CHAPTER 4

Aβ-oligomers quantification in CSF, and their clinical value
4.2

Amyloid-\(\beta\) oligomers relate to cognitive decline in Alzheimer’s disease

Submitted

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ABSTRACT

Objectives
1) Analyze the potential of amyloid beta (Aβ)-oligomer concentrations in cerebrospinal fluid (CSF) to diagnose and predict progression to Alzheimer’s disease (AD) in a large clinical study sample. 2) Monitor Aβ-oligomer concentrations over-time, both in early and advanced stages of AD. 3) Examine the relation between Aβ-oligomer levels in CSF and cognitive functioning.

Methods
24 Non-demented, 61 mild cognitive impairment and 64 AD-patients who underwent lumbar puncture and cognitive testing at baseline and follow-up were selected from the memory clinic based Amsterdam Dementia Cohort. CSF samples were analyzed for standard AD-biomarkers and Aβ-oligomer levels using a validated in-house Aβ-oligomer specific enzyme-linked immunosorbent assay. Aβ-oligomer levels were analyzed as indicators of disease progression (follow-up AD diagnosis) and cognitive decline, respectively.

Results
Patient groups did not differ in Aβ-oligomer concentrations at baseline or follow-up. Baseline CSF Aβ-oligomer levels were similar in MCI patients that develop AD as in stable MCI patients. Interestingly, both MCI and AD patients showed an annual decrease in Aβ-oligomer levels of 9.4% and 6.8%, respectively. A decrease in Aβ-oligomer levels over time was strongly associated with more severe cognitive decline in AD patients.

Conclusion
Despite the limited diagnostic potential of Aβ-oligomer levels in CSF to differentiate between patient groups, and between MCI-AD and MCI-stable patients, changes in CSF Aβ-oligomer levels were related to cognitive decline. Therefore, CSF Aβ-oligomers may aid in the selection of patients with a more aggressive disease course.
INTRODUCTION

An imbalance in the production and removal of the Aβ peptide can cause direct and indirect, through accumulation of Aβ into senile plaques and subsequent glial activation, neurodegenerative changes which ultimately lead to AD. Small, soluble aggregates of the Aβ peptide, called Aβ-oligomers, have gained substantial scientific interest due to the in vivo findings on their direct toxic effects at the synapse level, and their early involvement in AD pathogenesis. Interestingly, Aβ-oligomers have been detected in CSF and levels were suggested to reflect the AD-related pathological event in the brain parenchyma.

Publications in which Aβ-oligomer levels in CSF were examined as a potential - and much needed - biomarker for AD have reported promising findings. Although these cross-sectional studies focus on the direct diagnostic importance of Aβ-oligomers, longitudinal changes in Aβ-oligomer levels may herald additional personalized diagnostic or pathophysiological insight in AD. To the best of our knowledge, longitudinal studies of CSF Aβ-oligomer levels have not been reported yet.

In this study we aimed to 1) analyze the potential of Aβ-oligomer concentrations in CSF to diagnose and predict AD in a large clinical study sample, and 2) monitor Aβ-oligomer concentrations in time, both in early and advanced stages of AD 3) analyze the relation between Aβ-oligomer levels in CSF and cognitive functioning.

METHODS

Participants

The clinical study sample (149 individuals) has been described in an earlier study, in which we measured specific and a-specific AD biomarkers at baseline and follow-up in patients that were selected from the memory clinic based Amsterdam Dementia Cohort. Diagnosis of Mild Cognitive Impairment (MCI) (n=61) was made according to the criteria set by Petersen et al., and the diagnosis of AD (n=64) was made following the National Institute of Neurological and Communicative Disease and Stroke/AD and Related Disorders Association (NINCDS-ADRDA) guidelines. When cognitive screening, physical and neurological examination, laboratory tests, electroencephalogram (EEG), and magnetic resonance imaging (MRI) were normal, subjects were considered to have subjective complaints. The group of non-demented individuals (n=24) consisted of 20 patients with subjective complaints, two patients with a psychiatric disorder and two patients with temporal epilepsy. Clinical diagnoses were made by consensus of a multidisciplinary team and were independent of the biomarker concentrations in CSF in all cases. Cognitive performance of all participants was assessed at baseline and follow-up through scores on the Mini-Mental State Examination.

