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

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BACKGROUND: Cerebrospinal fluid (CSF) amyloid-β_{1-42} (Aβ42) reliably detects brain amyloidosis based on its high concordance with plaque burden at autopsy and with amyloid positron emission tomography (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.

METHODS: An LC–MS/MS reference method for Aβ42, modified by adding Aβ40 and Aβ38 peptides to calibrators, was used to analyze 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. Aβ42 calibrators' accuracy was established using CSF-based Aβ42 Certified Reference Materials (CRM).

RESULTS: CRM-adjusted Aβ42 calibrator concentrations were calculated using the 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 2 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 our modified LC–MS/MS reference method for 3 Aβ peptides. The improved diagnostic performance of the CSF ratio Aβ42/Aβ40 suggests that Aβ42 and Aβ40 should be measured together and supports 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 disease (AD) *(1)*. Pathological changes of Aβ42 are reflected in lowered concentrations in cerebrospinal fluid (CSF) and its deposition in amyloid plaques in the brain *(2–4)*. CSF Aβ42 concentrations show high concordance with plaque burden at autopsy *(5, 6)* and cortical amyloid ligand retention in positron emission tomography (PET) brain scans *(7–10)*.

Two shorter Aβ forms, Aβ40 and Aβ38, have also been measured in CSF by liquid chromatography with tandem mass spectrometric (LC–MS/MS) detection or immunoassays *(11–15)*. Similar to Aβ42, they are produced by Aβ precursor protein catabolism by the concerted actions of β-secretase (BACE1) and the γ-secretase protease complex *(16)*. One hypothesis posits that the concentration of Aβ42 in CSF depends on the

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A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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total amount of Aβ peptides present in addition to the pathophysiological Aβ status *(17)*. By normalizing to the concentration of Aβ40, the most abundant in the CSF, the ratio normalizes the differences in overall Aβ concentration, providing a better index of Aβ-related pathology. Recently, several studies reported that adding the CSF Aβ42/Aβ40 ratio to diagnostic tools might: (*a*) improve prediction accuracy of amyloid plaque burden in patients with mild cognitive impairment (MCI), (*b*) improve discrimination of AD from other forms of dementia, and (*c*) increase the concordance between CSF and PET amyloidosis *(7, 13, 17)*.

We developed an LC–MS/MS method for Aβ42 analysis in CSF *(18)*. This published method was subsequently transferred to more sensitive mass spectrometer, fully validated and recognized as a reference method by the JCTLM (ID no. C12RMP1). Full method validation included suitability assessment of a surrogate matrix for calibrator preparation and an interlaboratory study in addition to fundamental parameters like accuracy, precision, sensitivity, and selectivity. This reference method was modified by adding 2 Aβ peptides, Aβ40 and Aβ38, as additional calibrators, and used for analysis of 1445 CSF samples from the ADNIGO/2 projects. One lot of in-house calibrators was analyzed against CRM-based calibration curve and the resulting linear regression equation used to obtain accuracy-based concentrations of Aβ42 for ADNI samples.

In this paper we: (*a*) present the overall performance of our modified reference method and unique data for calibrators' lot-to-lot reproducibility, (*b*) describe value transfer from CRMs to calibrators, (*c*) discuss the results of Aβ peptides in ADNIGO/2 participants CSF, and (*d*) discuss the utility of the Aβ42/Aβ40 ratio for improved detection of amyloid plaque burden measured with PET.

Materials and Methods

ADNI STUDY PARTICIPANT DATA

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database *(19)*. The ADNI was launched in 2004 as a public-private partnership, led by principal investigator, Michael W. Weiner, MD and has undergone several phases (ADNI1, ADNIGO, ADNI2, and currently ADNI3). The primary goal of ADNI is to test whether serial magnetic resonance imaging, PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI to early AD. Clinical diagnoses were based on the absence (NC) or presence of a significant memory concern (EMCI, MCI, SMC, AD) together with meeting cut-off scores for the Mini-Mental State Examination (MMSE), Clinical Dementia Rating, and Logical Memory tests as defined in the ADNI2 protocol *(20)*. CSF samples obtained from ADNIGO/2 participants ($n = 1445$; ADNI2 $n = 1089$, ADNIGO $n = 151$, and ADNI1 $n = 205$ as part of longitudinal studies) were collected, processed according to ADNI2 Procedure Manual *(20)* and stored at −80°C. The range of storage times for CSF samples varied from 0.39 to 11.32 years (mean ± SD: 4.91 ± 2.07 years). Only aliquots which underwent a single freeze-thaw cycle prior to assay, were analyzed. Concurrent florbetapir amyloid PET results were available for 766 participants: 149 normal control (NC), 405 MCI, 87 subjective memory complaints (SMC), 125 AD (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 4 × 5min frames acquired at 50–70 minutes postinjection that were realigned, averaged, resliced to a common voxel size (1.5 mm^3), and smoothed to a common resolution of 8 mm^3 full-width at half maximum. MPRAGE images, acquired concurrently with baseline florbetapir images, were used as a structural template to define cortical composite regions (frontal, cingulate, temporal, parietal) and whole cerebellum (white + gray matter) in native space for each individual using Freesurfer (v.5.3.0) *(21)*.

