ADNI Biomarker Core

Leslie M Shaw and John Q Trojanowski
Department of Pathology & Laboratory Medicine Perelman School of Medicine
University of Pennsylvania

WWADNI meeting, Boston, MA, July 12, 2013

ADNI Biomarker core 2012-2013

- Biofluid report update (presented at ADNI SanDiego meeting, 3/17/2013)
- Studies approved by RARC/NIA/ADNI using ADNI biofluids
  - α-SYN & Hgb, JZhang, et al (ADNI I BL CSF)
  - C3 and factor H in ADNI I BL CSF-just uploaded, JZhang
  - BACE and sAPPβ in ADNI 1 BL CSF, Merck, poster Sunday
  - mrm/tandem mass spectrometry of tryptic peptides associated with 251 proteins, Caprion/FNIH/ADNI/PPSB (ADNI I BL CSF), underway
  - Autoantibody pilot study in serum from 100 ADNI GO/II subjects, Robert Nagele
- α-SYN in ADNI and PPMI
- Longitudinal CSF biomarkers
- ADNI 2 and GO CSF biomarker studies update
- mrm/tandem mass spectrometry reference method for Aβ1-42

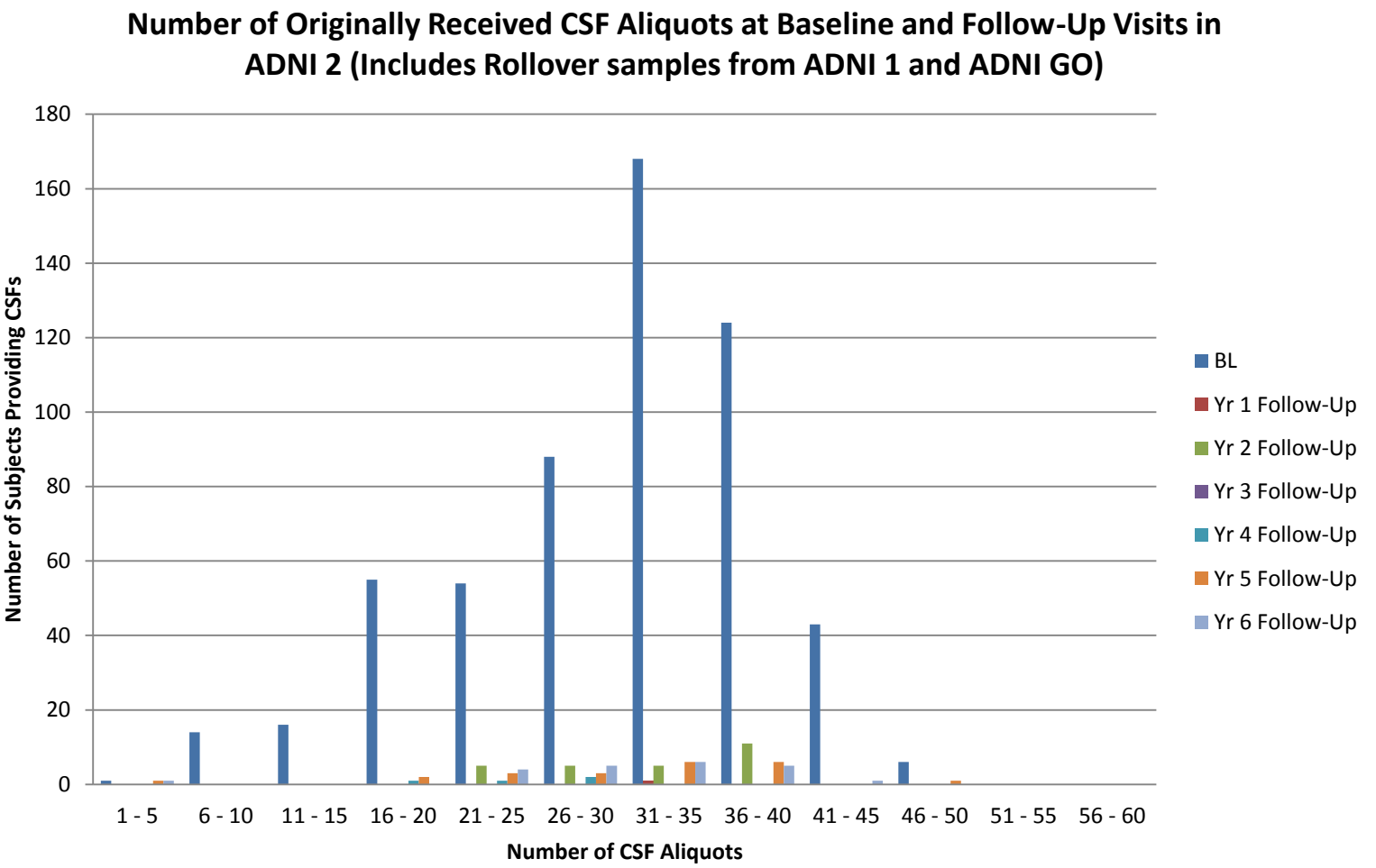
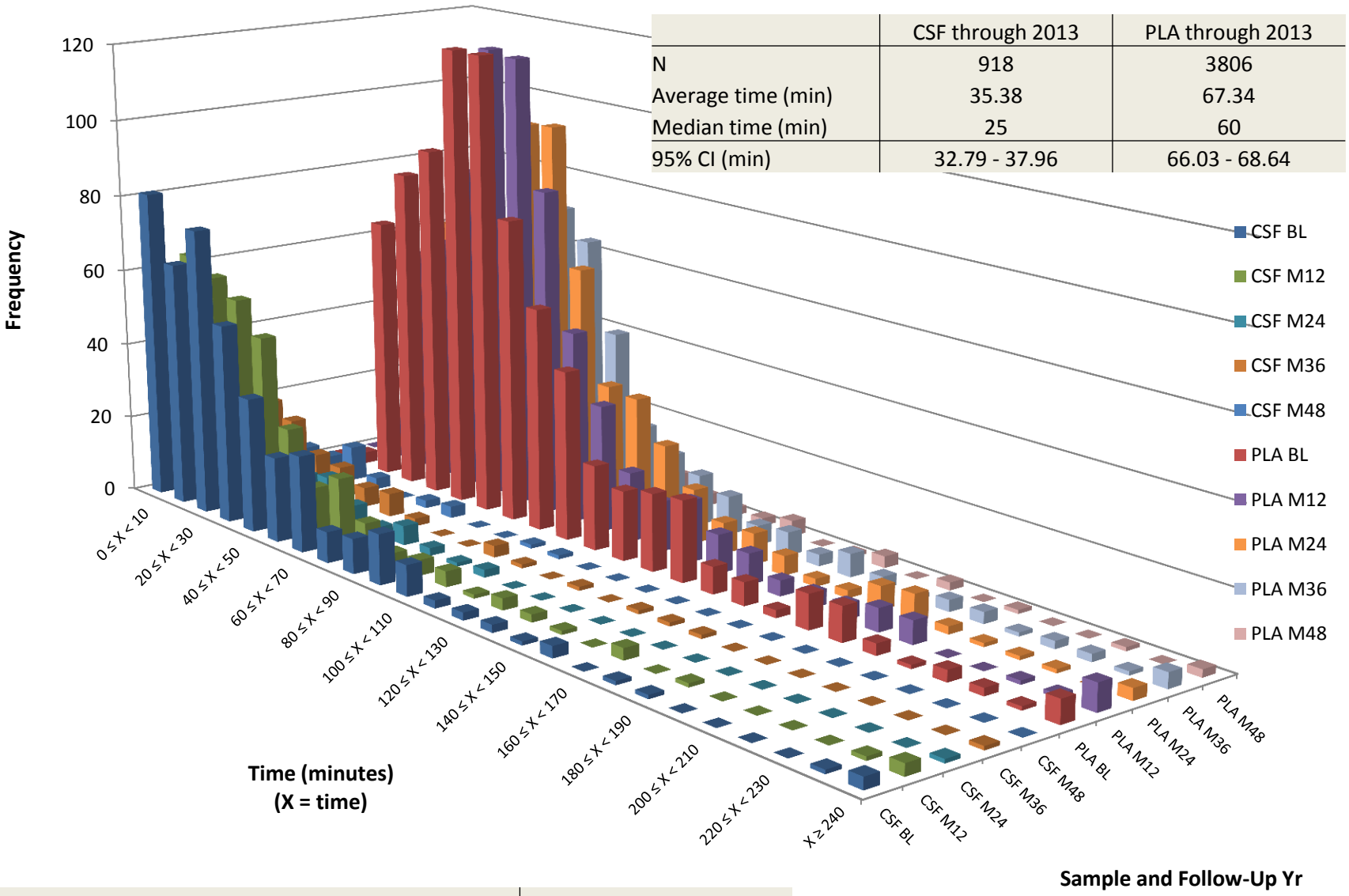


Figure 4a. Number of Originally Received CSF Aliquots for ADNI 2 Phase at Baseline and Follow-Up Visits (including Roll-over Subjects from ADNI 1 and ADNI GO). The graph above displays the number of subjects who have provided CSF samples during the ADNI 2 study phase at baseline and at follow-up visits. Subjects who provided CSF samples at baseline in ADNI 2 are those who were initially enrolled in ADNI 2. The follow-up visits here include subjects who rolled over into ADNI 2 from either ADN1 or ADNI GO. For example, we originally received between 21 and 25 CSF aliquots per subject from 54 individuals at baseline, compared to 0 individuals at 1-year follow-up, 5 individuals at 2-year follow-up, 0 individuals at 3-year follow-up, 1 individual at 4 year follow-up, 3 individuals at 5-year follow-up, and 4 individuals at 6-year follow-up.

