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Review

Cerebrospinal Fluid Biomarkers for Early and Differential Alzheimer's Disease Diagnosis

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Abstract. An accurate and early diagnosis of Alzheimer's disease (AD) is important to select optimal patient care and is critical in current clinical trials targeting core AD neuropathological features. The past decades, much progress has been made in the development and validation of cerebrospinal fluid (CSF) biomarkers for the biochemical diagnosis of AD, including standardization and harmonization of (pre-) analytical procedures. This has resulted in three core CSF biomarkers for AD diagnostics, namely the 42 amino acid long amyloid-beta peptide ($A\beta_{1\text{-}42}$), total tau protein (T-tau), and tau phosphorylated at threonine 181 ($P\text{-}tau_{181}$). These biomarkers have been incorporated into research diagnostic criteria for AD and have an added value in the (differential) diagnosis of AD and related disorders, including mixed pathologies, atypical presentations, and in case of ambiguous clinical dementia diagnoses. The implementation of the CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio in the core biomarker panel will improve the biomarker analytical variability, and will also improve early and differential AD diagnosis through a more accurate reflection of pathology. Numerous biomarkers are being investigated for their added value to the core AD biomarkers, aiming at the AD core pathological features like the amyloid mismetabolism, tau pathology, or synaptic or neuronal degeneration. Others aim at non-AD neurodegenerative, vascular or inflammatory hallmarks. Biomarkers are essential for an accurate identification of preclinical AD in the context of clinical trials with potentially disease-modifying drugs. Therefore, a biomarker-based early diagnosis of AD offers great opportunities for preventive treatment development in the near future.

Keywords: Alzheimer's disease, amyloid, biomarkers, cerebrospinal fluid, dementia, diagnosis, mild cognitive impairment, neuropathology, tau

INTRODUCTION

An accurate and early diagnosis of Alzheimer's disease (AD) is important to select the optimal patient care and is critical in current clinical trials targeting core AD neuropathological features. Its value will grow even more so when a disease modifying treatment is available. To date, diagnosis of AD is still based on a full clinical work-up, including neuropsychological testing [1] and brain imaging such as magnetic resonance imaging (MRI). However, clinical dementia diagnosis does not always correspond to the neuropathological definite diagnosis with clinical diagnostic accuracy levels ranging between 82% and

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84% [2, 3]. When a clinician should discriminate AD from a non-AD dementia relying on (non-biomarker based) clinical diagnostic criteria, 16% are misdiagnosed and 16% of the patients have a doubtful AD versus non-AD diagnosis [2, 4, 5]. Therefore, it is important to increase the clinical diagnostic accuracy, which will even be harder at an early stage of the disease. Since biochemical changes are believed to take place and be detectable through biomarkers around two decades before clinical symptom onset [6], they will be important tools in the clinical setup for early and differential diagnosis of AD.

Several attempts have been made to counteract the effects of amyloid-β (Aβ) mismetabolism, which is assumed to be one of the key pathogenic events in AD. However, all but one clinical trials have failed to reverse or slow down cognitive decline [7]. Possible reasons for the failures may be that non-homogeneous groups of patients have been included in the trials, the treatment has been administered too late in the course of the disease or has been too short, and/or that the Aβ aggregation is not the key event in AD.

Also driven by the need of clinical trials to select 'pure' AD subjects early in the course of the disease, much effort has been put in developing biomarkers for AD during the past two decades (see Alzforum biomarker database and meta-analyses at http://www.alzforum.org/alzbiomarker) [8]. This has resulted in three core cerebrospinal fluid (CSF) biomarkers for AD diagnostics, namely the 42 amino acid long amyloid-beta peptide ($A\beta_{1\text{-}42}$), total tau protein (T-tau), and tau phosphorylated at threonine 181 ($P\text{-}tau_{181}$) [9]. A biomarker classification scheme, which is based on the early findings and interpretations of the above CSF biomarkers by Blennow et al. [10], has been suggested as a tool to assess the pathophysiology in the brain independent of the clinical evaluation. This so called "A/T/N" system, captures the three main neuropathological findings related to AD. The A refers to the Aβ pathology measured by either amyloid PET or CSF $A\beta_{1\text{-}42}$, T represents tangle pathology and is assessed by either tau PET or CSF P-tau, and N stands for neurodegeneration or neuronal injury detected by either [18F]-fluorodeoxyglucose-PET, structural MRI, or CSF T-tau [11].

Biomarkers have been incorporated into research diagnostic criteria for AD [12–14] and, although the clinical examination (including full neuropsychological evaluation) is still the basis for AD diagnosis [1], these biomarkers are being introduced in daily clinical practice as *in vivo* surrogate markers for the confirmation of AD neuropathology. The core CSF AD biomarkers increase the diagnostic accuracy for diagnosing AD (mainly in cases with atypical presentations), also in its prodromal phase (mild cognitive impairment (MCI) due to AD) [15, 16] and are able to differentiate between AD and psychiatric disorders [17]. The CSF biomarkers are useful to diagnose AD in patients with ambiguous clinical dementia diagnoses [4] and in cases with mixed brain pathology like AD with cerebrovascular disease [5, 18, 19].

During ten years of CSF AD biomarker analyses, the number of samples referred to the BIODEM lab at UAntwerp from clinical centers has increased with 238% [20]. Due to the revisions of diagnostic criteria for AD diagnosis and the herein described use of CSF biomarkers [12–14], confidence in the importance of biomarkers has grown. Not only are they used more often by clinicians, they are also useful in clinical trials as enrichment strategy or outcome measures due to their *in vivo* pathophysiological characteristics [21]. However, there is a general shift from samples referred for neurochemical confirmation of AD diagnosis to referrals for differential AD versus non-AD dementia diagnosis. This may be due to the growing scientific support for biomarker-based differential diagnosis between AD and non-AD dementias [22], also in the prodromal phase [23].

BIOMARKER ASSOCIATION WITH NEUROPATHOLOGY AND DETECTION OF DEFINITE AND CLINICAL AD

A biomarker should be readily accessible, accurately indicating a biological or pathological state and preferably inexpensive. In case of neurodegenerative biomarkers, the choice of CSF over blood biomarkers, at least in the case of $A\beta_{1\text{–}42}$, is mainly based on the fact that the central nervous system is secluded from the systemic circulation, which precludes direct translation of biomarker findings of the brain to the periphery. This has been supported by studies in plasma showing conflicting results, and in the case of $A\beta_{1\text{–}42}$ for instance no overall difference between AD and controls were found in a meta-analysis [8] (http://www.alzforum.org/alzbiomarker). Furthermore, no correlation between plasma and CSF levels of the $A\beta_{1\text{–}42}$ biomarker has been found [24, 25]. Tau on the other hand have shown more potential as a plasma or serum biomarker for the differentiation between AD and controls [8] (http://www.alzforum.org/alzbiomarker);

however, speaking against a direct translation of CSF values into plasma is the finding that nor tau CSF levels correlate with its plasma counterpart [26]. Nevertheless in CSF, $A\beta_{1–42}$, T-tau, and P-tau_{181} have shown to be consistently altered in AD versus controls, also in the prodromal phase [8] (http://www.alzforum.org/alzbiomarker).

