



DR. LORENZO GAETANI | MD, PhD

Neurologist and Assistant Professor, University of Perugia, Italy
GM-54569. March 2025.

CSF BIOMARKERS IN ALZHEIMER'S DISEASE: FROM CURRENT CLINICAL APPLICATIONS TO FUTURE PERSPECTIVES



CSF=Cerebrospinal Fluid.

Lilly

Personal Disclosures



Dr. Gaetani served at scientific advisory boards and/or as a consultant for Almirall, Biogen, Fujirebio, Eli Lilly, Novartis.

Dr. Gaetani gave lectures in symposia sponsored by Eli Lilly, Fujirebio and Siemens Healthineers.

Dr. Gaetani received funding for traveling from Almirall, Biogen, Fujirebio, Eli Lilly, Merck, Mylan, Novartis, Roche, Sanofi, Siemens Healthineers and Teva.



Beach TG, et al.

**Accuracy of the
clinical diagnosis of
Alzheimer disease at
National Institute on
Aging Alzheimer
Disease Centers,
2005–2010**

Journal of
Neuropathology &
Experimental
Neurology.
2012¹

919 subjects

Clinical diagnoses
probable or possible AD

**Neuropathological
diagnoses**
4 levels of confidence




Sensitivity

70.9% to 87.3%



Specificity

44.3% to 70.8%



Neurologists of the NIA-ADCs had higher predictive accuracy when they diagnosed AD in subjects with dementia than when they diagnosed dementing diseases other than AD.

AD=Alzheimer's Disease; NIA-ADC=National Institute on Aging Alzheimer's Disease Center.

1. Beach TG, et al. Accuracy of the Clinical Diagnosis of Alzheimer Disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. *J Neuropathol Exp Neurol*. 2012;71(4):266-273.

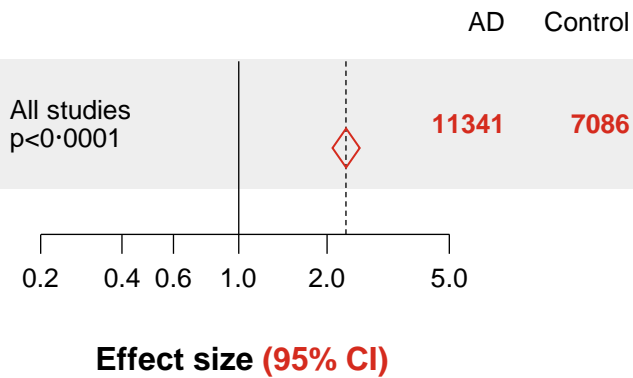
Lilly

CSF AD Core Biomarkers

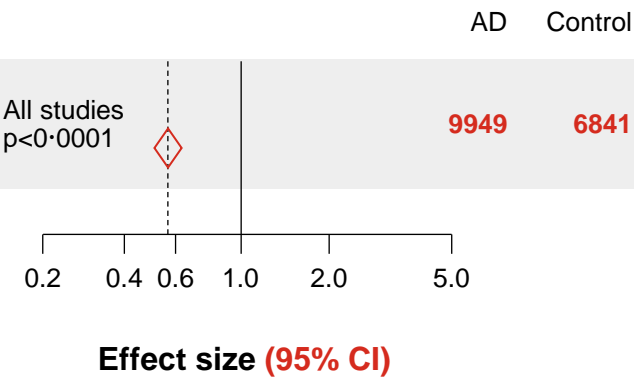


Olsson B, et al.
CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis
Lancet Neurology. **2016**¹

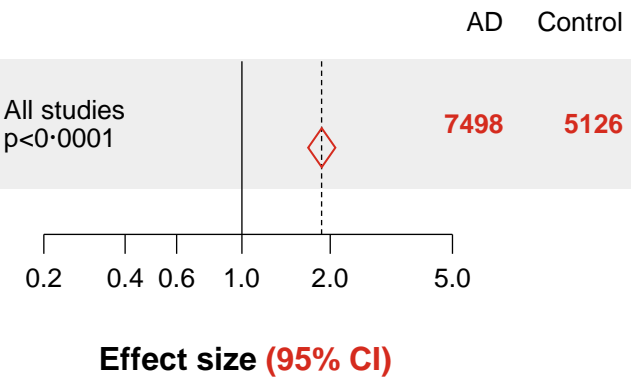
AD to control ratio for CSF T-tau*



AD to control ratio for CSF Aβ₄₂*



AD to control ratio for CSF P-tau*

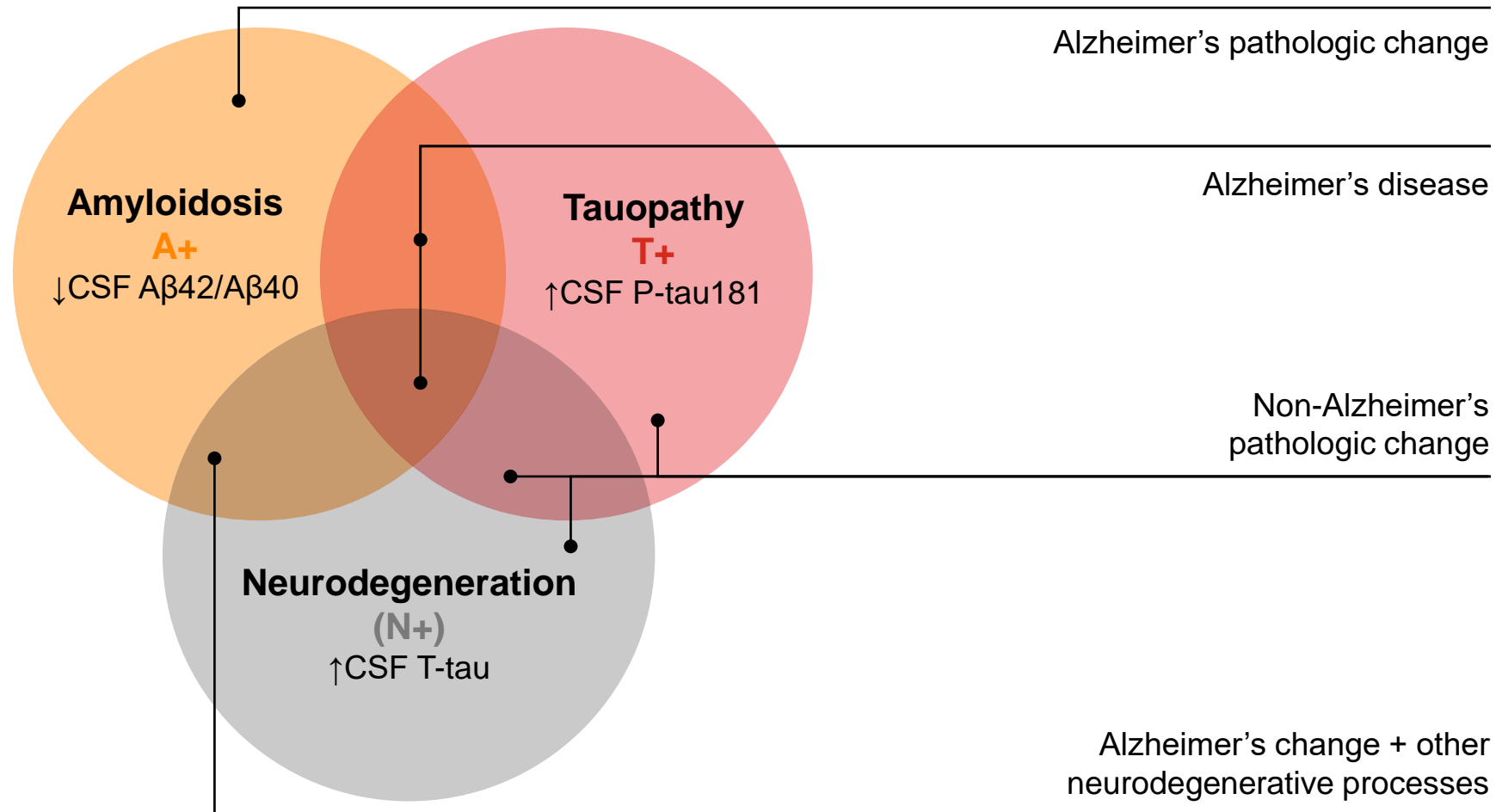


Modified from: Olsson B, et al. *Lancet Neurol.* 2016¹

*The solid line indicates a ratio of one and the dotted line indicates the average ratio.
Aβ=Amyloid-Beta; AD=Alzheimer's Disease; CI=Confidence Interval; CSF=Cerebrospinal Fluid; P-tau=Phosphorylated tau; T-tau=Total tau.
1. Olsson B, et al. CSF and Blood Biomarkers for the Diagnosis of Alzheimer's Disease: A Systematic Review and Meta-Analysis. *Lancet Neurol.* 2016;15(7):673-684.



