Journal of Alzheimer's Disease 53 (2016) 53–67
DOI 10.3233/JAD-151029
IOS Press

Review

Methods for the Specific Detection and Quantitation of Amyloid-β Oligomers in Cerebrospinal Fluid

Judith Schuster and Susanne Aileen Funke*
Bioanalytik, Hochschule für angewandte Wissenschaften Coburg, Coburg, Germany

Handling Associate Editor: Charlotte Teunissen

Accepted 28 March 2016

Abstract. Protein misfolding and aggregation are fundamental features of the majority of neurodegenerative diseases, like Alzheimer's disease (AD), Parkinson's disease, frontotemporal dementia, and prion diseases. Proteinaceous deposits in the brain of the patient, e.g., amyloid plaques consisting of the amyloid-β (Aβ) peptide and tangles composed of tau protein, are the hallmarks of AD. Soluble oligomers of Aβ and tau play a fundamental role in disease progression, and specific detection and quantification of the respective oligomeric proteins in cerebrospinal fluid may provide presymptomatically detectable biomarkers, paving the way for early diagnosis or even prognosis. Several studies on the development of techniques for the specific detection of Aβ oligomers were published, but some of the existing tools do not yet seem to be satisfactory, and the study results are contradicting. The detection of oligomers is challenging due to their polymorphous and unstable nature, their low concentration, and the presence of competing proteins and Aβ monomers in body fluids. Here, we present an overview of the current state of the development of methods for Aβ oligomer specific detection and quantitation. The methods are divided in the three subgroups: (i) enzyme linked immunosorbent assays (ELISA), (ii) methods for single oligomer detection, and (iii) others, which are mainly biosensor based methods.

Keywords: Alzheimer's disease, amyloid-β oligomers, biomarkers, diagnosis, protein misfolding diseases, therapy monitoring

INTRODUCTION

Alzheimer's disease (AD) is the most common cause for dementia and a major global cause of disability and dependency, raising significant personal and economic issues. In 2013, an estimated 44.4 million people worldwide suffered from dementia, and this number is expected to increase to 135.5 million in 2050 (http://www.alz.co.uk/research).

*Correspondence to: Susanne Aileen Funke, Hochschule für angewandte Wissenschaften Coburg, Friedrich-Streib-Str. 2, 96450 Coburg, Germany. Tel.: +49 9561 317 327; Fax: +49 9561 317 311; E-mail: aileen.funke@hs-coburg.de.

Still, definite diagnosis of AD requires postmortem examination of the brain, where amyloid plaques, consisting of the amyloid-β (Aβ) peptide, and tangles, composed of tau protein, are detected. Diagnosis of probable AD during lifetime is based on clinical criteria, including medical history, physical examination, neuropsychological tests, and is supported by ancillary examinations like *in vivo* imaging methods and laboratory tests, reaching a confidence level of approximately 80% [1].

Early symptoms of the disease are shared by a variety of dementia disorders and the specificity of early diagnosis is less accurate; furthermore, diagnosis based on clinical symptoms will never reveal

presymptomatic AD cases. Initial studies on familial AD as well as presymptomatic sporadic AD patients indicated that humans who will develop AD have detectable pathological abnormalities two decades or more before the first clinical symptoms can be perceived [2–5].

At present, the disease can only be treated symptomatically, but we move in the direction of disease modification and secondary prevention. Therefore, objective measures of core pathologic processes, which reflect the status of AD, are essential for early and definite diagnosis, prognosis, or for the monitoring of drug effects. A biomarker is defined as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention" [6].

One biomarker probably suited to fulfill the requirements is the Aβ peptide, as it is one of the earliest and most prominent players in AD, accumulating as fibers in amyloid plaques. Aβ is generated from the amyloid-β protein precursor (AβPP) by subsequent enzymatic cleavage of the β- and γ-secretase, respectively [7–9]. Several Aβ isoforms of various chain length are formed, but $A\beta_{42}$ has the highest propensity for aggregation and undergoes formation of oligomers and larger intermediate forms like protofibrils, insoluble fibrils, and plaques. A range of conformational subtypes, varying in number of monomers, secondary and tertiary structure, shape, size, and compactness has already been described *in vitro* and *in vivo* [10, 11].

It was suggested in the amyloid cascade hypothesis that fibrillar forms of Aβ, deposited in amyloid plaques, were responsible for neuronal dysfunction [12]. Today, soluble and diffusible Aβ oligomers are known to be the main synaptotoxic and pro-apoptotic species, inducing learning deficits and neuronal loss as well as inhibition of long-term potentiation [13–16].

Across a large number of studies, $A\beta_{42}$ monomer concentrations in cerebrospinal fluid (CSF) samples, determined by ELISA or similar methods, are consistently and significantly reduced by 50% in patients with AD compared to healthy controls. Sensitivity and specificity exceeded 80% in most of the studies, making CSF $A\beta_{42}$ one of the best established biochemical markers. It is shown that patients with mild cognitive impairment (MCI) and later conversion to AD have reduced CSF $A\beta_{42}$ levels as well. These reduced CSF $A\beta_{42}$ levels allowed an accurate prediction of development of AD in tested MCI subjects (sensitivity of 90%, specificity of 71%) [3, 17–20]. According to Jack and coworkers, the first biomarker change detectable with state-to-the-art methods is the decrease of CSF $A\beta_{42,}$ which might reflect the formation of Aβ plaques, as suggested by the "sink hypothesis" [21]. Neuritic plaque formation can be visualized using PIB-PET [22].

Recent biomarker studies often include measurements of other core biomarkers of AD other than $A\beta_{42}$ ($A\beta_{40}$, tau, phospho-tau), and the combination of results increased the current average sensitivity of AD biomarker detection slightly [17, 20]. However, these assays did not significantly improve the predictive value over present clinical tests and therefore, other biomarkers are needed to monitor fundamental disease features. Additionally, even if CSF $A\beta_{42}$ monomer is the best established neurochemical biomarker for AD, the usefulness in differential diagnosis is not well established, as decreased CSF $A\beta_{42}$ concentrations are also found, for example, in patients with Lewy body dementia, amyotrophic lateral sclerosis, and Creutzfeld-Jacob disease [23–25].

Aβ monomers are a physiological metabolic product, also abundant in healthy humans and a natural component of serum and plasma. They might even have a neuroprotective function and are not thought to be responsible for pathologic effects in AD [26]. As already described above, soluble Aβ oligomers were found to be fundamental for AD pathology, and in several preliminary studies, it was demonstrated that the concentration of Aβ oligomers is increased in AD patients. However, a few conflicting studies were published (reviewed in [27]). To verify whether Aβ oligomers are an appropriate biomarker for AD, a representatively large number of AD patient samples and controls have to be tested for their Aβ oligomer content. The detection of Aβ oligomers is technically challenging due to their polymorphous and metastable nature, their low concentration, the presence of competing proteins, and the respective protein monomers in body fluids.

Here, we review the current state of Aβ oligomer detection and focus on method development to reliably and routinely detect Aβ oligomers in CSF. Methods, which were developed and tested on brain extracts, but not applied to human CSF yet, as well as methods for the detection of Aβ oligomers in blood samples, are not discussed here. We have published reviews on the topic of oligomer detection earlier, but growing interest in oligomer detection has led to an substantial increase in the number of publications, evoking the need for an update [27–29].

METHODS FOR DETECTION OF Aβ OLIGOMERS IN CSF: ELISA

In recent years, a variety of ELISA-based tools were published. All studies reviewed in this chapter are summarized in Table 1. ELISA studies are comparably simple to perform, are well suited for high-throughput, and offer adequate sensitivity. In the ELISA-based techniques described up to date, Aβ oligomer specificity was provided either by usage of oligomer specific antibodies or by double usage of the same sequence specific antibody twice in the system, for capturing Aβ as well as for detection. This ensures that no monomers (here, the binding epitope would already be blocked by the capture antibody) are detected.