At follow-up (on average 45 months, with minimum of 6 months), 34 of the 61 MCI patients had progressed to AD, three to frontotemporal lobar degeneration, two to vascular dementia, one to dementia with Lewy bodies and one subject was diagnosed...
with normal pressure hydrocephalus. Six non-demented subjects had progressed to MCI, two progressed to AD and one was diagnosed with vascular dementia at follow-up. Demographic data of the cohort are depicted in Table 1. The VU University Medical Center ethics committee approved the study and all participants gave written informed consent.

**Sample handling**

CSF was obtained through lumbar puncture between the L3/L4 and L4/L5 intervertebral space, collected in 12 mL polypropylene tubes. Samples were centrifuged at 1800 x g for 10 minutes at 4°C within two hours after lumbar puncture. A fraction of the CSF was stored at -20°C for ELISA and routine analysis including total cell counts (for leucocytes and erythrocytes), total protein and glucose determination. CSF was aliquoted into 0.5 mL or 1.0 mL polypropylene tubes and stored at -80°C for further analysis in this study. Aβ42, Tau and pTau (181) concentrations were determined using the Innotest sandwich ELISA (Innotest β-amyloid1-42, htau Ag and Phospo-tau (181P) Innogenetics, Ghent, Belgium). Aβ40 levels were quantified using an in-house method. Staff involved in CSF analyses was blinded for clinical diagnosis.

**Aβ-oligomer specific enzyme-linked immunosorbent assay (ELISA)**

For the measurement of Aβ-oligomer levels in CSF we used an Aβ-specific sandwich ELISA (see Supplementary data for assay validation), in which a 96-wells Costar 9018 plate (Corning Inc., New York, USA) was coated with 100 µL/well of VU-17 antibody at

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-demented</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male/female)</td>
<td>24 (17/7)</td>
<td>61 (38/23)</td>
<td>64 (36/28)</td>
</tr>
<tr>
<td>Age - years</td>
<td>64 (10)</td>
<td>68 (8)a</td>
<td>65 (7)</td>
</tr>
<tr>
<td>Time to follow-up - months</td>
<td>48 (32)</td>
<td>45 (25)</td>
<td>38 (24)</td>
</tr>
<tr>
<td>Baseline cognition - MMSE</td>
<td>28.3 (1.8)</td>
<td>26.5 (2.5)a</td>
<td>21.7 (5.3)b</td>
</tr>
<tr>
<td>Follow-up cognition - MMSE</td>
<td>27.3 (2.8)</td>
<td>21.9 (5.5)a b</td>
<td>16.9 (6.6)b</td>
</tr>
<tr>
<td>Aβ40 - µg/L</td>
<td>9.7 (3.0)</td>
<td>9.5 (3.2)</td>
<td>8.5 (2.8)</td>
</tr>
<tr>
<td>Follow-up Aβ40 - µg/L</td>
<td>10.8 (3.2)</td>
<td>9.9 (3.1)</td>
<td>9.1 (3.4)</td>
</tr>
<tr>
<td>Aβ42 - ng/L</td>
<td>711 (276)</td>
<td>530 (243)ab</td>
<td>415 (141)b</td>
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<tr>
<td>Follow-up Aβ42 - ng/L</td>
<td>721 (300)</td>
<td>560 (253)ab</td>
<td>451 (174)b</td>
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<tr>
<td>Tau - ng/L</td>
<td>395 (258)</td>
<td>606 (495)</td>
<td>690 (360)b</td>
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<tr>
<td>Follow-up Tau - ng/L</td>
<td>443 (271)</td>
<td>649 (636)</td>
<td>678 (333)b</td>
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<tr>
<td>pTau - ng/L</td>
<td>58 (29)</td>
<td>78 (43)</td>
<td>87 (35)b</td>
</tr>
<tr>
<td>Follow-up pTau - ng/L</td>
<td>66 (33)</td>
<td>79 (42)</td>
<td>85 (36)</td>
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<tr>
<td>Aβ-oligomers - a.u.</td>
<td>1471 (574)</td>
<td>1651 (589)b</td>
<td>1581 (685)</td>
</tr>
<tr>
<td>Follow-up Aβ-oligomers - a.u.</td>
<td>1622 (555)</td>
<td>1427 (428)b</td>
<td>1520 (641)</td>
</tr>
</tbody>
</table>