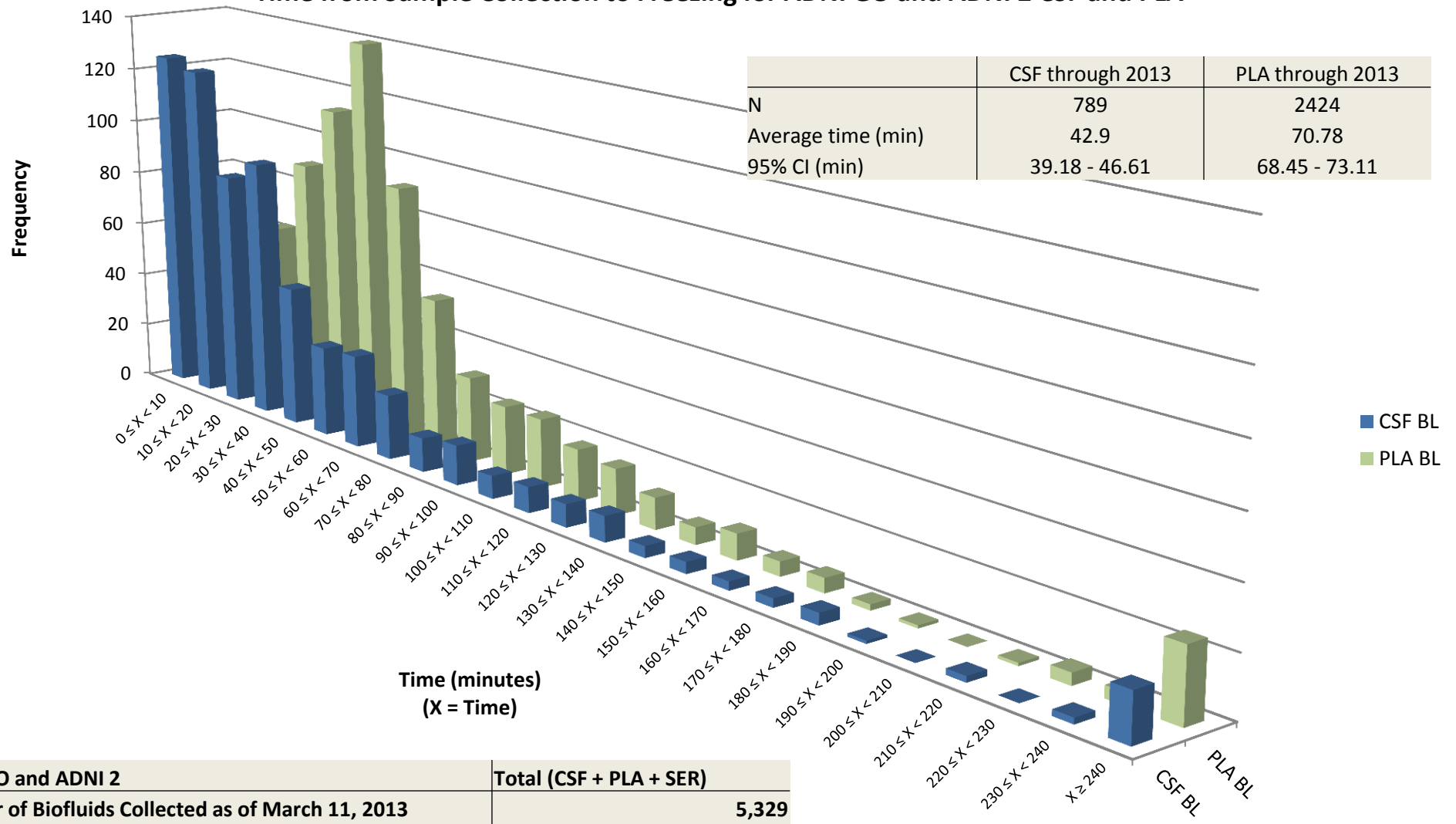
Time from Sample Collection to Freezing for ADNI 1 CSF and PLA

	CSF through 2013	PLA through 2013
N	918	3806
Average time (min)	35.38	67.34
Median time (min)	25	60
95% CI (min)	32.79 - 37.96	66.03 - 68.64



ADNI 1	Total (CSF + PLA + SER)
Number of Biofluids Collected as of March 11, 2013	8,601
Number of Aliquots in Biofluid Bank	141,978

Time from Sample Collection to Freezing for ADNI GO and ADNI 2 CSF and PLA



	CSF through 2013	PLA through 2013
N	789	2424
Average time (min)	42.9	70.78
95% CI (min)	39.18 - 46.61	68.45 - 73.11

ADNI GO and ADNI 2	Total (CSF + PLA + SER)
Number of Biofluids Collected as of March 11, 2013	5,329
Number of Aliquots in Biofluid Bank	94,290

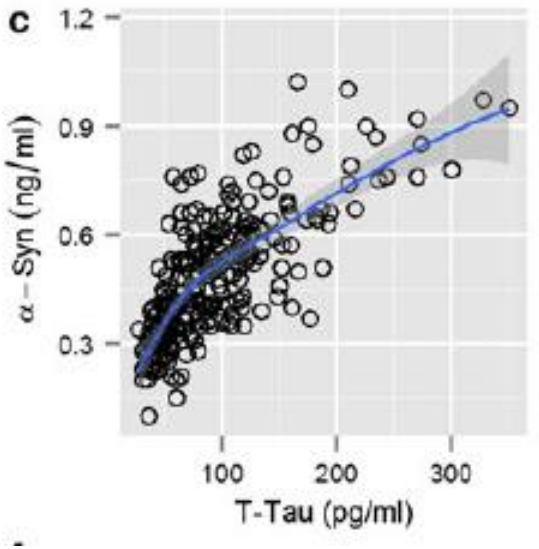
All ADNI	Grand Total (CSF + PLA + SER)
Number of Biofluids Collected as of March 11, 2013	13,930
Number of Aliquots in Biofluid Bank	236,268

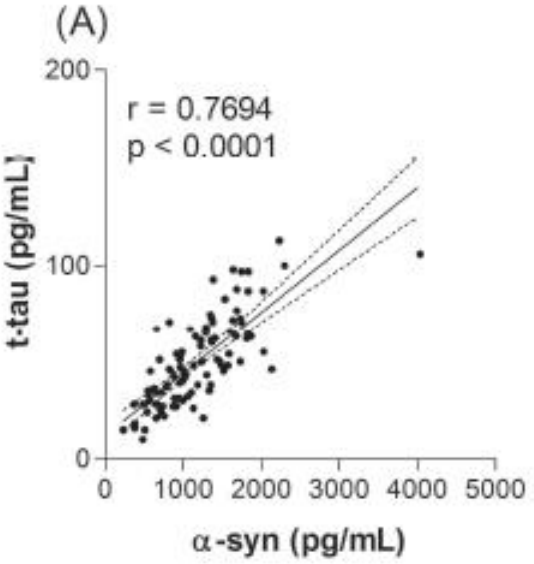
ADNI biofluid collections update

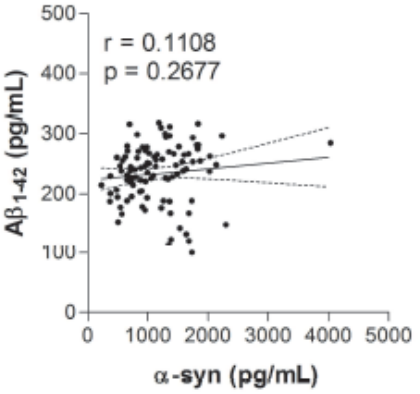
Update planned for upload on LONI ADNI site: 9/30/2013

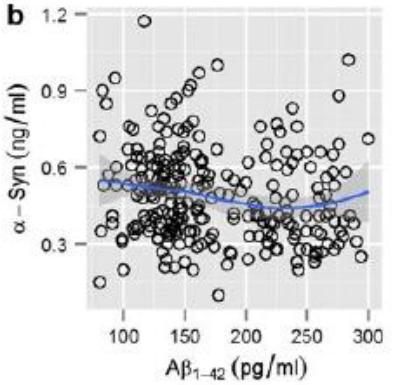
α-synuclein correlates with tau & p-tau$_{181}$ but not with Aβ$_{1-42}$

Toledo, J.B., Korff, A., Shaw, L.M., Trojanowski, J.Q., and Zhang, J. for the Alzheimer's Disease Neuroimaging Initiative. CSF α-synuclein improves diagnostic and prognostic performance of CSF tau and Aβ in Alzheimer's disease. Acta Neuropath, In press, 2013.









