Analytical and Clinical Performance of Amyloid-Beta Peptides Measurements in CSF of ADNIGO/2 Participants by an LC-MS/MS Reference Method.

Running title:

Amyloid beta in CSF of ADNIGO/2 participants

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Abbreviations:

Aβ42 – amyloid beta 1-42, AD – Alzheimer's disease, CSF – cerebrospinal fluid, PET – positron emission tomography, MCI – mild cognitive impairment, LC-MSMS- liquid chromatography with tandem mass spectrometric detection, JCTLM - Joint Committee for Traceability in Laboratory Medicine, ADNI – Alzheimer's Disease Neuroimaging Initiative, CRM – certified reference material, NC – normal controls, SMC – subjective memory complaints, LP – lumbar puncture, FBP – florbetapir, FWHM – full width at half maximum, MPRAGE - Magnetization Prepared Rapid Acquisition Gradient Echo, SUVRs – standardized uptake value ratios, EC-JRC – European Commission Joint Research Centre, IRMM- Institute for Reference Materials and Measurements, DMSO – dimethyl QC- quality control, BSA – bovine serum albumin, aCSF/*BSA* – artificial CSF with BSA, EMCI - early MCI, CV – coefficient of variation

Abstract

Background: CSF amyloid-β_{1-42} (Aβ42) reliably detects brain amyloidosis based on high concordance with plaque burden at autopsy and with amyloid PET ligand retention observed in several studies. Low CSF Aβ42 concentrations in normal aging and dementia are associated with the presence of fibrillary Aβ across brain regions detected by amyloid PET imaging.

Method: LC-MSMS reference method for Aβ42, modified by adding Aβ40 and Aβ38 peptides to calibrators, was used for analysis of 1445 CSF samples from ADNIGO/2 participants. Seventy runs were completed using 2 different lots of calibrators. For preparation of Aβ42 calibrators and controls spiking solution, reference Aβ42 standard with certified concentration was obtained from EC-JRC-IRMM (Belgium). Aβ40 and Aβ38 standards were purchased from rPeptide (USA). Aβ42 calibrators' accuracy was established using CSF-based Aβ42 Certified Reference Materials (CRM).

Results: CRM-adjusted Aβ42 calibrator concentrations were calculated using regression equation Y (CRM-adjusted) = 0.89X (calibrators) + 32.6. Control samples and CSF pools yielded imprecision ranging from 6.5 to 10.2% (Aβ42) and 2.2 to 7.0% (Aβ40). None of the CSF pools showed statistically significant differences in Aβ42 concentrations across two different calibrator lots. Comparison of Aβ42 with Aβ42/Aβ40 showed that the ratio improved concordance with concurrent [^{18}F]-florbetapir PET as a measure of fibrillar Aβ (n=766) from 81% to 88%.

Conclusions: Long term performance assessment substantiates the robustness of our modified LC-MSMS reference method for three Aβ peptides. The potential for improved diagnostic

performance of the CSF ratio Aβ42/Aβ40 suggests that these two Aβ peptides should be measured together and support the need for an Aβ40 CRM.

Introduction

The 42 amino acid form of Aβ, Aβ42, is a well characterized biomarker for brain amyloidosis associated with Alzheimer's disease (AD)(1). Pathological changes of Aβ42 are reflected in lowered concentration of this peptide in cerebrospinal fluid (CSF) and its deposition in amyloid plaques in the brain (2-5). Concentrations of Aβ42 in CSF show high concordance with plaque burden at autopsy (6, 7) and with cortical amyloid ligand retention in positron emission tomography (PET) brain scans (8-11).

Two shorter forms of Aβ, Aβ40 and Aβ38, have also been measured in CSF by at least two different techniques: liquid chromatography with mass spectrometric detection or immunoassays (12-17). Similar to Aβ42, they are produced from the catabolism of Aβ precursor protein by the concerted actions of β-secretase (BACE1) and the γ-secretase protease complex (18). One hypothesis posits that the concentration of Aβ42 in the CSF depends not only on the pathophysiological Aβ status but also on the total amount of Aβ peptides present (19). By normalizing to the concentration of the most abundant in the CSF Aβ40, the ratio removes the potential confound of differences in overall Aβ concentration and provides a better index of underlying Aβ-related pathology. Recently, a number of studies have reported that adding the CSF Aβ42/Aβ40 ratio to diagnostic tools might: 1) improve prediction accuracy of amyloid plaque burden in subjects with mild cognitive impairment (MCI), 2) improve discrimination of AD from other forms of dementia and 3) increase the concordance between CSF and PET amyloidosis (8, 14, 19).

