ADNI Alzheimer's Disease Neuroimaging Initiative

ADNI GO and ADNI 2 First batch analyses of CSF biomarkers: REVISED ANCHORING TO 2007 ADNI 1 BASELINE DATASET PROCEDURE

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This is a follow-up description of our ongoing work on the process of anchoring to the ADNI 1 2007 BASELINE CSF data the untransformed (raw) CSF $A\beta_{1-42}$, t-tau and p-tau_{181} concentration results for the first 390 BASELINE CSF samples, collected through 2/21/2012, for ADNI GO and ADNI 2. The analytical performance and untransformed CSF $A\beta_{1-42}$, t-tau and p-tau_{181} concentration results data for this series of analyses are reported in the "ADNI GO and ADNI 2 CSF Report for $A\beta_{1-42}$, t-tau and p-tau_{181}", dated 2012-06-04, and is available on the LONI web site. In this "REVISED ANCHORING OF 2012 BASELINE RAW CSF $A\beta_{1-42}$, t-tau and p-tau_{181} CONCENTRATION DATA TO ADNI 1 2007 BASELINE DATASET" Methods Report we first summarize key analytical performance results from the "ADNI GO and ADNI 2 CSF Report for $A\beta_{1-42}$, t-tau and p-tau_{181}", 2012-06-04, including:

I. **Longitudinal precision performance of an abnormal CSF pool (#53) and that of a normal CSF pool (#54) (Table 1 and Figure 1A & 1B)** provides a numerical and graphical summary of the longitudinal performance for the 2012 analytical runs. In addition we summarize the longitudinal precision performance for the analytical runs performed in 2013 that used different calibration standards & controls and immunoassay kit reagent lot numbers to document lot-to-lot longitudinal quality control experience in 2012 through 2013

Table 1. Longitudinal precision performance across reagents lot#s, 2012 - 2013

2012	Sample	N	Mean	SD	%CV	2013	Sample	N	Mean	SD	%CV
t-tau	CSF pool #53	13*	132	8.4	6.4	t-tau	CSF pool #53	25*	128	10.4	8.1
	CSF pool #54	12	70.3	3.1	4.4		CSF pool #54	25	65.9	6.0	9.2
$A\beta_{1-42}$	CSF pool #53	13	136	6.9	5.1	$A\beta_{1-42}$	CSF pool #53	25	148	9.4	6.3
	CSF pool #54	12	222	15	7.0		CSF pool #54	25	236	18	7.8
p-tau_{181}	CSF pool #53	13	25.4	1.4	5.6	p-tau_{181}	CSF pool #53	25	26.5	1.4	5.1
	CSF pool #54	12	19.1	1.0	5.1		CSF pool #54	25	19.1	1.2	6.1

2012: Stds/Controls and Kit reagents Lot#s S215382_K220093 **2013:** Stds/Controls and Kit reagents Lot#s S225736_K222545
*Each qc result for each analytical run is the mean value for duplicate assays as is the case for each patient result

Figure 1A. Longitudinal plots of CSF $A\beta_{1-42}$ concentration (pg/mL) for CSF pools #53 and #54 for 2012 and 2013.

