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 ADNI2 and ADNIGO CSF

Magdalena Korecka, Michal Figurski, Leona Fields, John Q Trojanowski, Leslie M Shaw
ADNI Biomarker Core at the University of Pennsylvania (UPenn)

Summary

The accompanying .CSV datafile, "UPENNMSMSABETA_ADNI2/GO", lists the concentration data for the amyloid-β peptides, $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ measured in 1445 BASELINE and available follow-up longitudinal CSF aliquot samples from ADNI2 and ADNIGO subjects. This number includes 205 CSF samples from ADNI1 participants who continue to provide longitudinal CSF samples. Another 615 CSF aliquot samples from DIAN subjects have been analyzed together with these ADNI CSF samples. As per DIAN protocols, a follow-up report and dataset that will include the results for the 615 CSFs from the DIAN cohort will be posted following internal review of the DIAN dataset by DIAN investigators.

The previously described reference methodology, 2D-UPLC-tandem mass spectrometry, for analysis of $A\beta_{1-42}$ (1,2) was modified by adding two additional peptides, $A\beta_{1-40}$, $A\beta_{1-38}$ and their internal standards to the protocol. This new method has been re-validated and compared with the original, reference method for $A\beta_{1-42}$ (Figure 1) and has fulfilled requirements for validation as a rugged and reliable procedure. Each reported value in the datafile is the average of analyses of duplicate 0.1 mL aliquots from each CSF sample. These analyses provide for the first time, in the ADNI2 and ADNIGO BASELINE and longitudinal CSF samples, mass spectrometry-based measurement of $A\beta_{1-42}$ and of $A\beta_{1-40}$ and $A\beta_{1-38}$. In this Methods document, we summarize the analytical method protocol, precision and accuracy performance, the overall data results distribution characteristics and comparison of $A\beta_{1-42}$ concentration with the Elecsys® β-amyloid(1-42) immunoassay (Roche)(3)(Figure 2). Recently a number of studies have reported that compared to CSF $A\beta_{1-42}$ alone, the CSF $A\beta_{1-42}$/ $A\beta_{1-40}$ ratio might improve: 1) prediction accuracy of Alzheimer's disease (AD) in MCI study participants, 2) discrimination of AD from other forms of dementia and 3) increased concordance between CSF and PET amyloidosis (4-7). In our statistical analyses of this data set, we focused on analyses of prediction accuracy and concordance between CSF $A\beta_{1-42}$ vs $A\beta_{1-42}/A\beta_{1-40}$ and Florbetapir PET.

Methodology

Table 1 below summarizes the major characteristics of this 2D-UPLC tandem mass spectrometry method. Detailed description of the method including: 1) treatment of CSF with highly concentrated guanidine HCl, 2) isolation of Aβ peptides from other endogenous compounds on mixed mode ion exchange microelution columns and 3) selection and assessment of a surrogate matrix composed of 4 mg/mL bovine serum albumin (BSA) in artificial CSF electrolyte mixture for standards preparation, as done previously (1,2,8-10). The analysis of the ADNI2 and ADNIGO CSF samples was completed during 70 runs performed from April to December 2017. For the $A\beta_{1-42}$ peptide, a reference standard material was provided by the EC-JRC-Institute for Reference Materials and Measurements (IRMM) (Belgium), with an assigned value of $A\beta_{1-42}$ concentration in the pure peptide solution of 84 mg/mL. **A final assignment of accuracy-based values for the calibration standards used in this reference**

method is planned for $A\beta_{1-42}$. This procedure will make use of the newly prepared and released human CSF-based Certified Reference Material for $A\beta_{1-42}$ that will be provided by the EC-JRC-IRMM (11). Thus at that future date we will perform a conversion of the mass spectrometry data for all ADNIGO/2 CSF data to the finalized accuracy-based $A\beta_{1-42}$ concentrations. These data will therefore differ from the current results by a constant value. We plan to do this in collaboration with the IFCC/CSF Workgroup members in the full spirit of harmonization across all analytical platforms for $A\beta_{1-42}$ accuracy based analyses (12). For $A\beta_{1-40}$ and $A\beta_{1-38}$ standard materials mass value assignments are those provided by the manufacturer, rPeptide (USA). **We expect that in the near future there will be an effort to prepare CRMs for $A\beta_{1-40}$ analogous to what was done for $A\beta_{1-42}$ in order to harmonize measurements for this analyte as well, and we will participate in this effort as well.**

Two stock solutions of $A\beta_{1-42}$ (50ng/mL and 500ng/mL), required for the preparation of spiking solutions for standards and quality control samples, were prepared using an analytical balance and their final concentration was determined by the weight. This manner of preparation is necessary to assure lot-to-lot results' reproducibility.

Table 1. Characteristics of the UPLC tandem mass spectrometry method for $A\beta_{1-42}$,$A\beta_{1-40}$ & $A\beta_{1-38}$.

