Selecting Aß isoforms for an Alzheimers disease cerebrospinal fluid biomarker panel

Selecting Aβ-isoforms for an Alzheimer's disease CSF biomarker panel

Charisse Somers[1], Joery Goossens[1], Sebastiaan Engelborghs[1,2], Maria Bjerke[1]

[1] Reference Center for Biological Markers of Dementia (BIODEM), Department of Biomedical Sciences, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium.

[2] Department of Neurology and Memory Clinic, Hospital Network Antwerp (ZNA) Middelheim and Hoge Beuken, Antwerp, Belgium.

Corresponding author:

Prof. Dr. Maria Bjerke, Reference Center for Biological Markers of Dementia (BIODEM), University of Antwerp, Universiteitsplein 1, BE-2610 Antwerp, Belgium (E: Maria.Bjerke@uantwerpen.be)

Abstract

Although the core cerebrospinal fluid (CSF) Alzheimer's disease (AD) biomarkers amyloid-β ($A\beta_{1-42}$) and tau show a high diagnostic accuracy, there are still limitations due to overlap in the biomarker levels with other neurodegenerative and dementia disorders.

During $A\beta_{1-42}$ production and clearance in the brain, several other Aβ peptides and APP fragments are formed that could potentially serve as biomarkers for this ongoing disease process. Therefore, this review will present the current status of the findings for APP and Aβ peptide isoforms in AD and clinically related disorders.

In conclusion, adding new Aβ-isoforms to the AD biomarker panel may improve early differential diagnostic accuracy and increase the CSF biomarker concordance with AD neuropathological findings in the brain.

Keywords

Alzheimer's disease – Amyloid – Biomarker – Cerebrospinal fluid – Diagnosis

- Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder in elderly, leading to dementia. An accurate and early diagnosis of AD is important to select the optimal patient care and will be critical in current clinical trials. 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 and positron emission tomography. However, clinical dementia diagnosis does not always correspond to the neuropathological definite diagnosis with clinical diagnostic accuracy levels ranging between 82 and 84% [2,3]. When a clinician should discriminate AD from a non-AD dementia relying on (non-biomarker based) clinical diagnostic criteria, 16% of the patients have a doubtful AD versus non-AD diagnosis [3–5]. Therefore it is important to increase the clinical diagnostic accuracy, which will be even 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 [6,7], they will be important tools in the clinical set-up for early dementia (differential) diagnosis.

Neuropathologically, AD is characterized by the formation of extracellular cortical senile plaques [8] and intracellular neurofibrillary tangles composed of hyperphosphorylated tau (P-tau) mainly found in the limbic and association cortices [9,10]. The core structure of the senile plaques mainly comprises the amyloid-β (Aβ) peptide, which is derived from the amyloid precursor protein (APP) [11]. While many different Aβ-isoforms are present in the brain, the hydrophobic 42 amino acid long Aβ ($A\beta_{1-42}$) peptide in particular is prone to self-accumulation into soluble oligomers. These oligomers further form larger fibrils and aggregate into insoluble extracellular plaques, both are believed to possess neurotoxic properties [12,13]. The exact cause of the accumulation and deposition of $A\beta_{1-42}$ into plaques is still unknown, though it has been hypothesized that an imbalance in production and clearance may be one of the reasons [14]. An imbalance in the regulation of protein kinases and phosphatases is believed to lead to hyperphosphorylation of microtubule-associated protein tau, which causes it to dissociate and accumulate into paired helical filaments that compose the intraneuronal neurofibrillary tangles [15].

Interestingly, it has been shown that patients that are clinically diagnosed with a high likelihood of AD due to a typical AD profile with an amnestic syndrome, portrait postmortem with a neuropathological tangle intensive (as graded by Braak staging) profile that involves hippocampus [16]. On the contrary, patients with a neuropathologically confirmed diagnosis of AD with a plaque intensive profile (as graded by the Consortium to Establish a Registry for Alzheimer's Disease; CERAD) were clinically diagnosed with a moderate likelihood of having AD due to an atypical clinical profile [16]. Together with the demand for early diagnosis, these findings support the need for *in vivo* biological verification of the disease [17]. Decades of research have resulted in the development of three core cerebrospinal fluid (CSF) biomarkers for the neurobiochemical diagnosis of AD [18] of which, especially in early diagnosis of atypical clinical cases, $A\beta_{1-42}$ may prove to be an invaluable tool for AD detection.

- The amyloid precursor protein and amyloid-β production

The prevailing theory of AD etiology, the amyloid cascade hypothesis, postulates a central role of $A\beta_{1-42}$ as an initiator of AD pathology. The toxic Aβ oligomers and/or senile plaques cause hyperphosphorylation of tau and formation of neurofibrillary tangles, activation of glia and neuroinflammation, synaptic loss and ultimately neuronal degeneration. The $A\beta_{1-42}$ is generated through the so called amyloidogenic pathway through sequential enzymatic cleavage of the APP protein by β-and γ-secretases. APP is a type-1 integral membrane protein and the β-secretase cleavage site (the β-site) is found in the extracellular amino terminal part of APP and thus the

extracellular soluble APP (sAPPβ) fragment is also liberated, while γ-secretases cleavage occurs in the membrane domain of APP which also leads to the liberation of an intracellular domain into the cytosol. An alternative pathway, referred to as the non-amyloidogenic pathway, involves cleavage of APP within the Aβ domain by α-secretase to preclude the formation of intact full-lenght $A\beta_{1-42}$. This pathway also liberates a 16 amino acid longer extracellular domain compared with the amyloidogenic pathway, an APP soluble fragment that has been termed sAPPα. There are several sites in the carboxyl terminal of Aβ domain for γ-secretase cleavage as well as many other possible cleavage sites for various enzymes in the Aβ sequence that may be involved in Aβ degradation and give rise to the multitude of Aβ peptides found in the brain [14,19].

- Biomarker characteristics and cerebrospinal fluid $A\beta_{1-42}$ reflecting AD pathology

For a biomarker to be an acceptable candidate for AD diagnosis it should be precise, reliable, inexpensive and non-invasive. In addition, the biomarker should be able to detect one of the pathological hallmarks of AD and this should be validated in neuropathologically confirmed cases. These biomarker features were established in the consensus paper by the Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group [20], which also stated that biomarker candidates should have a sensitivity of at least 80% for the detection of AD and a specificity for differentiation from other neurodegenerative disorders between 75% and 85%.

