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FEATURED ARTICLE

Plasma and CSF biomarkers in a memory clinic: Head-to-head comparison of phosphorylated tau immunoassays

Nicholas J. Ashton[1,2,3,4] | Albert Puig-Pijoan[5,6,7] | Marta Milà-Alomà[6,8,9,10] | Aida Fernández-Lebrero[5,6,8] | Greta García-Escobar[5] | Fernándo González-Ortiz[1,8] | Przemysław R. Kac[1] | Wagner S. Brum[1,11] | Andréa L. Benedet[1] | Juan Lantero-Rodriguez[1] | Theresa A. Day[12] | Jeroen Vanbrabant[13] | Erik Stoops[13] | Eugeen Vanmechelen[13] | Gallen Triana-Baltzer[14] | Setareh Moughadam[14] | Hartmuth Kolb[14] | Paula Ortiz-Romero[6,8] | Thomas K. Karikari[1,15] | Carolina Minguillon[6,8,10] | Juan José Hernández Sánchez[16] | Irene Navalpotro-Gómez[5,6,8] | Oriol Grau-Rivera[5,6,8,10] | Rosa María Manero[5,6] | Víctor Puente-Periz[5,6] | Rafael de la Torre[6,17,18] | Jaume Roquer[5,6,7] | Jeff L. Dage[19] | Henrik Zetterberg[1,20,21,22,23] | Kaj Blennow[1,20] | Marc Suárez-Calvet[5,6,8,10]

[1]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Sweden

[2]Wallenberg Centre for Molecular and Translational Medicine, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Sweden

[3]King's College London, Institute of Psychiatry, Psychology & Neuroscience, Maurice Wohl Clinical Neuroscience Institute, London, UK

[4]NIHR Biomedical Research Centre for Mental Health & Biomedical Research Unit for Dementia at South London & Maudsley NHS Foundation, London, UK

[5]Cognitive Decline and Movement Disorders Unit, Neurology Department, Hospital del Mar, Barcelona, Spain

[6]IMIM (Hospital del Mar Medical Research Institute), Barcelona, Spain

[7]Department of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

[8]Barcelonaβeta Brain Research Center (BBRC), Pasqual Maragall Foundation, Barcelona, Spain

[9]Universitat Pompeu Fabra, Barcelona, Spain

[10]Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable (CIBERFES), Madrid, Spain

[11]Graduate Program in Biological Sciences: Biochemistry, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

[12]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana, USA

[13]ADx NeuroSciences, Technologiepark 94, Ghent, Belgium

[14]Neuroscience Biomarkers Janssen Research & Development La Jolla California, USA

[15]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA, USA

[16]Laboratori de Referència de Catalunya, Barcelona, Spain

[17]Department of Experimental and Health Sciences, Universitat Pompeu Fabra (CEXS-UPF), Barcelona, Spain

[18]Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Madrid, Spain

[19]Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, Indiana, USA

[20]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden

Nicholas J. Ashton, Albert Puig-Pijoan, and Marta Milà-Alomà contributed equally to this work.

Kaj Blennow and Marc Suárez-Calvet are equal senior co-authors on this work.

[21]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK

[22]UK Dementia Research Institute at UCL, London, UK

[23]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China

Correspondence
Kaj Blennow, Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, and the Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, SE 43180, Gothenburg, Sweden.
Email: kaj.blennow@neuro.gu.se

Marc Suárez-Calvet, Fluid Biomarkers and Translational Neurology Research Group, Alzheimer Prevention Program – Barcelonaβeta Brain Research Center, Wellington 30, 08005, Barcelona, Spain.
Email: msuarez@barcelonabeta.org

Abstract

Introduction: Direct comparisons of the main blood phosphorylated tau immunoassays in memory clinic populations are needed to understand possible differences.

Methods: In the BIODEGMAR study, 197 participants presenting with cognitive complaints were classified into an Alzheimer's disease (AD) or a non-AD cerebrospinal fluid (CSF) profile group, according to their amyloid beta 42/ phosphorylated tau (Aβ42/p-tau) ratio. We performed a head-to-head comparison of nine plasma and nine CSF tau immunoassays and determined their accuracy to discriminate abnormal CSF Aβ42/p-tau ratio.

Results: All studied plasma tau biomarkers were significantly higher in the AD CSF profile group compared to the non-AD CSF profile group and significantly discriminated abnormal CSF Aβ42/p-tau ratio. For plasma p-tau biomarkers, the higher discrimination accuracy was shown by Janssen p-tau217 (r = 0.76; area under the curve [AUC] = 0.96), ADx p-tau181 (r = 0.73; AUC = 0.94), and Lilly p-tau217 (r = 0.73; AUC = 0.94).

Discussion: Several plasma p-tau biomarkers can be used in a specialized memory clinic as a stand-alone biomarker to detect biologically-defined AD.

KEYWORDS
biomarker, dementia, disease, phosphorylated tau, plasma, tau

Highlights

- Patients with an Alzheimer's disease cerebrospinal fluid (AD CSF) profile have higher plasma phosphorylated tau (p-tau) levels than the non-AD CSF profile group.
- All plasma p-tau biomarkers significantly discriminate patients with an AD CSF profile from the non-AD CSF profile group.
- Janssen p-tau217, ADx p-tau181, and Lilly p-tau217 in plasma show the highest accuracy to detect biologically defined AD.
- Janssen p-tau217, ADx p-tau181, Lilly p-tau217, Lilly p-tau181, and UGot p-tau231 in plasma show performances that are comparable to their CSF counterparts.

1 | BACKGROUND

Recent efforts have focused on developing blood biomarkers for neurodegenerative diseases, which have resulted in important advances.[1] We can now assert that—at least for research purposes—several blood biomarkers accurately detect Alzheimer's disease (AD) pathology. Cerebrospinal fluid (CSF) biomarkers and amyloid β (Aβ) positron emission tomography (PET) continue to be the established tests to support an in vivo diagnosis of AD,[2] which should always be interpreted in the context of a comprehensive neurological and neuropsychological assessment. Blood biomarkers offer major advantages over both of these tests as they are less invasive, cheaper, and have the potential of a higher scalability for widespread application. Due to this, the future implementation of blood biomarkers, as either screening or diagnostic tools, will naturally increase the diversity of the examined population, in both demographic composition and clinical presentations.

The most promising AD blood biomarkers include the measurement of Aβ species (by mass spectrometry or immunodetection methods),[3–8] tau (either phosphorylated tau [p-tau] or total tau [t-tau]),[9–14] the reactive astrogliosis marker glial fibrillary acidic protein (GFAP)[15] and, although not specific for AD, the neurodegeneration marker neurofilament light chain (NfL).[16] Several assays targeting p-tau in blood have been developed, with the best success achieved by assays measuring tau phosphorylated at threonine 181 (T181),[9,10,17] 217 (T217),[12,18,19]

or 231(T231).[14,20] Blood p-tau assays have demonstrated a high accuracy to discriminate asymptomatic and symptomatic AD from other neurological diseases and healthy controls.[9,10,12,14,17,18] The emergence of blood p-tau assays has occurred in parallel with the discovery of new CSF p-tau assays that quantify N-terminal tau species, thereby improving the diagnostic performance of the more widely used CSF assays targeting the mid-region tau and phosphorylation at T181.[13] It is important to note that commercial blood and CSF p-tau assays have also been recently developed and, hence, a number assays targeting tau will be soon widely available.

