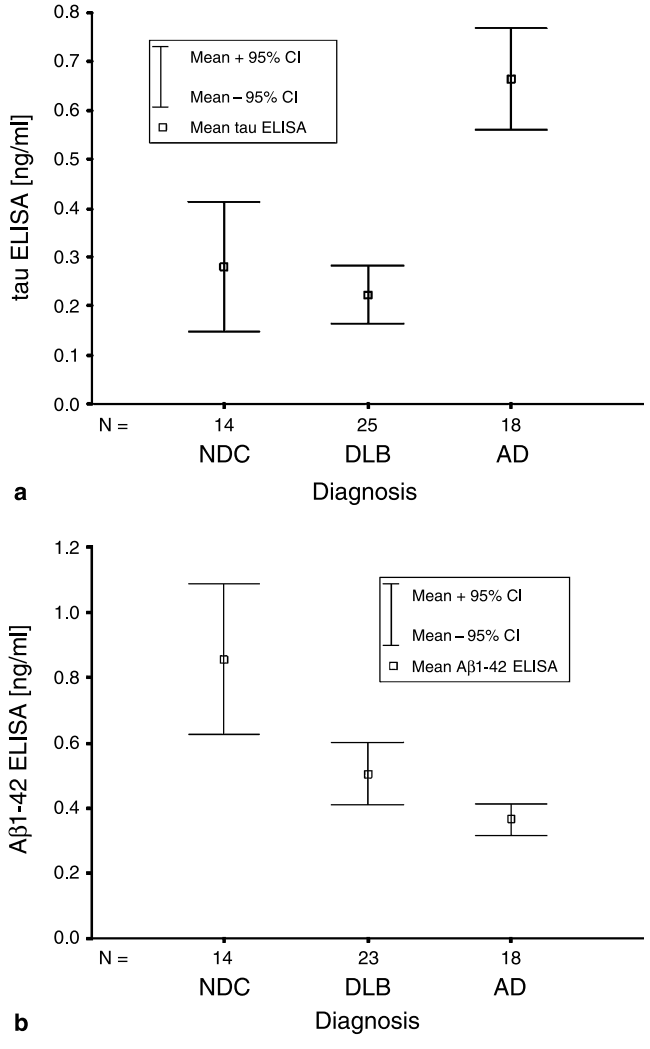

Fig. 2. Mean, 95% confidence interval (CI) of absolute tau (**a**) and Aβ1-42 (**b**) levels as measured by ELISA for each diagnostic group