As cerebrospinal fluid (CSF) is in direct contact with the brain interstitial fluid, its contents reflect the biochemical changes taking place during a disease process, making CSF biomarkers good diagnostic candidates for brain disorders. The contamination of the CSF proteome by blood proteins is prohibited by the blood-CSF/blood-brain barriers, obstructing possible degradation, binding or dilution of the biomarker of interest by other non-brain derived and non-AD pathology-specific proteins [21,22]. The choice of CSF biomarkers over blood biomarkers, at least in the case of $A\beta_{1-42}$, is supported by studies in plasma showing conflicting results but no overall difference between AD and controls when evaluated in a meta-analysis (22 study comparisons) [18]. Also, no correlation between plasma and CSF $A\beta_{1-42}$ biomarker have been found [23,24]. Furthermore, $A\beta_{1-42}$ in CSF 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 [25] as well as the *in vivo* correlation to cortical amyloid load as measured by amyloid positron emission tomography (PET) with Pittsburgh Compound B in patients with AD [26]. Moreover, the CSF $A\beta_{1-42}$ levels have also shown satisfactory sensitivity and specificity for the distinction between healthy subjects and AD cases in neuropathologically confirmed cohorts [3,27] as well as between pathologically confirmed AD and non-AD dementia cohorts [3,4,28,29]. Also, a recent meta-analysis of $A\beta_{1-42}$ in CSF shows that the sensitivity and specificity for differential diagnosis between clinical AD and non-AD dementias reached the consented level for an acceptable AD biomarker [30].

Due to its favorable 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 [31] as *in vivo* surrogate marker for plaque pathology. 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 when performed on assays utilizing different calibrators, major efforts are undertaken to overcome these problems by introducing a certified reference material that can be used for value assignment of the assay calibrators [32–35]. Also, the Alzheimer's Association external quality control (QC) program monitors site-to-site and batch-to-batch CSF test variation for the purpose of enabling the participating laboratories to synchronize their procedures [36]. These efforts

will lead to precise and reliable measurements between laboratories that will enable the introduction of a world-wide cut-off point for CSF $A\beta_{1-42}$ measurements for the purpose of clinical diagnostics and patient stratification in clinical trials.

- Cerebrospinal fluid $A\beta_{1-42}$ for early and differential AD diagnosis

The present core AD CSF biomarkers, including $A\beta_{1-42}$, total tau protein (T-tau), and tau phosphorylated at threonine 181 ($P\text{-}tau_{181}$), 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 $P\text{-}tau_{181}$ levels [17]. The inclusion of these biomarkers into the new criteria for AD diagnosis for research purposes is not only based on the correlation to the above mentioned imaging and neuropathology findings, but also on more than 100 studies showing the consistency of altered levels of the three core biomarkers in AD compared to controls, as well as over 10 papers showing the same alterations in patients with mild cognitive impairment (MCI) who later develop AD dementia compared to those who remain stable [18]. It should be emphasized that early 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 syndrome entities and it should be kept in mind that when solely clinically assessed, less than 50% of these subjects converted to dementia [37] even after an extensive follow-up period of 6 years. Even fewer (18%) of these SCD and MCI subjects specifically developed a dementia due to AD [37]. Particularly, CSF $A\beta_{1-42}$ is a more attractive biomarker for early AD detection than both CSF T-tau and $P\text{-}tau_{181}$ as tau alterations seem to occur at a later time point in the disease process [38]. Early detection is likely to become even more important as soon as disease modifying pharmacological treatment for AD will be available, since medications that halt or prevent the disease are likely to be most efficient in an early stage, when neurodegeneration has not become too severe. At present, low CSF $A\beta_{1-42}$ levels are 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 crucial for monitoring treatment effects [39].

Although there is strong evidence for the importance of CSF $A\beta_{1-42}$ as a biomarker for AD, 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 (subcortical) vascular dementia (VaD) [40–43], dementia with Lewy bodies (DLB) [44,45], Creutzfeldt-Jakob Disease (CJD) [46] and normal pressure hydrocephalus (NPH) [47,48] compared to healthy individuals. Though the $A\beta_{1-42}$ levels are most often still lower in AD compared to VaD and DLB, a significant overlap nevertheless limits their discrimination [40,41,43,49]. Conflicting results have been shown in frontotemporal lobar degeneration (FTLD) were either no changes in $A\beta_{1-42}$ levels or lower levels compared to controls have been found, though these levels were also still higher than in AD [50,51]. While AD pathology is often found to co-exist with both Parkinson's disease and DLB as well as with cerebrovascular disease [3,45,52,53], decreased $A\beta_{1-42}$ levels in CSF of patients with pure VaD (related to subcortical small vessel disease), CJD, NPH or FTLD are most likely not due to plaque pathology. Proposed causes for the decreased concentrations of $A\beta_{1-42}$ are a diminished production, an increased clearance or an enhanced binding to carriers that will mask the epitopes and therefor decrease the detection of $A\beta_{1-42}$ by immunoassays. For example, CJD is characterized by presence of prion protein (PrP^{Sc}) depositions and physiological PrP^{C} has been shown to promote the aggregation of $A\beta_{1-42}$ into plaques, therefore influencing Aβ metabolism and its presence in CSF. As for subcortical VaD and NPH, the decreased levels of $A\beta_{1-42}$ may be caused by

the inhibition of axonal transport of APP, causing a disturbed APP metabolism, or by the altered clearance of Aβ due to disrupted CSF dynamics or alterations in enzymatic systems.

- Additional APP/Aβ mediators to increase diagnostic specificity for AD

Cerebrospinal fluid $Aβ_{1-40}$ and $Aβ_{1-38}$ in AD and other dementia disorders

Both $Aβ_{1-40}$ and $Aβ_{1-38}$ are more abundant in CSF than $Aβ_{1-42}$. They have been less extensively studied compared to $Aβ_{1-42}$ as a biomarker for AD possibly due to early discouraging findings showing no difference between AD and controls [54,55]. Though there have been some conflicting results, the negative findings were confirmed in a recent meta-analysis showing only negligible overall decrease in $Aβ_{1-40}$ levels with a small effect size in AD compared with controls (25 comparisons). Furthermore, no significant difference was found in the same meta-analysis (three studies included) for the comparison of CSF $Aβ_{1-40}$ in patients with MCI that converted to AD at follow-up and subjects who maintained their MCI status [18]. It has also previously been shown that there was no difference between AD and non-AD neurodegenerative disorders (Mehta et al., 2000); however, the non-AD group contained small patient numbers in each scattered patient group precluding any subanalyses. Another Aβ peptide that was assessed in the meta analyses, summarized in the AlzBiomarker database (http://www.alzforum.org/alzbiomarker), that is also highly abundant in CSF and for which no differences between AD and controls have been found is $Aβ_{1-38}$ (8 studies included) [18]. Importantly, both $Aβ_{1-40}$ and $Aβ_{1-38}$ have been further investigated for their ability to differentiate between AD and non-AD brain disorders and it has been shown in several studies that lower levels of both $Aβ_{1-40}$ and $Aβ_{1-38}$ are found in FTLD, VaD and DLB/Parkinson's disease dementia (PDD) compared to AD and controls [56–60]. The differential diagnostic added value of $Aβ_{1-40}$ and $Aβ_{1-38}$ will be addressed below.

