Blood-Based Biomarkers for Alzheimer's disease

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Disclosures

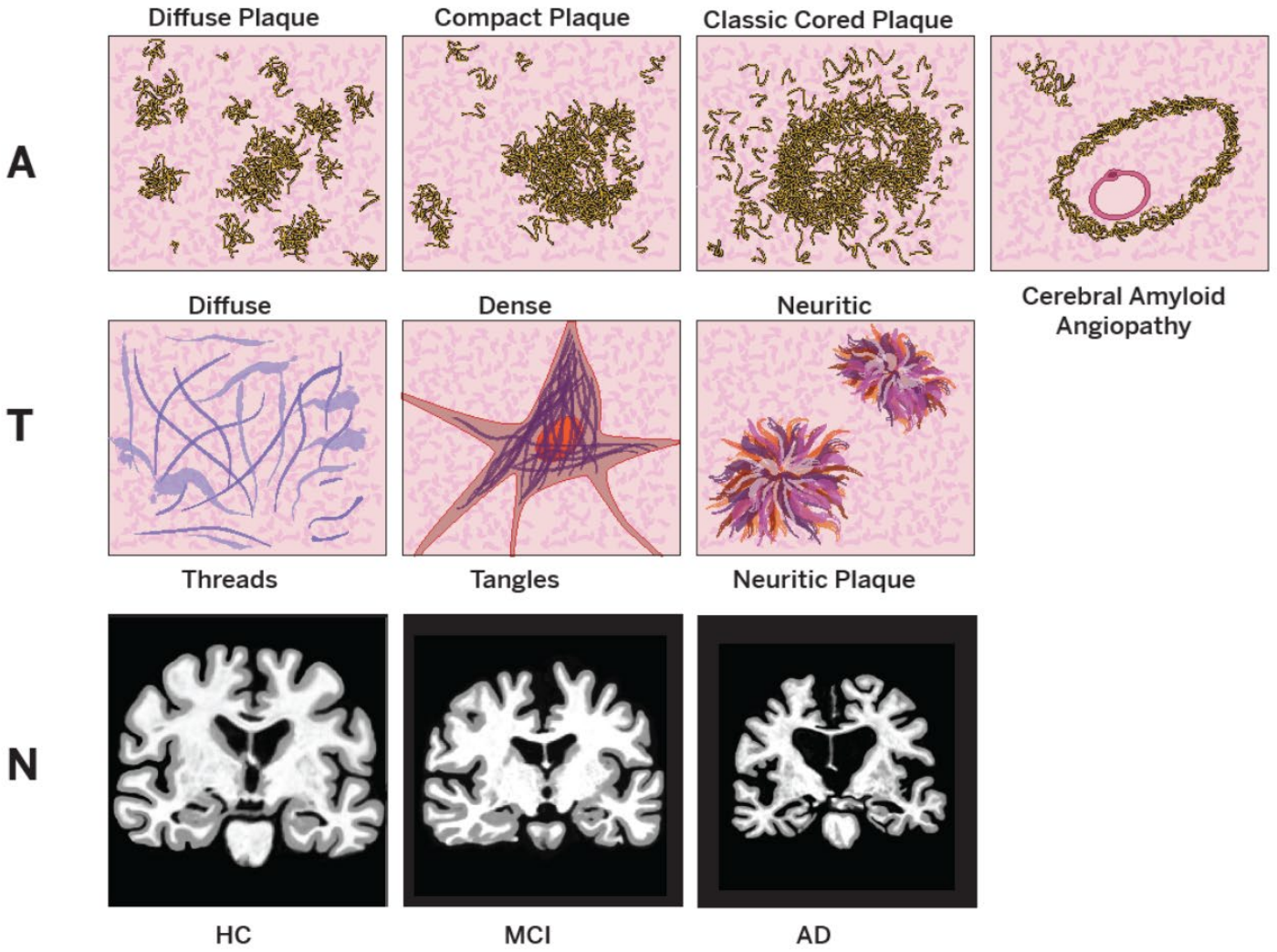
1. JLD is a minor shareholder of Eli Lilly and Company Stock
2. JLD is an inventor on patents associated with the P-tau217 assay discussed in this presentation
3. JLD received some support for his research from Eli Lilly, ADx Neurosciences, and Roche Diagnostics
4. JLD served as a consultant for Karuna Therapeutics, and Gates Ventures
5. JLD received speaker fees from Eli Lilly for Lilly sponsored seminars

Key Concepts

1. Update on Blood-based biomarkers for Amyloid, Tau, and Neurodegeneration

2. Need for Standardization/Harmonization

Blood-Based-Biomarkers for AD Pathology



Blood-Based-Biomarkers

Amyloid beta peptide Ratio (Aβ42/40)

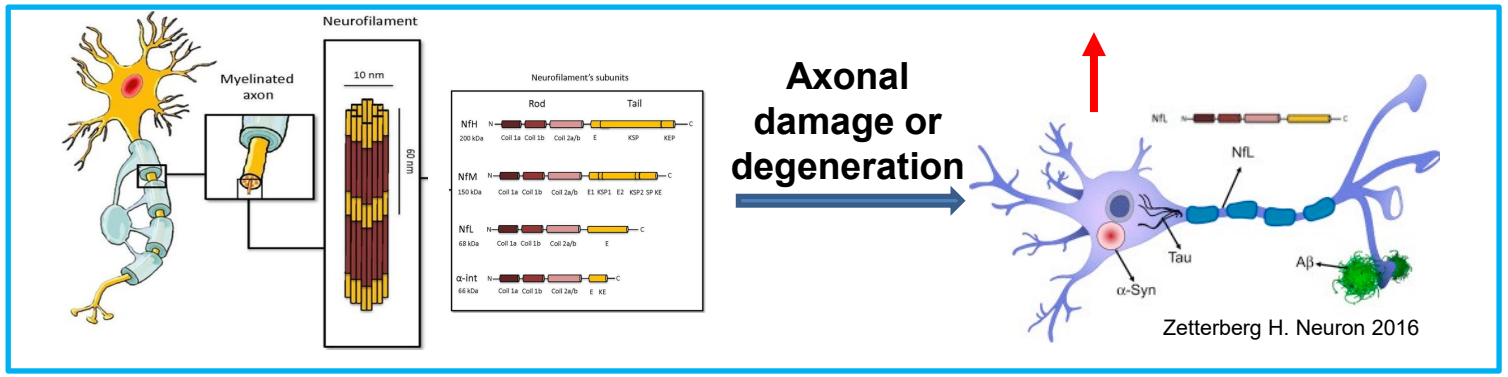
Phosphorylated Tau (P-tau)

Neurofilament Light Chain (NfL)

Neurofilament light chain (NF-L) is a fluid biomarker for neurodegeneration that reflects **ongoing** neuronal injury

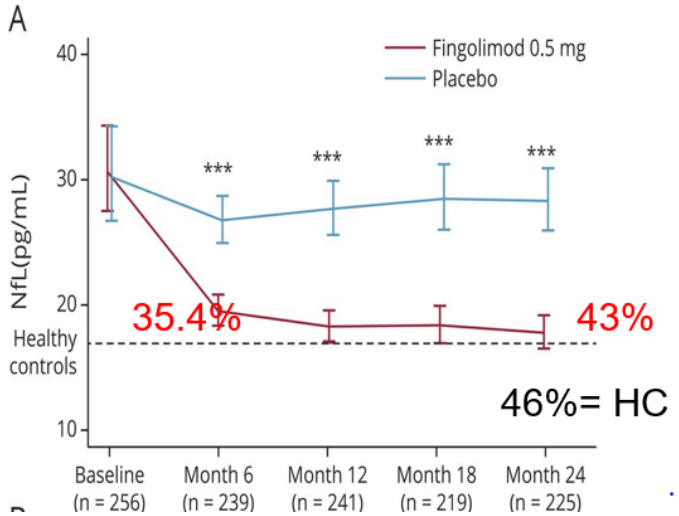
1. Bridel, C., et al., *Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. JAMA Neurol, 2019.*
2. Kuhle, J., et al., *Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology, 2019. 92(10): p. e1007-e1015.*
3. Miller, T., et al., *Phase 1-2 Trial of Antisense Oligonucleotide Tofersen for SOD1 ALS. N Engl J Med, 2020. 383(2): p. 109-119.*
4. Olsson, B., et al., *NFL is a marker of treatment response in children with SMA treated with nusinersen. J Neurol, 2019. 266(9): p. 2129-2136.*