CSF Biomarkers of AD in the 2018 NIA-AA Research Framework¹



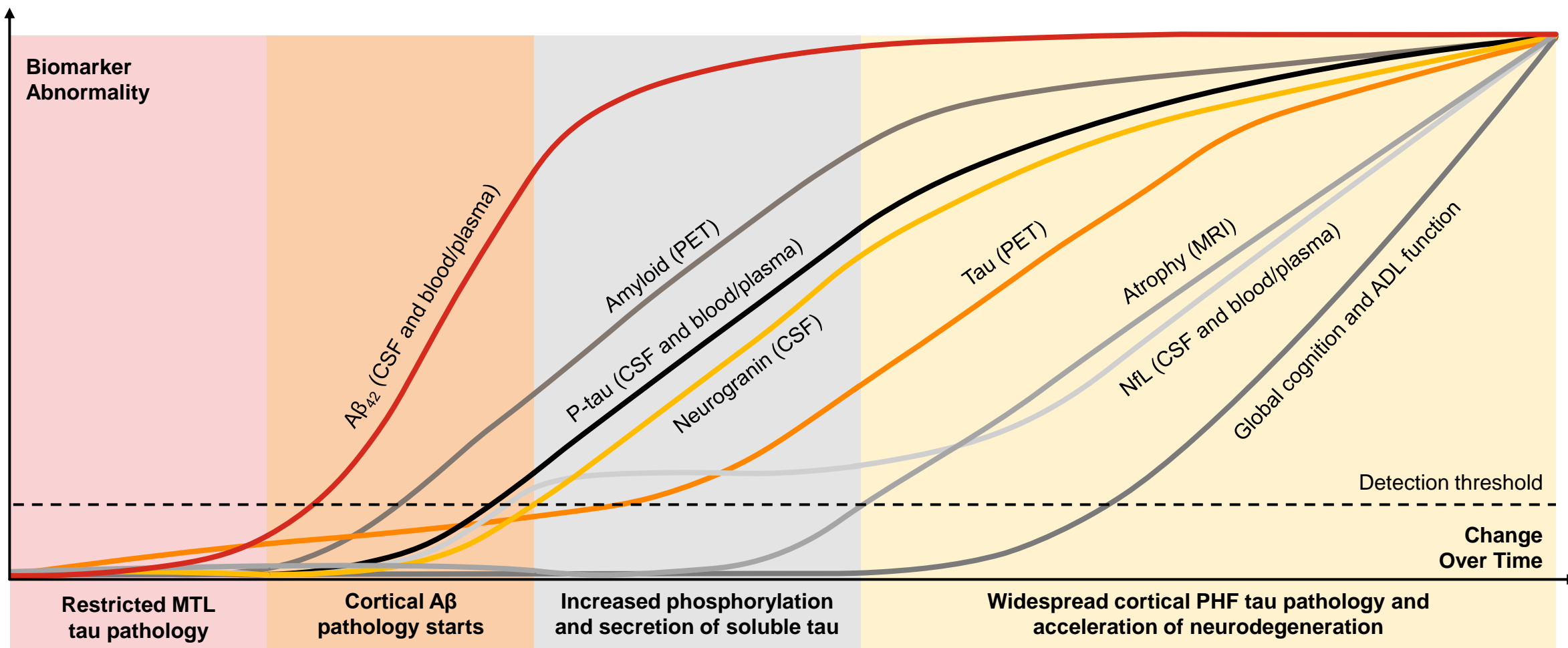
Modified from: Gaetani L, et al. *Expert Rev Mol Diagn.* 2023¹

A+=Amyloid-Beta Deposition Positive; Aβ=Amyloid-Beta; AD=Alzheimer's Disease; CSF=Cerebrospinal Fluid; N+=Neurodegeneration Positive; NIA-AA=National Institute on Aging-Alzheimer's Association; P-tau=Phosphorylated tau; T+=Pathologic Tau Positive; T-tau=Total tau.

1. Gaetani L, et al. Required Improvements for Cerebrospinal Fluid-Based Biomarker Tests of Alzheimer's Disease. *Expert Rev Mol Diagn.* 2023;23(12):1195-1207.



AD Pathological Cascade: Biomarker Trajectories¹



Modified from: Hansson O. *Nat Med.* 2021¹

A β =Amyloid-Beta; AD=Alzheimer's Disease; ADL=Activities of Daily Living; CSF=Cerebrospinal Fluid; MRI=Magnetic Resonance Imaging; MTL=Medial Temporal Lobe; NfL=Neurofilament Light Chain; P-tau=Phosphorylated tau; PET=Positron Emission Tomography; PHF=Paired Helical Filament.

