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Neurochemical dementia diagnostics: assays in CSF and blood

Abstract In this review, current neurochemical dementia diagnostics (NDD) procedures are presented with a focus on biomarkers in the cerebrospinal fluid (CSF) and blood: amyloid β peptides, tau protein, and its phosphorylated form (pTau). CSF analysis is an increasingly important tool for early and differential diagnosis of dementia syndromes. Although lumbar puncture is a mildly invasive procedure with a low incidence of complications, establishing blood assays capable of reliably measuring NDD biomarkers is an aim of several studies worldwide.

Key words dementia · cerebrospinal fluid · tau · amyloid · Alzheimer's disease

Introduction

Dementia is a rapidly growing challenge for the health care system worldwide. Regarding Alzheimer's disease (AD), however, the increasing number of patients is currently not met by increasingly accurate standards of *durante vitam* diagnosis. Although sensitivity of clinical diagnosis is relatively high (93%), its specificity may be considerably lower, e.g. reported as only 55% in a multi-center clinical-autopsy study [37]. The clinical characterization of AD is predictive of the AD neuropathology in 80–90% of cases when performed in expert centers, however, very early diagnosis of AD as well as the correct differential diagnosis of unusual presentations of patients with dementia remains difficult on pure clinical grounds. With the introduction of potentially successful treatment strategies against dementia (reviewed in [25]), the need for an early and differential diagnosis of dementia becomes even more urgent, and the aim of this review is to summarize the current state of the art in the field of neurochemical routine analysis of dementing conditions, followed by the discussion on challenging research perspectives.

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Neurochemical dementia diagnostics: assays in the CSF

Almost 10 years ago, requirements were proposed for a test to become an acceptable diagnostic parameter in AD [51], and a common consensus is that sensitivity and specificity of such a test should not be lower than approximately 85%, and 75–85%, respectively [51].

Since two groups of molecules, namely amyloid β peptides, and Tau proteins along with hyperphosphorylated forms of the latter, are directly involved in two major pathologic processes of AD: accumulation of plaques, and deposition of neurofibrillary tangles, respectively, it is not surprising that these two groups of biomarkers play the most important diagnostic role in AD and other dementias (Fig. 1). Moreover, there are accumulating evidences that the two metabolic pathways may interact with each other [40] with Wnt

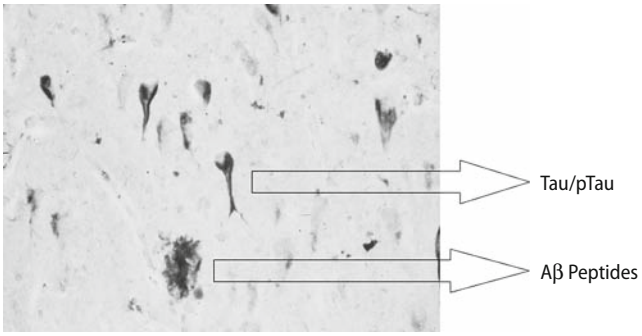

Fig. 1 Accumulation of plaques and deposition of neurofibrillary tangles in *post mortem* brain of Alzheimer's disease patient. The two most important CSF biomarkers: amyloid β peptides and Tau/pTau proteins are directly involved in these two pathologic events

pathway linking both amyloid plaques biogenesis and neurofibrillary changes [12].

Amyloid β peptides as biomarkers of AD

Amyloid β peptides result from enzymatic processing of Amyloid Precursor Protein (APP) [23] by α-, β-, and γ-secretases releasing several forms of amyloid β-peptides (Aβ), including Aβ1-37/38/39/40/42 ('Aβ quintet'). Interestingly, the discovery of amyloid β peptides ending at different C-termini leads to a conclusion that different γ-secretase activities may exist [9, 24], however, as an alternative explanation a different mechanism is postulated of the dependency of the cleavage site from the length of the intramembrane APP domain [36].