We developed a liquid chromatography tandem mass spectrometry method (LC-MSMS) for analysis of Aβ 42 in CSF (20) which has been recognized as a reference method by the JCTLM

and assigned the Identification Number : C12RMP1. This method was modified by adding two Aβ peptides, i.e. Aβ40 and Aβ38 as additional calibrators, and used for analysis of 1445 samples of CSF obtained from participants of the ADNIGO/2 projects. One lot of in–house calibrators was analyzed against CRM-based calibration curve and the resulting linear regression equation was used to get final, accuracy-based concentrations of Aβ42 for ADNI samples.

In this paper we: 1) present overall performance of our modified method and unique data for calibrators lot-to-lot reproducibility, 2) describe value transfer from CRMs to calibrators, 3) discuss the results of Aβ peptides in CSF obtained from ADNIGO/2 participants, 4) discuss the utility of Aβ42/Aβ40 ratio for improved detection of amyloid plaque burden measured with PET and therefore improved diagnosis of AD.

Materials and Methods

ADNI study participant data

CSF Aβ42, demographic, amyloid PET imaging and clinical diagnosis were obtained from the ADNI database (http://adniloni.usc.edu).

The ADNI study, with 59 sites across the United States and Canada, is an ongoing collaborative longitudinal study, launched in 2004 as a public–private partnership, across several phases (ADNI1, ADNIGO, ADNI2, currently ADNI3). The primary goal of ADNI has been to test whether serial magnetic resonance imaging, PET, other biological markers, clinical and neuropsychological assessments can be combined to measure the progression of MCI and used for the early diagnosis of AD. The ADNI principal investigator is Michael Weiner, MD, at VA MC and UCSF. For current ADNI information, see www.adni-info.org.

CSF samples obtained from ADNIGO/2 projects' participants (n=1445; ADNI2 n=1089, ADNIGO n=151 and ADNI1 n=205 as a part of longitudinal studies) were collected, processed

according to ADNI2 Procedure Manual (https://adni.loni.usc.edu/wp-content/uploads/2008/07/adni2-procedures-manual.pdf) and stored at -80°C. Only pristine aliquots, which underwent a single freeze-thaw cycle prior to assay, were used for analyses. Concurrent florbetapir amyloid PET results were available for 766 subjects (normal control (NC) n=149, MCI n=405, subjective memory complaints (SMC) n=87, AD n=125) (time interval of PET and LP ± 3 months for 762 participants, and between 98 and 154 days for 4 participants). Florbetapir (FBP) images consisted of 4X5min frames acquired at 50-70min post-injection which were realigned, averaged, resliced to a common voxel size ($1.5mm^3$), and smoothed to a common resolution of $8mm^3$ FWHM. MPRAGE images that were acquired concurrently with the baseline florbetapir images were used as a structural template to define cortical composite regions (frontal, cingulate, temporal, parietal) and whole cerebellum (white+grey matter) in native space for each individual using Freesurfer (v5.3.0) as described previously (21).

The baseline cortical summary florbetapir standardized value uptake ratios (SUVRs) were calculated by averaging across Freesurfer-defined cortical composite regional SUVR means, and dividing by the Freesurfer-defined whole cerebellum. An FBP positivity threshold of 1.11 was applied based on uptake in young, cognitively normal individuals (22) and which has also been autopsy-validated (23).

These studies were approved by the Institutional Review Boards, and written informed consent was obtained from all participants or authorized representatives at each site.

Chemicals and reagents

The method used for the current study is a modification of a previously validated LC-MSMS methodology (20). Therefore, we only describe the changes which were made to the previous protocol, and summarize the current procedure (Supplemental Table 1).

