Neurochemical dementia diagnostics: State of the art and research perspectives

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The aim of this review is to present current state of the art on the field of routine neurochemical dementia diagnostics (NDD) with a focus on cerebrospinal fluid (CSF) biomarkers: amyloid β peptides, tau protein, and its phosphorylated form (pTau). After several years of experience, it is reasonably to postulate that CSF biomarkers analysis is an increasingly important tool within the early and differential diagnosis of dementia syndromes. Actual research activities are briefly discussed, too, including: (i) possibilities and limitations of the diagnosis of incipient Alzheimer’s disease in preclinical stages (e.g., mild cognitive impairment), (ii) the role of multiplexing technologies in dementia biomarkers research, (iii) the role of biomarkers in differential diagnosis of dementia syndromes, (iv) approaches to improve analytical performance of available methods, and (v) research activities to identify dementia biomarkers in blood.

Keywords:
Alzheimer’s disease / Amyloid / Cerebrospinal fluid / Dementia / Tau

1 Introduction

Increasing life expectancy in Western industrialized countries results in an increased ratio of subjects afflicted with dementia. Already now, dementia is a rapidly growing challenge for the health care system. Regarding Alzheimer’s disease (AD), however, the increasing number of patients is currently not met by increasingly accurate standards of during 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 multicenter clinical-autopsy study [1]. 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 Ref. [2–4]), 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.

Since cerebrospinal fluid (CSF) is in direct contact with the environment of the CNS, it is obvious that any changes in biochemical composition of brain parenchyma should be predominantly reflected in the CSF. A review by Reiber [5] presents a complete concept of distinguishing diffusion of brain-derived proteins into CSF from diffusion of proteins from blood into CSF, allowing proteins that originate from the brain to be prioritized. Lumbar puncture is an easy procedure, with low incidence of complications. In a large study of Andreasen et al. [6] only 4.1% of all patients experienced postlumbar headache, and even a smaller ratio of 2% was reported in the study of Blennow et al. [7]. Thus, it is reason-
able to postulate that lumbar puncture is a feasible, moderately invasive procedure, and CSF analysis could possibly improve current clinic-, neuropsychology-, and neuroimaging-based diagnostic approaches.

2 Neurochemical dementia diagnostics: The state of the art in the routine

Almost 10 years ago, requirements were proposed for a test to become an acceptable diagnostic parameter in AD [8]. Ideally, such a test should be: (i) able to detect a fundamental feature of AD pathology, (ii) validated in neuropathologically confirmed cases, (iii) precise and (iv) reliable, (v) non-invasive, (vi) simple to perform, and (vii) inexpensive. A common consensus is that sensitivity and specificity of such a test should not be lower than approximately 85, and 75–85%, respectively [8].

2.1 Amyloid precursor protein (APP) – metabolism and impact on AD diagnosis

Amyloid plaques are composed mainly of peptides derived from enzymatic cut of β-APP [9]. This transmembrane protein is encoded in man by a gene on chromosome 21, and its alternative splicing results in at least eight forms. The form known as APP 695 (i.e., the one consisting of 695 amino acid residues) is expressed predominantly in the brain [10]. The physiological role of APP is not clear so far, however, an involvement in cell-to-cell and matrix interactions is postulated. APP is enzymatically processed by α-, β-, and γ-secretases to release several forms of amyloid β (Aβ) peptides, five of them: Aβ1–37/38/39/40/42 (“Aβ quintet”) are found consequently in human CSF of all subjects investigated by our group so far (Fig. 1). Interestingly, the discovery of Aβ peptides ending at different C-terminus leads to a conclusion that different γ-secretase activities may exist [11, 12], 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 [13].