In ADNI AD subjects

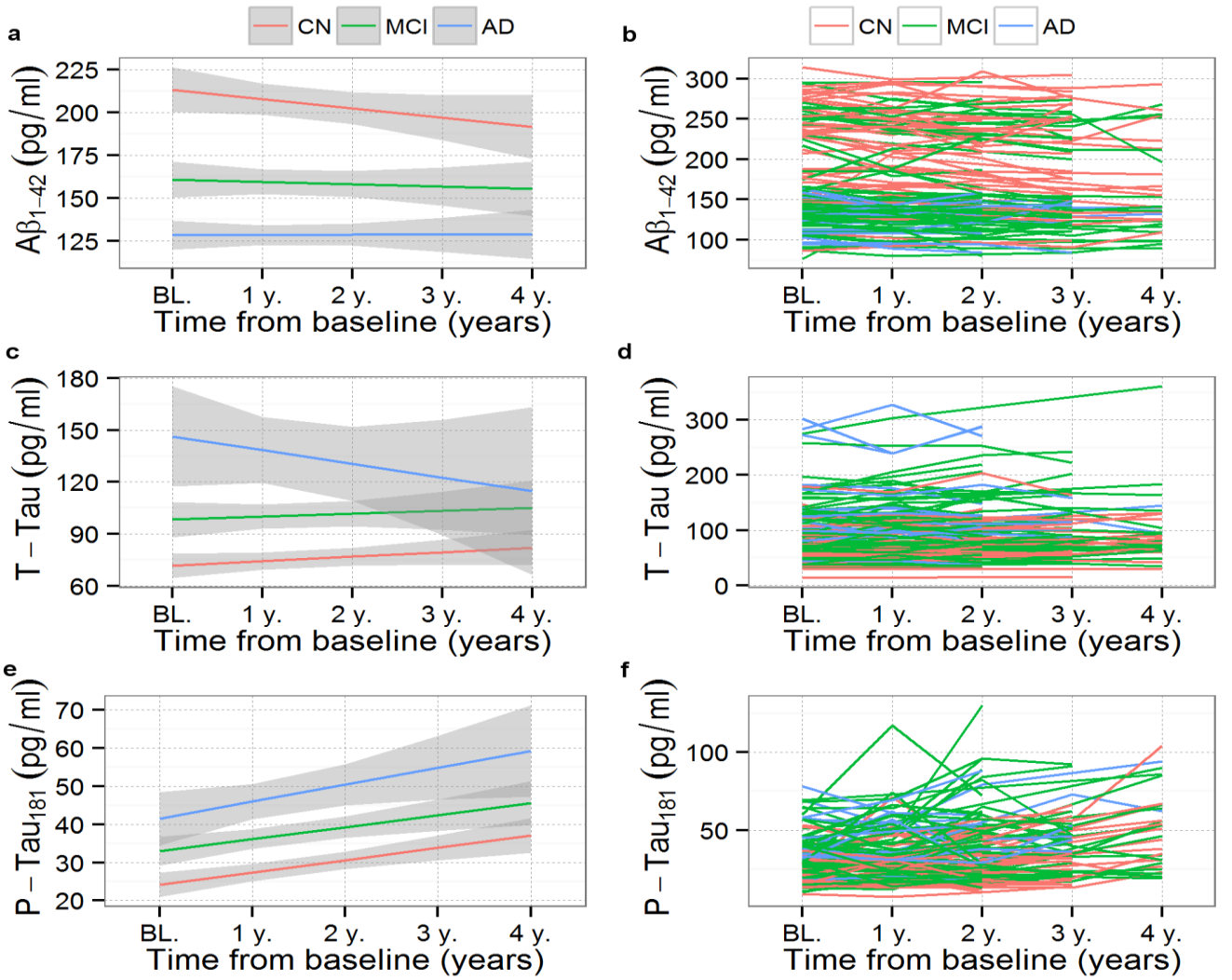
- ↑α-SYN in CSF
- In a subset of ADNI subjects there is an α-SYN-p-tau-Mismatch

ADNI(92 AD, 187 MCI, 119 CN)
Toledo JB, et al, Acta Neuropath, 2013

PPMI (63 PD, 39 CN)
Kang J-H, et al JAMA Neurol, 2013

In a subset of ADNI patients there is an α-SYN-p-tau-Mismatch, (a-SYN lower concentration in proportion to p-tau) and it is proposed that these are individuals likely to have Lewy Body pathology in addition to AD

ADNI I longitudinal CSF $A\beta_{1\text{-}42}$, t-tau & p-tau_{181}



Toledo J, et al, Acta Neuropath, 2013

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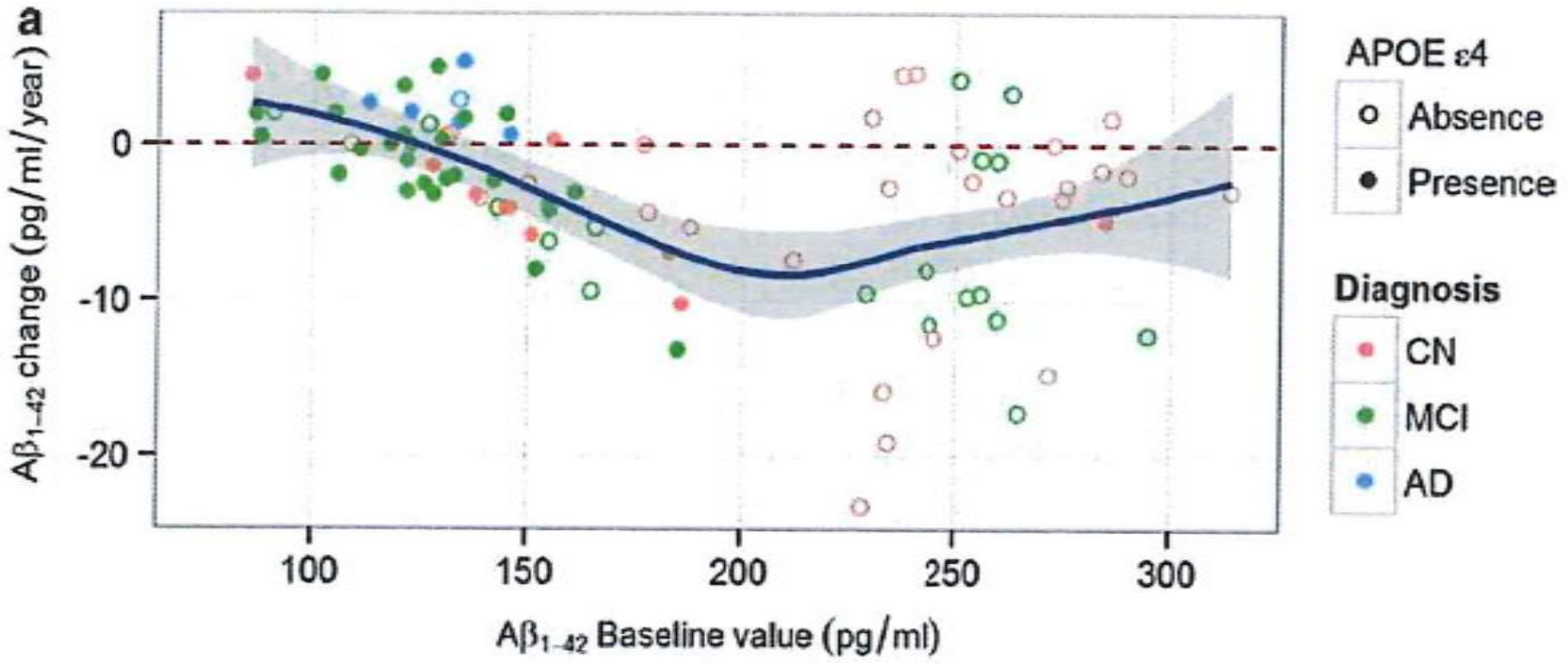
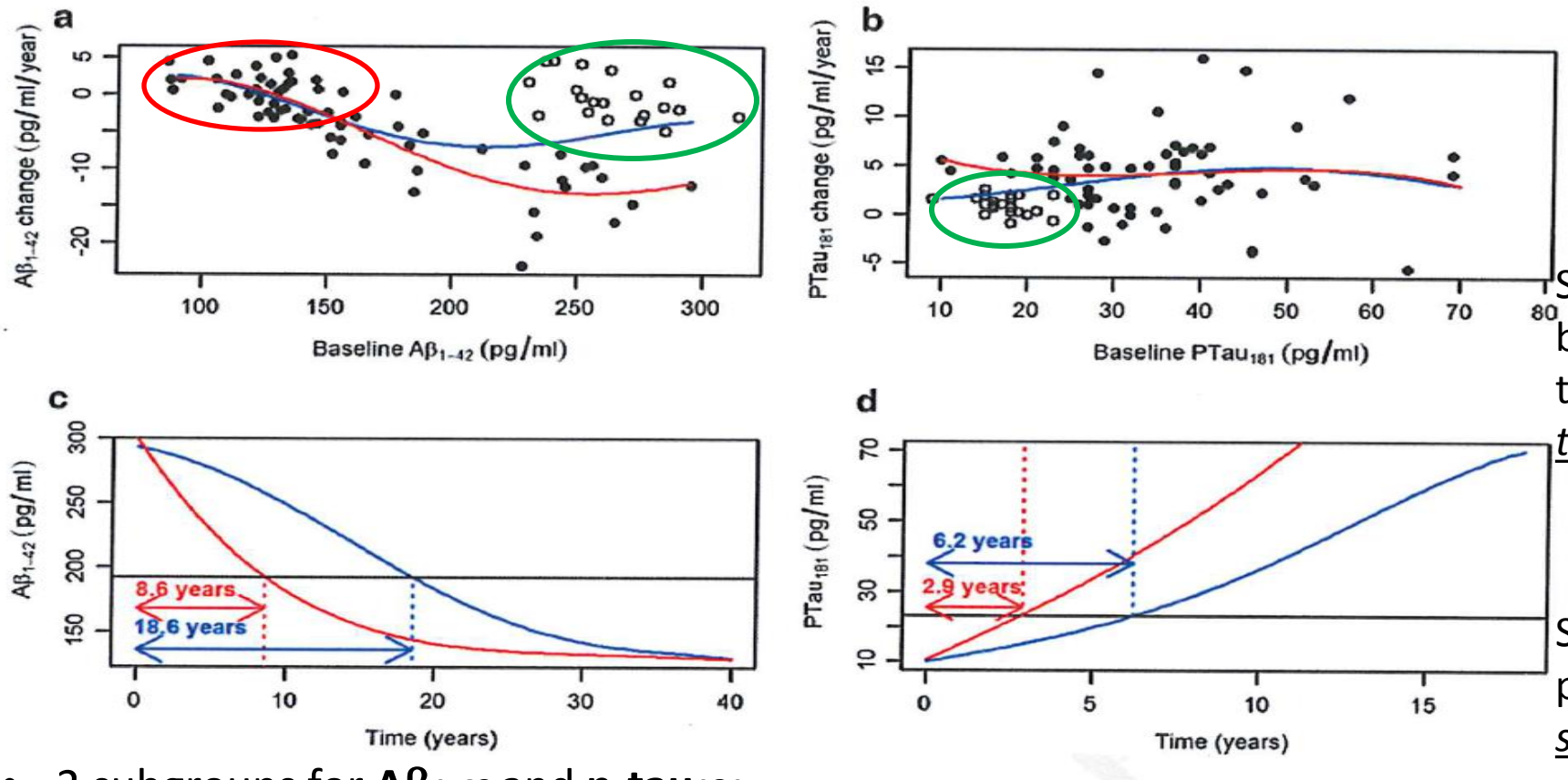