Reference standard and CRMs for CSF Aβ42 were obtained from EC-JRC-IRMM (Belgium). An assigned value for Aβ42 concentration in the reference standard was based on amino acid analysis (24). The concentration of Aβ42 for three CSF-based CRMs (450, 720 and 1220pg/mL, uncertainty 70, 110 and 180pg/mL, respectively) were obtained by LC-MSMS reference methods (20, 25). Two other Aβ peptides, Aβ40 and Aβ38 together with three internal standards, uniformly labelled with ^{15}N, Aβ42, Aβ40 and Aβ38, were purchased (rPeptide, USA). Two stock solutions of Aβ42 (500ng/mL and 50ng/mL), for calibrators and quality control (QC) samples spiking solutions, were prepared by diluting the solution of reference standard with DMSO and using an analytical balance to correct their final concentrations. This manner of preparation was necessary to assure reproducibility of results across different lots of calibrators when CRMs were not yet available. Each spiking solution for calibrators and QC samples contained 3 peptides at appropriate concentrations. Two different lots of calibrators were utilized for this project, #41717 (38 runs) and #92917 (32 runs).

The concentration of internal standards, 1ng/mL, is lower than in the original protocol due to the more sensitive mass spectrometer used in this study. In addition to three QC samples prepared in surrogate matrix (artificial CSF with 4% of BSA, [aCSF/*BSA*], Supplemental Table 1), five pools of human CSF served as biological controls. Four were prepared from CSF leftovers of ADNI1 subjects (pool 55, 56, 57, 58) and one from mixing residual CSF from discarded routine clinic patients at the hospital at the University of Pennsylvania (pool M).

Sample preparation and chromatography with mass spectrometric detection

There were no major changes in the sample preparation procedure (Supplemental Table 1) aside from reduction of volumes of some compounds. Since analysis of Aβ peptides was carried out on the more sensitive XEVO TQ-S mass spectrometer (Waters, USA), two changes were possible:

1) volume reduction of calibrators, QC and human CSF samples from 0.25 mL to 0.1 mL, and 2) injection volume decreased from 0.05mL to 0.025mL. The mass spectrometer interfaced with an AQUITY ultra performance liquid chromatograph (Waters) with: sample manager, two pumps and column oven, as previously described (20). Ion transitions for the 6 analytes (3 peptides and internal standards) are listed in Supplemental Table 1.

Study design

Imprecision and accuracy data using the current method for analysis of three Aβ peptides were collected during 70 runs, and completed on 5 pairs of columns; trap and analytical (Supplemental Table 1).

Eight samples were employed as QCs: three in surrogate matrix, aCSF/*BSA*, and five pools of human CSF. QC samples were analyzed in duplicate and six of them (3 in aCSF/*BSA* and 3 pools) were included in each analytical run.

The reference method for analysis of Aβ42 alone was modified for measurement of three Aβ peptides and re-validated by comparison with the reference method for analysis of Aβ42 alone (n=79 samples) and with the Elecsys® β-amyloid(1-42) immunoassay (Roche, Germany) (n=1439 samples).

We used CSF-based CRM, recently introduced by EC-JRC-IRMM, to establish the accuracy of Aβ42 concentrations in one, out of two lots of our in-house calibrators for the analysis of ADNIGO/2 samples. We applied the procedure of direct value transfer from CRM to in-house calibrators (26). Three CRMs, were used for calibration curve construction. Four pools of human CSF and three QC samples in aCSF/*BSA* were used for quality control assessments of these two runs in which the CRMs were used as calibrators. Seven Aβ42 calibrators, with concentrations of Aβ42 established by weight (C_A) were analyzed against the CRM-based calibration curve and

relative concentrations (C_R) of Aβ42 for all calibrators obtained. Linear regression analysis of C_A vs C_R resulted in a line which represents the relation of the concentrations of Aβ42 in the CRMs and calibrators. Target Aβ42 concentrations in our calibrators, C_T, were calculated from the regression equation:

$$C_T = \alpha \times C_R + b$$

where:

C_T- target concentration

α – the regression line slope

C_R – concentration of Aβ42 obtained from CRM calibration curve

b - the regression line intercept.

The equation was also used for recalculation of Aβ42 concentrations for ADNIGO/2 participants. New values for the Aβ42 cut off and concordance with FBP PET were obtained.

