ADNI Alzheimer's Disease Neuroimaging Initiative

2D-UPLC tandem mass spectrometry measurement of $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$ in ADNI1 BASELINE CSF

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Summary

The accompanying .CSV datafile, "UPENNMSMSABETA", lists the concentration data for the amyloid-β peptides, $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ measured in 400 ADNI1 subject BASELINE CSF samples. The 2D-UPLC-tandem mass spectrometry method used for these analyses has previously been described (1). This methodology has since been re-validated using a Waters UPLC system mated to a XEVO-TQ-S tandem mass spectrometer and has been recognized as an accepted reference method by the Joint Committee for Traceability in Laboratory Medicine (JCTLM). It has been published in the JCTLM database under the JCTLM Identification Number : C12RMP1. Each reported value in the datafile is the average of duplicate analyses of 0.1 mL CSF. In this Methods document we summarize the analytical method protocol, precision and accuracy performance, the overall data results distribution and the previously published comparison, using non-ADNI CSF, with the RUO AlzBio3 immunoassay. These analyses provide for the first time, in the ADNI1 BASELINE CSF samples, accuracy-based measurement of $A\beta_{1-42}$ and, in addition, provide measurement of $A\beta_{1-40}$ and $A\beta_{1-38}$. The latter amyloid β peptides are also produced from the catabolism of amyloid precursor protein, but their CSF concentrations are not appreciably diminished in Alzheimer's disease and are therefore regarded as useful for normalization of $A\beta_{1-42}$ concentrations, a procedure that has been recommended to improve upon the diagnostic utility of $A\beta_{1-42}$ concentration alone, especially in extreme cases of subjects who normally are "low producers" of $A\beta_{1-42}$ who might have falsely low(pathologic) values of this biomarker.

Methodology

Table 1 below summarizes major characteristics for the 2D-UPLC tandem mass spectrometry method. Key to the development of successful performance of this method was, firstly, the use of high concentration (5 Moles/L) guanidine HCl (GuCl) to release, presumably in denatured form, $A\beta_{1-42}$ from aggregates and complexes with other CSF proteins and with $A\beta_{1-42}$ itself, and, secondly separation of the $A\beta_{1-42}$ from other endogenous proteins and substances by running the GuCl-treated CSF samples through mixed bed microelution ion exchange columns at acidic pH (1-4). All calibration standards and controls are treated in the same manner. A third key to the evolution of this method was the development and validation of a suitable matrix for the calibrators that precluded aggregation and loss of $A\beta_{1-42}$. A surrogate matrix composed of 4 mg/mL bovine serum albumin (BSA) in artificial CSF electrolyte mixture (Table1) was developed and validated and shown to provide equivalent $A\beta_{1-42}$ concentrations when compared to human CSF as the calibrator matrix (Figure 1). In selection of the surrogate matrix an important consideration was use of materials that are readily available and reproducible. Ideally pooled CSF would be the selected matrix, but due to limited availability and the need to use a non-direct method for calibration [eg, the Method of Standard Additions (5)], we developed the surrogate matrix method. We have shown equivalence in CSF $A\beta_{1-42}$ concentrations using this surrogate matrix for 7 CSF pools as described above, direct comparison with another JCTLM-listed reference method(4,6) (Figure 2) that used pooled CSF as calibrator matrix, and the reverse-curve method for calibration, and comparisons to the Roche

Elecsys immunoassay (7). The original analyses of the ADNI1 BASELINE samples were completed in late 2014 using a reference preparation of $A\beta_{1-42}$ peptide, provided by the Institute for Reference Materials and Measurements, with a provisional mass value assignment, that was finalized in 2016 following extensive amino acid analyses. The value assigned in 2016 is 1.135 times higher than the provisional value provided in 2014. Thus the 2014 dataset for ADNI1 BASELINE CSF samples has been updated by multiplying each result by 1.135. We verified that this simple multiplication provides equivalent results to those produced by multiplying each of the original, 2014, calibrator values by 1.135 and re-calculating the concentrations in each of the ADNI1 BASELINE CSFs (see Figure 3). The $A\beta_{1-40}$ and $A\beta_{1-38}$ calibrant mass value assignments are those provided by the manufacturer, rPeptide in the 2014 analytical runs. Routine QC data for CSF Aβ1-42 are summarized in Table 2. Comparable QC performance data were obtained for Aβ1-40 and Aβ1-38.