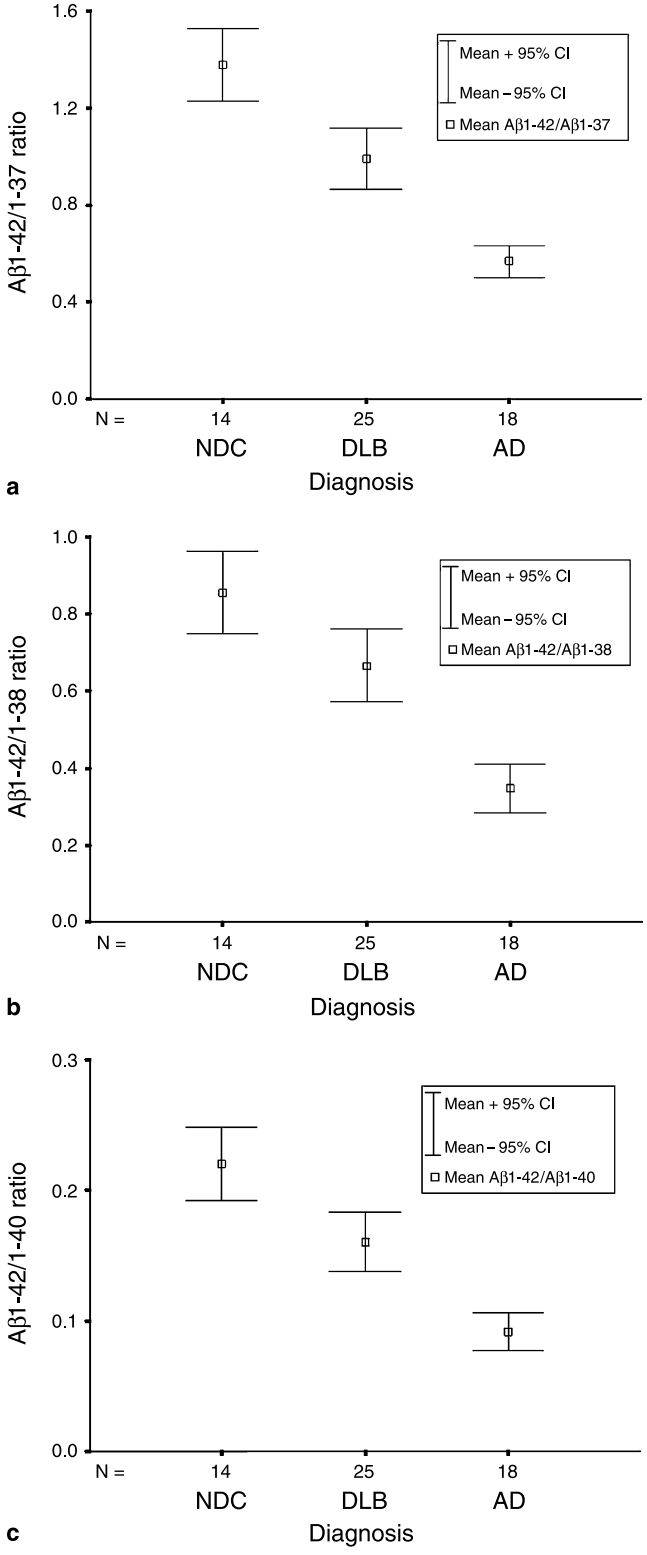

Fig. 3. Mean, 95% confidence interval (CI) of the ratios Aβ1-42/Aβ1-37 (**a**), Aβ1-42/Aβ1-38 (**b**) and Aβ1-42/Aβ1-40 (**c**) for each diagnostic group

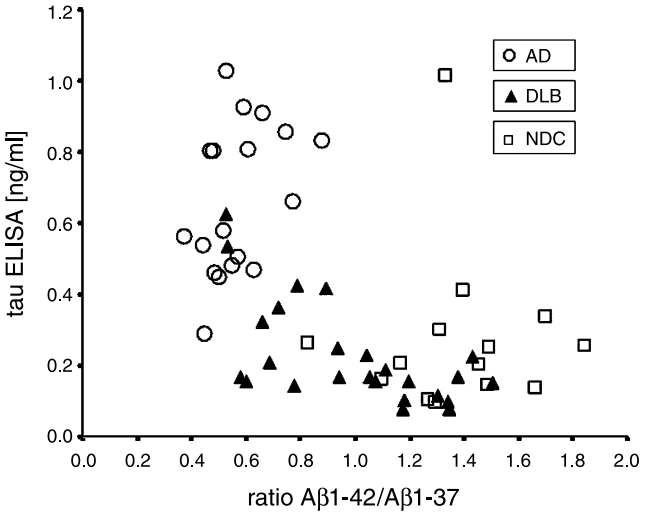

Fig. 4. Scatterplot of AD and DLB patients divided by their absolute tau levels and the ratio of Aβ1-42/Aβ1-37

DLB. Otherwise, AD displayed a slight increase in Aβ1-37, Aβ1-38 and Aβ1-40 as compared to DLB.

By introducing ratios of Aβ1-42 to Aβ1-37, Aβ1-38, Aβ1-39 and Aβ1-40, respectively, we found an improved differentiation among the diagnostic groups.

The ratio of Aβ1-42 to Aβ1-37 (Aβ1-42/Aβ1-37) discriminated NDC from AD and DLB at a highly significant level ($p = 8.48 \times 10^{-9}$ and $p = 1 \times 10^{-3}$, respectively). Moreover, the ratio highly significantly differentiated AD from DLB ($p = 6.6 \times 10^{-6}$). The ratio of Aβ1-42 to Aβ1-38 (Aβ1-42/Aβ1-38) discriminated NDC from AD and DLB at a level of $p = 1.3 \times 10^{-7}$ and $p = 1 \times 10^{-2}$, respectively. Again, the ratio highly significantly differentiated AD from DLB ($p = 7 \times 10^{-6}$). The ratio of Aβ1-42 to Aβ1-40 (Aβ1-42/Aβ1-40) discriminated NDC from AD and DLB at a highly significant level ($p = 3 \times 10^{-8}$ and

Table 1. *Absolute abundances of* Aβ peptide patterns, Aβ1-42 and tau in the CSF of the diagnostic groups