MRM-2D-UPLC parameters	
Peptide standards range (n=8 plus Blank)	$A\beta_{1-42}$: 100 - 3000 pg/mL; $A\beta_{1-40}$: 200 - 20000 pg/mL; $A\beta_{1-38}$: 100 - 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
Standards diluent	ACN:water:ammonia (50:49:1)
LC system	UPLC (Waters)
LC solvents	Mobile phase A: 0.1% ammonia in water, Mobile phase B: ACN:MeOH:TFE (70:25:5), Trap A: ACN:water:ammonia (98:2:0.1), Trap B: ACN: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 $A\beta_{1-42}$, $A\beta_{1-40}$, $A\beta_{1-38}$ & ^{15}N-labelled internal standards	$A\beta_{1-42}$ Precursor→fragment ions: *m/z* 1129.6→1079.0 $A\beta_{1-40}$ Precursor→fragment ions: *m/z* 1083.6→1054.0 $A\beta_{1-38}$ Precursor→fragment ions: *m/z* 1034.1→1000.0 ^{15}N-$A\beta_{1-42}$ Precursor→fragment ions: *m/z* 1142.5→1091.5 ^{15}N-$A\beta_{1-40}$ Precursor→fragment ions: *m/z* 1096.0→1066.5 ^{15}N-$A\beta_{1-38}$ Precursor→fragment ions: *m/z* 1046.0→1012.5
Calibration standards source	
$A\beta_{1-42}$	IRMM[a] - reference standard material with mass value of 84 mg/L
$A\beta_{1-40}$	rPeptide(Bogart, GA 30622)
$A\beta_{1-38}$	rPeptide(Bogart, GA 30622)
Standards 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 in artificial CSF	Cohn Fraction V, heat-shock treated, Dnase, Rnase and protease free, 4mg/mL
Sample preparation	
CSF	5M guanidine HCl in water; 0.1mL with 0.01mL of each internal standard per 0.1mL of CSF, incubation 45 min on Vortex, use supernatant for sample cleanup on solid phase mixed mode ion exchange cartridges.
Post-high concentration Guanidine HCL treatment	Microelution on solid phase mixed mode ion exchange cartridges in 96 well plates, acidify GuCl-treated CSF, add to cartridge, wash with acidic solution, followed by acetonitrile/water, elute with ammonium hydroxide in acetonitrile/water solution.
[a]-IRMM, Institute for Reference Materials and Measurements	

The modified method for analysis of three amyloid beta peptides was re-validated before using for analysis of ADNI2 and ADNIGO samples as a mandatory step of GCP/GLP regulations. For this purpose, the modified method was compared with the established reference method for analysis of $A\beta_{1-42}$ alone using leftover CSF samples (n=79) obtained from routine clinic patients at the hospital at the University of Pennsylvania (Figure 1). We also compared the results of $A\beta_{1-42}$ measured by UPLC-MS-MS with the results obtained from Elecsys® β-amyloid(1-42) immunoassay (Roche, Germany) for ADNI2 and ADNIGO samples (n=1438) (Figure 2). These comparisons were presented at AAIC in 2016 (3).

Statistically significant correlations between: 1) reference method and modified method and 2) UPLC/MSMS and Elecsys® β-amyloid(1-42) were obtained with r^2 values of 0.96 and 0.92, and slope values of 0.9985 and 1.020, respectively (see Figure 1 and Figure 2 for more details).