Statistical analyses

Data collected during this long term project were used for the following statistical analyses:

- long term assessment of imprecision and accuracy of measured concentrations of Aβ42, Aβ40 and Aβ38 in three QC samples prepared in aCSF/*BSA* and five pools of human CSF
- unique comparison of Aβ42 concentrations for three pools of human CSF analyzed using two different lots of in-house calibrators to evaluate lot-to-lot reproducibility
- comparison of Aβ42 concentrations obtained using the original reference method (Aβ42 alone) vs the modified method (three Aβ peptides)

- comparison of Aβ42 concentrations obtained using the modified method vs Aβ42 results obtained using Elecsys® β-amyloid (1-42) immunoassay
- assessment of the reference method stability over three years' time period based on the Aβ42 results for 46 pristine replicate aliquots analyzed in 2014 vs 2017.

This paper describes for the first time concentrations of CSF Aβ peptides by LC-MSMS for ADNIGO/2 subjects. An unpaired t-test was used to compare results between 5 clinically different groups of participants; NC, early MCI (EMCI), MCI, SMC and AD. These data were consequently used to examine agreement or disagreement with the reports that Aβ42/Aβ40 ratio improves concordance with amyloid PET and can improve the diagnosis of AD, by comparison of the concordance between FBP PET and CSF Aβ42 concentration vs the ratio Aβ42/Aβ40 for ADNIGO/2 subjects.

This is the first report of using CSF-based Aβ42 CRMs for amyloid beta 42 concentration value transfer to in-house calibrators.

Results

Analytical method evaluation

Imprecision and accuracy - for all three Aβ peptides inter-assay imprecision (%CV) for all but one control (10.2 %CV) was below 10% (Supplemental Table 2). The mean imprecision for duplicate analyses of the CSF samples was 4.5% (Aβ42), 3.0% (Aβ40) and 3.6% (Aβ38).

The accuracy for all 3 Aβ peptides for controls in aCSF/*BSA* was excellent, from 97.5% to 103.1%.

Lot-to lot reproducibility - three out of five pools of human CSF were analyzed using two different lots of in-house calibrators and based on this unique data we estimated between-lot

reproducibility. No statistically significant differences in Aβ42 concentrations were obtained across two different lots of calibrators (p=0.767, 0.256 and 0.45 for each pool) (Figure 1).

Method comparisons - the correlation between different methods was assessed by Deming regression (Microsoft R v 3.3.1 with Meth Comp v 1.22.2)(27). Aβ42 concentrations measured by the reference method (single analyte) and the modified reference method (triple analytes) showed a linear relationship with a correlation coefficient r^2=0.96, a slope of 0.999 (y = 0.999x + 13.46) and a mean error of 2.22% (n = 79) (Figure 2A).

The Deming regression plot between an established, highly automated method, Elecsys® β-amyloid(1-42) immunoassay (28) and our modified reference method showed also a linear relationship (y = 1.02x + 52.8) with r^2 of 0.92 and mean error of 9.27% (n =1439) (Figure 2B).

Method stability – Deming regression between two groups of results (from 2014 and 2017) showed great stability of our method over 3 years: correlation coefficient r^2=0.93 and the mean error of 5% (Figure 3).

Standards accuracy check against Aβ42 CRMs – For human CSF pools used to assess quality of the runs where in-house calibrators were analyzed against CRM-based calibration curve, accuracy was 96.1%-103.6%, and for the aCSF/*BSA* controls the mean accuracy was 94±3%. Linear regression analysis established a line y = 0.89x + 32.6, (Supplemental Figure 1); all calibrators' concentration of Aβ42 were recalculated to the new target values according to this equation.

This equation was also used to recalculate Aβ42 concentrations for ADNIGO/2 participants and these new values were used for assessment of the Aβ42, Aβ42/Aβ40 ratio cut offs and concordance with FBP PET (Figure 4).

Clinical utility of the method

CSF biomarkers for ADNIGO/2 samples, data overview

The concentrations of Aβ42, Aβ40 and the ratio of Aβ42/Aβ40 in all BASELINE CSF samples from ADNIGO/2 subjects are summarized in Table 1. The recalculated concentration values (CRM adjusted) for Aβ42 for ADNIGO/2 participants will be uploaded on the ADNI website in the near future.