Although physiological role of APP and Aβ peptides is unclear, several lines of evidence show links between Aβ peptides and pathophysiology of AD. Formation of senile plaques composed predominantly of Aβ peptides is one of the hallmark of the disease [14]. Studies on mice immunized against Aβ showed significantly decreased plaque load in these animals [15]. This observation lead to the initiation of a clinical study and demonstration that antibodies against Aβ are not only effective in slowing progression of AD [16], but also probably decrease amyloid plaque load in human brain [17]. Although this particular study had to be terminated due to unexpected side effects, it is speculated that immunization against Aβ (perhaps with different epitopes) will remain one of the major avenues in AD therapy research. Similarly, several other therapeutic studies, including γ-secretase inhibition, application of nonsteroid anti-inflammatory drugs, or Aβ cleavage by simulation of muscarinic acetylcholine-receptor agonists are based on the hypothesized role of Aβ metabolism in AD (for review, see Ref. [4]).

Therefore, it is not surprising that many studies, including those from our group, reported altered (namely, decreased) CSF concentration of Aβ peptides ending at the C-terminus of 42 (Aβ42) in AD patients ([18, 19], and reviews: [20–22]), whereas the total level of Aβ peptides remains unchanged [23]. 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 our 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 [24]. Similarly, decreased levels of CSF Aβ42 were recorded in bacterial meningitis [25], a disease with may cause chronic memory deficits but does not present with Aβ plaques. In fact, the lowest values found in a large multicenter study of Hulstaert et al. [26] were observed in two cases of subacute sclerosing panencephalitis and in one case of bacterial meningitis. 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. [26], and Galasko et al. [27] reported similar figures of 78 and 83% for sensitivity and specificity, respectively. In our study [18], application of CSF Aβ42 alone resulted in a correct classification of 87% of subjects when non-Alzheimer’s dementia and non-demented patients were treated as a control group for AD. Blennow et al. [20] analyzed data from eight studies with total number of n = 562 AD patients and n = 273 controls, and reported mean sensitivity and specificity of 85 and 84%, respectively.

2.2 Tau protein and its phosphorylated forms

Tau proteins belong to the family of microtubule-associated proteins found in neuronal and non-neuronal cells (reviewed in Ref. [28]). The human tau gene is located on the long arm of chromosome 17. Its alternative splicing leads to formation of six forms of the protein ranging from 352 to 441 amino acids. Studies on the role of tau proteins have revealed that their main function is to promote neuronal microtubule sta-
bility and assembly. Tau proteins are also involved in promoting microtubule nucleation, growth and bundling, and it is hypothesized that phosphorylation of the tau molecule is an important factor in regulating tau-microtubule interaction (reviewed in Ref. [20]). The phosphorylation status of tau is considered to change during development, with a relatively high degree of phosphorylation during the fetal phase followed by a steady decrease with age, most probably as a result of phosphatase activation [30, 31]. Total tau protein concentration has been extensively studied as an unspecific marker of neuronal destruction in neurodegeneration. The meta-analysis of Sunderland et al. [32] was based on the data from 17 reports on Aβ42 and 34 reports on CSF tau in AD, and all these studies reported increased CSF total tau in AD. Increased CSF total tau concentrations are observed in neuropsychiatric disorders other than AD, like, e.g., CJD and stroke [33, 34]. Nevertheless, given the fact that tau most likely can be used to monitor efficacy of the neuron protective drugs in AD patients, and that CJD and acute stroke are easily distinguishable from AD clinically, should not dampen the value of this marker.

While the 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 [35], and hyperphosphorylated molecules of tau form neurofibrillary tangles [36]. Tau can be phosphorylated at 79 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 [20]. Interestingly, Hu et al. [37] shown that phosphorylated tau (pTau)396/404 to total tau ratio in CSF could discriminate AD from other dementias and neurological disorders at sensitivity of 96% and specificity of 94%. These findings suggest that CSF analysis of tau phosphorylated at serine-396/404 might be more promising than some of the other sites reported to date. It must be also noted that while processes of hyperphosphorylation of tau dominate in AD, dephosphorylation of these molecules is supposed to happen as well, and the ratio of phosphorylated and dephosphorylated tau molecules must be considered dynamically. Moreover, dephosphorylation of hyperphosphorylated tau seems to be a promising therapeutic target in AD [38].