NF-L is an abundant axonal structural protein that is highly expressed in neurons

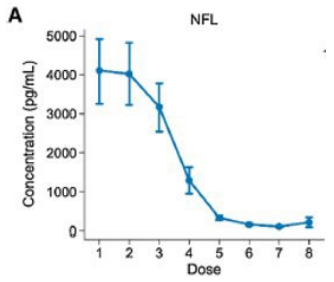


- In neurodegenerative conditions, NfL levels increase significantly (7.6x in ALS, 10.5x in ALS/FTD, 1.6-1.8x in RRMS, AD 1.4-1.8x). *Bridel C., AMA Neurol. 2019*

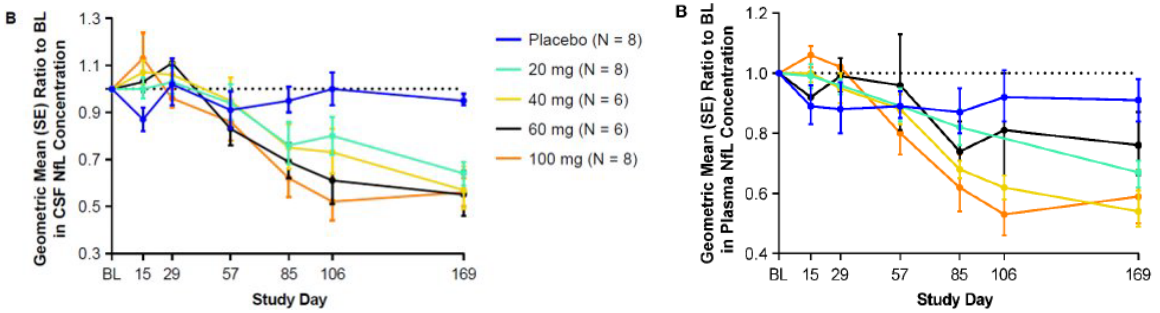
NF-L as an efficacy biomarker for neurodegenerative therapeutics



Nusinersen (SMA)



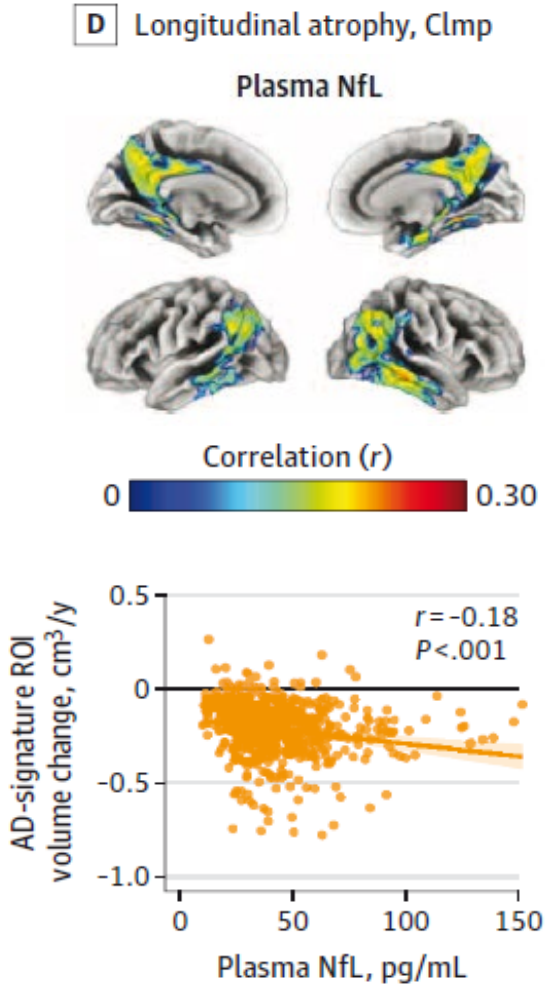
Tofersen (SOD1 in ALS)

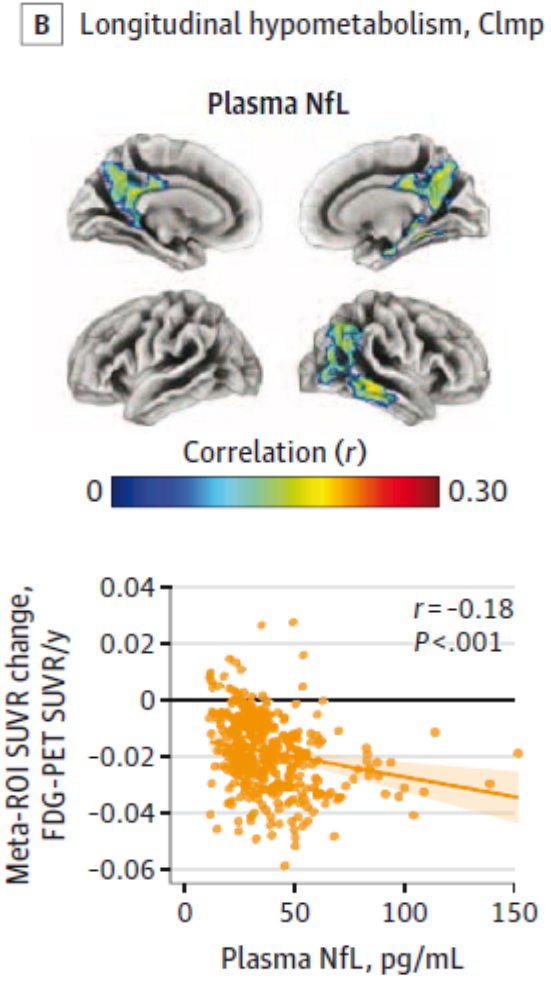


- *Plasma NfL levels reduced in response to treatment in Relapsing-Remitting MS patients (Kuhle J., Neurology. 2019)*
- *CSF NfL levels reduced in response to treatment*

NfL as a marker of neurodegeneration in Alzheimer's disease

1. NfL is a disease related non-specific marker of neurodegeneration
2. Associations with FDG-PET are weak
3. Associations with atrophy are weak



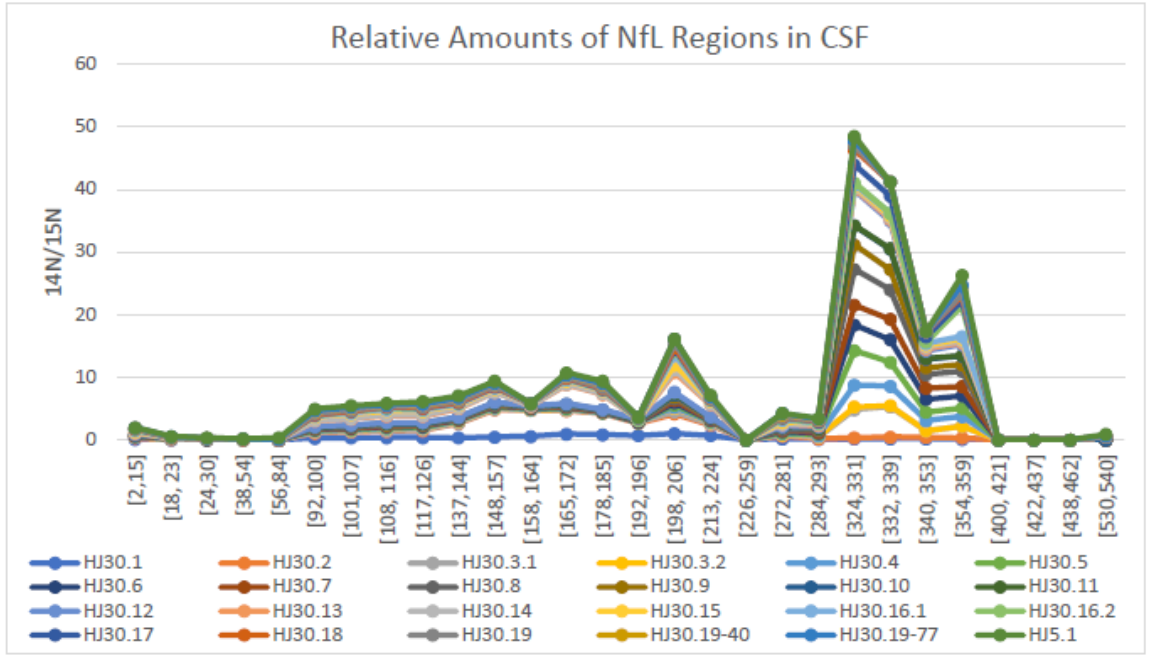


JAMA Neurology | Original Investigation

Longitudinal Associations of Blood Phosphorylated Tau181 and Neurofilament Light Chain With Neurodegeneration in Alzheimer Disease

Alexis Moscoso, PhD; Michel J. Grothe, PhD; Nicholas J. Ashton, PhD; Thomas K. Karikari, PhD; Juan Lantero Rodríguez, MSc; Anniina Snellman, PhD; Marc Suárez-Calvet, MD, PhD; Kaj Blennow, MD, PhD; Henrik Zetterberg, MD, PhD; Michael Schöll, PhD; for the Alzheimer's Disease Neuroimaging Initiative

Neurofilament Light Chain – Exploring the Forms of NfL in CSF by IP-LC/MS/MS



Supplemental Figure 2: Immunoprecipitation of native NfL from pooled CSF. Each of the 23 in-house NfL antibodies (HJ.30.x) and one negative control antibody against amyloid beta (HJ5.1) were assessed for their ability to immunoprecipitate NfL from CSF. Line colors correspond to individual antibodies and are noted in the figure legend. Antibodies appeared to target 3 different regions of NfL (see main text figure 1.)