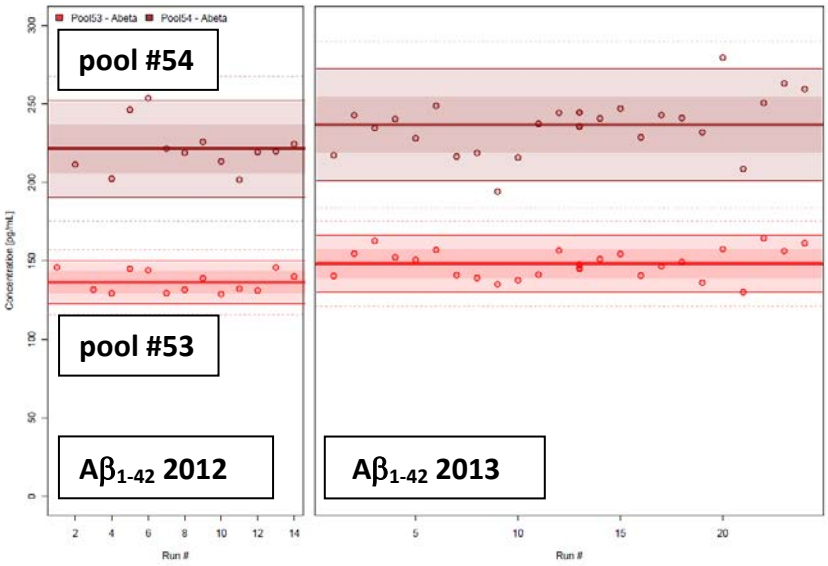
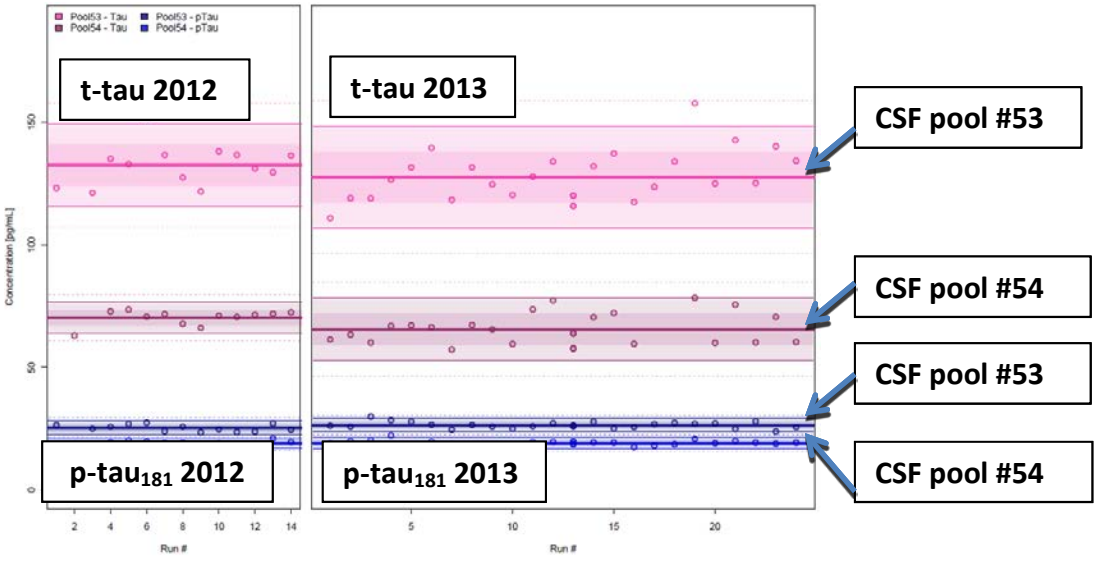


Figure 1B. Longitudinal plots of CSF t-tau and p-tau_{181}concentration (pg/mL) for CSF pools #53 and #54 for 2012 and 2013.



In addition to the earlier report from the ADNI Biomarker Core investigators and PPSB and academic center collaborators (Acta Neuropath, 2011, reference #3), this is the only detailed lot-to-lot performance reported in an AD CSF biomarker study that we are aware of in the published world's literature on this topic. The close agreement between the two lots (lot #id information in footnote, Table 1) provides an important basis for us to correct the anchoring methodology as described below.

The longitudinal precision performance summarized in Table 1 and accompanying plots in Figures 1A & 1B shows very good precision performance across the two series of runs, one in 2012 and the second a year later in 2013. Importantly the lot-to-lot performance across the two different lots of reagents used during these two time periods separated by one year shows comparable mean concentrations and precision for the two different lots of kit reagents.

The differences between the mean values [(Mean biomarker conc 2013-conc 2012/mean 2012)X100] are:

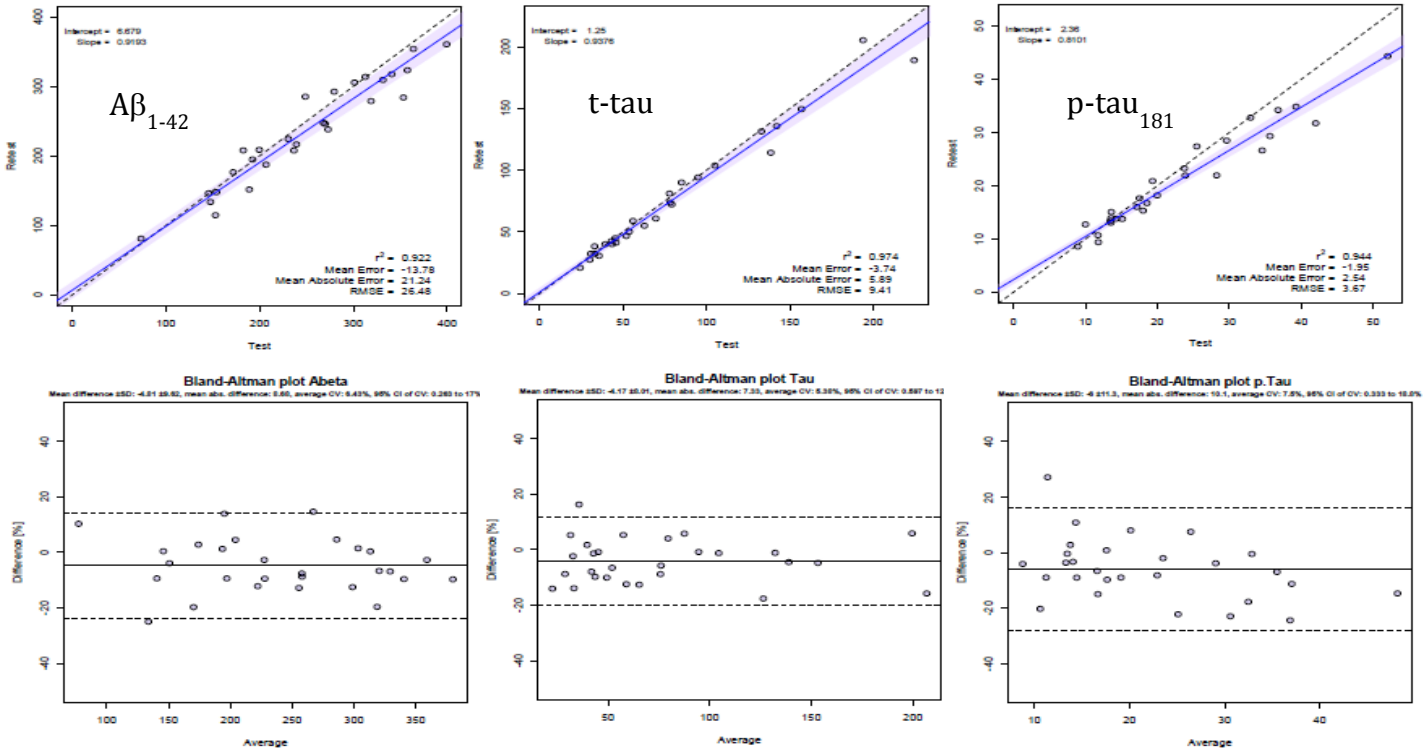
Pool #53		**Pool #54**	
t-tau	= -3.03%	t-tau	= -6.26%
$A\beta_{1-42}$	= 8.82%	$A\beta_{1-42}$	= 6.31%
p-tau_{181}	= 4.33%	p-tau_{181}	= 0%

Acceptance criteria for each run in 2012 and 2013 were used in accord with the ADNI Biomarker Core SOP for this immunoassay procedure, namely at least 3 of the 4 qc results (2 CSF pools and two kit aqueous controls) must be within ±3 SD using the mean and SD values, established for each CSF pool prior to performance of the batch set of CSF samples, and the values of the two kit controls must be within the ranges established by the manufacturer. Further details regarding the 2012 and the 2013 analytical performance are provided in the two analytical reports, "ADNI GO and ADNI 2 CSF Report for $A\beta_{1-42}$, t-tau and p-tau_{181}", 2012-06-04 and "ADNI I, GO and 2 CSF report for $A\beta_{1-42}$, t-tau and p-tau_{181}", 2013-05-22, both available on the LONI ADNI web site.

II. **Test/re-test precision performance for the 2012 analytical runs** is a second key analytical performance result from the "ADNI GO and ADNI 2 CSF Report for $A\beta_{1-42}$, t-tau and p-tau_{181}", 2012-06-04, summarized in Figure 2 showing performance consistent with our ongoing experience.

Test/re-test precision performance in 2012 is consistent with prior performance (1) and the overall mean precision values were 6.4%, 5.4% and 7.5% for $A\beta_{1-42}$, t-tau and p-tau_{181}, respectively (from the bias plots summaries in Figure 2).

Figure 2. Test/re-test performance for 2012 ADNI GO & ADNI II CSF $A\beta_{1-42}$, t-tau and p-tau_{181} analytical runs.



III. **Calibrator reproducibility and precision** of duplicate results, a third key analytical performance measure from the "ADNI GO and ADNI 2 CSF Report for $A\beta_{1-42}$, t-tau and p-tau_{181}", 2012-06-04 were at the same high level of performance as in prior studies and in 2013 and full details are in the "ADNI GO and ADNI 2 CSF Report for $A\beta_{1-42}$, t-tau and p-tau_{181}", 2012-06-04.