Table 1. Characteristics of the UPLC tandem mass spectrometry method for Aβ1-42,Aβ1-40 & Aβ1-38.

Mrm-2D-UPLC parameters	
Peptide standards	$A\beta_{1-42}$: 100, 200, 350, 500, 750, 1000, 3000pg/mL; $A\beta_{1-40}$: 200, 350, 500, 1000, 3000, 7500, 10000 pg/mL; $A\beta_{1-38}$: 100, 200, 350, 500, 1000, 3000, 7500 pg/mL
Internal standard and concentration	^{15}N-$A\beta_{1-38}$, ^{15}N-$A\beta_{1-40}$, ^{15}N-$A\beta_{1-42}$, , each at 1ng/mL of CSF
Calibrator diluent	Acetonitrile:water:ammonia (50:49:1)
HPLC system	UPLC (Waters)
HPLC solvents	Mobile phase A: 0.1% ammonia in water, Mobile phase B: ©:MeOH:TFE (70:25:5), Trap A: ©:water:ammonia (98:2:0.1), Trap B: ©:MeOH:IPA:water (65:25:10:5)
Column	analytical: BEH C18, 1.7μm, 2.1x150mm; trapping: Xbridge C18 3.5μm, 2.1x30mm
Mass spectrometer	XEVO TQ-S (Waters)
4+ charged Precursor and fragment ions for $A\beta_{1-42}$ & ^{15}N-labelled internal standard	$A\beta_{1-42}$ Precursor→fragment ions: m/z 1129.5→1079.1 ^{15}N-$A\beta_{1-42}$ Precursor→fragment ions: m/z 1142.5→1091.5
Calibration standards source	
$A\beta_{1-42}$	IRMM-prepared reference calibrant with mass value determined by amino acid analysis
$A\beta_{1-40}$	rPeptide(Bogart, GA 30622)
$A\beta_{1-38}$	rPeptide(Bogart, GA 30622)
Calbrator Matrix Composition	
Aqueous diluent composition	Artificial CSF: Na^+ 150 mM, K^+ 3.0 mM, Ca^{++} 1.4 mM, Mg^{++} 0.8 mM, P 1.0 mM, and Cl^- 155 mM
Albumin source and concentration	4mg/mL Cohn Fraction V, heat-shock treated, Dnase, Rnase and protease free, in Artificial CSF
Sample preparation	
CSF	5M guanidine HCl in water; 0.1mL per 0.1mL of CSF, mix 45 min, centrifuge, use supernatant for sample cleanup on solid phase mixed bed ion exchange cartridges.
Post-high concentration Guanidine HCL treatment	Microelution solid phase mixed-bed ion exchange cartridges on 96 well plates, acidify GuCl-treated/centrifuged CSF, add to cartridge, wash with acidic solution, followed by acetonitrile/water, elute with ammonium hydroxide in acetonitrile/water solution.

*IRMM, Institute for Reference Materials and Measurements

Figure 1. Correlation of Aβ1-42 results measured in 9 CSF pools using calibration curves prepared in surrogate human CSF matrix [aCSF with BSA (4 mg/mL)] vs measured concentrations using human CSF. For the latter, each of the 9 CSF pools served as calibrator matrix (method of standard additions)(described in Korecka, et al, 2014).