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 was autopsy-validated *(23)*.

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

CHEMICALS AND REAGENTS

The method used in this study is a modification of the published LC–MS/MS methodology *(18)* and of the JCTLM reference method, suitable for analysis of 2 additional Aβ peptides, Aβ40 and Aβ38. Therefore, we describe the changes made to the previously published protocol, and JCTLM reference method and summarize it in online Supplemental Table 1.

CSF Aβ42 reference standard and CRMs were obtained from EC-JRC-IRMM (Belgium). An assigned value for Aβ42 concentration in the reference standard was based on amino acid analysis *(24)*. Aβ42 concentrations in 3 CSF-based CRMs (450, 720, and 1220 pg/mL; uncertainty 70, 110, and 180 pg/mL, respectively) were measured by LC–MS/MS reference methods *(18, 25)*. Aβ peptides, Aβ40 and Aβ38 (their concentrations

established by amino acid analysis [personal communication]) together with 3 internal standards, Aβ42, Aβ40, and Aβ38, uniformly labeled with ^{15}N, were purchased (rPeptide). Two stock solutions of each Aβ peptide (500 and 50 ng/mL), for calibrators and quality control (QC) sample spiking solutions, were prepared by diluting the reference standard solution with DMSO and using an analytical balance to correct their final concentrations. The necessity of calibrator preparation on the balance was based on the experiment where 2 groups of calibrators used to measure Aβ42 concentrations in 3 pooled CSF samples were prepared with and without an analytical balance. For this experiment fresh lot of calibrators was prepared each day ($n = 3$ days) and each sample was analyzed 3 times per day against 2 different sets of calibrators (prepared with and without the analytical balance).

Calibrators and QC samples used for analysis of ADNIGO/2 samples were prepared on the day of analysis in surrogate matrix by spiking 0.95 mL of the matrix (artificial CSF with 4% of BSA, [aCSF/*BSA*], online Supplemental Table 1) with 0.05 mL of spiking solution. Further details about calibrators/QC samples preparation are in online Supplemental Table 1 and in our previous paper *(18)*. Each spiking solution for calibrators and QC samples contained 3 peptides at appropriate concentrations. Two different lots of calibrators were utilized for this project, no. 41717 (38 runs) and no. 92917 (32 runs).

Internal standards concentrations, 1 ng/mL, are lower than in the original protocol due to the more sensitive mass spectrometer used in this study. In addition to 3 surrogate matrix-QC samples, 5 pools of human CSF served as biological control samples.

SAMPLE PREPARATION AND CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION

There were no major changes in the sample preparation procedure (online 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), 2 changes were possible: (*a*) volume reduction of calibrators, QC and human CSF from 0.25 to 0.1 mL, and (*b*) injection volume decreased from 0.05 to 0.025 mL. The mass spectrometer was interfaced with an ACQUITY ultra performance liquid chromatograph (Waters), sample manager, 2 pumps, and column oven, as previously described *(18)*. Online Supplemental Table 1 summarizes ion transitions for the 3 peptides, internal standards, and 2 qualifier ion transitions.

STUDY DESIGN

Aβ peptide imprecision and accuracy data were collected during 70 runs, and completed on 5 pairs of trap and analytical columns (online Supplemental Table 1). QC samples (3 in aCSF/*BSA* and 3 pools) were analyzed in duplicate in each analytical run.

The modified reference method suitable for measurement of 3 Aβ peptides was 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) ($n = 1439$ samples).