We found significantly increased CSF concentrations of pTau181 in the group of AD patients with clinical diagnosis supported neurochemically by decreased Aβ42 in CSF [39]. This biomarker seems to be particularly interesting since pTau181 remains unchanged while total tau is increased after acute stroke [34], which may suggest that pTau181 is not only a marker of simple neuronal loss. Similarly, Vanmechelen et al. [40] reported significantly increased levels of CSF pTau181 in AD compared to all other groups studied (frontotemporal degeneration, dementia with Lewy bodies (DLB), Parkinson’s disease, multiple system atrophy, and progressive supranuclear palsy) except for corticobasal degeneration, and Parnetti et al. [41] confirmed that pTau181 was a useful biomarker to distinguish AD from DLB [41]. Regarding other phosphorylation sites, in an international multicenter project, Itoh et al. reported a significant overall increase of pTau199 in patients with AD compared to all other non-AD groups. In this study, both sensitivity and specificity of CSF pTau199 for discriminating AD from other studied groups yielded 85% at the cutoff level of 1.05 fmol/mL [42]. 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 Ref. [20]). 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 [43]. Interestingly, tau protein phosphorylated at both positions threonine 231 and serine 235 turned out to be increased in patients with mild cognitive impairment (MCI) who developed AD during follow up [44]. In this study, a simultaneous evaluation of total tau and pTau distinguished the group of patients at risk of developing AD from those who complained of having memory impairment but did not have objective memory loss.

When the three different forms of phospho-tau (pTau) forms were compared regarding their ability to distinguish patients with different forms of dementia as well as nondemented controls, it turned out that overall performance of pTau181 and pTau231 was equal, with somehow worse performance of pTau199. Interestingly, discrimination between AD and DLB was maximized using pTau181 at sensitivity of 94% and specificity of 64%, and pTau231 maximized group separation between AD and frontotemporal dementia with sensitivity of 88% and specificity of 92%. Therefore, there is some evidence, however so far failing the level of significance, for pTau proteins to perform differently in the discrimination of particular types of dementias [45].

### 2.3 Apolipoprotein E (APOE) genotype

Apolipoprotein E is a protein involved in the transport of cholesterol. Apart of the presence in plasma, it is also produced by astrocytes in CNS to support growth and repair of neurons. The APOE gene is localized on chromosome 19 with three alleles known (ε2, ε3, and ε4). A growing volume of evidence have been reported on association of APOE ε4 and late-onset familial AD (for review, [46]). As many as 40–50% of AD patients posses ε4 allele as compared to 15–25% of controls [47], which means that subjects homozygous for ε4 allele are reported to have a 6–8-fold increased risk of developing AD as compared to the risk for heterozygotic subjects which is increased 3–4-fold (reviewed by [46]). So far, the ε4 allele is identified as a major genetic risk factor, which is independent from gender, age, and ethnic origin of individuals [48]. In a large American study with 2188 patients, analysis of ε4 allele showed mild sensitivity and
specificity of 65 and 68\% respectively [49]. Thus, it is suggested that genotyping for APOE allele should be reserved for patients in early stages of dementia, since the presence of one or two APOE ε4 alleles does considerably improve the post hoc diagnostic specificity patients who otherwise fulfill the clinical criteria of AD. The mechanisms regulating increased risk of developing AD in cases carrying the ε4 allele are still unclear.