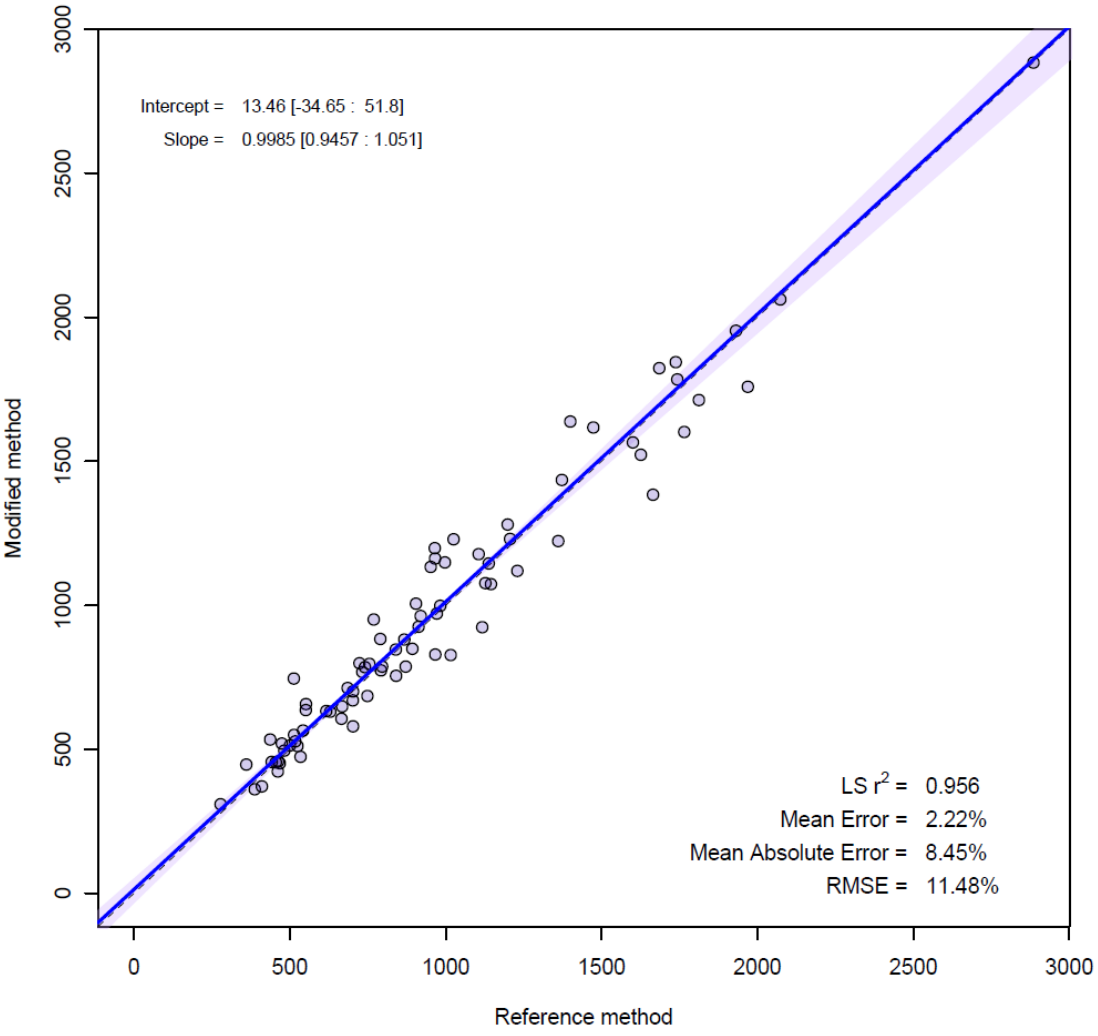

Figure 1. Correlation of $A\beta_{1\text{-}42}$ concentration results (pg/mL) measured in 79 routine clinic discarded patient CSF samples, using the reference method for analysis of single peptide (x axis) i.e. $A\beta_{1\text{-}42,}$ vs concentrations of $A\beta_{1\text{-}42}$ obtained using the modified method for analysis of three peptides (y axis). This analysis shows the comparability of the modified 3-amyloid-β peptide method and the single peptide reference method for $A\beta_{1\text{-}42}$ measurement

