Biomarker Core
ADNI Steering Committee
Boston, MA 4/24/2017

Leslie M Shaw

John Q Trojanowski

Perelman School of Medicine
University of Pennsylvania

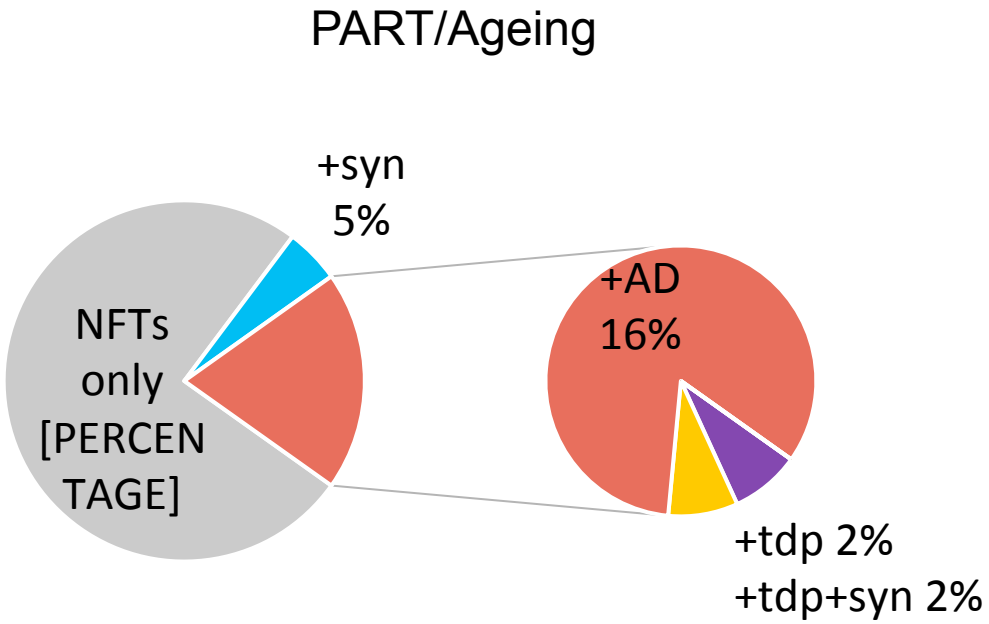
New biomarkers in NIA/ADNI/RARC-approved studies

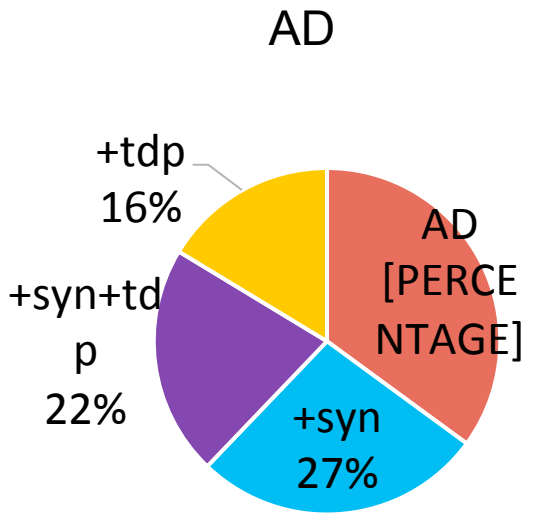
Biomarker	Fluid	#	ADNI study	Investigator
NFL & Tau diag/prognosis	Plasma	3,000+	ADNIGO/2 BL + longitudinal	HZetterberg;NMattsson;Kblennow; Sweden
sTREM2	CSF	1007	ADNI1/GO/2 BL + longitudinal – shared samples	MSuares-Calvert,MEwers; DZNE, Germany
sTREM2 levels & AD; IA	CSF	1007	ADNI1/GO/2 BL + longitudinal	Cruchaga; Wash U
Metabolic networks	Serum	905	Studies in ADNIGO/2 BL samples	RKaddoura-Daouk; Duke Univ,
Aβ1-42/1-40;ELISA	plasma	764	ADNI 1, GO, 2 in BL & longitudinal	ISherriff; Araclon Biotech
Metabolic networks	serum	833	Studies in ADNI1 BL samples; data uploaded	RKaddoura-Daouk; Duke Univ,
SNAP25 & neurogranin	CSF	612	Longitudinal samples	AFagan; Wash University
T-& Phos-α-SYN; IA	CSF	567	Longitudinal samples, to be uploaded	JZhang; University of Wash
Vilip 1; YKL-40; IA	CSF	612	Longitudinal samples, publication planned	AFagan; Wash University
Tau; IA	plasma	595	BL ADNI1; publication	KBlennow; Sahlgrenska UHosp
Neurogranin; NFL; IA	CSF	416	BL ADNI1; multiple publications	KBlennow; Sahlgrenska UHosp
Proteome/ MRM/MSMS	CSF	306	BL ADNI1; 221 proteins;publication;another planned	ADNI PPSB/FNIH; LHonigsberg
Proteome/RBM	plasma	1,065	BL & yr1; multiple publications	HSoares;Pfizer/PPSB/FNIH
Proteome/RBM	CSF	317	BL ADNI1; multiple publications	WPotter,etal/PPSB/FNIH
BACE & sAPP	CSF	402	BL ADNI1; recent publication	MSavage;merck/PPSB/FNIH
α-Synuclein;xMAP	CSF	390	BL ADNI1; several publications	JZhang; University of Wash

Alzheimer's Disease (AD) And Related Dementias (ADRD) Often Show Comorbid Multi-Proteinopathies

DISEASE	LESIONS	COMPONENTS
Alzheimer's Disease (AD) The most common multi-proteinopathy	**SPs (100%)** **NFTs (100%)** **LBs (-50%)** **TDP-43 (-50%)**	**Aβ** **Tau** **Alpha-synuclein** **TDP-43**
Frontotemporal Degeneration (FTD)	Inclusions	Tau (FTLD-Tau), TDP-43 (FTLD-TDP), FTLD-FUS
Amyotrophic Lateral Sclerosis (ALS)	Inclusions	TDP-43, FUS, Tau, SOD1
Parkinson's Disease (PD) -/+ Dementia (PDD) & Dementia With Lewy Bodies (DLB)	LBs, SPs, NFTs	Alpha-synuclein, Aβ, Tau
Multiple System Atrophy (MSA)	GCIs	Alpha-Synuclein
Prion Diseases	SPs	Prions, Tau, Aβ, Alpha-synuclein
Trinucleotide repeat diseases	Inclusions	Expanded polyglumatine repeats

Incidental Alpha-synuclein (Syn) And TDP-43 (TDP) Pathologies Are Rare In PART/NA, But Very Common In AD Such That Only 35% Of AD Patients In Our CNDR Brain Bank Have Pure Plaque And Tangle only AD.





TDP-43: Potential Use

Useful for diagnosis of AD and related dementias (ADRD)

Useful treatment selection for ADRD

Useful for formulating prognoses for ADRD

Useful for monitoring disease progression for ADRD

Useful for monitoring disease modifying therapies when available for ADRD

Acknowledgements: Many thanks to Linda K. Kwong, Yan Xu, Dawn M. Riddle and Virginia M.-Y. Lee for all their efforts on TDP-43 antibody production and ELISA development and to John Robinson, Maria Corrada and Claudia Kawas for their efforts on the comorbid pathology studies. These studies were made possible through grants from NIA/NINDS and the Koller Family Foundation as well as the support of the Families of our patients.

Why automation of CSF biomarkers?

- Eliminate as many manual steps as possible
- Promote best possible precision & accuracy
  - Within-lab
  - *Between-labs*
    - *using common samples, eg AlzAssn QC program*
    - *Same study population and pre-analytical protocol, eg, treatment trials*
    - *Different study populations and pre-analytical protocols, eg, ADNI, BioFINDER*
- Improved lot-to-lot performance
- Enable IVD test approval → clinical laboratory test
- Can provide both accurate and precise data
- Use in treatment trials, especially international where local laboratory is essential(eg, China).

Between-labs performance: Alz Association QC program

	Between laboratory CV (percent)					
	INNOTEST® β-AMYLOID (1-42) Fujirebio	EuroImmune / ADx β-amyloid (1-42)	AlzBio3 β-amyloid (1-42) Fujirebio	Meso-Scale Human Aβ42	Elecsys® β-Amyloid(1–42) Roche Diagnostics	Lumipulse® β-Amyloid(1–42) Fujirebio
	ELISA	ELISA	Luminex	ECL V-PLEX	Fully automated	Fully automated
Round						
2014-14A	18		16	low n	2,9	
2014-14B	21		19	low n	4,4	
2014-15A	15		7,1	12	4,6	
2014-15B	17		14	12	3,4	
2104-16A	27	57	40	13	3	
2014-16B	17	19	30	11	2,5	
2015-17A	19	6,5	17	21	1,9	
2015-17B	14	8,2	15	20	3,2	
2015-18A	13	22	25	10	7,2	
2015-18B	13	16	13	9,4	4,7	
2015-19A	13	13	40	10	3	
2015-19B	13	13	15	13	1,5	
2016-20A	17,4	18	ND	10,5	2	
2016-20B	21,1	15,4	ND	14	Level out of range	
2016-21A	15	18	22	8,1	10	6,3
2016-21B	11	15	29	7,4	5,2	4
2016-22A	14	12	28	39	5,9	7,5
2016-22B	19	13	24	37	6,7	9,4
MEAN	**16,5**	**17,6**	**22,1**	**15,5**	**4,2**	**6,8**

KBlennow

ADNI3 Aims for Biomarker Core

Aim 2: Provide highly standardized $A\beta_{1-42}$, t-tau and p-tau_{181} measurements on all ADNI subject CSF samples using the Roche automated immunoassay platform(Cobas e601) and immunoassay reagents. In addition provide immunoassay-independent measurements of Aβ species ($A\beta_{1-42}$, $A\beta_{1-40}$ and $A\beta_{1-38}$) using a validated reference 2D-UPLC/tandem mass spectrometry method in baseline and longitudinal CSF samples. Continue collaboration with other investigators to achieve harmonization of these measurements across centers and different platforms in support of their use in clinical trials.