In 2010, Fukumoto et al. reported on one of the first ELISAs, specific for high molecular weight (HMW) Aβ oligomers between 40 to 200 kDa. Monomers and lower molecular weight oligomer species were not detected, the latter probably not because of steric hindrance. The antibody BAN50 binds to a N-terminal sequential epitope of Aβ and was used for Aβ capturing, as well as for detection. The sensitivity of the assay was estimated by the authors to be at 0.1 pM (or 0.45 pg/ml). The authors detected significantly higher amounts of HMW Aβ oligomers in CSF samples from AD and MCI patients (at the mean level) compared to age matched controls. Additionally, the authors could demonstrate a negative correlation with Mini-Mental-State Examination (MMSE) scores in the AD/MCI group [30].

Later, Herskovits and coworkers adapted the ELISA described above to a Luminex platform. Luminex platforms have the advantages of simultaneous analyses of several biomarkers in one run, reduction of human error, potential of reduced reagent costs, and a modest reduction of sample volume when compared to classical ELISA [31]. The authors found that the BAN50 antibody based assay detected Aβ oligomers, but demonstrated also sAβPPα fragment sensitivity, probably due to the N-terminal epitope of BAN50. The authors tested CSF samples of 20 AD patients and found elevated Aβ oligomer levels in comparison to the 19 CSF control samples (with substantial overlap for single samples). The respective biomarker profiles for $A\beta_{42}$ monomers (tau and phospho-tau, as determined by the Alzbio3 assay) were consistent with prior studies for the AD group. A correlation between MMSE and Aβ oligomer content was less clear. However, the ratio of Aβ oligomers and $A\beta_{42}$ monomers was elevated. The ratio corresponded to a sensitivity of 90% and a specificity of 94.7%, but the values were mainly driven by $A\beta_{42}$, leading the authors to question the added value of the oligomer measurement, as judged by the diagnostic standpoint [32].

In 2010, Gao and coworkers used peptides specifically binding Aβ aggregates as a bait to capture Aβ aggregates in CSF to perform the so-called "misfolded protein assay" (MPA). After binding to beads, the immobilized Aβ was denatured and the concentration determined by ELISA specific for $A\beta_{40}$ and $A\beta_{42}$ monomers, respectively. In CSF samples of 26 AD patients an elevated concentration of $A\beta_{40}$ aggregates could be detected in comparison to the samples of 10 controls. Interestingly, similar results were not found for $A\beta_{42}$ aggregates [33].

In 2013, Tai et al. planned to address the hypothesis that reduced levels of soluble apolipoprotein E (apoE)/Aβ complexes and an increase of soluble oligomeric Aβ are associated with APOE4 and AD. They developed two ELISA to measure soluble apoE/Aβ and oligomeric Aβ (modified protocol by Xia et al. [34]). Here, we only focus on the results of soluble oligomeric Aβ. About ten CSF samples each, obtained at autopsy, were tested for the control AD-APOE3, and the AD-APOE4 group, respectively. Aβ oligomer levels were significantly increased in AD patients compared to controls, and Aβ oligomer levels were increased in the AD-APOE4 group compared to the AD-APOE3 group, however, with a great overlap between single results of the different groups [35].

Höltta et al. evaluated Aβ oligomers in the CSF by investigation of four different patient material collections from different clinical centers (199 AD patients, 148 cognitively healthy controls, 165 followed-up MCI patients). They developed a highly sensitive ELISA with a lower limit of quantification of 2 pg/ml (using synthetic Aβ dimers for the standard curve), which detected synthetic Aβ oligomers with a molecular weight above 10 kDa using the N-terminal monoclonal antibody 82E1 for capture and detection. The influence of heterophilic antibodies was evaluated and excluded. Aβ oligomer levels were increased in three AD/control comparisons, but with a substantial overlap between the single patients of different groups. No correlation between Aβ oligomers and $A\beta_{42}$ was found. MCI patients who later converted to AD had increased levels of Aβ oligomers compared to controls, while patients with stable MCI did not differ. Interestingly, patients with different severity of dementia, based on MMSE examination results, were tested. It was found that AD patients with mild

Table 1
Summary of methods for single oligomer detection: ELISA

Oligomer selection method	Oligomer species detected	Oligomer results	Detection limit	Standard preparation	Read-out	Assay validation	Pre-analytical sample handling	Ref.
N-terminal binding BAN50 for capture and detection	HMW oligomers, 40 to 200 kDa	Elevated in 18 AD and 7 MCI samples versus 25 controls, inverse correlation to MMSE	0.1 pM or 0.45 pg/ml*, monomer equivalent	ADDLs, characterized by SEC and PAGE	a.u.	Dilution linearity, intra- and interassay variability <9%, cross reactivity of sAβPPα	LP am, samples immediately frozen at –80°C	[30]
N-terminal binding BAN50 for capture and detection	n.d.	Elevated levels in 20 AD patients versus 19 controls, correlation to MMSE less clear	n.d.	ADDLs, additional separation by SEC	a.u.	Dilution linearity, cross reactivity with aggregated AβPP proteolytic fragments	LP am, overnight fasting, CSF transferred into PP tubes, storage at –70°C within 15 min	[32]
Aggregate specific capture, dissolution, determination of monomer concentration	n.d.	Higher concentration of $Aβ_{40}$ aggregates in 26 AD samples versus 10 controls	5 pg/ml	Globular oligomers [70]. HFIP pre-treated Aβ dissolved in PBS and SDS, centrifuged before dialysis	a.u.	Dilution linearity	CSF collected into PP tubes, centrifugation, storage at –80°C	[33]
N-terminal binding MOAB-2 for capture and detection	Aβ oligomers	Levels increased in 10 AD-APOE4 patients versus 10 AD-APOE3 patients versus 10 controls	n.d.	ADDLs	ng/ml,	Dilution linearity	Samples obtained at autopsy	[35]
N-terminal binding 82E1 for capture and detection	Aβ oligomers with molecular weight above 10 kDa	Levels increased in AD patients and MCI-AD converter group, no correlation to $Aβ_{42}$, MMSE correlation less clear	200 fg/ml or 0.2 pg/ml	Synthetic Aβ dimers, $Aβ_{1–11}$ with added C-terminal cysteine	fg/ml	Dilution linearity, intra- and interassay variation (7 and <20%, respectively), cross-reactivity with heterophilic antibodies	SOPs, LP performed in standard interspace, collection in PP tubes, centrifugation, mixing, aliquotation, storage at –80°C	[36]
Oligomer specific antibody as capture, sequence specific antibody for detection; for both: 3D6/3D6B, NAB61/ 3D6B	Aβ dimers to HMW oligomers	No oligomers detected in 89 AD CSF samples	6–12 pg/ml	Synthetic Aβ dimers $(Aβ\ S26C)_2$, purified by SEC	pg/ml	Dilution linearity, reproduceability, linearity, cross-reactivity sAβPPα and β, spiking recovery	SOPs, precooled PP tubes, mixing, centrifugation, aliquotation, storage at –80°C	[37]
N-terminal binding HJ3.4 for capture and detection	Aβ oligomers	No oligomers detected in 20 CSF samples of mild AD	1.56 pg/ml; lower limit of quantification: 6.25 pg/ml	Synthetic Aβ dimers ($Aβ_{40}$S26C), purified by SEC	Dimer equivalents	Dilution linearity, intra- and interassay variability, precision, spiking recovery	SOPs, LP performed am, overnight fasting, centrifugation, aliquotation into EDTA containing PP tubes, stored at –84°C	[38]

