

## Supplementary Online Content

Gonzalez MC, Ashton NJ, Gomes BF, et al; European–Dementia With Lewy Bodies (E-DLB) Consortium. Association of plasma p-tau181 and p-tau231 concentrations with cognitive decline in patients with probable dementia with Lewy bodies. *JAMA Neurol*. Published online November 22, 2021. doi:10.1001/jamaneurol.2021.4222

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This supplementary material has been provided by the authors to give readers additional information about their work.

## **eMethods.**

### **Diagnostic Examination program**

We diagnosed probable DLB using the International Consensus Criteria<sup>1</sup>. Based on the clinical examination, we evaluated the core diagnostic features (fluctuating cognition, REM sleep abnormalities, Parkinsonism, and recurrent visual hallucinations) as previously described<sup>2-5</sup>. An abnormal dopamine transporter SPECT in 88 patients supported the diagnosis. PD cases fulfilled UK Brain Bank criteria<sup>6</sup> and AD cases were defined according to the most recent international consensus criteria<sup>7</sup>. Local reference values for CSF A $\beta$ 42 concentrations are in [supplementary table 2](#). Healthy controls were subjects without any neurodegenerative disease. Participants with acute delirium, terminal illness, those recently diagnosed with a major somatic illness, and patients with other previous major psychiatric or neurological disorders were excluded from the study as well as patients with a clinical diagnosis of AD but with normal abeta42 levels on CSF. We show the final number of included patients at each center in the [e-supplementary table 1](#).

### **Phosphorylated tau blood measurements**

Plasma samples were collected in each center. Details of collection procedures in each center are available in [e-supplementary table 2](#). All plasma p-tau biomarkers were assessed using in-house Simoa methods on the HD-X instrument (Quanterix) at the Clinical Neurochemistry Laboratory Sahlgrenska University Hospital, Mölndal, Sweden as previously reported<sup>8,9</sup>. The analytical variation of the internal quality control (iQC) samples were <10% for p-tau181 and p-tau231 across the whole study ([e-supplementary table 3](#)). All measurements were performed using one batch of reagents and randomized samples with the analysts blinded to clinical data. We excluded plasma values under the lower limit of quantification (LLOQ).

### **Statistics**

All statistical analyses were performed using SPSS 23.0 (IBM Corp., Armonk, NY) and R (4.0.5) statistical software. We reported baseline characteristics of the cohorts using proportions for categorical variables, means and standard deviations for continuous variables, and normality distribution assumption was checked with the Shapiro-Wilk test. For sex groups, we used the Chi-squared test. We estimated the effect size of the differences with Cohen's d (d). We considered P-value statistically significant at 0.05.

**eTable 1.** Overview of Diagnostic Groups According to Centers

Center	DLB patients	AD patients	PD patients	HC
Neuróloga at Fundació ACE Institut Català de Neurociències Aplicades Spain	31	63	0	19
Alzheimer Centre, Dept Neuro, VU Med Centre, Amsterdam	40	40	0	40
Department of Neuroscience and Imaging and Aging Research Centre, G. d'Annunzio University, Chieti, Italy	22	6	0	0
Cog Center, University of Paris	33	4	0	0
University of Brescia, Italy	41	7	204	97
Dept of Neurology, University Medical Center- Ljubljana, Slovenia	17	0	0	0
Helse Stavanger - Stavanger universitetssjukehus	5	1	0	1
University Medical Center Göttingen-Paracelsus-Elena-Klinik	54	0	0	0
University Hospitals Leuven	20	10	0	18
Strasbourg Resource and Research Memory Centre	108	76	0	30
<b>Total</b>	<b>371</b>	<b>207</b>	<b>204</b>	<b>205</b>

Abbreviations: (DLB) Dementia with Lewy bodies, (AD) Alzheimer's disease, (PD) Parkinson's disease, (HC) Healthy control