Since there is no reference standard for these CSF biomarkers to permit manufacturers to calibrate their kit standards against, we in the ADNI Biomarker Core use a "reference set" of samples for our studies to enable anchoring each data set to a common standard (BASELINE ADNI 1 CSF biomarker concentrations). These CSF samples are precious material and the selection of 2007 ADNI 1 BASELINE samples to use for anchoring has been done thoughtfully and judiciously to assure conservation of this resource while addressing this critical need to anchor these data across time and lots of reagents. At the conclusion of this report we will recommend no further use of 2007 ADNI 1 BASELINE samples for this purpose thereby preserving them for use in studies approved by the NIA RARC/ADNI. Instead, we will use the 2013 ADNI BASELINE samples, which are in more plentiful supply, and are therefore preferred for anchoring and we will do this by using only those samples with 20 or more aliquots for the purpose of anchoring future datasets to ADNI 2 BASELINE, which provides an equivalent reference standard to ADNI 1 BASELINE CSF biomarker data.

The procedure in 2012 for anchoring the 2012 raw concentration data to 2007 ADNI 1 BASELINE CSF $A\beta_{1-42}$, t-tau and p-tau_{181} concentrations was based on the use of a limited number of never before thawed 2007 BASELINE CSF aliquots. Thus, a total of 12 2007 aliquots were selected based on two criteria: 1)at least 20 aliquots were available for each selected patient CSF; 2) selection of six sets of two aliquots was based on ascending $A\beta_{1-42}$ concentrations over the range observed for the 2007 BASELINE samples. We included these 12 CSF aliquot samples in one analytical run performed in early 2012 and used the Passing-Bablok (P-B) regression line to anchor the data set to 2007. At the time we did these analyses we assumed that the relationship between the 2012 and 2007 studies captured by analysis of these 12 samples in one analytical run would be representative of the whole set of 2007 ADNI 1 BASELINE samples. However, as shown in Figure 3 this assumption was not correct, having undertransformed all 3 biomarkers, but there was no precedent upon which to base our initial selection of the number of replicates and runs to use in these studies. Hence, the findings shown in Figure 3 were unexpected, and, in light of the current data, however, we conclude that to capture the natural variance in $A\beta_{1-42}$, t-tau and p-tau_{181} concentration measurements and achieve accurate anchoring to the 2007 ADNI 1 BASELINE set a larger # CSF samples and runs are needed.

The revised strategy for anchoring the 2012 CSF BASELINE dataset (n=390) that we believe provides a reasonable approximation to the 2007 ADNI 1 BASELINE CSF results is to use the P-B regression equation produced by the transformation of the 2013 ADNI CSF data set using the sixty-two 2007 CSF samples that were assayed in 2013 as part of longitudinal studies. Since the lot-to-lot performance was acceptable between 2012 and 2013 (Table 1 & Figure 1) we believe this is the best approach and there are no other published data on these analytes that offer more specific guidelines for this process. The regression analysis results for the 2012 CSF biomarker transformed data using this approach ("2012 T62") shown in Figure 5 confirm the success of this transformation.

Figure 3. Performance assessment of 12-sample transformation: comparison of 25 CSF samples assayed and transformed in 2012 ("2012 T12") vs 2013 that were transformed using 62 ADNI I BASELINE CSF samples ("2013 T62").

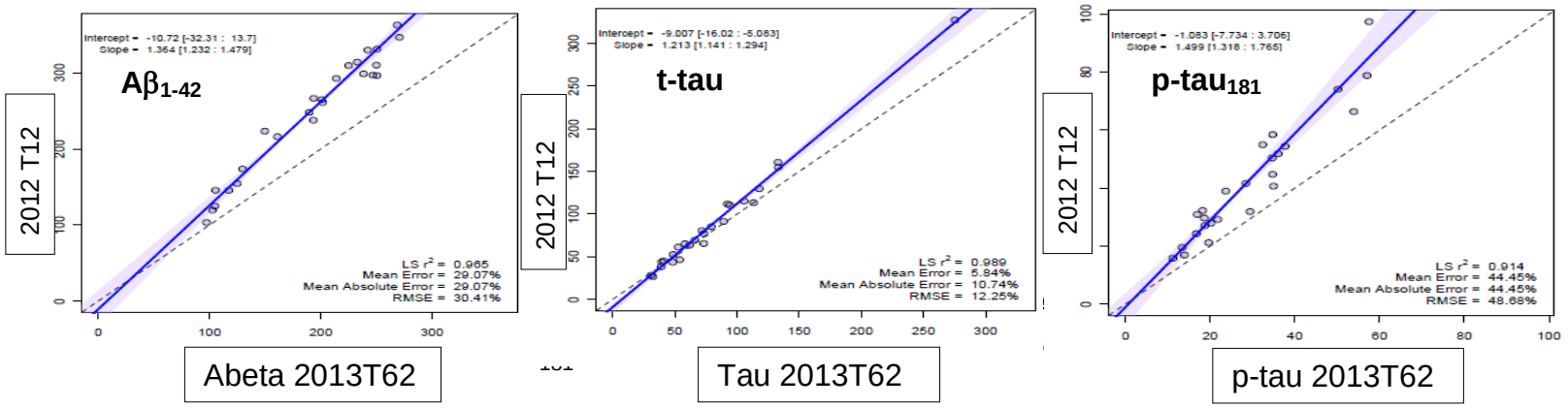


Figure 4A. The 62-sample transformation for 2013 → 2007 to define the P-B transformation linear regression parameters for anchoring 2013 Baseline datasets for $A\beta_{1-42}$, t-tau and p-tau_{181}.