- ***Change:*** from manual RUO immunoassay to fully automated immunoassay platform for ADNI 3:
- ***Due diligence:*** started Q4, 2014, in consultation with ADNI Exec Comm & NIA & PPSB/BBWG/ DDWG.
- ***Selection:*** in consultation with ADNI PPSB/BBWG/DDWG, chaired by Johan Luthman.
- ***Roche Elecsys:*** validation for $A\beta_{1-42}$ in CSF completed.
- ***External QC:*** Participation in the AlzAssn CSF QC program for $A\beta_{1-42}$
- ***Validation of t-tau and p-tau_{181}:*** completed FALL, 2016
- ***Analyses of all ADNI CSFs:*** *late* FALL, 2016-early WINTER, 2017
- ***Continued collaboration:*** with Kaj Blennow & AlzAssn and IFCC CSF WGs to produce certified reference CSF pools with assigned reference $A\beta_{1-42}$ concentration values, measured with reference 2D-UPLC/tandem mass spectrometry, to provide certified reference materials for validation of $A\beta_{1-42}$ calibrators--promoting harmonization across assay platforms.
- ***Review & participate in:*** studies of pre-analytical factors for CSF collection.

Analysis of 2401 ADNI1/GO/2 CSF samples

2401 ADNI pristine CSFs, collected from 9/7/2005 to 7/25/2016 were analyzed in 36 analytical runs at UPenn from 11-17-2016 to 1-20-2017:

- 402 ADNI1 BASELINE; 819 ADNIGO/2 BASELINE
- ADNI1: 112 HC, 192 MCI, 98 AD
- ADNIGO/2: 160 HC, 96 SMC, 277 EMCI, 154 LMCI, 132 AD

Analyses of ADNI1/GO/2 CSF $A\beta_{1-42}$, t-tau, p-tau_{181} using the Roche Elecsys fully automated immunoassay platform

- Rationale for moving from RUO to full automation
- Validation of $A\beta_{1-42}$ for precision, accuracy, and clinical performance
- General statistics for $A\beta_{1-42}$, t-tau, p-tau_{181}, t-tau/$A\beta_{1-42}$, p-tau_{181}/$A\beta_{1-42}$ in the ADNI1/GO/2 CSF samples
- Histogram distributions for $A\beta_{1-42}$, t-tau/$A\beta_{1-42}$, p-tau_{181}
- Distributions based on FBP amyloid-β PET + or –
- Cutpoint determinations
- Collaborative study with BioFINDER
- Concordance with FBP amyloid-β PET
- Prediction of cognitive decline(CDRsob)
- Summary

Method validation studies at UPenn: Roche Elecsys immunoassay

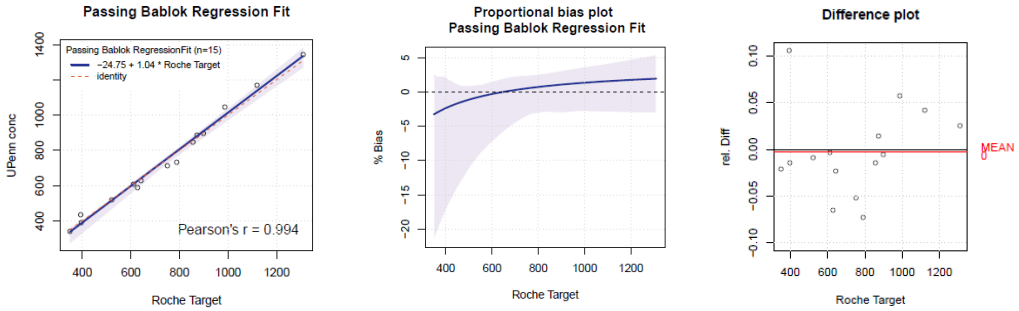
CSF Aβ1-42:

- Analytical studies
  - Short and long-term precision studies
  - Linearity
  - Comparison of Elecsys between UPenn and Roche
  - Comparison with a reference mrm/mass spectrometry method
  - Comparison with the RUO AlzBio3 immunoassay
  - Two sets of non-ADNI CSF samples utilized(250 residual CSF from routine clinic patients; 129 CSFs from the UPenn ADRC)
- ROC analyses for AD vs HC in 129 CSFs from the UPenn ADRC(62 AD, 67 HC)

SUMMARY

UPENN/Roche comparison (both use Roche Elecsys,15 CSF pools): PB regression—Y = 1.04X - 24.8; Pearson‘s r = 0.994

- Bias at cut-off <10%
- Slope is within 1.0 ± 0.1



Elecsys, AlzBio3 and LC-MS Abeta(1-42) measurements were performed for 250 samples from data set A and 129 samples from data set B

Data set A and B were not pooled as AlzBio3 measurements differed between the two sample sets

Correlation between

Elecsys and AlzBio3: Spearman‘s rho 0.86(A)/0.82(B); some non-linearity
Elecsys and LC-MS: Spearman‘s rho 0.95(A)/0.96(B); Linear relationship
LC-MS and AlzBio3: Spearman‘s rho 0.87(A)/0.77(B); some non-linearity

} details in following ppt.

ROC-AUC analysis within the data set B(AD vs HC): equivalent performance of all 3 methods

**Toronto 2016 AAIC meeting poster & included in an AAIC symposium talk.*

Figure 2: Comparison and analysis of individual Aβ(1–42) concentrations obtained for each sample for the three methods. The shaded area of the proportional bias graph (panel B) represents the 95% confidence interval.

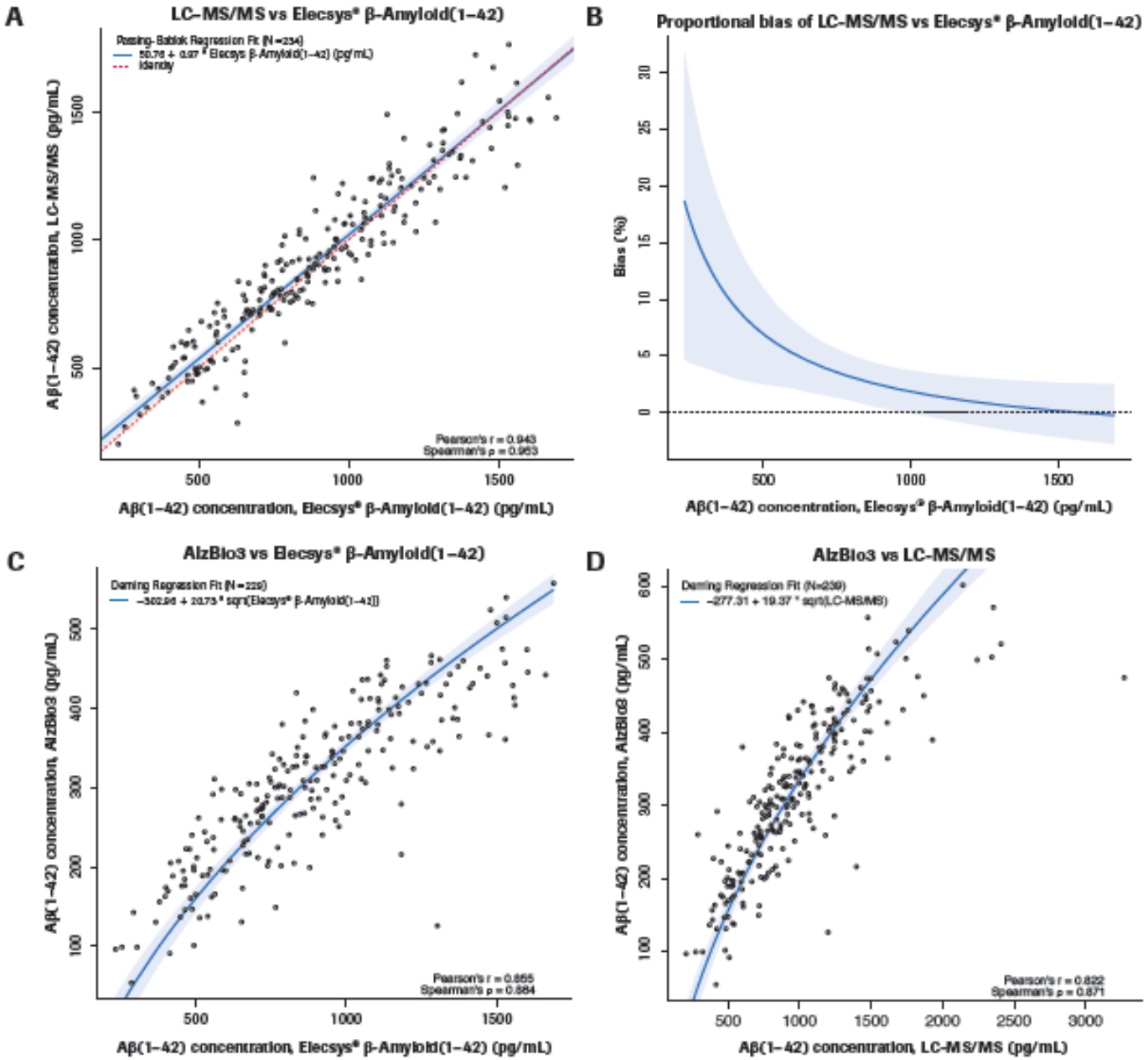
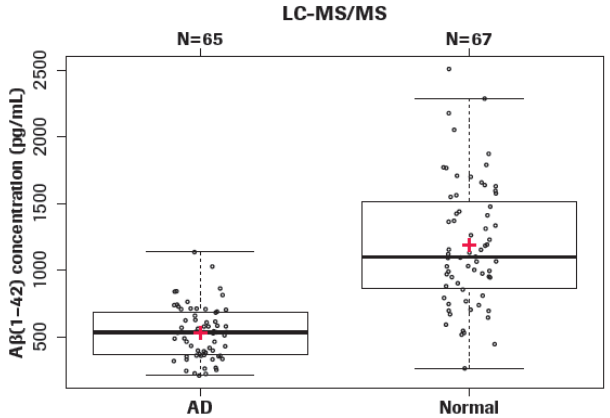
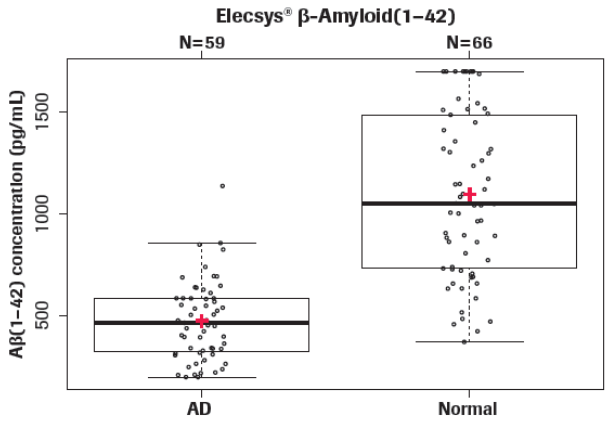
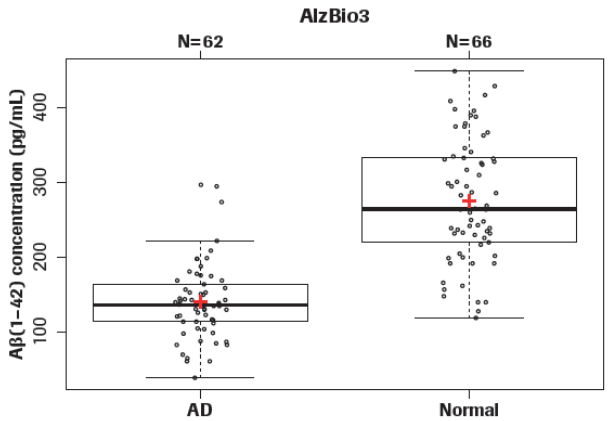
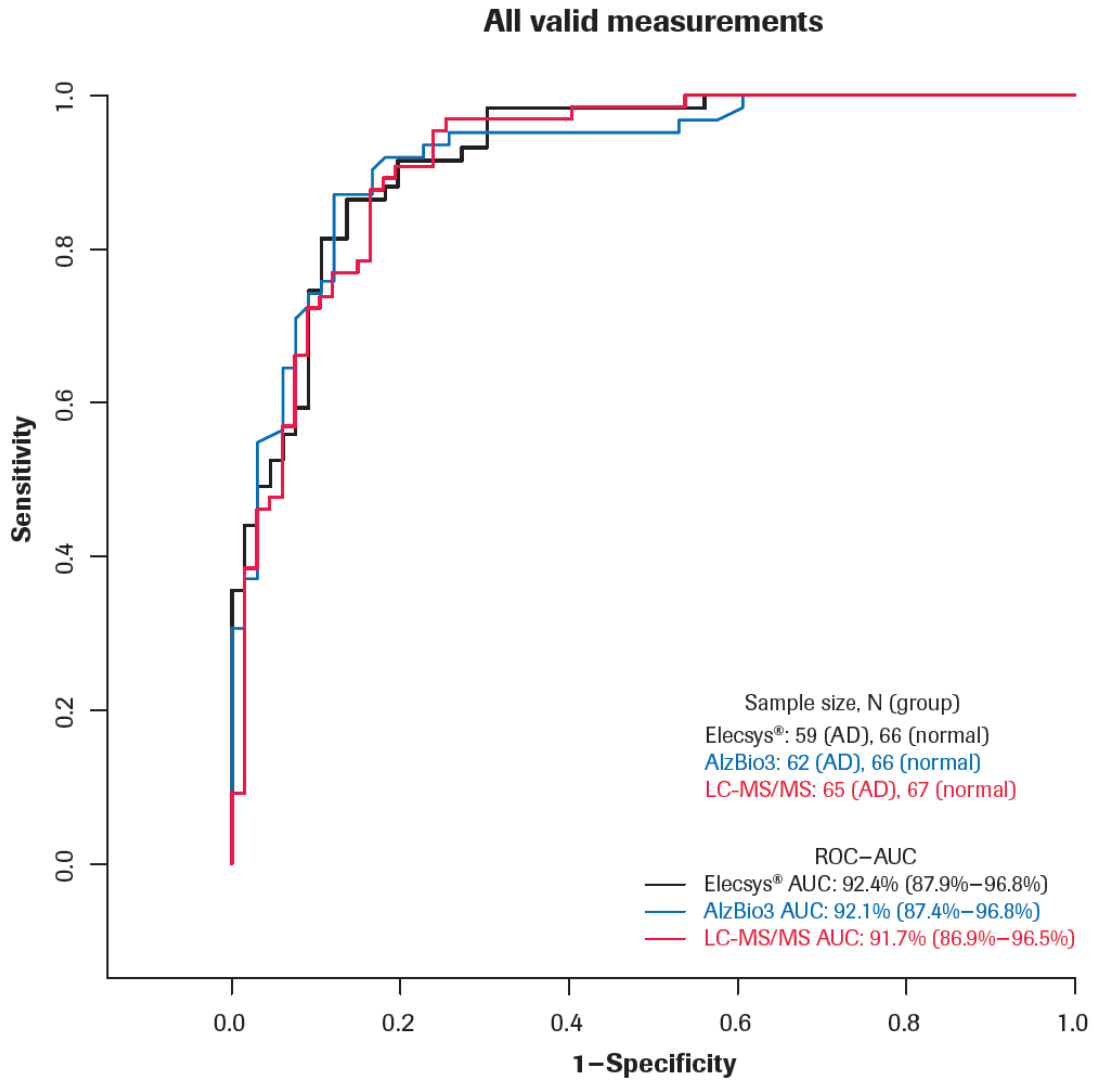