The BIODEM lab was the first to demonstrate the diagnostic value of the CSF biomarkers $A\beta_{1\text{-}42}$, T-tau, and P-tau_{181} in clinical AD using the neuropathological diagnosis as a reference [2, 27]. The study demonstrated that all three biomarkers provide useful information, showing promising sensitivity and specificity values that systematically exceed the 80% threshold. The use of a biomarker-based model in patients with a clinically ambiguous diagnosis, resulted in a correct diagnosis in the majority of autopsy-confirmed AD and non-AD cases, indicating that biomarkers have an added diagnostic value in these cases [1, 2, 4].

Moreover, $A\beta_{1–42}$ has proven its potential to mirror the build-up of plaques, which is supported by the inverse correlation between the CSF $A\beta_{1–42}$ levels and the amount of amyloid plaques found at neuropathological examination of AD brains [28] as well as the *in vivo* association with cortical amyloid load as measured by amyloid PET in patients with AD [29–31]. The present core AD CSF biomarkers, including $A\beta_{1–42}$, T-tau, and P-tau_{181}, have recently been incorporated into the research diagnostic criteria of AD, with a CSF profile suggestive for AD being low $A\beta_{1–42}$ in combination with high T-tau and/or P-tau_{181} levels [14].

THE ADDED VALUE OF CSF BIOMARKERS FOR EARLY AND DIFFERENTIAL AD DIAGNOSIS

It should be emphasized that biomarkers are especially important for the selection of preclinical AD subjects (who are asymptomatic at risk for AD or subjects who suffer from subjective cognitive decline [SCD] due to AD) and for the selection of patients in the earliest symptomatic stages of AD (prodromal AD or MCI due to AD). SCD and MCI are both very heterogeneous syndromes and when assessed in a large clinical cohort less than 25% of the subjects converted to dementia after an extensive follow-up period of 6 years. The majority of these SCD and MCI subjects (42%) developed dementia due to AD, while subjects with mixed AD with cerebrovascular disease and pure vascular dementia (VaD) were the second most common diagnoses representing about a quarter each of the converters [32]. In this particular setting, CSF $A\beta_{1–42}$ would portrait as a very attractive biomarker for early AD detection since both CSF T-tau and P-tau_{181} alterations seem to occur at a later time point in the disease process closer to clinically detectable dementia [33]. However, it has previously been suggested and shown that a combination of the core AD biomarkers is superior compared with the single biomarkers alone, especially for differential diagnosis [2]. Investigating autopsy-confirmed AD and non-AD dementia patients has also improved our knowledge and insights with regard to the differential diagnostic value of the existing AD CSF biomarkers. An added discriminatory value for AD versus non-AD dementia was shown for P-tau_{181} to the panel of $A\beta_{1\text{-}42}$ and T-tau [34, 35] and, furthermore, that the ratio of $A\beta_{1\text{-}42}$/P-tau_{181} has shown a higher diagnostic accuracy than $A\beta_{1\text{-}42}$, T-tau, and P-tau_{181} alone, but also than the $A\beta_{1\text{-}42}$/T-tau ratio to discriminate between AD and non-AD dementias. This clearly shows the importance of P-tau_{181} in the biomarker panel for differential dementia diagnosis [2, 4, 20, 35].

Although there is strong evidence for the importance of CSF $A\beta_{1–42}$ as a biomarker for early AD detection, there are still limitations to be overcome. One such limitation is the overlap of CSF $A\beta_{1–42}$ between different neurodegenerative disorders. For instance, decreased CSF levels of $A\beta_{1–42}$ have also been observed in patients with prodromal and manifest (subcortical) VaD [34, 36, 37], dementia with Lewy bodies (DLB) [34, 38], Creutzfeldt–Jakob disease (CJD) [34, 39], and frontotemporal lobar degeneration (FTLD) [34, 40] compared with healthy individuals. Though the $A\beta_{1–42}$ levels are most often still lower in AD compared with VaD and DLB, a significant overlap nevertheless limits the discrimination. Concomitant AD pathology in DLB has been shown to occur in 72% of autopsy-confirmed DLB patients, which was reflected by low CSF $A\beta_{1\text{-}42}$ values [38]. While AD pathology is often found in combination with both DLB and cerebrovascular disease [2, 38], decreased $A\beta_{1–42}$ levels in CSF of patients with pure VaD (related to subcortical small vessel disease), CJD or FTLD may on the other hand be related to other pathophysiological characteristics than plaque burden.

The introduction of Aβ peptide ratios was proposed already in the late 1990s to improve the AD differential diagnosis [41, 42]. It has previously been

shown that CSF $A\beta_{1–42}$ and florbetapir-PET showed a nonlinear association with pathological values of CSF $A\beta_{1–42}$ preceding PET abnormalities [29] and it has been suggested that CSF $A\beta_{1–42}$ is an earlier marker of brain amyloid pathology compared with PET [43]. However, this has more or less been disputed by the finding that the CSF $A\beta_{1–42}/A\beta_{1–40}$ ratio shows a higher concordance with amyloid load in the brain as assessed by PET compared with $A\beta_{1–42}$ alone [31, 44, 45]. The $A\beta_{1–42}/A\beta_{1–40}$ ratio is decreased in AD and more accurately differentiates between AD and controls, FTLD, VaD, and DLB, due to the latter four groups having ratios that approach control levels because of decreased concentrations in both $A\beta_{1–42}$ and $A\beta_{1–40}$. This finding supports the increased association of the CSF $A\beta_{1–42}/A\beta_{1–40}$ ratio to pathology as the above non-AD dementias are not affected by plaque pathology in their purest forms [44]. Furthermore, the ratio shows a high concordance with PET, also in subjects with subjective cognitive decline and mild cognitive impairment (AUC $\geq$ 0.93), indicating a high agreement at the early stages of disease [46]. However, more comparative studies are needed to investigate the biomarker characteristics for early pre-clinical detection of amyloid pathology with regard to the timing of CSF $A\beta_{1–42}/A\beta_{1–40}$ ratio and amyloid PET. The $A\beta_{1–42}/A\beta_{1–40}$ ratio has also been shown to perform equally well as the combination of $A\beta_{1–42}$, P-tau_{181}, and T-tau in differentiating between AD and other non-AD dementias [47]. In another study, it was shown that adding the Aβ ratio to the core biomarkers improved the accuracy when distinguishing between definite AD and non-AD dementias in cases with intermediate P-tau_{181} [48], indicating its potential usefulness as a biomarker for differential dementia diagnosis. Along the same line, the added value to the core biomarkers has also been assessed in a clinical setting where it was shown that in cases with a discrepancy in the AD core biomarker profile, the $A\beta_{1–42}/A\beta_{1–40}$ ratio was in agreement with the clinical diagnosis in over 50% of the cases [49]. All together, these findings speak in favor of the added value of the $A\beta_{1–42}/A\beta_{1–40}$ ratio for differential diagnosis, when alterations in CSF tau are yet to be seen. Other ratios that have been less well investigated but still show potential as biomarkers are $A\beta_{1–42}/A\beta_{1–38}$ and $A\beta_{1–42}/A\beta_{1–37}$ [35]. In this study, it was concluded that $A\beta_{1–42}/A\beta_{1–38}$ ratio performed the best for the separation between AD and DLB, and that it outperformed the single AD biomarkers. Also, $A\beta_{1–42}/A\beta_{1–37}$ was shown to have an added value for the differentiation between AD and FTLD [35]. More studies are needed in order to determine which Aβ peptide ratios achieve the best separation in different diagnostic settings.