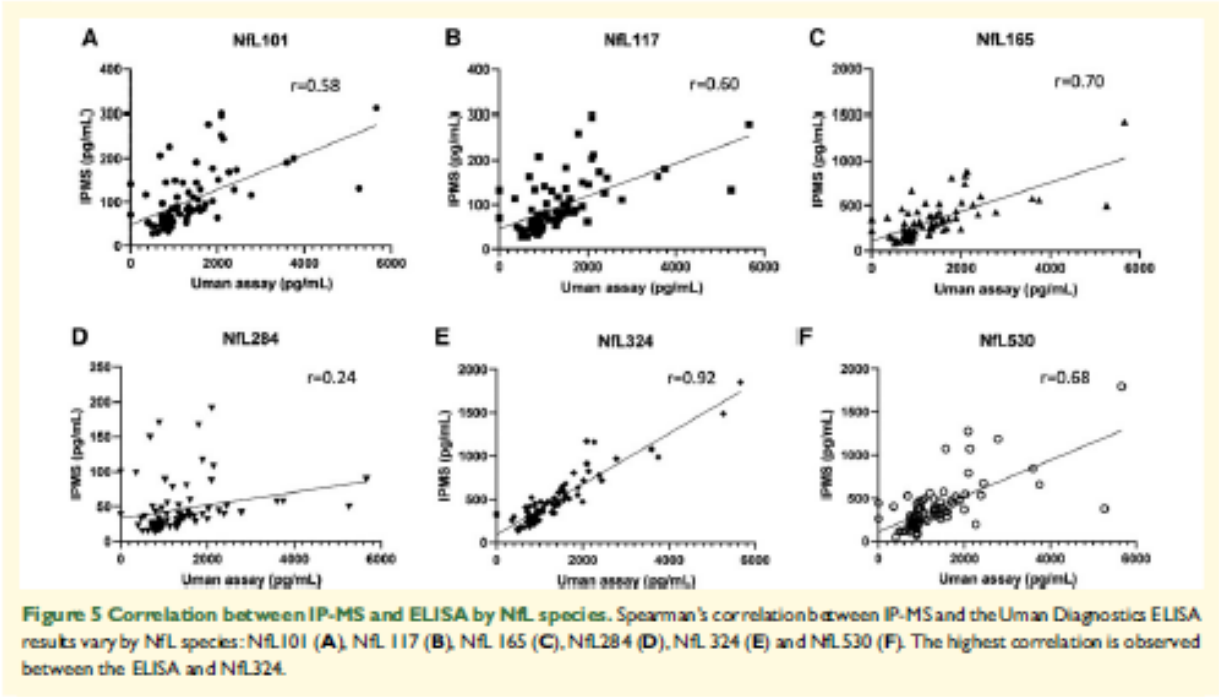


Figure 5 Correlation between IP-MS and ELISA by NfL species. Spearman's correlation between IP-MS and the Uman Diagnostics ELISA results vary by NfL species: NfL101 (**A**), NfL 117 (**B**), NfL 165 (**C**), NfL284 (**D**), NfL 324 (**E**) and NfL530 (**F**). The highest correlation is observed between the ELISA and NfL324.

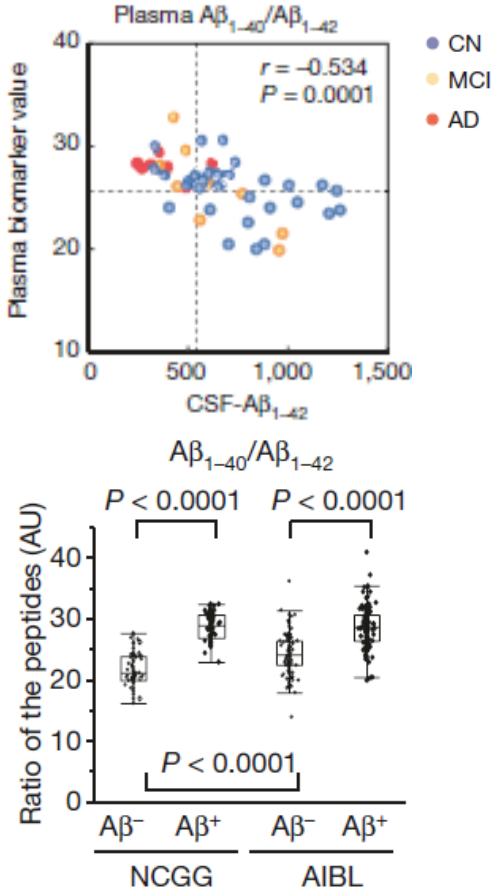
Budelier MM, He Y, Barthelemy NR, Jiang H, Li Y, Park E, Henson RL, Schindler SE, Holtzman DM, Bateman RJ. A map of neurofilament light chain species in brain and cerebrospinal fluid and alterations in Alzheimer's disease. Brain Commun. 2022;4(2):fcac045. Epub 20220222. doi: 10.1093/braincomms/fcac045. PubMed PMID: 35415607; PMCID: PMC8994116.

Summary of NfL

- NfL is a measure of neurodegeneration with broad application across indications
- NfL can be measured in preclinical models and may inform on molecule specific expectations
- Substantial data exists to design trials with NfL as an exploratory endpoint as measured in CSF or Plasma
- The utility of NfL in AD clinical trials has yet to be realized. Perhaps due to a lack of robust efficacy directly targeted at neurodegeneration