Figure 3: Clinical performance of Aβ(1–42) measurements of the three methods.
Left panel: Box plots of Aβ(1–42) measurements analysed according to clinical diagnosis information (AD vs controls).
Right panel: ROC analysis (sensitivity vs 1-specificity).









AUC = area under the curve; ROC = receiver operating characteristic

ADNI Alzheimer's Disease Neuroimaging Initiative

ADNI 3: Batch analyses of $A\beta_{1-42}$, t-tau and p-tau_{181} in ADNI1 and ADNIGO/2 CSF using the fully automated Roche Elecsys and cobas e immunoassay analyzer system

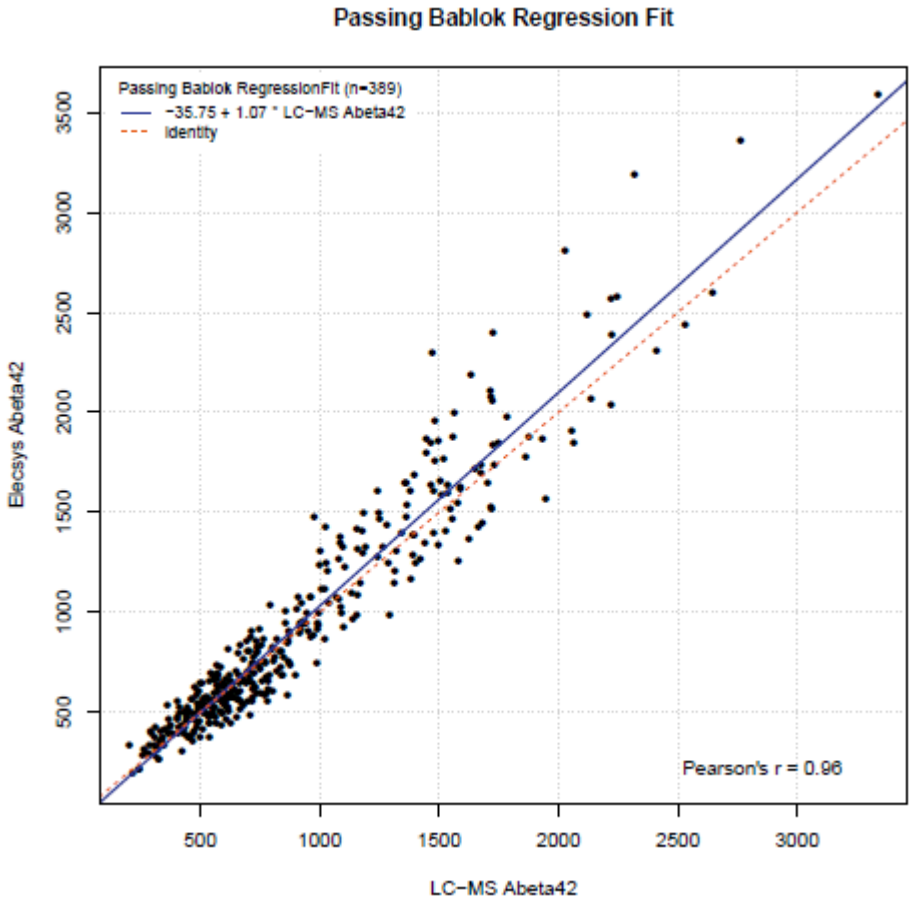
Leslie M Shaw, Michal Figurski, Leona Fields and John Q Trojanowski

ADNI Biomarker Core, Department of Pathology & Laboratory Medicine and Center for Neurodegenerative Diseases Research, Perelman School of Medicine University of Pennsylvania (UPenn)

Please note:

The Elecsys β-Amyloid(1-42) CSF immunoassay in use is not a commercially available IVD assay. It is an assay that is currently under development and for investigational use only. The measuring range of the assay is 200 (lower technical limit) – 1700 pg/mL (upper technical limit). The performance of the assay beyond the upper technical limit has not been formally established. Therefore use of values above the upper technical limit, which are provided based on an extrapolation of the calibration curve, is restricted to exploratory research purposes and is excluded for clinical decision making or for the derivation of medical decision points.

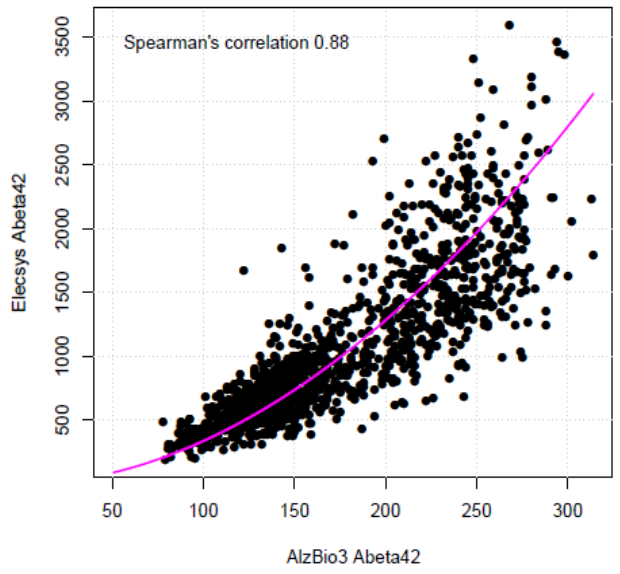
Roche Elecsys versus LC/MS for ADNI1 BASELINE CSF $A\beta_{1\text{-}42}$



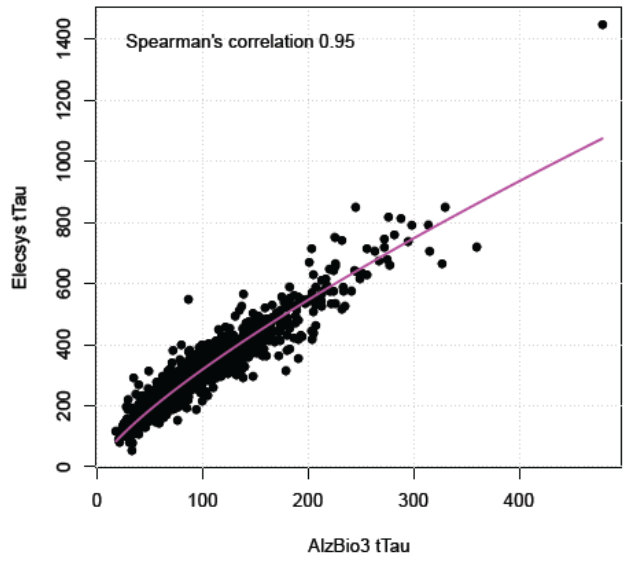
→ Confirms finding from UPenn Method Comparison study: linear relationship and approximately 1:1

Comparisons between Roche Elecsys & AlzBio3 immunoassays for ADNI1/GO/2 CSFs.