Age, time, MMSE and AD-biomarkers are depicted as mean (standard deviation). Compared to AD a P <0.05 Compared to non-demented b P <0.05. Missing data on one AD and two MCI patients.
1 µg/mL in 100 mM carbonate buffer at pH 9.6, overnight at 4°C on a shaking platform (600rpm). After coating, the plate was washed once with PBS and block buffer (PBS with 1% BSA) was added for two hours at RT. The plate was washed three times with wash buffer (20mM Tris-HCl, 0.05% Tween at pH 7.5). An aliquot of Aβ-oligomer enriched Aβ42, which contained a total of 500 ng/mL synthetic Aβ42 was diluted in assay buffer (20mM Tris-HCl, 50 mM NaCl, 0.1% BSA and 0.1% Tween20 at pH 7.5) to reference values of 15, 3, 1.5, 0.5, 0.25 and 0.125 ng/mL. CSF samples were added at four times dilution and incubated for two hours at RT at 600rpm. The plate was washed three times with wash buffer followed by detection with 100 µL/well biotinylated VU-17 (1 mg/mL) diluted 1:2500 in assay-buffer for one hour at RT at 600rpm. Next, the plate was washed three times with wash buffer followed by incubation with 100µL Streptavidin/poly-HRP conjugate (1 mg/mL) diluted 1:10,000 in PBS for 30 minutes at RT. The plate was washed four times with wash buffer. Then, 100 µL/well of TMB in substrate buffer (100 µL of 10 mg/mL TMB was added to 11 mL citric acid/acetate buffer at pH 4.0 with 10 µL 30% H2O2) was added and incubated at RT. After 10 minutes the reaction was stopped with 100 µL sulfuric acid at 1M, followed by detection at 530nm using a spectrophotometer (Bio-Tek Synergy HT, Winooski, VT, U.S.A). Due to uncertainty concerning the exact size of the oligomers in the solution used to prepare the calibration curve, the concentration was based on the molecular size of a single Aβ42 peptide (molecular weight of 4514 dalton). The lower limit of detection of the Aβ-oligomer ELISA was 9 pg/mL. See Supplementary data for additional assay characteristics.

We used an oligomer-enriched preparation with a known start concentration of amyloid beta as standard material, but did not verify the stringent levels of oligomers within this preparation. Therefore, we present our data as arbitrary units (a.u.) throughout the manuscript.

**Statistical analyses**

Group comparisons were performed using one-way analysis of variance (ANOVA). Pearson’s correlation coefficient was used to assess the correlation between continuous variables. Age- and sex-adjusted linear mixed models were applied to assess baseline effects for diagnosis and changes over time in CSF Aβ-oligomer levels by diagnosis. In this model, Aβ-oligomer concentration was the dependent variable, while diagnosis and time (in years; treated as a continuous variable) and interaction between diagnosis and time were independent variables. Furthermore, we calculated the annualized changes of CSF biomarker level and MMSE, with the formula value of measurement at follow-up minus value of measurement at baseline, divided by follow-up period in years. Associations between annualized change in CSF biomarker levels and annualized change in MMSE-scores (data available for 130 patients) were assessed using multiple linear regression, stratified for diagnoses and adjusted for sex and age. A priori power analysis, with power set at 0.8, showed that at least 15 patients were required per group to detect the expected differences between AD and controls. SPSS Statistics package 19.0 (IBM, New York, USA) for OS X Lion was used for the statistical analyses and the generation of graphs. P-values below 0.05 were considered significant.
RESULTS

Group comparison
Levels of CSF Aβ-oligomers did not differ between the groups of non-demented, MCI and AD neither at baseline (Figure 1A), nor at follow-up (Figure 1B). Aβ-oligomer levels decreased significantly at an annual rate of 9.4% (155 a.u., p-value < 0.05) in MCI and 6.8% (108 a.u., p-value < 0.05) in AD patients. The Aβ-oligomer levels in CSF of non-demented subjects did not change over time.

Prediction of AD in MCI patients
Within the group of MCI patients (n = 61) a total of 34 patients had progressed to AD (MCI-AD) at follow-up clinical assessment. To examine the predictive value of Aβ-oligomers, we compared the baseline Aβ-oligomer concentrations in CSF of MCI-AD patients and those that did not progress to AD (MCI-stable). As shown in Figure 2, there...
was no difference in Aβ-oligomer levels between MCI-AD (1690 ± 571 a.u.) and MCI-stable patients (1571 ± 629 a.u.).