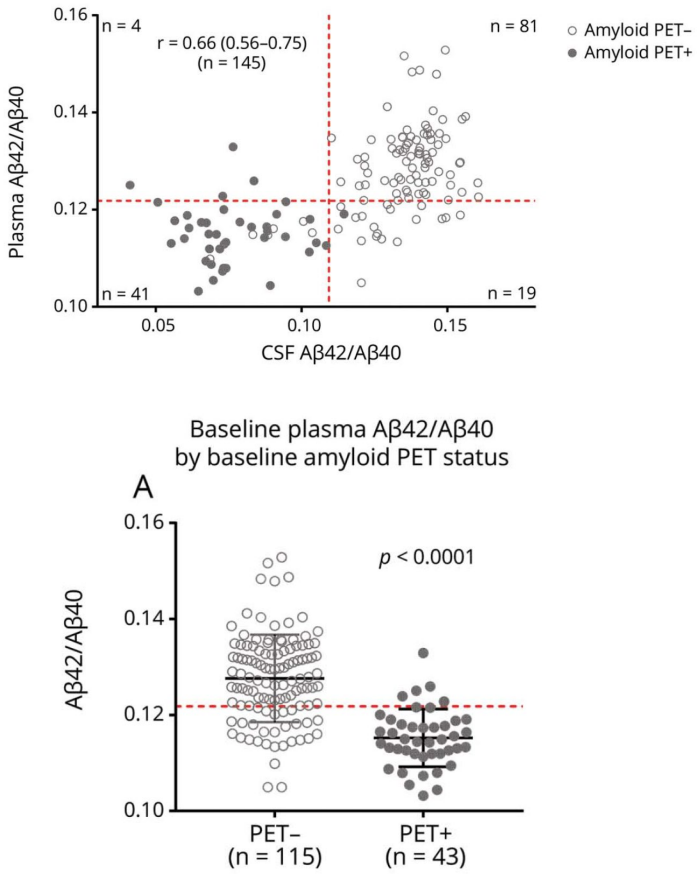
Blood-based tests for Aβ Peptides

Mass spectrometry



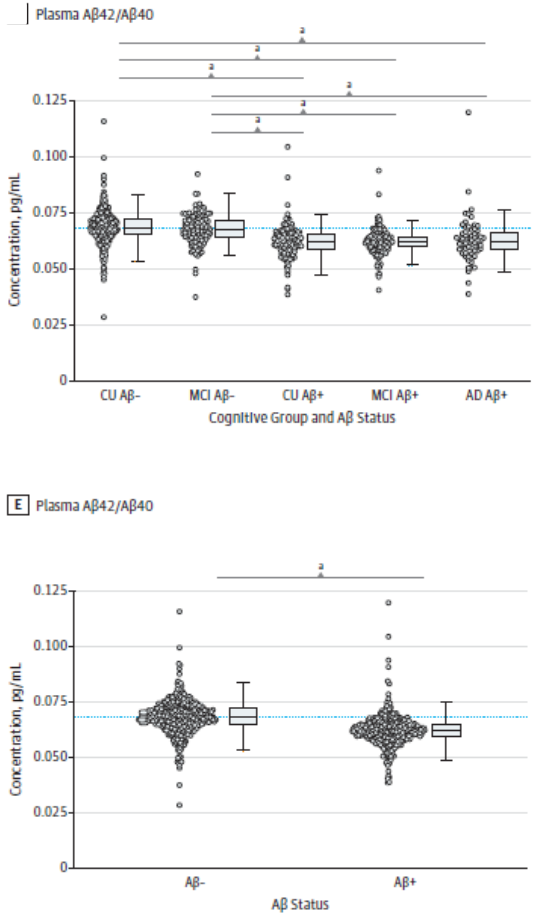
Nakamura et al. Nature, 2018

Mass spectrometry



Schindler et al., Neurology, 2019

Elecsys immunoassay



Palmqvist, Janelidze et al., JAMA Neurology, 2019

- Many measurement platforms and assays are available
- Each assay has variability unrelated to the pathology that contributes to measurement error

The biological effect size:

Plasma Aβ 42/40 is reduced by 10-15%

Blood-based tests for Aβ Peptides

Two cohorts

- 292 subjects – Biofinder Study and 122 subjects ADNI

Main Findings

- Wide-ranging diagnostic accuracy between assays [AUC], 0.86-0.64
- The results were similar when using Aβ-PET or CSF as outcome measures
- Assays showed significant wide-ranging coefficients for correlations with CSF Aβ42/40 (Spearman rho range, 0.15-0.65)

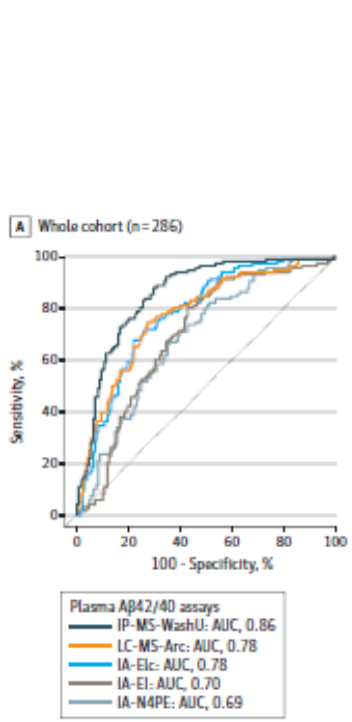
Plasma Aβ42/40 Assay	AUC (95% CI)[b] CSF Aβ42/40	Aβ-PET
Entire cohort		
Aβ+, No.	118	110
Aβ−, No.	168	176
IP-MS-WashU	0.855 (0.810-0.899)	0.833 (0.787-0.879)
IA-EIc	0.778 (0.725-0.832)[c]	0.727 (0.669-0.784)[d]
LC-MS-Arc	0.776 (0.721-0.830)[c]	0.753 (0.696-0.811)[c]
IA-EI	0.697 (0.635-0.758)[d]	0.672 (0.609-0.735)[d]
IA-N4PE	0.687 (0.626-0.748)[d]	0.655 (0.591-0.719)[d]
Subcohort with IP-MS-Shim Aβ42/40[a]		
Aβ+, No.	86	86
Aβ−, No.	114	114
IP-MS-WashU	0.872 (0.824-0.920)	0.872 (0.824-0.920)
IP-MS-Shim	0.825 (0.767-0.882)	0.825 (0.767-0.882)
LC-MS-Arc	0.775 (0.711-0.839)[c]	0.775 (0.711-0.839)[c]
IA-EIc	0.773 (0.709-0.837)[c]	0.773 (0.709-0.837)[c]
IA-EI	0.704 (0.631-0.777)[d]	0.704 (0.631-0.777)[d]
IA-N4PE	0.679 (0.605-0.753)[d]	0.679 (0.605-0.753)[d]
Subcohort with IP-MS-UGOT and IA-Quan Aβ42/40		
Aβ+, No.	91	86
Aβ−, No.	136	141
IP-MS-WashU	0.838 (0.785-0.891)	0.814 (0.760-0.868)
IA-EIc	0.795 (0.738-0.853)	0.728 (0.663-0.793)[c]
LC-MS-Arc	0.763 (0.700-0.827)[f]	0.742 (0.676-0.809)[f]
IA-N4PE	0.706 (0.639-0.773)[c]	0.649 (0.577-0.721)[d]
IA-EI	0.697 (0.628-0.767)[d]	0.667 (0.596-0.738)[d]
IP-MS-UGOT	0.678 (0.605-0.750)[d]	0.632 (0.557-0.707)[d]
IA-Quan	0.636 (0.563-0.709)[d]	0.600 (0.525-0.675)[d]

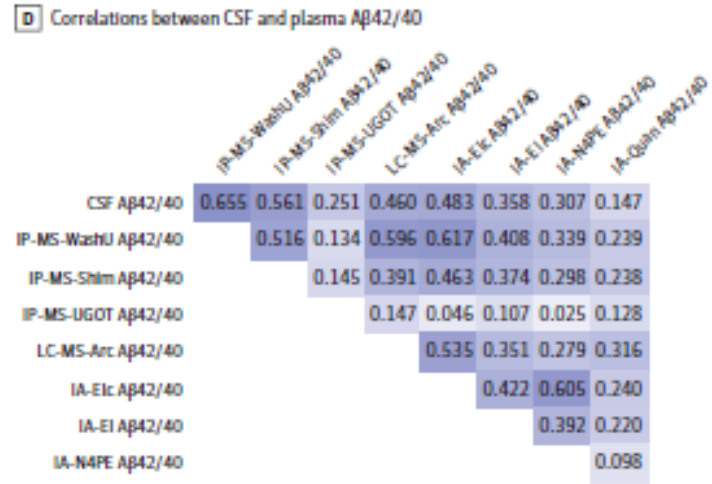
JAMA Neurology | Original Investigation

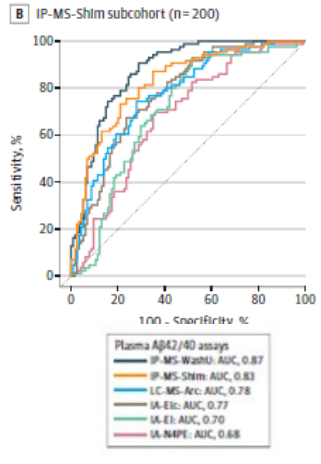
Head-to-Head Comparison of 8 Plasma Amyloid-β 42/40 Assays in Alzheimer Disease

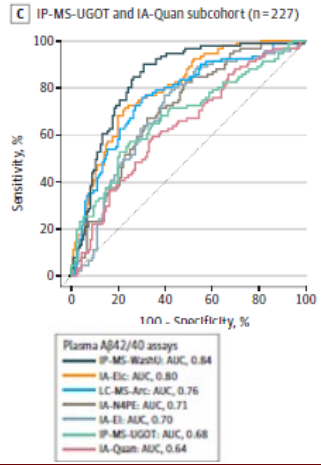
Shorena Janelidze, PhD; Charlotte E. Teunissen, PhD; Henrik Zetterberg, MD, PhD; José Antonio Allué, PhD; Leticia Sarasa, PhD; Udo Eichenlaub, PhD; Tobias Bittner, PhD; Vitally Ovod, MSs; Inge M. W. Verberk, MSs; Kenji Toba, MD, PhD; Akinori Nakamura, MD, PhD; Randall J. Bateman, MD, PhD; Kaj Blennow, MD, PhD; Oskar Hansson, MD, PhD

JAMA Neurol. 2021;78(11):1375-1382. doi:10.1001/jamaneurol.2021.3180
Published online September 20, 2021. Last corrected on November 22, 2021.