2.4 Combined analysis of CSF parameters

There are many examples of CNS diseases where a combination of more than one CSF parameter significantly improves the accuracy of the diagnosis. Neuroinflammatory diseases, like neuroborreliosis [50] or multiple sclerosis [51] are representative examples. Senile plaques, composed mostly of A\(β\) peptides, and neurofibrillary tangles containing hyperphosphorylated forms of tau proteins are the two neuropathologic hallmarks of AD. Hence, it is not surprising that the biomarkers related to these alterations play crucial role in the neurochemical diagnosis of this disorder. Since clinical praxis, as well as studies summarized in this chapter show that both, A\(β\) peptides and tau/pTau proteins have their analytical advantages and disadvantages, we believe that only analysis of biomarkers of the two pathophysiological groups can assure the highest diagnostic performance to be obtained currently. For example, whereas total tau is a sensitive marker of neurodegeneration, it is not specific at all, and must be accompanied by analysis of pTau, and vice versa, analysis of pTau without tau and A\(β\) would lead to false negative diagnosis of many cases of non-AD neurodegeneration and dementias. In line with this hypothesis, we have seen in the everyday lab routine many AD patients with apparently normal tau and pTau but significantly decreased A\(β\)42 and/or A\(β\) ratio or vice versa: patients with unaltered A\(β\) peptides, but increased pTau181. A question still remains open how importance of particular biomarkers should be weighted,” and how to prepare a simple diagnostic algorithm.

In an international multicenter project, combined analysis of A\(β\)1–42 and tau protein showed 85\% diagnostic sensitivity and 58\% specificity to distinguish AD from non-Alzheimer types of dementia [26]. 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 [18], we have found slightly better discrimination of patients with AD, non-Alzheimer's dementia and controls when A\(β\)42 was combined with A\(β\)40 (i.e., a concentration quotient of A\(β\)42/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 A\(β\) peptides in human CSF is not altered in pathological conditions ([18, 19, 52], and reviewed in Ref. [53]), the issue how the total CSF A\(β\) load influences the reliability of the neurochemical dementia diagnosis has not been tested so far. We addressed this question by measuring corresponding A\(β\) biomarkers, and relating them to the concentration of A\(β\)x-40, i.e., the most abundant peptide in human CSF, closely correlating and thus reflecting the total load of CSF A\(β\) peptides [54]. As independent biomarkers describing the second neuropathological hallmark of AD (i.e., hyperphosphorylation of tau and formation of neurofibrillary tangles), we applied the CSF concentration of tau proteins (pTau181 and total-tau). In patients with a low CSF load of total A\(β\) peptides a true non-AD subject is at risk to be erroneously classified as false AD patient, if only A\(β\)1–42 is measured. Since in the latter case also the concentration of CSF Aβ1–40 is low the ratio of A\(β\)42/A\(β\)40 is not decreased, which identifies the subject correctly as a non-AD case. The opposite holds true for “high CSF A\(β\) load patients.” In this case A\(β\)1–42 will have selectively dropped (at unchanged high A\(β\)1–40) during the preclinical course of AD. However, due to the high baseline value the decreased A\(β\) CSF concentration is still above the cut-off value of the total study population. In contrast to A\(β\)1–42, the ratio A\(β\)42/A\(β\)40 is decreased since A\(β\)1–40 stays at the high baseline value. Since the total load of CSF A\(β\) peptides does follow a normal distribution this classification error due to divergent CSF loads of total A\(β\) is expected to affect at least five percent of subjects beyond the two-fold SD at each side of the normal distribution, i.e., one out of ten patients. To validate our finding we applied CSF pTau181 and tau: patients with high A\(β\)x-40 and pathologically decreased A\(β\) ratio (i.e., lower than 0.11, [18]) do present with elevated pTau181 and total-tau CSF values (indicating true AD cases) as compared to the group of patients with low A\(β\)x-40 and normal A\(β\) ratio. This indicates that in the latter patient groups the A\(β\) ratio does reflect more adequately the tau-dependent neuropathology, and it may indicate that the CSF concentrations of A\(β\)x-42 is not only influenced by the aggregation-promoting dyscatabolism observed in AD but that it is also determined in sporadic AD by the rate of APP metabolism of yet unknown background. Currently pathomechanisms of such differences in APP processing rate can only be speculated, but there are evidence that a genetic background could be responsible for it [55]. Andreasen et al. [6] reported 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 A\(β\)1–42. Specificity in this study was high to differentiate AD from psychiatric disorders and nondemented subjects (100 and 89\%, respectively), however, low concentrations of A\(β\)1–42 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. A study of Kanai et al. [56] reported
similar figures of 71 and 83% for diagnostic sensitivity and specificity, respectively, of simultaneous tau/Aβ1–42 analysis.