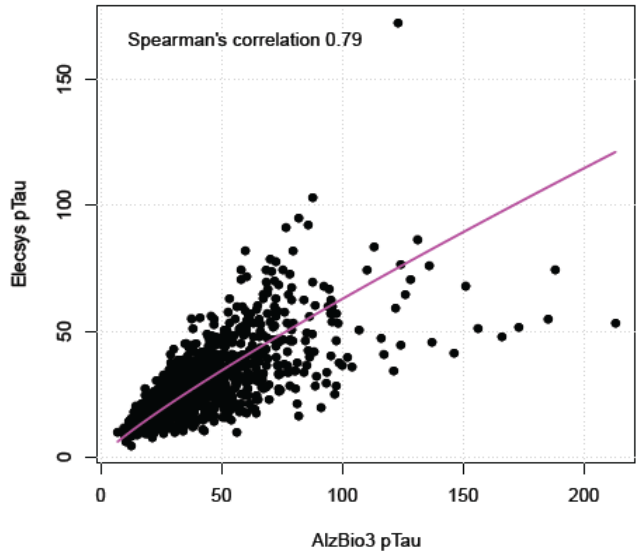
$A\beta_{1-42}$



t-tau



$p\text{-}tau_{181}$

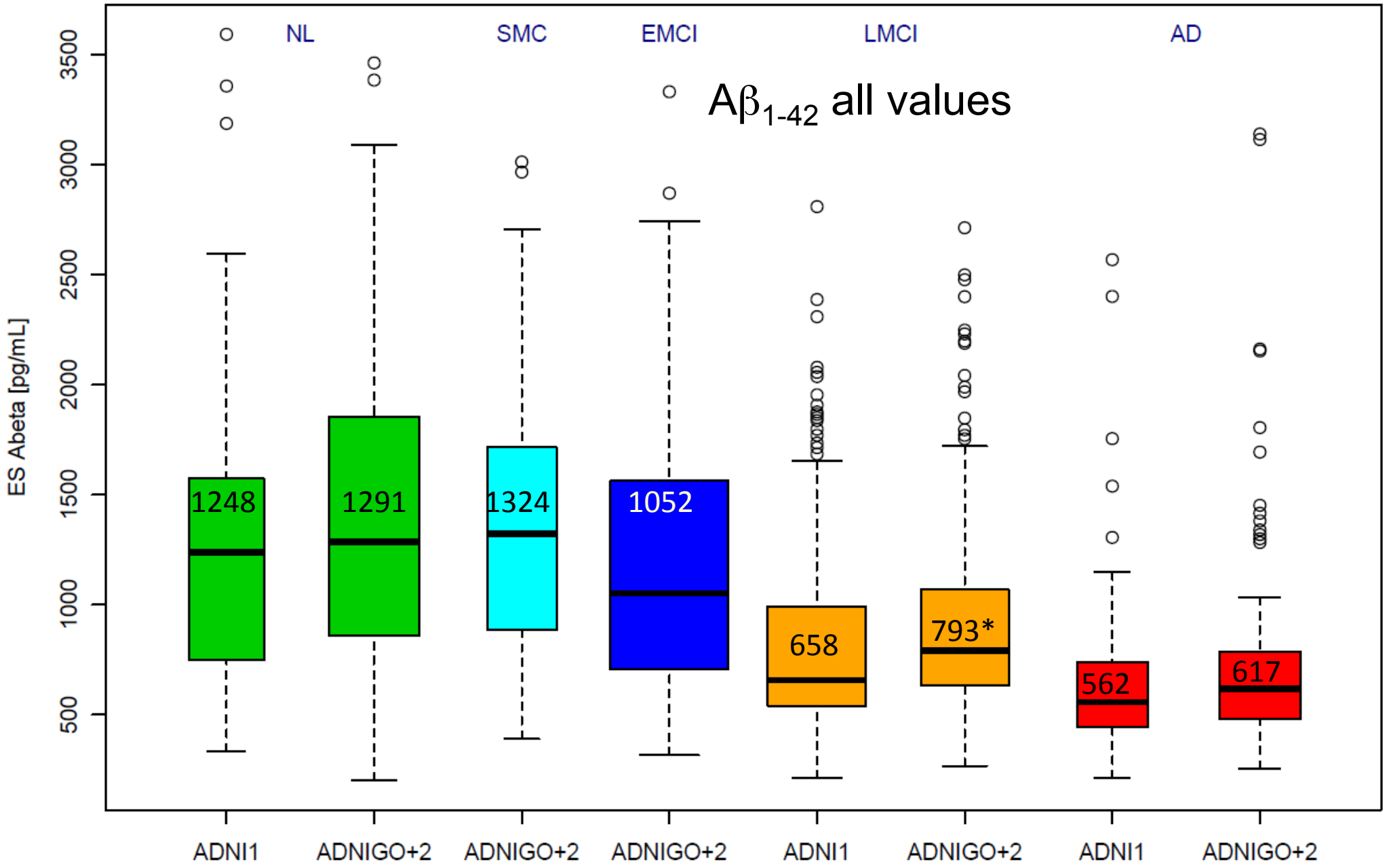


ADNI1 BASELINE CSF $A\beta_{1-42}$, t-tau, p-tau_{181}& ratios

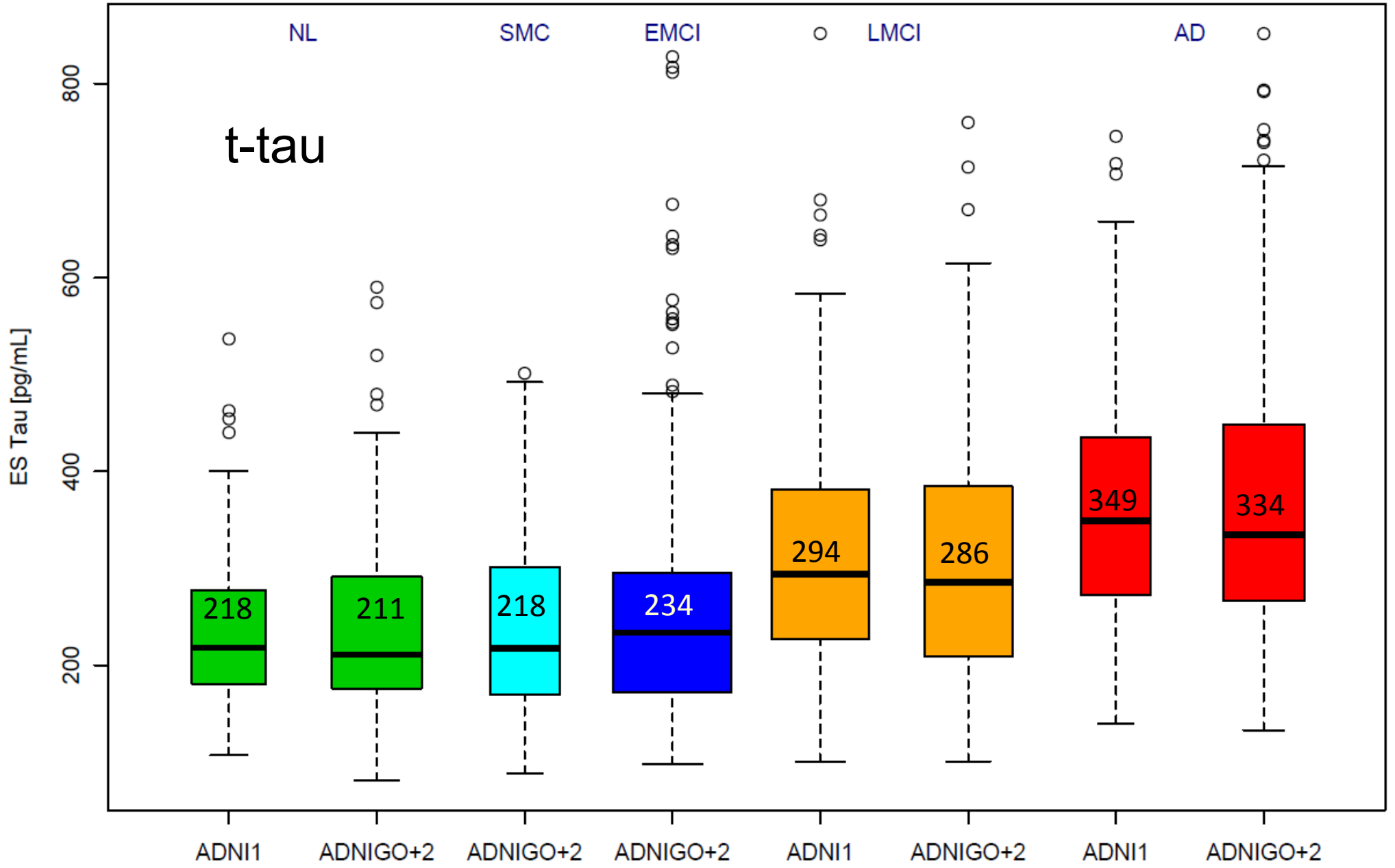
ADNI1	$A\beta_{1-42}$	t-tau	p-tau_{181}	t-tau/$A\beta_{1-42}$	p-tau_{181}/$A\beta_{1-42}$	% ε4+
	(pg/mL)	(pg/mL)	(pg/mL)			
AD						72.6
Median	**548**	**349**	**34**	**0.62**	**0.063**	
N=95 mean±SD	610±242	359±130	36±15	0.65±0.28	0.066±0.032	
95% CI	305-1125	154-687	13-73	0.15-1.42	0.012-0.14	
MCI						56.6
Median	**633**	**294**	**28**	**0.50**	**0.050**	
N=176 mean±SD	741±338	312±124	31±14	0.51±0.30	0.052±0.033	
95% CI	292-1624	140-599	12-63	0.12-1.22	0.010-0.13	
NC						26.4
Median	**989**	**218**	**20**	**0.18**	**0.017**	
N=91 mean±SD	1018±397	239±84	22±9	0.27±0.18	0.026±0.019	
95% CI	394-1640	112-444	11-43	0.11-0.73	0.0089-0.079	