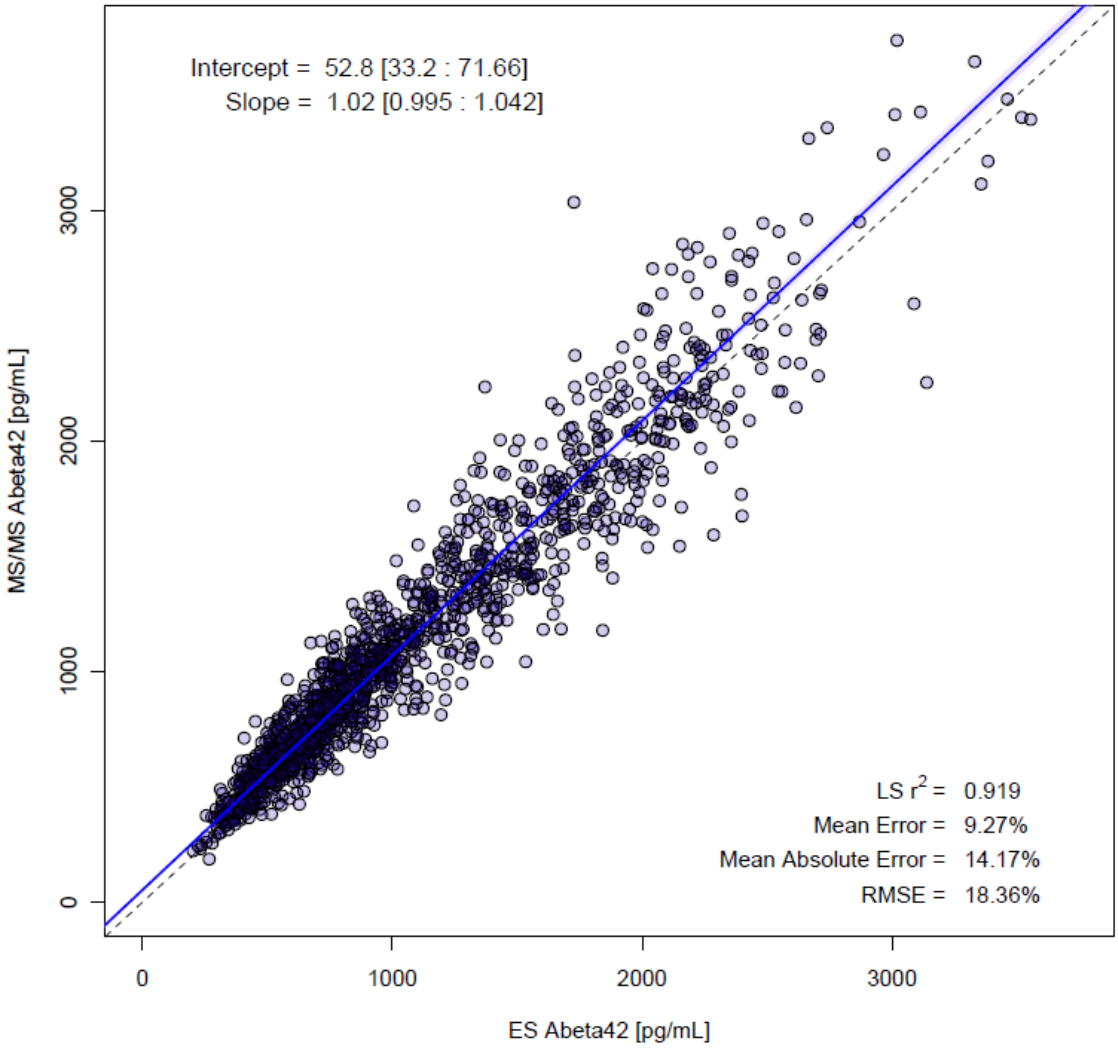

Figure 2. Results of $A\beta_{1-42}$ from 2D UPLC-MS-MS method for 3 peptides vs results obtained from Elecsys® β-amyloid(1-42) immunoassay for 1438 ADNI2 and ADNIGO samples. For mass spec data each result is the mean value for two measurements, for Elecsys® β-amyloid(1-42) this is a single result.

Five different CSF pools were used as biological controls and additionally three quality controls prepared in artificial CSF/BSA were included in each analytical run. Table 2 summarizes QCs performance data obtained for $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$.
Three out of five CSF pools were run with two different lots of standards. For $A\beta_{1-42}$ none of the CSF pools showed statistically significant differences when two lots of standards were compared (n=38 runs using lot#41717, and n=32 runs using lot#92917)(Figure 3).