1. Hansson O. Biomarkers for Neurodegenerative Diseases. *Nat Med.* 2021;27(6):954-963.



Evolution of AD Biomarkers Before Diagnosis

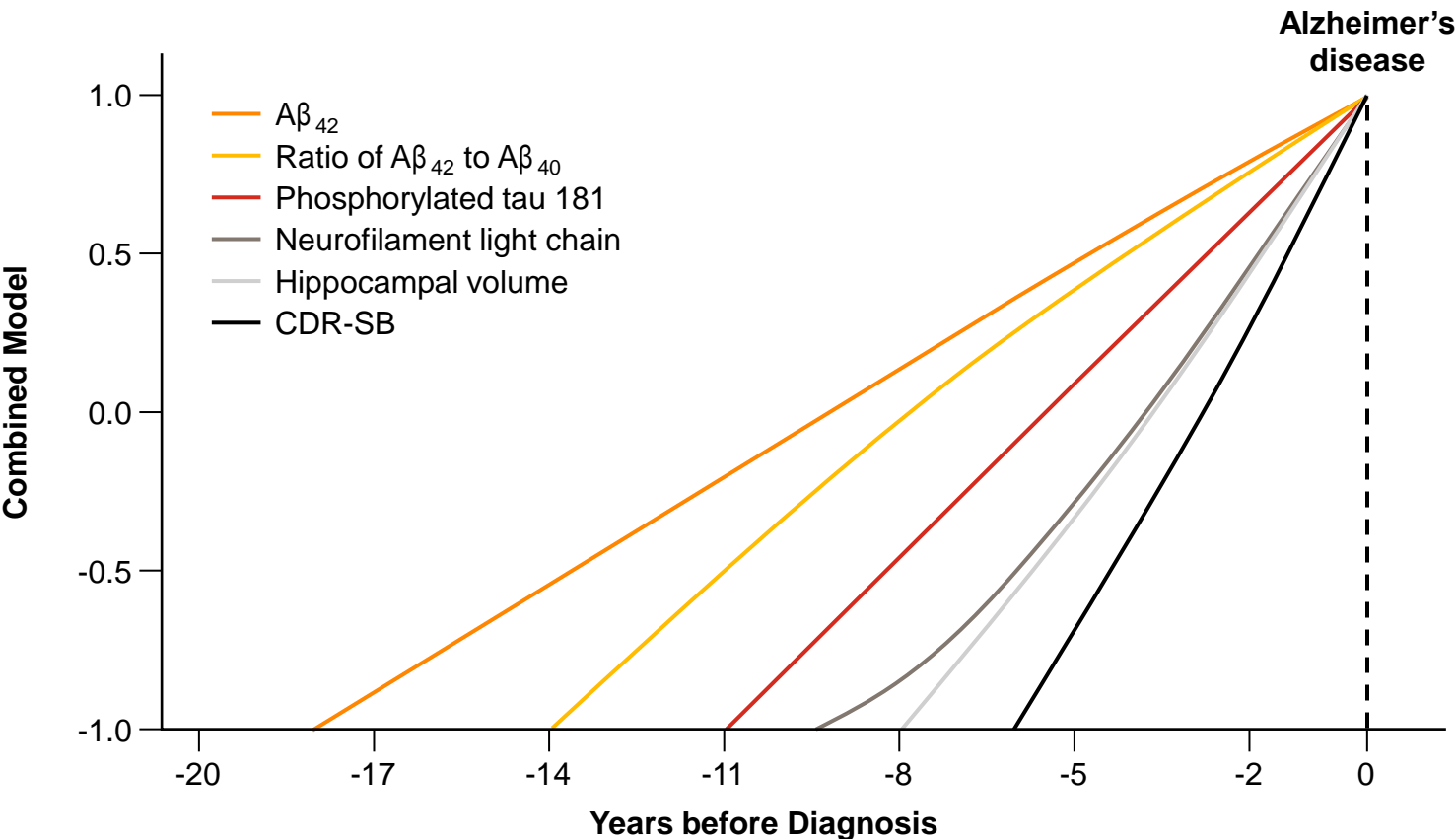


Combined model of individual biomarker trajectories before diagnosis¹

648 subjects

**CSF, cognitive assessments,
and structural imaging:**
2-year to 3-year intervals

Median follow-up:
19.9 years



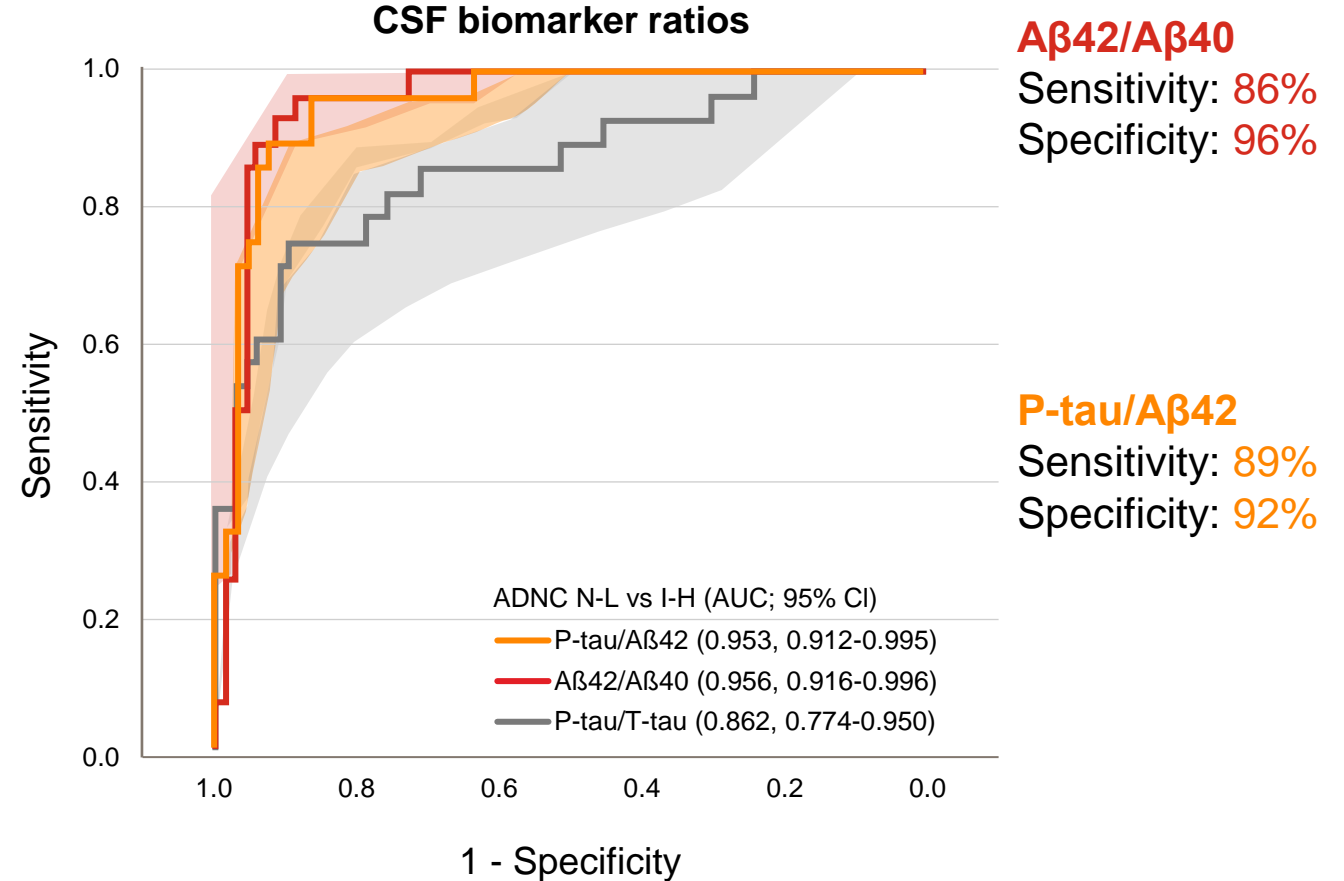
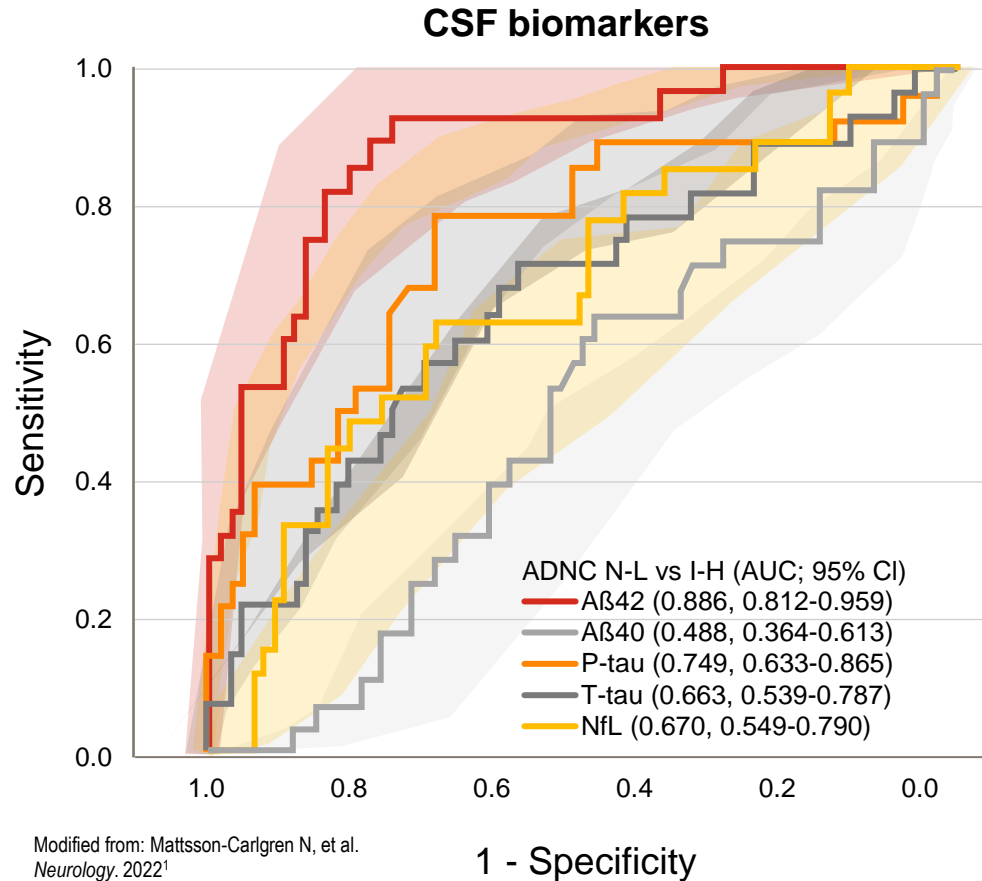
Modified from Jia J, et al. *N Engl J Med*. 2024¹

Aβ=Amyloid-Beta; AD=Alzheimer's Disease; CDR-SB=Clinical Dementia Rating - Sum of Boxes; CSF=Cerebrospinal Fluid.
1. Jia J, et al. Biomarker Changes During 20 Years Preceding Alzheimer's Disease. *N Engl J Med*. 2024;390(8):712-722.