The CSF $A\beta_{1–42}/A\beta_{1–40}$ ratio has been shown to be superior to $A\beta_{1–42}$ alone when concerned with the distinction between MCI patients who progress to AD dementia and MCI patients who remain stable [50]. The early diagnostic value of the existing biomarker panel might be improved by the addition of $A\beta_{1\text{-}40}$, since formation of amyloid-plaques is believed to be initiated up to twenty years before the onset of the clinical symptoms. The use of low CSF $A\beta_{1\text{-}42}$ and the CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio opens new possibilities for the accurate identification of 'asymptomatic at risk for AD' subjects for clinical trials.

NEW AD AND NON-AD RELATED BIOMARKERS FOR IMPROVED DIFFERENTIAL DIAGNOSIS

Numerous biomarkers have been investigated for their added value to the core AD biomarkers in early detection or differential diagnosis of AD. Several of those have aimed at the AD core pathological features like the amyloid mismetabolism, tau pathology, or synaptic or neuronal degeneration. Others have aimed at non-AD neurodegenerative, vascular, or inflammatory hallmarks.

More or less overlapping results have been found for the differentiation of AD and non-AD dementias including DLB, FTLD, and VaD using the core AD biomarkers $A\beta_{1\text{-}42}$, T-tau, and P-tau_{181}. When concerned with FTLD-tau, tau CSF levels are usually intermediate ranging from normal to abnormal, precluding its usability as a biomarker for differential diagnosis [51]. Other tau species, with for instance different modifications, may be more specific for either AD or FTLD-tau pathology increasing its clinical utility. Based on this thought, an assay measuring non-phosphorylated tau at positions T175 and T181 was developed, which was highly accurate at detecting MCI due to AD when compared to healthy controls [52]. However, there was no added value compared with the core AD biomarkers for the differential diagnosis of AD with regard to non-AD neurodegenerative diseases, including FTLD and DLB. Neither was there any added value for the differentiation of AD or FTLD from healthy controls [40].

Other core AD biomarker than $A\beta_{1\text{-}42}$ representing Aβ or amyloid-β protein precursor (AβPP) metabolism have also been investigated for their ability to discriminate AD from healthy controls, such as $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}38}$ peptides as well as soluble AβPPα and β protein fragments. They have shown to be unaltered in AD [8, 53, 54], but decreased levels have been associated with inflammation, cerebrovascular disease, and white matter lesions [54, 55]. As mentioned before, the CSF $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio is likely to perform with a higher specificity than for instance $A\beta_{1\text{-}42}$/P-tau_{181} to discriminate between AD and VaD [56] as well as FTLD and DLB [35]. These findings are of outermost importance for the accuracy of detection of amyloid plaque pathology that characterizes AD, both for clinical practice as well as for patient inclusion into clinical trials. Furthermore, these biomarkers may contribute to detect cerebrovascular disease that may also affect the trajectory of AD under the influence of mixed pathology.

As synaptic and neuronal degeneration is assumed to correlate with cognitive decline in AD, being able to monitor neurodegeneration through synaptic function would be an important advantage, both clinically and in clinical trials to detect disease progression or treatment efficacy. For this purpose, the postsynaptic protein neurogranin has been investigated and was found to be increased in CSF of patients with MCI and dementia due to AD as compared with healthy controls [26, 57], while in paired plasma samples no difference was found [26]. As CSF neurogranin and tau has been found to correlate strongly, its added value to the core biomarkers needs further attention as well as the relation to clinical parameters [26, 57].

Neurofilament light (Nf-L) is another marker of neuronal integrity reflecting axonal damage of the subcortical white matter. Nf-L is therefore considered as a candidate biomarker for subcortical small vessel disease and related dementia [58–60] as it has been found to be associated with white matter hyperintensities [55, 61]. Nf-L has been found to be elevated in CSF from patients with subcortical VaD and mixed AD with subcortical small vessel disease as well as in FTLD [36, 37, 62]. However, it has also been proposed as a biomarker for AD [8], if Nf-L in this case is related to concomitant cerebrovascular disease remains to be clarified. The relation to cerebrovascular disease has also been corroborated by findings of increased levels in stroke [63]. So far, Nf-L is the only marker that has been shown to be directly transferrable from CSF to plasma and show potential as a clinical tool to predict cognitive decline and brain atrophy in AD [64]. Another marker reflecting axonal damage or remodeling of the myelin sheet is myelin basic protein (MBP), which has also been found to be increased in subcortical VaD and stroke [37, 63] and associated with white matter lesions [55]. If MBP more specifically reflects white matter lesions related to subcortical VaD than Nf-L remains to be shown.

Other markers that have been proposed to improve the differential diagnosis between AD and (subcortical) VaD are biomarkers reflecting blood-brain barrier (BBB) dysfunction, such as albumin ratio, and markers related to inflammation and opening of the BBB [58, 59]. The albumin ratio has consistently been found to be increased in subcortical VaD compared with AD and healthy controls [36, 37, 62]. Moreover, matrix metalloproteinases (MMP-9) known to be associated with BBB opening has been found to be increased in subcortical VaD together with its endogenous inhibitor, the tissue inhibitor of metalloproteinases 1 (TIMP-1) compared with AD and healthy controls [37]. TIMP-1 has also been shown to correlate with white matter lesion volume and albumin ratio, while MMP-9 has been shown to be associated with white matter lesion progression [37, 55]. These markers and other markers related to inflammation and cerebrovascular disease needs further attention for their role to reflect subcortical small vessel disease [60].

Major molecular pathologies underlying FTLD include aggregation of transactive response DNA-binding protein of 43 kDa (TDP-43, FTLD-TDP), and tau (FTLD-tau) [65]. Having the potential to be a specific biomarker for FTLD-TDP, a lot of research was done on TDP-43. Because of low absolute levels, quantitative analysis of TDP-43 in biofluids will require a very sensitive immunoassay, preferably specific for pathological TDP-43 [66]. So far, research has not been translated into a sensitive and specific biomarker for TDP-43. The only FTLD-specific biomarker is progranulin, showing decreased concentrations in serum or plasma of subjects with *GRN* mutation-related FTLD (a subgroup of FTLD-TDP) [67, 68]. The diagnostic value of CSF progranulin levels is a matter of debate.