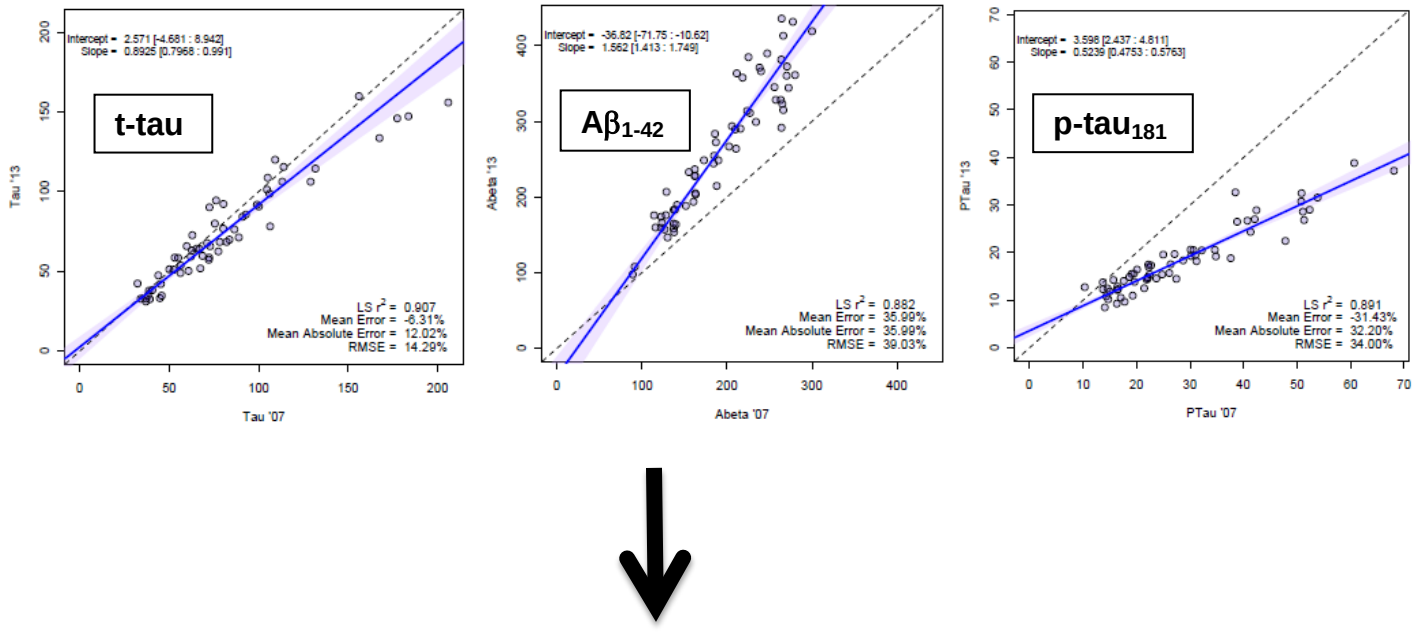


Figure 4B. The P-B linear regression plots of CSF transformed $A\beta_{1-42}$, t-tau and p-tau_{181} (Y axis), the 2013 data transformed using the P-B linear regression parameters defined by P-B regression defined in Fig4A, vs ADNI 1 BASELINE 2007 results for sixty-two ADNI 1 aliquot samples.