By plotting concentrations of Aβ1–42 versus tau, Motter et al. [23] found 96% predictive value to have AD for subjects with high tau/low Aβ1–42, and 100% predictive value not to have AD for subjects with low tau/high Aβ1–42. Similar figures, with positive and negative predictive values of 90 and 95%, respectively, at a prevalence of probable AD of 44% were obtained also by Andreasen et al. [6]. To evaluate data of simultaneous analysis of CSF Aβ1–42 and tau, Galasko et al. [27] used the binary tree-structured classification system obtaining 85.2% of correct diagnosis with specificity and sensitivity of 90 and 80%, respectively. A combination of low CSF Aβ42 and high CSF pTau181 allowed early onset AD patients to be distinguished from these with frontotemporal lobar degeneration with a sensitivity of 72% and a specificity of 93% [57].

In a recently published report, Engelborghs et al. [58] tested 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 specificity of 86% and 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%.

Somewhat discrepant results have been presented when CSF tau or CSF pTau was related to APOE genotype. While Arai et al. [59] reported no correlation of total tau to the number of APOE ε4 allele, and Ihb et al. [42] reported a similar finding regarding pTau199. Golombowski et al. [60] and Blomberg et al. [61] found that AD patients with ε4 allele had higher values of CSF tau than these without it. Riemenschneider et al. [62], Galasko et al. [27], and Hulstaert et al. [26] reported highest Aβ42 peptide’s level in AD patients with no ε4 allele, intermediate in ε 4 heterozygous, and lowest in ε4 homozygous. As a possible explanation of this correlation, a high-affinity of binding Aβ1–42 to ApoE is suggested [63]. Moreover, sensitivity for the combination of CSF-tau and CSF Aβ1–42 in patients possessing ε4 allele increased from 94 to 99% for probable and from 88 to 100% for possible AD [6]. On the other hand, in a study including more that 400 AD cases, no effect on CSF tau levels was found for the ε4 allele [64]. These observations lead to the conclusion that APOE genotype should be taken into consideration in interpretation of Aβ1–42 levels, and that combination of APOE genotyping with other parameters may significantly improve specificity and sensitivity of the diagnosis.

3 Research perspectives

3.1 Early diagnosis in preclinical stages

One of the most demanding aspects of neurochemical analysis of dementia disorders is to find biomarkers capable to predict development of AD in patients with MCI. Such a prediction (preclinical) diagnosis is hoped to offer preventive therapeutic interventions. According to the epidemiological data of Petersen et al. [65], approximately 10–15% of MCI subjects develop dementia within 1 year. Andreasen et al. [66] reported increased positive likelihood ratio for total tau (8.45), pTau (7.49), and Aβ42 (8.2) in the 1-year follow up of 44 MCI patients progressing to AD. These data suggest that these biomarkers are already altered in an early phase of dementia and that they may help to identify MCI subjects who will progress to AD. Similarly, Andreasen et al. [6] showed elevated tau and decreased Aβ42 levels in MCI patients at baseline. In a study addressing a combination of three CSF biomarkers, namely 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 a hope to discriminate the subgroup of patients with MCI who would eventually develop AD from those who would not offer early treatment for the subjects at risk [67]. In a recently published paper [68], 137 MCI patients, who underwent successful lumbar puncture at baseline, were followed clinically for 4–6 years with 39 healthy individuals, cognitively stable over 3 years, serving as controls, and a combination of CSF tau and Aβ1–42 at baseline yielded a sensitivity of 95% and a specificity of 83% for detection of incipient AD in patients with MCI, showing increased relative risk of progression to AD in MCI cases with pathologic tau and Aβ1–42 at the baseline (hazard ratio 17.7, p<0.0001). The combination of tau and Aβ1–42/ pTau181 ratio yielded closely similar results (sensitivity 95%, specificity 87%, hazard ratio 19.8). In our recently published study, based on multiplexing technology (see also below), 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 characterizing with increased risk to develop AD from a subgroup of MCI subjects without such a risk [69].