Accuracy of CSF Biomarkers: Concordance With Neuropathology

CSF Biomarkers for ADNC Classification¹



Modified from: Mattsson-Carlgren N, et al.
Neurology. 2022¹

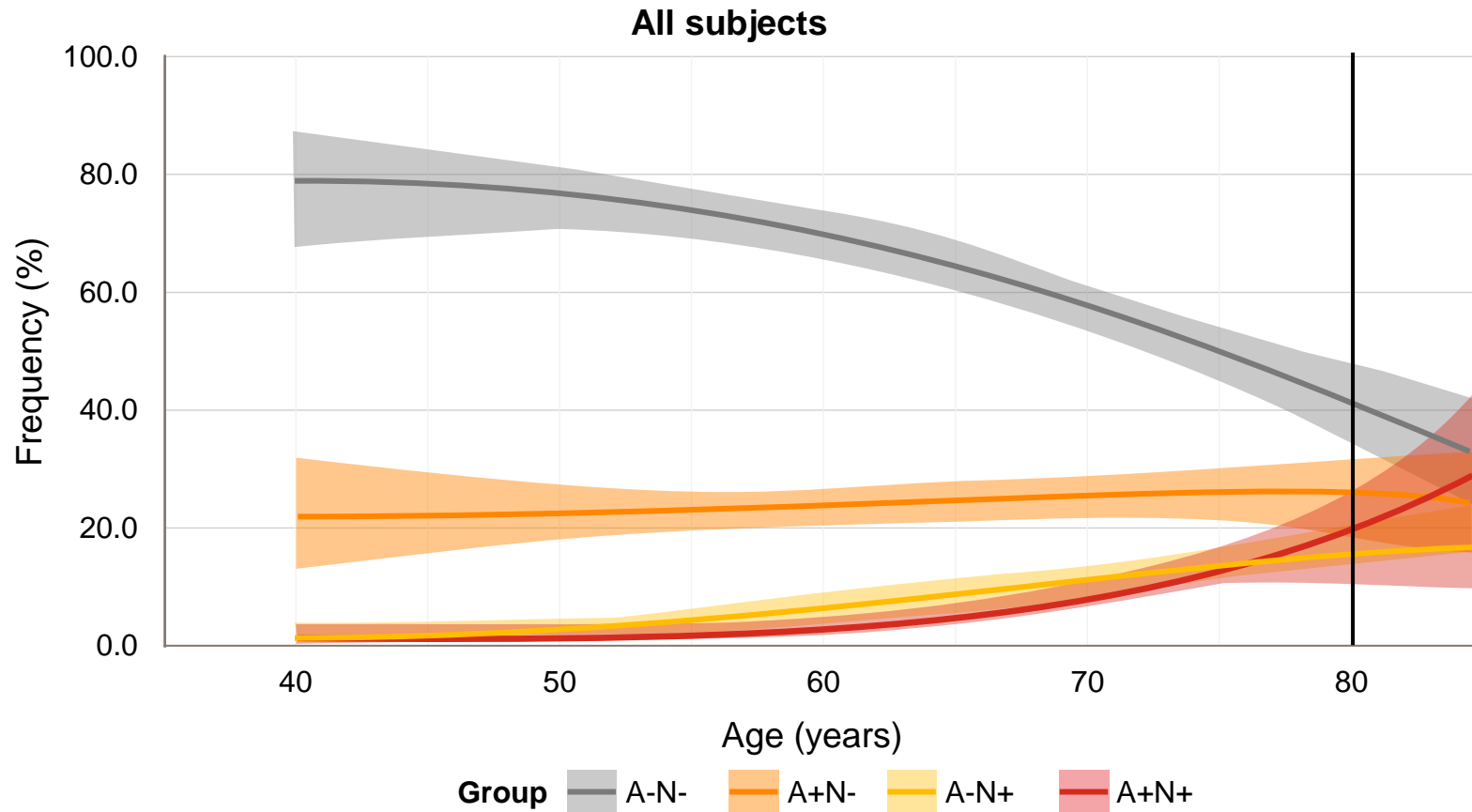
Aβ=Amyloid-Beta; ADNC=Alzheimer's Disease Neuropathological Change; AUC=Area Under the Curve; CI=Confidence Interval; CSF=Cerebrospinal Fluid; I-H=Intermediate-High; N-L=None-Low; NfL=Neurofilament Light Chain; P-tau=Phosphorylated tau; T-Tau=Total tau.

1. Mattsson-Carlgren N, et al. Cerebrospinal Fluid Biomarkers in Autopsy-Confirmed Alzheimer Disease and Frontotemporal Lobar Degeneration. *Neurology*. 2022;98(11):e1137-e1150.



AD CSF Biomarkers in Cognitively Normal Individuals

Estimated frequency of A β (A) and neurodegeneration (N) categories according to the age of individuals¹



Modified from: Toledo JB, et al. *Brain*. 2015¹

A=Amyloid; A β =Amyloid-Beta; AD=Alzheimer's Disease; CSF=Cerebrospinal Fluid; N=Neurodegeneration.
1. Toledo JB, et al. Alzheimer's Disease Cerebrospinal Fluid Biomarker in Cognitively Normal Subjects. *Brain*. 2015;138:2701-2715.



AT(N) in the Clinical Context

F – 71 years old

History of major depression

Memory disturbances over the last year

Brain MRI: normal

CSF analysis

Measured
value*

Cutoff
value**

A β 42/40

0.115

>0.072

P-tau181

33 pg/mL

<50

T-tau

229 pg/mL

<392

A-/T-/ N-

F – 70 years old

History of diabetes and hypertension

Memory disturbances over the last 2 years

Brain MRI: vascular white matter changes

CSF analysis

Measured
value*

Cutoff
value**

A β 42/40

0.040

>0.072

P-tau181

61.9 pg/mL

<50

T-tau

430 pg/mL

<392

A+/T+/N+

*CLEIA analysis on Lumipulse® G1200. **Cutoff values from Bellomo G, et al. *Front Neurosci.* 2021. Cutoff values for the three core AD biomarkers with their 95% CI were calculated by maximizing the Youden's index between samples belonging to the AD clusters and control clusters in this study.¹

A β =Amyloid-Beta; AT(N)=Amyloid-Beta Deposition, Pathologic Tau, Neurodegeneration; CI=Confidence Interval; CLEIA=Chemiluminescent Enzyme Immunoassay; CSF=Cerebrospinal Fluid; MRI=Magnetic Resonance Imaging; P-tau=Phosphorylated tau; T-tau=Total tau.

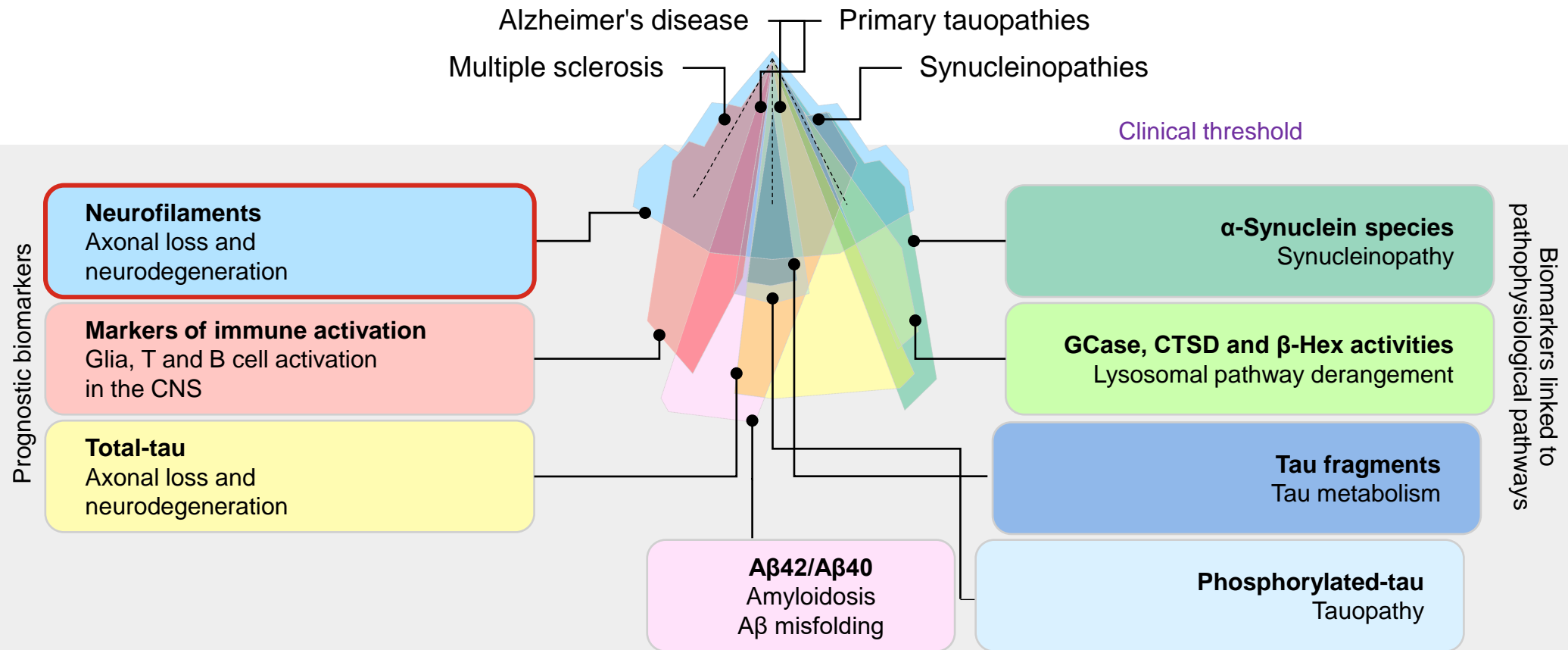
1. Bellomo G, et al. Machine Learning Driven Profiling of Cerebrospinal Fluid Core Biomarkers in Alzheimer's Disease and Other Neurological Disorders. *Front Neurosci.* 2021;15:647783.