We used CSF-based CRM, from EC-JRC-IRMM, to establish the accuracy of Aβ42 concentrations in one lot of our in-house calibrators for analysis of ADNIGO/2 samples. In 2 replicate runs, 7 Aβ42 calibrators with Aβ42 concentrations established by weight (C_A) were analyzed against the CRM-based calibration curve and relative concentrations (C_R) of Aβ42 for all calibrators obtained by direct value transfer methodology *(26)*. Linear regression analysis of C_A vs C_R resulted in a line that represents the relation of the concentrations of Aβ42 in the CRMs and calibrators. Target Aβ42 calibrator concentrations, C_T, were calculated from the regression equation:

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

where: C_T is the target concentration, α is the regression line slope, C_R – concentration of Aβ42 obtained from CRM calibration curve, b is 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

Statistical analyses performed on the data collected during this long-term project include:

- assessment of imprecision and accuracy of measured concentrations of A*β*42, A*β*40, and A*β*38 in 3 QC samples prepared in aCSF/*BSA* and 5 pools of human CSF
- comparison of A*β*42 concentrations for 3 human CSF pools analyzed using 2 different lots of in-house calibrators to evaluate lot-to-lot reproducibility
- comparison of A*β*42 concentrations obtained using the reference method (A*β*42 alone) vs the modified method (3 A*β* peptides)
- comparison of A*β*42 concentrations obtained using the modified method vs A*β*42 results from the Elecsys *β*-amyloid (1-42) immunoassay
- assessment of the reference method stability based on A*β*42 results for 46 replicates of one-freeze–thaw-cycle aliquots analyzed in 2014 vs 2017
- comparison of results between the 5 clinical cohorts: NC, early MCI (EMCI), MCI, SMC, and AD by unpaired *t*-test
- comparison of the concordance between FBP PET and CSF A*β* comparison of the concordance A*β*42/A*β*40 for ADNIGO/2 participants.

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

Results

ANALYTICAL METHOD EVALUATION

Imprecision and accuracy

For all 3 Aβ peptides interassay imprecision (CV) for all but one control participant (10.2 CV) was below 10% (online Supplemental Table 2). Importantly, for Aβ42 concentrations below the cut-off value of approximately 1000 pg/mL, the CV was between 7.4 and 7.6% (based on QC 2 and Pool 58 with Aβ42 concentrations of 778 and 935 pg/mL, respectively). Mean imprecision expressed as CV for duplicate analyses of the CSF samples was 4.5% (Aβ42), 3.0% (Aβ40), and 3.6% (Aβ38).

Accuracy for all 3 Aβ peptides for control participants in aCSF/*BSA* was excellent, 97.5 to 103.1%. More details of the Aβ40 method validation studies are in the online Supplemental Data.

Lot-to-lot reproducibility

No statistically significant differences in Aβ42 concentrations were found across 2 different lots of calibrators ($P = 0.767$, 0.256, and 0.45 for each of 3 CSF pools) (Fig. 1). Our calibrators were prepared using an analytical balance since this preparation technique resulted in lower between days ($n = 3$) CV values for Aβ42 concentrations in 3 human CSF pools compared to the data obtained using calibrators prepared without analytical balance (3.3, 2.0, and 3.1% vs 7.4, 4.6, and 6.4%, respectively) (online Supplemental Table 3). This preparation procedure assured reproducibility of results across different lots of calibrators.

Method comparisons

Aβ42 concentrations measured by the reference method (single analyte) and the modified reference method (triple analytes) showed a linear relationship, by Deming regression *(27)* with a correlation coefficient $r^2 = 0.96$, slope of 0.999 ($y = 0.999x + 13.46$), and mean error of 2.22% ($n = 79$) (Fig. 2A).

The regression plot between a highly automated method, Elecsys β-amyloid(1-42) immunoassay *(28)* and CRM-adjusted results also showed a linear relationship ($y = 0.913x + 73.63$) with r^2 of 0.92 and mean error of 1.30% ($n = 1439$) (Fig. 2B).

Method stability

Deming regression between 2 groups of results (2014 and 2017) showed excellent stability of our method over 3 years: correlation coefficient $r^2 = 0.93$ and mean error of 5% (Fig. 3). More details of the Aβ40 method validation studies are in the online Supplemental Data.