Toledo J, et al, Acta Neuropath, 2013

ADNI I longitudinal CSF $A\beta_{1-42}$, t-tau & p-tau_{181}

$A\beta_{1-42}$ p-tau_{181}



Subjects with normal baseline $A\beta_{1-42}$ or p-tau_{181} **and** *stable trajectory*

Subjects with pathological $A\beta_{1-42}$ **and** *stable trajectory*

- 2 subgroups for **$A\beta_{1-42}$** and **p-tau_{181}**

$A\beta_{1-42}$

(1) $A\beta_{1-42}$ ***"Stable Group"*** : **-0.5 pg/mL/yr**
(2) $A\beta_{1-42}$ ***"Decrease Group"*** : **-9.2 pg/mL/yr**

p-tau_{181}

(1) p-tau_{181} ***"Stable Group"*** : **+1.5 pg/mL/yr**
(2) p-tau_{181} ***"Increase Group"*** : **+5.1 pg/mL/yr**

UPENNBIOMK4 dataset
N=142 (50 CN, 74 MCI, 18 AD)

- 4-7 samples/subject
- all individual subject CSFs run on the same plate
- all concentration results anchored to 2007 BASELINE

ADNI I longitudinal CSF $A\beta_{1\text{-}42}$, t-tau & p-tau_{181}

- Using an unsupervised statistical modeling approach two distinct subgroups in the ADNI population were detected
  - One subset of individuals: stable $A\beta_{1\text{-}42}$ (-0.5 pg/mL/yr) and p-tau_{181} (+1.5 pg/mL/yr)
  - The other subset of individuals: ↓ $A\beta_{1\text{-}42}$ (mean -9.2 pg/mL/yr) or ↑ p-tau_{181} (mean +5.1 pg/mL/yr)
- low BASELINE $A\beta_{1\text{-}42}$ associated with longitudinal ↑p-tau_{181}
- High BASELINE p-tau_{181} did not predict longitudinal changes in $A\beta_{1\text{-}42}$
- When subjects with normal BL biomarkers and stable concentrations were excluded, the expected time to reach abnormal CSF AD-like concentrations was significantly shortened
- These longitudinal findings support the hypothesis that CSF $A\beta_{1\text{-}42}$ changes precede p-tau_{181} changes

ADNI UPenn Biomarker Core Laboratory

ADNI 1, GO AND 2 CSF REPORT

$A\beta_{1-42}$, $t-Tau$ AND $p-Tau_{181}$

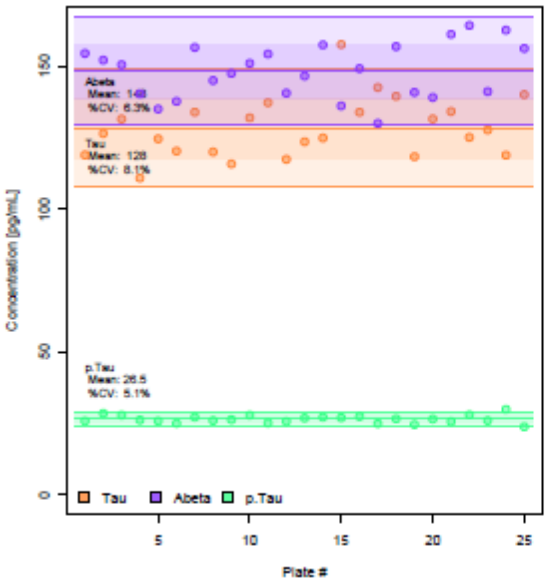
Contents

2013-05-22

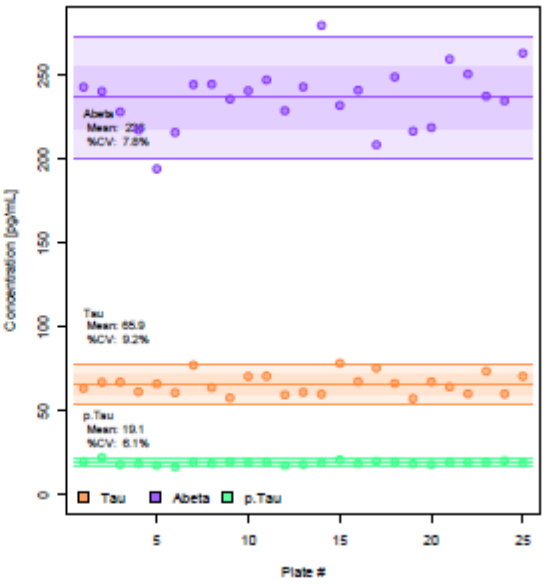
Precision performance of AlzBio3 immunoassay 2013
25 analytical runs