Diagnosis	NDC (n = 14)		DLB (n = 25)		AD (n = 18)	
	MW	±SD	MW	±SD	MW	±SD
age	67.3	6.6	72.0	7.5	69.7	10.6
MMSE	27.67	2.84	18.79	4.89	18.39	4.52
Tau ELISA	0.86	0.38	0.50	0.22	0.36	0.10
Aβ1-42 ELISA	0.28	0.22	0.22	0.14	0.67	0.20
Aβ1-37	1.10	0.60	0.95	0.35	1.06	0.47
Aβ1-38	1.71	0.78	1.45	0.57	1.77	0.74
Aβ1-39	0.86	0.44	0.83	0.31	0.83	0.32
Aβ1-40	6.56	2.86	5.79	1.77	6.52	2.38
Aβ1-42	1.53	0.90	0.93	0.40	0.60	0.28
total Aβ[1]	11.83	5.49	10.04	3.19	10.88	3.98

[1]Total Aβ peptide concentration as measured by the sum of all investigated Aβ peptides.

Table 2. *Cut off points, sensitivities and specificities, Youden index of the best discriminating single marker,* Aβ peptide ratio and marker combination, respectively, for each differential diagnostic testing

Differential diagnosis	Parameter	Cut off	Sensitivity (%)	Specificity (%)	Youden index
AD versus NDC	tau	0.431	94	93	0.87
AD versus NDC	Aβ1-42-ELISA	0.559	100	79	0.79
AD versus NDC	Aβ1-42-ELISA/tau	1.554	100	93	0.93
AD versus NDC	Aβ1-42/Aβ1-37	0.797	94	100	0.94
AD versus NDC	Aβ1-42/Aβ1-37/tau	2.317	100	93	0.93
AD versus NDC	Aβ1-42/Aβ1-38	0.46	83	100	0.83
AD versus NDC	Aβ1-42/Aβ1-38/tau	0.817	94	100	0.94
AD versus NDC	Aβ1-42/Aβ1-40	0.149	100	93	0.93
AD versus NDC	Aβ1-42/Aβ1-40/tau	0.428	100	93	0.93
AD versus DLB	tau	0.435	94	92	0.86
AD versus DLB	Aβ1-42-ELISA	0.475	48	94	0.42
AD versus DLB	Aβ1-42-ELISA/tau	1.011	94	91	0.85
AD versus DLB	Aβ1-42/Aβ1-37	0.659	83	84	0.67
AD versus DLB	Aβ1-42/Aβ1-37/tau	1.709	100	92	0.92
AD versus DLB	Aβ1-42/Aβ1-38	0.438	83	84	0.67
AD versus DLB	Aβ1-42/Aβ1-38/tau	1.034	100	92	0.92
AD versus DLB	Aβ1-42/Aβ1-40	0.145	100	68	0.68
AD versus DLB	Aβ1-42/Aβ1-40/tau	0.307	100	88	0.88
AD versus DLB and NDC	tau	0.435	94	92	0.86
AD versus DLB and NDC	Aβ1-42-ELISA	0.541	100	54	0.54
AD versus DLB and NDC	Aβ1-42-ELISA/tau	1.011	94	92	0.86
AD versus DLB and NDC	Aβ1-42/Aβ1-37	0.772	94	82	0.76
AD versus DLB and NDC	Aβ1-42/Aβ1-37/tau	1.709	100	92	0.92
AD versus DLB and NDC	Aβ1-42/Aβ1-38	0.438	83	90	0.73
AD versus DLB and NDC	Aβ1-42/Aβ1-38/tau	1.034	100	92	0.92
AD versus DLB and NDC	Aβ1-42/Aβ1-40	0.145	100	77	0.77
AD versus DLB and NDC	Aβ1-42/Aβ1-40/tau	0.307	100	90	0.90

$p = 1.4 \times 10^{-3}$, respectively). The ratio highly significantly differentiated AD from DLB ($p = 7.4 \times 10^{-5}$) (Fig. 3). The ratio of Aβ1-42 to Aβ1-39 (Aβ1-42/Aβ1-39) discriminated NDC from AD and DLB at a level of $p = 2.8 \times 10^{-7}$ 10^{-7} and 3.6×10^{-4}, respectively. The ratio highly significantly differentiated AD from DLB ($p = 2.6 \times 10^{-4}$).