Standards accuracy check against A*β*42 CRMs

Accuracy was 96.1–103.6% for CSF pools that assessed run quality where in-house calibrators were analyzed

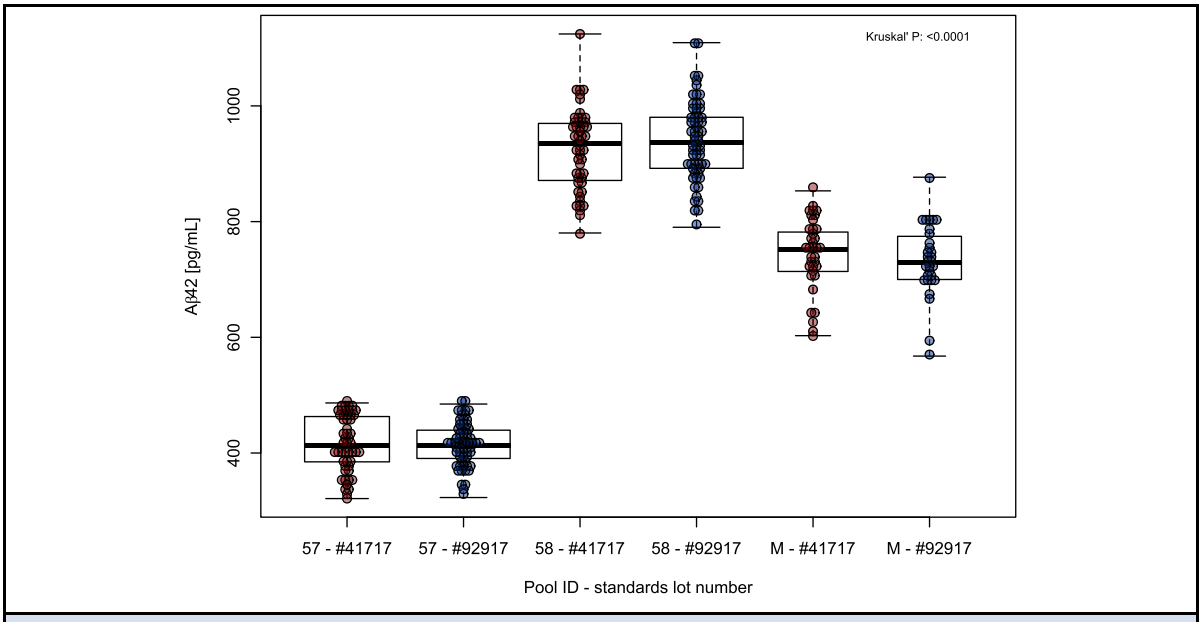

Fig. 1. In-house calibrators lot-to-lot reproducibility of Aβ42 concentration for 3 pooled CSFs (no. 57, 58, and M). For pools 57 and 58, 27 runs were completed with lot no. 41717, and 32 with lot no. 92917, for pools M, 18 and 52, respectively.