These promising and consistent results prompt the implementation of p-tau biomarkers in clinical settings and in clinical trials. Nonetheless, a comparison of the performances of a wide range of p-tau assays in a single study is lacking. Most available data have been published in different research cohorts, which may differ in the characteristics of the participants and pre-analytical and analytical procedures. In addition, biomarker measurements in the same cohort have occurred at different times, according to the evolution of biomarker development, which may add further variability. Therefore, a direct comparison of the discrimination accuracy of each of the p-tau biomarkers cannot be inferred based on these currently available results. Moreover, from the biochemical point of view, it remains unanswered whether a certain p-tau epitope (T181, T217, T231), assay composition, and/or platform has a superior accuracy. In addition, studies in patients routinely assessed at memory clinics are lacking. Many studies have been performed in highly specialized research cohorts, and it needs to be determined whether blood p-tau biomarkers also have a good performance in a real-world population of a memory clinic, which has a higher heterogeneity in patient demographics, co-morbidities, and disease presentations.

To this end, the main aim of this study was to perform a head-to-head and blinded comparison of plasma and CSF tau immunoassays and determine their accuracy to discriminate between biologically defined AD (prodromal or dementia stage) from non-AD in symptomatic individuals in a clinical setting.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using traditional sources (e.g., PubMed). Several publications investigating blood phosphorylated tau (p-tau) biomarkers have been published in recent years. Yet, few studies have performed head-to-head comparisons at memory clinics. These publications are properly cited throughout the manuscript.
2. **Interpretation**: In this cross-sectional observational study in a memory clinic population, several plasma p-tau biomarkers, with differing epitope targets and analytical platforms, showed very high accuracy to discriminate patients with biologically defined Alzheimer's disease (AD) from non-AD. Several of these assays demonstrated comparable performance to cerebrospinal fluid (CSF) p-tau assays.
3. **Future directions**: Our findings support the utility of plasma p-tau biomarkers as diagnostic tools in a heterogeneous memory clinic population. Further studies are needed to precisely determine the role of plasma biomarkers and their implementation for the assessment of cognitive decline complaints in clinical settings.

2 | METHODS

2.1 | Participants

This is a cross-sectional study that included participants of the BIODEGMAR cohort, an observational longitudinal study that enrolls individuals with cognitive decline and/or neurodegenerative diseases visiting the Cognitive Decline and Movement Disorders Unit of Hospital del Mar (Barcelona, Spain).[21] Participants from the BIODEGMAR cohort donated a blood sample and underwent a detailed neurological and neuropsychological evaluation, a brain magnetic resonance imaging (MRI) study, and a lumbar puncture. All participants included in the present study had a Global Deterioration Score (GDS) >1.[22] Core AD CSF biomarkers (Aβ42/40, p-tau, and t-tau) were measured with Lumipulse immunoassays (Fujirebio, Belgium). Participants were classified as AD CSF profile if the CSF Aβ42/p-tau ratio was <10.25.[21]

A comprehensive description of the BIODEGMAR cohort, the inclusion and exclusion criteria, the core AD CSF biomarkers measurements, and cutoffs determination can be found in the Methods section in the supporting information.

2.2 | Plasma and CSF tau biomarkers measurements

A total of nine immunoassays measuring plasma tau (8× p-tau and 1× t-tau) and corresponding CSF counterparts were investigated (Figure 1A). The design of p-tau Lumipulse immunoassay employed as the core AD CSF biomarker is displayed in Figure 1B. A detailed description of the immunoassays can be found in the Methods section and in Table S1 in the supporting information, and their analytical precision and sensitivity for this study are described in Table S2 in the supporting information. All plasma and CSF samples collected were treated identically. Polypropylene tubes of 1.8 mL of plasma or CSF collected in Barcelona were shipped to the University of Gothenburg with dry ice. In Gothenburg, the blinded samples were aliquoted into smaller volumes in polypropylene tubes and frozen at −80°C. Subsequently, one aliquot was shipped to each of the collaborators with dry ice (i.e., ADx, Janssen, Lilly), whereas two aliquots remained at the University of Gothenburg, where the Quanterix p-tau181 and the University of Gothenburg in-house immunoassays (p-tau181 and p-tau231) were performed. Each participating center sent their results