ADNIGO/2 CSF BASELINE $A\beta_{1-42}$, t-tau, p-tau_{181} & ratios

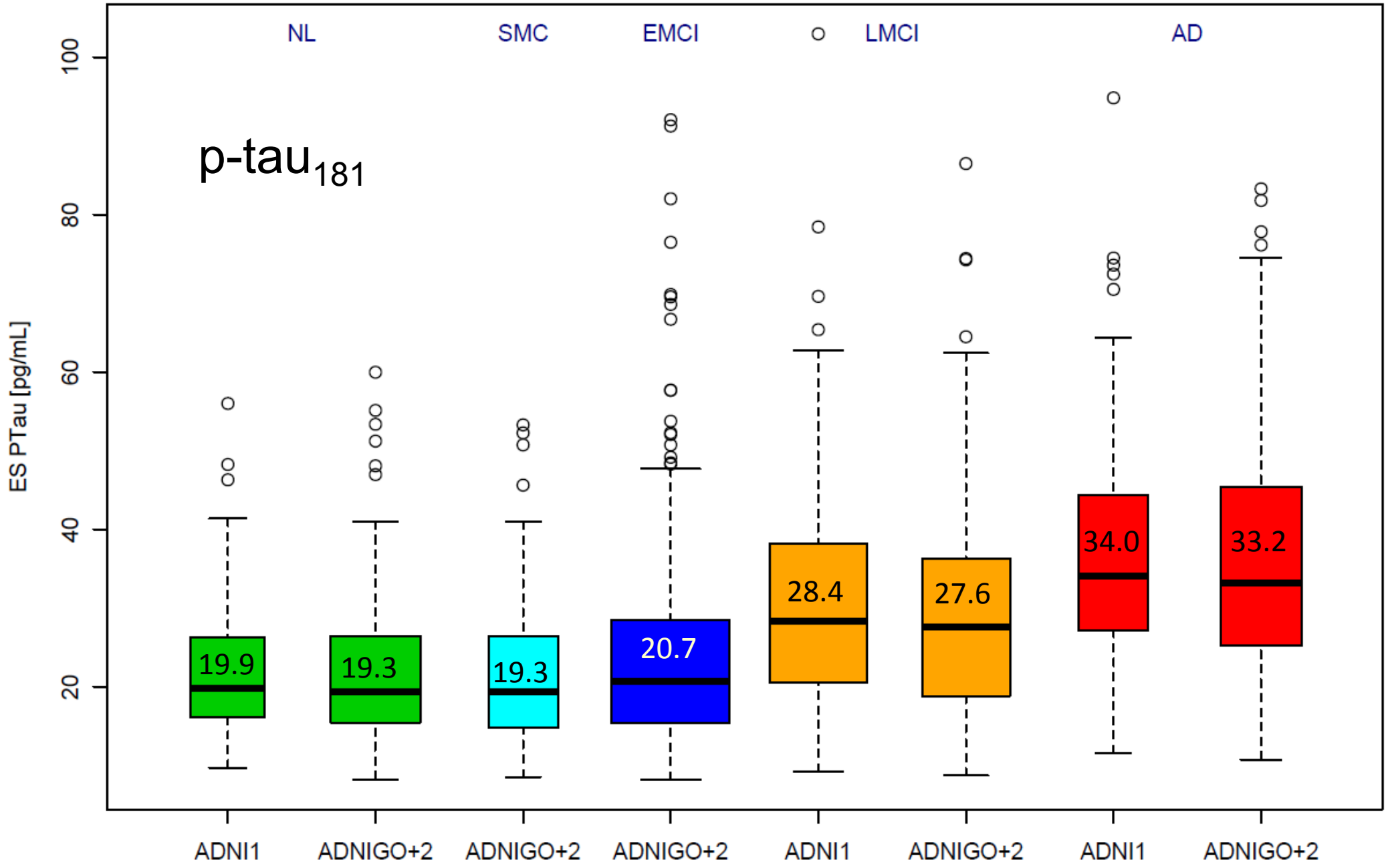
ADNIGO/2		$A\beta_{1-42}$	t-tau	p-tau_{181}	t-tau/$A\beta_{1-42}$	p-tau_{181}/$A\beta_{1-42}$	% ε4+
		(pg/mL)	(pg/mL)	(pg/mL)			
AD	Median	**594**	**334**	**33**	**0.58**	**0.058**	68.5
N=127	mean±SD	649±257	375±155	37±16	0.64±0.32	0.064±0.034	
	95% CI	309-1375	170-750	15-76	0.18-1.42	0.014-0.15	
LMCI	Median	**756**	**286**	**28**	**0.50**	**0.050**	60.9
N=138	mean±SD	800±285	308±136	30±15	0.51±0.30	0.052±0.033	
	95% CI	340-1457	115-577	10-63	0.12-1.22	0.010-0.13	
EMCI	Median	**865**	**234**	**20**	**0.27**	**0.025**	49.4
N=122	mean±SD	943±355	256±122	22±9	0.33±0.26	0.033±0.029	
	95% CI	382-1659	117-582	11-43	0.09-0.95	0.0082-0.106	
SMC	Median	**1111**	**218**	**19**	**0.19**	**0.017**	43.7
N=71	mean±SD	1079±374	241±94	22±10	0.25±0.16	0.024±0.017	
	95% CI	454-1670	107-462	10-49	0.10-0.67	0.0084-0.071	
NC	Median	**974**	**211**	**19**	**0.21**	**0.020**	33.0
N=109	mean±SD	1013±379	238±92	22±9	0.27±0.18	0.0260.019	
	95% CI	342-1686	110-469	10-48	0.09-0.69	0.0086-0.073	



Numbers inside the boxes are the respective median values for BL $A\beta_{1-42}$ in pg/mL placed above the median value horizontal line.
*p<0.005 for LMCI ADNIGO+2 vs ADNI1; p=0.11 for NL ADNIGO+2 vs ADNI1; p=0.23 for AD ADNIGO+2 vs ADNI1

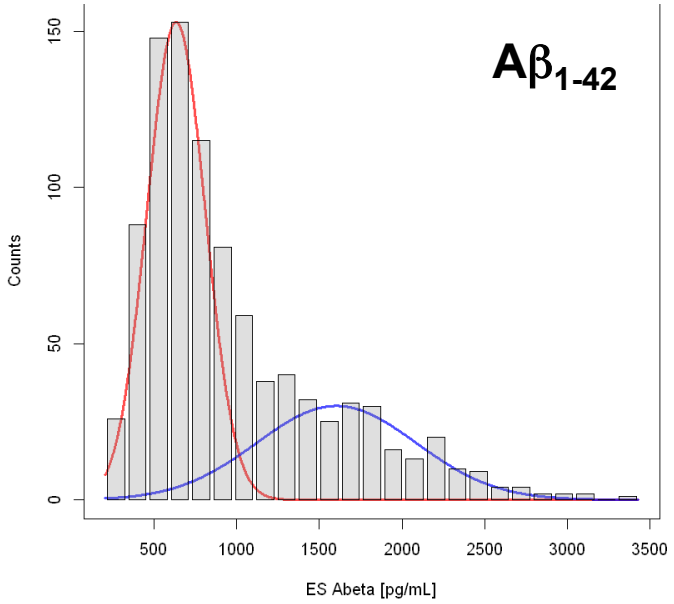


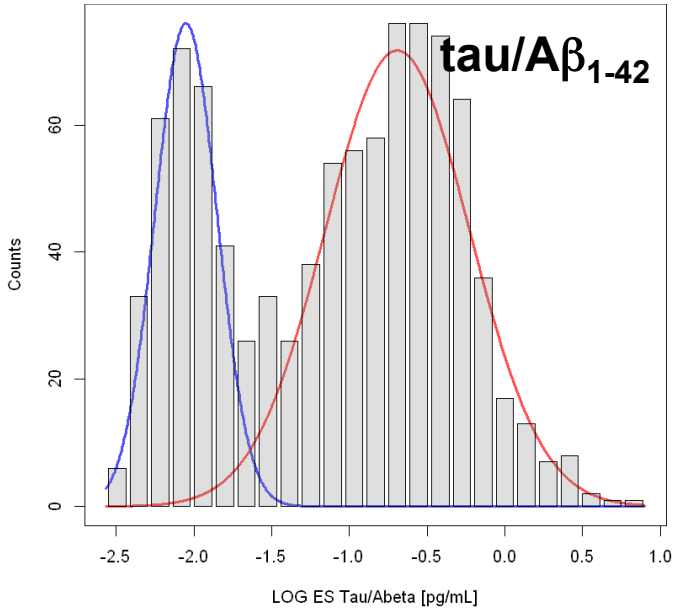
Numbers inside the boxes are the respective median values for BL t-tau in pg/mL placed above the median value horizontal line.
P=0.81 for NL ADNIGO+2 vs ADNI1; p=0.51 for MCI ADNIGO+2 vs ADNI1; p=0.81 for AD ADNIGO+2 vs ADNI1

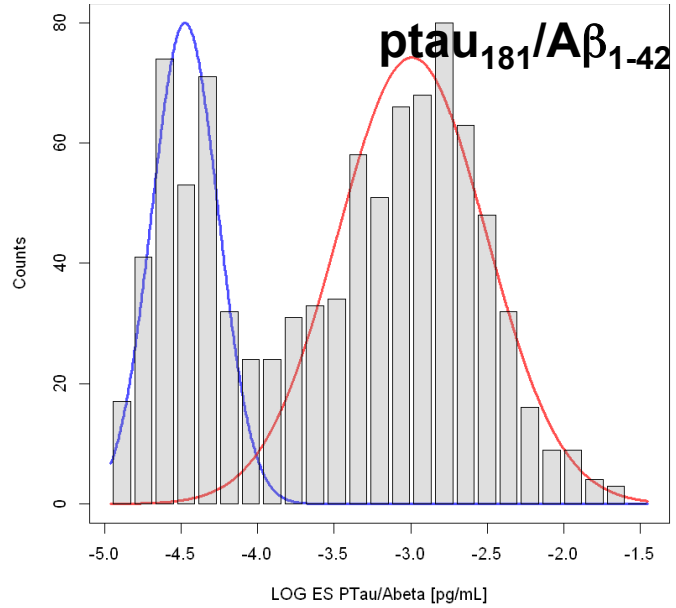


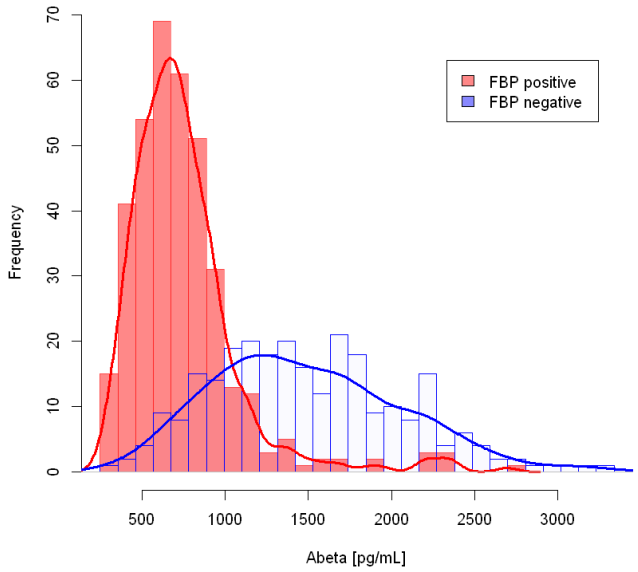
Numbers inside the boxes are the respective median values for p-tau$_{181}$ in pg/mL placed above the median value horizontal line.
*p=0.71 for ADNIGO+2 vs ADNI1; p=0.43 for MCI ADNIGO+2 vs ADNI1; p=0.88 for AD ADNIGO+2 vs ADNI1.