This case presentation discusses Dr. Lorenzo Gaetani's professional experience. Individual results might vary, and the experience discussed may not reflect the results seen in all patients.

Lilly

Toward a Biological Definition of Neurological Diseases

Biomarkers and their roles in central nervous system neuroinflammatory and neurodegenerative diseases¹



β-Hex=β-Hexosaminidase; Aβ=Amyloid-Beta; CNS=Central Nervous System; CTSD=Cathepsin D; GCase=Glucocerebrosidase.

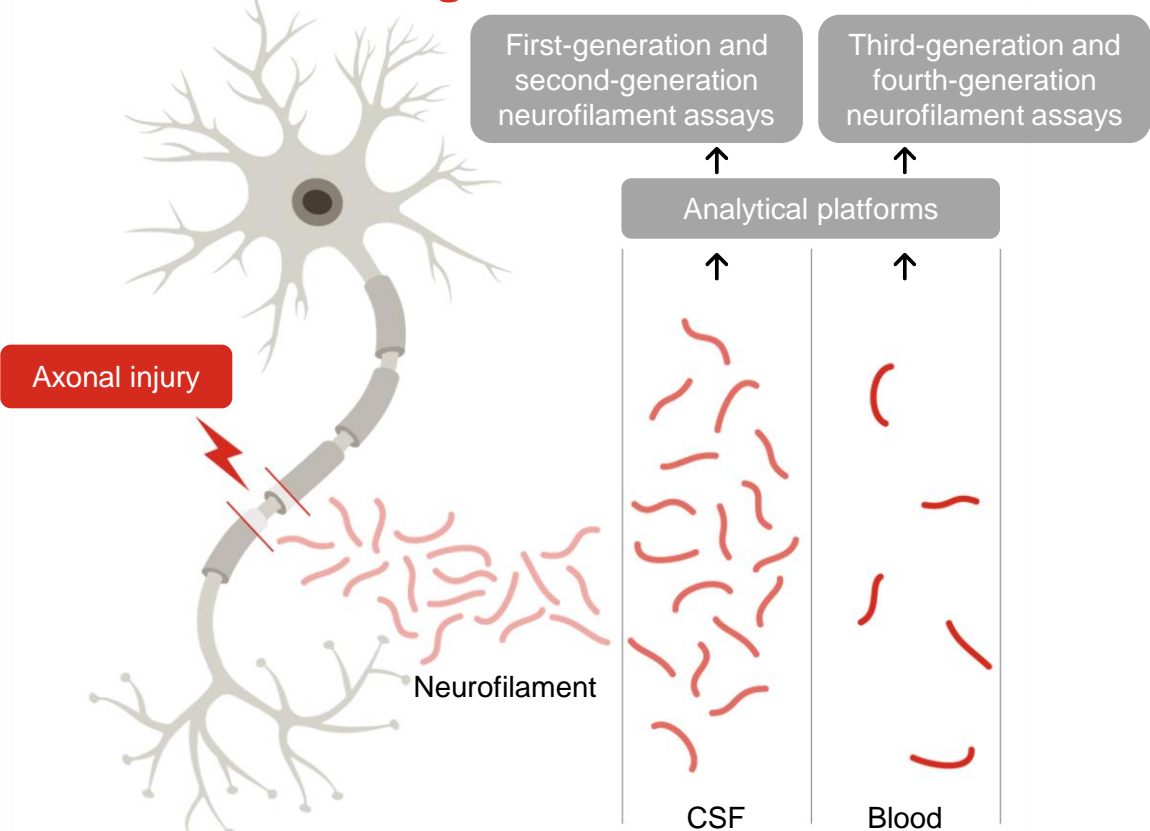
1. Gaetani L, et al. CSF and Blood Biomarkers in Neuroinflammatory and Neurodegenerative Diseases: Implications for Treatment. *Trends Pharmacol Sci.* 2020;41(12):1023-1037.

Modified from: Gaetani L, et al. *Trends Pharmacol Sci.* 2020¹



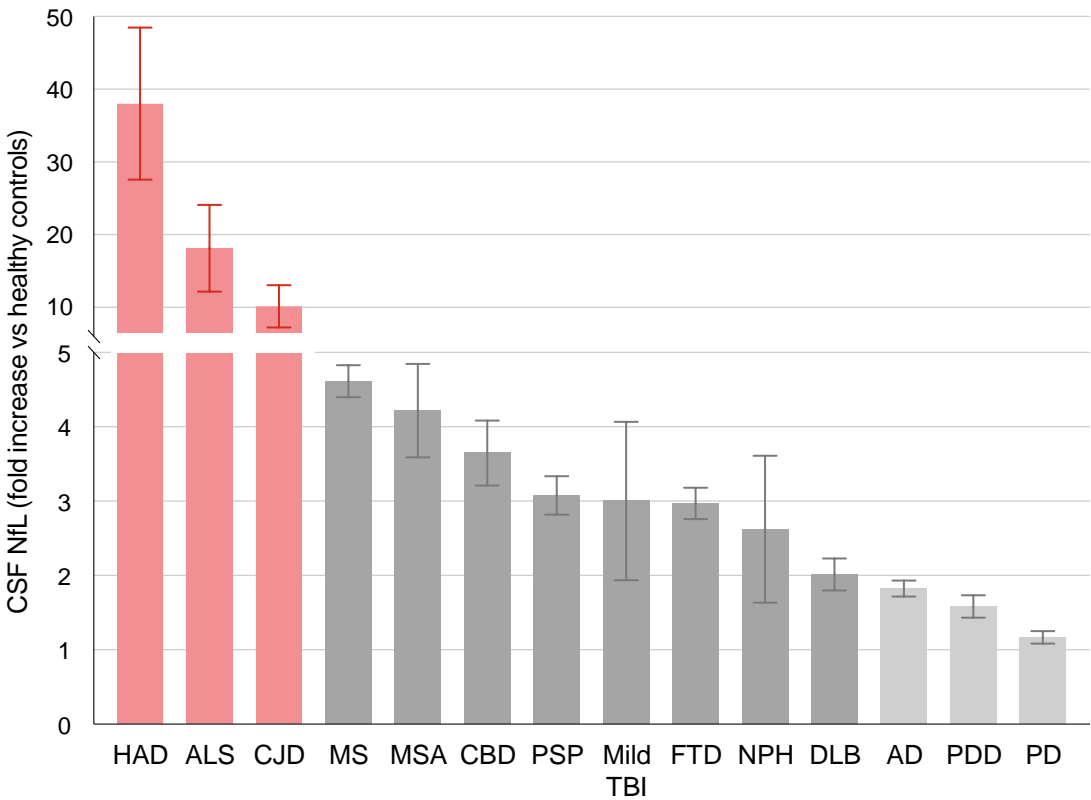
Neurofilaments as Biomarkers in Neurological Disorders

Neurofilament release after axonal damage¹



Modified from: Khalil M, et al. *Nat Rev Neurol*. 2018¹

Increase of CSF NfL in a variety of neurological diseases associated with axonal damage²



Modified from: Gaetani L, et al. *J Neurol Neurosurg Psychiatry*. 2019²

ALS=Amyotrophic Lateral Sclerosis; AD=Alzheimer's Disease; CBD=Corticobasal Degeneration; CJD=Creutzfeldt-Jakob Disease; CSF=Cerebrospinal Fluid; DLB= Dementia with Lewy Bodies; FTD=Frontotemporal Dementia; HAD=HIV (Human immunodeficiency virus)-Associated Dementia; MS=Multiple Sclerosis; NfL=Neurofilament Light Chain; NPH=Normal Pressure Hydrocephalus; PD=Parkinson's Disease; PDD=Parkinson's Disease Dementia; PSP=Progressive Supranuclear Palsy; TBI=Traumatic Brain Injury.
 1. Khalil M, et al. Neurofilaments as Biomarkers in Neurological Disorders. *Nat Rev Neurol*. 2018;14(10):577-589. 2. Gaetani L, et al. Neurofilament Light Chain as a Biomarker in Neurological Disorders. *J Neurol Neurosurg Psychiatry*. 2019;90(8):870-881.