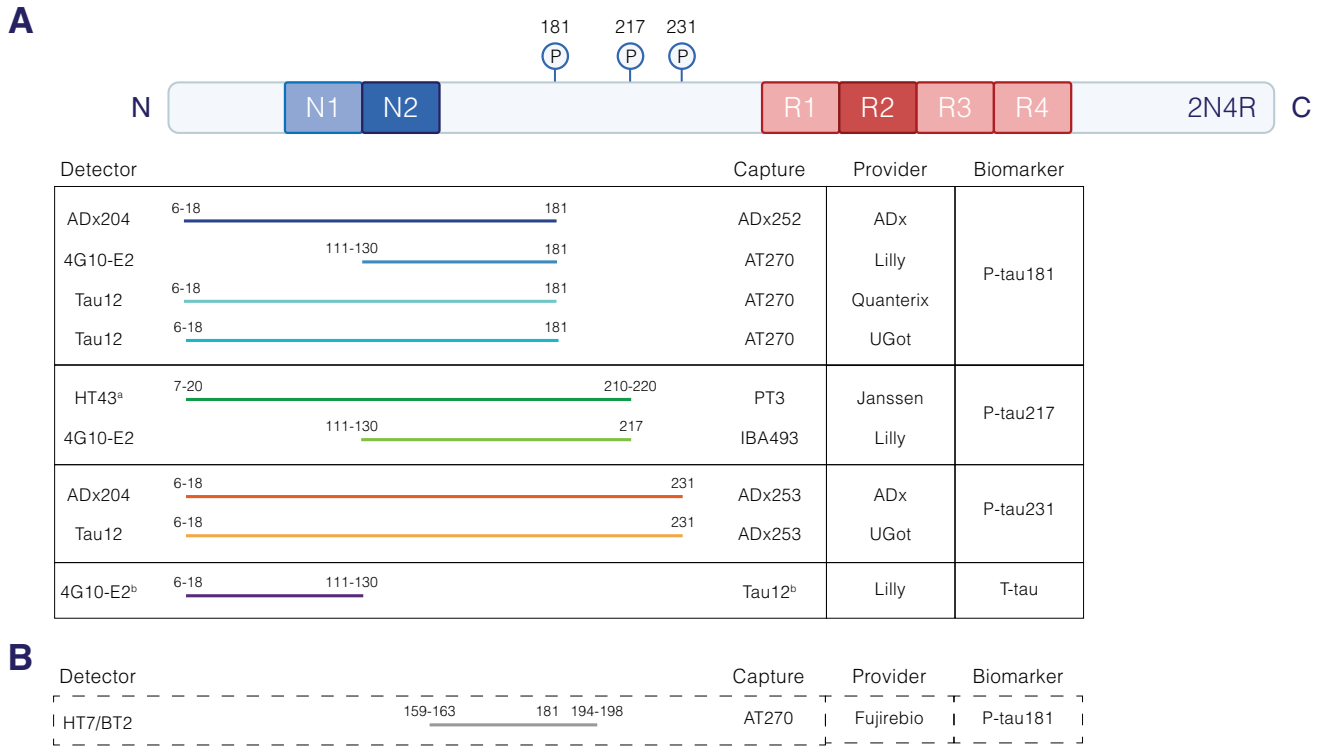

FIGURE 1 Diagram of tau depicting the antibodies and the epitopes recognized in each plasma immunoassay. Schematic diagram of the tau isoform (2N4R) of 441 amino acids, which comprises two N-terminal domains (N) and the four microtubule-binding domains (R). The phosphorylation sites T181, T217, and T231 are also shown. (A) The tau protein fragments recognized by the combination of antibodies of the immunoassays tested in this study. (B) The combination of antibodies employed in Lumipulse cerebrospinal fluid (CSF) phosphorylated tau (p-tau) assay, which was used to determine the Alzheimer's disease (AD) CSF profile (amyloid beta (Aβ)42/p-tau ratio). The epitopes recognized by each antibody are shown. [a]CSF Janssen p-tau217 used PT82 as detector antibody, which targets aa119-126 (Janssen R&D, Springhouse, PA). [b]Plasma Lilly t-tau assay, the capture antibody is Tau12, and detector antibody is 4G10-E2.

directly to the study coordinators at Barcelonaβeta Brain Research Center (BBRC), who independently performed the data unblinding and the analysis. In addition, the same immunoassays in plasma were analyzed in paired CSF samples, to deduce the concordance when using the same antibody pairs in different matrices.

2.3 | Statistical analyses

Demographical and clinical data of the AD CSF profile group and the non-AD CSF profile group were compared with a *t*-test for continuous variables and a Pearson's chi-square test for categorical variables. We used a non-parametric test because most of the plasma and CSF tau biomarkers did not meet the assumption that the underlying residuals are normally distributed. Comparisons between the AD CSF profile group and non-AD CSF profile group were tested with a Mann-Whitney *U* test. Plasma and CSF tau biomarkers levels are presented as medians and interquartile ranges. The effect sizes of the comparisons (*r*) were calculated by dividing the absolute (positive) standardized test statistic *Z* by the square root of the total number of individuals.[23] We tested the accuracy of plasma and CSF tau biomarkers to discriminate between AD CSF profile and non-AD CSF profile with receiver-operating characteristic (ROC) analyses. We computed areas under the curve (AUCs) and their 95% confidence intervals (CIs). The AUCs of two ROC curves were compared using the DeLong test, and we applied a false discovery rate (FDR) multiple comparison correction.[24] Optimal cutoffs for each tau biomarker were calculated at the highest Youden's index (sensitivity + specificity − 1). We used a Spearman rank-order correlation to test the correlations between biomarkers. The analyses in the main text were performed in the whole cohort study and using CSF Aβ42/p-tau as a reference method for the biological diagnosis of AD.[21] Sensitivity analyses were performed in those patients with a syndromic diagnosis of subjective cognitive decline (SCD), mild cognitive impairment (MCI), and dementia, or only predementia stages (SCD and MCI). Additional analyses were performed using CSF Aβ42/40 as a reference method.

3 | RESULTS

3.1 | Patient characteristics

From April 27, 2017 to July 24, 2020, a total of 233 participants were included in the BIODEGMAR cohort at the Cognitive Decline and Movement Disorders Unit of Hospital del Mar (Barcelona, Spain). In the present study, we excluded 13 participants with normal cognition and

TABLE 1 Participants' characteristics in the BIODEGMAR cohort

	Non-AD CSF profile ($n = 70$, 35.5%)	AD CSF profile ($n = 127$, 64.5%)	Total ($n = 197$)	*P*-value
Age, years	70.7 (6.47)	73.2 (5.28)	72.3 (5.83)	0.008
Female, *n* (%)	34 (48.6)	75 (59.1)	109 (55.3)	0.16
Education, years	8.39 (4.46)	8.29 (4.23)	8.33 (4.30)	0.879
APOE ε4 carriers, *n* (%)	10 (15.9)	75 (63.6)	85 (47)	<0.001
Age at symptom onset, years	67 (7.15)	69.4 (5.8)	68.5 (6.40)	0.17
Symptom duration, years	3.37 (2.94)	3.33 (2.9)	3.35 (2.91)	0.925
MMSE	25 (22–28)	21 (18–24)	23 (19–26)	<0.001
AD CSF core biomarkers (Lumipulse)				
Aβ42/40	0.092 (0.071–0.10)	0.043 (0.037–0.050)	0.050 (0.040–0.078)	<0.001
p-tau181 (pg/ml)	40.4 (28.3–55.8)	109.6 (79.9–140)	81.8 (48.6–126.3)	<0.001
t-tau (pg/ml)	298 (209–425)	649 (484–879)	515 (322–753)	<0.001

Note: Data are expressed as mean (*M*) and standard deviation (SD) [age, education, age at symptom onset, symptom duration], median (M) and interquartile range (IQR) [MMSE, AD CSF core biomarkers] or number of participants (*n*) and percentage (%) [sex, *APOE* ε4 carrier AD]. AD CSF profile was defined by a CSF Aβ42/p-tau181 ratio < 10.25 (as measured by Lumipulse G600II, Fujirebio). MMSE was not available in 23 (11.8%) individuals. *APOE* ε4 genotype was not available in 16 (8.1%) individuals. *P*-values tested the difference between AD CSF core biomarkers profile groups and were computed with a *t*-test (age, education, age at symptom onset, symptom duration), a Mann-Whitney *U* test (MMSE, AD CSF core biomarkers), or a chi-square (sex, *APOE* ε4 carrier status). Clinical diagnoses of the non-AD CSF group ($n = 70$): 14 SCD, 33 MCI, 6 AD dementia, 3 LBD, 1 VCID, 2 PSP/CBS, 5 bvFTD, 5 PA, 1 CAA. Clinical diagnoses o the AD CSF group ($n = 127$): 4 SCD, 49 MCI, 54 AD dementia, 1 LBD, 3 VCID, 5 PSP/CBS, 7 PA, 2 CAA, 2 unclassifiable dementia. Clinical Dementia Rating (CDR) of the non-AD CSF group ($n = 70$): 16 CDR = 0, 34 CDR = 0.5, 12 CDR = 1, 7 CDR = 2, 1 CDR = 3. CDR of the AD CSF group ($n = 127$): 4 CDR = 0, 43 CDR = 0.5, 43 CDR = 1, 32 CDR = 2, 5 CDR = 3.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; bvFTD, behavioral variant of Frontotemporal dementia; CBS, corticobasal syndrome; CCA, cerebral amyloid angiopathy; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; LBD, Lewy body dementia; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PA, progressive aphasia; PSP, progressive supranuclear palsy; p-tau181, tau phosphorylated at threonine 181; SCD, subjective cognitive decline; t-tau, total tau; VCID, vascular cognitive impairment and dementia.