So far, immunoassays have mainly been used for quantitative assessments of $Aβ_{1-42}$, $Aβ_{1-40}$ and $Aβ_{1-38}$ in human CSF, but it has also been shown that established quantitative methods based on mass spectrometry (MS) perform very well [61–64] and the measurements correlate highly with immunobased assays [33,65]. The interchangeable use of the methods for Aβ measurements is advantageous and an even more important role for MS is expected in the search for new biomarkers and new possible Aβ peptides that may contribute to the differential diagnosis of AD, but for which there are no (specific) antibodies available.

Cerebrospinal fluid Aβ peptides in AD

One of the first attempts to characterize Aβ species in human CSF was undertaken in the early 1990s when there was still much doubt about its existence in CSF. The peptides identified by laser desorption MS confirmed the presence of Aβ species, all beginning with aspartic acid in the Aβ (Asp 1) carboxyl-terminus (C-terminal), containing 27, 28, 30, 34, 35, 40, 42, or 43 amino acids [66]. Through a further refined method that employed a combination of immunoprecipitation (with 6E10 and 4G8 antibodies) and MS (IP-MS) it was shown that a whole range, including the above mentioned species, of Aβ fragments (1-13 – 1-20, 1-32 - 1-34, 1-37 - 1-42) truncated in the amino-terminus (N-terminal) were captured, but also many shorter C-terminally truncated peptides (2-, 3-, 4-, 5-, 6-, -8, 11-, 12-, 14-, 15-, 16-, and 17-) of different lengths [67]. This method has since paved the way for characterization of Aβ fragments in human CSF. While CSF candidates for the differentiation between AD and controls, such as $Aβ_{1-16}$, $Aβ_{1-33}$, and $Aβ_{1-39}$, have been lifted forward using IP (antibodies 6E10 and 4G8)-MS with label free (semi) quantification [68] most of these peptides have not yet been quantitatively assessed and the results have not been verified by others. The combined levels of $Aβ_{1-15/16}$ were investigated further but no difference was found between AD and controls, while there was a significant decrease in Parkinson's disease (PD) and PDD, multiple system atrophy and progressive supranuclear palsy compared to controls [69]. One argument in support of the above

$A\beta_{1-16}$ findings is if increased levels of $A\beta_{1-16}$ were masked by a concomitant decrease in the concentration of $A\beta_{1-15}$ in AD, this would render the overall change non-significant with this assay set-up. However, $A\beta_{1-15}$ was not suggested to be decreased in AD in above study. Also, no significant changes in the above peptides ($A\beta_{1-15}$, $A\beta_{1-16}$, $A\beta_{1-33}$, or $A\beta_{1-39}$) or any other Aβ peptides assessed ($A\beta_{1-13}$, $A\beta_{1-14}$, $A\beta_{1-17}$, $A\beta_{1-19}$, $A\beta_{1-20}$, $A\beta_{1-30}$, $A\beta_{1-34}$, $A\beta_{1-37}$, $A\beta_{1-38}$ or $A\beta_{1-40}$) except for $A\beta_{1-42}$ were seen between sporadic AD and healthy controls in another IP-MS attempt [70]. Another candidate that was lifted forward by IP (antibodies 6E10, 4G8 and antibody directed against $A\beta_{21-34}$)-MS using isobaric quantification was $A\beta_{1-34}$ (slightly decreased, but not significant after Bonferroni correction), while in another MS study that also employed isobaric labelling $A\beta_{22-40}$ was found to be reduced in AD compared to controls [71]. Neither study verified the other study findings. Thus, there are so far no other Aβ peptides except for $A\beta_{1-42}$ that seem to be specific for AD versus controls.

Cerebrospinal fluid Aβ-oligomers in AD

The interest in Aβ-oligomers as a biomarker arose when it was shown that they may be toxic and cause synaptic dysfunction and inhibit long term potentiation. Since oligomers are, according to the amyloid cascade hypothesis, the preceding step to the formation of amyloid plaques it was hoped that they would serve as even earlier biomarkers than $A\beta_{1-42}$. Although significantly higher levels of high molecular weight CSF Aβ-oligomers (40-200kDa) were found in AD compared to controls [72], and in AD and MCI due to AD compared to controls [73], contradictory results have been shown by another study [74], were no differences were found for Aβ-oligomers in CSF between MCI patients that developed AD, MCI patients that remained stable or non-demented controls. This discrepancy could be due to differences in assay design and performance that favor detection of different analytes or Aβ-oligomers. It was further stated in the above study, were differences between patient groups were found, that Aβ oligomers have not potential as biomarkers due to a high degree of overlap between the diagnostic groups and the oligomers did not perform better than CSF $A\beta_{1-42}$. Furthermore, there was no correlation at all to $A\beta_{1-42}$ levels, indicating other possible mechanisms for the aggregation of $A\beta_{1-42}$ into plaques [73].

Cerebrospinal fluid sAPPα and sAPPβ in AD and other dementia disorders

As the extracellular part of the APP protein is being released by β- and α-secretases as soluble APP fragments (sAPPβ and sAPPα, respectively) these were proposed to be able to serve as upstream biomarkers for the amyloid and non-amyloid pathways in CSF. Unfortunately, it has been shown in the AlzBiomarker database meta-analysis that neither sAPPβ nor sAPPα holds the potential to serve as AD biomarkers, since the levels are not significantly different in AD compared to controls (10 publications for sAPPβ and 9 for sAPPα) or in MCI patients that developed AD at follow-up compared to those who were stable in their MCI syndrome (3 publications including sAPPβ and sAPPα) [18]. It should be noted that for the measurement of sAPPα many of the assays do not specifically capture fragments cleaved at the α-secretase cleavage site and therefore may include both fragments that are shorter and longer than intended.