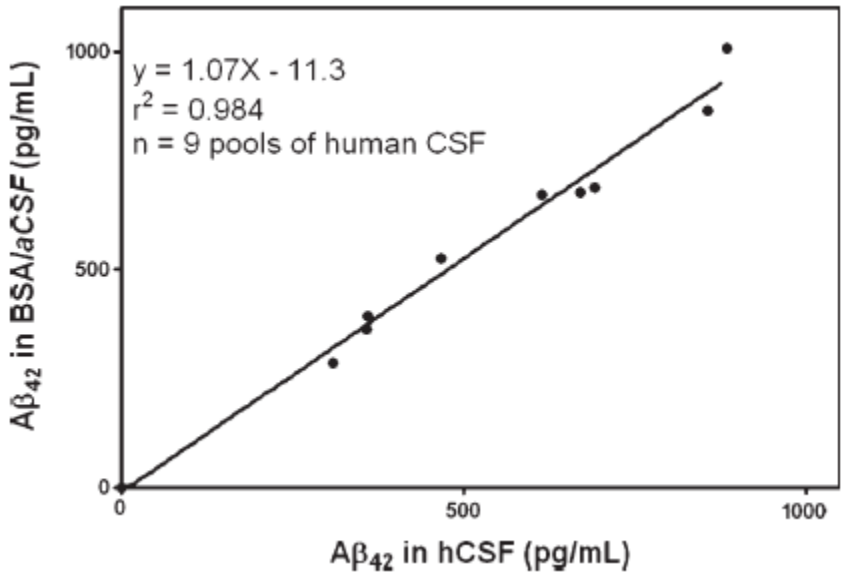


Figure 2. Linear regression comparison between two HPLC/MSMS reference methods. Ten CSF pools prepared from residual CSF samples were analyzed in 3 replicate runs in the two laboratories performing the testing (Univ of Gothenberg and University of Pennsylvania). The primary standard $A\beta_{1-42}$ used for calibration by the two laboratories was provided by IRMM. Each result is the mean value for the 3 replicate runs.