3.2 Multiplexing

The growing number of potentially important biomarkers to be analyzed in a limited volume of the CSF makes techniques of a simultaneous analysis of several parameters in a single small-volume CSF sample a method of choice in the future. Such technologies are generally defined as “multiplexing” independently of the physical–chemical background of the method of measurement. The flow cytometric-based Luminex xMAP® technology (Luminex, Austin, TX) involves
coupling of specific monoclonal capturing antibodies to the surface of microsphere sets uniquely identified with a combination of two fluorescence dyes [70, 71]. This allows a simultaneous reaction with up to 100 (theoretical limit) antigens in a single sample assuming no crossreactivity of the particular antibodies. Two studies by Olsson et al. [72] and Vanderstichele et al. [73] reported a successful application of this technology for analysis of the three crucial AD biomarkers: Aβ1–42, total tau, and pTau181 with the assay of Innogenetics (Ghent, Belgium). In our recently published multicenter study [69], the multiplexing results of total tau and pTau181 measured in the group of 233 patients correlated very well with the results obtained with the corresponding ELISAs, whereas the correlation for Aβ1–42 was clearly lower, which might result from capturing antibodies used in both applications. Overall result of this study, however, clearly confirmed results of the two previous showing that this technology might be expected to replace conventional ELISAs in the future.

In this context, it is worth mentioning that electrochemiluminescence-based multiplexing method of Meso Scale Discovery (Gaithersburg, USA) offers similar opportunity to simultaneously measure dementia biomarkers in a small volume of human body fluids.

### 3.3 Differential diagnosis of dementia syndromes

Similarly to early diagnosis of dementia, differential diagnosis of different dementing conditions is a very challenging task.

The discrimination among AD, DLB, and Parkinson's disease dementia (PDD) is quite difficult. In a recently published study, we used previously reported by our group method of Aβ-SDS-PAGE/immunoblot [19, 52] to investigate the Aβ peptides pattern in the CSF of patients with AD, DLB, and PDD, and we observed disease-specific variations of the CSF Aβ patterns: 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, and additionally, a novel peptide with Aβ-like immunoreactivity was observed constantly in the CSF of all 88 investigated patients, and its pronounced percentage increase in the samples from DLB allowed a highly significant discrimination of this disorder from PDD. Using a cut-off point of 0.954%, this novel marker yielded a diagnostic sensitivity and specificity of 81 and 71%, respectively. From several lines of indication, we consider this peptide to represent an oxidized α-helical form of Aβ1–40 (Aβ1–40α) [74].

Similarly, we observed a significant decrease of CSF Aβ-37, Aβ1–38, and Aβ42 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 [75, 76].

A pattern of CSF biomarkers with extremely high concentration of total tau (higher than 1200 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 differential diagnostic item [24, 33]. Additionally, CSF 14-3-3 proteins should be measured by Western-immunoblot when the high-throughput capable and quantitative ELISA or multiplex assays do indicate possible CJD, since these proteins are elevated in CJD [77].