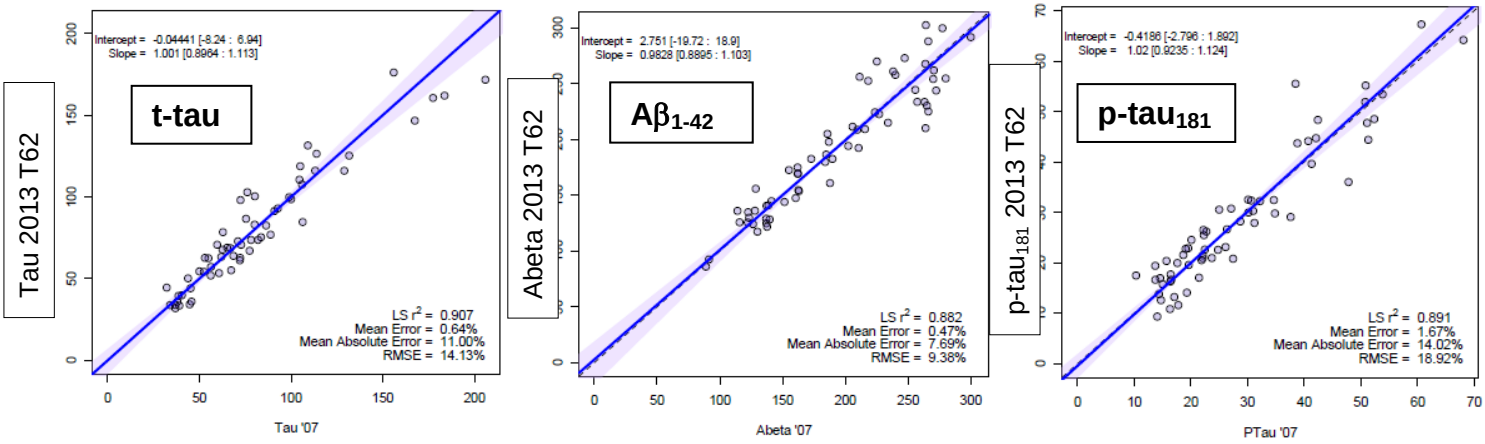
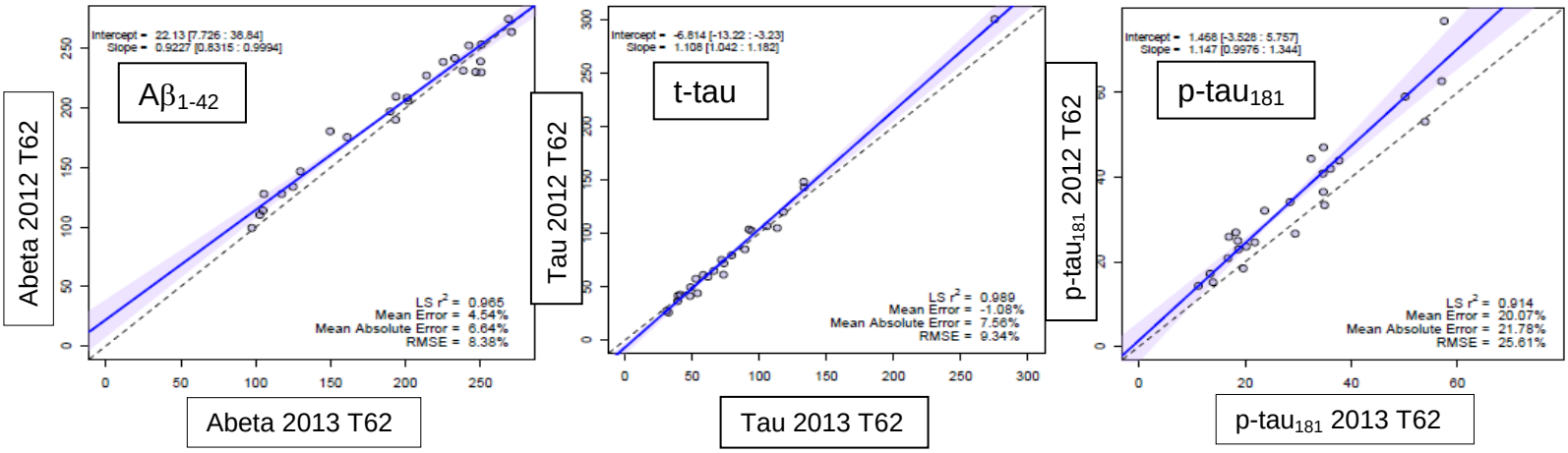


Table 2. . P-B linear regression equation parameters defined by anchoring 2013 analyses of sixty-two ADNI 1 CSFs to 2007 BASELINE data (Fig 4B). Ninety-five % confidence intervals are provided for the slope and intercept values.