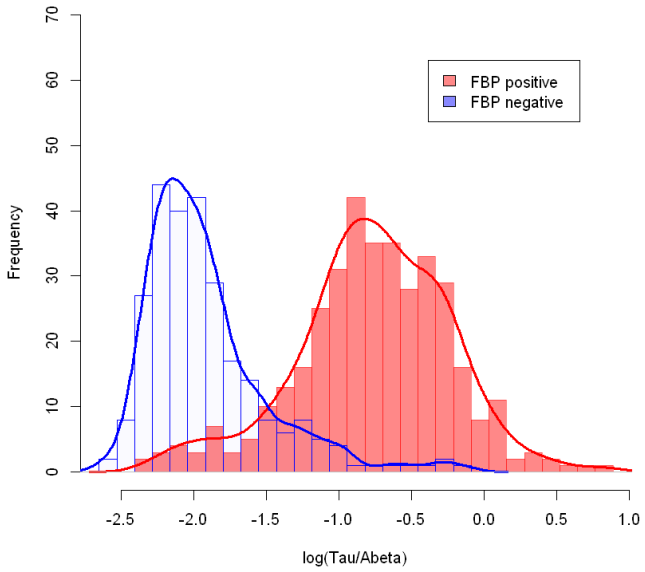
Frequency distribution plots: upper are mixture model plots, lower are FBP+ and FBP- for ADNI SMC/EMCI/LMCI/AD

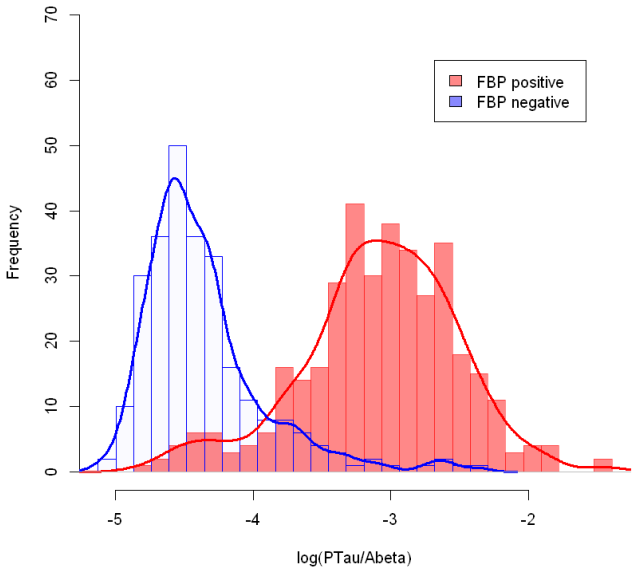




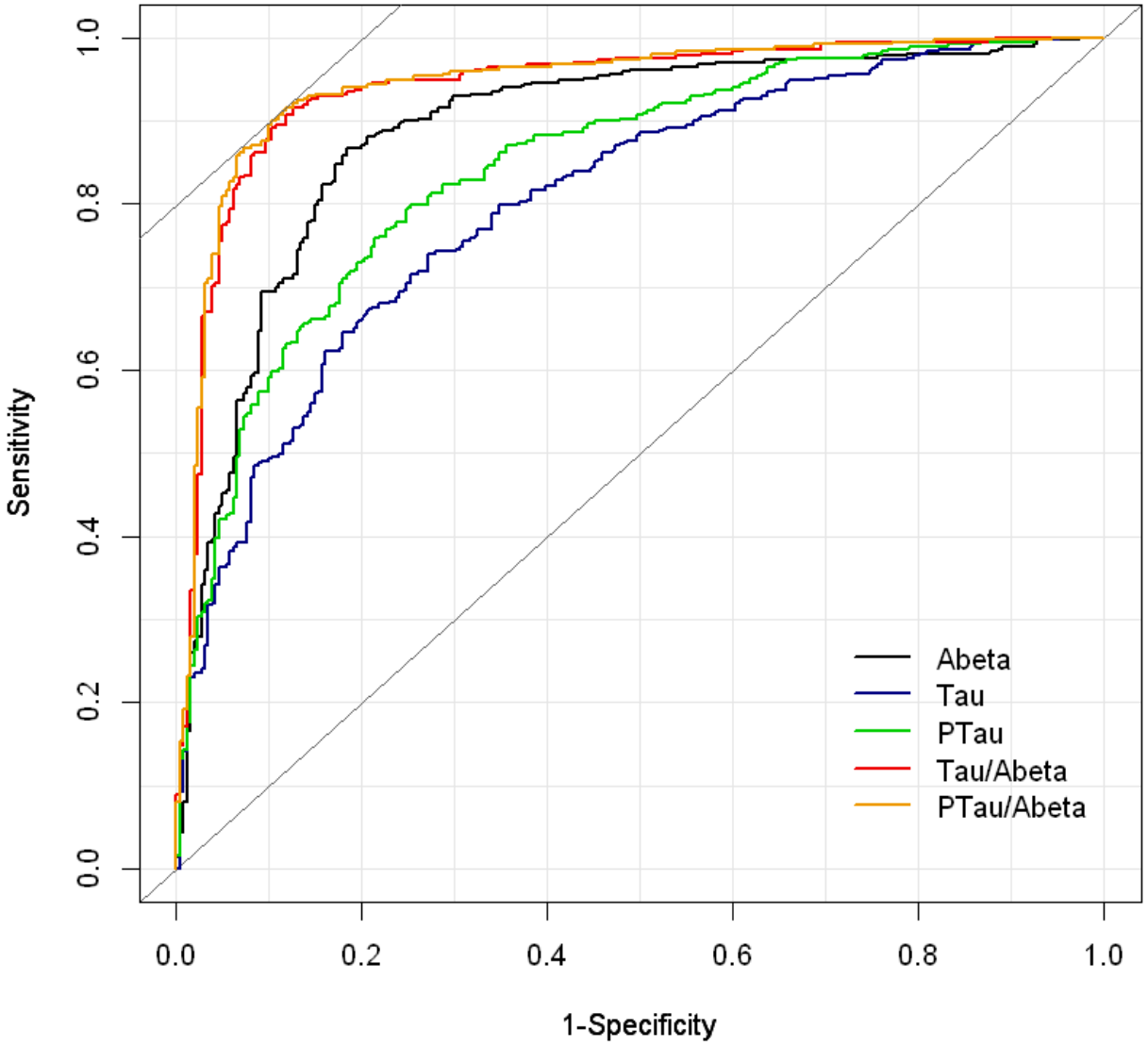








ROC Curves for SMC+EMCI+LMCI+AD CSF biomarkers using FBP PET+/- as the clinical endpoint*



AUC values:		Sens	Spec	Eff
p-tau/$A\beta_{1-42}$	0.944	91.3%	88.5%	90.2%
t-tau/$A\beta_{1-42}$	0.940	91.6%	87.4%	89.9%
$A\beta_{1-42}$	0.889	86.7%	81.7%	84.6%
p-tau_{181}	0.845	79.9%	74.8%	77.8%
t-tau	0.803	74.0%	72.9%	73.5%

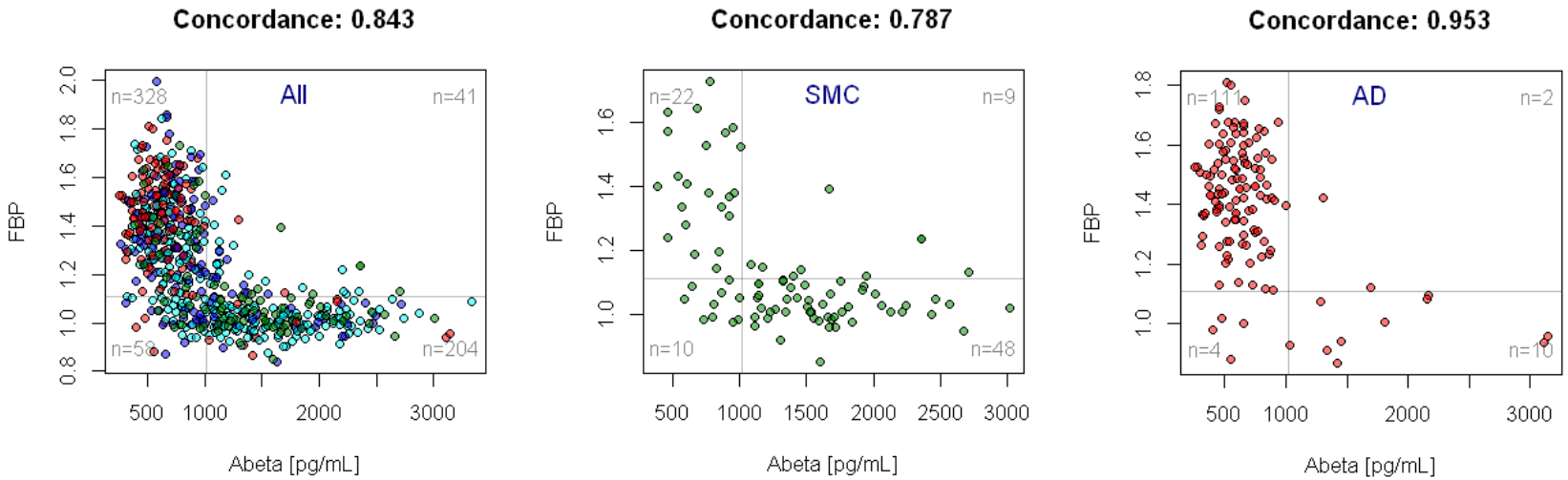
Cutpoint values:	
p-tau/$A\beta_{1-42}$	0.021
t-tau/$A\beta_{1-42}$	0.222
$A\beta_{1-42}$	980 pg/mL
p-tau_{181}	21.8 pg/mL
t-tau	245 pg/mL