Several studies, including these from our groups (for review see [35, 42]), reported decreased CSF concentration of amyloid β peptides ending at the C-terminus of 42 (Aβ42) in AD patients, whereas the total level of Aβ peptides remains unchanged. Mechanisms leading to decreased concentrations of Aβ42 in CSF of patients with AD are not clarified so far. Accumulation of the peptide in the plaques is suggested by some investigators, however, this hypothesis cannot explain the results of a selective decrease of the concentration of this Aβ peptide species in the CSF of the subgroup of patients with Creutzfeldt-Jakob disease (CJD) who did not develop any amyloid plaques at all [43, 57]. Similarly, decreased levels of CSF Aβ42 were recorded in bacterial meningitis [49], and subacute sclerosing panencephalitis [20]. Sensitivity and specificity of Aβ1-42 alone to distinguish AD from elderly controls were 78% and 81%, respectively, in the study of Hulstaert et al. [20], and Galasko et al. reported similar figures of 78 and 83% for sensitivity and specificity, respectively [13]. Blennow et al. analyzed data from eight studies with a total number of n = 562 AD patients and n = 273 controls, and reported mean sensitivity and specificity of 85 and 84%, respectively [7]. Moreover, in our recently published study we have found that, in addition to Aβ peptides, also soluble forms of their precursor, namely CSF sAPPα and sAPPβ, may be candidate biomarkers of AD [32].

Amyloid β peptides seem also to be attractive candidates for differential diagnosis among dementias. Recently, we investigated the Aβ peptides pattern in the CSF of patients with AD, DLB, and PDD, and we observed that the ratio of the differentially altered Aβ1-42 to the Aβ1-37 levels subsequently discriminated all diagnostic groups from each other at a highly significant level, except DLB from PDD [3]. Similarly, a significant decrease was observed of CSF Aβ1-37, Aβ1-38, and Aβ1-42 in patients with frontotemporal lobe degeneration, and decreased CSF concentration of Aβ1-37 and Aβ1-38 might represent an interesting differential biomarker candidate for this disease [4, 5].

Tau and phospho-Tau proteins

While increase in the total tau CSF concentration is considered to reflect unspecific disruption of nerve cells, abnormal hyperphosphorylation of tau is a hallmark of AD [22], and hyperphosphorylated molecules of tau form neurofibrillary tangles [15]. Tau can be phosphorylated at seventy-nine putative positions, serine and threonine being predominant. In studies available so far, mean sensitivity and specificity of tau phosphorylated at different positions varied in the ranges of 44–94%, and 80–100%, respectively [7].

We found significantly increased CSF concentrations of pTau181 in the group of AD patients with clinical diagnosis supported neurochemically by decreased Aβ42 in CSF [29]. This form seems to be particularly interesting since pTau181 remains unchanged while total tau is increased after acute stroke [19, 41]. Similarly, Vanmechelen et al. [54] reported significantly increased levels of CSF pTau181 in AD compared to all other groups studied except for corticobasal degeneration, and Parnetti et al. confirmed that pTau181 was a useful biomarker to distinguish AD from DLB [46]. Tau phosphorylated at threonine 231 (pTau231) seems to help in the differentiation of AD from relevant diseases, i.e. frontotemporal dementia, vascular dementia, and DLB (reviewed in [7]). A follow up study revealed increased CSF concentration of pTau231 at the onset of the disease followed by decreasing concentrations of pTau231 but not total tau in a group of untreated AD patients, which in turn may suggest a possible role of this form in tracking a natural course of the disease [16].

When the three different phospho-tau proteins (pTau199, pTau181, and pTau231) were compared regarding their ability to distinguish patients with different forms of dementia as well as non-demented controls, it turned out that overall performance of pTau181 and pTau231 was equal, with somehow worse performance of pTau199 [17].

Combination of CSF biomarkers

Since amyloid β peptides and Tau/pTau proteins represent two pathologic processes in AD, with still unclear cross-talk between each other, one might expect that their simultaneous analysis synergistically complements each other in terms of diagnostic accuracy.

In an international multicenter project, combined analysis of Aβ1-42 and tau protein showed 85% diagnostic sensitivity and 58% specificity to distinguish AD from non-Alzheimer types of dementia [20]. In this study, the mean sensitivity and specificity levels of the individual markers were significantly improved from 74–79 to 86% if both markers were considered simultaneously. In our study [31], we have found slightly better discrimination of patients with AD, non-Alzheimer's dementia and controls when Aβ42 was combined with Aβ40 (i.e. a concentration quotient of Aβ42/Aβ40). This discrimination was further slightly improved by a simultaneous evaluation of CSF total Tau concentration, and combination of all these three parameters resulted in a correct separation of 94% of subjects in our study.