The discovery of α-synuclein (α-syn) as a major component of Lewy bodies, which is the neuropathological hallmark of Parkinson's disease (PD) and DLB, initiated research on α-syn as a potential CSF biomarker. α-syn is as well a constituent of glial inclusions in multiple system atrophy (MSA). Large variations in the absolute levels of α-syn in CSF and serum have bene revealed, even when the same

types of α-syn isoforms were detected [69]. Differences in values are related to differences in analytical procedures, stressing the need for standardization of procedures [70]. CSF α-syn levels are decreased in PD and DLB but also in MSA [69] as compared to AD where increased levels have been reported, correlating with tau and thus possibly with neurodegeneration [71]. These data show the (differential) diagnostic potential of α-syn as a biomarker.

Fast progressive AD phenotypes often pose a diagnostic challenge and may be confused clinically with CJD. The major biological diagnostic biomarker for identifying CJD, 14-3-3 protein in CSF, unfortunately lacks specificity when confronted with a rapid dementia presentation [72, 73]. Very high T-tau concentrations may be found in AD in CSF (>1200 pg/ml), but are also indicative of CJD. In case of suspicion of CJD, analysis of the total concentration of prion protein (t-PrP) in CSF has been shown to be useful to increase the diagnostic accuracy [39]. The use of CSF t-PrP levels may be beneficial in clinical practice in addition to the current classic biomarkers.

IMPROVEMENT AND HARMONIZATION OF AD CSF BIOMARKER MEASUREMENTS

Even with an elevated confidence in the use of CSF biomarkers for the clinical work-up of dementia diagnosis, multicenter trials have shown that there is a substantial center-to-center variation [9].

Due to its favorable diagnostic characteristics and the relative inexpensive costs, much effort has been put into making $A\beta_{1–42}$ manageable as an AD biomarker worldwide to be used in daily clinical dementia practice as *in vivo* surrogate marker for plaque pathology [20]. Though the absolute measurements of $A\beta_{1–42}$ in CSF show inter-laboratory variability, mainly due to differences in pre-analytical and analytical procedures, which also includes the performance of different assays utilizing different calibrators. This causes an important concern as direct comparisons of measurements between laboratories and across techniques are not reliable, hampering biomarker development and their utility for clinical routine diagnosis. Storage in different tubes, different aliquot volumes, and number of freeze-thaw cycles are factors that significantly influence CSF biomarker concentrations, stressing the need for standard operating procedures for pre-analytical sample handling [74–76]. However, it has previously been suggested [77] and verified by us that the $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio is a more robust measurement compared with $A\beta_{1\text{-}42}$ alone as it corrects for intra-individual confounding factors and minimizes variability due to for instance adsorption of Aβ peptides to storage tubes [76]. An exploratory study in MCI patients, on the other hand, with clinical follow-up and autopsy-confirmed AD patients provided evidence that, for a specific context of use, the impact on clinical diagnostic accuracy of biomarker concentration shifts might be lower than originally expected [78]. However, standardization of (pre)analytical sample handling as well as the cut-off thresholds should be accomplished as they influence biomarker results [74–76, 79].

Major efforts are undertaken to overcome these problems by introducing a certified reference material and a certified reference method that can be used for value assignment of the assay calibrators [79–81]. Also, the Alzheimer's Association external quality control (QC) program monitors site-to-site and batch-to-batch CSF measurement variability for the purpose of enabling the participating laboratories to synchronize their procedures [82]. These efforts will lead to precise and reliable measurements between laboratories that will enable the introduction of a worldwide cut-off point for CSF $A\beta_{1–42}$, T-tau and P-tau_{181} measurements for the purpose of clinical diagnostics and patient stratification in clinical trials.

With this in mind, several programs for standardization and harmonization were set up, such as the QC program of the Alzheimer's Association, as well as the Alzheimer Biomarker Standardization Initiative (ABSI) and the JPND BIOMARKAPD consortium [79, 82–84].

CONCLUDING REMARKS

Over the past decades, much progress has been made in the development and validation of three core CSF biomarkers for the biochemical diagnosis of AD, including standardization and harmonization of (pre-) analytical procedures. One of the most important recommendations with a significant effect on pre-analytical variability is likely to be the implementation of the $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ into the clinical diagnostic work-up. This is especially important since $A\beta_{1\text{-}42}$ has proven to be the earliest marker to reflect AD related pathological changes take place in the brain and the ratio show a higher concordance with amyloid pathology. At present, low CSF $A\beta_{1\text{-}42}$

concentration is an inclusion criterion for several clinical trials with potential disease-modifying drugs that target AD in its earliest (and even preclinical) stages. Hence, biomarkers reflecting the pathology targeted by specific clinical trials are essential for inclusion but also to monitor treatment effects. Early clinical detection is also likely to become more important as soon as disease-modifying pharmacological treatment for AD is available as medications that halt or prevent the disease are likely to be most effective at an early stage, when neurodegeneration has not become too severe. The validated core AD CSF biomarkers have an added value in the early and differential diagnosis of AD and related disorders, including mixed pathologies, atypical presentations of AD, and in case of ambiguous dementia diagnosis.

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REFERENCES

[1] Bier JC, Verschraegen J, Vandenberghe R, Guillaume B, Picard G, Otte G, Mormont E, Gilles C, Segers K, Sieben A, Thiery E, Ventura M, De Deyn P, Deryck O, Versijpt J, Salmon E, Engelborghs S, Ivanoiu A (2015) Clinical utility and applicability of biomarker-based diagnostic criteria for Alzheimer's disease: A BeDeCo survey. *Acta Neurol Belg* **115**, 547-555.

[2] 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.

[3] Brunnstrom H, Englund E (2009) Clinicopathological concordance in dementia diagnostics. *Am J Geriatr Psychiatry* **17**, 664-670.

[4] Le Bastard N, Martin JJ, Vanmechelen E, Vanderstichele H, De Deyn PP, Engelborghs S (2010) Added diagnostic value of CSF biomarkers in differential dementia diagnosis. *Neurobiol Aging* **31**, 1867-1876.

[5] Niemantsverdriet E, Feyen BF, Le Bastard N, Martin JJ, Goeman J, De Deyn PP, Engelborghs S (2015) Overdiagnosing vascular dementia using structural brain imaging for dementia work-up. *J Alzheimers Dis* **45**, 1039-1043.

[6] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR Jr, Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH (2011) Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 280-292.

[7] Sevigny J, Chiao P, Bussiere T, Weinreb PH, Williams L, Maier M, Dunstan R, Salloway S, Chen T, Ling Y, O'Gorman J, Qian F, Arastu M, Li M, Chollate S, Brennan MS, Quintero-Monzon O, Scannevin RH, Arnold HM, Engber T, Rhodes K, Ferrero J, Hang Y, Mikulskis A, Grimm J, Hock C, Nitsch RM, Sandrock A (2016) The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. *Nature* **537**, 50-56.