CSF Pools

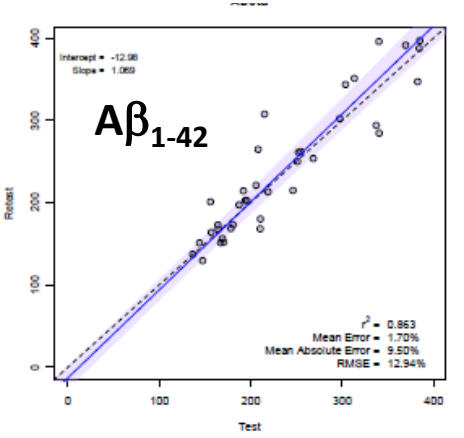
Abnormal

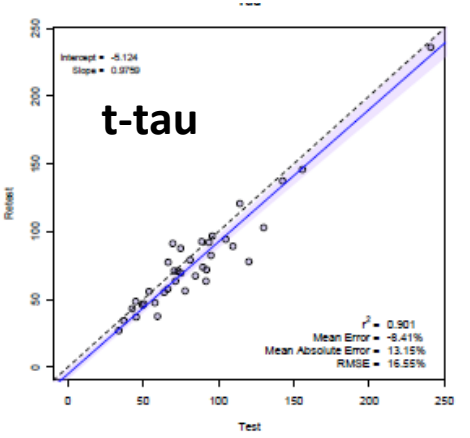


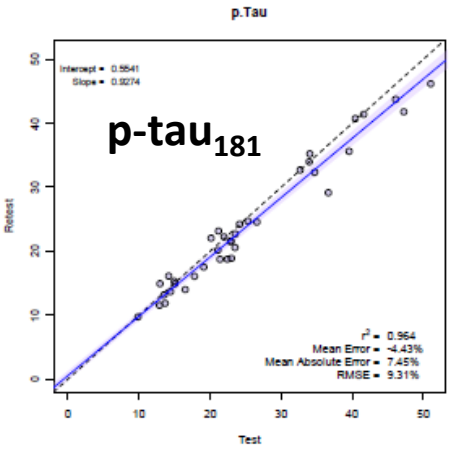
Normal



Test-retest performance

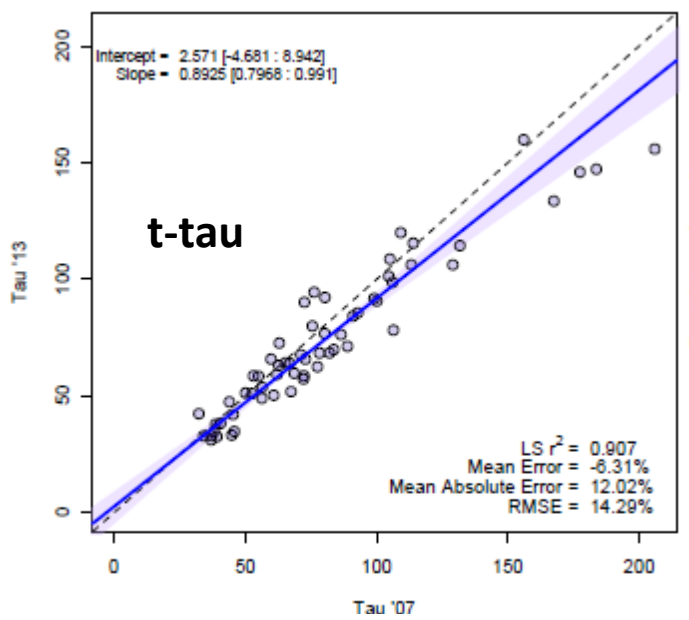


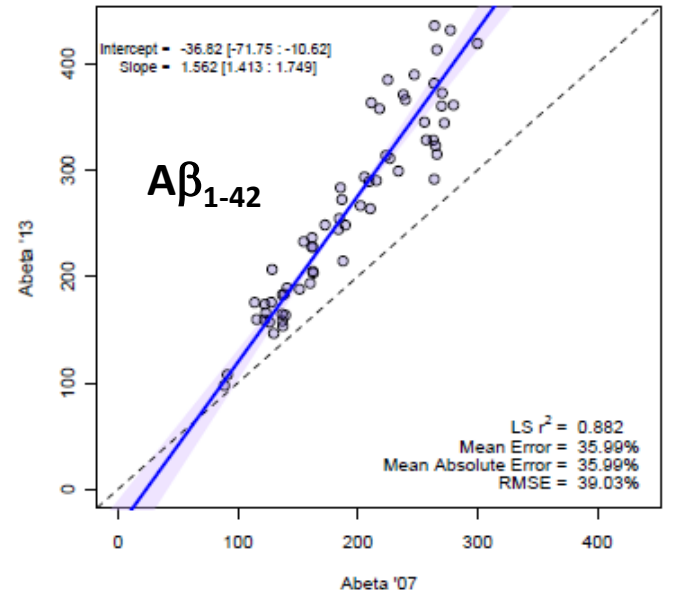




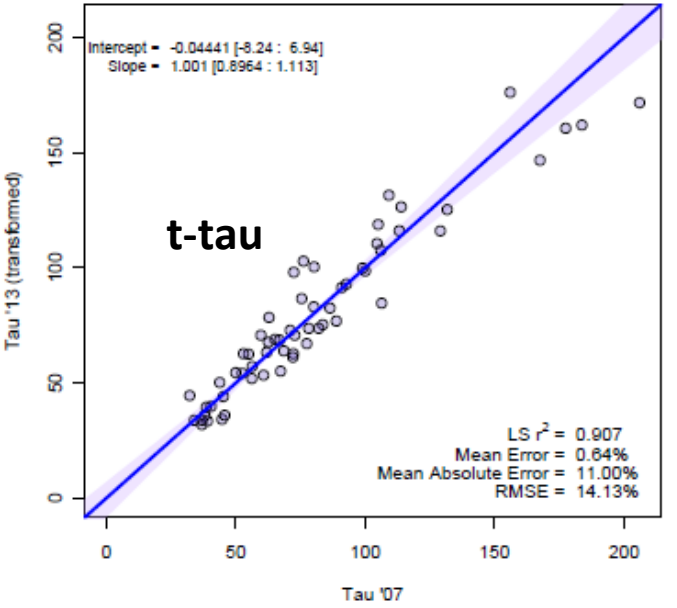
Anchoring 2013 CSF biomarkers to BASELINE 2007