no subjective cognitive decline (GDS = 1), 1 participant with a normal pressure hydrocephalus, and 22 participants with either no CSF and/or plasma sample remaining for one or more tested immunoassay. Thus, a total of 197 individuals were included herein. Participants had the following syndromic diagnosis: 18 subjective cogntive decline (SCD), 82 mild cognitive impairment (MCI), 60 Alzheimer's disease (AD) dementia, 4 Lewy body dementia (LBD), 4 vascular cognitive impairment and dementia (VCID) with predominant small vessel disease, 3 with radiological evidence of cerebral amyloid angiopathy (CAA) 7 progressive supranuclear palsy or corticobasal syndrome (PSP, CBS), 5 behavioral variant of frontotemporal dementia (bvFTD), 12 progressive aphasia (PA), and 2 unclassified dementia syndromes. Regardless of the syndromic diagnosis, all patients were classified based on their AD CSF core biomarker profile (i.e., CSF Aβ42/p-tau ratio), and their characteristics are summarized in Table 1. Participants with an AD CSF profile ($n = 127$) were significantly older and had a lower Mini-Mental Status Examination (MMSE) score compared with the non-AD CSF profile ($n = 70$). There was a significantly higher percentage of apolipoprotein E (*APOE*) ε4 carriers in the AD CSF profile group.

3.2 | Levels of tau plasma and CSF biomarkers

Table 2 shows the levels of the plasma and CSF tau biomarkers. Mann-Whitney *U* tests indicated that all plasma and CSF tau biomarkers were significantly greater in the AD CSF profile group than in the non-AD CSF profile group, but with varying effect sizes (Figure 2, Table 2; Figure S1 in supporting information). A large effect size was found for plasma ADx p-tau181 ($r = 0.73$; $P < 0.001$), Janssen p-tau217 ($r = 0.76$; $P < 0.001$), Lilly p-tau181 ($r = 0.68$; $P < 0.001$), Lilly p-tau217 ($r = 0.73$; $P < 0.001$), and UGot p-tau231 ($r = 0.63$; $P < 0.001$). A medium effect size was found in plasma UGot p-tau181 ($r = 0.50$; $P < 0.001$), Quanterix p-tau181 ($r = 0.49$; $P < 0.001$), and Lilly t-tau ($r = 0.38$; $P < 0.001$), and a small effect was found in plasma ADx p-tau231 ($r = 0.26$; $P < 0.001$). All effect sizes of the CSF tau biomarkers were large ($r > 0.50$). The magnitude of the difference, as measured by the percentage increase of the median in the AD CSF profile group compared to the non-AD CSF profile group, is also shown in Figure 2, Figure S1 in supporting information, and Table 2. The results remained similar when the comparisons were adjusted by the effect of age (Table S3 in supporting information).

3.3 | Discrimination of AD CSF biomarker profile status

We next investigated how plasma and CSF tau biomarkers discriminate between patients with an AD CSF profile group from those with a non-AD CSF profile, regardless of the clinical diagnosis, in an ROC curve analysis (Figure 3, Table 3; Figure S2 in supporting information).

TABLE 2 Plasma and CSF tau biomarkers

Tau biomarkers (pg/ml)	Non-AD CSF profile ($n = 70$, 35.5%)	AD CSF profile ($n = 127$, 64.5%)	Total ($n = 197$)	*P*-value	*r*	%Inc.
ADx						
Plasma p-tau181	7.78 (4.84–12.68)	27.1 (20.0–37.1)	20.05 (9.12–30.4)	<0.001	0.73	248.3
CSF p-tau181	229.5 (163.6–360.8)	1095.1 (721–1406.6)	692.3 (303.5–225.1)	<0.001	0.75	377.2
Plasma p-tau231	3.70 (2.50–5.22)	5.13 (3.49–7.46)	4.63 (2.94–6.60)	<0.001	0.26	38.6
CSF p-tau231	48.3 (35.2–74.2)	173.4 (125–227.5)	123.4 (62.8–196.7)	<0.001	0.70	259
Janssen						
Plasma p-tau217	0.023 (0.014–0.039)	0.120 (0.070–0.201)	0.070 (0.029–0.146)	<0.001	0.76	421.7
CSF p-tau217	2.13 (0.80–3.41)	18.17 (11.3–30.1)	14.4 (5.37–24.8)	<0.001	0.72	753.1
Lilly						
Plasma p-tau181	0.61 (0.52–0.86)	1.58 (1.15–2.13)	1.18 (0.72–1.85)	<0.001	0.68	159
CSF p-tau181	20.3 (14.5–31.6)	66.8 (46.5–89.0)	47.8 (27.3–76.4)	<0.001	0.74	229.1
Plasma p-tau217	0.15 (0.12–0.20)	0.49 (0.36–0.74)	0.36 (0.18–0.59)	<0.001	0.73	226.7
CSF p-tau217	4.50 (2.85–7.34)	32.3 (20.3–44.9)	20.07 (6.26–38.4)	<0.001	0.79	618
Plasma t-tau	26.7 (22.9–35.0)	36.4 (29.4–49.6)	32.5 (25.8–44.1)	<0.001	0.38	36.3
CSF t-tau	1022.2 (763–1495)	2012.2 (1401.6–2496.1)	1568.8 (1147.6–2260.4)	<0.001	0.57	96.8
Quanterix						
Plasma p-tau181	2.65 (1.71–3.53)	4.40 (3.35–5.54)	3.67 (2.70–5.05)	<0.001	0.49	66
CSF p-tau181	33.6 (25.3–55.1)	168.8 (120.9–224.5)	120.7 (45.4—192.4)	<0.001	0.76	402.4
U Got (in house)						
Plasma p-tau181	11.2 (10.1–14.2)	15.5 (13.5–18.2)	14.7 (12.2–17.7)	<0.001	0.50	38.4
CSF p-tau181	381.4 (325.5–455.8)	801.3 (642.9–950.6)	652.3 (411.7–855.9)	<0.001	0.73	110.1
Plasma p-tau231	6.46 (4.82–8.79)	12.6 (9.48–15.9)	10.7 (7.37–14.4)	<0.001	0.63	95

P-values were computed with a Mann-Whitney *U* test for all plasma or CSF biomarkers. The effect sizes of the comparisons were shown as *r* (which is calculated by dividing the absolute standardized test statistic *Z* by the square root of the total number of individuals) and with the percentage (%) increase of the tau biomarker median in the AD CSF group compared to the non-AD group.