**Correlation of Aβ-oligomer levels with AD-biomarkers levels in CSF**
Aβ-oligomers and pTau levels correlated in non-demented subjects, but not in MCI or AD patients, at baseline \( (r = 0.38, \ p\text{-value} = 0.07) \) and at follow-up \( (r = 0.54, \ p\text{-value} = 0.01) \). Aβ-oligomer concentrations were unrelated to total Tau, Aβ40 or Aβ42 in any of the diagnostic groups.

**Longitudinal CSF Aβ-oligomer and AD-biomarkers levels**
We assessed the relation between the annualized changes of Aβ-oligomer content in CSF with annualized changes in Aβ40, Aβ42, Tau and pTau levels. An increase in Aβ-oligomer levels was positively associated with Tau \( (r = 0.27, \ p\text{-value} < 0.05) \) and pTau levels \( (r = 0.31, \ p\text{-value} = 0.02) \) in AD patients. A positive relation was found in longitudinal CSF Aβ-oligomer levels and Tau levels in MCI patients \( (r = 0.37, \ p\text{-value} = 0.005) \). No relation was found between longitudinal changes in Aβ-oligomer concentration and the established AD-biomarkers in non-demented subjects.

**Correlation with cognition**
There was no association between Aβ-oligomer levels in CSF and cognitive performance at baseline in any of our patient groups. There was, however, a strong relation between the yearly decrease in Aβ-oligomer levels and cognitive decline, as reflected by yearly decrease in MMSE-scores, in subjects with AD \( (\beta = 1.42 \cdot 10^{-3}, \ p\text{-value} = 0.02, \ \text{corrected for age and sex}) \) and a trend in MCI patients \( (\beta = 2.27 \cdot 10^{-3}, \ p\text{-value} = 0.06, \ \text{corrected for age and sex}) \). As visualized in Figure 3, subjects with AD that had the sharpest decrease in Aβ-oligomer levels (at least 267 a.u.) had the most pronounced cognitive decline. Fluctuations in Aβ-oligomer levels over-time were not associated with cognitive decline in the non-demented subjects.
DISCUSSION

The neurotoxic and synaptotoxic effects of Aβ-oligomers in in vitro and animal model studies, point to a role for Aβ-oligomers in the neurodegenerative changes and associated cognitive decline in AD. In the present longitudinal study, we have investigated if Aβ-oligomer levels in CSF are associated with AD clinical phenotype and with cognitive decline. No differences in CSF Aβ-oligomer concentrations were observed between non-demented, MCI patients and AD patients at baseline. Neither could we distinguish MCI-AD from MCI-stable patients on the basis of the Aβ-oligomer content in CSF. Based on these findings we conclude that the Aβ-oligomer levels in CSF have limited use as a direct diagnostic tool.

However, when baseline and follow-up (mean 43 months) concentrations were compared, a decrease was observed in MCI and AD cases and not in non-demented subjects (Figure 1). These longitudinal changes in CSF Aβ-oligomers in MCI and AD were associated with the rate of cognitive decline, independent from baseline cognition. Interestingly, others found specific isoforms of Aβ (Aβ*56) to be reduced in AD brain compared to controls, and the reduced levels were associated with neuronal loss. Although our assay is unlikely to quantify Aβ*56 oligomers specifically, it is conceivable that decreased levels of Aβ-oligomers and consequent neurodegeneration are the underlying mechanisms for cognitive decline in AD patients. In contrast to Aβ-oligomers,
the levels of established AD-biomarkers Aβ₁₋₄₂, total Tau or p-Tau were not associated with cognitive decline in any subject group. These findings indicate that Aβ-oligomers are an independent entity, which is separate from Aβ₁₋₄₂ or Tau, which may be used to evaluate within-subject disease progression.