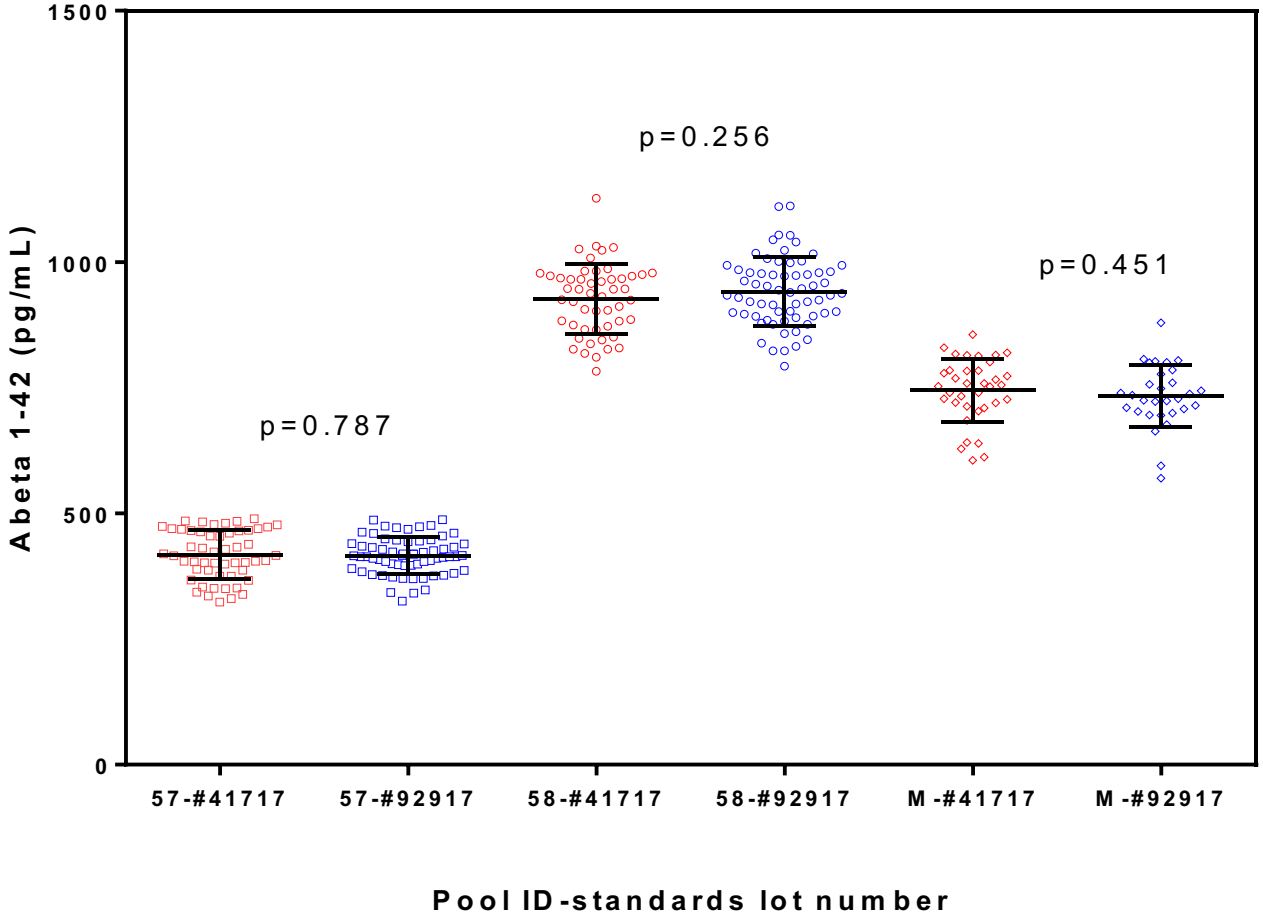

Figure 3. Reproducibility of $A\beta_{1-42}$ for three CSF pools between two different lots of in house standards. *p* values show that there is no statistically significant difference in $A\beta_{1-42}$ concentration obtained when two different lot of standards were used for preparation of calibration curve.

Table 2. Overall performance of biological controls and controls prepared in artificial CSF/BSA for $A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$. Seventy runs were performed from April to December 2017 to analyze 1445 samples of CSF from ADNI2 and ADNIGO participants. Samples were run in duplicate. A total of five analytical columns and two different lots of in house standards were utilized over the course of these 70 runs.

Abeta 1-42								
Parameter	**QC 1**	**QC 2**	**QC 3**	**Pool 55 (NC)**	**Pool 56 (AD)**	**Pool M**	**Pool 57 (AD)**	**Pool 58 (NC)**
Mean conc.* (pg/mL)	230.8	778.4	1197.9	**882.1**	**496.8**	**739.9**	**416.8**	**935.2**
Accuracy (%)	100.3	96.9	99.4	NA	NA	NA	NA	NA
SD(conc.)	17.9	58.9	113	64	32.3	62.4	42.5	68.8
CV (%)	**7.7**	**7.6**	**9.4**	**7.3**	**6.5**	**8.4**	**10.2**	**7.4**
n	60	60	63	25	26	66	117	115
Abeta 1-40								
Mean conc. (pg/mL)	1232.9	4994.5	9939.6	**5212.7**	**5729.2**	**4639.2**	**5301.9**	**5686.0**
Accuracy (%)	102.7	99.9	99.4	NA	NA	NA	NA	NA
SD(conc.)	63.3	318.6	693.2	176	123.6	326.3	347.1	332.2
CV (%)	**5.1**	**6.4**	**7.0**	**3.4**	**2.2**	**7.0**	**6.6**	**5.8**
n	140	143	137	29	32	72	118	116
Abeta 1-38								
Mean conc. (pg/mL)	802.0	1237.3	2993.1	**1187.5**	**1308.6**	**1085.8**	**1369.2**	**1426.3**
Accuracy (%)	100.3	103.1	99.8	NA	NA	NA	NA	NA
SD(conc.)	50.0	97.5	239.3	75.7	74.9	8.2	89.3	84.5
CV (%)	**6.2**	**7.9**	**8.0**	**6.4**	**5.7**	**7.6**	**6.5**	**5.9**
n	138	139	131	25	28	70	119	115

* For $A\beta_{1-42}$ QC samples based on artificial CSF/BSA the results are for standards' lot number 92917.

Statistical analyses

Statistical analyses of our data focused on confirming the recent reports and main findings of this study:

1. Improvement of amyloid pathology detection when using CSF $A\beta_{1-42}/A\beta_{1-40}$ vs CSF $A\beta_{1-42}$ alone based on better concordance between CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio and PET-amyloid than CSF $A\beta_{1-42}$ alone and PET-amyloid.
2. Assessment of the diagnostic utility of $A\beta_{1-42}/A\beta_{1-38}$.

Numerous papers report that the concordance between amyloid-PET and cerebrospinal fluid amyloid beta increases when the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio is used as compared to CSF $A\beta_{1-42}$ alone (4-7). Analysis of BASELINE CSF from ADNI2 and ADNIGO participants with concurrent Florbetapir amyloid PET (n=766) comparing $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio confirms these reports. We observed that the $A\beta_{1-42}/A\beta_{1-40}$ ratio improved concordance from 83% to 89% (ROC analysis was done using Florbetapir PET (FBP)+ or – as the endpoint for determining cut-points used to categorize results in the scatterplots (13))(Figure 4A and 4B). Additionally based on further data analysis we report here that the $A\beta_{1-42}/A\beta_{1-38}$ ratio equally well improved concordance also to 89% (Figure 4C). These two observations suggest that two peptides, $A\beta_{1-42}$ and $A\beta_{1-40}$ should be both measured and used for the detection of amyloid pathology and that the comparative utility of $A\beta_{1-38}$ can be followed up in future investigations. The ROC analyses provided the following values for cut points: for $A\beta_{1-42}$: 1079 pg/mL, for the $A\beta_{1-42}/A\beta_{1-40}$ ratio: 0.133 and 0.627 for the $A\beta_{1-42}/A\beta_{1-38}$ ratio.