Statistical analysis of our data revealed that concentrations of Aβ42 are significantly lower in the AD (n=130), MCI (n=171) and EMCI (n=268) groups when compared with NC (n=177), as expected ($p<0.0001$, $p<0.0001$ and $p<0.05$, respectively). In addition, Aβ42 concentrations are significantly lower in AD vs MCI, EMCI and SMC (n=95) ($p<0.0001$). The concentrations of Aβ40 in AD and MCI, but not in EMCI ($p=0.389$), are also significantly lower compared to NC ($p<0.005$, $p<0.05$). Furthermore, Aβ40 concentrations are significantly lower in AD vs EMCI and SMC ($p<0.05$ and $p<0.005$, respectively) but not vs MCI ($p=0.232$).

The values of the Aβ42/Aβ40 ratio in AD and MCI but not in EMCI are significantly lower when compared with NC. In AD the ratio Aβ42/Aβ40 is significantly lower than the MCI, EMCI and SMC groups ($p<0.0001$).

There was no difference between Aβ42, Aβ40 and the Aβ42/Aβ40 ratios between subjects in the NC vs SMC ($p=0.601$, 0.773 and 0.721, respectively), a finding consistent with a previous report using an automated immunoassay (11).

Concordance between amyloid PET and concentration of Aβ peptides in CSF

The relationship between CSF biomarkers and cortical florbetapir SUVRs are shown in Figure 4. Based on this first-time analysis of data obtained from ADNIGO/2 participants by LC-MSMS reference method, concordance for Aβ42 and florbetapir-PET was 81% and for CSF Aβ42/Aβ40 ratio the concordance increased to 88%.

Mixture Modeling analyses of Aβ42 concentration and Aβ42/Aβ40 ratio values provided the following cut-point values: 1096pg/mL (Aβ42) and 0.138 (Aβ42/Aβ40). ROC analysis using amyloid PET as the standard of truth afforded cut-off values of 992.9pg/mL and 0.124 (Aβ42 and Aβ42/Aβ40, respectively) (Supplemental Figures 2-3).

Frequency distribution histogram plots of Aβ42 concentration and the Aβ42/Aβ40 ratio for the 766 participants of ADNIGO/2 with cortical Aβ deposition, measured by florbetapir PET, are presented on Figure 5. These plots show two overlapping distributions, PET-positive and PET-negative amyloid deposition. The Aβ42/Aβ40 ratio clearly better separates PET (+) from PET (-) participants, than Aβ42 alone.

Discussion

In this paper we describe the analytical and clinical performance of a modified reference procedure for the analysis of Aβ peptides in CSF by LC-MSMS. We present data for distribution of Aβ peptides and the Aβ42/Aβ40 ratio for participants of ADNIGO/2 projects and based on statistical analyses we discuss the potential utility of the Aβ42/Aβ40 ratio for improved diagnosis of AD.

We also describe the procedure for using Aβ42 CRMs for assignment of target values for Aβ42 concentrations for in-house calibrators.

This analysis of three Aβ peptides in CSF was used for almost 5 months in 2017, employed 5 pairs of columns, analytical and trapping, and two lots of in-house calibrators. Four batches of samples, calibrators and QCs were analyzed weekly and the entire system was continuously operated Monday-Friday without any need for in-between-run cleaning. This observation highlights the effectiveness of sample preparation and robustness of the entire system.

This long term project permitted collection of a large dataset and based on this we can report that this procedure has very good characteristics with respect to imprecision, accuracy and precision between duplicate measurements for all three Aβ peptides. Concentrations of Aβ42 obtained by the modified method correlate very well with the results obtained using both, the reference method for Aβ42 alone (slope 0.999, $r^2 = 0.96$), and Elecsys® β-amyloid (1-42) immunoassay (29) (slope 1.02, $r^2 = 0.92$). There is an urgent need to harmonize the assays across different platforms and this finding demonstrates the feasibility for success in this effort. Furthermore earlier studies documented the commutability of CSF-based reference materials (24, 30). Final assignment of accuracy-based values for one lot of our standards used in ADNIGO/2 project, was performed using CSF-based Aβ42 CRM. The new values of Aβ42 concentrations for ADNIGO/2 subjects were obtained and will be uploaded on LONI webpage in the near future.