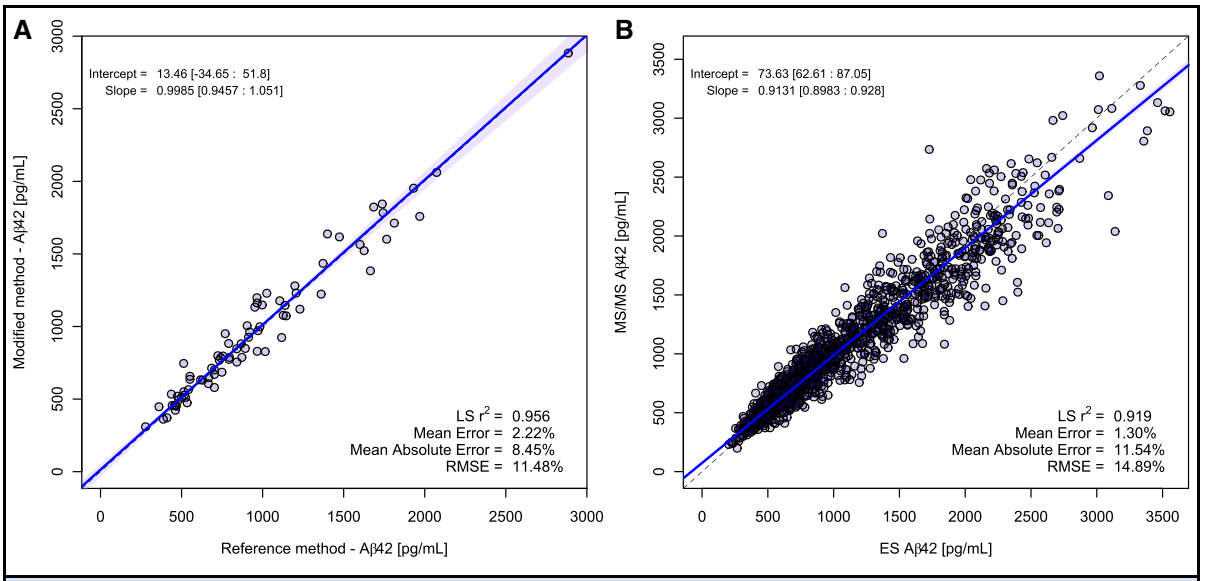

Fig. 2. (A) Methods comparison of Aβ42 concentration by modified method for simultaneous analysis of 3 Aβ peptides vs reference method for analysis of Aβ42 alone (β = 79), and (B) Aβ42 concentration by modified LC-MS/MS method for simultaneous analysis of 3 Aβ peptides vs Elecsys immunoassay (ES) ($n = 1439$).

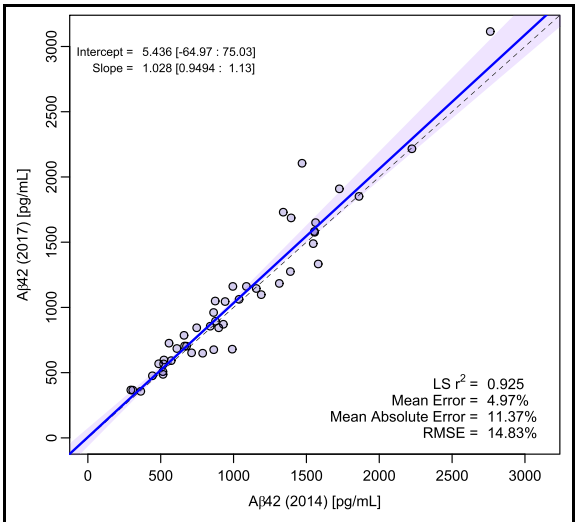

Fig. 3. Comparison of Aβ42 concentration by modified method for simultaneous analysis of 3 Aβ peptides performed in 2017 and 2014 ($n = 46$).

against CRM-based calibration curve; mean accuracy was 94 ± 3% for the aCSF/*BSA* controls. Linear regression analysis established a line $y = 0.89x + 32.6$ (online Supplemental Fig. 1); all calibrator concentrations 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 to assess the Aβ42, Aβ42/Aβ40 ratio cut offs, and concordance with FBP PET (Fig. 4).

CLINICAL UTILITY OF THE METHOD

CSF biomarkers for ADNIGO/2 samples, data overview

Concentrations of Aβ42, Aβ40, and the ratio Aβ42/Aβ40 in all ADNIGO/2 participant BASELINE CSF samples are summarized in Table 1. Statistical analyses revealed that Aβ42 concentrations were significantly lower in the AD ($n = 130$), MCI ($n = 171$), and EMCI ($n = 268$) groups compared with NC ($n = 177$), as expected ($P < 0.0001$, $P < 0.0001$, and $P < 0.05$, respectively). In addition, Aβ42 concentrations were significantly lower in AD vs MCI, EMCI, and SMC ($n = 95$) ($P < 0.0001$). Aβ42 concentrations in AD and MCI, but not in EMCI ($P = 0.389$), were also significantly lower compared to NC ($P < 0.005$, $P < 0.05$). Furthermore, Aβ40 concentrations were significantly lower in AD vs EMCI and SMC ($P < 0.05$ and $P < 0.005$, respectively) but not vs MCI ($P = 0.232$).

Values of the Aβ42/Aβ40 ratio in AD and MCI but not in EMCI were significantly lower compared with NC. In AD the ratio Aβ42/Aβ40 was significantly lower than the MCI, EMCI, and SMC groups ($P < 0.0001$).

There was no difference between Aβ42, Aβ40, and the Aβ42/Aβ40 ratios in the NC vs SMC ($P = 0.601$,