Although there have been several reports showing that the total concentration of Aβ peptides in human CSF is not altered in pathological conditions [21, 31, 56], the issue of how the total CSF Aβ load influences the reliability of the neurochemical dementia diagnosis has not been tested so far. We addressed this question by measuring corresponding Aβ biomarkers, and relating them to the concentration of Aβx-40, i.e. the most abundant peptide in human CSF, closely correlating and thus reflecting the total load of CSF Aβ peptides [55], and we found that Aβ ratio correlates better with 'independent' AD biomarkers: Tau and pTau181 than 'raw' Aβ42 concentration in patients with high- and low- Aβ load.

Andreasen et al. reported a sensitivity of 94% in the group of 105 probable AD, and 88% in the group of 58 possible AD when analysis of CSF total Tau was accompanied by Aβ1-42 [2]. Specificity in this study was high to differentiate AD from psychiatric disorders and non-demented subjects (100 and 89%, respectively), however, low concentrations of Aβ1-42 found in several cases of DLB resulted in lower specificity to discriminate this disease. The lowest specificity (48%) was found to discriminate AD from vascular dementia, probably because these patients had concomitant pathological features of AD.

In a recently published report, Engelborghs et al. [11] tested the diagnostic performance of the CSF biomarkers: Aβ1-42, total tau, and pTau181 on the ground of autopsy-controlled cases, and concluded that dementia could be discriminated from controls with a sensitivity of 86% and a specificity of 89%. Tau and Aβ1-42 optimally discriminated AD from other dementias and controls (with sensitivity of 90% and specificity of 89%). AD was optimally discriminated from non-AD using pTau181 and Aβ1-42 (sensitivity of 80% and specificity of 93%). This leads to the conclusion that the value of biomarkers in differential dementia diagnosis can be shown, using pathological findings as a reference, whereby the newly developed models achieve sensitivity, specificity, and diagnostic accuracy levels consistently exceeding 80%.

One of the most demanding aspects of the neurochemical analysis of dementia disorders is to find biomarkers capable of predicting the development of AD in patients with mild cognitive impairment. Such a predictive (preclinical) diagnosis is hoped to open the way to preventive therapeutic interventions. In a study addressing a combination of the three CSF biomarkers Tau, pTau181 and Aβ42, incipient AD could be detected among patients fulfilling the criteria for MCI with a sensitivity of 68% (95% CI 45–86%) and a specificity of 97% (95% CI 83–100%), therefore suggesting effective discrimination between subgroups of patients with MCI who would eventually develop AD from those who will not proceed to AD in order to offer early treatment for the subjects at risk [59]. Recently, these observations were confirmed with much bigger disease groups and longer observation period [18]. In our recently published study, based on multiplexing technology, the cut off levels of Aβ1-42 and pTau181 derived from the differential analysis of early dementia patients allowed correct definition of a subgroup of MCI subjects characterized by an increased risk to develop AD from a subgroup of MCI subjects without such a risk [33].

It is unclear whether the concentrations of the CSF biomarkers reflect the degree of dementia. Although correlation of the CSF biomarkers with the stage of the disease, at least as measured with MMSE, was found neither by us [29, 31], nor by other investigators [39], there are data in the literature suggesting that such a correlation may exists, at least in some subgroups of AD patients [48]. In this respect, interesting information comes from the observation that the highest concentrations of Tau in the CSF are observed in neurodegenerative processes with high dynamics, for example CJD or acute stroke, which certainly highlights the correlation between the ratio of neuronal death and the concentration of the biomarker [6].

As a matter of fact, a pattern of CSF biomarkers with an extremely high concentration of total Tau (higher than 1,200 pg/ml), normal or only slightly increased pTau, and sometimes slightly decreased Aβ42 concentrations is quite characteristic for rapidly progressing neurodegeneration, and in all such cases CJD should be considered as a differential diagnostic item [44, 57]. Additionally, CSF 14-3-3 proteins should be measured by western-immunoblot when the high-throughput quantitative ELISA or multiplex assays do indicate possible CJD, since these proteins are elevated in CJD [58].