The availability of validated reference methods, CRMs for Aβ42 in CSF and a practical effective procedure for target value assignment are key elements that underpin the prospect for successful global standardization of assays used for the measurement of CSF Aβ42.

In this paper for the first time we described the reproducibility data for Aβ42 concentration in CSF pools analyzed with two different lots of in-house calibrators and showed lack of statistically significant differences between Aβ42 concentrations across these two lots of calibrators. Since the stock solutions for Aβ42 calibrators were prepared using an analytical balance for weighing both, the primary standard material and diluent, and the final concentrations were corrected based on the obtained weight, we concluded that using the analytical balance is mandatory for sustaining reproducibility between different lots of calibrators. This observation is crucial at a time when the efforts on developing reference systems for CSF biomarker measurements are in progress (31-33).

Forty-six samples had two Aβ42 concentration results; first from 2014 while we were analyzing baseline samples of ADNI1 subjects and the second from the current project, as a result of our decision to include replicate aliquots for these samples as part of longitudinal study. We used these data to assess long term method stability and the result of this evaluation is excellent (slope 1.03, $r^2 = 0.93$). Lack of difference between the results from 2014 vs 2017 additionally supports our documentation of lot-to-lot reproducibility.

In the clinical section of this study we describe for the first time profiles of Aβ peptides in CSF for 1445 participants of the ADNIGO/2 study and provide the incidence of Alzheimer's pathologic change, defined as decreased CSF Aβ42 concentration, or positive amyloid PET imaging test (34) across the AD, MCI, EMCI, SMC and NC subgroups.

The CSF levels of Aβ40 for the AD and MCI group were also significantly lower compared to NC subjects, while there was no statistically significant difference in CSF Aβ40 concentration between AD and MCI. Decreased CSF levels of Aβ40 together with a discussion about the possible mechanisms of that change such as reduced neuronal numbers and/or viability were previously reported for AD and non-AD patients when compared to controls (35), frontotemporal dementia subjects (36), vascular dementia cases and dementia with Lewy bodies (37). In other studies that examined CSF levels of Aβ40 in AD and NC, no significant differences were found (38) or Aβ40 concentrations in the AD-MCI group turned out to be significantly higher compared to the controls (15). Results are not consistent and more work is required on Aβ40, paying special attention to classification of subjects and taking into consideration developing Aβ40 reference material and method standardization.

As reported in previous studies the CSF Aβ42/Aβ40 ratio is a better predictor of brain amyloid deposition in prodromal AD than CSF Aβ42 alone and better differentiates AD dementia from

non-AD dementias (8, 14, 19, 35, 39). Based on our finding in 766 ADNIGO/2 participants of improved concordance with PET from 81% to 88% we can confirm these reports. Comparable concordance results were obtained using cutoffs based on ROC analysis (83% and 89% concordance values respectively). Our method measures both peptides, Aβ42 and Aβ40 from the same sample minimizing methodological variability as a source of discordance between CSF and cortical amyloid. We suggest that these two peptides should be both measured and used for amyloid burden detection in the diagnosis of AD. For our group of subjects the number of cases with abnormal/low Aβ42 and normal PET (Figure 4A; lower left quadrant) was higher than the number of cases with normal Aβ42 and abnormal PET (Figure 4A; upper right quadrant), consistent with previous reports (19). When Aβ42/Aβ40 ratio was used as a diagnostic tool the number of cases with abnormal/low Aβ42 and normal PET decreased by 43 % (42 cases were moved to lower right quadrant; normal Aβ42 and normal PET) (Figure 4B), and the number of cases with normal Aβ42 and abnormal PET dropped by 32% (16 cases were moved to upper left quadrant; abnormal Aβ42 and abnormal PET) (Figure 4B). Thus using Aβ42/Aβ40 ratio improved diagnostic accuracy for 7.6% of participants. An hypothesis-driven explanation that the concentration of Aβ42 in the CSF depends not only on the amyloid status of a given subject but also on the total amount of the Aβ peptides present has been described elsewhere (40).