Frequency distribution histogram plots of $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ for the group of 766 participants. Each result was color coded, red if FBP+, blue if FBP-, with the resulting color-coded frequency plots providing further evidence for the improved separation of FBP+ from FBP- afforded by the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio over $A\beta_{1-42}$ alone as a marker of amyloid-positivity by PET (Figure 5). In the same figure we also present a frequency distribution histogram plot of $A\beta_{1-42}/A\beta_{1-38}$ ratio since it also improves separation of PET (+) from PET(-) subjects when compared with $A\beta_{1-42}$ alone.

A well-known hypothesis explains that the concentration of $A\beta_{1-42}$ in the CSF depends not only on the physiological amyloid status of a given individual (presence or absence of amyloid pathology) but also on the total amount of Aβ peptides in each CSF sample. By normalizing to the concentration of the most abundant Aβ peptide in the CSF, $A\beta_{1-40}$, the ratio removes the potential confound of differences in overall amyloid beta concentration and provides a better index of underlying amyloid-related pathology (4).

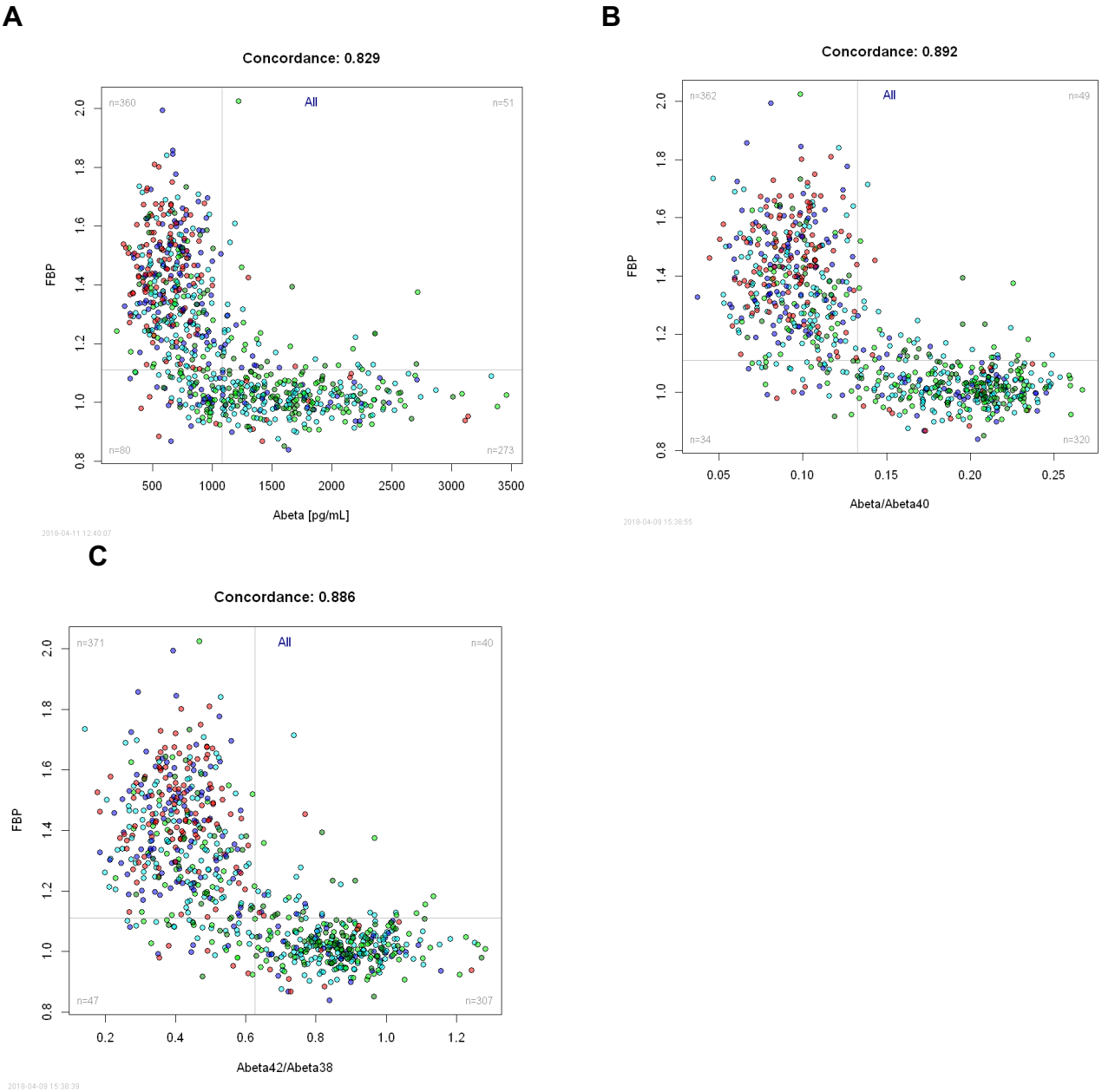

Figure 4. Scatterplots of cortical amyloid florbetapir PET and concentrations of CSF $A\beta_{1-42}$ (A), the $A\beta_{1-42}/A\beta_{1-40}$ ratio (B) and $A\beta_{1-42}/A\beta_{1-38}$ ratio (C). Our data confirms reports that $A\beta_{1-42}/A\beta_{1-40}$ improves concordance with amyloid PET. ROC analyses was done to determine the cut-points used (vertical and horizontal lines) in each figure, using package 'Epi', version 2.16 (13)