Clinically useful diagnostic AD-biomarkers should be able to discriminate AD patients from subjects with minor cognitive complaints rather than healthy controls, since these subjects reflect the population that visits the memory clinic for medical diagnosis. Therefore, participants with subjective complaints (SC) were used as non-AD reference group in this study. A drawback of the use of SC as a control group is that differences in biomarker levels compared to AD may be less obvious as with real controls, since SC subjects are known to have an increased risk of developing AD. Indeed, six non-demented subjects had progressed to MCI and two to AD at follow-up clinical assessment. On the other hand, studying SC may give insight in the earliest events in AD pathology. Therefore, further studies aim to elucidate the role of low-molecular weight CSF Aβ-oligomers in the earliest stages of AD-pathology in SC cases without detectable cognitive deficits.

Whereas we found no difference in Aβ-oligomer levels between AD, MCI and non-demented cases, others have reported increased levels in AD. This discrepancy may be due to the use of different detection platforms, and pre-analytical procedures that favor detection of distinct species of Aβ-oligomers. Our assay is most sensitive to smaller aggregates (Supplementary Figure 1), while others almost exclusively quantified larger Aβ aggregates for example, or did not identify their molecular targets. The dynamic nature of Aβ-aggregation limits the stringency with which the total level of Aβ-oligomers can be determined, and restricts the analysis to relative differences among subjects and groups. Because of the intrinsic unstable nature of the Aβ-oligomers, we used CSF that was acquired under uniform conditions, was processed within two hours and directly stored at -80°C until further analysis. Therefore, we are confident that our study describes biologically relevant differences in actual Aβ-oligomer content. With respect to multicenter and large-scale studies, implementation of strict and concordant (pre-) analytical protocols is pivotal for reliable assessments of Aβ-oligomer concentrations in CSF.

It important to realize that distinct biological effects have been described for Aβ aggregates of varying sizes. Particularly the small and soluble amyloid aggregates have a profound impact on neuronal long-term potentiation, neuronal dystrophy and degeneration as well as tau phosphorylation. In line with these studies, we found that the concentration of low-molecular weight Aβ-oligomer species is related to cognitive deterioration. The mechanisms underlying the increased cognitive deficits remain to be investigated. Interestingly, the Aβ*56 oligomers have recently been proposed to play a pathogenic role very early in AD, and were found to be correlated with pathological forms of tau in healthy individuals. The authors speculate that the Aβ*56 oligomers may underlie subtle cognitive deficits and impaired brain metabolism in healthy individuals at risk for AD. Our data partly support their hypothesis, as we observed a relation between pTau and Aβ-oligomers in individuals with subjective complaints, which was uncoupled in MCI and AD.
In conclusion, our results indicate that quantification of CSF Aβ-oligomer does not provide additional diagnostic information over and above the established CSF AD-biomarkers in a cross-sectional setting. However, we did observe a decrease in CSF Aβ-oligomer concentrations over-time, which was strongly associated to cognitive decline in AD patients. The association with cognitive decline points to a role of Aβ-oligomers as a disease-stage marker, in contrast to CSF Aβ42 and Tau levels. More specifically, assessment of fluctuations in Aβ-oligomer levels might aid in targeting patients with a more progressive disease course.

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REFERENCES


SUPPLEMENTAL DATA

Preparation and stabilization of Aβ-oligomers
Aβ1-42 (Bachem) was dissolved in hexafluoroisopropanol (Sigma), aliquotted, dried in a speed-vacuum and stored at -80°C until use. 700 µg of Aβ1-42 was dissolved in phosphate-buffered saline (0.01M phosphate buffered saline at pH 7.2) and allowed to aggregate at room temperature for 24 hours. This sample was treated with photo-induced cross-linking of unmodified proteins (PICUP) to snap-freeze the current state of aggregation, essentially as described before.1,2 In short, we added 100µL 3.1mM ruthenium (II) (Sigma-Aldrich, St. Louis, USA) and 100µL 62mM ammonium persulfate to

Supplementary Figure e1 A/B
Specificity of the Oligomer-assay
Figure e1a) Western blot, using 6E10 antibody, of stabilized Aβ-oligomers separated into fractions 1-11 by size-exclusion chromatography on a Superdex 75 column. The fractions were enriched for different sizes of Aβ-aggregates, ranging from monomers to fibrillar Aβ. Figure e1b) The Aβ-oligomer ELISA specifically recognizes Aβ-oligomer species present in fractions 2-6, but not fibrils (fraction 1) or monomers and dimers (fractions 7-11).
the Aβ-oligomer enriched sample in a darkroom. The sample was then illuminated for one second using a cold light fountain (Type 482, Karl Storz KG, Tuttingen, Germany). The cross linking reaction was quenched by adding 10µL DTT (1M) followed immediately by vortexing. Following the PICUP reaction, Aβ-oligomers were heated at 95°C before centrifugation at 10,000 x g. Aβ was collected in the precipitate, which was dissolved in 700µL sodium phosphate buffer containing 0.2% SDS.