Two important pre-analytical confounding factors must be considered: (a) that these proteins/peptides

have a tendency to stick to the walls of test tubes made of glass and hard plastic, resulting in falsely low levels [1, 27], and (b) that the concentration of Aβ peptides tend to have lower values following repeated freeze/thaw cycles [53]. Therefore, it is important to collect CSF into non-absorbing test tubes made of polypropylene, and to analyze them without prior freezing or froze them once [34]. Another important point is an obvious lack of systematic inter-laboratory quality control surveys: in a quality control pilot study recently coordinated by our laboratory, we observed inter-laboratory coefficients of variation of biomarkers in the range of 20–30%, which clearly points at the necessity of further optimization of measurements [28]. Therefore, we regard it as mandatory that clinicians who have ordered CSF-based neurochemical dementia diagnostics (CSF-NDD) obtain the results not only in terms of 'raw' concentrations, but as an integrated report containing interpretation of the results.

Neurochemical dementia diagnostics: assays in blood

Lumbar puncture is a relatively safe and uncomplicated procedure, and only a small ratio of AD patients complains about post-puncture complications [2, 8]. However, re-punctures and follow-up measurements of CSF parameters are generally considered as inconvenient for patients, and thus there is a need to search for alternative body fluids as a possible source of relevant biomarkers. There are several hypothesis-driven rationale to speculate that blood, and blood-derived fluids (serum and plasma) would fulfill criteria of such a source (reviewed in [30]), and moreover, evidence exists that Aβ peptides may be released from other tissue in addition to the brain [10, 26].

At least three large (hundreds to thousands of subjects) prospective studies published in the last 2–3 years addressed alterations of plasma Aβ peptides as potential biomarkers of AD. Van Oijen et al. [52] showed in a large population-based prospective study that high plasma concentrations of Aβ1-40 were associated with an increased risk of dementia, especially for people who have concomitant low concentrations of Aβ1-42. Individuals with high concentrations of Aβ1-40 combined with low concentrations of Aβ1-42 had an over tenfold increased risk of dementia compared with people with low concentrations of both Aβ1-40 and Aβ1-42. Interestingly, these findings were independent of the presence of an APOE ε4 allele. Similarly, Mayeux et al. [38] reported higher baseline plasma Aβ1-40 and Aβ1-42 concentrations in individuals who developed AD than in elderly people who did not over an observation period of five years. Moreover, after adjusting for Aβ1-40 concentration, Aβ1-42 concentrations remained significantly different.

Graff-Radford et al. [14] measured plasma Aβ40 and Aβ42 concentrations at baseline in a elderly cohort of 563 volunteers followed up for 2–12 years. In this cohort, 53 subjects developed MCI or AD, and the subjects with plasma Aβ42/Aβ40 ratios in the lower quartiles turned out to have significantly greater risk of MCI or AD. Comparison of subjects with plasma Aβ42/Aβ40 ratios in the lowest and the highest quartile gave a relative risk of 3.1, and this observation suggests that the plasma Aβ42/Aβ40 ratio may be a useful biomarker for identifying cognitively normal elderly subjects at increased risk to develop cognitive disturbances.

In a recently published, large, cross-sectional study, Sun et al. [50] found a very interesting association between decreased plasma Aβ42 concentration and depression, suggesting the presence of at least two depression subtypes from the cognitive perspective: (a) amyloid-associated depression, which is associated with poor memory and other cognitive dysfunction, and (b) non-amyloid depression, which is associated with only visuospatial and executive dysfunction. Their finding of decreased plasma Aβ42 in patients with depression and memory complaints but not in patients with depression and without cognitive disturbances, taken together with the fact that depression is quite often a prodromal stage of dementia [45], might actually suggest that they found a group of patients with depression as the first symptom of incipient dementia.

A different approach to AD diagnosis based on biomarkers in blood was suggested by Ray and colleagues [47], who measured abundance of 120 signaling proteins in samples from 259 subjects with different stages of AD and in controls. Dividing the samples into a training set and two test sets, they defined 18 proteins that could classify unknown samples from AD and controls with 95% of positive and 83% of negative agreement. Moreover, they were able to identify MCI subjects who progressed to dementia within 2–6 years.