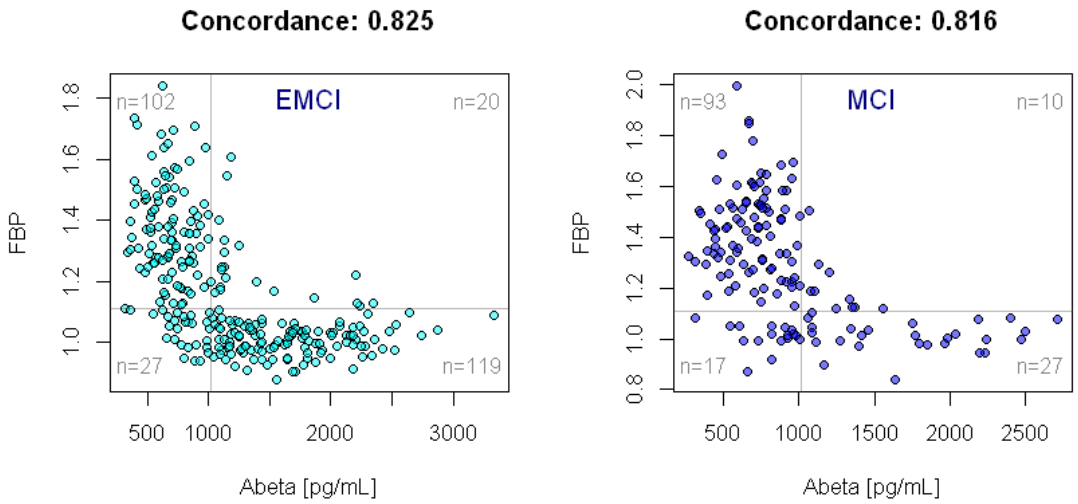
*SUVR of 1.1 used: Landau and Jagust

Cutpoint assessments for CSF $A\beta_{1-42}$, t-tau & $p\text{-}tau_{181}$ in ADNI

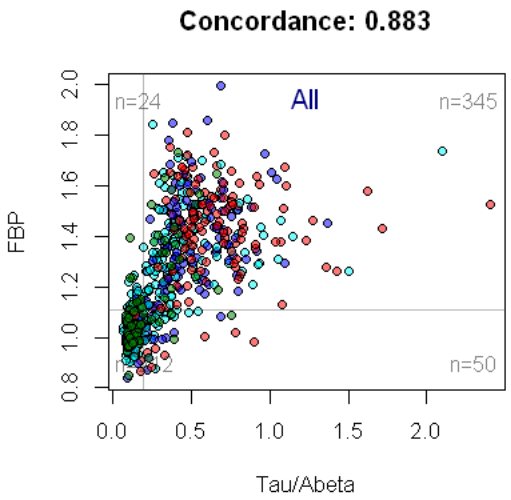
- ROC with FBP PET as the endpoint:
  - $A\beta_{1-42}$, 980 pg/mL
  - t-tau, 245 pg/mL
  - $p\text{-}tau_{181}$, 21.8 pg/mL
  - t-tau/$A\beta_{1-42}$, 0.22
  - $p\text{-}tau_{181}/A\beta_{1-42}$, 0.021
- Disease-independent mixture modeling
  - $A\beta_{1-42}$, 1016 pg/mL
  - t-tau, NA
  - $p\text{-}tau_{181}$, NA
  - t-tau/$A\beta_{1-42}$, 0.19
  - $p\text{-}tau_{181}/A\beta_{1-42}$, 0.018
- Prediction from BioFINDER study based on pre-analytic differences
  - $A\beta_{1-42}$, 880 pg/mL
  - t-tau, 270 pg/mL
  - $p\text{-}tau_{181}$, 24 pg/mL
  - t-tau/$A\beta_{1-42}$, 0.33
  - $p\text{-}tau_{181}/A\beta_{1-42}$, 0.028

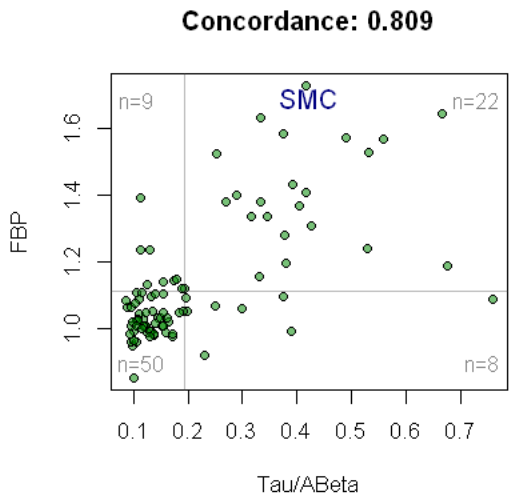
Concordance plots for FBP vs CSF $A\beta_{1\text{-}42}$ in ADNIGO/2 SMC, EMCI, LMCI & AD participants at BASELINE

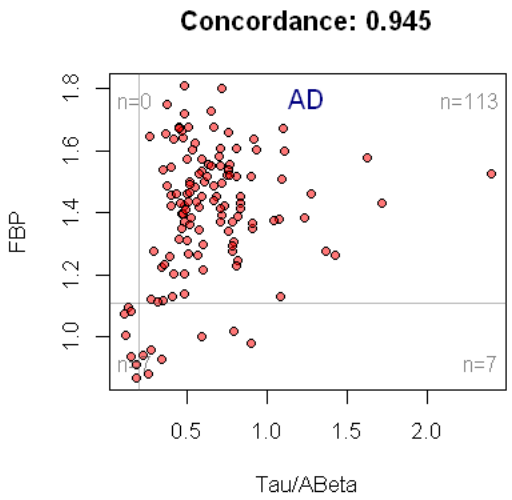


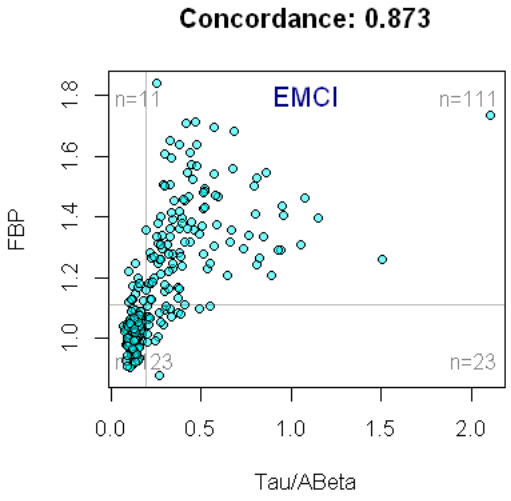


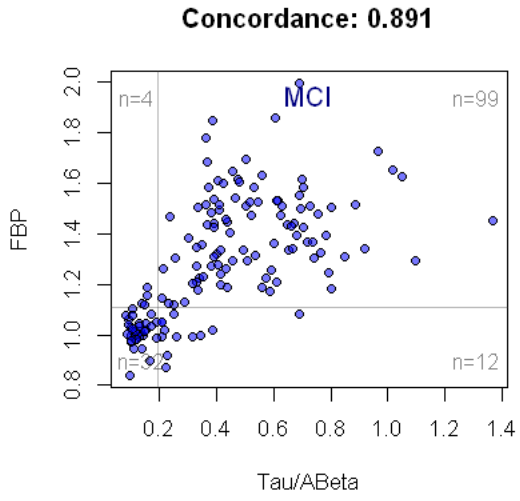
Concordance plots for FBP vs CSF tau/A$\beta_{1\text{-}42}$ in ADNIGO/2 SMC, EMCI, LMCI & AD participants at BASELINE



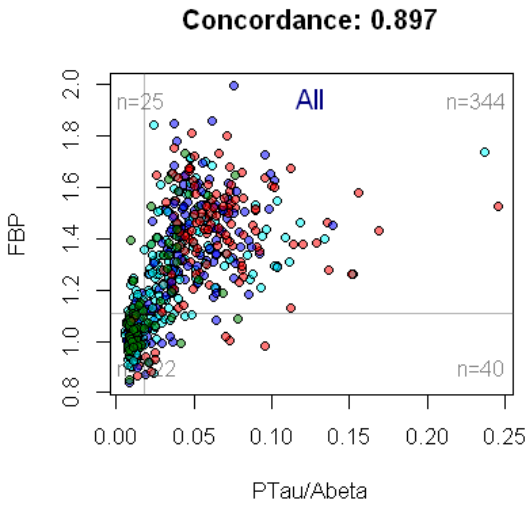


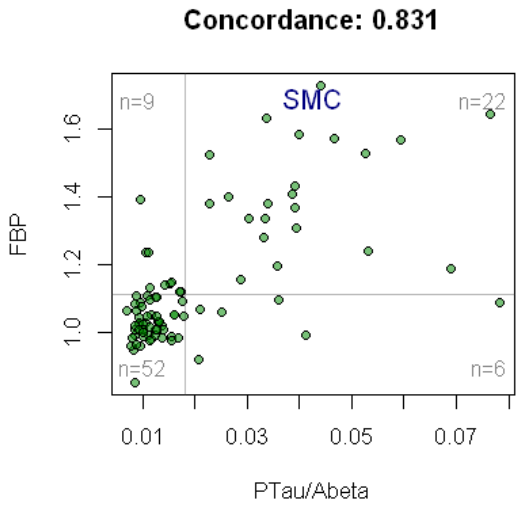


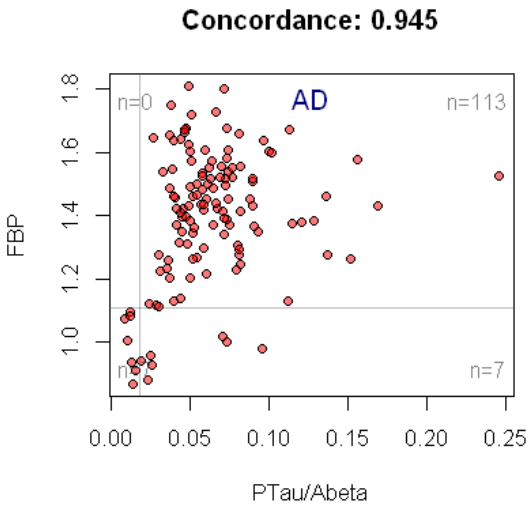


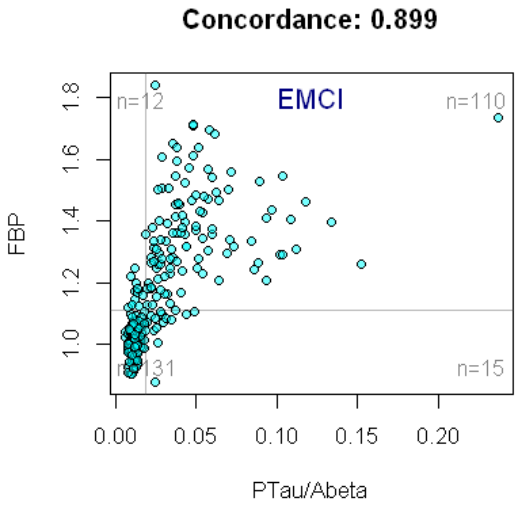


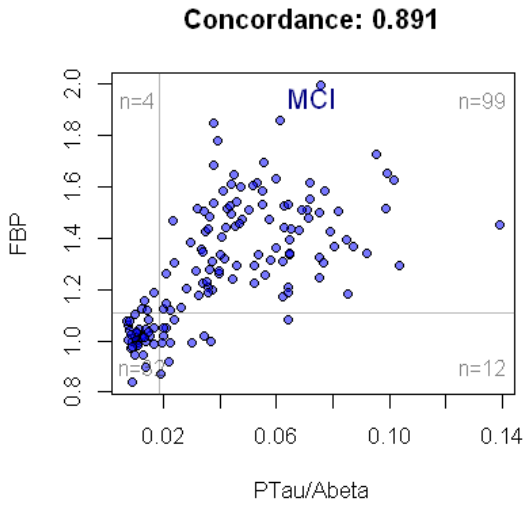
Concordance plots for FBP vs CSF ptau/Aβ_{1-42} in ADNIGO/2 participants at BASELINE