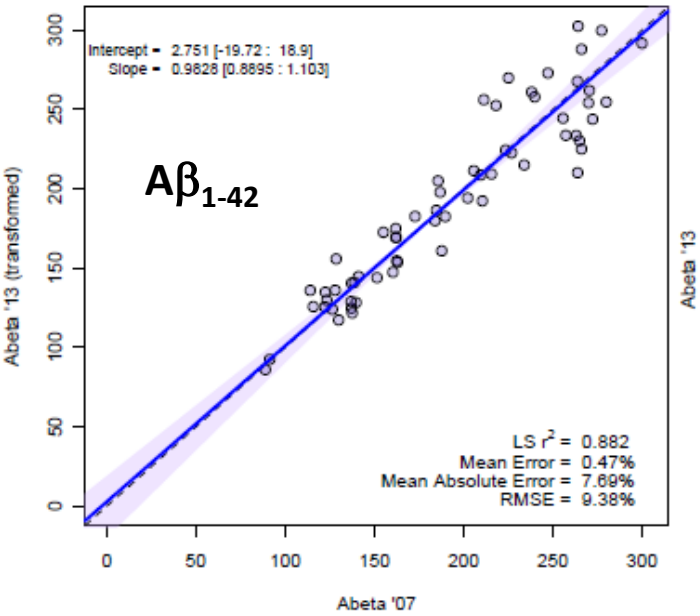
2013 raw vs 2007



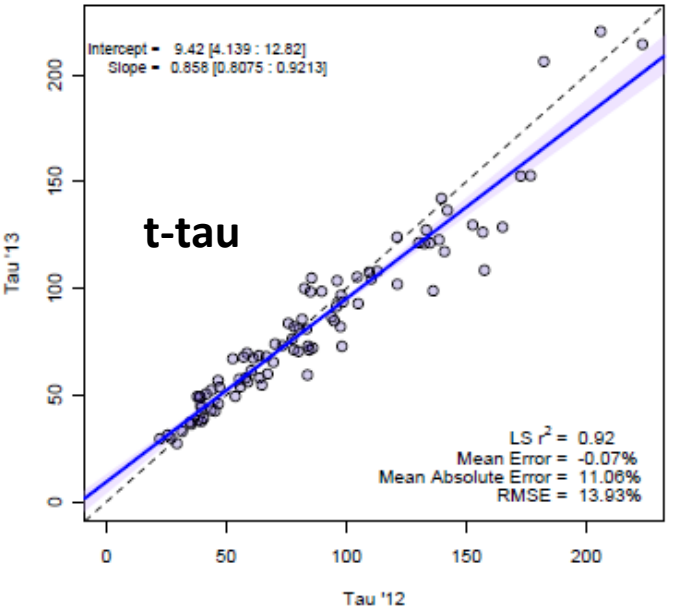


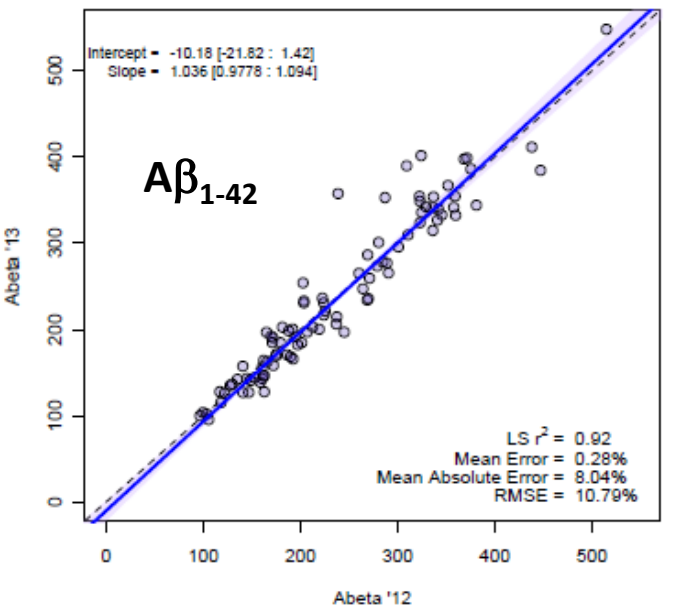
2013 anchored to 2007



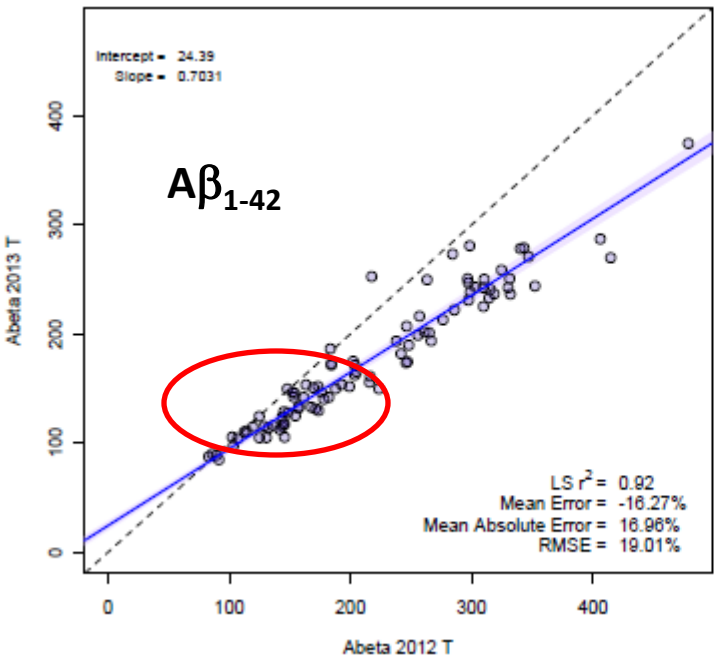


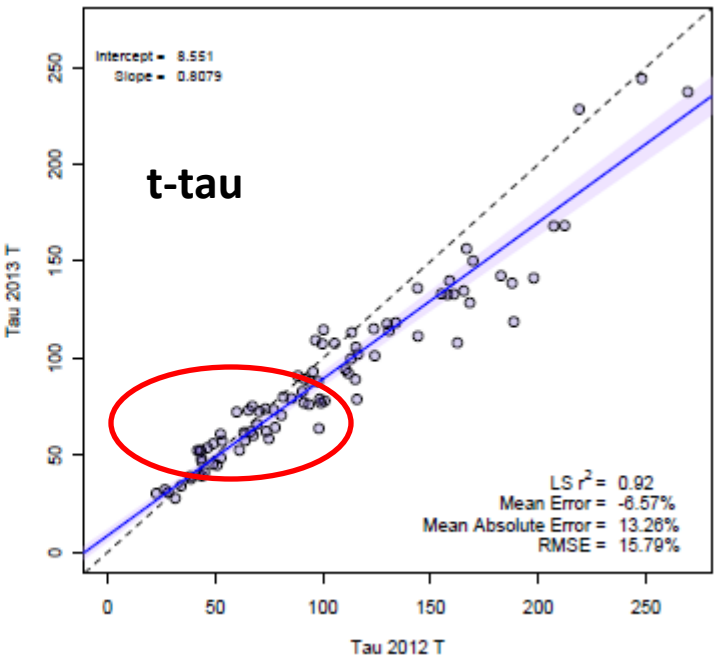
2013 raw vs 2012 raw





Comparison of concentrations in CSF for pristine aliquots anchored using 12 samples and one analytical run in 2012 vs using 62 samples and multiple runs in 2013



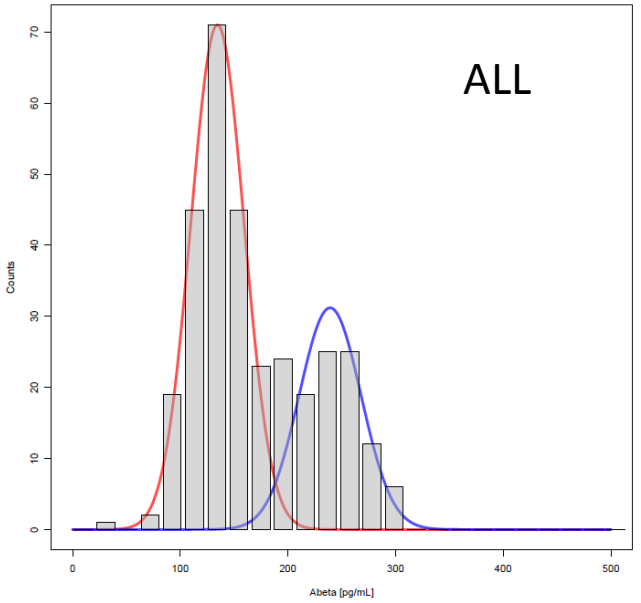


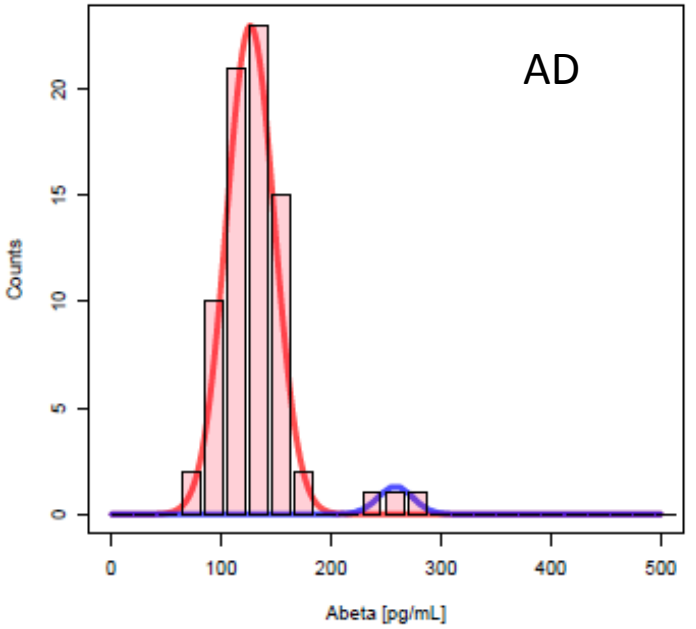
2013 BASELINE ADNI II CSF $A\beta_{1-42}$, t-tau, p-tau_{181}, ratios & logistic regression model (mean±SD)

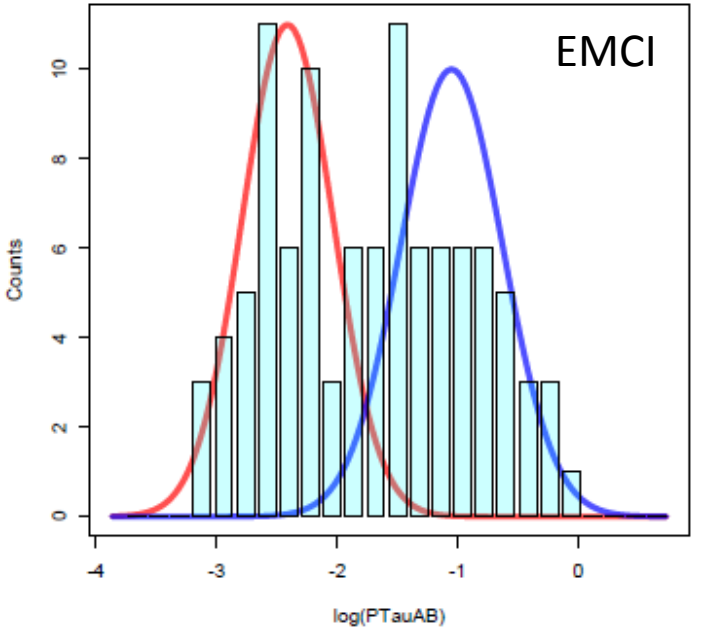
	APOE ε4	$A\beta_{1-42}$	t-tau	p-tau_{181}	t-tau/ $A\beta_{1-42}$	LRTAA2i
	(%)	(pg/mL)	(pg/mL)	(pg/mL)		
AD (76)	74	132±34	138±65	58±31	1.12±0.63	0.84±0.25
LMCI (87)	57	163±51	104±58	45±24	0.73±0.50	0.61±0.39
EMCI (101)	49	184±56*	80±46**	36±20***	0.51±0.44****	0.41±0.37*****
NC (53)	38	209±54	68±39	36±25	0.36±0.28	0.30±0.34

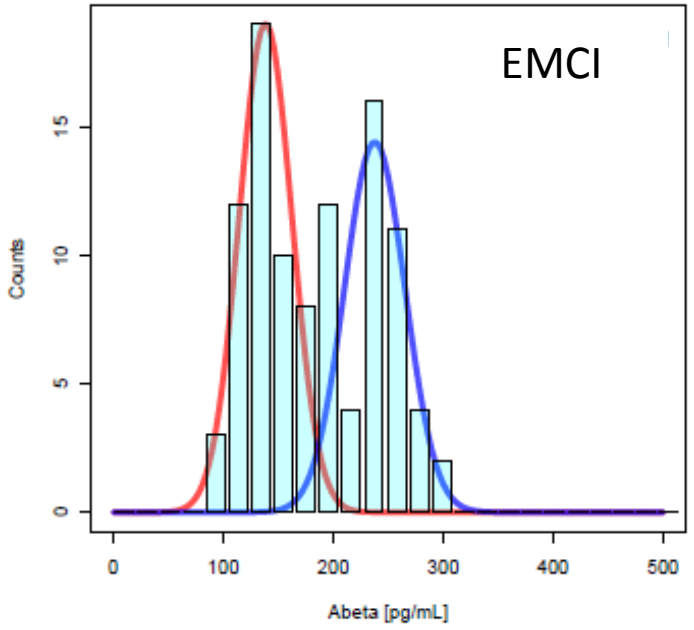
*$A\beta_{1-42}$: p<0.000001 vs AD; p<0.05 vs LMCI or NC. **t-tau: p<0.000001 vs AD; p<0.01 vs LMCI; p=0.11 vs NC
***p-tau_{181}: p<0.000001 vs AD; p<0.01 vs LMCI; p=0.60 vs NC. ****t-tau/ $A\beta_{1-42}$: p<0.000001 vs AD; p<0.001 vs LMCI; p=0.08 vs NC
*****LRTAA2i: p<0.000001 vs AD; p<0.0005 vs LMCI; p=0.11 vs NC;
LRTAA2i: logistic regression model including t-tau, $A\beta_{1-42}$, #APOE ε4 allele counts(0,1 or2), age, gender.