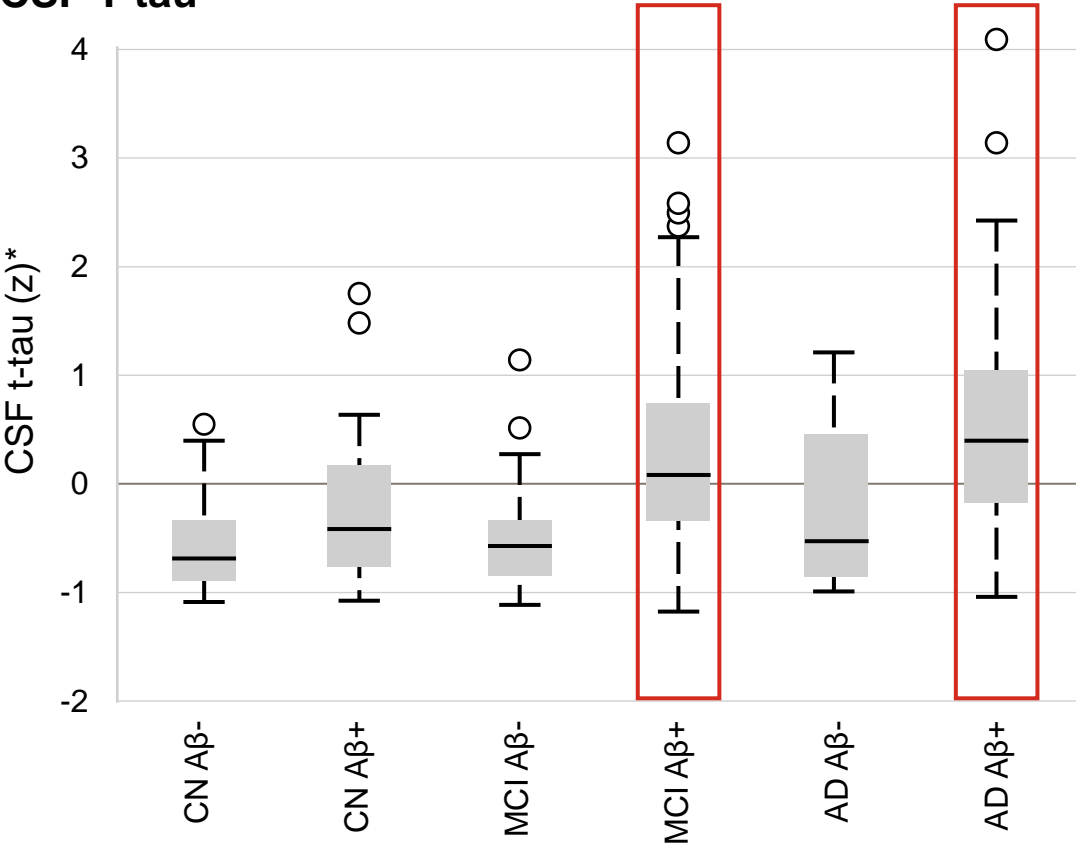


CSF T-tau and NfL in Alzheimer's Disease

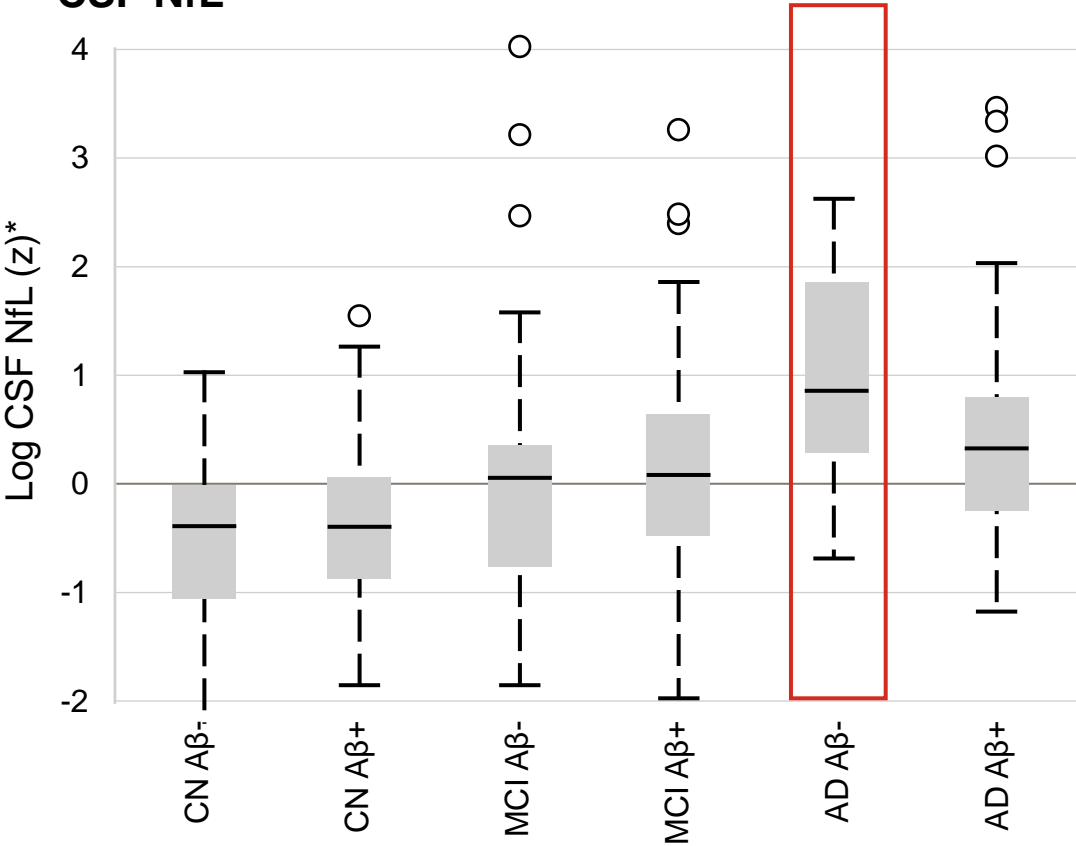


Biomarkers by diagnosis and amyloid pathology¹

CSF T-tau¹



CSF NfL¹



Modified from: Mattsson N, et al. *EMBO Mol Med.* 2016¹

*Biomarker levels are standardized to z-scores and shown in box plots (indicating median and interquartile ranges; whiskers are defined as quartiles 1 and 3 \pm 1.5 \times interquartile range, respectively).
Aβ=Amyloid-Beta; AD=Alzheimer's Disease; CN=Cognitively Normal; CSF=Cerebrospinal Fluid; MCI=Mild Cognitive Impairment; NfL=Neurofilament Light Chain; T-tau=Total tau.
1. Mattsson N, et al. Cerebrospinal Fluid Tau, Neurogranin, and Neurofilament Light in Alzheimer's Disease. *EMBO Mol Med.* 2016;8(10):1184-1196.



AT(N) in the Clinical Context

F – 71 years old

History of major depression

Memory disturbances over the last year

Brain MRI: normal

CSF analysis	Measured value*	Cutoff value**
A β 42/40	0.115	>0.072
P-tau181	33 pg/mL	<50
T-tau	229 pg/mL	<392
NfL	430 pg/mL	<5 th percentile for controls***

A-/T-/N-/low NfL

F – 70 years old

History of diabetes and hypertension

Memory disturbances over the last 2 years

Brain MRI: vascular white matter changes

CSF analysis	Measured value*	Cutoff value**
A β 42/40	0.040	>0.072
P-tau181	61.9 pg/mL	<50
T-tau	430 pg/mL	<392
NfL	1664 pg/mL	>95 th percentile for controls, 50–75 th percentile for AD***