Both sAPPβ and sAPPα have also been investigated for their potential as differential diagnostic tools. In one study, patients with clinical dementia disorders with either an AD supportive or an AD dismissive CSF profile were investigated for their sAPP profiles. Decreased levels of both both sAPPβ and sAPPα were found in the dementia patients with a negative AD CSF profile compared to those with a CSF profile indicative of AD [75]. Further characterization of sAPP alterations in non-AD dementias have shown sAPPβ to be significantly decreased in FTLD compared with AD [60,76], while no difference was observed between the levels in AD and DLB or PDD [77]. Furthermore, CSF sAPPβ has been shown to be inversely correlated with white matter lesion volume in patients with cerebrovascular disease [78] and acute stroke [79] possibly indicating dysfunctional axonal transport

in patients with small vessel disease. Moreover, sAPPα and sAPPβ levels have been shown to be decreased in CSF in NPH compared with healthy subjects [80] and to be strong markers for the differentiation between AD and NPH [47]. The alterations in sAPPα and sAPPβ have been shown to be independent of Aβ pathology, though it may be that changes take place much earlier in the cascade causing a disconnection to current tissue pathology, and it has been suggested that sAPP may rather reflect metabolic impairment in the brain tissue possibly caused by ischemia [81].

Aβ peptide ratios to increase diagnostic accuracy for AD

It has been shown previously that a combination of the core AD biomarkers are superior compared to the single biomarkers alone; however, it has also been shown that tau does not perform as well as $A\beta_{1-42}$ in the early stages of disease [38]. Thus if there are other amyloid metabolites in CSF that can be added to the AD biomarker panel to improve early differential diagnostic accuracy this would be immensely important.

The introduction of Aβ ratios may prove to be of importance for early differential AD diagnosis. First of all, it has been demonstrated that the $A\beta_{1-42}/A\beta_{1-40}$ ratio shows better concordance with amyloid load in the brain as assessed by PiB-PET than $A\beta_{1-42}$ alone [56,82,83]. The $A\beta_{1-42}/A\beta_{1-40}$ ratio has been revealed to be decreased in AD compared with controls, FTLD, VaD, DLB and PDD, the latter 4 groups being inseparable, and was more accurate for differentiating AD from the other types of dementia than $A\beta_{1-42}$ alone [56,57,84,85]. Also, the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio has been shown to be superior or equal 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 [86,87] and the $A\beta_{1-42}/A\beta_{1-40}$ ratio has been shown to perform equally well as the combination of $A\beta_{1-42}$, P-tau181 and T-tau in differentiating between AD and other non-AD dementias [85,88]. In another study, it was shown that adding the Aβ ratio to the core biomarkers (T-tau not included) improved the accuracy when distinguishing between AD and non-AD dementias in cases with intermediate $P\text{-}tau_{181}$ [89]. 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 pointed in the direction in over 50% of the cases to be in agreement with the clinical diagnosis [90]. These findings speak in favor of the added value of the $A\beta_{1-42}/A\beta_{1-40}$ ratio for early dementia differential diagnosis, when alterations in CSF tau are yet to be seen. The influence of the ratio could possibly be contributed to the fact that $A\beta_{1-40}$ closely represents the total cerebral Aβ load and thus eliminates inter-individual differences in total Aβ concentrations.

Other ratios that have been less well investigated but still show potential as biomarkers are $A\beta_{1-42}/A\beta_{1-38}$ and $A\beta_{1-42}/A\beta_{1-37}$ [88,91–93]. These studies concluded that $A\beta_{1-42}/A\beta_{1-38}$ was the best ratio for the separation between AD and DLB and that it outperformed the single AD biomarkers. Also, $A\beta_{1-42}/A\beta_{1-37}$ has been shown to have an additive value for the differentiation between AD and FTLD [88]. More studies are needed to in order to determine which Aβ peptide ratios achieve the best separation in different diagnostic setting.

- Conclusion

In this review we summarize the possible added value of CSF APP and Aβ metabolites as biomarkers for early and differential AD diagnosis. It can be concluded that CSF $A\beta_{1-42}$ is the superior AD biomarker that has consistently proven to be altered in AD compared to controls compared to other Aβ peptides or APP fragments. Additionally, both sAPPβ and various Aβ peptides (e.g. $A\beta_{1-40}$, $A\beta_{1-38}$ and $A\beta_{1-37}$) are altered in several non-AD neurodegenerative and dementia disorders and may therefore prove to be valuable tools for differential diagnosis, most likely combined in a ratio with $A\beta_{1-42}$. However, it is far too early to state which specific Aβ peptide ratio combinations may prove to be the most accurate predictors when concerned with early differential diagnosis. Importantly, the

Aβ ratio needs to capture the AD neuropathological changes taking place in the brain that leads to the build-up of plaques better than $A\beta_{1-42}$. In the case of the $A\beta_{1-42}/A\beta_{1-40}$ ratio the concordance between CSF findings and amyloid load measured by PET imaging is higher than for $A\beta_{1-42}$ alone and this seems to be a general feature for all the neurodegenerative and dementia diseases assessed. These findings are immensely important for patient selection to reach improved treatment effects when concerned with amyloid-based therapy.

- Future perspectives

With the prospective of disease modifying treatment for AD becoming available in the near future, an accurate diagnosis that reflects the neuropathology will be of great importance for selecting patients that will benefit from the treatment. Though a combination of the current AD core CSF biomarkers shows high accuracy for AD, they may be less efficient in the really early phases of disease due to the time dependence of tau to symptom onset. The Aβ-ratio may be a future substitute for early detection of AD amyloid pathology, since the decrease of CSF $A\beta_{1-42}$ alone overlaps with other neurodegenerative and dementia diseases known to be less affected by plaque pathology. The concordance between CSF and amyloid PET imaging increases with the introduction of the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio, which speaks in favor of its clinical utility as an inexpensive *in vivo* biomarker of plaque pathology. Its potential of reflecting pathology should also be assessed in autopsy confirmed cases with and without plaque pathology, preferably including cases with other dementia diseases and mixed AD pathologies. Furthermore, the potential as an early differential biomarker must be evaluated in more longitudinal studies that include early MCI cases. Other Aβ ratios, with peptides such as $A\beta_{1-38}$ and $A\beta_{1-37}$, should also be included to assess their performance in comparison to $A\beta_{1-42}/A\beta_{1-40}$. The implementation of the $A\beta_{1-42}/A\beta_{1-40}$ ratio (or possibly another ratio) or the substitution of $A\beta_{1-42}$ by the ratio will most likely increase the core AD biomarker accuracy and great focus should therefore be put on its validation in the very near future.