**eTable 2.** Overview of Plasma Sample Collection, Handling, and Storage and CSF A $\beta$ 42 Cutoff Values

Center	Collection	Centrifuging	Storage	A $\beta$ 42 Analysis Essay	Cut off values [pg/mL]
Neuróloga at Fundació ACE Institut Català de Neurociències Aplicades Spain	EDTA-tubes	Centrifuged at 2000g for 10 minutes at 4°C	Stored in polypropylene tubes at -80°C	Lumipulse	A $\beta$ 42 < 796
Alzheimer Centre, Dept Neuro, VU Med Centre, Amsterdam.	EDTA-tubes	Centrifuged at 1800 x g for 10 minutes at room temperature	Stored in polypropylene tubes at -80°C	INNOTEST Double sandwich ELISAs Elecsys Roche Cobas	A $\beta$ 42 < 813 A $\beta$ 42 < 1092
Department of Neuroscience and Imaging and Aging Research Centre, G. d'Annunzio University, Chieti, Italy	EDTA-tubes	Centrifuged at 1600 rpm for 10 minutes at room temperature	Stored at -80°C within 30 minutes.	INNOTEST Double sandwich ELISAs	A $\beta$ 42: <800
Université de Paris APHP Laiboisière Cognitive Neurology Center	EDTA-tubes	Centrifuged at 2000g for 20 minutes at 4°C	Stored in polypropylene tubes at -80°C	INNOTEST Double sandwich ELISAs Roche Cobas	A $\beta$ 42 < 730 A $\beta$ 42 < 860
University of Brescia, Italy.	EDTA-tubes	Centrifuged at 1800g for 15 minutes at 4°C	Stored in polypropylene tubes at -80°C	INNOTEST Double sandwich ELISAs	A $\beta$ 42: <650
Dept of Neurology, University Medical Center Ljubljana	EDTA-tubes	Centrifuged at 1500g for 15 minutes	Stored in polypropylene tubes at -80°C	INNOTEST Double sandwich ELISAs	A $\beta$ 42: <550
Stavanger SUS	EDTA-tubes	Centrifuged at 2000g for 10 minutes at 4°C	Stored in polypropylene tubes at -80°C	Biosource Europe S.A.	A $\beta$ 42: <482
University Medical Center Göttingen-Paracelsus-Elena-Klinik	EDTA-tubes	Centrifuged at 2500g for for 10 min at 20°C	Stored in polypropylene tubes within 30 min at -80°C	INNOTEST Double sandwich ELISAs	A $\beta$ 42: <450
University Hospitals Leuven	EDTA-tubes	Centrifuged at 2880g for 10 minutes at 4°C	Stored in cryo tubes Nunc 1.8ml at -80°C	No A $\beta$ 42 confirmation	No A $\beta$ 42 confirmation
Strasbourg Resource and Research Memory Centre	EDTA-tubes	Centrifuged at 1700g for 15 minutes at 4°C	Stored in polypropylene tubes at -80°	INNOTEST Double sandwich ELISAs	A $\beta$ 42: <500

**Abbreviations: (DLB) dementia with Lewy bodies, (A $\beta$ 42) amyloid- $\beta$ 42, (EDTA) ethylenediamine tetraacetic acid, (RPM) revolutions per minute. Samples were shipped from each center to Sweden by a competent courier on sufficient dry ice**

**eTable 3.** Analytical Performance of the p-tau181 and p-tau231 Assays

	<b>iQC values</b>	<b>With-in run % CV</b>	<b>Between run % CV</b>
p-tau181	36.5 pg/mL	4.2%	4.7%
	20.9 pg/mL	5.2%	5.7%
	17.8 pg/mL	3.9%	6.4%
p-tau231	25.5 pg/mL	6.5%	6.5%
	16.2 pg/mL	6.5%	7.5%
	7.7 pg/mL	5.7%	8.8%