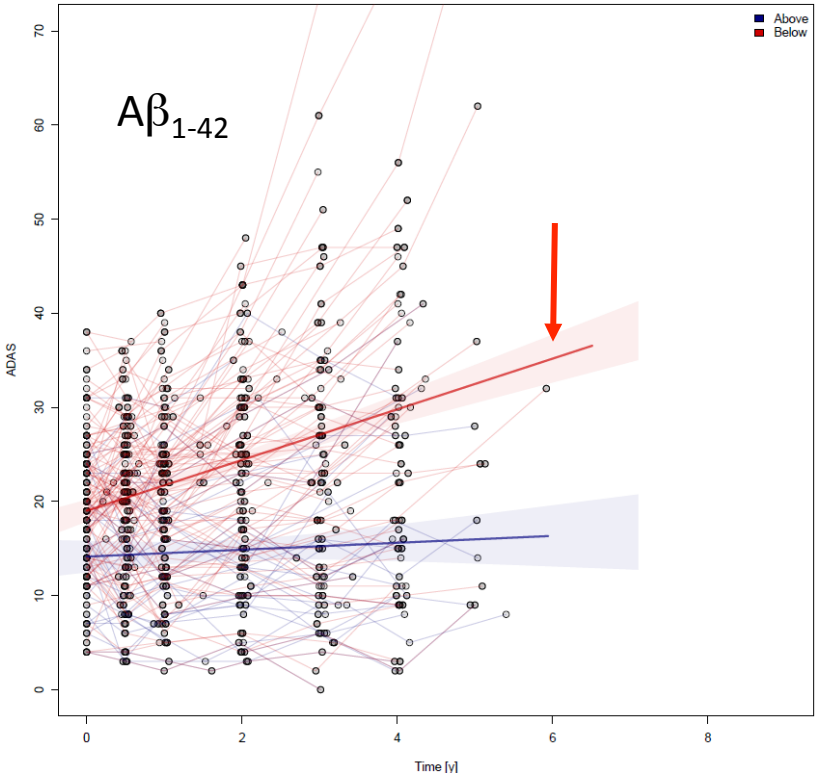


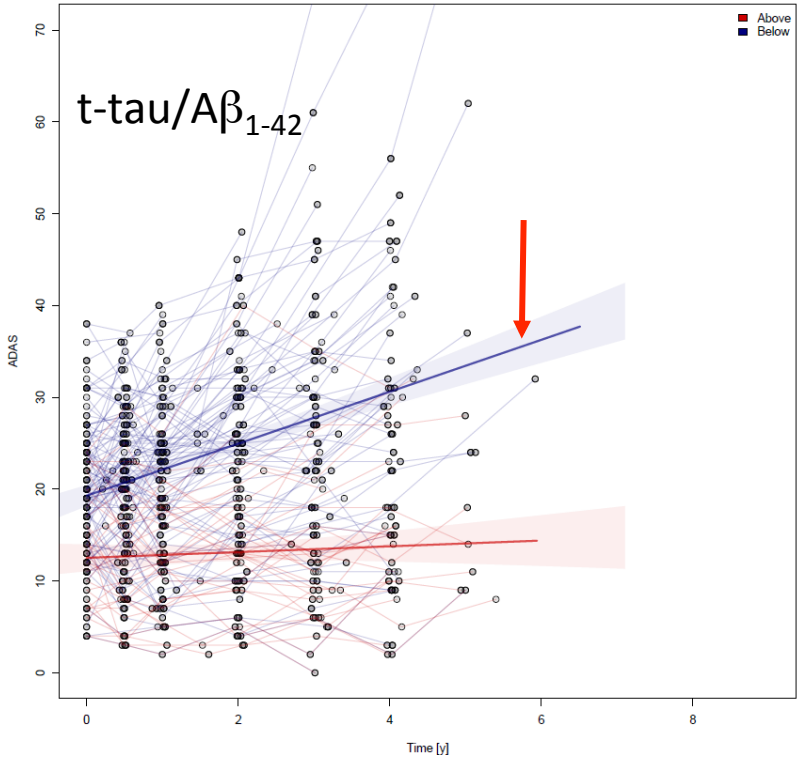


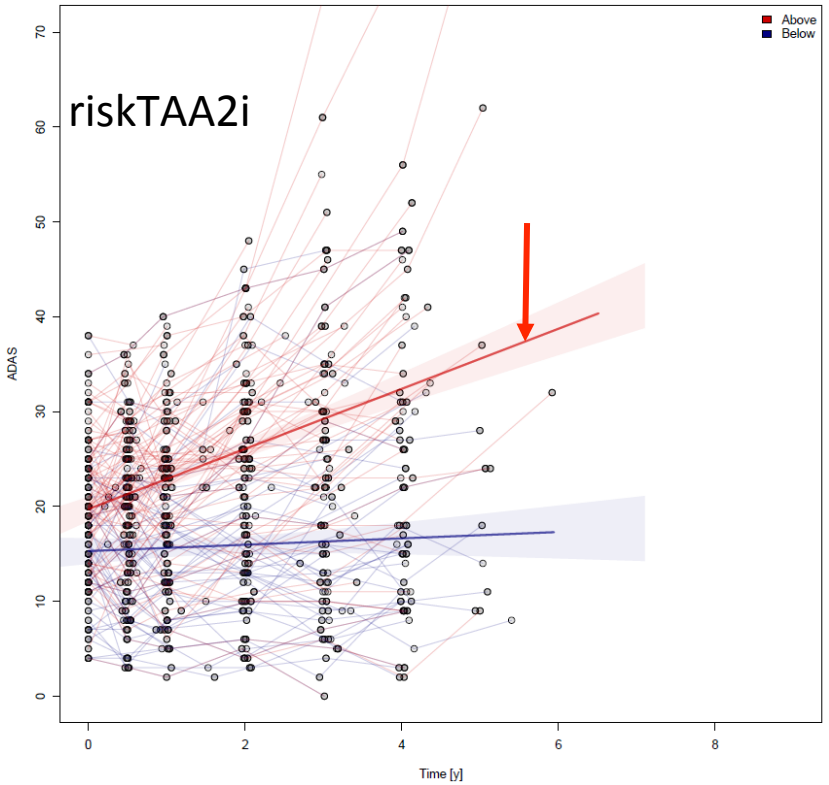




Prediction of cognitive decline(CDRsob) in ADNIGO/2 LMCI subjects







Vertical red arrow points to regression line for CDRsob values associated with $A\beta_{1\text{-}42}$ values below cutpoint value, t-tau/$A\beta_{1\text{-}42}$ values above cutpoint value, and logistic regression model(includes $A\beta_{1\text{-}42}$, t-tau and *APOE ε4* allele # as covariates) values above cutpoint value.

Summary

- Roche Elecsys immunoassays for Abeta1-42, t-tau and p-tau181 completed for 2401 ADNI1/GO/2 CSFs, and uploaded on the ADNI/LONI website, March 2017
- Precision and accuracy validations completed according to CLSI EP05
- General stats, Frequency distributions, mixture modeling & ROC with FBP PET as endpt described
- The t-tau/$A\beta_{1\text{-}42}$ and p-tau_{181}/$A\beta_{1\text{-}42}$ ratios outperformed $A\beta_{1\text{-}42}$ alone for clinical utilities based on:
  - Comparisons to FBP PET in ROC analyses
  - Concordance with FBP PET
  - Disease-independent mixture modeling
  - This observation is consistent with the BioFINDER study(using Roche platform/flutemetmol PET) as well as multiple other studies that used other immunoassay platforms and clinical endpoints:
    - Seeburger, 2015(OPTIMA study, N=227, autopsy-based diagnosis); Fagan, 2011(HASD, PIB PET based endpoint, N=103); Palmqvist, 2015(BioFINDER, Flutemetamol PET, N=366)
  - Mechanism possibilities: normalization of variance; tau abnormality adds to predictive performance, further studies needed
- Cutpoint assessments: ROC with FBP as endpoint; disease independent mixture modeling; extrapolation from BioFINDER study based on pre-analytical differences
- Prediction performance of BASELINE CSF AD biomarkers for cognitive decline documentation
- Continue ongoing work with ADNI and other studies toward goal of defining universal cutpoints for $A\beta_{1\text{-}42}$, t-tau and p-tau_{181} including use of autopsy-based cases and age-matched controls.
- Continue to work with colleagues on pre-analytical and other factors to help minimize and control these sources of variability
- Implement in ADNI3
- Collaboration on multimodal studies that include CSF, imaging, genetic, clinical parameters

ACKNOWLEDGEMENTS

Biomarker Research Lab

Magdalena Korecka
Michal Figurski
Magdalena Brylska
Teresa Waligorska
Leona Fields
Jacob Alexander
Ju Hee Kang

CNDR/ADRC

John Trojanowski
Virginia M-Y Lee
Steve Arnold
Murray Grossman
Jon Toledo
Alice Chen-Plotkin

William Hu
Anne Fagan
Hugo Vanderstichele
Kaj Blennow
Henrik Zetterberg
Chris Clark*
Manu Vandijck
John Lawson
Udo Eichenlaub
Tobias Bittner
The Roche team

*Deceased

Robert Dean
Holly Soares
Adam Simon
Eric Siemers
Piotr Lewczuk
William Potter
Rand Jenkins
Erin Chambers

Supported by the NIH/NIA & families of our patients

MJ Fox Fdn for PD research

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