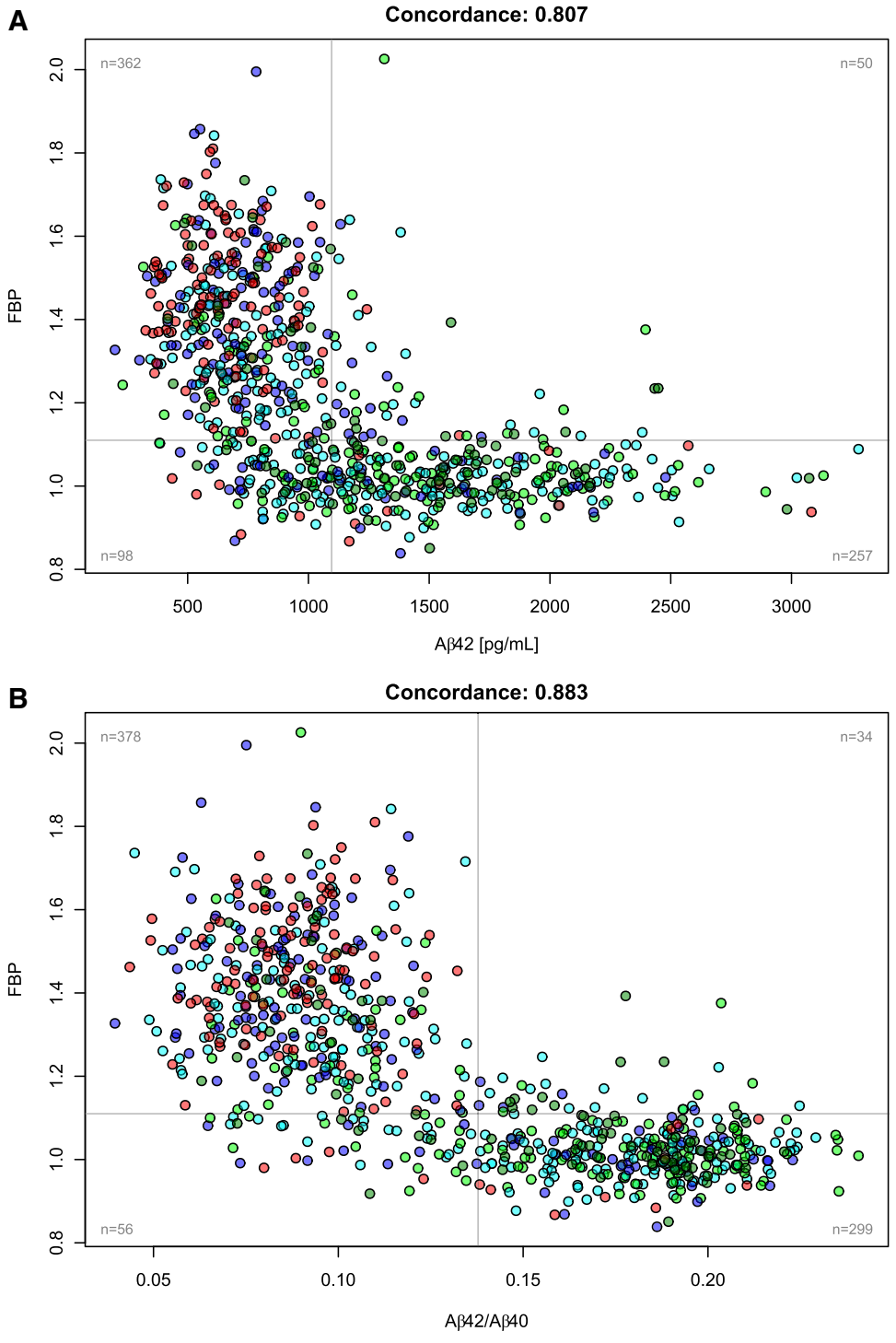

Fig. 4. Scatterplots of florbetapir amyloid PET and CSF Aβ42 (A) and Aβ42/Aβ40 ratio (B). Vertical lines represent cut-off values for Aβ42 (1096 pg/mL) and Aβ42/Aβ40 ratio (0.138) determined by mixture-modeling (Supplemental Fig. 2). Based on baseline Aβ42 concentration and concurrent florbetapir amyloid 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).

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.
Abbreviations: EMCI–early MCI, SMC–subjective memory complaints.

0.773, and 0.721, respectively), consistent with a previous report using an automated immunoassay *(10)*.

Concordance between amyloid PET and concentration of Aβ peptides in CSF

The relationships between CSF biomarkers and cortical florbetapir SUVRs are shown in Fig. 4. Based on this first-time analysis of ADNIGO/2 participant data by LC–MS/MS reference method, the concordance for Aβ42 and florbetapir PET was 81%, and increased to 88% for the CSF Aβ42/Aβ40 ratio.