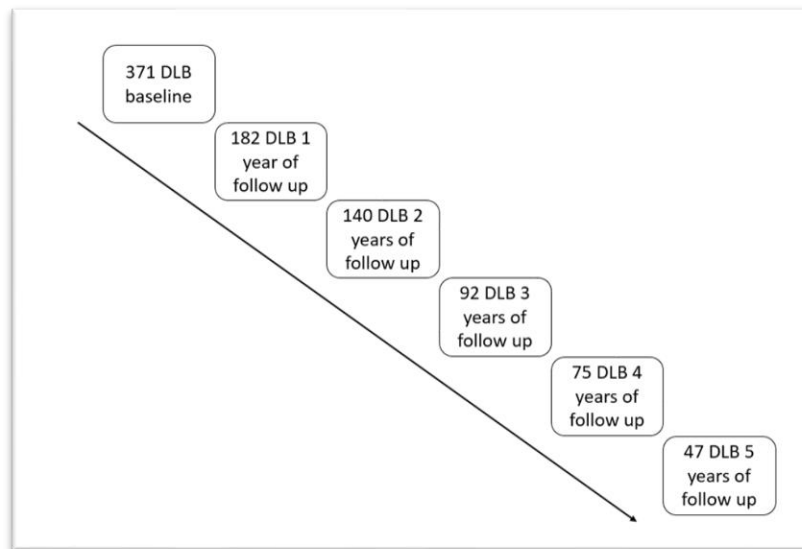
**Abbreviations:** (pg/mL) picograms per milliliter, (iQC) Internal quality control, (CV) coefficient of variation

**eTable 4.** MMSE Average Change per Group for Each Increased Unit (pg/mL) of Plasma p-tau at Baseline

Groups	MMSE Average change			
	p-tau181 pg/ml	CI 95%	p-tau231 pg/ml	CI 95%
AD	-0.095	[-0.126, -0.069]	-0.170	[-0.209, -0.135]
DLB	-0.092	[-0.129, -0.060]	-0.166	[-0.216, -0.122]
HC	-0.044	[-0.074, -0.022]	-0.083	[-0.127, -0.049]
PD	-0.053	[-0.082, -0.032]	-0.097	[-0.135, -0.065]

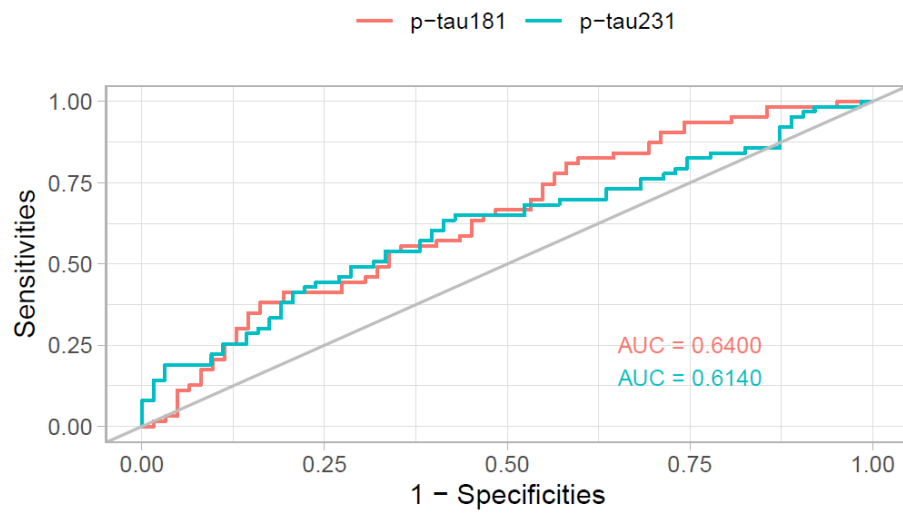
Abbreviations: (MMSE) Mini-Mental State Examination, (DLB) Dementia with Lewy bodies, (AD) Alzheimer’s disease, (PD) Parkinson’s disease, (HC) Healthy control, (CI) Confidence interval

**eFigure 1.** Flowchart of Number of DLB Patients Completing Annual Follow-up Assessments



**Abbreviations: (DLB) Dementia with Lewy bodies**

**eFigure 2.** Plasma p-tau231 and p-tau181 Performance Across Confirmed AD Pathology  
Based on Reduced A $\beta$ 42 on CSF in DLB



The AUC values of the ROC curves indicate the overall biomarker performance across confirmed AD pathology based on reduced A $\beta$ 42 on CSF in DLB. The change in the AUC of both biomarkers was not statistically significant.

Abbreviations: (AUC) Area under the curve, (CSF) Cerebrospinal fluid



## eReferences.

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