[8] Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, Holtta M, Rosen C, Olsson C, Strobel G, Wu E, Dakin K, Petzold M, Blennow K, Zetterberg H (2016) CSF and blood biomarkers for the diagnosis of Alzheimer's disease: A systematic review and meta-analysis. *Lancet Neurol* **15**, 673-684.

[9] Mattsson N, Zetterberg H, Blennow K (2010) Lessons from multicenter studies on CSF biomarkers for Alzheimer's disease. *Int J Alzheimers Dis* **2010**, 610613.

[10] Blennow K, Vanmechelen E, Hampel H (2001) CSF total tau, Abeta42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. *Mol Neurobiol* **24**, 87-97.

[11] Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, Hampel H, Jagust WJ, Johnson KA, Knopman DS, Petersen RC, Scheltens P, Sperling RA, Dubois B (2016) A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* **87**, 539-547.

[12] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Gamst A, Holtzman DM, Jagust WJ, Petersen RC, Snyder PJ, Carrillo MC, Thies B, Phelps CH (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 270-279.

[13] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* **7**, 263-269.

[14] Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, DeKosky ST, Gauthier S, Selkoe D, Bateman R, Cappa S, Crutch S, Engelborghs S, Frisoni GB, Fox NC, Galasko D, Habert MO, Jicha GA, Nordberg A, Pasquier F, Rabinovici G, Robert P, Rowe C, Salloway S, Sarazin M, Epelbaum S, de Souza LC, Vellas B, Visser PJ, Schneider L, Stern Y, Scheltens P, Cummings JL (2014) Advancing research diagnostic criteria for Alzheimer's disease: The IWG-2 criteria. *Lancet Neurol* **13**, 614-629.

[15] 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.

[16] Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, Herukka SK, van der Flier WM, Blankenstein MA, Ewers M, Rich K, Kaiser E, Verbeek M, Tsolaki M, Mulugeta E, Rosen E, Aarsland D, Visser PJ, Schroder J, Marcusson J, de Leon M, Hampel H, Scheltens P, Pirttila T, Wallin A, Jonhagen ME, Minthon L, Winblad B, Blennow K (2009) CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* **302**, 385-393.

[17] Niemantsverdriet E, Valckx S, Bjerke M, Engelborghs S (2017) Alzheimer's disease CSF biomarkers: Clinical indications and rational use. *Acta Neurol Belg* **117**, 591-602.

[18] Engelborghs S, Le Bastard N (2012) The role of CSF biomarkers in the diagnostic work-up of mixed vascular-degenerative dementia. *J Neurol Sci* **322**, 197-199.

[19] Engelborghs S (2013) Clinical indications for analysis of Alzheimer's disease CSF biomarkers. *Rev Neurol (Paris)* **169**, 709-714.

[20] Somers C, Struyfs H, Goossens J, Niemantsverdriet E, Luyckx J, De Roeck N, De Roeck E, De Vil B, Cras P, Martin JJ, De Deyn PP, Bjerke M, Engelborghs S (2016) A decade of cerebrospinal fluid biomarkers for Alzheimer's disease in Belgium. *J Alzheimers Dis* **54**, 383-395.

[21] Lleo A, Cavedo E, Parnetti L, Vanderstichele H, Herukka SK, Andreasen N, Ghidoni R, Lewczuk P, Jeromin A, Winblad B, Tsolaki M, Mroczko B, Visser PJ, Santana I, Svenningsson P, Blennow K, Aarsland D, Molinuevo JL, Zetterberg H, Mollenhauer B (2015) Cerebrospinal fluid biomarkers in trials for Alzheimer and Parkinson diseases. *Nat Rev Neurol* **11**, 41-55.

[22] Simonsen AH, Herukka SK, Andreasen N, Baldeiras I, Bjerke M, Blennow K, Engelborghs S, Frisoni GB, Gabryelewicz T, Galluzzi S, Handels R, Kramberger MG, Kulczynska A, Molinuevo JL, Mroczko B, Nordberg A, Oliveira CR, Otto M, Rinne JO, Rot U, Saka E, Soininen H, Struyfs H, Suardi S, Visser PJ, Winblad B, Zetterberg H, Waldemar G (2017) Recommendations for CSF AD biomarkers in the diagnostic evaluation of dementia. *Alzheimers Dement* **13**, 274-284.

[23] Herukka SK, Simonsen AH, Andreasen N, Baldeiras I, Bjerke M, Blennow K, Engelborghs S, Frisoni GB, Gabryelewicz T, Galluzzi S, Handels R, Kramberger MG, Kulczynska A, Molinuevo JL, Mroczko B, Nordberg A, Oliveira CR, Otto M, Rinne JO, Rot U, Saka E, Soininen H, Struyfs H, Suardi S, Visser PJ, Winblad B, Zetterberg H, Waldemar G (2017) Recommendations for cerebrospinal fluid Alzheimer's disease biomarkers in the diagnostic evaluation of mild cognitive impairment. *Alzheimers Dement* **13**, 285-295.

[24] Le Bastard N, Aerts L, Leurs J, Blomme W, De Deyn PP, Engelborghs S (2009) No correlation between time-linked plasma and CSF Abeta levels. *Neurochem Int* **55**, 820-825.

[25] Le Bastard N, Leurs J, Blomme W, De Deyn PP, Engelborghs S (2010) Plasma amyloid-beta forms in Alzheimer's disease and non-Alzheimer's disease patients. *J Alzheimers Dis* **21**, 291-301.

[26] De Vos A, Jacobs D, Struyfs H, Fransen E, Andersson K, Portelius E, Andreasson U, De Surgeloose D, Hernalsteen D, Sleegers K, Robberecht C, Van Broeckhoven C, Zetterberg H, Blennow K, Engelborghs S, Vanmechelen E (2015) C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease. *Alzheimers Dement* **11**, 1461-1469.

[27] Engelborghs S, Sleegers K, Cras P, Brouwers N, Serneels S, De Leenheir E, Martin JJ, Vanmechelen E, Van Broeckhoven C, De Deyn PP (2007) No association of CSF biomarkers with APOEepsilon4, plaque and tangle burden in definite Alzheimer's disease. *Brain* **130**, 2320-2326.

[28] Strozyk D, Blennow K, White LR, Launer LJ (2003) CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology* **60**, 652-656.

[29] Toledo JB, Bjerke M, Da X, Landau SM, Foster NL, Jagust W, Jack C Jr, Weiner M, Davatzikos C, Shaw LM, Trojanowski JQ, Alzheimer's Disease Neuroimaging Initiative (2015) Nonlinear association between cerebrospinal fluid and Florbetapir F-18 beta-amyloid measures across the spectrum of Alzheimer disease. *JAMA Neurol* **72**, 571-581.

[30] Leuzy A, Chiotis K, Hasselbalch SG, Rinne JO, de Mendonca A, Otto M, Lleo A, Castelo-Branco M, Santana I, Johansson J, Anderl-Straub S, von Arnim CA, Beer A, Blesa R, Fortea J, Herukka SK, Portelius E, Pannee J, Zetterberg H, Blennow K, Nordberg A (2016) Pittsburgh compound B imaging and cerebrospinal fluid amyloid-beta in a multicentre European memory clinic study. *Brain* **139**, 2540-2553.