Summary of Plasma Aβ 42/40

- Plasma Aβ 42/40 reduces with development of amyloid pathology by approximately 10-15%
- Data from round robin studies suggest existing assays are not highly correlated
- If an assay (from sample collection to result) can deliver high precision, is robust to interferences, and can be kept in control for the duration of enrollment, the plasma Aβ 42/40 ratio may be useful for enriching for amyloid positive subjects in patient registries
- Implementation requires careful choice of assay, validation of key assay characteristics and monitoring of performance

Measuring P-tau in Plasma

Bayoumy et al.
Alzheimer's Research & Therapy (2021) 13:198
https://doi.org/10.1186/s13195-021-00939-9

Alzheimer's Research & Therapy

RESEARCH Open Access

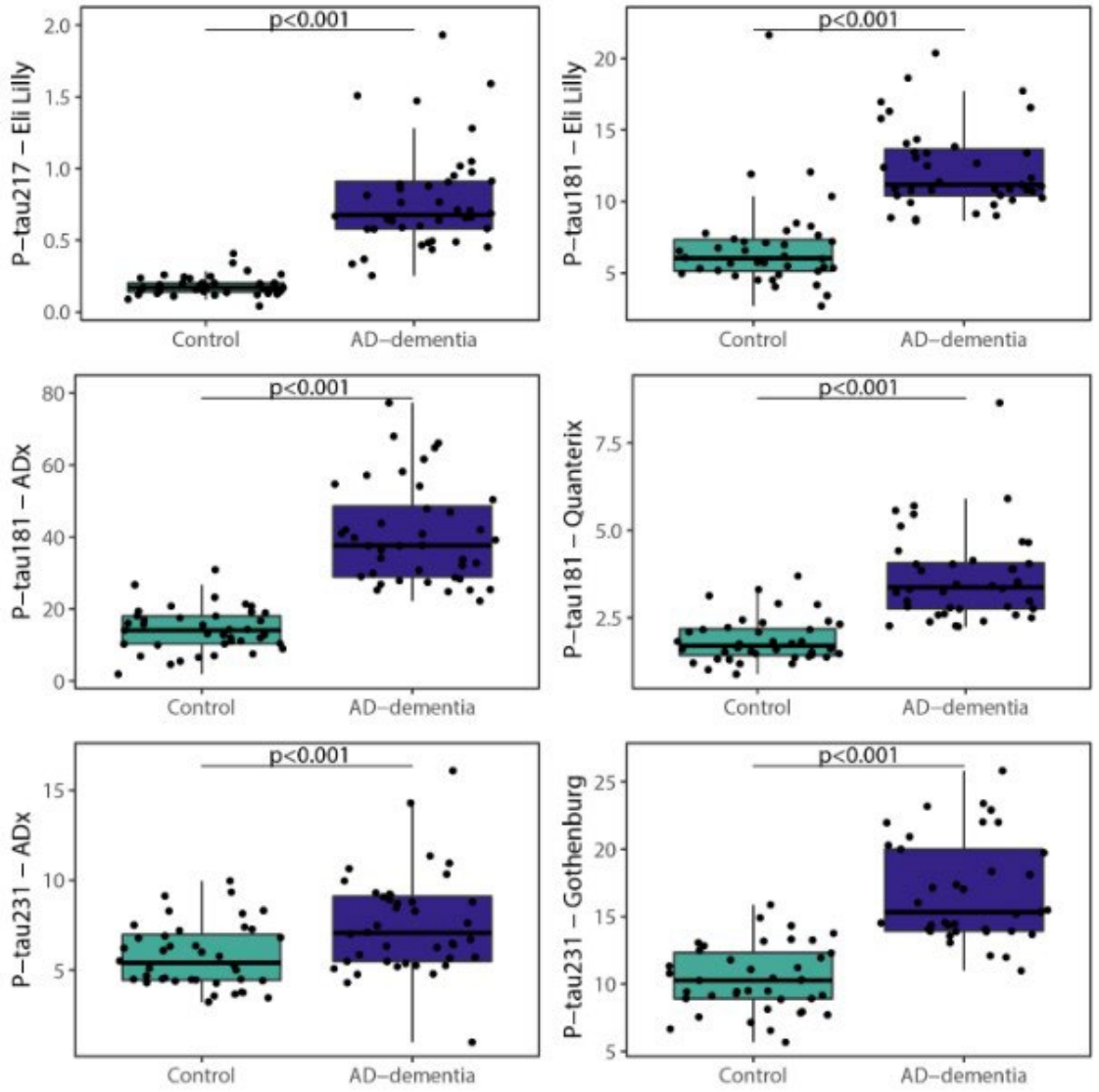
Clinical and analytical comparison of six Simoa assays for plasma P-tau isoforms P-tau181, P-tau217, and P-tau231

Sherif Bayoumy[1*†], Inge M. W. Verberk[1†], Ben den Dulk[1], Zulaiga Hussainali[1], Marissa Zwan[2], Wiesje M. van der Flier[2,3], Nicholas J. Ashton[4,5,6,7], Henrik Zetterberg[4,8,9,10,11], Kaj Blennow[4,8], Jeroen Vanbrabant[12], Erik Stoops[12], Eugeen Vanmechelen[12], Jeffrey L. Dage[13,14] and Charlotte E. Teunissen[1]

Diagnostic Accuracy and performance of the six P-tau assays

	AD-dementia	Controls	Differentiation AD-dementia versus controls				
	Median [IQR]	Median	Fold change	AUC (95% CI)	Cutoff	%Sens	%Spec
P-tau181 Eli Lilly	11.1 [10.4-13.6]	6.1 [5.1-7.4]	1.8	0.938 (0.872-1.000)	8.6	100	89
P-tau181 ADx	37.6 [28.8-48.6]	13.2 [10.3-17.6]	2.9	0.988 (0.969-1.000)	24	100	92
P-tau181 Quanterix	3.4 [2.7-4.1]	1.6 [1.4-2.2]	2.0	0.936 (0.885-0.987)	2.2	100	78
P-tau217 Eli Lilly	0.7 [0.6-0.9]	0.17 [0.14-0.2]	4.1	0.995 (0.987-1.000)	0.42	92.5	100
P-tau231 ADx	7.3 [5.6-9.1]	5.5 [4.5-6.9]	1.3	0.719 (0.607-0.831)	4.7	95	43
P-tau231 Gothenburg	15.3 [13.9-19.8]	10.3 [8.9-11.9]	1.5	0.943 (0.896-0.991)	13.4	89	90