Note: Data are expressed as median (M) and interquartile range (IQR). AD CSF profile was defined by a CSF Aβ42/p-tau181 ratio < 10.25 (as measured by Lumipulse G600II, Fujirebio).

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; p-tau181, tau phosphorylated at threonine 181; p-tau217, tau phosphorylated at threonine 217; p-tau231, tau phosphorylated at threonine 231; t-tau, total tau.

In plasma, the calculated AUCs were the following (from highest to lowest): Janssen p-tau217 (0.96, 95% CI 0.93–0.99), ADx p-tau181 (0.94, 95% CI 0.91–0.97), and Lilly p-tau217 (0.94, 95% CI 0.90–0.98), Lilly p-tau181 (0.91, 95% CI 0.86–0.96), UGot p-tau231 (0.88, 95% CI 0.83–0.93), Quanterix p-tau181 (0.80, 95% CI 0.73–0.87), and UGot p-tau181 (0.80, 95% CI 0.73–0.87), Lilly t-tau (0.73, 95% CI 0.65–0.81), and ADx p-tau231 (0.66, 95% CI 0.58–0.74). DeLong tests between AUCs showed that Janssen p-tau217, ADx p-tau181, Lilly p-tau217, Lilly p-tau181, and UGot p-tau231 had significantly better discriminative accuracy than Quanterix p-tau181, UGot p-tau181, Lilly t-tau, or ADx p-tau231. All of the AUC comparisons using the DeLong test within plasma tau biomarkers or within CSF tau biomarkers are shown in Table 3.

In addition, we tested whether the AUC of a plasma biomarker differed from its CSF counterpart. The discriminative accuracy of ADx p-tau231, Lilly t-tau, Quanterix p-tau181, and UGot p-tau181 immunoassays in plasma were significantly lower than those in CSF. The rest of the tau immunoassays (ADx p-tau181, Janssen p-tau217, Lilly p-tau181, Lilly p-tau217, and UGot p-tau231) did not have a different discriminative accuracy when performed in plasma or in CSF (Table 3). The tau biomarkers cutoff points using Youden's index and the resulting values of sensitivity and specificity are shown in Table S4.

Considering that this is a diverse cohort that includes syndromic diagnoses, not typically caused by AD, we performed additional analyses by only including patients with a clinical syndrome of SCD, MCI, and AD dementia ($n = 160$; Figure S3 and Table S5) or only including the pre-dementia (i.e., SCD and MCI) clinical syndromes ($n = 100$; Figure S4 and Table S6). The AUCs calculated in these subsets of patients were very similar to those calculated in the entire sample (Tables S7 and S8).

Finally, we also repeated the ROC analyses but using the CSF Aβ42/40 ratio as a reference standard (AD CSF profile if CSF Aβ42/40 < 0.062; Table S9 for the participants' characteristics). The results were similar, but most plasma and CSF tau immunoassays had a slightly lower AUC than when using the CSF Aβ42/p-tau ratio (Tables S10 and S11).