N-terminal antibody Nab228 for capture and detection	Different oligomers, 10–25 kDa (HMW)-oligomers generate highest signals	No difference of oligomer concentration in AD versus control CSF	2.2 ng/L, monomer equivalent	Synthetic oligomers, Aβ after HFIP treatment and DMSO dissolution dissolved in phosphate buffered saline	ng/mg total protein	Influence of heterophilic antibodies, dilution linearity, inter- and intraassay coefficients <18%	SOPs, collection in PP tubes, centrifugation, storage at –80°C	[39]
Oligomer specific antibody 19.3 as capture, sequence specific 82E1 for detection		Increase in 63 AD CSF samples versus 54 controls, inverse correlation to MMSE	0.09 pg/ml, limit of reliable quantification 0.18 pg/ml	ADDLs, Standard reproduceability and stability was assayed	pg/ml	Dilution linearity, spike recovery, intra- and interassay precision at mean level ≤17%	CSF stored in 1 ml aliquots at –70°C, addition of Tween 20 to the samples after thawing before testing to avoid PP binding	[41]
Capture: 1C22 (aggregate specific) Detection: 3D6B (N-terminal epitope)	40 to 600 kDa, preferentially large oligomers	37 control, 20 AD, 31 MCI samples, no correlation	0.15 pg/ml (ADDL standard); 40.6 pg/ml ($A\beta_{40}$S26-dimer standard); 375 pg/ml (DiY standard)	ADDLs, $A\beta_{40}$S26-dimer and DiY were prepared as described before [71]	pg/ml	Dilution linearity, spike recovery, intra- and interassay precision at mean level ≤11%	SOPs, LP performed in standard interspace, collection in PP tubes, centrifugation, aliquotation, aliquoted, stored at –80°C, slightly different procedures for the cohorts	[42]
VU-17 (N-terminal epitope) for capture and detection	Middle range oligomers	24 controls, 61 MCI, 64 AD, no difference between groups, decrease of oligomer levels over time in AD and MCI	0.9 a.u.; limit of quantification 0.11 a.u.; here one a.u. corresponds to 1 ng/ml total Aβ present in the Aβ oligomer enriched fraction	ADDLs centrifugation, photo induced crosslinking to stabilize ADDLs	a.u.	Spike recovery, dilution linearity	SOPs, LP from standard interspace, collected in PP tubes, centrifugation, aliquotation, storage at –80°C	[43]

Definitions of different Aβ multimer species: Aβ oligomers, non fibrillar, soluble low-molecular weight oligomers. Designations describing certain Aβ species were taken from the reviewed methods; ADDLS, Aβ derived diffusible ligands: small soluble Aβ oligomers with molecular weights between 17 and 42 kDa. Synthetic Aβ after HFIP dilution and evaporation, DMSO dissolution and dilution in F12 medium [40]; a.u., arbitrary units; DiY, stable $A\beta_{40}$ dimer, formed by covalent coupling of the two tyrosine-10 residues; DMSO, dimethyl sulfoxide; HFIP, 1,1,1,3,3,3-Hexafluoro-2-propanol; HMW, high molecular weight; LP, lumbar puncture; n.d., not determined; MMSE, Mini-Mental State Examination; PMCA, protein misfolding cyclic amplification assay; PP, polypropylene; SEC, size exclusion chromatography; standard interspace, L3-L4 or L4-L5 interspace. *calculated by the authors for easier comparison.

and moderate dementia had higher Aβ oligomer levels compared to controls, while patients with severe dementia did not [36].

However, some ELISA studies were published in which Aβ oligomers either could not be detected in CSF or no difference was found in oligomer concentration of AD patient samples and control groups. One assay, published by the group of Selkoe, employed a highly sensitive electroluminescence system [37]. This was either based on an Aβ aggregate specific capturing antibody combined with an antibody for detection binding to an N-terminal epitope of Aβ, or the Aβ specific antibody was used for both. The system detected sensitively synthetic Aβ oligomers (with detection limits of approximately 10 pg/ml) and Aβ oligomers in extracts of AD brains, but no oligomers could be detected in 89 human CSF samples [37].

Esparza et al. developed a plate-based immunoassay for Aβ oligomers. Measurements of eluted antibody conjugates were performed on the Erenna platform. In typical applications, the Erenna platform uses spot illumination and single photon counting to detect paramagnetic microparticle based antibody fluorescence emission events. The system allows a 20- to 100-fold increase in sensitivity compared to classical detection systems. Microparticle/capture antibody/antigen/fluorescent detection antibody complexes are passed through a capillary, where a laser induces light emission, which is detected after passing through a confocal microscope lens. In the assay described by Esparza et al., no microparticles were used, but after a conventional ELISA, the antibody-antigen-antibody sandwich was eluted and the fluorescent-labeled antibody was counted on the Erenna system. The same N-terminal antibody HJ3.4 was used as capture and for detection, achieving a limit of detection of 1.56 pg/ml, determined using $A\beta_{1-40}$Ser26Cys dimer as standard. The specificity for Aβ oligomers was demonstrated, the assay did not detect oligomers formed by other peptides. Additionally, truncated and modified Aβ oligomers were not detected. Aβ oligomers could be detected in brain tissue samples, but not in 20 CSF samples of patients with mild AD [38].

Bruggink and coworkers developed an oligomer specific ELISA which used the same N-terminal epitope binding antibody as capture and for detection. The assay predominantly recognized relatively small Aβ oligomers between 10 and 25 kDa with a calculated limit of detection of 2.2 ng/l or 2.2 pg/ml. For measurements in human samples, depletion of human anti-mouse antibodies (HAMAs) was necessary. Aβ oligomers could be detected, but no difference in concentration was detected between each 25 samples of AD patients and controls, whereas a significantly higher concentration of Aβ monomers was found in hippocampal extracts of AD patients compared to controls. The authors hypothesized that the levels of small oligomers might not be suitable as a biomarker [39].

Savage et al. also employed the Erenna platform already used by Esparza et al. (see above) for specific detection of Aβ in CSF, but a different antibody combination was used. Antibody 19.3 with selectivity for Aβ oligomers was used as capture, while antibody 82E1, specific for an N-terminal epitope of Aβ, was used for detection. The authors demonstrated a limit of detection (synthetic ADDL standard, ADDL: "Aβ derived diffusible ligands", in the original paper defined as small soluble Aβ oligomers with molecular weights between 17 and 42 kDa [40] in CSF samples) of 0.09 pg/ml, 2500-fold selectivity for Aβ oligomers over $A\beta_{40}$ monomers and adequate inter- and intra-assay precision, spike recovery and dilution linearity. In this study, AD patients could be distinguished from controls in three commercially available cohorts (in total 63 AD and 54 controls). AD CSF samples contained significantly higher monomer and lower $A\beta_{42}$ concentrations as controls and other diagnostic groups. Aβ oligomer levels did not strongly correlate with age and gender, but had an inverse correlation with MMSE score [41].

In 2015 Yang et al. tried to confirm and extend the study by Savage et al. Novel antibody pairs were tested for usage in combination with the Erenna platform and a combination of 1C22 (aggregate specific, also detects fibrils and plaques) for capture and 3D6B (N-terminal specific) for detection yielded a quantification limit of 0.15 pg/ml, similar to the one reported by Savage et al. [42]. The assay detected oligomers of sizes spanning 40 kDa to 600 kDa, preferentially large versus small Aβ oligomers, as tested with three different standards: ADDLs versus S26C-dimer versus DiY, an $A\beta_{40}$ dimer formed by covalent coupling of the two tyrosine-10 residues. DiY does not further aggregate under the conditions that were used, whereas the S26C-dimer does. The authors tested CSF samples of three cohorts (together 20 AD, 37 controls, 33 MCI samples) and found detectable Aβ oligomer values, but AD and controls did overlap. MCI had modest elevation by mean value. The established biomarkers for AD ($A\beta_{42}$,

tau, and phospho-tau) distinguished control and AD patients better [42].

In 2015, a paper reported on a longitudinal study of CSF Aβ oligomer levels. CSF of 24 non-demented, 61 MCI, and 64 AD patients was tested as baseline and after follow up (on average 45 months, on minimum 6 months) using an ELISA based on VU-17 antibody with N-terminal epitope for capture and detection. The ELISA detected middle range oligomers. The oligomer concentrations did not differ between groups, but MCI and AD patients showed a decrease of oligomer levels, strongly associated with severe cognitive decline [43].