In summary, CSF-based neurochemical dementia diagnostics can be regarded as a diagnostic tool in everyday medical routine. Moreover, a rapidly expanding research in this area will hopefully result in defining and optimizing additional promising assays in the near future. It seems that a lack of standardized, commercially available and reliable assays to measure AD biomarkers in blood is currently the main limitation disabling the comparison of the results obtained by different research groups.

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References

1. Andreasen N, Minthon L, Clarberg A, Davidsson P, Gottfries J, Vanmechelen E, Vanderstichele H, Winblad B, Blennow K (1999) Sensitivity, specificity, and stability of CSF-tau in AD in a community-based patient sample. Neurology 53:1488–1494
2. Andreasen N, Minthon L, Davidsson P, Vanmechelen E, Vanderstichele H, Winblad B, Blennow K (2001) Evaluation of CSF-tau and CSF-Aβ42 as diagnostic markers for Alzheimer disease in clinical practice. Arch Neurol 58:373–379
3. Bibl M, Mollenhauer B, Esselmann H, Lewczuk P, Klafki HW, Sparbier K, Smirnov A, Cepek L, Trenkwalder C, Ruther E, Kornhuber J, Otto M, Wiltfang J (2006) CSF amyloid-beta-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia. Brain 129:1177–1187
4. Bibl M, Mollenhauer B, Lewczuk P, Esselmann H, Wolf S, Trenkwalder C, Otto M, Stiens G, Ruther E, Kornhuber J, Wiltfang J (2007) Validation of amyloid-beta peptides in CSF diagnosis of neurodegenerative dementias. Mol Psychiatry 12:671–680
5. Bibl M, Mollenhauer B, Wolf S, Esselmann H, Lewczuk P, Kornhuber J, Wiltfang J (2007) Reduced CSF carboxyterminally truncated Abeta peptides in frontotemporal lobe degenerations. J Neural Transm 114:621–628
6. Blennow K, Hampel H (2003) CSF markers for incipient Alzheimer's disease. Lancet Neurol 2:605–613
7. Blennow K, Vanmechelen E, Hampel H (2001) CSF total tau, Aβ42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. Mol Neurobiol 24:87–97
8. Blennow K, Wallin A, Hager O (1993) Low frequency of post-lumbar puncture headache in demented patients. Acta Neurol Scand 88:221–223
9. Citron M, Diehl TS, Gordon G, Biere AL, Seubert P, Selkoe DJ (1996) Evidence that the 42- and 40-amino acid forms of amyloid beta protein are generated from the beta-amyloid precursor protein by different protease activities. Proc Natl Acad Sci USA 93:13170–13175
10. Di Luca M, Pastorino L, Bianchetti A, Perez J, Vignolo LA, Lenzi GL, Trabucchi M, Cattabeni F, Padovani A (1998) Differential level of platelet amyloid β precursor protein isoforms: an early marker for Alzheimer disease. Arch Neurol 55:1195–1200
11. Engelborghs S, De Vreese K, Van de Casteele T, Vanderstichele H, Van Everbroeck B, Cras P, Martin JJ, Vanmechelen E, De Deyn PP (2008) Diagnostic performance of a CSF-biomarker panel in autopsy-confirmed dementia. Neurobiol Aging 29:1143–1159
12. Fuentealba RA, Farias G, Scheu J, Bronfman M, Marzolo MP, Inestrosa NC (2004) Signal transduction during amyloid-beta-peptide neurotoxicity: role in Alzheimer disease. Brain Res Brain Res Rev 47:275–289
13. Galasko D, Chang L, Motter R, Clark CM, Kaye J, Knopman D, Thomas R, Kholodenko D, Schenk D, Lieberburg I, Miller B, Green R, Basherad R, Kertiles L, Boss MA, Seubert P (1998) High cerebrospinal fluid tau and low amyloid β42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E genotype. Arch Neurol 55:937–945
14. Graff-Radford NR, Crook JE, Lucas J, Boeve BF, Knopman DS, Ivnik RJ, Smith GE, Younkin LH, Petersen RC, Younkin SG (2007) Association of low plasma abeta42/abeta40 ratios with increased imminent risk for mild cognitive impairment and Alzheimer disease. Arch Neurol 64:354–362
15. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI (1986) Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci USA 83:4913–4917
16. Hampel H, Buerger K, Kohnken R, Teipel SJ, Zinkowski R, Moeller HJ, Rapoport SI, Davies P (2001) Tracking of Alzheimer's disease progression with cerebrospinal fluid tau protein phosphorylated at threonine 231. Ann Neurol 49:545–546
17. Hampel H, Buerger K, Zinkowski R, Teipel SJ, Goernitz A, Andreasen N, Sjoegren M, DeBernardis J, Kerkman D, Ishiguro K, Ohno H, Vanmechelen E, Vanderstichele H, McCulloch C, Moller HJ, Davies P, Blennow K (2004) Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. Arch Gen Psychiatry 61:95–102
18. Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L (2006) Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 5:228–234
19. Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E, Blennow K (2001) Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci Lett 297:187–190
20. Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, Bancher C, Cras P, Wiltfang J, Mehta PD, Iqbal K, Pottel H, Vanmechelen E, Vanderstichele H (1999) Improved discrimination of AD patients using β-amyloid$_{(1-42)}$ and tau levels in CSF. Neurology 52:1555–1562
21. Ida N, Hartmann T, Pantel J, Schröder J, Zerfass R, Förstl H, Sandbrink R, Masters CL, Beyreuther K (1996) Analysis of heterogeneous βA4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. J Biol Chem 271:22908–22914
22. Iqbal K, Grundke-Iqbal I, Zaidi T, Merz PA, Wen GY, Shaikh SS, Wisniewski HM, Alafuzoff I, Winblad B (1986) Defective brain microtubule assembly in Alzheimer's disease. Lancet 2:421–426
23. Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Muller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325:733–736
24. Klafki H, Abramowski D, Swoboda R, Paganetti PA, Staufenbiel M (1996) The carboxyl termini of beta-amyloid peptides 1–40 and 1–42 are generated by distinct gamma-secretase activities. J Biol Chem 271:28655–28659
25. Klafki HW, Staufenbiel M, Kornhuber J, Wiltfang J (2006) Therapeutic approaches to Alzheimer's disease. Brain 129: 2840–2855
26. Kuo YM, Kokjohn TA, Watson MD, Woods AS, Cotter RJ, Sue LI, Kalback WM, Emmerling MR, Beach TG, Roher AE (2000) Elevated Aβ42 in skeletal muscle of Alzheimer disease patients suggests peripheral alterations of AβPP metabolism. Am J Pathol 156:797–805
27. Lewczuk P, Beck G, Esselmann H, Bruckmoser R, Zimmermann R, Fiszer M, Bibl M, Maler JM, Kornhuber J, Wiltfang J (2006) Effect of sample collection tubes on cerebrospinal fluid concentrations of Tau proteins and amyloid β peptides. Clin Chem 52:332–334
28. Lewczuk P, Beck G, Ganslandt O, Esselmann H, Deisenhammer F, Regeniter A, Petereit HF, Tumani H, Gerritzen A, Oschmann P, Schroder J, Schonknecht P, Zimmermann K, Hampel H, Burger K, Otto M, Haustein S, Herzog K, Dannenberg R, Wurster U, Bibl M, Maler JM, Reubach U, Kornhuber J, Wiltfang J (2006) International quality control survey of neurochemical dementia diagnostics. Neurosci Lett 409:1–4
29. Lewczuk P, Esselmann H, Bibl M, Beck G, Maler JM, Otto M, Kornhuber J, Wiltfang J (2004) Tau protein phosphorylated at threonine 181 in CSF as a neurochemical biomarker in Alzheimer's disease: original data and review of the literature. J Mol Neurosci 23:115–122
30. Lewczuk P, Esselmann H, Bibl M, Paul S, Svitek J, Miertschischk J, Meyrer R, Smirnov A, Maler JM, Klein C, Otto M, Bleich S, Sperling W, Kornhuber J, Ruther E, Wiltfang J (2004) Electrophoretic separation of amyloid beta peptides in plasma. Electrophoresis 25:3336–3343
31. Lewczuk P, Esselmann H, Otto M, Maler JM, Henkel AW, Henkel MK, Eikenberg O, Antz C, Krause WR, Reulbach U, Kornhuber J, Wiltfang J (2004) Neurochemical diagnosis of Alzheimer's dementia by CSF Aβ42, Aβ42/Aβ40 ratio and total tau. Neurobiol Aging 25:273–281