Executive summary

Introduction

- There is a need to improve early accurate clinical diagnosis of AD to improve the concordance with neuropathology diagnosis.
- The amyloid plaque is one of the neuropathological hallmarks of AD and its major constituent, $A\beta_{1-42}$, is an *in vivo* biological marker of AD.

The amyloid precursor protein and amyloid-β production

- The hydrophobic $A\beta_{1-42}$ peptide is produced during amyloid precursor protein (APP) metabolism along with soluble APP fragments and a multitude of Aβ-isoforms.
- Amyloid plaques and Aβ-oligomers are presumed to have neurotoxic effects ultimately causing synaptic dysfunction and neurodegeneration.

Biomarker characteristics and cerebrospinal fluid $A\beta_{1-42}$ reflecting AD pathology

- A biomarker should reflect disease specific neuropathological hallmarks.
- The favorable characteristics of CSF $A\beta_{1-42}$ as *in vivo* biomarker for amyloid plaque pathology and as a CSF differential biomarker for AD diagnosis have led to great efforts for measurement standardization for the biomarker implementation into clinical routine.

Cerebrospinal fluid $A\beta_{1-42}$ for early and differential AD diagnosis

- Of the three core biomarkers, $A\beta_{1-42}$ is the most attractive for early disease detection.
- The CSF $A\beta_{1-42}$ biomarker value for AD differential diagnosis is hampered due to its overlap in concentration with other neurodegenerative disorders.

Additional APP/Aβ mediators to increase diagnostic specificity for AD

- Introducing Aβ peptide ratios (e.g. $A\beta_{1-42}/A\beta_{1-40}$) improve AD differential diagnostic accuracy and concordance between CSF measurements and amyloid PET imaging.

Conclusion

- The CSF $A\beta_{1-42}$ peptide as a single marker is superior compared to other Aβ peptides and APP fragments alone for AD diagnosis. It is consistently altered in AD compared to controls.
- One limitation for CSF $A\beta_{1-42}$ as a biomarker is the overlap between AD and other dementia diseases.
- Additional sAPP fragments or Aβ peptides (e.g. $A\beta_{1-40}$, $A\beta_{1-38}$ and $A\beta_{1-37}$) that are altered in several non-AD neurodegenerative and dementia disorders may improve the accuracy of the AD biomarker panel.
- The $A\beta_{1-42}/A\beta_{1-40}$ ratio (or possibly other Aβ ratios) seems to improve the accuracy for early AD differential diagnosis and better capture the AD neuropathological changes compared with $A\beta_{1-42}$ alone.

Future perspectives

- The improved accuracy of the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio compared to $A\beta_{1-42}$ alone to reflect plaque pathology should be further assessed both in combination with amyloid imaging and in autopsy confirmed cases with and without plaque pathology, importantly including cases with other dementia diseases and mixed AD pathologies.
- Validation of the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio as an early differential biomarker must be evaluated in more large longitudinal cohorts that include early MCI cases as well as differential diagnoses. Additional Aβ ratios, with peptides such as $A\beta_{1-38}$ and $A\beta_{1-37}$, should be assessed alongside for their performance in comparison to $A\beta_{1-42}/A\beta_{1-40}$.
- The implementation of the $A\beta_{1-42}/A\beta_{1-40}$ ratio (or possibly another ratio), or the substitution of $A\beta_{1-42}$ by the ratio, will most likely increase the core biomarker accuracy for the detection of AD, thus great focus should therefore be put on its clinical and analytical validation in the very near future.

- References (papers of special interest have been highlighted with an asterisk (*))

1. Bier J-C, Verschraegen J, Vandenberghe R, *et al.* Clinical utility and applicability of biomarker-based diagnostic criteria for Alzheimer's disease: a BeDeCo survey. *Acta Neurol. Belg.* 115(4), 547–55 (2015).

2. Brunnström H, Englund E. Clinicopathological Concordance in Dementia Diagnostics. *Am. J. Geriatr. Psychiatry*. 17(8), 664–670 (2009).

3. Engelborghs S, De Vreese K, Van de Casteele T, *et al.* Diagnostic performance of a CSF-biomarker panel in autopsy-confirmed dementia. *Neurobiol. Aging*. 29(8), 1143–59 (2008).

4. Le Bastard N, Martin J-J, Vanmechelen E, Vanderstichele H, De Deyn PP, Engelborghs S. Added diagnostic value of CSF biomarkers in differential dementia diagnosis. *Neurobiol. Aging*. 31(11), 1867–76 (2010).

5. Niemantsverdriet E, Feyen BFE, Le Bastard N, *et al.* Overdiagnosing Vascular Dementia using Structural Brain Imaging for Dementia Work-Up. *J. Alzheimers. Dis.* 45(4), 1039–43 (2015).

6. Reiman EM, Quiroz YT, Fleisher AS, *et al.* Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. *Lancet Neurol.* 11(12), 1048–1056 (2012).

7. Sperling RA, Aisen PS, Beckett LA, *et al.* Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging and the Alzheimer's Association workgroup. *Alzheimer's Dement.* 7(3), 280–292 (2011).

8. Thal DR, Rüb U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology*. 58(12), 1791–800 (2002).

9. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U. S. A.* 83(13), 4913–4917 (1986).

10. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* , 239–259 (1991).

11. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 82(12), 4245–4249 (1985).

12. Burdick D, Soreghan B, Kwon M, *et al.* Assembly and aggregation properties of synthetic Alzheimer's A4/β amyloid peptide analogs. *J. Biol. Chem.* 267(1), 546–554 (1992).

13. Jarrett JT, Berger EP, Lansbury PT. The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: Implications for the pathogenesis of Alzheimer's disease. *Biochemistry*. 32(18), 4693–4697 (1993).

14. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol. Med.* 8(6), 595–608 (2016).

15. Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc. Natl. Acad. Sci. U. S. A.* 98(12), 6923–8 (2001).

16. Nelson PT, Kukull WA, Frosch MP. Thinking outside the box: Alzheimer-type neuropathology that does not map directly onto current consensus recommendations. *J. Neuropathol. Exp. Neurol.* 69(5), 449–454 (2010).