METHODS FOR DETECTION OF SINGLE Aβ OLIGOMERS IN CSF

ELISA methods, as described above, offer adequate sensitivity for detection of Aβ oligomers, and quantification is possible using standard Aβ oligomer preparations. Nevertheless, only one signal for all oligomers in the sample is provided and the oligomers cannot be characterized in more detail (aggregate size distribution, real number). In recent years, some methods were described which claim single molecule sensitivity. These methods are based on fluorescence correlation spectroscopy (FCS), flow cytometry, and confocal laser scanning microscopy (LSM). In theory, these methods should be even more sensitive than the methods described in the previous chapter. All methods reviewed in this chapter are summarized in Table 2.

In 1998, Pitschke et al. came up with the first method for single Aβ aggregate detection. Labeled Aβ monomers were added to pre-existing Aβ oligomers in the CSF sample. Based on the principle of seeded polymerization, the labeled monomers rapidly bound to the pre-existing seeds, while under the given conditions monomer aggregation was delayed in time. Detection of the labeled Aβ oligomers was based on FCS, a method in which particles can be detected in concentrations of less than 100 nM, due to confocal laser optics and extremely sensitive detectors. The method was originally developed for high-resolution spatial and temporal analysis of extremely low concentrated biomolecules. The assay was linear between 20 ng and 1000 ng Aβ aggregates (prepared from synthetic Aβ, solubilized in DMSO and diluted and incubated in phosphate buffer). CSF samples of 19 non-AD controls resulted in a relatively low and fluctuating fluorescence signal, whereby fluctuations represented the number of fluorophores in the confocal volume. In contrast samples from 15 AD patients produced fluctuating signals with high-intensity fluorescence bursts, probably due to the addition of fluorescence labeled Aβ monomers to seeds in the sample [44].

In 2007, Henkel et al. used a related method with improved sensitivity combined with molecular imaging to investigate CSF samples of AD patients ($n = 14$), patients with other dementias ($n = 13$), and non-demented controls ($n = 6$) for large Aβ-peptide binding particles (LAPs) and their diagnostic potential. In contrast to the study described above, large individual differences could be detected between the patients, but no significant differences in LAP frequency were detected between the groups. The LAPs could be classified into four subtypes depending on their area, brightness, shape, and texture. So called LAP-4 particles consisted of Aβ and IgGs and could not be detected in any of the AD patients, but they were virtually present in 42% of all non-AD cases, pointing at the role of the immune system in regulating Aβ homeostasis [45].

Santos et al. reported on an approach for the detection of single ADDLs in CSF by combining fluorescence resonance energy transfer (FRET) and flow cytometry. The ADDLs in the CSF sample were labeled by two Aβ antibodies (4G8 and 6E10, 6E10 with N-terminal sequence specific epitope, 4G8 binds a central region of Aβ (amino acids 17 to 24)), coupled to Alexa-Fluor 488 and Alexa-Fluor 594, respectively. Binding of both antibodies to one ADDL brought the fluorescence dyes in close spatial distance (less than 10 nm), and excitation of Alexa 488 and subsequent emission of fluorescent light led to excitation of the other dye, enabling the detection of FRET events by a fluorescence-activated cell sorter (FACS). Monomers were not detected, as binding of one antibody hinders the other one from binding the same monomer. The assay was linear over a range of 10 pM to 2.5 nM (or 45.14 pg/ml to 11.28 ng/ml), referring to the Aβ monomer concentration of ADDL preparations. For the beginning, 174 CSF samples of non-demented individuals were analyzed, revealing a large variation in the concentration of the detected oligomers and a weak correlation between the age of the individuals and the ADDL concentration [46].

In 2007, Funke et al. reported on a method, denominated sFIDA (surface fluorescence intensity distribution analysis), for the detection of Aβ aggregates in CSF samples, based on FCS [47, 48]. The aggregates were immobilized on the surface of

Table 2
Summary of methods for single Aβ oligomer detection

Method	Oligomer selection method	Oligomer species detected	Oligomer results	Detection limit	Standard preparation	Read-out	Assay validation	Pre-analytical sample handling	Ref.
FCS	Principle of seeded polymerization in combination with FCS	Aβ multimers	Fluorescence bursts in 15 AD samples versus 19 controls	20 ng/20μl sample volume	Dilution of Aβ in DMSO and phosphate buffer, incubation for 16 h	Number of fluorescence bursts	Dilution linearity tested, data not shown	Samples frozen within two hours after LP, stored at –70°C	[44]
FCS and LSM	Principle of seeded polymerization in combination with FCS and LSM	Aβ binding particles (LAPs)	No correlation found in 14 AD patient CSF samples and controls	–	–	Lap frequency	–	Samples centrifuged and immediately frozen at –80°C	[45]
FRET	Dual color FRET detection with flow cytometry in solution, 4G8 and 6E10 sequence specific antibodies	ADDLs and other oligomers	Aβ oligomers in CSF of 174 controls	fM range, calculation based on monomers used for oligomer formation	ADDLs	FRET events	Dilution linearity	Samples frozen within two hours after LP, stored at –85°C	[46]
Surface-FIDA	Dual color single aggregate FCS or LSM detection after immobilization of aggregates (6E10, Nab228 sequence specific antibodies)	Aβ aggregates	Increased in 12 AD patient CSF samples, correlation to MMSE	pM range, calculation based on monomers used for oligomer formation	Aβ oligomers, prepared by SEC	FIDA read-out	Dilution linearity	Samples purchased, stored at –80°C	[50, 47, 48]

Definitions of different Aβ multimer species: Aβ oligomers, non fibrillar, soluble low-molecular weight oligomers. Aβ species designations are used as by the authors of the reviewed methods; ADDLS, Aβ derived diffusible ligands: small soluble Aβ oligomers with molecular weights between 17and 42 kDa. Synthetic Aβ after HFIP dilution and evaporation, DMSO dissolution and dilution in F12 medium [40]; a.u., arbitrary units; FCS, fluorescence correlation spectroscopy; FIDA, fluorescence intensity distribution analysis; FRET, fluorescence resonance energy transfer; LP, lumbar puncture; LSM, laser scanning microscopy; MMSE, Mini-Mental State Examination.

a glass chip using the N-terminal sequence specific Aβ antibody 6E10. Subsequently, the aggregates were labeled by two different, fluorescence dye coupled antibodies (6E10-Alexa 488 and Nab-Alexa 633, specific for a similar epitope at the N-terminal). Then, the surface of the glass chip was scanned by the laser focus of a FCS system. Similar to the method of Pitschke et al., labeled aggregates led to comparably high bursts in fluorescence intensity. In addition, monomer detection was excluded by application of the same antibody for capturing and detection. Later, the biochemical steps of the assay were improved and LSM devices were used for the measurements instead of FCS methods, leading to further improvement of sensitivity and the option for virtual characterization of single aggregates [49]. In 2013, the Aβ aggregate content in CSF samples of 14 AD patients and 12 age-matched controls was determined. The AD group had a significantly higher level of Aβ aggregates and the sFIDA readout correlated negatively to the MMSE score [50].

METHODS FOR DETECTION OF OTHER Aβ OLIGOMERS IN CSF

Despite the progress already achieved in the method development described above, drawbacks may occur in some of the methods in terms of daily handling, critical steps in the procedure which could influence Aβ oligomerization status, and time-consuming washing and incubation steps. Some alternative assay procedures were already developed, but especially biosensors techniques may provide an elegant and sensitive alternative to ELISA and single molecule detection techniques, and were already applied in a small number of studies for the detection of Aβ or α-synuclein oligomers. Methods like surface plasmon resonance (SPR) or electrochemical impedance spectroscopy (EIS) sensors were already combined with oligomer specific recognition systems like antibodies or peptides. Biosensors have the potential to provide rapid, real-time, and accurate results, have been used for a variety of diagnostic applications and have already been considered as "point of care testing = POCT" devices [51]. Double antibody binding as well as antibody labeling can be avoided, reducing costs and time demand.