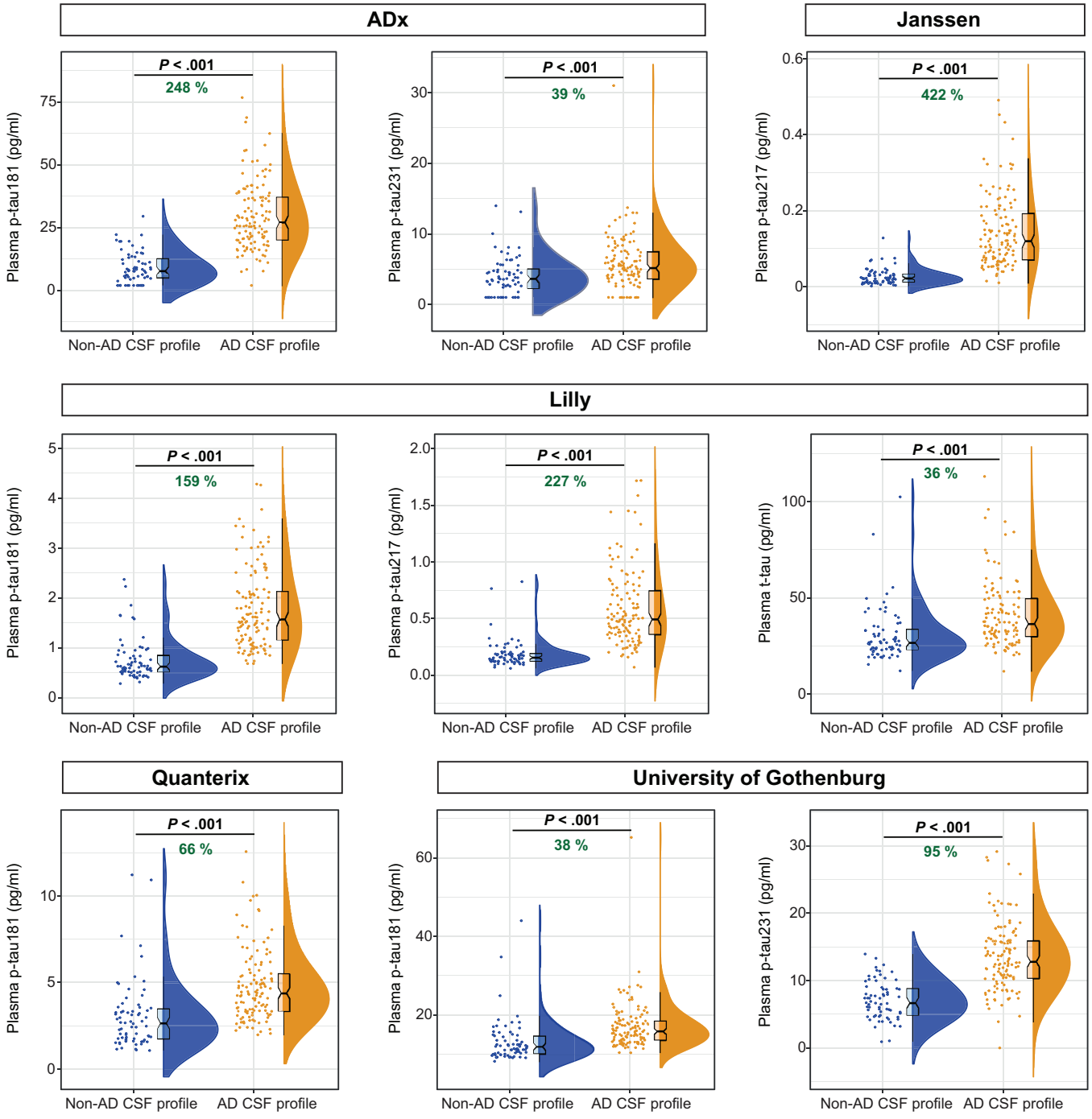

FIGURE 2 Levels of the plasma tau biomarkers in the non-AD versus the AD cerebrospinal fluid (CSF) profile groups, visualized as raincloud plots. Each point indicates a tau measurement of an individual. The box plot indicates the median (horizontal line), interquartile range (box), and 1.5× interquartile range (whiskers). The width of the shaded area (violin plot) represents the proportion of the data located there. AD CSF profile was defined by a CSF amyloid beta (Aβ)42/p-tau181 ratio < 10.25 (as measured by Lumipulse, Fujirebio). *P*-values were computed with a Mann-Whitney *U* test for all plasma biomarkers. The percentage increase of the tau biomarkers in the AD CSF profile from the non-AD profile is shown in green.

3.4 | Correlations between tau biomarkers

We tested the correlation between plasma and CSF for each immunoassay (Figure S5). There was a strong correlation between plasma and CSF for ADx p-tau181 ($r = 0.67$; $P < 0.001$), Janssen p-tau217 ($r = 0.69$; $P < 0.001$), Lilly p-tau181 ($r = 0.64$; $P < 0.001$), and Lilly p-tau217 ($r = 0.72$; $P < 0.001$). There was a moderate correlation for Quanterix p-tau181 ($r = 0.52$; $P < 0.001$), UGot p-tau181 ($r = 0.44$; $P < 0.001$), and UGot p-tau231 ($r = 0.54$; $P < 0.001$), and a weak correlation for ADx p-tau231 ($r = 0.22$; $P = 0.003$) and

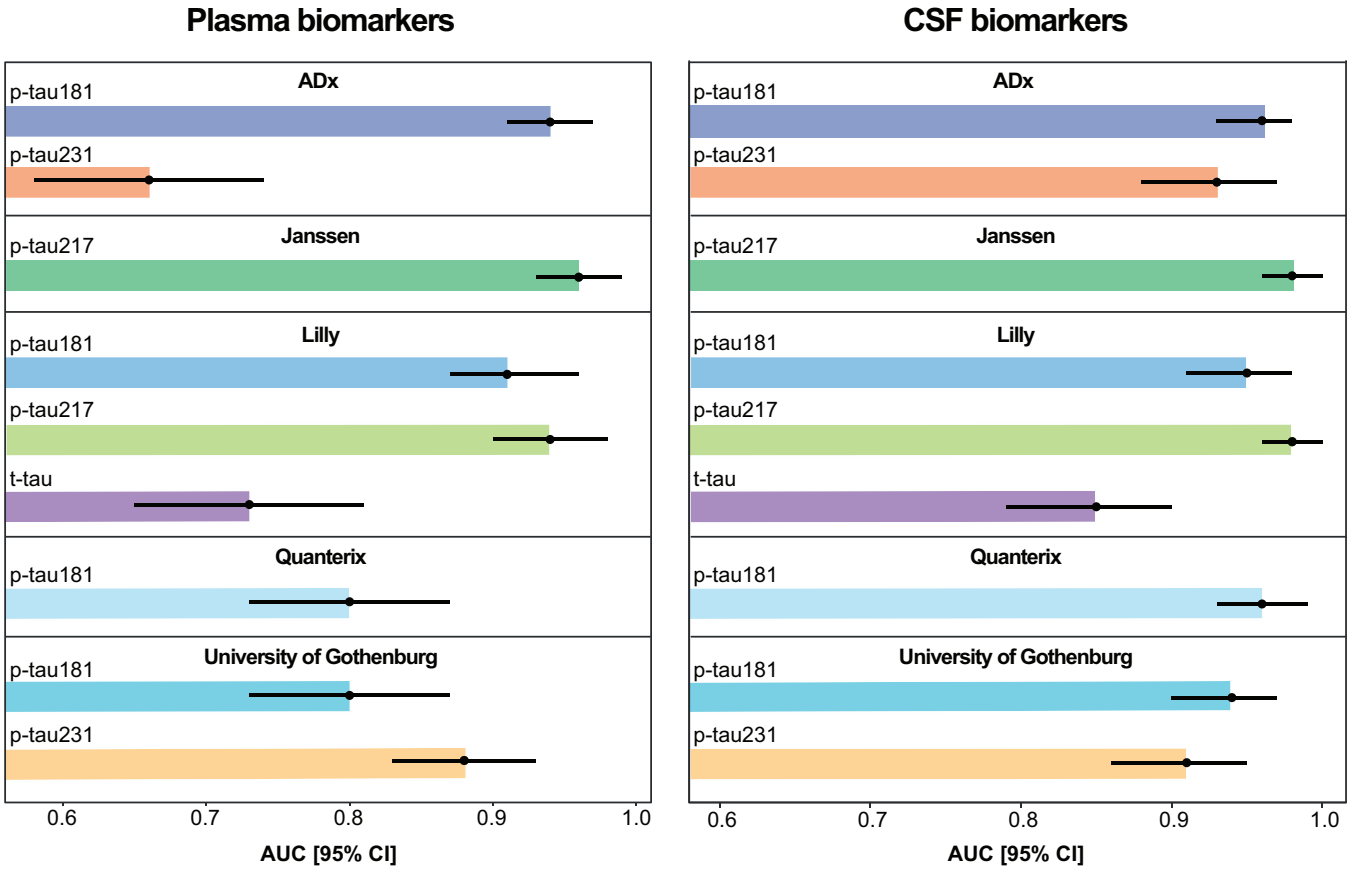

FIGURE 3 Discrimination accuracy (receiver-operating characteristic [ROC] analyses) of plasma and cerebrospinal fluid (CSF) tau immunoassays. Forest plot depicting the area under the curve (AUC) and the 95% confidence interval (CI) of each tau biomarker to discriminate between the Alzheimer's disease (AD) CSF profile group form the non-AD CSF profile groups. AD CSF profile was defined by a CSF amyloid beta (Aβ)42/p-tau181 ratio < 10.25 (as measured by Lumipulse G600II, Fujirebio). CI, confidence interval.

Lilly t-tau ($r = 0.23$; $P = 0.002$). Correlations stratified by AD CSF status are shown in Figure S6. In addition, we tested the correlations between all tau biomarkers and the results are summarized in Figures S7, S8, and S9.

4 | DISCUSSION

Plasma biomarkers, in particular p-tau, have the potential to transform the process in which the pathology that underlies AD can be identified in primary and secondary care.[1,25,26] This will be of significant importance given the emergence of disease-modifying treatments for AD. Despite the numerous recent publications on blood biomarkers for AD, it is still to be determined if high accuracies of p-tau plasma biomarkers translate to a heterogenous memory clinic setting. In addition, it is to be determined whether a superiority of a specific p-tau epitope is due to immunoassay design, or platform. To answer this, a tightly controlled head-to-head study of tau biomarkers in a memory clinic population was needed.