A+/T+/N+/high NfL

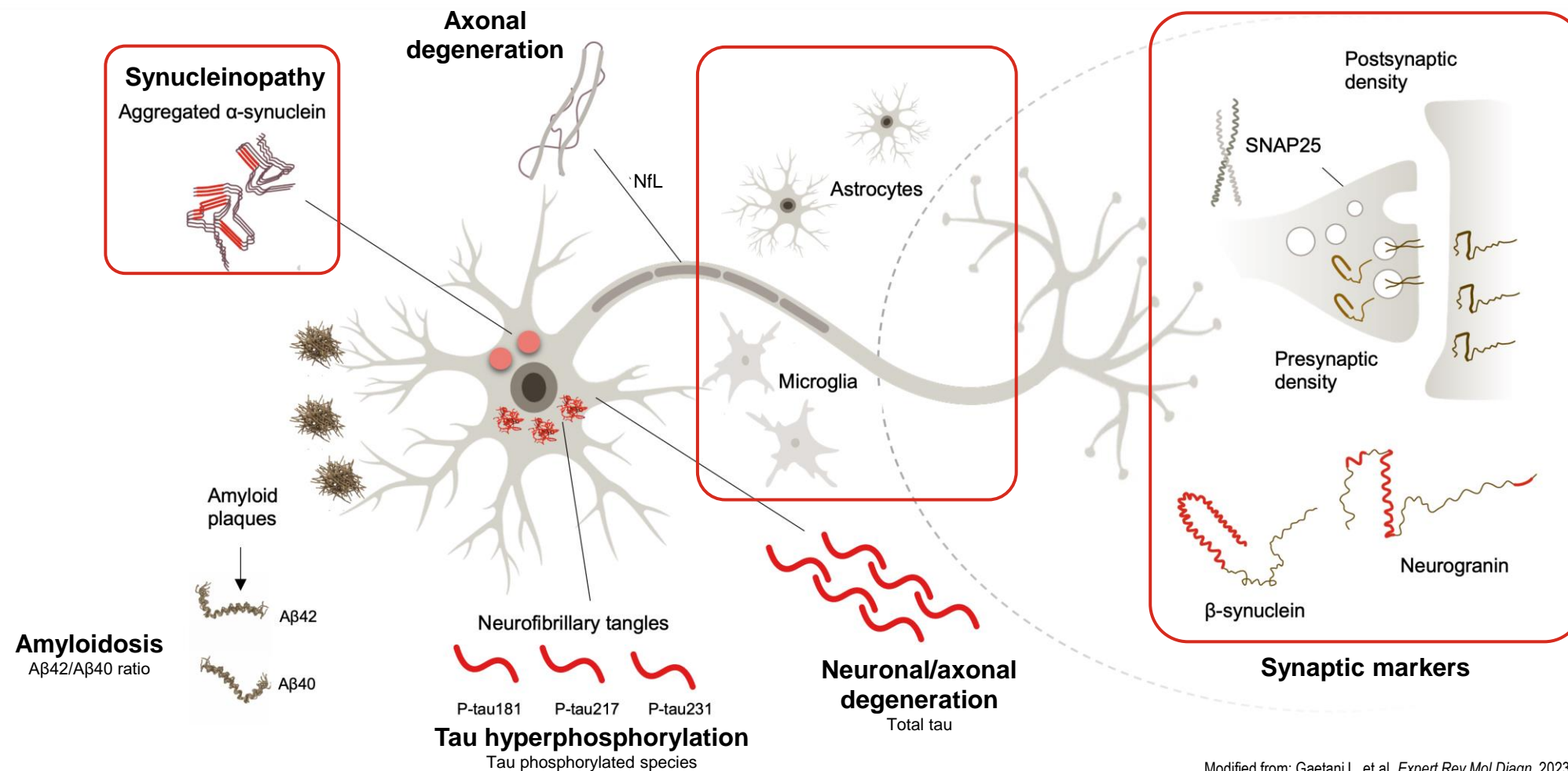
*CLEIA analysis on Lumipulse® G1200. **Cutoff values from Bellomo G, et al. *Front Neurosci.* 2021. Cutoff values for the three core AD biomarkers with their 95% CI were calculated by maximizing the Youden's index between samples belonging to the AD clusters and control clusters in this study.¹ ***Percentiles of reference values based on a study by Vermunt L, et al. *Ann Clin Transl Neurol.* 2022.² Value assessments from this study for percentiles according to the age of the patients are available through *NfL interface for physicians.*³ A β =Amyloid-Beta; AD=Alzheimer's Disease; AT(N)=Amyloid-Beta Deposition, Pathologic Tau, Neurodegeneration; CI=Confidence Interval; CLEIA=Chemiluminescent Enzyme Immunoassay; CSF=Cerebrospinal Fluid; MRI=Magnetic Resonance Imaging; NfL=Neurofilament Light Chain; P-tau=Phosphorylated tau; T-tau=Total tau.

1. Bellomo G, et al. Machine Learning Driven Profiling of Cerebrospinal Fluid Core Biomarkers in Alzheimer's Disease and Other Neurological Disorders. *Front Neurosci.* 2021;15:647783. 2. Vermunt L, et al. Age- and Disease-Specific Reference Values for Neurofilament Light Presented in an Online Interactive Support Interface. *Ann Clin Transl Neurol.* 2022;9(11):1832-1837. 3. NfL Interface for Physicians (Version 2.5.3). 2023. Available from: <https://mybiomarkers.shinyapps.io/Neurofilament/>. Accessed February 2025.

This case presentation discusses Dr. Lorenzo Gaetani's professional experience. Individual results might vary, and the experience discussed may not reflect the results seen in all patients.

Lilly

Alzheimer's Disease Pathophysiology and Related Biomarkers¹



Modified from: Gaetani L, et al. *Expert Rev Mol Diagn.* 2023¹

$A\beta$ =Amyloid-Beta; NfL=Neurofilament Light Chain; P-tau=Phosphorylated tau; SNAP25=Synaptosomal-Associated Protein 25.

1. Gaetani L, et al. Required Improvements for Cerebrospinal Fluid-Based Biomarker Tests of Alzheimer's Disease. *Expert Rev Mol Diagn.* 2023;23(12):1195-1207.

Lilly

Alzheimer’s Association Revised Criteria for Diagnosis and Staging of Alzheimer’s Disease¹

Categorization of fluid analytes and imaging biomarkers

Biomarker category	CSF or plasma	Imaging
Core biomarkers		
Core 1		
A (Aβ proteinopathy)	Aβ42	Amyloid PET
T1 (phosphorylated and soluble AD tau)	P-tau217, P-tau181, P-tau231	
Core 2		
T2 (AD tau proteinopathy)	MTBR-tau243, other phosphorylated tau forms (e.g., p-tau205), non-phosphorylated mid-region tau fragments*	Tau PET
Biomarkers of non-specific processes involved in AD pathophysiology		
N (injury, dysfunction, or degeneration of neuropil)	NfL	Anatomic MRI, FDG PET
I (Inflammation - astrocytic activation)	GFAP	
Biomarkers of non-AD co-pathology		
V (Vascular brain injury)		Infarction on MRI or CT, WMH
S (α-synuclein)	α-synuclein seed amplification assays	



Potential intended uses ^{1,*}
Amyloid PET: diagnosis – staging – prognosis
Plasma P-tau217: diagnosis – staging – prognosis – indicator of biological treatment effect
Staging – prognosis – indicator of biological treatment effect
Anatomic MR and FDG PET: Staging – prognosis – indicator of biological treatment effect
Staging – prognosis – indicator of biological treatment effect
Identification of co-pathology
Identification of co-pathology

*P-tau231, P-tau205, MTBR-tau243, and non-phosphorylated tau fragments have not undergone the same level of validation testing as other Core biomarkers.
Aβ= Amyloid-Beta; AD=Alzheimer’s Disease; CSF=Cerebrospinal Fluid; CT=Computed Tomography; FDG=Fluorodeoxyglucose; GFAP=Glial Fibrillary Acidic Protein; MRI=Magnetic Resonance Imaging; MTBR-tau243=Microtubule-Binding Region-243; NfL=Neurofilament Light Chain; P-tau=Phosphorylated tau; PET=Positron Emission Tomography; WMH=White Matter Hyperintensities.
1. Jack CR Jr, et al. Revised Criteria for Diagnosis and Staging of Alzheimer’s Disease: Alzheimer’s Association Workgroup. *Alzheimer’s Dement.* 2024;20(8):5143-5169.

Modified from: Jack CR Jr, et al. *Alzheimer’s Dementia.* 2024¹



Automated Platforms for Biomarker Measurement

Overview of CSF AD Biomarkers¹

			Automated platforms available		Possibility to measure in blood
				Label	
	Pathophysiological mechanisms	Biomarker change	Biomarker name	EU	
Established biomarkers	Amyloidosis	↓ Aβ42	Lumipulse	CE marked	Yes*
			Elecsys	IVD	
	Tauopathy	↓ Aβ42/Aβ40	Lumipulse	IVD	Yes*
			Lumipulse	CE marked	Yes*
	Neurodegeneration	↑ P-tau181	Elecsys	IVD	
			Lumipulse	CE marked	No**
		↑ T-tau	Elecsys	IVD	
Novel biomarkers	Tauopathy	↑ P-tau217	N/A	-	Yes***
		↑ P-tau231	N/A	-	Yes***
	Neurodegeneration and axonal loss	↑ NfL	Lumipulse	RUO	Yes*
	Synaptic damage	↑ Neurogranin	N/A	-	No
		↑ SNAP25	N/A	-	No
		↑ β-synuclein	N/A	-	Yes***

Modified from: Gaetani L, et al. *Expert Rev Mol Diagn.* 2023¹

*Automated Lumipulse assays available under the RUO label in EU and US for blood measurement. **Measurement through ultrasensitive assays possible, but no difference between AD and controls has been documented in blood due to extra-CNS source of t-tau. ***Measurement through ultrasensitive, non-fully automated, platforms.
Aβ=Amyloid-Beta; AD=Alzheimer’s Disease; CE=European Conformity; CNS=Central Nervous System; EU=European Union; IVD=In Vitro Diagnostic Device; N/A=Not Applicable; NfL=Neurofilament Light Chain; P-tau=Phosphorylated tau; RUO=Research Use Only; SNAP25=Synaptosomal-Associated Protein 25; T-tau=Total tau; US=United States.