By now, none of the methods described in this chapter was tested in longitudinal studies or on large cohorts, but studies are ongoing. All methods reviewed in this subchapter are summarized in Table 3, with exception for the methods which were not tested with real CSF samples yet.

Georganopoulou et al. developed an ultrasensitive barcode assay for the nanoparticle-based detection of ADDLs. Oligonucleotide (="barcode")-modified gold nanoparticles as well as magnetic microparticles were functionalized with anti-ADDL antibodies to form DNA-ADDL-magnet complexes, which could be extracted after incubation with the samples by a magnet. The number of barcodes, representing the number of ADDLs, could be determined after signal-amplifying hybridization by scanometric detection methods. As stated by the authors, the detection limit of the semi-quantitative assay was determined to be 100 aM (or 0.45 fg/ml); the assay was linear up to concentrations of 500 fM (or 2.25 pg/ml). ADDL concentrations of the CSF samples of 15 AD cases were tested to be significantly higher than CSF samples of age matched controls [52].

In 2014, Salvadores et al. reported on the protein misfolding cyclic amplification assay (Aβ-PMCA). The assay is based on the seeding nucleation mechanism, see above [53]. Aβ oligomers, already abundant in the sample, act as seed for monomeric $A\beta_{42}$ in an experimental environment where spontaneous Aβ oligomerization is excluded. About 3 fmol Aβ synthetic oligomers could be detected in a sample by thioflavin T fluorescence. In the CSF of 50 AD patients, 39 controls and 37 patients with non-AD dementia, the assay reached an overall sensitivity of 90% and specificity of 92% [54].

The first biosensor method was developed by Haes et al. in 2005, who established a nanoscale optical biosensor based on localized surface plasmon resonance (LSPR). The signal transduction mechanism of this technique is based on the sensitivity of the plasmon frequency to changes in local index of refraction at the surface of nanoparticles [55]. ADDL-specific polyclonal antibodies M71 were immobilized on surface-confined silver nanoparticles. After incubation with the sample, the nanoparticle-antibody-ADDL complexes were again incubated with ADDL antibodies. This sandwich principle resulted in signal amplification. Transmission UV-vis spectroscopy was applied to investigate the optical properties of the loaded nanoparticles. The extinction spectra of the nanoparticles shifted in correspondence to small changes in the refractive index within the electromagnetic field which surrounds the nanoparticles. In a very preliminary study, the CSF of one AD patient had a higher concentration of ADDLs than the CSF of one control sample.

Table 3
Other methods for Aβ oligomer detection

Method	Oligomer selection method	Oligomer species detected	Oligomer results	Detection limit	Standard preparation	Read-out	Assay validation	Pre-analytical sample handling	Ref.
Bio-barcode assay	20C2, polyclonal ADDL specific antibody	ADDLs	Higher in 15 AD CSF samples versus 15 controls	aM range	ADDLs	a.u.	Dilution linearity	Kept frozen until used	[52]
PMCA	–	–	CSF of 50 AD patients, 39 controls, 37 patients with non-AD dementia, overall sensitivity of 90%, specificity of 92%	fM range	Aβ oligomers, seed-free Aβ incubated for certain time	Lag phase [hr]	–	SOPs, LP performed in standard interspace, overnight fasting, collection in PP tubes, centrifugation, aliquotation, stored at –80°C, integrity of blood brain barrier tested	[54]
LSPR	Polyclonal anti-oligomer M71 antibody for immobilization and read-out	ADDLs	Higher in one AD sample	fM range	ADDLs	LSPR shift	Dilution linearity	Kept frozen until used	[72]
EIS	A4 nanobody	Small oligomeric Aβ	CSF of 3 AD patients, 3 PD-like patients and 3 controls distinguished	fM range	–	% impedance change	–	Post-mortem samples were collected, diluted and frozen	[57]

Definitions of different Aβ species. ADDLs, Aβ derived diffusible ligands: small soluble Aβ oligomers with molecular weights between 17and 42 kDa. Synthetic Aβ after HFIP dilution and evaporation, DMSO dissolution and dilution in F12 medium [40]; a.u., arbitrary units; Aβ oligomers, non fibrillar, soluble low-molecular weight oligomers. Designations describing certain Aβ species are used as by the authors of the reviewed methods; EIS, electrochemical impedance spectrometry; LP, lumbar puncture; LSPR, localized surface plasmon resonance; PP, polypropylene; PMCA, protein misfolding cyclic amplification assay; standard interspace, L3-L4 or L4-L5 interspace.

Sierks et al. used the EIS based NanoMonitor assay, capable of ultrasensitive biomarker detection of the low ng/ml down to the pg/ml level, in combination with selective morphology specific nanobodies to investigate if different aggregate species of Aβ (and α-synuclein, implicated in Parkinson's disease) have the potential to be recruited as biomarkers in CSF to study and diagnose AD and other neurodegenerative diseases. EIS measures the dielectric properties of a medium as a function of frequency. Impedance changes to the electrical double layer at the solid-liquid interface, induced, e.g., by protein-protein interactions, can be measured and provide detailed information about the amount of bound proteins, linear correlating to the concentration of respective protein. The authors could demonstrate that detection of certain Aβ oligomers using the A4 nanobody [56] distinguished postmortem CSF samples of three AD patients, three PD-like patients, and three age-matched controls. Using an additional α-synuclein specific nanobody, AD, PD, and control samples could be distinguished specifically [57].

In recent years, at least three further novel biosensor methods for the specific detection of Aβ oligomers were developed. So far, none of them was tested with real CSF samples, but real samples are under investigation. Li et al. developed an electrochemical biosensor which made use of the oligomer-binding induced change of surface electron transfer and square wave voltammetry frequency. Oligomers were captured on a gold disk electrode using a linear peptide probe I10 (11-mercaptoundecanoic acid MUA-RGTWEGKWK-Ferrocene) which recognizes a linear motif on the surface of Aβ oligomers [58]. The critical point might be the target incubation time of 20 h, but oligomer concentrations as low as 240 pM (or 1.08 pg/ml) produced detectable signal response [59]. Stravalaci et al. described a novel SPR based immunoassay, in which the antibody 4G8 served as biorecognition element. 4G8 recognizes a central region of Aβ (amino acids 17–21, amino acids LVFFA) and a specific signal was evoked by transient oligomers, which were proven to disturb the integrity of artificial lipid bilayers and inhibit the physiological pharyngeal contractions in a *Caenorhabditis elegans* model. 4G8 bound Aβ oligomers in which the epitope might be exposed in a pseudo-irreversible manner, whereas Aβ monomers where bound with faster dissociation rates [60]. Rushworth at al. developed an EIS gold electrode biosensor, which employed a synthetic peptide, a part of the cellular prion protein (PrP^C, residues 95 to 110) as a biorecognition element. PrP^C is a natural receptor expressed in the brain. It is implicated in AD pathology and binds Aβ oligomers with residues 95 to 110, located in the unstructured N-terminal region of the receptor [61, 62]. Aβ oligomers down to a concentration of 0.5 pM (or 2.25 pg/ml) could be detected and the biosensor was validated using cell-culture derived Aβ oligomers [63].

DISCUSSION AND OUTLOOK

Very recently, the interest of the AD research community to detect Aβ oligomers in CSF grew substantially. Aβ oligomer species might have the potential to be a biomarker for disease severity and progression, probably even more than the currently examined monomeric CSF $A\beta_{42}$. Due to the close proximity to the brain, CSF is a valuable surrogate for detection of neuropathological changes occurring during AD progression [64]. However, the development of techniques for detection and quantitation of Aβ oligomers in CSF is hampered, e.g., due to the heterogeneity of the oligomers and their unstable nature. Additionally, monomeric Aβ competes for the binding spaces in immunoassays where no oligomer specific biorecognition elements are used. Furthermore, Aβ oligomers are expected to be low in abundance. Whereas Aβ monomers can be detected in concentrations of less than 500 pg/ml [65], the concentration of Aβ oligomers in CSF was reported to be between 0.1 and 10 pg/ml [36, 41], as quantified by ELISA.