32. Lewczuk P, Kamrowski-Kruck H, Peters O, Heuser I, Jessen F, Popp J, Burger K, Hampel H, Frolich L, Wolf S, Prinz B, Jahn H, Luckhaus C, Perneczky R, Hull M, Schroder J, Kessler H, Pantel J, Gertz H-J, Klafki H-W, Kolsch H, Reulbach U, Esselmann H, Maler JM, Bibl M, Kornhuber J, Wiltfang J (2008) Soluble amyloid precursor proteins in the cerebrospinal fluid as novel potential biomarkers of Alzheimer's disease: a multicenter study. Mol Psychiatry (in press)
33. Lewczuk P, Kornhuber J, Vanderstichele H, Vanmechelen E, Esselmann H, Bibl M, Wolf S, Otto M, Reulbach U, Kolsch H, Jessen F, Schroder J, Schonknecht P, Hampel H, Peters O, Weimer E, Perneczky R, Jahn H, Luckhaus C, Lamla U, Supprian T, Maler JM, Wiltfang J (2008) Multiplexed quantification of dementia biomarkers in the CSF of patients with early dementias and MCI: a multicenter study. Neurobiol Aging 29:812–818
34. Lewczuk P, Kornhuber J, Wiltfang J (2006) The german competence net dementias: standard operating procedures for the neurochemical dementia diagnostics. J Neural Transm 113:1075–1080
35. Lewczuk P, Wiltfang J (2008) Neurochemical dementia diagnostics: state of the art and research perspectives. Proteomics 8:1292–1301
36. Lichtenthaler SF, Beher D, Grimm HS, Wang R, Shearman MS, Masters CL, Beyreuther K (2002) The intramembrane cleavage site of the amyloid precursor protein depends on the length of its transmembrane domain. Proc Natl Acad Sci USA 99:1365–1370
37. Mayeux R (1998) Evaluation and use of diagnostic tests in Alzheimer's disease. Neurobiol Aging 19:139–143
38. Mayeux R, Honig LS, Tang MX, Manly J, Stern Y, Schupf N, Mehta PD (2003) Plasma Aβ40 and Aβ42 and Alzheimer's disease: relation to age, mortality, and risk. Neurology 61:1185–1190
39. Mehta PD, Pirttila T, Patrick BA, Barshatzky M, Mehta SP (2001) Amyloid [beta] protein 1–40 and 1–42 levels in matched cerebrospinal fluid and plasma from patients with Alzheimer disease. Neurosci Lett 304:102–106
40. Mudher A, Lovestone S (2002) Alzheimer's disease-do tauists and baptists finally shake hands? Trends Neurosci 25:22–26
41. Otto M, Esselmann H, Schulz-Shaeffer W, Neumann M, Schroter A, Ratzka P, Cepek L, Zerr I, Steinacker P, Windl O, Kornhuber J, Kretzschmar HA, Poser S, Wiltfang J (2000) Decreased β-amyloid1-42 in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Neurology 54:1099–1102
42. Otto M, Lewczuk P, Wiltfang J (2008) Neurochemical approaches of cerebrospinal fluid diagnostics in neurodegenerative diseases. Methods 44:289–298
43. Otto M, Wiltfang J, Cepek L, Neumann M, Mollenhauer B, Steinacker P, Ciesielczyk B, Schulz-Schaeffer W, Kretzschmar HA, Poser S (2002) Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. Neurology 58:192–197
44. Otto M, Wiltfang J, Tumani H, Zerr I, Lantsch M, Kornhuber J, Weber T, Kretzschmar HA, Poser S (1997) Elevated levels of tau-protein in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Neurosci Lett 225:210–212
45. Ownby RL, Crocco E, Acevedo A, John V, Loewenstein D (2006) Depression and risk for Alzheimer disease: systematic review, meta-analysis, and metaregression analysis. Arch Gen Psychiatry 63:530–538
46. Parnetti L, Lanari A, Amici S, Gallai V, Vanmechelen E, Hulstaert F (2001) CSF phosphorylated tau is a possible marker for discriminating Alzheimer's disease from dementia with Lewy bodies. Phospho-Tau International Study Group. Neurol Sci 22:77–78