17. Dubois B, Feldman HH, Jacova C, *et al.* Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol.* 13(6), 614–29 (2014).

18. Olsson B, Lautner R, Andreasson U, *et al.* CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet. Neurol.* 15(7), 673–84 (2016).

19. Andreasson U, Portelius E, Andersson ME, Blennow K, Zetterberg H. Aspects of β-amyloid as a biomarker for Alzheimer's disease. *Biomark. Med.* 1(1), 59–78 (2007).

20. The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Consensus Report of the Working Group on: " Molecular and Biochemical Markers of Alzheimer's Disease ." *Neurobiol. Aging*. 19(2), 109–116 (1998).

21. Shibata M, Yamada S, Ram Kumar S, *et al.* Clearance of Alzheimer's amyloid-β1-40 peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J. Clin. Invest.* 106(12), 1489–1499 (2000).

22. Biere AL, Ostaszewski B, Stimson ER, Hyman BT, Maggio JE, Selkoe DJ. Amyloid beta-peptide is transported on lipoproteins and albumin in human plasma. *J. Biol. Chem.* 271(51), 32916–22 (1996).

23. Mehta PD, Pirttilä T, Mehta SP, Sersen E a, Aisen PS, Wisniewski HM. Plasma and cerebrospinal fluid levels of amyloid beta proteins 1-40 and 1-42 in Alzheimer disease. *Arch. Neurol.* 57(1), 100–105 (2000).

24. Le Bastard N, Aerts L, Leurs J, Blomme W, De Deyn PP, Engelborghs S. No correlation between time-linked plasma and CSF Aβ levels. *Neurochem. Int.* 55(8), 820–825 (2009).

25. Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology*. 60(4), 652–6 (2003).

26. Fagan AM, Mintun MA, Mach RH, *et al.* Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Aβ 42 in humans. *Ann. Neurol.* 59(3), 512–519 (2006).

27. Shaw LM, Vanderstichele H, Knapik-czajka M, *et al.* Cerebrospinal Fluid Biomarker Signature in Alzheimer's Disease Neuroimaging Initiative Subjects. *Pathology*. 65(4), 403–413 (2009).

28. Irwin DJ, McMillan CT, Toledo JB, *et al.* Comparison of Cerebrospinal Fluid Levels of Tau and Aβ 1-42 in Alzheimer Disease and Frontotemporal Degeneration Using 2 Analytical Platforms. *Arch. Neurol.* 69(8), 1018–1025 (2012).

29. Struyfs H, Niemantsverdriet E, Goossens J, *et al.* Cerebrospinal Fluid P-Tau181P: Biomarker for Improved Differential Dementia Diagnosis. *Front. Neurol.* 6(June), 1–8 (2015).

30. Mo J-A, Lim J-H, Sul A-R, Lee M, Youn YC, Kim H-J. Cerebrospinal fluid β-amyloid1-42 levels in the differential diagnosis of Alzheimer's disease--systematic review and meta-analysis. *PLoS One*. 10(2), e0116802 (2015).

31. Somers C, Struyfs H, Goossens J, *et al.* A Decade of Cerebrospinal Fluid Biomarkers for Alzheimer's Disease in Belgium. *J. Alzheimer's Dis.* 54(1), 383–395 (2016).

32. Bjerke M, Portelius E, Minthon L, *et al.* Confounding factors influencing amyloid Beta concentration in cerebrospinal fluid. *Int. J. Alzheimers. Dis.* 2010, 1–11 (2010).

33. Bjerke M, Andreasson U, Kuhlmann J, *et al.* Assessing the commutability of reference material formats for the harmonization of amyloid-β measurements. *Clin. Chem. Lab. Med.* 54(7), 1177–1191 (2016).

34. Kuhlmann J, Andreasson U, Pannee J, *et al.* CSF Aβ1-42 - an excellent but complicated Alzheimer's biomarker - a route to standardisation. *Clin. Chim. Acta.* [Epub ahea (2016).

35. Le Bastard N, De Deyn PP, Engelborghs S. Importance and Impact of Preanalytical Variables on

Alzheimer Disease Biomarker Concentrations in Cerebrospinal Fluid. *Clin. Chem.* 61(5), 734–743 (2015).

36. Mattsson N, Andreasson U, Persson S, *et al.* The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimer's Dement.* 7(4), 386–395 (2011).

37. Wallin A, Nordlund A, Jonsson M, *et al.* 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(1), 114–131 (2016).

38. Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O. Cerebrospinal fluid levels of β-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(1), 98–106 (2012).

39. Timmers M, Van Broeck B, Ramael S, *et al.* Profiling the dynamics of CSF and plasma Aβ reduction after treatment with JNJ-54861911, a potent oral BACE inhibitor. *Alzheimer's Dement. Transl. Res. Clin. Interv.* in press.

40. Bjerke M, Andreasson U, Rolstad S, *et al.* Subcortical vascular dementia biomarker pattern in mild cognitive impairment. *Dement. Geriatr. Cogn. Disord.* 28(4), 348–356 (2009).

41. Bjerke M, Zetterberg H, Edman Å, Blennow K, Wallin A, Andreasson U. Cerebrospinal fluid matrix metalloproteinases and tissue inhibitor of metalloproteinases in combination with subcortical and cortical biomarkers in vascular dementia and Alzheimer's disease. *J. Alzheimer's Dis.* 27(3), 665–676 (2011).

42. Kaerst L, Kuhlmann A, Wedekind D, Stoeck K, Lange P, Zerr I. Cerebrospinal fluid biomarkers in Alzheimer's disease, vascular dementia and ischemic stroke patients: a critical analysis. *J. Neurol.* 260(11), 2722–2727 (2013).

43. Tang W, Huang Q, Wang Y, Wang Z-Y, Yao Y-Y. Assessment of CSF Aβ42 as an aid to discriminating Alzheimer's disease from other dementias and mild cognitive impairment: a meta-analysis of 50 studies. *J. Neurol. Sci.* 345(1–2), 26–36 (2014).

44. Gómez-Tortosa E, Gonzalo I, Fanjul S, *et al.* Cerebrospinal Fluid Markers in Dementia With Lewy Bodies Compared With Alzheimer Disease. *Arch. Neurol.* 60(9), 1218–1222 (2003).

45. Slaets S, Theuns J, Sleegers K, *et al.* Amyloid pathology influences Aβ1-42 cerebrospinal fluid levels in dementia with lewy bodies. *J. Alzheimer's Dis.* 35(1), 137–146 (2013).