To date, a variety of methods to detect Aβ oligomers were published, but still, challenges with regard to reproducibility, reliability, and real quantification occur, and will be discussed in more detail below. To provide the reader with details about assay fitness and reliability, information like standard preparation, detection limit, assay read-out, the availability of assay validation data, and pre-analytical sample handling procedures are listed in Tables 1–3 and are additionally discussed here.

The studies reviewed here investigated the concentrations of Aβ oligomers in CSF employing a diverse range of techniques to detect different or uncharacterized Aβ oligomer species. The results are variable or even contradicting, probably due to several reasons: i) in most studies only a small number of clinical samples was tested; ii) variable diagnostic platforms with variable reactions and incubation conditions were applied; iii) usage of different antibodies

or Aβ oligomer binding substances as biorecognition elements, therefore; iv) detection of different species and conformers; v) different readouts (only in ELISA studies real quantities were given, most authors only give relations) and vi) different oligomer standards for validation and quantification.

In most of the studies, detection limits are given in pg/ml. However, some of the authors calculate molarities (often expressed via monomer equivalents). This might not be feasible, as most of the oligomer preparations will span a range of molecular weights, monomers and insoluble conformers might be included. In addition, a part of total Aβ in the preparation will be removed by centrifugation, chromatography, or similar methods, leading to wrong estimation of the oligomer concentration in the standard preparation. Oligomers for standardization and determination of detection limit were often prepared from synthetic monomeric Aβ after hexafluoro-2-propanol (HFIP) treatment, dissolution in dimethyl sulfoxide (DMSO), and further dilution in phenol red free F12 culture medium [30, 32], HFIP pretreatment and incubation, or HFIP pretreatment and size exclusion chromatography [50]. For direct comparison of standard generation procedures, see Tables 1–3. Some groups used Aβ dimers as standards [38]. In the study of Yang et al., three different standards were used to determine the assay characteristics: ADDLs (containing comparably large oligomers) versus $A\beta_{40}$S26C-dimer (aggregates further into smaller oligomers) versus DiY (does not aggregate further under the reaction conditions). A clear dependency of the detection limit on the aggregated size was shown, as expected [42]. In future studies, the standard should be carefully chosen for the respective assay to be validated. Kasai et al. recently reported on synthetic peptide standards in which a branching lysine core connected multiple BAN50 epitopes [66]. Similar standards might help to allow reliable standard curves and therefore quantification in future assays.

Preanalytical factors might also influence assay performance. It is already known that different test tube materials, specific buffers, heat incubation, freeze and thaw cycles, and others can confound $A\beta_{42}$ values [67]. Salvadores et al. reported that the Aβ-PMCA assay did not work on samples coming from another location than the samples that did perform well. Here, Aβ did not aggregate as expected, even after spiking the samples with synthetic Aβ oligomers. This was discussed to be due to preanalytical factors [54]. Höltta et al. reported that some of the AD patient samples had undetectable Aβ oligomer levels, whereas control samples had a relatively high oligomer concentration. It could not be excluded that sample storage conditions and collection materials could affect Aβ oligomer concentration. Freeze/thaw cycles did not affect assay results, as tested in five cycles [36]. As can be clearly seen in Tables 1–3, CSF sample generation is far away from being standardized, which leads to further reduced comparability of the single assay formats.

Further research to develop sensitive and reliable analytical methods and standards for the respective oligomers need to be established before evaluation of Aβ oligomer concentrations can become a routine diagnostic method. A much larger samples size will need to be analyzed before Aβ oligomers can be established as a biomarker for AD. The biorecognition element has to be carefully chosen. Not each antibody for Aβ oligomers is specific for Aβ oligomers, some also detect other oligomeric proteins, i.e., α-synuclein, polyglutamate, or heat shock proteins [68, 69]. N-terminally binding antibodies will not allow differentiation between oligomers formed by $A\beta_{42}$ or A_{40} or any other. Another important aspect is the size/form of the oligomers, which are detected by the respective assay. As can be seen in Tables 1–3, different methods detected different oligomers with heterogeneous molecular weights. The question will be how the same amount of input Aβ in ng/ml will be detected by the different assays in dependence of oligomer size. At the moment, it is not clear which Aβ conformer is best suitable as a biomarker. In addition, the size of the conformer to be found in CSF might be dependent of the time course of the disease. Oligomer-specific assays might help to evaluate the time dependence of the occurrence of certain oligomer species. As an example, Hölta et al. evaluated if Aβ oligomers were elevated especially in the early phases of AD and investigated MCI to AD converters in comparison to stable MCI patients. In contrast to the stable MCI group, the MCI-AD group was shown to have increased Aβ oligomer levels in comparison to the control group, but with a substantial overlap leading to a specificity of 85% [36]. Interestingly, patients with severe dementia did not differ from the control group while patients with mild and moderate AD did [36]. The reverse hypothesis about the potential release of Aβ oligomers in the last stages of the disease because the plaques act as a reservoir for Aβ oligomers could not be supported.

Most of the studies reviewed here state an increase of Aβ oligomer concentration in CSF of the AD

patient group, but in a number of studies with a substantial overlap between individuals. The results of Aβ oligomer detection should be compared to $A\beta_{42}$, tau, and phospho-tau outcome as well as the results of PET with any amyloid specific ligand. This will lead to the elucidation of correlations between plaque load, Aβ monomer concentration reduction, and probably increased concentration of Aβ oligomers. All assay parameters, which could influence Aβ oligomerization like pH, temperature, incubation times, and so on should be carefully controlled. Antibodies used in the assay should be tested for any cross reactivity toward Aβ monomers or other protein oligomers. Full validation of the assays, including inter- and intraassay variability, spike recovery, dilution linearity, and limits for detection and quantification should be given.

DISCLOSURE STATEMENT

Authors' disclosures available online (http://j-alz.com/manuscript-disclosures/15-1029r2).

REFERENCES

[1] Santacruz KS, Swagerty D (2001) Early diagnosis of dementia. *Am Fam Physician* **63**, 703–713, 717-718.

[2] 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 M, Jicha GA, Nordberg A, Pasquier F, Rabinovici G, Robert P, Rowe C, Salloway S, Sarazin M, Epelbaum S, de Souza, Leonardo C, 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.

[3] Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC (2012) Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* **367**, 795-804.

[4] Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* **9**, 119-128.

[5] Price JL, McKeel DW, Buckles VD, Roe CM, Xiong C, Grundman M, Hansen LA, Petersen RC, Parisi JE, Dickson DW, Smith CD, Davis DG, Schmitt FA, Markesbery WR, Kaye J, Kurlan R, Hulette C, Kurland BF, Higdon R, Kukull W, Morris JC (2009) Neuropathology of nondemented aging: Presumptive evidence for preclinical Alzheimer disease. *Neurobiol Aging* **30**, 1026-1036.

[6] Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, Oates JA, Peck CC, Schooley RT, Spilker BA, Woodcock J (2001) Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Biomarkers Definitions Working Group. *Clin Pharmacol Ther* **69**, 89-95.

[7] Haass C, Selkoe DJ (1993) Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide. *Cell* **75**, 1039-1042.

[8] Weidemann A, König G, Bunke D, Fischer P, Salbaum JM, Masters CL, Beyreuther K (1989) Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein. *Cell* **57**, 115-126.

[9] Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Müller-Hill B (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325**, 733-736.

[10] Bemporad F, Chiti F (2012) Protein misfolded oligomers: Experimental approaches, mechanism of formation, and structure-toxicity relationships. *Chem Biol* **19**, 315-327.