Size exclusion chromatography
To separate the Aβ monomers and oligomers according to molecular size, the Aβ-oligomer sample was applied to a Superdex 75 column (GE Healthcare Bio-Science AB, Uppsala, Sweden) for size-exclusion chromatography on an AKTA-purifier. The flow-through was collected in 250µL fractions, which were snap-frozen in liquid nitrogen immediately after collection and stored at -80°C for later analysis. The optical density of the flow-through was continuously monitored at 280nm (UNICORN 5.11 software, GE Healthcare), which was used to determine the total amount of peptides in each of the fractions (Figure e1b). Fractions 1-11 were analyzed using our Aβ-oligomer ELISA.

SDS-PAGE and western blot
Aβ-oligomers in each of the SEC fractions were analyzed by sodium dodecyl sulfate polyacrylamide gelelectrophoresis, using a 4-12% Nu-PAGE pre-cast gel (Invitrogen, Carlsbad, CA, U.S.A). Next, the proteins were electro blotted onto 0.2µM PVDF membranes (Millipore Corp., Bedford, MA, U.S.A) in a Trans-blot SD semi-dry transfer cell (Bio-Rad, U.S.A) at 0.11mA for 60 minutes. Immunodetection was performed with mouse monoclonal 6E10 antibody (Sigma-Aldrich, St. Louis, MO, U.S.A; at 200ng/mL), which recognizes the n-terminal amino acids of the Aβ peptides and a secondary peroxidase-linked goat-anti-mouse antibody (Dako, Glostrup, Denmark; at 0.5µg/mL). Peptides were visualized through ECL+ chemiluminescence (GE Healthcare, Munich, Germany), and luminescence was detected using a Fujifilm LAS-3000 camera and Imagereader LAS-3000 Lite software (Fujifilm Holdings Corporation., Dusseldorf, Germany). The western blot of fractions 1-11 is shown in Figure e1a.

Supplementary Table e1. Spike-recovery of the Aβ-oligomer ELISA

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Low (1ng/mL)</th>
<th>Medium (2ng/mL)</th>
<th>High (3ng/mL)</th>
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</thead>
<tbody>
<tr>
<td>Number of CSF (n)</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Expected (ng/mL)</td>
<td>0.42</td>
<td>1.52</td>
<td>1.81</td>
</tr>
<tr>
<td>Observed (ng/mL)</td>
<td>0.32</td>
<td>0.85</td>
<td>1.35</td>
</tr>
<tr>
<td>Recovery</td>
<td>76.7%</td>
<td>49.1%</td>
<td>58.3%</td>
</tr>
</tbody>
</table>

Table e-1 shows the spike-recovery data from low, medium and high concentrations of untreated Aβ-oligomer preparations (as used in the standard material for the assay).
ELISA specificity
Fractions 1-11 were analyzed using our Aβ-oligomer ELISA. The ELISA signal is compared to the total amount of peptides present in each fraction. As shown in Supplementary Figure 1a, the ELISA signal is strongly increased in fractions 2-6, which all contain mid-range oligomers, and not in fractions 1 (fibrils) or 8-11 (dimers and monomers). (Figure 1b).

Spike-recovery of the Aβ-oligomer ELISA
To determine the spike-recovery percentage of the Aβ-oligomer ELISA, a total of seven human CSF samples were analyzed in the ELISA. To each of these CSF samples spikes of 1, 2 and 3 ng/mL of non-stabilized Aβ-oligomers were added, which reflect the low, but biologically relevant concentrations of Aβ-oligomers in human CSF. The ELISA read-out of each of the spiked samples was corrected for their endogenous (non-spiked) Aβ-oligomer concentrations. Using this approach, the spike-recovery of our Aβ-oligomer ELISA was determined at 61.4% (See Table e1). The relatively low recovery percentages are possibly attributed to the dynamic nature of Aβ-oligomers and factors in CSF that can bind and/or dissociate the unstable oligomers used for spiking.