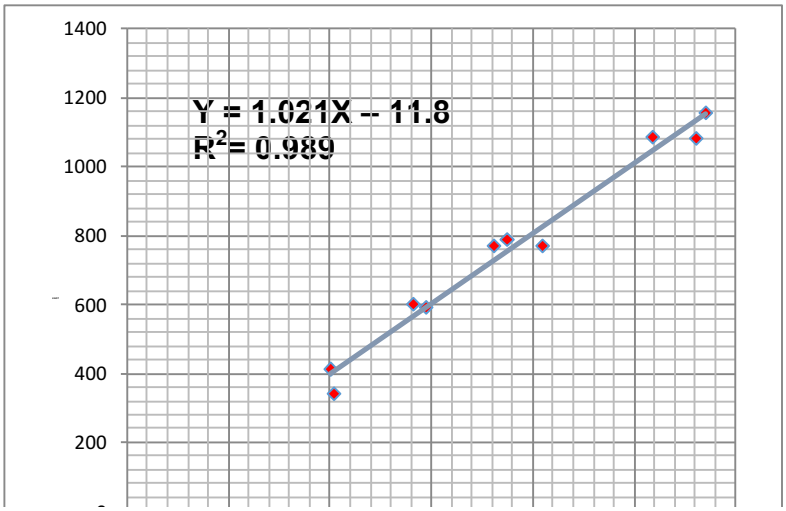
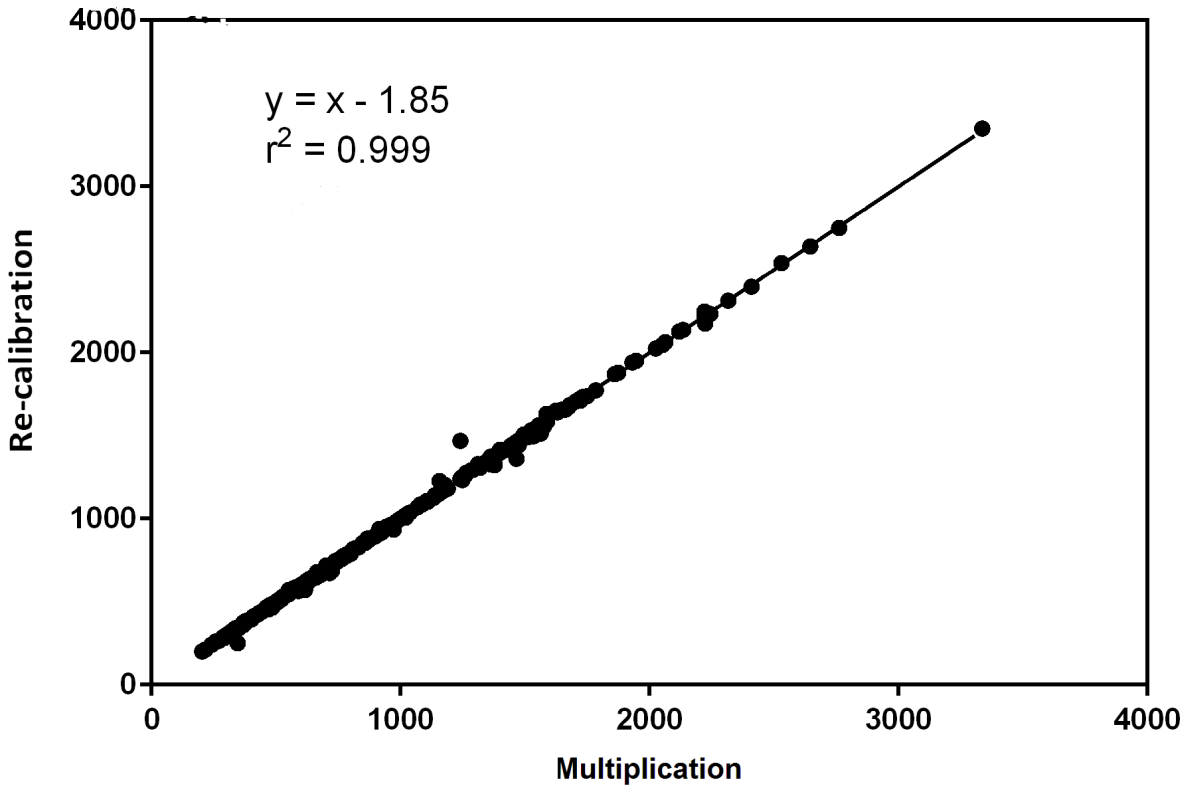
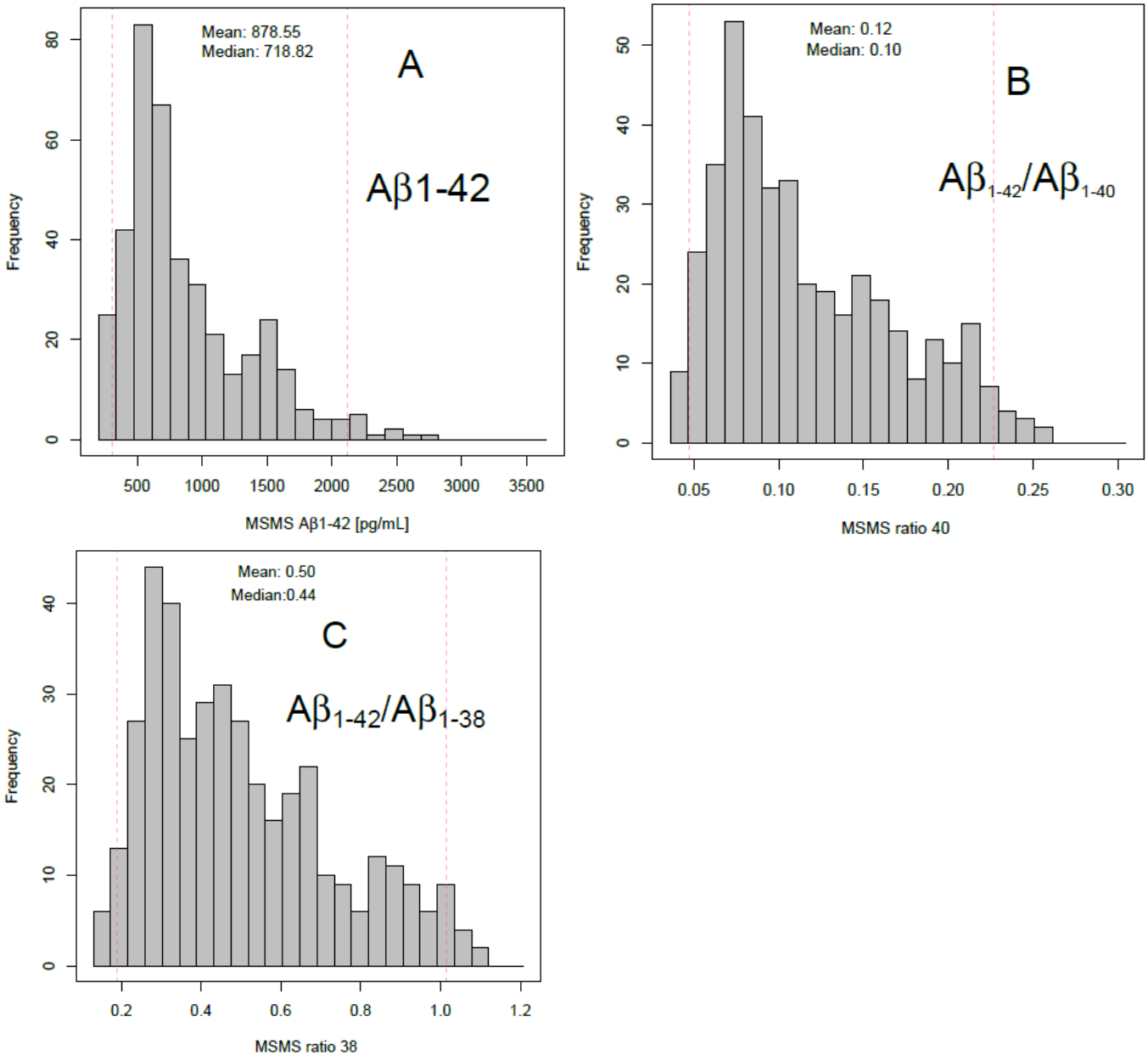