[11] Finder VH, Glockshuber R (2007) Amyloid-beta aggregation. *Neurodegener Dis* **4**, 13-27.

[12] Hardy JA, Higgins GA (1992) Alzheimer's disease: The amyloid cascade hypothesis. *Science* **256**, 184-185.

[13] Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rowan MJ, Lemere CA, Regan CM, Walsh DM, Sabatini BL, Selkoe DJ (2008) Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med* **14**, 837-842.

[14] Haass C, Selkoe DJ (2007) Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol* **8**, 101-112.

[15] Walsh DM, Klyubin I, Fadeeva JV, Rowan MJ, Selkoe DJ (2002) Amyloid-beta oligomers: Their production, toxicity and therapeutic inhibition. *Biochem Soc Trans* **30**, 552-557.

[16] Bhatia R, Lin H, Lal R (2000) Fresh and globular amyloid beta protein (1-42) induces rapid cellular degeneration: Evidence for AbetaP channel-mediated cellular toxicity. *FASEB J* **14**, 1233-1243.

[17] Blennow K, Hampel H, Weiner M, Zetterberg H (2010) Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol* **6**, 131-144.

[18] Hertze J, Minthon L, Zetterberg H, Vanmechelen E, Blennow K, Hansson O (2010) Evaluation of CSF biomarkers as predictors of Alzheimer's disease: A clinical follow-up study of 4.7 years. *J Alzheimers Dis* **21**, 1119-1128.

[19] Diniz BSO, Pinto Júnior JA, Forlenza OV (2008) Do CSF total tau, phosphorylated tau, and beta-amyloid 42 help to predict progression of mild cognitive impairment to Alzheimer's disease? A systematic review and meta-analysis of the literature. *World J Biol Psychiatry* **9**, 172-182.

[20] 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.

[21] DeMattos RB, Bales KR, Parsadanian M, O'Dell MA, Foss EM, Paul SM, Holtzman DM (2002) Plaque-associated disruption of CSF and plasma amyloid-beta (Abeta) equilibrium in a mouse model of Alzheimer's disease. *J Neurochem* **81**, 229-236.

[22] Jack CR, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, Shaw LM, Vemuri P, Wiste HJ, Weigand SD, Lesnick TG, Pankratz VS, Donohue MC, Trojanowski JQ (2013) Tracking pathophysiological processes

in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. *Lancet Neurol* **12**, 207-216.

[23] Kasuga K, Tokutake T, Ishikawa A, Uchiyama T, Tokuda T, Onodera O, Nishizawa M, Ikeuchi T (2010) Differential levels of alpha-synuclein, beta-amyloid42 and tau in CSF between patients with dementia with Lewy bodies and Alzheimer's disease. *J Neurol Neurosurg Psychiatr* **81**, 608-610.

[24] Sjögren M, Davidsson P, Wallin A, Granérus A, Grundström E, Askmark H, Vanmechelen E, Blennow K (2002) Decreased CSF-beta-amyloid 42 in Alzheimer's disease and amyotrophic lateral sclerosis may reflect mismetabolism of beta-amyloid induced by disparate mechanisms. *Dement Geriatr Cogn Disord* **13**, 112-118.

[25] Otto M, Esselmann H, Schulz-Shaeffer W, Neumann M, Schröter A, Ratzka P, Cepek L, Zerr I, Steinacker P, Windl O, Kornhuber J, Kretzschmar HA, Poser S, Wiltfang J (2000) Decreased beta-amyloid1-42 in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. *Neurology* **54**, 1099-1102.

[26] Puzzo D, Arancio O (2013) Amyloid-β peptide: Dr. Jekyll or Mr. Hyde? *J Alzheimers Dis* **33**, S111-120.

[27] Funke SA (2011) Detection of soluble amyloid-β oligomers and insoluble high-molecular-weight particles in CSF: Development of methods with potential for diagnosis and therapy monitoring of Alzheimer's disease. *Int J Alzheimers Dis* **2011**, 151645.

[28] Funke SA, Birkmann E, Willbold D (2013) Detection and quantitation of Amyloid-β aggregates in body fluids may be suitable for early diagnosis of Alzheimer's disease. *In: Advances in Alzheimer Research.* Bentham Science Ebooks.

[29] Funke SA, Birkmann E, Willbold D (2009) Detection of Amyloid-beta aggregates in body fluids: A suitable method for early diagnosis of Alzheimer's disease? *Curr Alzheimer Res* **6**, 285-289.

[30] Fukumoto H, Tokuda T, Kasai T, Ishigami N, Hidaka H, Kondo M, Allsop D, Nakagawa M (2010) High-molecular-weight beta-amyloid oligomers are elevated in cerebrospinal fluid of Alzheimer patients. *FASEB J* **24**, 2716-2726.

[31] Kang J, Vanderstichele H, Trojanowski JQ, Shaw LM (2012) Simultaneous analysis of cerebrospinal fluid biomarkers using microsphere-based xMAP multiplex technology for early detection of Alzheimer's disease. *Methods* **56**, 484-493.

[32] Herskovits AZ, Locascio JJ, Peskind ER, Li G, Hyman BT (2013) A Luminex assay detects amyloid β oligomers in Alzheimer's disease cerebrospinal fluid. *PLoS One* **8**, e67898.

[33] Gao CM, Yam AY, Wang X, Magdangal E, Salisbury C, Peretz D, Zuckermann RN, Connolly MD, Hansson O, Minthon L, Zetterberg H, Blennow K, Fedynyshyn JP, Allauzen S (2010) Aβ40 oligomers identified as a potential biomarker for the diagnosis of Alzheimer's disease. *PLoS One* **5**, e15725.

[34] Xia N, Liu L, Harrington MG, Wang J, Zhou F (2010) Regenerable and simultaneous surface plasmon resonance detection of Aβ(1-40) and Aβ(1-42) peptides in cerebrospinal fluids with signal amplification by streptavidin conjugated to an N-terminus-specific antibody. *Anal Chem* **82**, 10151-10157.

[35] Tai LM, Bilousova T, Jungbauer L, Roeske SK, Youmans KL, Yu C, Poon WW, Cornwell LB, Miller CA, Vinters HV, Van Eldik, Linda J, Fardo DW, Estus S, Bu G, Gylys KH, Ladu MJ (2013) Levels of soluble apolipoprotein E/amyloid-β (Aβ) complex are reduced and oligomeric Aβ increased with APOE4 and Alzheimer disease in a transgenic mouse model and human samples. *J Biol Chem* **288**, 5914-5926.

[36] Hölttä M, Hansson O, Andreasson U, Hertze J, Minthon L, Nägga K, Andreasen N, Zetterberg H, Blennow K (2013) Evaluating amyloid-β oligomers in cerebrospinal fluid as a biomarker for Alzheimer's disease. *PLoS One* **8**, e66381.

[37] Yang T, Hong S, O'Malley T, Sperling RA, Walsh DM, Selkoe DJ (2013) New ELISAs with high specificity for soluble oligomers of amyloid β-protein detect natural Aβ oligomers in human brain but not CSF. *Alzheimers Dement* **9**, 99-112.

[38] Esparza TJ, Zhao H, Cirrito JR, Cairns NJ, Bateman RJ, Holtzman DM, Brody DL (2013) Amyloid-β oligomerization in Alzheimer dementia versus high-pathology controls. *Ann Neurol* **73**, 104-119.

[39] Bruggink KA, Jongbloed W, Biemans EALM, Veerhuis R, Claassen JAHR, Kuiperij HB, Verbeek MM (2013) Amyloid-β oligomer detection by ELISA in cerebrospinal fluid and brain tissue. *Anal Biochem* **433**, 112-120.

[40] Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A* **95**, 6448-6453.

[41] Savage MJ, Kalinina J, Wolfe A, Tugusheva K, Korn R, Cash-Mason T, Maxwell JW, Hatcher NG, Haugabook SJ, Wu G, Howell BJ, Renger JJ, Shughrue PJ, McCampbell A (2014) A sensitive aβ oligomer assay discriminates Alzheimer's and aged control cerebrospinal fluid. *J Neurosci* **34**, 2884-2897.