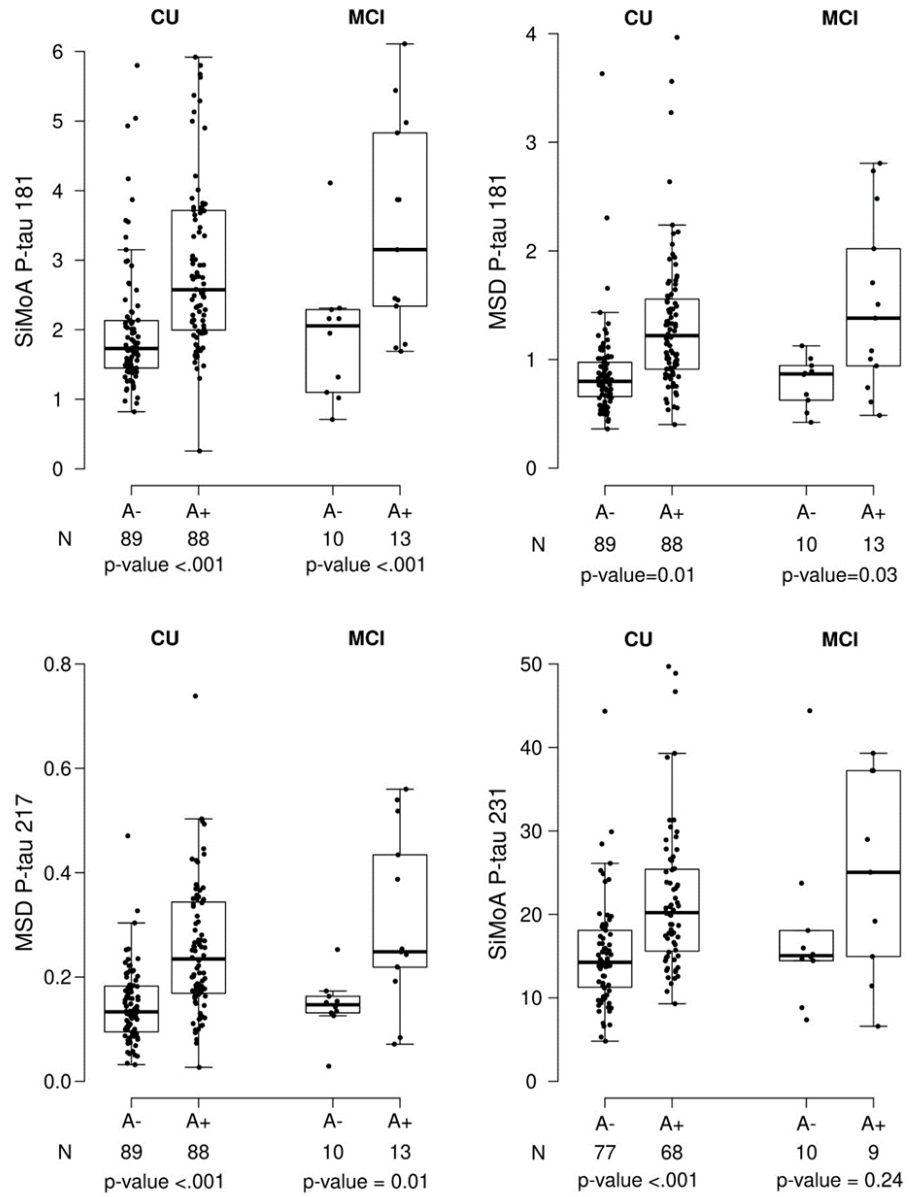
Median concentrations are in pg/mL. Fold-change was calculated by dividing the median concentration in the AD-dementia group over the median concentration in the control group. AUCs were derived from ROC analysis. All group comparisons were significant with p-values below the Bonferroni-adjusted p-value cutoff of 0.0083. P-tau cutoff was specified at the Youden's index (maximum sum of sensitivity and specificity). P-tau=phosphorylated tau, AD=Alzheimer's disease, IQR=interquantile range, AUC=area under the curve, sens=sensitivity, spec=specificity.



Plasma P-tau in Cognitively Unimpaired Subjects

The amyloid pathology related difference in plasma P-tau in CU subjects has minor differences between P-tau isoforms:

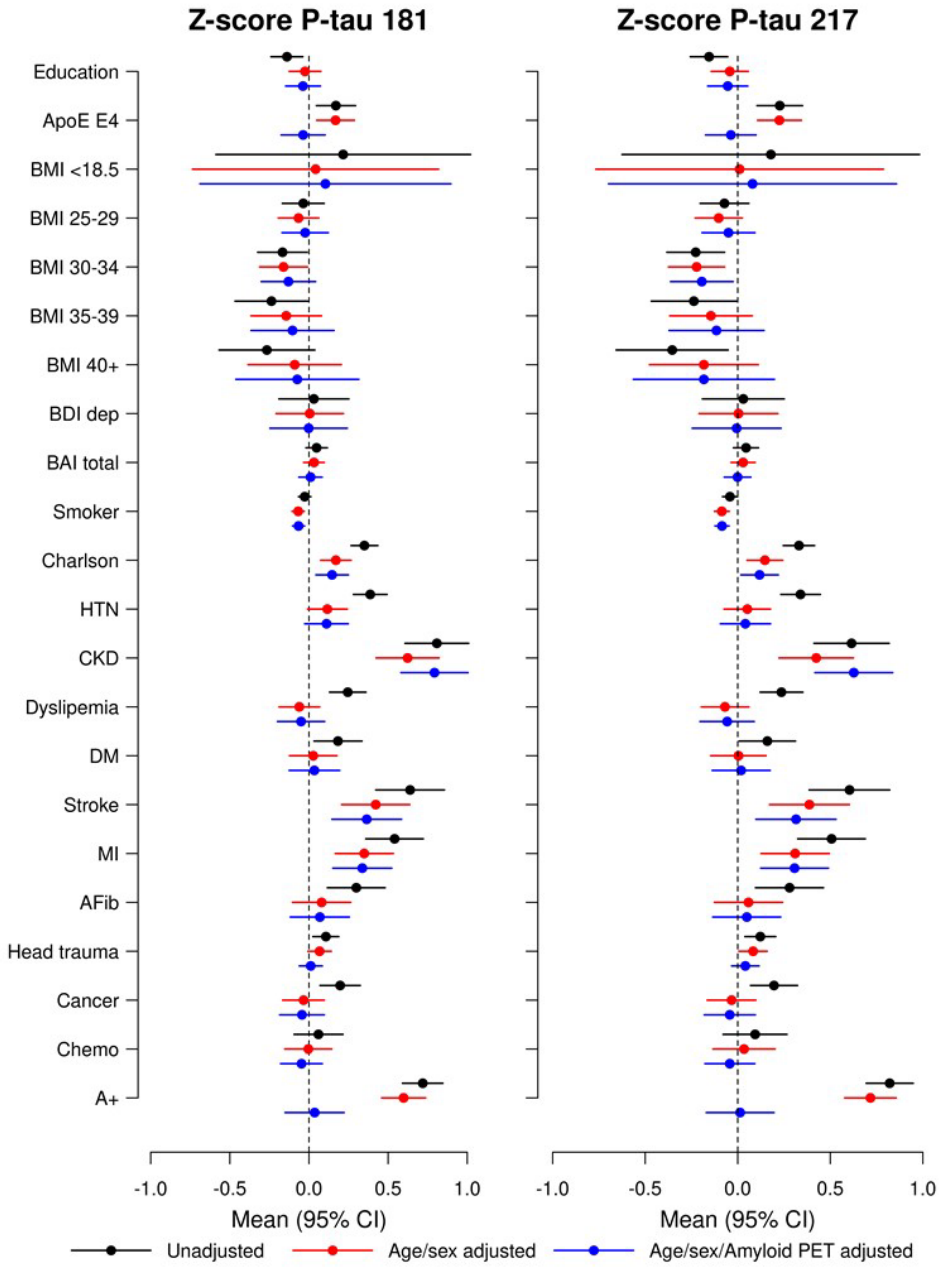
- In cognitively unimpaired subjects from a recent study in Mayo Clinic Study of Aging
- Quanterix P-tau181 +49%
- University of Gothenburg P-tau231 +42%
- Lilly P-tau181 +53%
- Lilly P-tau217 +77%



Mielke MM, Frank RD, Dage JL, et al. Comparison of Plasma Phosphorylated Tau Species With Amyloid and Tau Positron Emission Tomography, Neurodegeneration, Vascular Pathology, and Cognitive Outcomes. JAMA Neurol 2021;78:1108-1117.

P-Tau Levels are influenced by multiple factors in the community

- Influence of some factors are lost when adjusting for age and sex (Education, Diabetes, Dyslipemia, Hypertension, Cancer)
- Further adjustment for Amyloid status eliminates significance of APOE
- BMI, Smoking, Chronic Kidney Disease, Stroke, Myocardial infarction all remain significant after adjustments
- The prevalence and incidence of these factors can differ by race/ethnicity, geography, and socioeconomic status