[31] Niemantsverdriet E, Ottoy J, Somers C, De Roeck E, Struyfs H, Soetewey F, Verhaeghe J, Van den Bossche T, Van Mossevelde S, Goeman J, De Deyn PP, Marien P, Versijpt J, Sleegers K, Van Broeckhoven C, Wyffels L, Albert A, Ceyssens S, Stroobants S, Staelens S, Bjerke M, Engelborghs S (2017) The cerebrospinal fluid Abeta1-42/Abeta1-40 ratio improves concordance with amyloid-PET for diagnosing Alzheimer's disease in a clinical setting. *J Alzheimers Dis* **60**, 561-576.

[32] Wallin A, Nordlund A, Jonsson M, Lind K, Edman A, Gothlin M, Stalhammar J, Eckerstrom M, Kern S, Borjesson-Hanson A, Carlsson M, Olsson E, Zetterberg H, Blennow K, Svensson J, Ohrfelt A, Bjerke M, Rolstad S, Eckerstrom C (2016) The Gothenburg MCI study: Design and distribution of Alzheimer's disease and subcortical vascular disease diagnoses from baseline to 6-year follow-up. *J Cereb Blood Flow Metab* **36**, 114-131.

[33] Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O (2012) Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch Gen Psychiatry* **69**, 98-106.

[34] Koopman K, Le Bastard N, Martin JJ, Nagels G, De Deyn PP, Engelborghs S (2009) Improved discrimination of autopsy-confirmed Alzheimer's disease (AD) from non-AD dementias using CSF P-tau(181P). *Neurochem Int* **55**, 214-218.

[35] Struyfs H, Niemantsverdriet E, Goossens J, Fransen E, Martin JJ, De Deyn PP, Engelborghs S (2015) Cerebrospinal fluid P-Tau181P: Biomarker for improved differential dementia diagnosis. *Front Neurol* **6**, 138.

[36] Bjerke M, Andreasson U, Rolstad S, Nordlund A, Lind K, Zetterberg H, Edman A, Blennow K, Wallin A (2009) Subcortical vascular dementia biomarker pattern in mild cognitive impairment. *Dement Geriatr Cogn Disord* **28**, 348-356.

[37] Bjerke M, Zetterberg H, Edman A, Blennow K, Wallin A, Andreasson U (2011) Cerebrospinal fluid matrix metalloproteinases and tissue inhibitor of metalloproteinases in combination with subcortical and cortical biomarkers in vascular dementia and Alzheimer's disease. *J Alzheimers Dis* **27**, 665-676.

[38] Slaets S, Le Bastard N, Theuns J, Sleegers K, Verstraeten A, De Leenheir E, Luyckx J, Martin JJ, Van Broeckhoven C, Engelborghs S (2013) Amyloid pathology influences

abeta1-42 cerebrospinal fluid levels in dementia with lewy bodies. *J Alzheimers Dis* **35**, 137-146.

[39] Dorey A, Tholance Y, Vighetto A, Perret-Liaudet A, Lachman I, Krolak-Salmon P, Wagner U, Struyfs H, De Deyn PP, El-Moualij B, Zorzi W, Meyronet D, Streichenberger N, Engelborghs S, Kovacs GG, Quadrio I (2015) Association of cerebrospinal fluid prion protein levels and the distinction between Alzheimer disease and Creutzfeldt-Jakob disease. *JAMA Neurol* **72**, 267-275.

[40] Goossens J, Bjerke M, Struyfs H, Niemantsverdriet E, Somers C, Van den Bossche T, Van Mossevelde S, De Vil B, Sieben A, Martin JJ, Cras P, Goeman J, De Deyn PP, Van Broeckhoven C, van der Zee J, Engelborghs S (2017) No added diagnostic value of non-phosphorylated tau fraction (p-taurel) in CSF as a biomarker for differential dementia diagnosis. *Alzheimers Res Ther* **9**, 49.

[41] Kanai M, Matsubara E, Isoe K, Urakami K, Nakashima K, Arai H, Sasaki H, Abe K, Iwatsubo T, Kosaka T, Watanabe M, Tomidokoro Y, Shizuka M, Mizushima K, Nakamura T, Igeta Y, Ikeda Y, Amari M, Kawarabayashi T, Ishiguro K, Harigaya Y, Wakabayashi K, Okamoto K, Hirai S, Shoji M (1998) Longitudinal study of cerebrospinal fluid levels of tau, A beta1-40, and A beta1-42(43) in Alzheimer's disease: A study in Japan. *Ann Neurol* **44**, 17-26.

[42] Shoji M, Matsubara E, Kanai M, Watanabe M, Nakamura T, Tomidokoro Y, Shizuka M, Wakabayashi K, Igeta Y, Ikeda Y, Mizushima K, Amari M, Ishiguro K, Kawarabayashi T, Harigaya Y, Okamoto K, Hirai S (1998) Combination assay of CSF tau, A beta 1-40 and A beta 1-42(43) as a biochemical marker of Alzheimer's disease. *J Neurol Sci* **158**, 134-140.

[43] Palmqvist S, Mattsson N, Hansson O, Alzheimer's Disease Neuroimaging Initiative (2016) Cerebrospinal fluid analysis detects cerebral amyloid-beta accumulation earlier than positron emission tomography. *Brain* **139**, 1226-1236.

[44] Janelidze S, Zetterberg H, Mattsson N, Palmqvist S, Vanderstichele H, Lindberg O, van Westen D, Stomrud E, Minthon L, Blennow K, Swedish BioFINDER study group, Hansson O (2016) CSF Abeta42/Abeta40 and Abeta42/Abeta38 ratios: Better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol* **3**, 154-165.

[45] Lewczuk P, Matzen A, Blennow K, Parnetti L, Molinuevo JL, Eusebi P, Kornhuber J, Morris JC, Fagan AM (2017) Cerebrospinal fluid Abeta42/40 corresponds better than Abeta42 to amyloid PET in Alzheimer's disease. *J Alzheimers Dis* **55**, 813-822.

[46] Janelidze S, Pannee J, Mikulskis A, Chiao P, Zetterberg H, Blennow K, Hansson O (2017) Concordance between different amyloid immunoassays and visual amyloid positron emission tomographic assessment. *JAMA Neurol* **74**, 1492-1501.

[47] Struyfs H, Van Broeck B, Timmers M, Fransen E, Sleegers K, Van Broeckhoven C, De Deyn PP, Streffer JR, Mercken M, Engelborghs S (2015) Diagnostic accuracy of cerebrospinal fluid amyloid-beta isoforms for early and differential dementia diagnosis. *J Alzheimers Dis* **45**, 813-822.

[48] Slaets S, Le Bastard N, Martin JJ, Sleegers K, Van Broeckhoven C, De Deyn PP, Engelborghs S (2013) Cerebrospinal fluid Abeta1-40 improves differential dementia diagnosis in patients with intermediate P-tau181P levels. *J Alzheimers Dis* **36**, 759-767.