In the present study, we performed such a head-to-head comparison and found that the majority of p-tau biomarkers demonstrated a high accuracy to identify biologically defined AD in a clinical population of patients with diverse clinical presentations. Yet, it was observed that some plasma p-tau biomarkers (ADx p-tau181; Janssen p-tau217; Lilly p-tau181; and Lilly p-tau217) performed exceptionally well and demonstrated AUCs > 0.9, which did not differ significantly from those of the same biomarker in the CSF. Plasma p-tau231 from UGot (AUC = 0.88) also showed no significant difference from the same biomarker in the CSF. Other p-tau181 immunoassays, that is, UGot p-tau181 and Quanterix p-tau181, performed well (AUCs = 0.80), but these results were significantly lower than the equivalent immunoassay in CSF.

Different immunoassays targeting distinct phosphorylation sites (T181, T217, and T231) had high AUCs. Both of the two p-tau217 immunoassays investigated herein (Janssen p-tau217 and Lilly p-tau217) had exceedingly high AUCs (≥ 0.94). The p-tau181 with the highest AUC was ADx p-tau181 (0.94), followed by Lilly p-tau181 (0.91) and, with a statistically significantly lower AUC, the Quanterix p-tau181 and UGot p-tau181 immunoassays (0.80). Although the UGot p-tau231 immunoassay had an AUC of 0.88, the ADx p-tau231 was significantly lower in performance and had an AUC of 0.66. Of note, ADx p-tau231 immunoassay in CSF performed particularly well (AUC = 0.93), which may suggest that the modest performance of the plasma immunoassay is related with the matrix (plasma) but not

TABLE 3 Discrimination accuracy (ROC analyses) of plasma and CSF tau immunoassays in the BIODEGMAR cohort

Tau biomarkers AUC (95% CI)	Plasma	CSF	*P*-value[a]
ADx			
p-tau181	0.94 (0.91–0.97)[a,b,c,d,e]	0.96 (0.93–0.98)[o,p,q,r]	0.50
p-tau231	0.66 (0.58–0.74)[d,f,g,h,i,j]	0.93 (0.88–0.97)[o,s,t]	<0.001
Janssen			
p-tau217	0.96 (0.93–0.99)[c,d,e,j,k]	0.98 (0.96–1.00)[q,u,v,w,x]	0.23
Lilly			
p-tau181	0.91 (0.86–0.96)[c,e,l]	0.95 (0.91–0.98)[q,s,v]	0.23
p-tau217	0.94 (0.90–0.98)[c,d,e]	0.98 (0.96–1.00)[q,v,x,y]	0.14
t-tau	0.73 (0.65–0.81)[j]	0.85 (0.79–0.90)[r,w,z]	0.041
Quanterix			
p-tau181	0.80 (0.73–0.87)[m]	0.96 (0.93–0.99)[r]	<0.001
UGot (in house)			
p-tau181	0.80 (0.73–0.87)[n]	0.94 (0.90–0.97)	<0.001
p-tau231	0.88 (0.83–0.93)	0.91 (0.87–0.95)	0.33

Significant differences between plasma tau immunoassays:	Significant differences between CSF tau immunoassays:
[a] $P < 0.001$ vs. Plasma p-tau231 (ADx)	[o] $P < 0.05$ vs CSF p-tau217 (Janssen)
[b] $P < 0.05$ vs. plasma p-tau217 (Jansen)	[p] $P < 0.05$ vs CSF p-tau217 (Lilly)
[c] $P < 0.001$ vs. plasma t-tau (Lilly)	[q] $P < 0.001$ vs CSF t-tau (Lilly)
[d] $P < 0.001$ vs. plasma p-tau181 (Quanterix)	[r] $P < 0.05$ vs CSF p-tau231 (UGot)
[e] $P < 0.001$ vs. plasma p-tau181 (UGot)	[s] $P < 0.01$ vs CSF p-tau217 (Lilly)
[f] $P < 0.001$ vs. plasma p-tau217 (Jansen)	[t] $P < 0.01$ vs CSF t-tau (Lilly)
[g] $P < 0.001$ vs. plasma p-tau181 (Lilly)	[u] $P < 0.01$ vs CSF p-tau181 (Lilly)
[h] $P < 0.001$ vs. plasma p-tau 217 (Lilly)	[v] $P < 0.05$ vs CSF p-tau181 (Quanterix)
[i] $P < 0.01$ vs. plasma p-tau181 (UGot)	[w] $P < 0.001$ vs CSF p-tau181 (UGot)
[j] $P < 0.001$ vs. plasma p-tau231 (UGot)	[x] $P < 0.001$ vs CSF p-tau231 (UGot)
[k] $P < 0.05$ vs. plasma p-tau181 (Lilly)	[y] $P < 0.01$ vs CSF p-tau181 (UGot)
[l] $P < .01$ vs. plasma p-tau181 (Quanterix)	[z] $P < 0.001$ vs CSF p-tau181 (Quanterix)
[m] $P < 0.01$ vs. plasma p-tau231 (UGot)	
[n] $P < .005$ vs. plasma p-tau231 (UGot)	

[a] *P*-value of the comparison between the AUC of plasma and CSF of the same immunoassay (DeLong test).
We also assessed the AUCs differences (DeLong test) between the plasma tau immunoassays or CSF tau immunoassays.

with the epitope itself. Yet, it must be noted that most plasma p-tau biomarkers had only modest correlations with the same immunoassay in CSF. This observation is particularly important for the use and interpretation of these biomarkers in clinical trials. Although plasma p-tau biomarkers have a high accuracy for detecting biologically defined AD (and hence enriching clinical trials with AD patients), it remains to be determined whether they are useful as pharmacodynamic biomarkers to monitor the effects of an intervention, since there is a modest—or weak, in some cases—linear relationship between plasma and CSF.

We also included a t-tau immunoassay that had the lowest AUC in CSF (0.85) and did not reach the high performance of most p-tau immunoassays when measured in plasma (AUC = 0.73). Given that this t-tau immunoassay has a configuration similar to that of Lilly p-tau immunoassays, it demonstrates the importance of p-tau-specific antibodies for greater disease specificity and diagnostic accuracy. Although more immunoassays targeting each of these post-translational modifications should be assessed, our results indicate that phosphorylations at T181, T217, or T231 can all be excellent biomarkers in plasma. All analytical platforms provided at least one immunoassay with satisfactory accuracies. Altogether, our results suggest that there is not a sole phosphorylation site and/or immunoassay platform or immunoassay design that is clearly superior to the other.