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

Frequency distribution histogram plots of Aβ42 concentration and the Aβ42/Aβ40 ratio in 766 participants of ADNIGO/2 with cortical Aβ deposition, measured by florbetapir PET, are presented in Fig. 5. These plots show 2 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 analysis of Aβ peptides in CSF by LC–MS/MS. We present data for the distribution of Aβ peptides and the Aβ42/Aβ40 ratio for ADNIGO/2 participants and based on statistical analyses we discuss the potential utility of the Aβ42/Aβ40 ratio for improved detection of amyloid pathology, which is important for accurate diagnosis of AD. We focused on Aβ42, Aβ40, and their ratio, however, our modified method can assess the possible use(s) of Aβ38 measurements in future studies. We also describe the procedure using Aβ42 CRMs for assignment of target values of Aβ42 concentrations for in-house calibrators.

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

Based on this long-term experience we report that this procedure has very good characteristics for imprecision, accuracy, and duplicate measurement precision for all three Aβ peptides. Concentrations of Aβ42 obtained by the modified method correlate very well with 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 0.913, $r^2 = 0.92$). The Elecsys β-amyloid (1-42) immunoassay calibrators were standardized to the same primary Aβ42 standard material we used in this and another interlaboratory study *(24)* and this manufacturer worked collaboratively with others to study the commutability of CSF-based reference materials. These studies were of fundamental value to the work of producing the now-available CRMs *(24, 30)*. There is an urgent need to harmonize assays across different platforms and this finding demonstrates the feasibility for success in this effort. In this paper, for the first time we describe reproducibility data for Aβ42 concentration in CSF pools analyzed with two different lots of in-house calibrators. 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 corrected based on the obtained weight since, as described in Results,

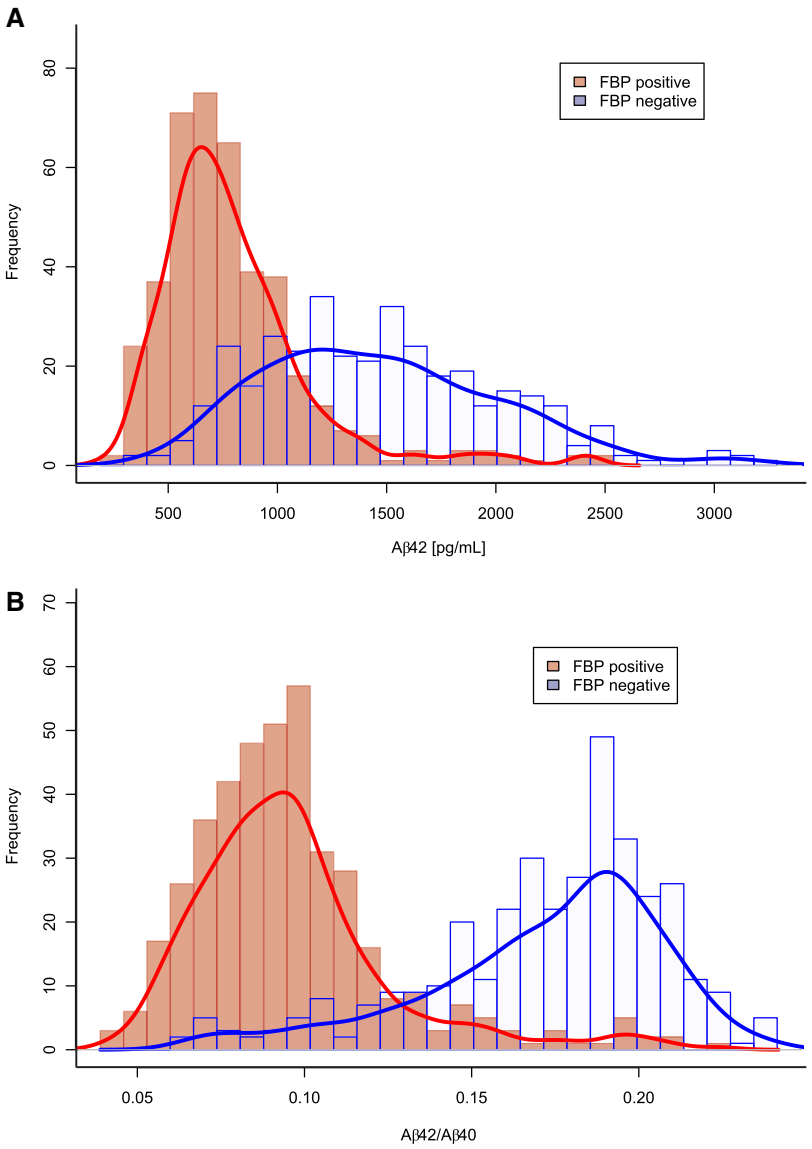

Fig. 5. Frequency distribution histogram plots of Aβ42 (A) and Aβ42/Aβ40 ratio (B) of ADNIGO/2 participants with cortical amyloid beta deposition measured by florbetapir PET ($n = 766$). The red curves are locally estimated scatterplot smoothing (LOESS) regression plots of the CSF Aβ42 (A) or Aβ42/Aβ40 (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 participants than Aβ42 alone, a finding consistent with concordance improvement for the ratio.