Figure 3. ADNI1 BASELINE CSF Aβ1-42 results re-calculated from 2014 data using the IRMM amino acid analysis-based mass assignment to the Aβ1-42 standard used for calibration of each of the 16 runs involved in the analyses of the 400 ADNI1 CSF samples. Re-calculation was done by two methods. One method was to multiply each calibrator by the 1.135 increased mass value(as compared to the provisional value assigned in 2014) and re-calculate each CSF Ab1-42 result using these updated calibrator concentrations. The second method was multiplication of each 2014 Aβ1-42 result by 1.135. The linear regression plot compares the first method ("re-calibration", Y axis) to the second method ("multiplication", X axis).



Frequency distribution histogram plots for ADNI1 CSF Aβ1-42, Aβ1-40, Aβ1-38 and the ratio values Aβ1-42/Aβ1-40 and Aβ1-42/Aβ1-38 are shown in Figure 4A-C. Aβ1-40 concentration values have been proposed to be of interest as a means to "normalize" Aβ1-42 concentration values in the extreme cases of subjects who normally are "low producers" of Aβ1-42 and who therefore might have falsely low(pathologic) values of this biomarker that gets corrected when normalized by calculating its ratio to that of Aβ1-40 (8-13). Thus these data will be of interest in the ADNI1 CSFs to determine the comparative diagnostic utilities of Aβ1-42, Aβ1-42/Aβ1-40 and Aβ1-42 measured by the AlzBio3 immunoassay.

Figure 4. Histogram frequency plots for 400 BASELINE ADNI1 CSFs Ab1-42 (A), Ab1-42/Ab1-40 ratio (B) and Ab1-42/Ab1-38 (C).