Tau levels were increased in AD as compared to NDC ($p = 1.1 \times 10^{-5}$) and DLB ($p = 4 \times 10^{-7}$). There was no significant elevation of tau-levels in DLB as compared to NDC ($p > 0.05$) (Fig. 2).

A subgroup analysis among NDC revealed no significant difference in any of the investigated peptides between depressive patients ($n = 10$) and organic brain disorders ($n = 4$).

The absolute abundances of Aβ peptides and tau of each diagnostic group are summarized in Table 1.

The cut off points, sensitivities and specificities as well as the maximum Youden index of the best discriminating single marker, Aβ peptide ratio and marker combination, respectively, are summarized in Table 2.

Discussion

Commercially available ELISAs for Aβ1-42, total tau and the recently established quantitative Aβ-SDS-PAGE/immunoblot (Wiltfang et al., 2002, 2003; Bibl et al., 2004) were applied to investigate the CSF samples of 57 patients suffering from AD, DLB and other neurological and psychiatric diseases without dementia for tau levels and disease-specific patterns of Aβ peptides.

Tau levels were significantly elevated and Aβ1-42 levels showed a pronounced drop in AD as compared to the DLB and NDC group, respectively. Additionally, the Aβ-SDS-PAGE/immunoblot demonstrated that the reduction of Aβ1-42 was not paralleled by a decrease of any of the other regularly abundant CSF Aβ peptide species (Aβ1-37, 1-38, 1-39 and 1-40) to the same degree in AD.

The concept of decreased Aβ1-42 and elevated tau levels as a typical alteration of brain specific proteins in the CSF of AD is widely accepted (Verbeek et al., 2003; Lewczuk et al., 2004) and can be considered as an applicable routine biomarker for AD (Andreasen et al., 2001). The reduction of Aβ1-42 levels in AD has long been explained by an increased clearance of the peptide from CSF into senile amyloid plaques (Motter et al., 1995). Other studies indicate the existence of alternative mechanisms, including the formation of SDS-stable oligomers (Podlisny et al., 1995), supramolecular aggregates of Aβ peptides (Pitschke et al., 1998) and chaperone complexes of Aβ peptides with specific carrier proteins (Wiltfang et al., 2002, 2003; Bibl et al., 2004). These mechanisms seem to be restricted to Aβ1-42 in AD but not in all neurodegenerative diseases, as reduced overall Aβ peptide levels aside a decrease of Aβ1-42 have been found in CJD (Wiltfang et al., 2003) and the moderate decrease of Aβ1-42 in DLB was accompanied by a slight drop of the carboxyterminally shorter Aβ peptides. The differentiation of AD from DLB remains crucial, mainly due to decreased Aβ1-42 levels in both dementias (Verbeek et al., 2003). The common significant reduction of Aβ1-42 in the CSF of AD and DLB is linked to the neuropathological finding of mixed pathologies for both neurodegenerative diseases (Merdes et al., 2003). Although the relationship between decreased levels of Aβ1-42 in CSF and the occurrence of senile amyloid plaques has yet to be clarified, the overlap of neuropathological and neurochemical phenotype may indicate that AD and DLB share common pathophysiological pathways. This hypothesis is supported by the lack of tauopathy in DLB (Hansen et al., 1993), which is paralleled by normal tau levels in CSF. In contrast, tau levels beyond 1300 pg/ml have been described for Creutzfeldt-Jakob disease without the occurrence of any neurofibrillary tangles (Otto et al., 2002), indicating that increased tau levels may simply reflect neurodegeneration in this case. Despite the distinct alterations of tau in AD and DLB, reported sensitivities for AD detection and specificities for DLB exclusion, respectively, did not exceed 75% in a combined assay with Aβ1-42 ELISA (Andreasen et al., 2001). In the present study the diagnostic accuracy of this assay was considerably higher and predominantly based on elevated tau in AD, whilst the combination with Aβ1-42 ELISA failed to improve the predictive value of tau protein alone. This may be attributed to the fact that the present study was restricted to probable DLB, where tau levels tend to be lower than in possible DLB (Mollenhauer et al., 2005).

To the best of our knowledge, the present study is the first report of a combined assay evaluating Aβ peptide patterns and tau levels in CSF for the differential diagnosis of AD and DLB. The introduction of ratios of the differentially altered Aβ peptide species relative to Aβ1-42 measured by the Aβ-SDS-PAGE/immunoblot improved the diagnostic test accuracy for each differential diagnostic question as compared to the sole measurement of Aβ1-42.