### 3.4 Improvement of analytical performance: Preanalytic and quality control

Several preanalytical and biological confounding factors may influence the analytical outcome of the CSF analyses, such as concentration gradients of the protein along the spinal cord, influence of hemorrhage, presence of the protein in plasma and passage over the blood–brain and/or blood–CSF barrier, and degradation or loss of the protein in vitro after the CSF tap. For CSF tau and Aβ, extremely important preanalytical confounding factors are: (i) 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 [64, 78], and (ii) that the concentration of Aβ peptides tend to have lower values following repeated freeze/thaw cycles [79]. Therefore, it is important to tap CSF into nonabsorbing test tubes made of polypropylene. Storage of CSF for up to 3 days does not influence levels of these biomarkers, thus, CSF samples can be sent to the laboratory at room temperature, after which all CSF samples should be frozen before assay. In any case, it is crucial to establish and consequently fulfill one sample handling protocol [80].

Another important point is an obvious lack of systematic interlaboratory quality control surveys. Recently our group coordinated a pilot study with 14 academic and private laboratories in Germany, Austria, and Switzerland asking them to measure routinely performed neurochemical dementia diagnostics (NDD) biomarkers in a CSF sample obtained from our laboratory [81]. We observed interlaboratory coefficients of variation of biomarkers in the range of 20–30%, which clearly points at necessity of further optimization of measurements. Currently we regard it as mandatory that clinicians who have ordered CSF-based NDD (CSF-NDD) do obtain from laboratories the results not only in terms of “raw” biomarkers’ concentrations, but as an integrated report which does also control for preanalytical sample handling, and which does consider the concomitant routine CSF analysis (e.g., evidence for neuroinflammation, blood–CSF barrier dysfunction), and most importantly laboratory-specific cut off values. Actually, CSF-NDD broken down to a sole measurement of dementia biomarkers (e.g., due to commercial reasons) and without adequate standard operating procedure (SOPs) for preanalytical sample handling would significantly endanger the further success of this rapidly developing and promising novel application of clinical neurochemistry.
3.5 Dementia biomarkers in blood

Lumbar puncture is a relatively safe and uncomplicated procedure, and only small ratio of AD patients complain about postpuncture complications [6, 7]. However, repunctures 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: (i) since the CSF stays in the direct contact with blood on the way of its flow in the spinal canal, several brain-derived factors have been found present also in the blood [5]; (ii) according to the concept of Felgenhauer, brain-derived factors released in the regions of brain distant from the ventricles may predominantly leave the brain parenchyma through the blood–brain barrier instead of through the brain–CSF barrier into the CSF [82]; (iii) parenteral administration of Aβ-specific mAb facilitates a rapid efflux of brain-derived Aβ40 and Aβ42 into the plasma in the transgenic mice model expressing human mutated APP gene [83, 84]; (iv) Aβ seems to be eliminated from the human brain parenchyma primarily into the blood via the leptomeningeal arteries [85]. Moreover, evidence exist that Aβ peptides may be released from other tissue in addition to the brain [86, 87], and it is certainly interesting to speculate that changes of Aβ peptides release from nonbrain tissue(s) followed by alterations in their plasma concentration might play a diagnostic role in AD, too. In our pilot study with Aβ-SDS-PAGE/immunoblot, we observed in plasma the presence of all Aβ peptides known to constantly occur in CSF (i.e., 1–37/38/39/40/42), as well as one “plasma-specific” peptide of yet unknown nature (most probably Aβ2–40), however, in that study we did not observe AD-specific blood Aβ peptide signatures [88].

Three large prospective studies addressed alterations of plasma Aβ42/Aβ40 ratio. In one of them, a decreased ratio was reported as a predictor of MCI/AD conversion [89], while in another study an increased ratio was a predictor of dementia [90]. Finally, Mayeux et al. [91] reported significantly higher plasma Aβ42 but not Aβ40 in patients with AD at baseline and those who developed AD during the follow-up observation compared to individuals who never developed AD, and interestingly, the highest plasma Aβ42 levels were observed in patients with AD who died during the follow-up.

Summarizing these reports, it must be stressed that although clinical variables such as the study population, the follow-up period, the endpoint could account for such differences, there is an obvious need for a standardized large-scale study to evaluate clinical utility of plasma biomarkers in the diagnosis of dementia.

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