In conclusion, the current study documents the long term analytical performance and substantiates the robustness of our modified LC-MSMS reference method. We highlighted the needs for: 1) use of an analytical balance to maintain reproducibility between different lots of calibrators, 2) developing CRMs for Aβ40 and 3) supporting the standardization process with the currently available three CRMs for Aβ42 in CSF. From the clinical diagnostic perspective, these

results for ADNIGO/2 participants show that the Aβ42/Aβ40 ratio improves concordance with amyloid PET.

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Table 1. The results of CSF biomarkers (Aβ42, Aβ40 and Aβ42/Aβ40) at BASELINE for ADNIGO/2 participants.

ADNIGO/2 participants	Aβ42 (pg/mL) mean±SD	Aβ40 (pg/mL) mean±SD	Aβ42/Aβ40 mean±SD	n
Normal (NC)	1303±573	8718±2555	0.149±0.05	177
EMCI	1167±566	8506±2518	0.138±0.05	268
MCI	915±434	8176±2195	0.111±0.05	171
AD	751±397	7841±2548	0.096±0.03	130
SMC	1342±581	8811±2488	0.151±0.05	95
t-test values. Aβ42: p<0.0001, <0.0001 and <0.05 comparing NC to AD, MCI and EMCI respectively; p<0.0001 for AD vs MCI, EMCI and SMC. Aβ40: p<0.005, <0.05 and p=0.389 for NC vs AD, MCI and EMCI, respectively; p<0.05, <0.005 and p=0.232 for AD vs EMCI, SMC and MCI, respectively; Aβ42/Aβ40: p<0.0001, <0.0001, <0.05 for NC vs AD, MCI and EMCI, respectively; p<0.0001 for AD vs MCI, EMCI and SMC. For NC vs SMC, p=0.601, 0.773 and 0.721 for Aβ42, Aβ40 and Aβ42/Aβ40, respectively.				

MCI – mild cognitive impairment
EMCI - early MCI
SMC - subjective memory complaints
AD – Alzheimer's disease

Figure Captions

Figure 1. In-house calibrators lot-to-lot reproducibility of Aβ42 concentration for 3 pooled CSFs (#57, 58 and M).
For pools 57 and 58 twenty- seven runs were completed with lot #41717,
and thirty-two with lot #92917, for pool M eighteen and fifteen, respectively.

Figure 2. (A) Methods comparison of Aβ42 concentration by modified method for simultaneous analysis of three abeta peptides vs reference method for analysis of Aβ42 alone (n=79),

and (B) Aβ42 concentration by modified LC-MS-MS method for simultaneous analysis of three abeta peptides vs Elecsys immunoassay (n=1439).

Figure 3. Comparison of Aβ42 concentration by modified method for simultaneous analysis

of three abeta peptides performed in 2017 and 2014 (n=46).

Figure 4. Scatterplots of florbetapir amyloid PET and CSF Aβ42 (A) and Aβ42/Aβ40 ratio (B). Vertical lines represent cutoff values for Aβ42 (1096pg/mL) and Aβ42/Aβ40 ratio (0.138) determined by mixture-modeling (Supplemental Figure 2) . Based on baseline Aβ42 concentration and concurrent florbetapir amyloid PET the concordance was 81%. When the CSF Aβ42/Aβ40 ratio was utilized we observed an increase of concordance to 88%. (light green – NC, dark green – SMC, light blue – EMCI, dark blue – MCI, red – AD).

Figure 5. Frequency distribution histogram plots of Aβ42 (A) and Aβ42/Aβ40 ratio (B) of ADNIGO/2 subjects with cortical amyloid beta deposition measured by florbetapir PET (n=766).

The red curves are locally estimated scatterplot smoothing (LOESS) regression plots of the CSF Aβ42(A) or A42/A40(B) frequency distributions for participants whose florbetapir PET SUVR values were positive (>1.11) and the blue LOESS plots are for participants whose florbetapir PET SUVR values were negative (<1.11). Visual inspection shows that the ratio better separates PET positive from PET negative subjects than Aβ42 alone, a finding consistent with concordance improvement for the ratio.