1. Gaetani L, et al. Required Improvements for Cerebrospinal Fluid-Based Biomarker Tests of Alzheimer’s Disease. *Expert Rev Mol Diagn.* 2023;23(12):1195-1207.



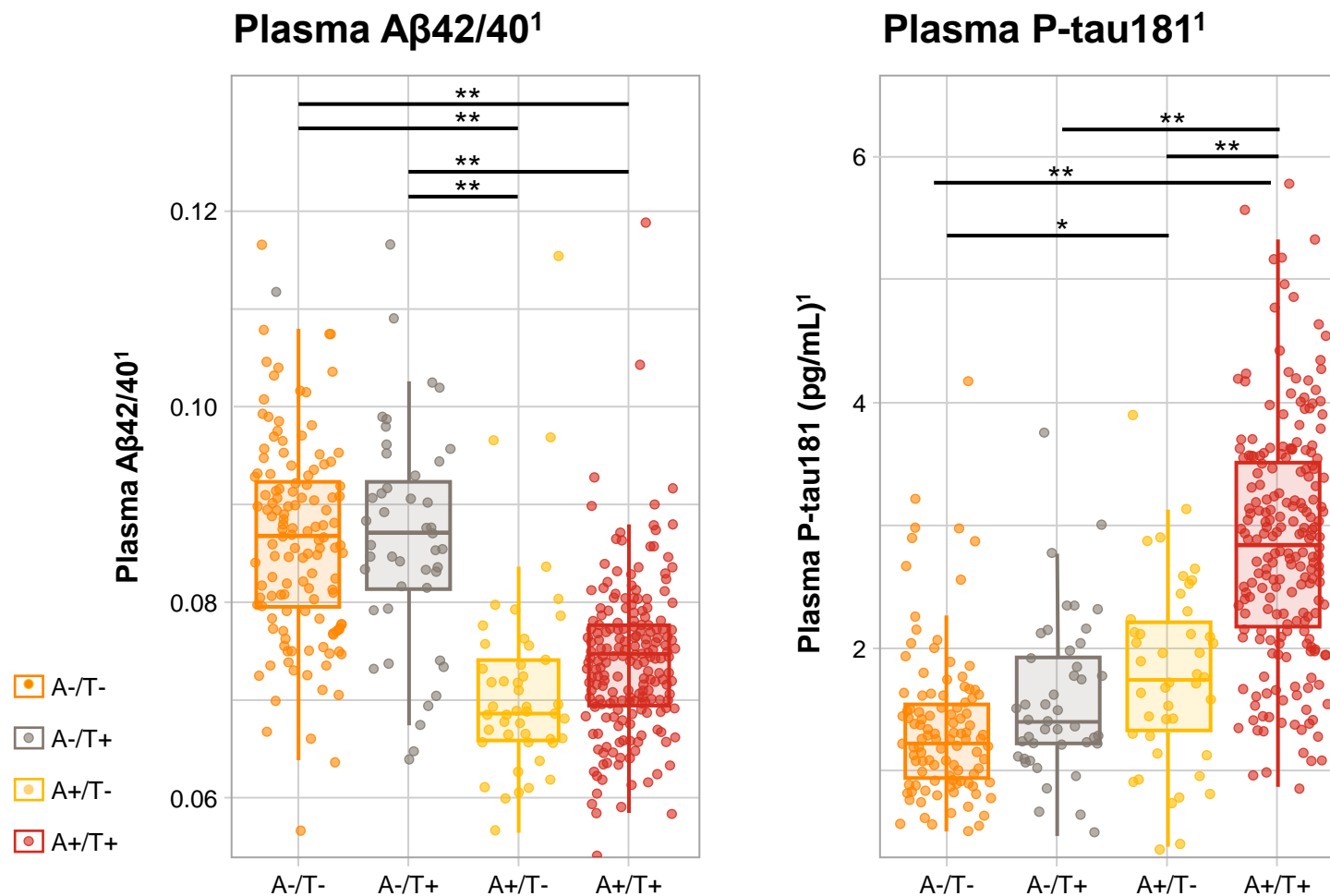
Plasma A β 42/40 and P-tau181 Across CSF A/T Categories

Fully automated measurement of plasma A β 42/40 and P-tau181¹

Two cohorts

Perugia and Amsterdam: total 450 patients

Plasma A β 42, A β 40, and P-tau181 were measured with a fully automated CLEIA



*0.001 \leq p adj. <0.01; **p adj. \leq 0.0001

A/T=Amyloid-Beta Deposition, Pathologic Tau; A β =Amyloid-Beta; CLEIA=Chemiluminescent Enzyme Immunoassay; CSF=Cerebrospinal Fluid; P-tau=Phosphorylated tau.

1. Bellomo G, et al. Fully Automated Measurement of Plasma A β 42/40 and P-tau181: Analytical Robustness and Concordance with Cerebrospinal Fluid Profile Along the Alzheimer's Disease Continuum in Two Independent Cohorts. *Alzheimers Dement.* 2024;20(4):2453-2468.

Lilly

Takeaways



Diagnosis of Alzheimer's disease is expanding to include a clinical-biological assessment.¹ CSF analysis with validated cutoffs is key in memory unit evaluations to confirm the presence of AD pathology.²



CSF diagnostic accuracy improves with specific biomarker ratios (e.g., $A\beta_{42}/A\beta_{40}$) or combinations (e.g., P-tau or T-tau and $A\beta_{42}$).²



CSF NfL is increased in many neurodegenerative diseases and can potentially be combined with core AD biomarkers to improve the prognostic evaluation.²⁻⁴



Blood-based biomarkers for AD are rapidly developing and are part of the ongoing Alzheimer's Association revised criteria for AD diagnosis.^{5,6}

$A\beta$ =Amyloid-Beta; AD=Alzheimer's Disease; CSF=Cerebrospinal Fluid; e.g.=For Example; NfL=Neurofilament Light Chain; P-tau=Phosphorylated tau; T-tau=Total tau.

1. Hampel H, et al. Designing the Next-Generation Clinical Care Pathway for Alzheimer's Disease. *Nat Aging*. 2022;2(8):692-703. 2. Gaetani L, et al. Required Improvements for Cerebrospinal Fluid-Based Biomarker Tests of Alzheimer's Disease. *Expert Rev Mol Diagn*. 2023;23(12):1195-1207. 3. Khalil M, et al. Neurofilaments as Biomarkers in Neurological Disorders. *Nat Rev Neurol*. 2018;14(10):577-589. 4. Gaetani L, et al. Neurofilament Light Chain as a Biomarker in Neurological Disorders. *J Neurol Neurosurg Psychiatry*. 2019;90(8):870-881. 5. Hansson O, et al. The Alzheimer's Association Appropriate Use Recommendations for Blood Biomarkers in Alzheimer's Disease. *Alzheimers Dement*. 2022;18(12):2669-2686. 6. Alzheimer's Association. October 2023. Revised Criteria for Diagnosis and Staging of Alzheimer's | AAIC. Available from: <https://aaic.alz.org/diagnostic-criteria.asp>. Accessed February 2025.





Section of Neurology

Clinical Neurochemistry Laboratory

Prof. Lucilla Parnetti
Dr. Giovanni Bellomo
Dr. Federico Paolini Paoletti
Dr. Giovanna Nardi
Dr. Alfredo Megaro
Marta Ferrettini

Memory Clinic

Prof. Lucilla Parnetti
Dr. Federico Paolini Paoletti
Dr. Chiara Montanucci
Dr. Nicola Salvadori
Dr. Andrea Toja
Dr. Elisa Siena
Dr. Claudio Verderosa
Dr. Edoardo Guido Torrigiani
Dr. Francesco Maria Paradiso
Dr. Davide Aprile

Section of Biochemistry

Prof. Davide Chiasserini

lorenzo.gaetani@unipg.it