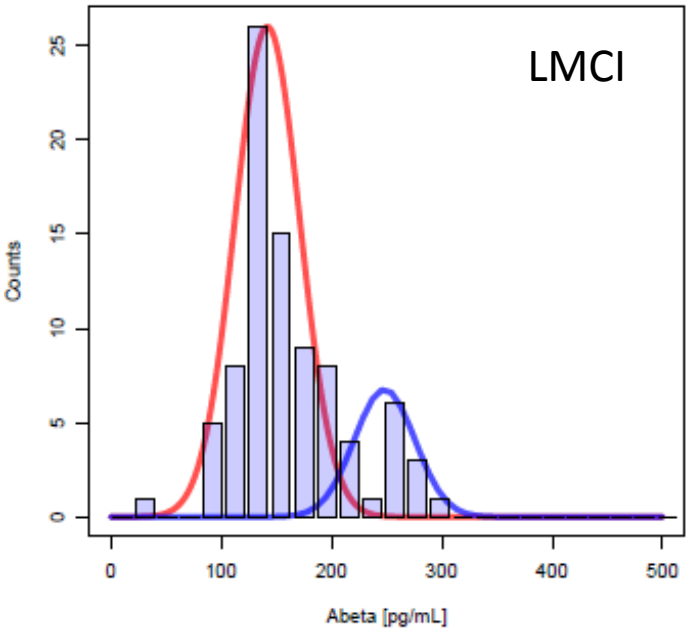
ADNI II 2013 BASELINE $A\beta_{1-42}$ & p-tau_{181}/ $A\beta_{1-42}$

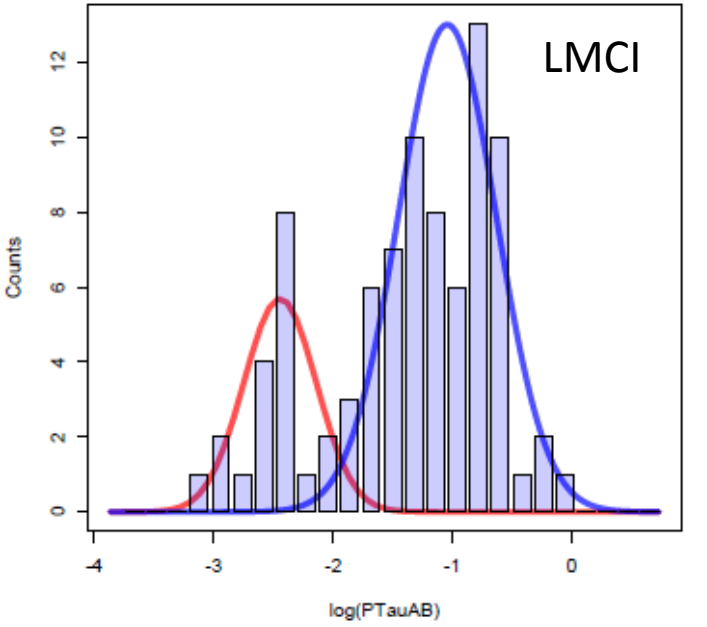












Anchoring CSF biomarkers to a “gold standard”

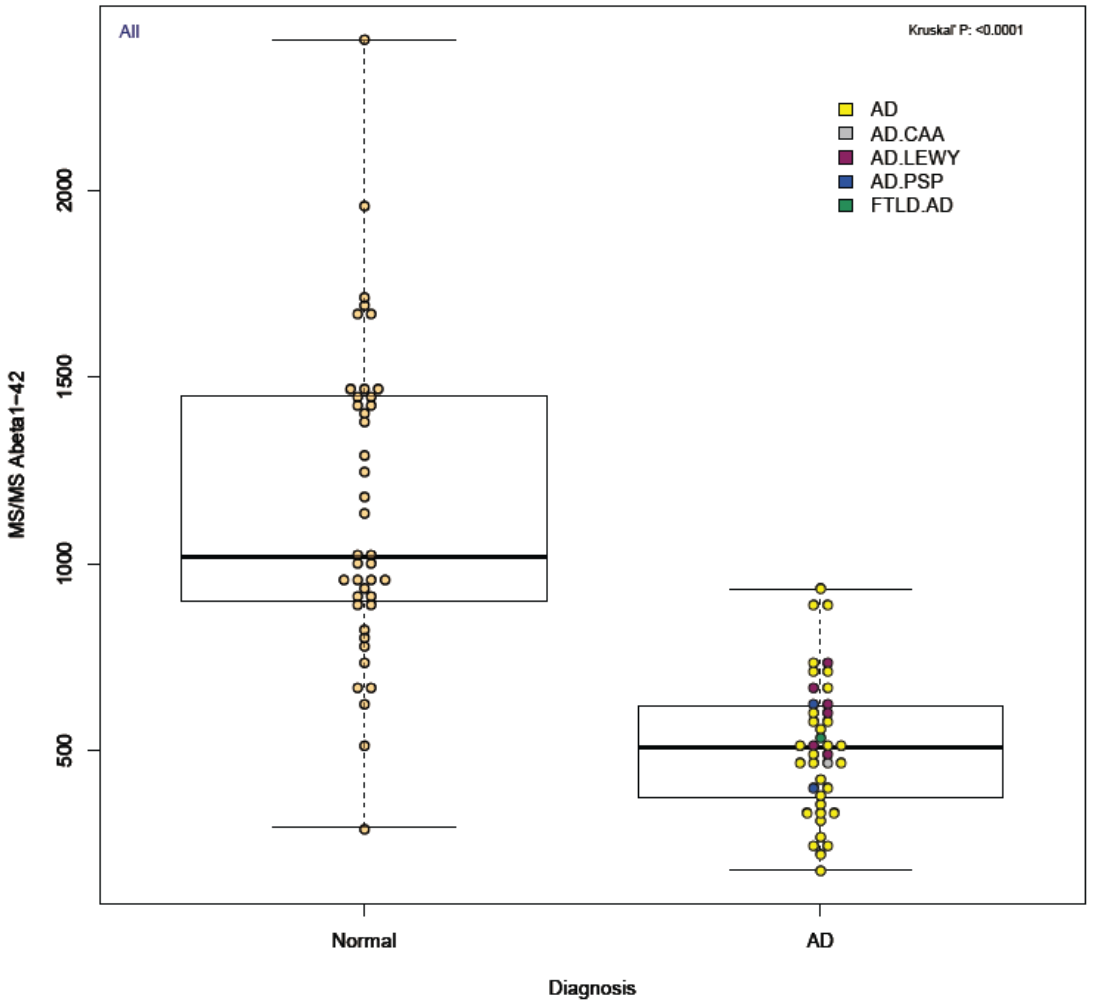
- Currently there is no reference standard for CSF biomarkers
- In the ADNI study we use a “reference set” of samples to enable anchoring the data to a common standard(BASELINE ADNI I CSFs)
- From our experience best results achieved when a sufficient # of “reference” samples/analytical runs are utilized
- For the 2013 dataset (ADNI II, GO, and ADNI I carryovers) we used the 62 2007 BASELINE CSFs that were included as part of the longitudinal sets of samples to anchor 2013 to 2007
- The 2013 BASELINE samples can be used as the reference standard in the future thereby sparing 2007 BASELINE samples
- We are further evaluating the anchoring process for the 2012 ADNI GO & 2 dataset & believe the selected set of 12 2007 BASELINE samples was inadequate to reasonably anchor 2012 to 2007
- A likely solution is to use the anchoring regression based on 62 ADNI I BASELINE samples run in 2007 and in 2013 on all 2012 and 2013 ADNI II and GO since the lot to lot performance across those two timepoints is tight.

Protocol for development & validation of the UPenn/ADNI UPLC-srm/tandem mass spectrometry method