First, this may be due to disease specific interactions of each ongoing neurodegenerative dementia process with APP metabolism, which cannot be adequately represented by the sole measurement of absolute Aβ1-42 levels (Wiltfang et al., 2001). The absolute levels of Aβ1-37, Aβ1-38 and Aβ1-40 were elevated in AD as compared to DLB, although this failed the level of significance. Speculatively,

the pronounced drop of Aβ1-42 may be counteracted by an upregulation of carboxyterminally shortened Aβ peptides, whereas this is not the case in DLB. Second, the introduction of ratios of Aβ1-42 to Aβ1-37, 1-38, 1-39 and 1-40, respectively, led to a reduced interindividual variance of values (Wiltfang et al., 2003) and subsequently enabled a higher accuracy of differentiation among the diagnostic groups than the absolute Aβ1-42 levels alone. This may be explained by the assumption that the abundances of single Aβ peptide species are closely correlated to each other and are thus regulated in narrow limits, whereas the total amount of Aβ peptides varies interindividually (Wiltfang et al., 2002, 2003). Accordingly, the ratios Aβ1-42/Aβ1-37, Aβ1-42/Aβ1-38 and Aβ1-42/Aβ1-40 improved the differential diagnosis of AD and DLB as compared to absolute Aβ1-42 levels and all diagnostic groups could be discriminated from each other at a highly significant level by these ratios. The evaluation of Aβ peptide patterns in combination with the tau protein levels (e.g. Aβ1-42/Aβ1-37/tau) exhibited an excellent diagnostic power and misclassified only two DLB subjects as AD at a sensitivity of 100% for AD detection. It is most noteworthy that one of these patients was an autopsy confirmed case of mixed AD and DLB pathology. Otherwise, the relative gain in sensitivity from tau levels alone to the ratio Aβ1-42/Aβ1-37/tau was not statistically significant. Thus, there is no sufficient evidence from the data that Aβ peptide patterns provide an additional value to absolute tau levels alone for the differential diagnosis of AD and probable DLB. Although the combination of both assays yielded the best accuracy, the sole measurement of tau readily satisfied the criteria recommendations for applicable biological markers (i.e. both sensitivity and specificity beyond 85%) according to an international consensus group (Wiltfang et al., 2005). In contrast, sole assessment of Aβ peptide patterns comes closest, but fails to fulfil the requirements in order to discriminate AD from probable DLB patients. Nevertheless, the evaluation of Aβ peptide patterns was superior in the differential diagnosis of dementias as compared to absolute Aβ1-42 levels, which confirms previous results (Wiltfang et al., 2002, 2003). Other studies have shown sensitivities for AD detection and specificities for DLB exclusion below 80% in a combined assay of tau and Aβ1-42 ELISA (Andreasen et al., 2001). The major impact of tau on the discrimination of AD and DLB may be contributed to the careful selection of probable DLB patients in the current study. Tau levels tend to be lower in probable than in possible DLB (Mollenhauer et al., 2005). Accordingly, the gain of diagnostic accuracy from combining tau and Aβ peptide patterns may have been more striking in order to discriminate AD among possible DLB patients. Thus, the diagnostic value of Aβ peptide patterns can still be considered to be relevant in discriminating AD and DLB.

However, the power of the presented data is limited by the study size and the reliance on clinical diagnosis, which is reported to misclassify 15–20% of dementia cases (Blacker et al., 1994). Moreover, the Aβ-SDS-PAGE/immunolblot is a quite work-consuming method in its current version, which may limit its application for routine diagnostic testing. The standardization of this method for routine diagnostic use is currently under further investigation.

We conclude that tau levels and Aβ peptide patterns represent a disease-specific neurochemical phenotype in CSF and seem to be linked to disease-specific pathophysiological pathways. Studies with larger and neuropathologically defined patient groups will have to be arranged to further elucidate the meaning of our promising data for the neurochemically supported differential diagnosis of dementias.

Acknowledgement

MB, PL, HE, JK, MO and JW are supported by grants from the German Federal Ministry of Education and Research (Competence Net Dementia, grant O1 GI 0420), MB is supported by the Research program, Faculty of Medicine, Georg-August-Universität Göttingen; JW and PL are supported by grants from the German Federal Ministry of Education and Research CJK (01 GI 0301) and HBPP-NGFN2 (01 GR 0447). The authors would like to thank Sabine Paul, Birgit Otte and Heike Zech for excellent technical assistance.

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