[49] Dumurgier J, Schraen S, Gabelle A, Vercruysse O, Bombois S, Laplanche JL, Peoc'h K, Sablonniere B, Kastanenka KV, Delaby C, Pasquier F, Touchon J, Hugon J, Paquet C, Lehmann S (2015) Cerebrospinal fluid amyloid-beta 42/40 ratio in clinical setting of memory centers: A multicentric study. *Alzheimers Res Ther* **7**, 30.

[50] Hansson O, Zetterberg H, Buchhave P, Andreasson U, Londos E, Minthon L, Blennow K (2007) Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. *Dement Geriatr Cogn Disord* **23**, 316-320.

[51] Pijnenburg YA, Verwey NA, van der Flier WM, Scheltens P, Teunissen CE (2015) Discriminative and prognostic potential of cerebrospinal fluid phosphoTau/tau ratio and neurofilaments for frontotemporal dementia subtypes. *Alzheimers Dement (Amst)* **1**, 505-512.

[52] Lewczuk P, Lelental N, Lachmann I, Holzer M, Flach K, Brandner S, Engelborghs S, Teunissen CE, Zetterberg H, Molinuevo JL, Mroczko B, Blennow K, Popp J, Parnetti L, Chiasserini D, Perret-Liaudet A, Spitzer P, Maler JM, Kornhuber J (2017) Non-phosphorylated tau as a potential biomarker of Alzheimer's disease: Analytical and diagnostic characterization. *J Alzheimers Dis* **55**, 159-170.

[53] Brinkmalm G, Brinkmalm A, Bourgeois P, Persson R, Hansson O, Portelius E, Mercken M, Andreasson U, Parent S, Lipari F, Ohrfelt A, Bjerke M, Minthon L, Zetterberg H, Blennow K, Nutu M (2013) Soluble amyloid precursor protein alpha and beta in CSF in Alzheimer's disease. *Brain Res* **1513**, 117-126.

[54] Somers C, Goossens J, Engelborghs S, Bjerke M (2017) Selecting Abeta isoforms for an Alzheimer's disease cerebrospinal fluid biomarker panel. *Biomark Med* **11**, 169-178.

[55] Bjerke M, Jonsson M, Nordlund A, Eckerstrom C, Blennow K, Zetterberg H, Pantoni L, Inzitari D, Schmidt R, Wallin A (2014) Cerebrovascular biomarker profile is related to white matter disease and ventricular dilation in a LADIS substudy. *Dement Geriatr Cogn Dis Extra* **4**, 385-394.

[56] Le Bastard N, Van Buggenhout M, De Leenheir E, Martin JJ, De Deyn PP, Engelborghs S (2007) LOW specificity limits the use of the cerebrospinal fluid Abeta1-42/P-TAU181P ratio to discriminate Alzheimer's disease from vascular dementia. *J Gerontol A Biol Sci Med Sci* **62**, 923-924; author reply 924-925.

[57] Thorsell A, Bjerke M, Gobom J, Brunhage E, Vanmechelen E, Andreasen N, Hansson O, Minthon L, Zetterberg H, Blennow K (2010) Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. *Brain Res* **1362**, 13-22.

[58] Wallin A, Ohrfelt A, Bjerke M (2012) Characteristic clinical presentation and CSF biomarker pattern in cerebral small vessel disease. *J Neurol Sci* **322**, 192-196.

[59] Rosenberg GA, Bjerke M, Wallin A (2014) Multimodal markers of inflammation in the subcortical ischemic vascular disease type of vascular cognitive impairment. *Stroke* **45**, 1531-1538.

[60] Wallin A, Kapaki E, Boban M, Engelborghs S, Hermann DM, Huisa B, Jonsson M, Kramberger MG, Lossi L, Malojcic B, Mehrabian S, Merighi A, Mukaetova-Ladinska EB, Paraskevas GP, Popescu BO, Ravid R, Traykov L, Tsivgoulis G, Weinstein G, Korczyn A, Bjerke M, Rosenberg G (2017) Biochemical markers in vascular cognitive impairment associated with subcortical small vessel disease – A consensus report. *BMC Neurol* **17**, 102.

[61] Jonsson M, Zetterberg H, Rolstad S, Edman A, Gouw AA, Bjerke M, Lind K, Blennow K, Pantoni L, Inzitari D, Wallin A (2012) Low cerebrospinal fluid sulfatide predicts progression of white matter lesions: The LADIS study. *Dement Geriatr Cogn Disord* **34**, 61-67.

[62] Wallin A, Nordlund A, Jonsson M, Blennow K, Zetterberg H, Ohrfelt A, Stalhammar J, Eckerstrom M, Carlsson M, Olsson E, Gothlin M, Svensson J, Rolstad S, Eckerstrom C, Bjerke M (2016) Alzheimer's disease–subcortical vascular disease spectrum in a hospital-based setting: Overview of results from the Gothenburg MCI and dementia studies. *J Cereb Blood Flow Metab* **36**, 95-113.

[63] Hjalmarsson C, Bjerke M, Andersson B, Blennow K, Zetterberg H, Aberg ND, Olsson B, Eckerstrom C, Bokemark L, Wallin A (2014) Neuronal and glia-related biomarkers in cerebrospinal fluid of patients with acute ischemic stroke. *J Cent Nerv Syst Dis* **6**, 51-58.

[64] Zetterberg H, Skillback T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, Weiner MW, Blennow K, Alzheimer's Disease Neuroimaging Initiative (2016) Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. *JAMA Neurol* **73**, 60-67.

[65] Sieben A, Van Langenhove T, Engelborghs S, Martin JJ, Boon P, Cras P, De Deyn PP, Santens P, Van Broeckhoven C, Cruts M (2012) The genetics and neuropathology of frontotemporal lobar degeneration. *Acta Neuropathol* **124**, 353-372.

[66] Goossens J, Vanmechelen E, Trojanowski JQ, Lee VM, Van Broeckhoven C, van der Zee J, Engelborghs S (2015) TDP-43 as a possible biomarker for frontotemporal lobar degeneration: A systematic review of existing antibodies. *Acta Neuropathol Commun* **3**, 15.

[67] Sleegers K, Brouwers N, Van Damme P, Engelborghs S, Gijselinck I, van der Zee J, Peeters K, Mattheijssens M, Cruts M, Vandenberghe R, De Deyn PP, Robberecht W, Van Broeckhoven C (2009) Serum biomarker for progranulin-associated frontotemporal lobar degeneration. *Ann Neurol* **65**, 603-609.

[68] Ghidoni R, Benussi L, Glionna M, Franzoni M, Binetti G (2008) Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. *Neurology* **71**, 1235-1239.

[69] Simonsen AH, Kuiperij B, El-Agnaf OM, Engelborghs S, Herukka SK, Parnetti L, Rektorova I, Vanmechelen E, Kapaki E, Verbeek M, Mollenhauer B (2016) The utility of alpha-synuclein as biofluid marker in neurodegenerative diseases: A systematic review of the literature. *Biomark Med* **10**, 19-34.