46. Mollenhauer B, Esselmann H, Roeber S, *et al.* Different CSF β-amyloid processing in Alzheimer's and Creutzfeldt-Jakob disease. *J. Neural Transm.* 118(5), 691–7 (2011).

47. Miyajima M, Nakajima M, Ogino I, Miyata H, Motoi Y, Arai H. Soluble amyloid precursor protein α in the cerebrospinal fluid as a diagnostic and prognostic biomarker for idiopathic normal pressure hydrocephalus. *Eur. J. Neurol.* 20(2), 236–242 (2013).

48. Jeppsson A, Höltta M, Zetterberg H, Blennow K, Wikkelsø C, Tullberg M. Amyloid mis-metabolism in idiopathic normal pressure hydrocephalus. *Fluids Barriers CNS*. 13(1), 13 (2016).

49. Mollenhauer B, Esselmann H, Trenkwalder C, *et al.* CSF amyloid-β peptides in neuropathologically diagnosed dementia with Lewy bodies and Alzheimer's disease. *J. Alzheimers. Dis.* 24(2), 383–91 (2011).

50. Sjögren M, Minthon L, Davidsson P, *et al.* CSF levels of tau, β-amyloid 1-42 and GAP-43 in frontotemporal dementia, other types of dementia and normal aging. *J. Neural Transm.* 107(5), 563–579 (2000).

51. Bibl M, Gallus M, Welge V, *et al.* Cerebrospinal fluid amyloid-β 2-42 is decreased in

Alzheimer's, but not in frontotemporal dementia. *J. Neural Transm.* 119(7), 805–13 (2012).

52. De Reuck J, Deramecourt V, Cordonnier C, *et al.* The incidence of post-mortem neurodegenerative and cerebrovascular pathology in mixed dementia. *J. Neurol. Sci.* 366, 164–166 (2016).

53. Toledo JB, Gopal P, Raible K, *et al.* Pathological α-synuclein distribution in subjects with coincident Alzheimer's and Lewy body pathology. *Acta Neuropathol.* 131(3), 393–409 (2016).

54. Shoji M, Matsubara E, Kanai M, *et al.* 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(2), 134–140 (1998).

55. Kanai M, Matsubara E, Isoe K, *et al.* 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(1), 17–26 (1998).

56. Janelidze S, Zetterberg H, Mattsson N, *et al.* CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: better diagnostic markers of Alzheimer disease. *Ann. Clin. Transl. Neurol.* 3(3), 154–65 (2016).

57. Pijnenburg YAL, Schoonenboom SNM, Mehta PD, *et al.* Decreased cerebrospinal fluid amyloid beta (1-40) levels in frontotemporal lobar degeneration. *J. Neurol. Neurosurg. Psychiatry*. 78(7), 735–737 (2007).

58. Bibl M, Mollenhauer B, Lewczuk P, *et al.* Validation of amyloid-beta peptides in CSF diagnosis of neurodegenerative dementias. *Mol. Psychiatry*. 12(7), 671–680 (2007).

59. Bibl M, Mollenhauer B, Lewczuk P, *et al.* Cerebrospinal fluid tau, p-tau 181 and amyloid-β38/40/42 in frontotemporal dementias and primary progressive aphasias. *Dement. Geriatr. Cogn. Disord.* 31(1), 37–44 (2011).

60. Gabelle A, Roche S, Gény C, *et al.* Decreased sAβPPβ, Aβ38, and Aβ40 cerebrospinal fluid levels in frontotemporal dementia. *J. Alzheimers. Dis.* 26(3), 553–563 (2011).

61. Oe T, Ackermann BL, Inoue K, *et al.* Quantitative analysis of amyloid beta peptides in cerebrospinal fluid of Alzheimer's disease patients by immunoaffinity purification and stable isotope dilution liquid chromatography/negative electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 20(24), 3723–35 (2006).

62. Pannee J, Portelius E, Oppermann M, *et al.* A selected reaction monitoring (SRM)-based method for absolute quantification of Aβ38, Aβ40, and Aβ42 in cerebrospinal fluid of Alzheimer's disease patients and healthy controls. *J. Alzheimers. Dis.* 33(4), 1021–32 (2013).

63. Mawuenyega KG, Kasten T, Sigurdson W, Bateman RJ. Amyloid-beta Isoform Metabolism Quantitation by Stable Isotope Labeled Kinetics. *Anal Biochem*. 440(1), 56–62 (2013).

64. Korecka M, Waligorska T, Figurski M, *et al.* Qualification of a surrogate matrix-based absolute quantification method for amyloid-β_{42} in human cerebrospinal fluid using 2D UPLC-tandem mass spectrometry. *J. Alzheimers. Dis.* 41(2), 441–51 (2014).

65. Pannee J, Gobom J, Shaw LM, *et al.* Round robin test on quantification of amyloid-β 1-42 in cerebrospinal fluid by mass spectrometry. *Alzheimers. Dement.* 12(1), 55–9 (2016).

66. Vigo-Pelfrey C, Lee D, Keim P, Lieberburg I, Schenk DB. Characterization of beta-amyloid peptide from human cerebrospinal fluid. *J. Neurochem.* 61(5), 1965–8 (1993).

67. Wang R, Sweeney D, Gandy SE, Sisodia SS. The profile of soluble amyloid beta protein in cultured cell media. Detection and quantification of amyloid beta protein and variants by immunoprecipitation-mass spectrometry. *J. Biol. Chem.* 271(50), 31894–902 (1996).

68. Portelius E, Zetterberg H, Andreasson U, *et al.* An Alzheimer's disease-specific beta-amyloid fragment signature in cerebrospinal fluid. *Neurosci. Lett.* 409(3), 215–9 (2006).

69. Nutu M, Bourgeois P, Zetterberg H, *et al.* Aβ1-15/16 as a Potential Diagnostic Marker in Neurodegenerative Diseases. *NeuroMolecular Med.* 15(1), 169–179 (2013).

70. Portelius E, Andreasson U, Ringman JM, *et al.* Distinct cerebrospinal fluid amyloid beta peptide signatures in sporadic and PSEN1 A431E-associated familial Alzheimer's disease. *Mol. Neurodegener.* 5(1), 2 (2010).

71. Hölttä M, Minthon L, Hansson O, *et al.* An integrated workflow for multiplex CSF proteomics and peptidomics-identification of candidate cerebrospinal fluid biomarkers of Alzheimer's disease. *J. Proteome Res.* 14(2), 654–63 (2015).