	$A\beta_{1-42}$	**t-tau**	**p-tau_{181}**
P-B reg eqn	Y = 0.98X + 2.75	Y = 1.001X - 0.044	Y = 1.02X - 0.47
Y intercept	2.751(-19 - 18.9)	-0.044(-8.2 - 6.9)	-0.419(-2.8 - 1.8)
Slope	0.983(0.89 - 1.10)	1.001(0.90 - 1.11)	1.02(0.92 - 1.12)
r^2	0.88	0.91	0.89

Figure 5. Shown below are 3 P-B linear regression plots of the twenty-five CSF $A\beta_{1-42}$, t-tau and p-tau_{181} results from 2012 ("2012 T62", Y axis) vs ("2013 T62", X axis). Each untransformed concentration value was transformed using the P-B linear regression parameters defined in Figure 4A above. These regression equation results confirm the very good agreement between 2012 biomarker concentrations, anchored to ADNI 1 BASELINE as described in the text, for each of the three biomarkers



Further support for this process of anchoring the 2012 and 2013 datasets to the ADNI 1 BASELINE dataset is provided in the groupwise comparison Table 3 below. This provides a summary of the comparison of results, in the NL, EMCI, LMCI and AD groups, of 2012 T62 with 2013 T62 CSF $A\beta_{1-42}$, t-tau and p-tau_{181}. The expected overall very good agreement of mean values for these biomarkers for each of the four ADNI cohorts was obtained, consistent with achievement of equivalent bridging of the 2012 and 2013 datasets to the ADNI 1 BASELINE dataset.

Table 3. Comparison of mean ± SD results for NL, EMCI, LMCI and AD for transformed 2012 & 2013 ADNI CSF $A\beta_{1-42}$, t-tau and p-tau_{181} results. The P-B equations used to bridge 2012 and 2013 data to ADNI 1 results were derived from the linear regression analyses of the sixty-two 2013 untransformed results vs ADNI 1 results in these sixty-two aliquot samples determined in 2007 shown in Figure 4A.

	2012T	**2013T**	***P****
Biomarker	**Mean ± SD**		
NL	(n=108)	(n=85)	
$A\beta_{1-42}$	187±48	211±54	*0.002*
t-tau	67.9±31	69.5±35	*0.746*
p-tau_{181}	34±15	32±21	*0.523*
EMCI	(n=188)	(n=103)	
$A\beta_{1-42}$	185±49	184±56	*0.814*
t-tau	76±49	81±50	*0.375*
p-tau_{181}	36±21	36.5±20	*0.956*
LMCI	(n=65)	(n=118)	
$A\beta_{1-42}$	152±46	164±51	*0.101*
t-tau	95±51	100±55	*0.525*
p-tau_{181}	51±31	42±23	*0.033*
AD	(n=25)	(n=76)	
$A\beta_{1-42}$	134±34	132±34	*0.812*
t-tau	125±53	138±65	*0.336*
p-tau_{181}	56±25	58±31	*0.847*
*Student's t test, 2-tailed, with Welch's approximation of the degrees of freedom.			

Summary

We describe the re-anchoring of the 2012 ADNI 2 and GO 390 untransformed BASELINE CSF biomarker dataset. The re-anchoring procedure takes advantage of the very good lot-to-lot performance approximately one year apart for the 2012 and 2013 CSF analyses using AlzBio3 immunoassay and the fact that a total of sixty-two 2007 BASELINE pristine ADNI 1 aliquots were analyzed in 2013. The total of sixty-two 2007 BASELINE ADNI 1 CSF samples that were analyzed over 10 analytical runs in 2013, as part of the study of the longitudinal behavior of $A\beta_{1-42}$, t-tau and p-tau_{181}, provided a sufficient number of samples to capture the natural variance observed over multiple analytical runs thereby providing a more accurate anchoring to 2007 BASELINE data. The slope and intercept data and respective 95% confidence interval data, shown in Figure 4B, fully support the accuracy, using 2007 ADNI 1 BASELINE data as reference standard, of the anchoring procedure for the 2013 samples. Thus the combination of very good lot-to-lot performance one year apart (2012 and 2013) and the successful anchoring of the 2013 data set to 2007 are the basis for the re-anchoring of the 2012 data set. Two objective measurements to verify the accuracy of the anchoring procedure for the 2012 dataset were (1) comparison of the twenty-five CSF $A\beta_{1-42}$, t-tau and p-tau_{181} concentration data-2012 transformed results-to the 2013 anchored to 2007 data (Figure 5) and (2) the group-wise comparability of the $A\beta_{1-42}$, t-tau and p-tau_{181} concentration results for the NL, EMCI, LMCI and AD groups tested in 2013 vs those in 2012 (Table 3).

A very valuable outcome of this is that future anchoring of ADNI CSF data to 2007 ADNI 1 BASELINE data will no longer be required as this can be done using 2013 ADNI 2 BASELINE CSF biomarker data. In the longer term, once the planned international reference material, based on pooled CSF and accuracy-based reference method mass spectrometry measurement of $A\beta_{1-42}$ concentration, becomes available, via the IFCC/IRMM standardization effort led by Kaj Blennow (5) that we participate in, we hope to create a series of pools across a range of $A\beta_{1-42}$ concentrations that can be used for future anchoring of ADNI CSF $A\beta_{1-42}$ datasets.