Comparison of Aβ1-42 measured by UPLC/MSMS vs AlzBio3 xMAP immunoassay was done and results recently published (1). In this study a direct comparison between the mass spectrometry-based method and the AlzBio3 immunoassay was performed using 41 AD(autopsy-confirmed cases from the Upenn AD Core Center (ADCC) and 41 age matched living cognitively normal controls. A statistically significant correlation between UPLC/MSMS and AlzBio3 was obtained with an r^2 value of 0.67 and the mass spectrometry values were ~4.5 times higher than the immunoassay values. Aβ1-42 calibrators were prepared using high purity material from rPeptide (Bogart, GA). ROC analyses were done and showed equivalent AUC values of 0.94 and 0.90, respectively (Figure 5), for the mass spectrometry method and the immunoassay. These were not statistically significantly different (p=0.2229)(1). These analyses were done in early 2013.

Figure 5. Comparison of ROC curves for the UPLC-tandem mass spectrometry method and the AlzBio3 Luminex immunoassay. For this study CSF samples were from 41 AD (autopsy confirmed) and 41 age-matched living cognitively normal controls(all provided by the Upenn ADCC). AUC values were 0.938 and 0.90, respectively, for the mass spectrometry method and the AlzBio3 immunoassay.

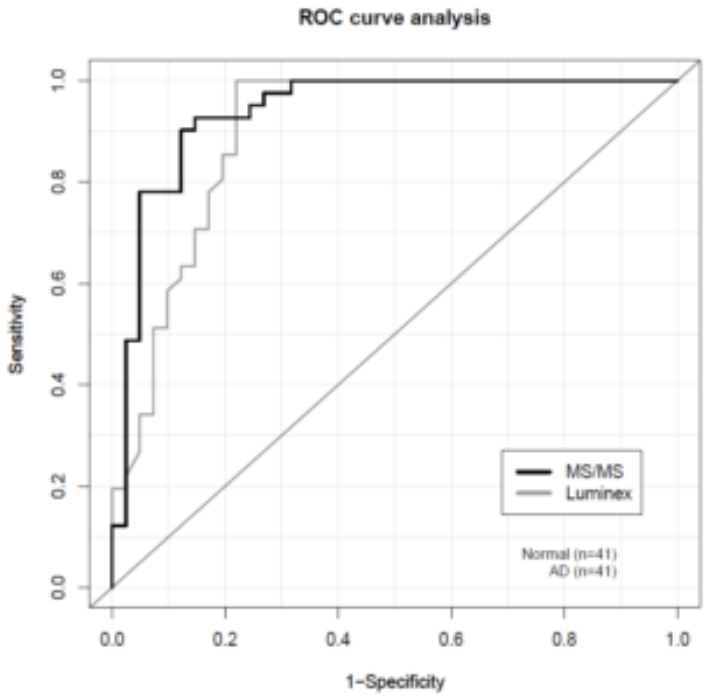


Table 2. quality control data acquired during the analyses of ADNI1 BASELINE CSF aliquots.							
Aβ1-42	QC 1 284 pg/mL	QC 2 908 pg/mL	QC 3 1362 pg/mL	CSF pool 55	CSF pool 56	CSF pool J	CSF pool K
Mean conc, pg/mL	290	935	1368	910	475	486	637
Accuracy(%)	102	103	100	--	--	--	--
SD	19.7	62.2	110.9	98.1	60.8	52.5	65.4
CV(%)	6.8	6.7	8.1	10.8	12.8	10.8	10.3
N	14	15	15	14	14	14	14
QC 1, 2 and 3 are BSA, 4 mg/mL, in artificial CSF electrolyte, solutions with the indicated spiked-in concentrations of the IRMM-provided reference Aβ1-42 preparation. CSF pools 55 and 56 were prepared from residual ADNI1 CSF samples and pools J and K were prepared from discarded CSF from routine clinic patients.							

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