[42] Yang T, O'Malley TT, Kanmert D, Jerecic J, Zieske LR, Zetterberg H, Hyman BT, Walsh DM, Selkoe DJ (2015) A highly sensitive novel immunoassay specifically detects low levels of soluble Aβ oligomers in human cerebrospinal fluid. *Alzheimers Res Ther* **7**, 14.

[43] Jongbloed W, Bruggink KA, Kester MI, Visser P, Scheltens P, Blankenstein MA, Verbeek MM, Teunissen CE, Veerhuis R (2015) Amyloid-β oligomers relate to cognitive decline in Alzheimer's disease. *J Alzheimers Dis* **45**, 35-43.

[44] Pitschke M, Prior R, Haupt M, Riesner D (1998) Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. *Nat Med* **4**, 832-834.

[45] Henkel AW, Dittrich PS, Groemer TW, Lemke EA, Klingauf J, Klafki HW, Lewczuk P, Esselmann H, Schwille P, Kornhuber J, Wiltfang J (2007) Immune complexes of autoantibodies against A beta 1-42 peptides patrol cerebrospinal fluid of non-Alzheimer's patients. *Mol Psychiatry* **12**, 601-610.

[46] Santos AN, Torkler S, Nowak D, Schlittig C, Goerdes M, Lauber T, Trischmann L, Schaupp M, Penz M, Tiller F, Böhm G (2007) Detection of amyloid-beta oligomers in human cerebrospinal fluid by flow cytometry and fluorescence resonance energy transfer. *J Alzheimers Dis* **11**, 117-125.

[47] Funke SA, Birkmann E, Henke F, Görtz P, Lange-Assch enfeldt C, Riesner D, Willbold D (2008) An ultrasensitive assay for diagnosis of Alzheimer's disease. *Rejuvenation Res* **11**, 315-318.

[48] Funke SA, Birkmann E, Henke F, Görtz P, Lange-Assch enfeldt C, Riesner D, Willbold D (2007) Single particle detection of Abeta aggregates associated with Alzheimer's disease. *Biochem Biophys Res Commun* **364**, 902-907.

[49] Funke SA, Wang L, Birkmann E, Willbold D (2010) Single-particle detection system for Abeta aggregates: Adaptation of surface-fluorescence intensity distribution analysis to laser scanning microscopy. *Rejuvenation Res* **13**, 206-209.

[50] Wang-Dietrich L, Funke SA, Kühbach K, Wang K, Besmehn A, Willbold S, Cinar Y, Bannach O, Birkmann E, Willbold D (2013) The amyloid-β oligomer count in cerebrospinal fluid is a biomarker for Alzheimer's disease. *J Alzheimers Dis* **34**, 985-994.

[51] Gruhl FJ, Rapp BE, Länge K (2013) Biosensors for diagnostic applications. *Adv Biochem Eng Biotechnol* **133**, 115-148.

[52] Georganopoulou DG, Chang L, Nam J, Thaxton CS, Mufson EJ, Klein WL, Mirkin CA (2005) Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. *Proc Natl Acad Sci U S A* **102**, 2273-2276.

[53] Soto C, Saborio GP, Anderes L (2002) Cyclic amplification of protein misfolding: Application to prion-related disorders and beyond. *Trends Neurosci* **25**, 390-394.

[54] Salvadores N, Shahnawaz M, Scarpini E, Tagliavini F, Soto C (2014) Detection of misfolded Aβ oligomers for sensitive biochemical diagnosis of Alzheimer's disease. *Cell Rep* **7**, 261-268.

[55] Unser S, Bruzas I, He J, Sagle L (2015) Localized surface plasmon resonance biosensing: Current challenges and approaches. *Sensors* **15**, 15684-15716.

[56] Zameer A, Kasturirangan S, Emadi S, Nimmagadda SV, Sierks MR (2008) Anti-oligomeric Abeta single-chain variable domain antibody blocks Abeta-induced toxicity against human neuroblastoma cells. *J Mol Biol* **384**, 917-928.

[57] Sierks MR, Chatterjee G, McGraw C, Kasturirangan S, Schulz P, Prasad S (2011) CSF levels of oligomeric alpha-synuclein and beta-amyloid as biomarkers for neurodegenerative disease. *Integr Biol* **3**, 1188-1196.

[58] Sato T, Kienlen-Campard P, Ahmed M, Liu W, Li H, Elliott JI, Aimoto S, Constantinescu SN, Octave J, Smith SO (2006) Inhibitors of amyloid toxicity based on beta-sheet packing of Abeta40 and Abeta42. *Biochemistry* **45**, 5503-5516.

[59] Li H, Cao Y, Wu X, Ye Z, Li G (2012) Peptide-based electrochemical biosensor for amyloid β 1-42 soluble oligomer assay. *Talanta* **93**, 358-363.

[60] Stravalaci M, Bastone A, Beeg M, Cagnotto A, Colombo L, Di Fede G, Tagliavini F, Cantú L, Del Favero E, Mazzanti M, Chiesa R, Salmona M, Diomede L, Gobbi M (2012) Specific recognition of biologically active amyloid-β oligomers by a new surface plasmon resonance-based immunoassay and an *in vivo* assay in Caenorhabditis elegans. *J Biol Chem* **287**, 27796-27805.

[61] Laurén J (2014) Cellular prion protein as a therapeutic target in Alzheimer's disease. *J Alzheimers Dis* **38**, 227-244.

[62] Laurén J, Gimbel DA, Nygaard HB, Gilbert JW, Strittmatter SM (2009) Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* **457**, 1128-1132.

[63] Rushworth JV, Ahmed A, Griffiths HH, Pollock NM, Hooper NM, Millner PA (2014) A label-free electrical impedimetric biosensor for the specific detection of Alzheimer's amyloid-beta oligomers. *Biosens Bioelectron* **56**, 83-90.

[64] Zetterberg H, Blennow K (2008) Biological CSF markers of Alzheimer's disease. *Handb Clin Neurol* **89**, 261-268.

[65] Hampel H, Bürger K, Teipel SJ, Bokde ALW, Zetterberg H, Blennow K (2008) Core candidate neurochemical and imaging biomarkers of Alzheimer's disease. *Alzheimers Dement* **4**, 38-48.

[66] Kasai T, Tokuda T, Taylor M, Nakagawa M, Allsop D (2012) Utilization of a multiple antigenic peptide as a calibration standard in the BAN50 single antibody sandwich ELISA for Aβ oligomers. *Biochem Biophys Res Commun* **422**, 375-380.

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

[68] Yoshiike Y, Minai R, Matsuo Y, Chen Y, Kimura T, Takashima A (2008) Amyloid oligomer conformation in a group of natively folded proteins. *PLoS One* **3**, e3235.

[69] Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* **300**, 486-489.

[70] Barghorn S, Nimmrich V, Striebinger A, Krantz C, Keller P, Janson B, Bahr M, Schmidt M, Bitner RS, Harlan J, Barlow E, Ebert U, Hillen H (2005) Globular amyloid beta-peptide oligomer - a homogenous and stable neuropathological protein in Alzheimer's disease. *J Neurochem* **95**, 834-847.

[71] O'Malley TT, Oktaviani NA, Zhang D, Lomakin A, O'Nuallain B, Linse S, Benedek GB, Rowan MJ, Mulder, Frans A A, Walsh DM (2014) Aβ dimers differ from monomers in structural propensity, aggregation paths and population of synaptotoxic assemblies. *Biochem J* **461**, 413-426.

[72] Haes AJ, Chang L, Klein WL, Van Duyne RP (2005) Detection of a biomarker for Alzheimer's disease from synthetic and clinical samples using a nanoscale optical biosensor. *J Am Chem Soc* **127**, 2264-2271.