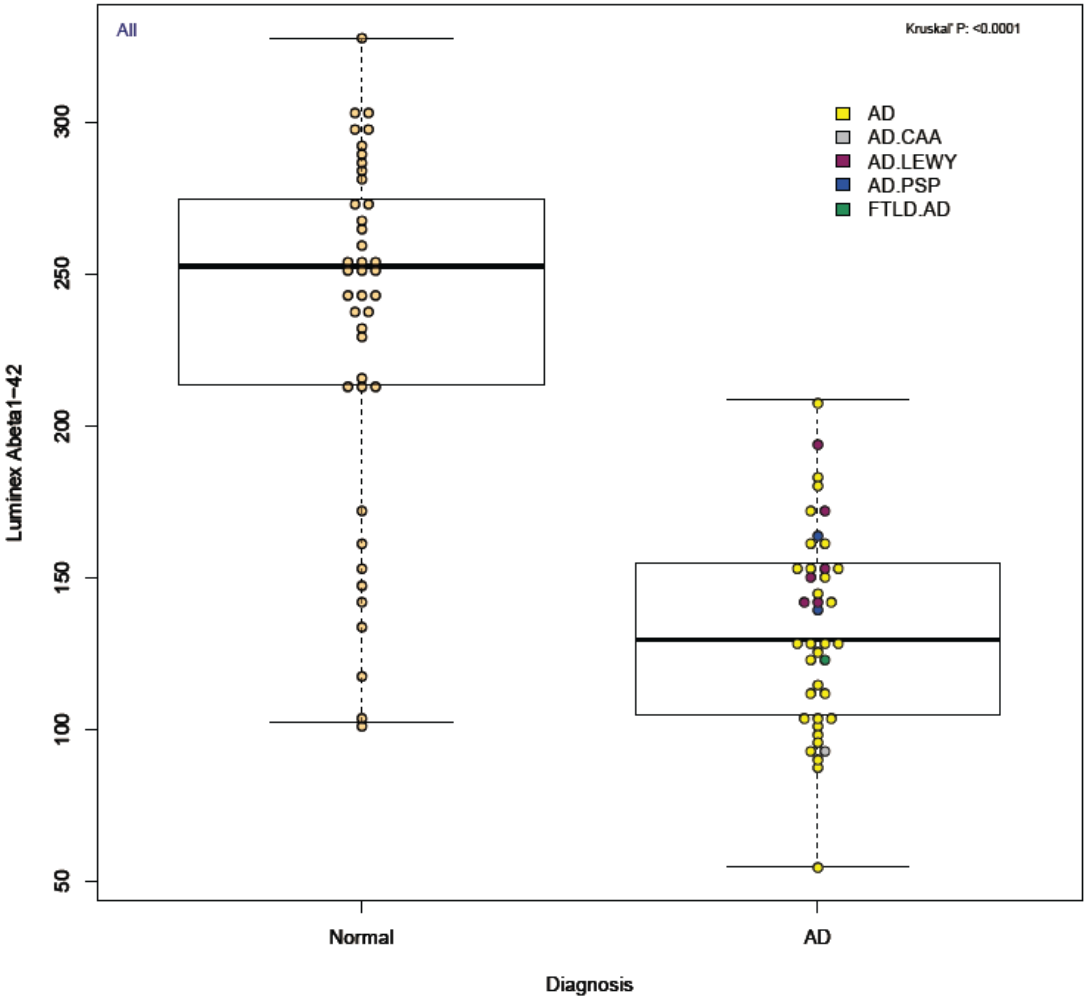
- High conc Guanidine HCl(5 M) to release $A\beta_{1\text{-}42}$ from aggregates, oligomers
- Mixed bed ion exchange 96 well format for 1st step sample cleanup
- Use of a surrogate matrix with equivalent performance to CSF as a calibration matrix: describe all constituents and sources of these
- Use high quality $A\beta_{1\text{-}42}$ standard and cross-check performance of several lots of this material; use uniformly N^{15}-labelled IS
- Waters ACUITY 2D HPLC + API 5000 tandem mass spectrometer
- Employ quality controls: *aCSF* (4 mg/mL BSA + electrolytes + $A\beta_{1\text{-}42}$) & CSF pools throughout
- Define all major analytical parameters as defined in US FDA Guidance and CLSI guidelines
  - Determine LLOQ & ULOQ
  - Linearity
  - Calibrators' precision and accuracy within- and between-day
  - *aCSF* spiked qc samples (3 spike levels) & 10 CSF pools
  - Spike recoveries from CSF pools and from *aCSF* containing 4 mg/mL BSA
  - Carryover
  - Selectivity (measurement of Aβ1-42 in the absence & presence of high concentrations of Aβ1-38 and 1-40)
  - Check for "ion suppression" (matrix interference in ionization intensity)
  - Short term stability of calibrators, qc samples & CSF pools (4 hr at room temp)
  - Long-term stability of all qc samples (two CSF pools & 2 *aCSF* spiked controls) over a two year period (ongoing)
- Analysis of AD and control CSF samples in non-ADNI CSF sample aliquots

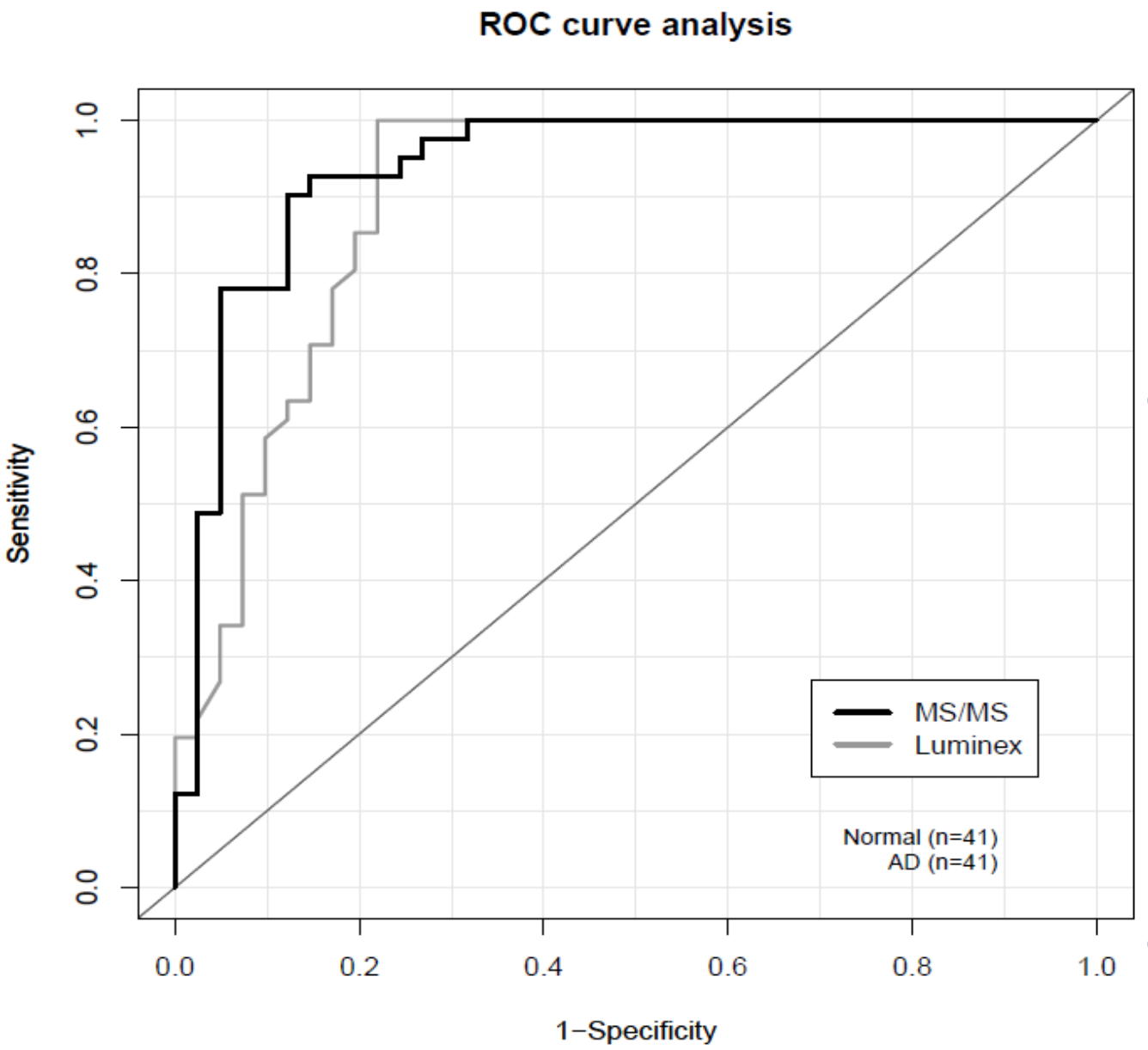
$A\beta_{1-42}$ concentrations measured in 41 autopsy-confirmed AD & 41 age-matched controls

SRM/tandem mass spectrometry



AlzBio3 *xMAP™* immunoassay





ROC analyses

Clinical performance using 41 AD, 41 age-matched cog normal controls for the mrm/MSMS method:

Sensitivity: 92.7%
Specificity: 85.4%
PPV: 86.4%
NPV: 92.1%
Test accuracy: 89%
AUC: 0.94

Clinical performance using the same 41 AD and 41 controls for the AlzBio3 Luminex Immunoassay:

Sensitivity: 100%
Specificity: 78%
PPV:82%
NPV:100%
Test accuracy: 89%
AUC: 0.90

p=0.2229

UPenn/ADNI 2D-HPLC/tandem mass spectrometry method for Aβ peptides

- Sample preparation based on Lame, et al, 2011
- Surrogate *aCSF* matrix(contains 4 mg/mL BSA) performance equivalent to hCSF
- Analytical qualification has been conducted
- Assessment of clinical performance using autopsy confirmed AD diagnosis shows at least equivalent performance compared to a qualified immunoassay
- Will use this method for ADNI CSF samples
- A candidate accuracy-based method
- One of 4 labs in an interlab study sponsored by the Alzheimer's Assn & collaboration with Kaj Blennow in an IFCC supported effort to develop a standard reference material for Aβ1-42, plans developed at a meeting in Milan May 2013.

It takes a great team effort!

John Q Trojanowski
Virginia M-Y Lee
Chris Clark*
Steve Arnold
Hugo Vanderstichele
Magdalena Korecka
Margaret Knapik-Czajka
Magdalena Brylska
Teresa Waligorska
Michal Figurski
Leona Fields
Sarah Pan

*Deceased

William Hu
Ju Hee Kang
Jon Toledo
Anne Fagan
Uwe Christians
Kaj Blennow
Erik Portelius
Jing Zhang
Henrik Zetterberg
Holly Soares
Adam Simon
Robert Dean
Eric Siemers
Piotr Lewczuk
William Potter

Rand Jenkins
Erin Chambers

Supported by the NIH/NIA and families of our patients

ADNI investigators include: (complete listing available at www.loni.ucla.edu\ADNI\Collaboration\ADNI_Manuscript_Citations.pdf).