An important aspect of our study is that it was performed in patients visited in a specialized memory clinic setting, and not in a research cohort. The BIODEGMAR cohort reflects the diversity of patients who come to a specialized unit consulting for cognitive complaints after being referred by their primary care physician, with whom they

consulted due to cognitive complaints. This study demonstrates that, in this setting, plasma p-tau biomarkers can accurately discriminate biologically defined AD from other neurological or medical conditions presenting with cognitive symptoms. Hence, we show that the accuracy of these novel plasma biomarkers is not restricted only to research cohorts but is also extended to a diverse population of a specialized memory clinic. Given the clinical heterogeneity, we performed further analyses after excluding patients who already have a clinical diagnosis of a non-AD neurodegenerative disease. We first restricted the analyses to those clinical syndromes that could fall into the continuum of AD (SCD, MCI, and dementia) and, second, in pre-dementia patients (SCD and MCI), and the results remained similar.

While we were preparing this article, a study focusing on the analytical comparisons of six Simoa p-tau immunoassays was published.[27] In line with our results, ADx p-tau181, Lilly p-tau181, Lilly p-tau217, Quanterix p-tau181, and UGot p-tau231 had a high diagnostic accuracy for AD dementia (AUC > 0.93), whereas ADx p-tau231 had a more modest AUC (0.72). The main differences of these reported results with our study involve the sample size, availability of underlying biological confirmation, and type of participants included. Although Bayoumy et al. selected 40 AD dementia patients and 40 age- and sex-matched controls from a different cohort, we studied patients prospectively presenting with cognitive complaints in a specialized memory clinic, all of them with CSF core biomarkers. Furthermore, we included three additional biomarkers: Janssen p-tau217, Lilly t-tau, and UGot p-tau181.

Our study is not free of limitations. First, neither neuropathological confirmation nor amyloid PET was available; yet it is important to remark that the AD CSF core biomarkers are widely accepted as biomarkers for routine clinical diagnosis of AD, and are the biomarkers routinely used in Hospital del Mar. Second, the number of non-AD neurodegenerative diseases was limited. Finally, these results need to be confirmed in other clinical cohorts to assess whether they are generalizable.

Our study has several strengths. First, BIODEGMAR is a well-characterized clinical cohort, and all patients had CSF biomarkers. Second, we included a remarkable number of plasma tau immunoassays and, also, their CSF counterparts. Third, all measurements were performed not only in the same patients but also in the exact same original aliquot of plasma or CSF, which were re-aliquoted and stored until the immunoassays were performed; thus, all samples were treated identically and the comparability between immunoassays were more reliable. Fourth, all measurements were performed blinded, and the statistical analyses were independently conducted at BBRC.

In conclusion, we demonstrate that there are several plasma p-tau biomarkers that can be used as accurate stand-alone diagnostic tests to detect biologically defined AD in a specialized memory clinic. In our opinion, precisely determining the role of plasma biomarkers in the assessment of cognitive complaints in specialized centers is the vital next step and in accordance with the recent appropriate use recommendations.[28] Our data suggest that some of these immunoassays can be a sole biological test, coupled with neurological assessment, to support the diagnosis of AD. However, a more conservative alternative could be using them as pre-screening tools to select those patients who will undergo a biomarker assessment by CSF or amyloid PET. It also remains to be answered whether this pre-screening can be done in primary care and how plasma biomarkers can be applied in population-based studies. These promising results urge the medical, scientific community and all related stakeholders to consider and discuss a possible implementation of AD blood biomarkers at the earliest clinical setting.

AUTHOR CONTRIBUTIONS

Nicholas J. Ashton, Albert Puig-Pijoan, and Marta Milà-Alomà are co-first authors. Kaj Blennow and Marc Suárez-Calvet are co-senior authors. Kaj Blennow and Marc Suárez-Calvet had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Nicholas J. Ashton, Kaj Blennow, and Marc Suárez-Calvet. Acquisition, analysis, or interpretation of data: Nicholas J. Ashton, Albert Puig-Pijoan, Marta Milà-Alomà, Aida Fernández-Lebrero, Greta García-Escobar, Fernando González-Ortiz, Przemysław R. Kac, Wagner S. Brum, Andréa L. Benedet, Juan Lantero-Rodriguez, Theresa A. Day, Jeroen Vanbrabant, Erik Stoops, Eugeen Vanmechelen, Gallen Triana-Baltzer, Setareh Moughadam, Hartmuth Kolb, Paula Ortiz-Romero, Thomas Karikari, Carolina Minguillon, Juan José Hernández Sánchez, Irene Navalpotro-Gómez, Oriol Grau-Rivera, Rosa María Manero, Víctor Puente-Periz, Rafael de la Torre, Jaume Roquer, Jeff L. Dage, Henrik Zetterberg, Kaj Blennow, and Marc Suárez-Calvet. Drafting of the manuscript: Nicholas J. Ashton, Albert Puig-Pijoan, Kaj Blennow, and Marc Suárez-Calvet. Statistical analysis: Albert Puig-Pijoan, Marta Milà-Alomà, Aida Fernández-Lebrero, Greta García-Escobar, and Marc Suárez-Calvet. Obtained funding: Irene Navalpotro-Gómez, Carolina Minguillón, Jaume Roquer, Henrik Zetterberg, Kaj Blennow, and Marc Suárez-Calvet. Administrative, technical, or material support: Aida Fernández-Lebrero, Greta García-Escobar, Fernando González-Ortiz, Przemysław R. Kac, Wagner S. Brum, Juan Lantero-Rodriguez, Paula Ortiz-Romero, and Carolina Minguillon. Supervision: Nicholas J. Ashton, Kaj Blennow, and Marc Suárez-Calvet. All authors critically reviewed and approved the final manuscript.

ADDITIONAL INFORMATION

All requests for raw and analyzed data and materials will be reviewed promptly by the senior authors to verify whether the request is subject to any intellectual property or confidentiality obligations. Bulk anonymized data can be shared by request from any qualified investigator for the sole purpose of replicating procedures and the results presented in the article, providing data transfer agrees with EU legislation and decisions by the institutional review board (IRB) of each participating center.

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CONFLICTS OF INTEREST

Theresa A. Day is an employee and shareholder of Eli Lilly and Company. Jeroen Vanbrabant and Erik Stoops are employees of ADx NeuroSciences. Eugeen Vanmechelen is co-founder of ADx NeuroSciences. Gallen Triana-Baltzer, Setareh Moughadam, and Hartmuth Kolb are employees of Janssen Research and Development. Jeff L. Dage is an inventor on patents associated with reagents used in the Lilly assays, is a minor shareholder of Eli Lilly and Company stock and receives support from Eli Lilly and Company and Roche Diagnostics. Henrik Zetterberg has served on scientific advisory boards for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Kaj Blennow has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg. Marc Suárez-Calvet has served as a consultant and on advisory boards for Roche Diagnostics International Ltd and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U and Roche Farma, S.A. Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

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