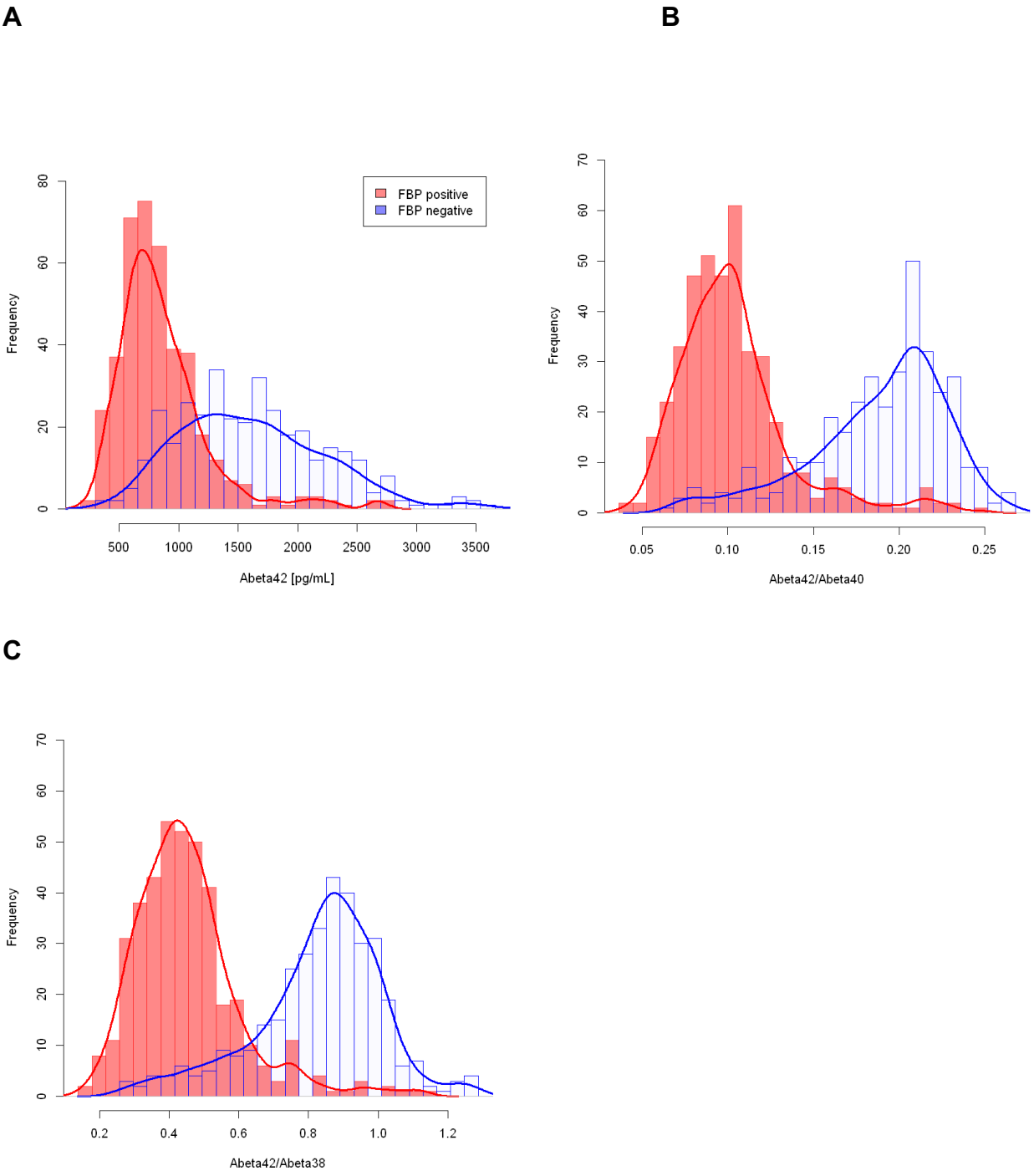

Figure 5. Distribution of $A\beta_{1-42}$ (A), $A\beta_{1-42}/A\beta_{1-40}$ (B) and $A\beta_{1-42}/A\beta_{1-38}$ (C) for group of 766 BASELINE ADNI2 and ADNIGO participants with concurrent florbetapir amyloid PET.