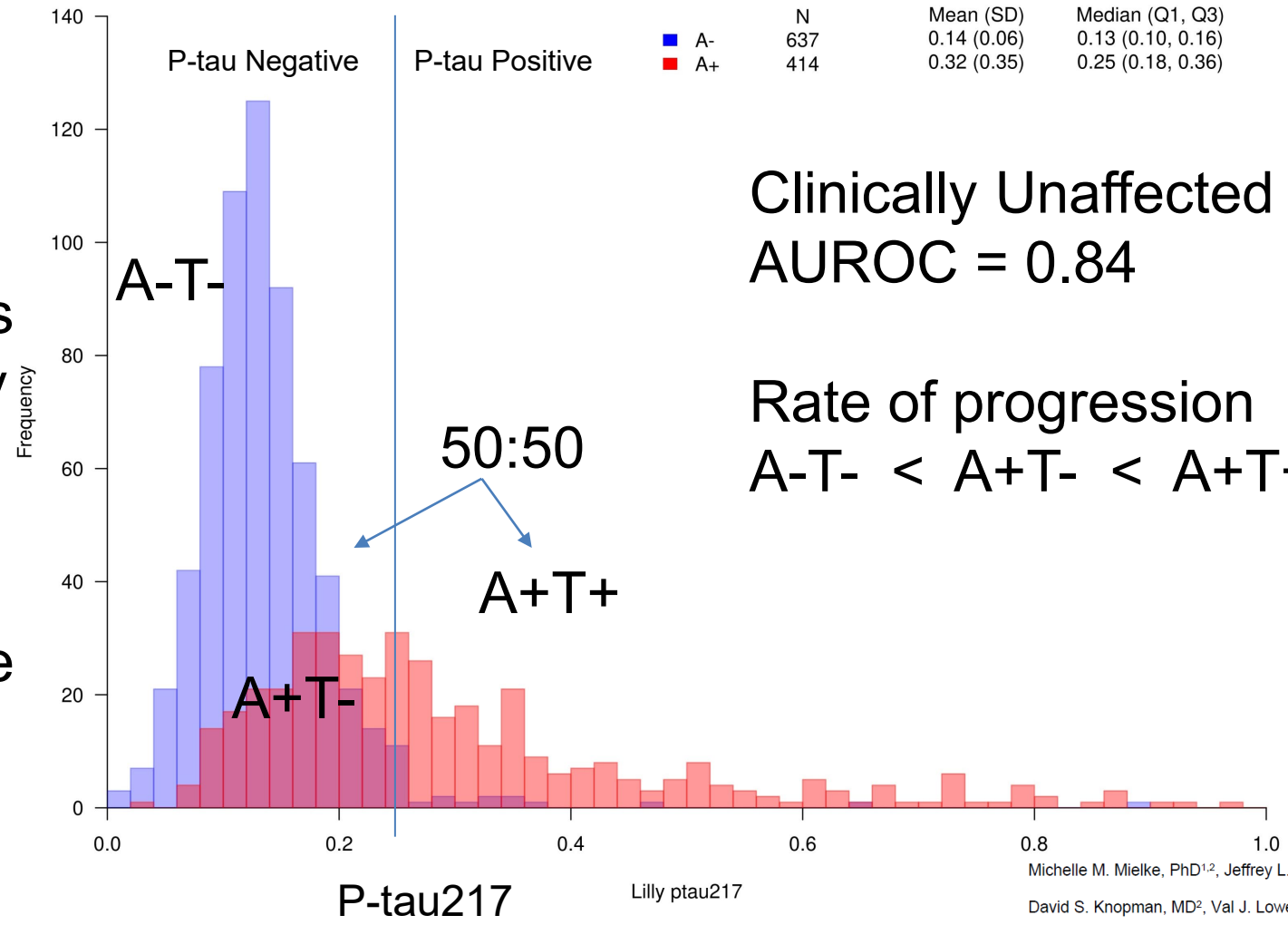


Michelle M. Mielke, PhD[1,2], Jeffrey L. Dage, PhD[3,4], Ryan D. Frank, MS[5], Alicia Algeciras-Schimnich, PhD[6], David S. Knopman, MD[2], Val J. Lowe, MD[7], Guojun Bu, PhD[8], Prashanthi Vemuri, PhD[7], Jonathan Graff-Radford, MD[2], Clifford R. Jack Jr, MD[7], Ronald C. Petersen, MD, PhD[1,2]

Accepted

Distribution of P-tau levels by amyloid status

- Earliest disease stage
- Identifies Subjects with AD pathology
- Missing subjects with Alzheimer's pathologic change



Clinically Unaffected Population Study
AUROC = 0.84

Rate of progression
A-T- < A+T- < A+T+

Accepted

Michelle M. Mielke, PhD[1,2], Jeffrey L. Dage, PhD[3,4], Ryan D. Frank, MS[5], Alicia Algeciras-Schimnich, PhD[6], David S. Knopman, MD[2], Val J. Lowe, MD[7], Guojun Bu, PhD[8], Prashanthi Vemuri, PhD[7], Jonathan Graff-Radford, MD[2], Clifford R. Jack Jr, MD[7], Ronald C. Petersen, MD, PhD[1,2]

Relationship between Blood and Imaging Measures of NFT pathology

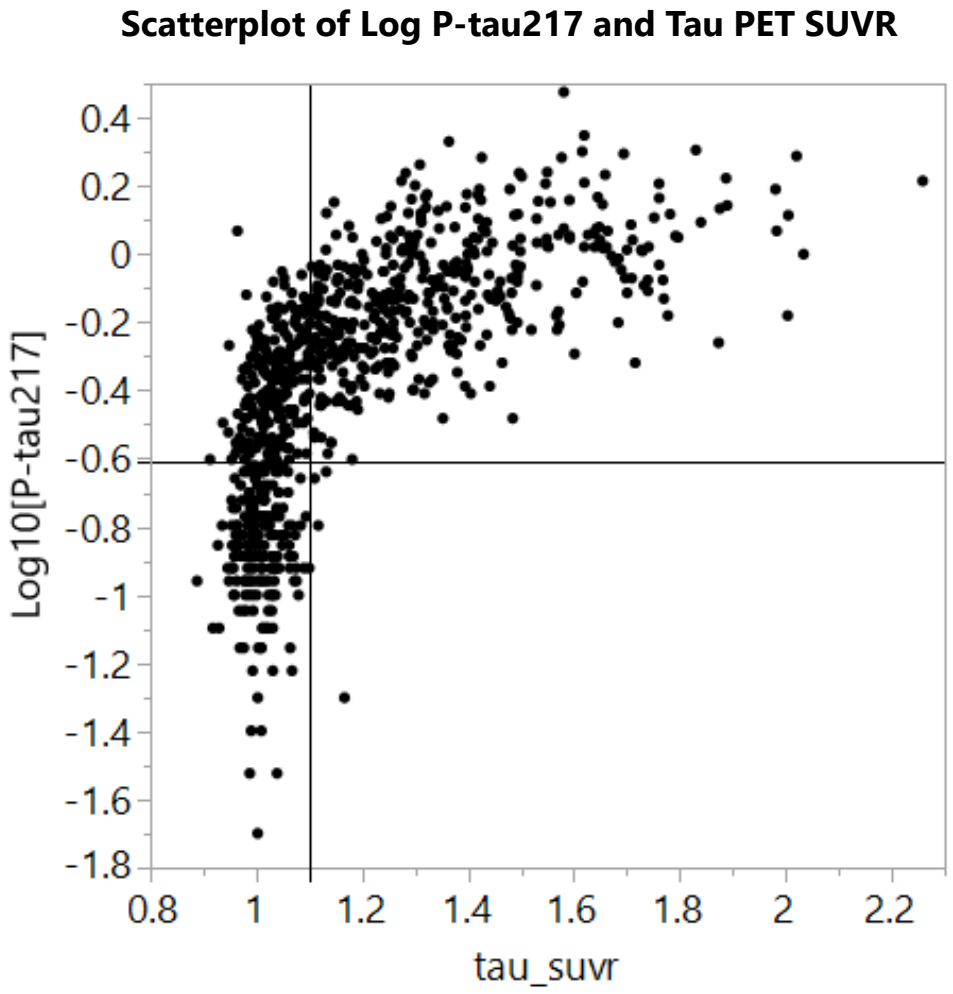
Samples Collected During Screening for Early Symptomatic Alzheimer's disease (NCT03518073)