47. Ray S, Britschgi M, Herbert C, Takeda-Uchimura Y, Boxer A, Blennow K, Friedman LF, Galasko DR, Jutel M, Karydas A, Kaye JA, Leszek J, Miller BL, Minthon L, Quinn JF, Rabinovici GD, Robinson WH, Sabbagh MN, So YT, Sparks DL, Tabaton M, Tinklenberg J, Yesavage JA, Tibshirani R, Wyss-Coray T (2007) Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. Nat Med 13:1359–1362
48. Riemenschneider M, Schmolke M, Lautenschlager N, Vanderstichele H, Vanmechelen E, Guder WG, Kurz A (2002) Association of CSF apolipoprotein E, A[beta]42 and cognition in Alzheimer's disease. Neurobiol Aging 23:205–211
49. Sjögren M, Gisslen M, Vanmechelen E, Blennow K (2001) Low cerebrospinal fluid β-amyloid 42 in patients with acute bacterial meningitis and normalization after treatment. Neurosci Lett 314:33–36
50. Sun X, Steffens DC, Au R, Folstein M, Summergrad P, Yee J, Rosenberg I, Mwamburi DM, Qiu WQ (2008) Amyloid-associated depression: a prodromal depression of Alzheimer disease? Arch Gen Psychiatry 65:542–550
51. The Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease" (1998) Consensus report of the working group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Neurobiol Aging 19:109–116
52. van Oijen M, Hofman A, Soares HD, Koudstaal PJ, Breteler MM (2006) Plasma Abeta(1–40) and Abeta(1–42) and the risk of dementia: a prospective case-cohort study. Lancet Neurol 5:655–660
53. Vanderstichele H, Van Kerschaver E, Hesse C, Davidsson P, Buyse MA, Andreasen N, Minthon L, Wallin A, Blennow K, Vanmechelen E (2000) Standardization of measurement of beta-amyloid(1–42) in cerebrospinal fluid and plasma. Amyloid 7:245–258
54. Vanmechelen E, Van Kerschaver E, Blennow K, De Deyn PP, Gartner FH, Parnetti L, Sindic CJM, Arai H, Riemenschneider M, Hampel H, Pottel H, Valgaeren A, Hulstaert F, Vanderstichele H (2001) CSF-Phospho-tau (181P) as a promising marker for discriminating Alzheimer's disease from dementia with Lewy bodies. In: Iqbal K, Sisodia SS, Winblad B (eds) Alzheimer's disease: advances in etiology, pathogenesis and therapeutics. Wiley, Chichester, pp 285–291
55. Wiltfang J, Esselmann H, Bibl M, Hull M, Hampel H, Kessler H, Frolich L, Schroder J, Peters O, Jessen F, Luckhaus C, Perneczky R, Jahn H, Fiszer M, Maler JM, Zimmermann R, Bruckmoser R, Kornhuber J, Lewczuk P (2007) Amyloid beta peptide ratio 42/40 but not A beta 42 correlates with phospho-Tau in patients with low- and high-CSF A beta 40 load. J Neurochem 101:1053–1059
56. Wiltfang J, Esselmann H, Cupers P, Neumann M, Kretzschmar H, Beyermann M, Schleuder D, Jahn H, Rüther E, Kornhuber J, Annaert W, De Strooper B, Saftig P (2001) Elevation of β-amyloid peptide 2–42 in sporadic and familial Alzheimer's disease and its generation in PS1 knockout cells. J Biol Chem 276:42645–42657
57. Wiltfang J, Esselmann H, Smirnov A, Bibl M, Cepek L, Steinacker P, Mollenhauer B, Buerger K, Hampel H, Paul S, Neumann M, Maler M, Zerr I, Kornhuber J, Kretzschmar HA, Poser S, Otto M (2003) β-amyloid peptides in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Ann Neurol 54:263–267
58. Zerr I, Bodemer M, Gefeller O, Otto M, Poser S, Wiltfang J, Windl O, Kretzschmar HA, Weber T (1998) Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. Ann Neurol 43:32–40
59. Zetterberg H, Wahlund LO, Blennow K (2003) Cerebrospinal fluid markers for prediction of Alzheimer's disease. Neurosci Lett 352:67–69