References

1. Korecka M, Waligorska T, Figurski M, Toledo JB, Arnold SE, Grossman M, Trojanowski JQ, Shaw LM. Qualification of a surrogate matrix-based absolute quantification method for Aβ1-42 in human cerebrospinal fluid using 2D UPLC-tandem mass spectrometry. J Alzheimers Dis 2014; 41:441-451.
2. JCTLM database under the JCTLM Identification Number : C12RMP1.
3. Shaw LM, Korecka M, Waligorska T, Trojanowski JQ, Allegranza D, Bittner T, He Y, Morgan K, Rabe C. Aβ1-42 measured in human CSF samples by liquid chromatography tandem mass spectrometry, INNOBIA AlzBio3 assay and the Elecsys® β-amyloid(1-42) assay. AAIC 2016 poster
4. Lewczuk P, Matzen A, Blennow K, Parnetti L, Molinuevo JL, Eusebi P, Kornhuber J, Morris JC, Fagan AM. Cerebrospinal fluid Ab42/40 corresponds better than Ab42 to amyloid PET in Alzheimer's disease. J AlzheimersDis 2017;55:813-822
5. Janeldze S, Pannee J, Mikulskis A, Chiao P, Zetterberg H, Blennow K, Hansson O. Concordance between different amyloid immunoassays and visual amyloid positron emission tomographic assessment. JAMA Neurol 2017;74:1492-1501
6. Janeldze S, Zetterberg H, Mattsson N, Palmqvist S, Vanderstichele H, Lindberg O, van Westen D, Stomrud E, Minthon L, Blennow K, for the Swedish BioFINDER study group and Hansson O. CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: better diagnostic markers of Alzheimer's disease. Ann Clin Transl Neurol 2016; 3: 154-165.
7. Niemantsverdriet E, Ottoy J, Somers C, De Roeck E, Struyfs H, Soetewey F, Verhaeghe J, Van den Bossche T, Van Mossevelde S, Goeman J, De Deyn PP, Mariën P, Versijpt J, Sleegers K, Van Broeckhoven C, Wyffels L, Albert A, Ceyssens S, Stroobants S, Staelens S, Bjerke M, Engelborghs S. The Cerebrospinal Fluid Aβ1-42/Aβ1-40 Ratio Improves Concordance with Amyloid-PET for Diagnosing Alzheimer's Disease in a Clinical Setting. J Alzheimers Dis. 2017;60:561-576
8. Lame ME, Chambers EE, Blatnik M. Quantitation of amyloid beta peptides Abeta(1-38), Abeta(1-40), and Abeta(1-42) in human cerebrospinal fluid by ltraperformance liquid chromatography-tandem mass spectrometry. Anal Biochem 2011; 419: 133-139.
9. Pannee J, Portelius E, Oppermann M, Atkins A, Hornshaw M, Zegers I, H¨ojrup P, Minthon L, Hansson O, Zetterberg H, Blennow K, Gobom J (2013) A selected reaction monitoring (SRM)-based method for absolute quantification of Aβ38, Aβ40 and Aβ42 in CSF of Alzheimer's disease patients and healthy controls. J Alzheimers Dis 2013; 33: 1021-1032.
10. Leinenbach A, Panee J, Dulffer T, Huber A, Bittner T, Andreasson U, Gobom J, Zetterberg H, Kobold U, Portelius, Blennow K. Mass spectrometry-based candidate reference measurement procedure for quantification of amyloid-beta in cerebrospinal fluid. Clin Chem, 2014. 60(7): p. 987-94.
11. Kuhlmann J, Boulo S, Andreasson U, Bjerke M, Pannee J, Charoud-Got J, Mazoua S, Trapmann S, Schimmel H, Emons H, Florian D, Quaglia M, Portelius E, Korecka M, Shaw LM, Lame M, Chambers E, Vanderstichele H, Stoops E, Leinenbach A, Bittner T, Jenkins RG, Kostanjavecki V, Lewczuk P, Zettterberg H, Zegers I, blennow K. The certification of Amyloid

b1-42 in CSF in ERM®-DA480/IFCC, ERM®-DA481/IFCC and ERM®. 2018 Reference Materials Report, JRC 107381.
12. Kuhlmann J, Andreasson U, Pannee J, Bjerke M, Portelius E, Leinenback A, Bittner T, Korecka M, Jenkins RG, Vanderstichele H, Stoops E, Lewczuk P, Shaw LM, Zegers I, Schimmel H, Zetterberg H, Blennow K. on behalf of the IFCC Working Group on Standardization of CSF proteins (WG-CSF). CSF Aβ1-42 – an excellent but complicated Alzheimer's biomarker – a route to standardization. Clin Chim Acta 2017; 467:27-33.
13. Bendix Carstensen, Martyn Plummer, Esa Laara, Michael Hills (2017). Epi: A Package for Statistical Analysis in Epidemiology. R package version 2.16. URL https://CRAN.R-project.org/package=Epi

About The Authors

The authors Magdalena Korecka, Teresa Waligorska, and Michal Figurski are staff members in the ADNI Biomarker Core Laboratory and Leslie M Shaw and John Q Trojanowski, co-direct the ADNI Biomarker Core in the Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania. For more information please contact Leslie Shaw by phone at 215-662-6575 or email: Les.Shaw@uphs.upenn.edu or John Trojanowski at 215-662-6399 or email: Trojanow@mail.med.upenn.edu.