[70] Kruse N, Persson S, Alcolea D, Bahl JM, Baldeiras I, Capello E, Chiasserini D, Bocchio Chiavetto L, Emersic A, Engelborghs S, Eren E, Fladby T, Frisoni G, Garcia-Ayllon MS, Genc S, Gkatzima O, Heegaard NH, Janeiro AM, Kovacech B, Kuiperij HB, Leitao MJ, Lleo A, Martins M, Matos M, Mollergard HM, Nobili F, Ohrfelt A, Parnetti L, de Oliveira CR, Rot U, Saez-Valero J, Struyfs H, Tanassi JT, Taylor P, Tsolaki M, Vanmechelen E, Verbeek MM, Zilka N, Blennow K, Zetterberg H, Mollenhauer B (2015) Validation of a quantitative cerebrospinal fluid alpha-synuclein assay in a European-wide interlaboratory study. *Neurobiol Aging* **36**, 2587-2596.

[71] Slaets S, Vanmechelen E, Le Bastard N, Decraemer H, Vandijck M, Martin JJ, De Deyn PP, Engelborghs S (2014) Increased CSF alpha-synuclein levels in Alzheimer's disease: Correlation with tau levels. *Alzheimers Dement* **10**, S290-S298.

[72] 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.

[73] Van Everbroeck B, Quoilin S, Boons J, Martin JJ, Cras P (2003) A prospective study of CSF markers in 250 patients with possible Creutzfeldt-Jakob disease. *J Neurol Neurosurg Psychiatry* **74**, 1210-1214.

[74] Bjerke M, Portelius E, Minthon L, Wallin A, Anckarsater H, Anckarsater R, Andreasen N, Zetterberg H, Andreasson U, Blennow K (2010) Confounding factors influencing amyloid Beta concentration in cerebrospinal fluid. *Int J Alzheimers Dis* **2010**, 986310.

[75] Le Bastard N, De Deyn PP, Engelborghs S (2015) Importance and impact of preanalytical variables on Alzheimer disease biomarker concentrations in cerebrospinal fluid. *Clin Chem* **61**, 734-743.

[76] Willemse E, van Uffelen K, Brix B, Engelborghs S, Vanderstichele H, Teunissen C (2017) How to handle adsorption of cerebrospinal fluid amyloid-beta (1-42) in laboratory practice? Identifying problematic handlings and resolving the issue by use of the Abeta42/Abeta40 ratio. *Alzheimers Dement* **13**, 885-892.

[77] 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 beta peptides. *Clin Chem* **52**, 332-334.

[78] Niemantsverdriet E, Goossens J, Struyfs H, Martin JJ, Goeman J, De Deyn PP, Vanderstichele H, Engelborghs S (2016) Diagnostic impact of cerebrospinal fluid biomarker (pre-)analytical variability in Alzheimer's disease. *J Alzheimers Dis* **51**, 97-106.

[79] Bjerke M, Andreasson U, Kuhlmann J, Portelius E, Pannee J, Lewczuk P, Umek RM, Vanmechelen E, Vanderstichele H, Stoops E, Lewis J, Vandijck M, Kostanjevecki V, Jeromin A, Salamone SJ, Schmidt O, Matzen A, Madin K, Eichenlaub U, Bittner T, Shaw LM, Zegers I, Zetterberg H, Blennow K (2016) Assessing the commutability of reference material formats for the harmonization of amyloid-beta measurements. *Clin Chem Lab Med* **54**, 1177-1191.

[80] Mattsson N, Zegers I, Andreasson U, Bjerke M, Blankenstein MA, Bowser R, Carrillo MC, Gobom J, Heath T, Jenkins R, Jeromin A, Kaplow J, Kidd D, Laterza OF, Lockhart A, Lunn MP, Martone RL, Mills K, Pannee J, Ratcliffe M, Shaw LM, Simon AJ, Soares H, Teunissen CE, Verbeek MM, Umek RM, Vanderstichele H, Zetterberg H, Blennow K, Portelius E (2012) Reference measurement procedures for Alzheimer's disease cerebrospinal fluid biomarkers: Definitions and approaches with focus on amyloid beta42. *Biomark Med* **6**, 409-417.

[81] Kuhlmann J, Andreasson U, Pannee J, Bjerke M, Portelius E, Leinenbach A, Bittner T, Korecka M, Jenkins RG, Vanderstichele H, Stoops E, Lewczuk P, Shaw LM, Zegers I, Schimmel H, Zetterberg H, Blennow K, IFCC Working Group on Standardization of CSF proteins (WG-CSF) (2017) CSF Abeta1-42 – an excellent but complicated Alzheimer's biomarker – a route to standardisation. *Clin Chim Acta* **467**, 27-33.

[82] Mattsson N, Andreasson U, Persson S, Arai H, Batish SD, Bernardini S, Bocchio-Chiavetto L, Blankenstein MA, Carrillo MC, Chalbot S, Coart E, Chiasserini D, Cutler N, Dahlfors G, Duller S, Fagan AM, Forlenza O, Frisoni GB, Galasko D, Galimberti D, Hampel H, Handberg A, Heneka MT, Herskovits AZ, Herukka SK, Holtzman DM, Humpel C, Hyman BT, Iqbal K, Jucker M, Kaeser SA, Kaiser E, Kapaki E, Kidd D, Klivenyi P, Knudsen CS, Kummer MP, Lui J, Llado A, Lewczuk P, Li QX, Martins R, Masters C, McAuliffe J, Mercken M, Moghekar A, Molinuevo JL,

Montine TJ, Nowatzke W, O'Brien R, Otto M, Paraskevas GP, Parnetti L, Petersen RC, Prvulovic D, de Reus HP, Rissman RA, Scarpini E, Stefani A, Soininen H, Schroder J, Shaw LM, Skinningsrud A, Skrogstad B, Spreer A, Talib L, Teunissen C, Trojanowski JQ, Tumani H, Umek RM, Van Broeck B, Vanderstichele H, Vecsei L, Verbeek MM, Windisch M, Zhang J, Zetterberg H, Blennow K (2011) The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimers Dement* **7**, 386-395 e386.

[83] Molinuevo JL, Blennow K, Dubois B, Engelborghs S, Lewczuk P, Perret-Liaudet A, Teunissen CE, Parnetti L (2014) The clinical use of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: A consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement* **10**, 808-817.

[84] Vanderstichele H, Bibl M, Engelborghs S, Le Bastard N, Lewczuk P, Molinuevo JL, Parnetti L, Perret-Liaudet A, Shaw LM, Teunissen C, Wouters D, Blennow K (2012) Standardization of preanalytical aspects of cerebrospinal fluid biomarker testing for Alzheimer's disease diagnosis: A consensus paper from the Alzheimer's Biomarkers Standardization Initiative. *Alzheimers Dement* **8**, 65-73.