N=860 with both P-tau and Tau PET

Plasma P-tau is associated with Tau PET

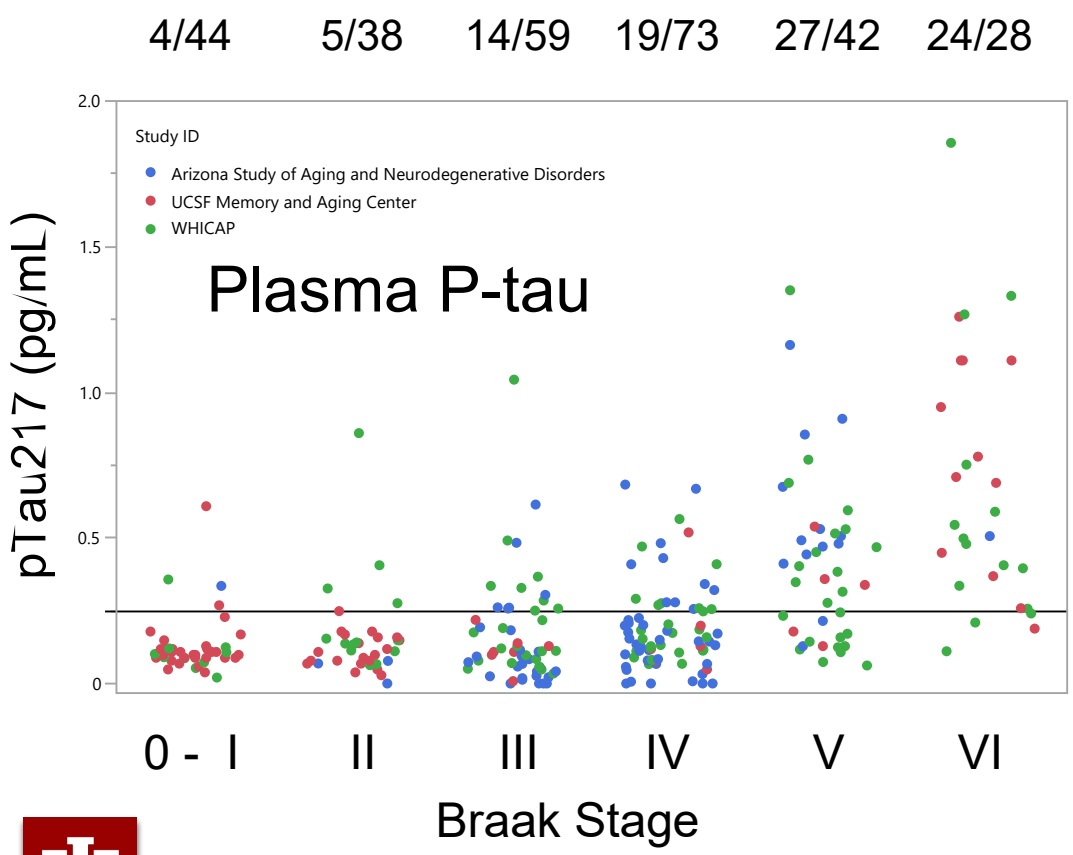
Plasma P-tau changes earlier than Tau PET

High Negative Predictive Value

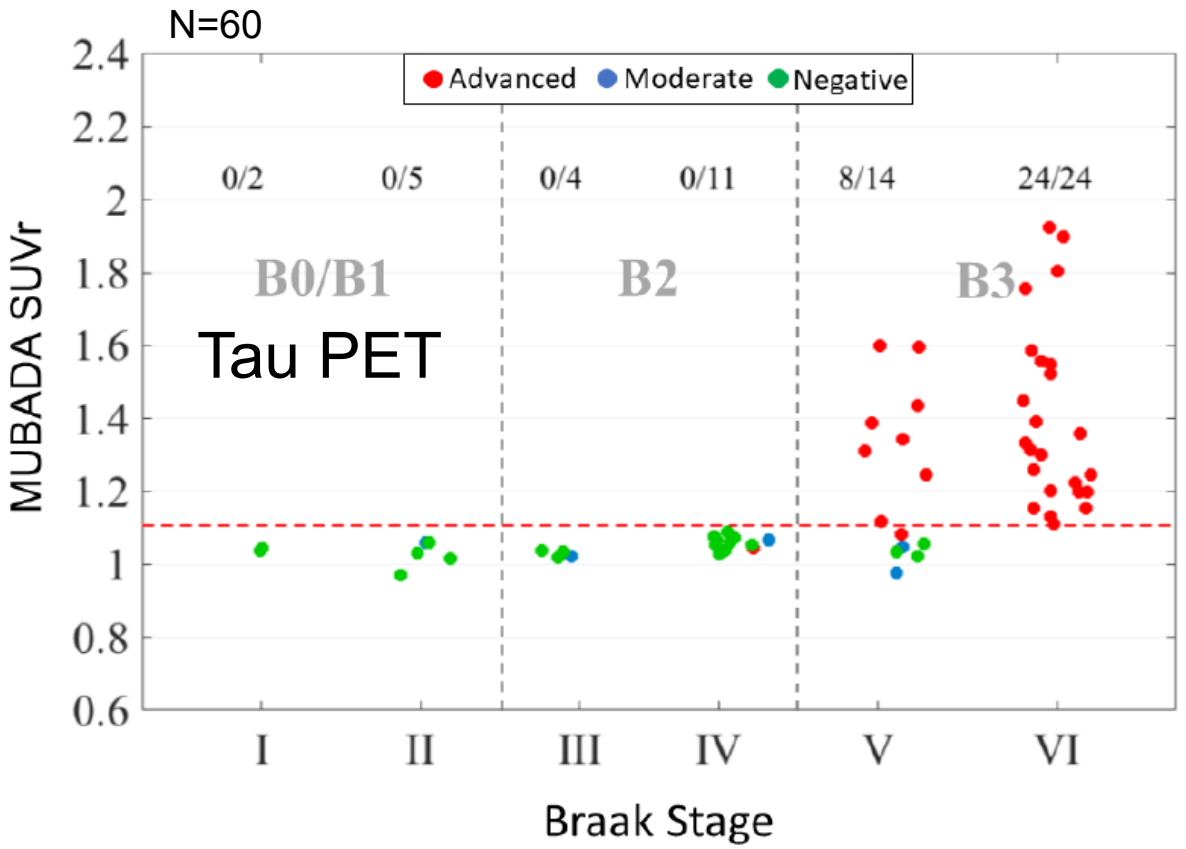


Plasma P-tau or [18F]Flortaucipir and Postmortem Assessment of Tau Pathology by Braak Stage

Unpublished Meta Analysis (N=284) of antemortem plasma P-tau217 values by neuropathology assigned NFT Braak Stage.



Fleisher et al. JAMA Neurol. 2020 - Supplemental



Jack CR Jr, et al. Alzheimers Dement. 2018 Apr;14(4):535-562. doi: 10.1016/j.jalz.2018.02.018. PMID: 29653606; PMCID: PMC5958625.

Cognitive Stages and Biomarkers

NIA Research Framework - 2018

Plasma P-tau can identify Alzheimer's disease across all cognitive stages

Risk of short-term cognitive decline based on the biomarker profile and cognitive stage

			Syndromal Cognitive Stage		
			Cognitively unimpaired	**MCI**	**dementia**
P-tau -	Biomarker Profile	$A^-T^-(N)^-$	normal AD biomarkers, cognitively unimpaired	normal AD biomarkers with MCI	normal AD biomarkers with dementia
		$A^+T^-(N)^-$	Preclinical Alzheimer's pathologic change	Alzheimer's pathologic change with MCI	Alzheimer's pathologic change with dementia
		$A^+T^-(N)^+$	Alzheimer's and concomitant suspected non Alzheimer's pathologic change, cognitively unimpaired	Alzheimer's and concomitant suspected non Alzheimer's pathologic change with MCI	Alzheimer's and concomitant suspected non Alzheimer's pathologic change with dementia
P-tau +		$A^+T^+(N)^-$	Preclinical Alzheimer's disease	Alzheimer's disease with MCI (Prodromal AD)	Alzheimer's disease with dementia
		$A^+T^+(N)^+$			

Non-Alzheimer's continuum profiles are not included in table because the risk associated with different combinations of T+(N)-, T+(N)+, T-(N)+ among A- individuals has not been established

□ rate of short term clinical progression expected to be low

■ rate of short term clinical progression expected to be high

Summary of Plasma – P-Tau

- Plasma P-tau increases ahead of clinical symptoms and is associated with tau pathology as measured by PET or neuropathology
- Assays for different proteoforms are correlated and have similar performance that is dependent on assay design
- Plasma P-tau levels are associated with tau haplotype in symptomatic AD
- Plasma P-tau levels are associated with co-morbidities that need to be considered as the biomarker moves into clinical use and community-based cohorts

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- Lilly P-tau217 development team

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Collaborators

Mayo Clinic	Lund University	UCSF	Columbia	Banner Sun Health Research Institute
Michelle Mielke	Oskar Hansson	Adam Boxer	Richard Mayeux	Eric Reiman
Ryan Frank	Shorena Janelidze	Elisabeth Thijssen	Adam Brickman	Tom Beach
	Sebastian Palmqvist			
	Niklas Mattsson-Carlgren			

ADRC Biomarker Cores Discussion

1. Depositing Blood-Based Biomarker Data at NACC
2. Sample collection and biomarker assay standardization or harmonization
   - NCRAD - Alzheimer Disease Center Fluid Biomarker Initiative (ADCFB)
   - Priority of assays harmonization and potential for standardization (P-tau, Abeta, NfL)
3. Harmonization of cut points and for which contexts of use
   - Diagnosis, Pathology, Prognosis, Other?