72. Fukumoto H, Tokuda T, Kasai T, *et al.* High-molecular-weight beta-amyloid oligomers are elevated in cerebrospinal fluid of Alzheimer patients. *FASEB J.* 24(8), 2716–2726 (2010).

73. Hölttä M, Hansson O, Andreasson U, *et al.* Evaluating amyloid-β oligomers in cerebrospinal fluid as a biomarker for Alzheimer's disease. *PLoS One*. 8(6), e66381 (2013).

74. Jongbloed W, Bruggink KA, Kester MI, *et al.* Amyloid-β oligomers relate to cognitive decline in Alzheimer's disease. *J. Alzheimers. Dis.* 45(1), 35–43 (2015).

75. Lewczuk P, Kamrowski-Kruck H, Peters O, *et al.* Soluble amyloid precursor proteins in the cerebrospinal fluid as novel potential biomarkers of Alzheimer's disease: a multicenter study. *Mol. Psychiatry*. 15(2), 138–145 (2010).

76. Alcolea D, Carmona-Iragui M, Suárez-Calvet M, *et al.* Relationship between γ-Secretase, Inflammation and Core Cerebrospinal Fluid Biomarkers for Alzheimer's Disease. *J. Alzheimer's Dis.* 42(1), 157–167 (2014).

77. Mulugeta E, Londos E, Hansson O, *et al.* Cerebrospinal Fluid Levels of sAPPα and sAPPβ in Lewy Body and Alzheimer's Disease: Clinical and Neurochemical Correlates. *Int. J. Alzheimers. Dis.* 2011, 495025 (2011).

78. Bjerke M, Jonsson M, Nordlund A, *et al.* Cerebrovascular Biomarker Profile Is Related to White Matter Disease and Ventricular Dilation in a LADIS Substudy. *Dement. Geriatr. Cogn. Dis. Extra*. 4(3), 385–94 (2014).

79. Selnes P, Blennow K, Zetterberg H, *et al.* Effects of cerebrovascular disease on amyloid precursor protein metabolites in cerebrospinal fluid. *Cerebrospinal Fluid Res.* 7, 10 (2010).

80. Jeppsson A, Zetterberg H, Blennow K, Wikkelsø C. Idiopathic normal-pressure hydrocephalus: pathophysiology and diagnosis by CSF biomarkers. *Neurology*. 80(15), 1385–92 (2013).

81. Pyykkö OT, Lumela M, Rummukainen J, *et al.* Cerebrospinal fluid biomarker and brain biopsy findings in idiopathic normal pressure hydrocephalus. *PLoS One*. 9(3) (2014).

82. Leuzy A, Chiotis K, Hasselbalch SG, *et al.* Pittsburgh compound B imaging and cerebrospinal fluid amyloid-β in a multicentre European memory clinic study. *Brain*. , aww160 (2016).

83. Lewczuk P, Matzen A, Blennow K, *et al.* Cerebrospinal Fluid Aβ42/40 Corresponds Better than Aβ42 to Amyloid PET in Alzheimer's Disease. *J. Alzheimers. Dis.* [Epub ahea (2016).

84. Nutu M, Zetterberg H, Londos E, *et al.* Evaluation of the Cerebrospinal Fluid Amyloid β1-42/Amyloid-β1-40 Ratio Measured by Alpha-LISA to Distinguish Alzheimer's Disease from Other Dementia Disorders. *Dement. Geriatr. Cogn. Disord.* 36(1–2), 99–110 (2013).

85. Spies PE, Slats D, Sjögren JMC, *et al.* The cerebrospinal fluid amyloid beta42/40 ratio in the differentiation of Alzheimer's disease from non-Alzheimer's dementia. *Curr. Alzheimer Res.* 7(5), 470–476 (2010).

86. Parnetti L, Chiasserini D, Eusebi P, *et al.* Performance of Aβ1-40, Aβ1-42, total tau, and phosphorylated tau as predictors of dementia in a cohort of patients with mild cognitive

impairment. *J. Alzheimer's Dis.* 29(1), 229–238 (2012).

87. Hansson O, Zetterberg H, Buchhave P, *et al.* Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. *Dement. Geriatr. Cogn. Disord.* 23(5), 316–20 (2007).

88. Struyfs H, Van Broeck B, Timmers M, *et al.* Diagnostic Accuracy of Cerebrospinal Fluid Amyloid-β Isoforms for Early and Differential Dementia Diagnosis. *J. Alzheimers. Dis.* 45(3), 813–22 (2015).

89. Slaets S, Le Bastard N, Martin J-J, *et al.* Cerebrospinal fluid Aβ1-40 improves differential dementia diagnosis in patients with intermediate P-tau181P levels. *J. Alzheimers. Dis.* 36(4), 759–767 (2013).

90. Dumurgier J, Schraen S, Gabelle A, *et al.* Cerebrospinal fluid amyloid-β 42/40 ratio in clinical setting of memory centers: a multicentric study. *Alzheimers. Res. Ther.* 7(1), 30 (2015).

91. Bibl M, Mollenhauer B, Esselmann H, *et al.* CSF amyloid-beta-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia. *Brain*. 129(Pt 5), 1177–87 (2006).

92. Bibl M, Gallus M, Welge V, Esselmann H, Wiltfang J. Aminoterminally truncated and oxidized amyloid-β peptides in the cerebrospinal fluid of alzheimer's disease patients. *J. Alzheimer's Dis.* 29(4), 809–816 (2012).

93. Mulugeta E, Londos E, Ballard C, *et al.* CSF amyloid β38 as a novel diagnostic marker for dementia with Lewy bodies. *J. Neurol. Neurosurg. Psychiatry*. 82(2), 160–4 (2011).

- Financial disclosure/acknowledgements

This research was funded in part by the University of Antwerp Research Fund; unrestrictive research grants from Janssen Pharmaceutica NV and ADx Neurosciences; the Alzheimer Research Foundation (SAO-FRA, http://alzh.org); the Institute Born-Bunge; the Belgian Science Policy Office Interuniversity Attraction Poles (IAP) program (BELSPO, www.belspo.be); the Flemish Government initiated Methusalem excellence grant (EWI, www.ewi-vlaanderen.be); the Flanders Impulse Program on Networks for Dementia Research (VIND); the Agency for Innovation by Science and Technology (IWT, www.iwt.be); and the Research Foundation Flanders (FWO, www.fwo.be).