we demonstrated that using an analytical balance improved reproducibility between different calibrator lots. This observation is critical at a time when efforts on developing reference systems for CSF biomarker measurements are in progress *(31–33)*.

Forty-six samples had two Aβ42 concentration results, first from analyses in 2014 ADNI1 participant samples and the second from the current project that included replicate aliquots for these samples as part of a longitudinal study. These data provided strong support for long-term method stability (slope 1.03, $r^2 = 0.93$). Lack of difference between the results from 2014 vs 2017 additionally supports documentation of lot-to-lot reproducibility and CSF sample stability.

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

The CSF concentration of Aβ40 for the AD and MCI group were also significantly lower compared to NC participants, while there was no statistically significant difference in CSF Aβ40 concentration between AD and MCI. Decreased CSF concentration 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 patients with AD and non-AD patients when compared to control participants *(35)*, and patients with frontotemporal dementia *(36)*, vascular dementia, and dementia with Lewy bodies *(37)*. Other studies examined CSF concentrations of Aβ40 in AD and NC, but either found no significant differences *(38)* or Aβ40 concentrations in the AD-MCI group turned out to be significantly higher compared to the control participants *(14)*. More work is therefore required on Aβ40 paying special attention to classification of participants and development of Aβ40 reference material and method standardization.

As previously reported 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 *(7, 13, 17, 35, 39)*. Based on our finding in 766 ADNIGO/2 participants of improved concordance with PET from 81 to 88% we 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 both be measured and used for amyloid burden detection. For our study participants, the number of cases with abnormal/low Aβ42 and normal PET (Fig. 4A; lower left quadrant) was higher than the number of cases with normal Aβ42 and abnormal PET (Fig. 4A; upper right quadrant), consistent with previous reports *(17)*. When the 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) (Fig. 4B), and the number of cases with normal Aβ42 and abnormal PET dropped by 32% (16 cases were moved to the upper left quadrant; abnormal Aβ42 and abnormal PET) (Fig. 4B). Thus, using the Aβ42/Aβ40 ratio improved concordance with amyloid PET for 7.6% of participants. A hypothesis-driven explanation that the concentration of Aβ42 in the CSF depends not only on the amyloid status of a given participant but also on the total amount of the Aβ peptides present has been described elsewhere *(40)*. We tested for a possible influence of *APOE e4* genotype on the concordance results and found (online Supplemental Figure 4), that participants with no *e4* alleles had improved concordance for the ratio vs Aβ42 alone, whereas the concordance values were comparable for participants with 1 or 2 alleles. Further studies are required to address the mechanistic basis for this observation.

In conclusion, the current study documents long-term analytical performance and substantiates the robustness of our modified LC–MS/MS reference method. We highlighted the needs for: (*a*) use of an analytical balance to maintain reproducibility between different lots of calibrators, (*b*) developing CRMs for Aβ40, and (*c*) 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.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard abbreviations: Aß42, amyloid b1-42; aCSF/BSA, artificial CSF with BSA; AD, Alzheimer disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; BSA, bovine serum albumin; CDR, clinical dementia rating; CRM, certified reference material; CSF, cerebrospinal fluid; CV, coefficient of variation; DMSO, dimethyl sulfoxide; EC-JRC, European Commission Joint Research Centre; EMCI, early MCI; FBP, florbetapir; IRMM, Institute for Reference Materials and Measurements; JCTLM, Joint Committee for Traceability in Laboratory Medicine; LC–MS/MS, liquid chromatography with tandem mass spectrometric detection; LP, lumbar puncture; MCI, mild cognitive impairment; MMSE, mini-mental state examination; MPRAGE, Magnetization Prepared Rapid Acquisition Gradient Echo; NC, normal controls; PET, positron emission tomography; QC, quality control; SMC, subjective memory complaints; SUVRs, standardized uptake value ratios

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