Method

The xMAP Luminex platform and Innogenetics/Fujirebio AlzBio3 Research Use Only immunoassay kits were used following the SOP in place at the UPenn/ADNI Biomarker Laboratory, according to the kit manufacturer's instructions, as described in previous publications (1-3) and most recently studied across three centers each following our detailed "unified SOP" and reported at the 2013 AAIC meeting (6). The ADNI 2012 batch analyses were performed in a series of 15 runs using a 96 well plate format, over the time period of February 21 through March 16, 2012. The acceptance criteria, documented in the UPenn/ADNI Biomarker Core SOP, were followed for run acceptance of these analyses.

Each of the 15 analytical runs met acceptance criteria for calibrator precision and accuracy(back calculated concentration result vs nominal concentration result) and quality control results were within stated limits (detailed data in "ADNI GO and ADNI 2 CSF

report for $A\beta_{1-42}$, t-tau and p-tau_{181}" , dated 2012-06-04, and available on the LONI web site). Individual ADNI CSF sample results were acceptable in all cases except where noted and those are reported as "NA" in the corrected CSV file "BIOMARK5".

Summary of batch runs of ADNI CSF samples

1. BASELINE CSFs ("adni_upennbiomk_.csv"), batch tested, uploaded on ADNI website: $A\beta_{1-42}$, t-tau, $pTau_{181}$ data early 2008, (REPORTED in Annals of Neurology, 2009). Data were reported on 410 subjects as described in the Annals of Neurology publication (reference #2). These BASELINE CSF $A\beta_{1-42}$, t-tau, $pTau_{181}$ data have served as the reference set of data for subsequent batch runs. A report on the quality control performance over time, using abnormal and normal CSF pools, and over different reagent lot #'s for reagents, calibrators and AlzBio3 kit controls can be found in reference #3.
2. BASELINE and YR1, batch tested, reported in: "adni_upennbiomk2_.csv", uploaded dataset Feb 2009, without $pTau_{181}$, and were "bridged" to 2007 data. These data contain a large set of longitudinal data for BASELINE and yr1; N=328 BL and YR1 pairs of ADNI subject data. For this set of longitudinal samples, as well as for all subsequent longitudinal datasets, all longitudinal CSF sample sets for each ADNI subject were run on the same analytical plate in order to preclude run-to-run variance as a contributor to within-subject analysis variability.
3. Tested 91 sets of "triplet" CSF samples (BASELINE, YR1 and 24 or 36 month CSFs) and were "bridged" to ADNI 1 BASELINE data. These results are reported in the .csv file, "adni_upennbiomk3_.csv". These data were uploaded in the 4th quarter of 2009 and were the first report of the add-on ADNI Longitudinal CSF biomarker study, funded by an anonymous donor.
4. Test results for 142 ADNI 1 study subjects with 3-5 visits each are reported in "adni_upennbiomk4_.csv". This dataset includes CSF $A\beta_{1-42}$, t-tau and p-tau_{181} results for 142 subjects and this dataset was uploaded in October, 2011. These are the study subjects included in Toledo J, et al, ref #7. Bridging to ADNI 1 BASELINE data was done as above.
5. In "adni_upennbiomk6_.csv" are included a dataset of CSF $A\beta_{1-42}$, t-tau, $pTau_{181}$ results that included 63 longitudinal ADNI 1 "carryover" subject sets that extend the longitudinal trajectory out as far as 6-7 yrs in some subjects and in addition 26 GO BASELINE + 1yr longitudinal samples. This data set, uploaded in July 2013, contains the longest interval of time longitudinal ADNI CSF sample sets. Individuals whose longitudinal data were provided in item 4 above were included in this series if additional longitudinal samples had been collected. Individuals were not included in this set of analyses if no additional longitudinal samples accrued as of the end of the sample accrual time window of 1/18/2013. The dataset includes 309 BASELINE ADNI 2 visit samples.

   For studies of ADNI GO + 2 subjects that aim to assess BASELINE-only CSF $A\beta_{1-42}$, t-tau, $pTau_{181}$ for predictive performance we recommend combining ADNI 2 BASELINE data from the dataset, "adni_upennbiomk6_.csv" together with the

ADNI 2 and GO BASELINE data in the corrected upennbiomk5 dataset that is described in this Methods report.

For studies of longitudinal changes in CSF $A\beta_{1-42}$, t-tau, $pTau_{181}$ we recommend use of "adni_upennbiomk6" dataset together with the subjects not included in the latter whose CSF samples were analyzed as part of "adni_upennbiomk4_.csv". This provides longitudinal samples for the ADNI study with the longest trajectories.

References

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