Neurobiology of Aging 143 (2024) 30–40

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging.org

Plasma p-tau181 and amyloid markers in Alzheimer's disease: A comparison between Lumipulse and SIMOA

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ARTICLE INFO

Keywords:
Plasma markers
Alzheimer's disease
Lumipulse
SIMOA
CSF
Validation

ABSTRACT

Aim of the project was to evaluate the technical and clinical validity of plasma Lumipulse p-tau, Aβ42 and Aβ40 species and their correlation with CSF core Alzheimer's Disease (AD) markers; a method comparison with SIMOA was also performed. One-hundred-thirthy-three participants, namely 55 A+T+N+ AD, 28 Neurodegenerative disorders (NDD) and 50 controls were enrolled for the study. Lumipulse technical validity showed high stability for p-tau181, Aβ42, and Aβ40, with higher stability of p-tau to repeated freezing thaw cycles. p-tau181 levels detected by both techniques were higher in AD compared to both NDD/controls and exhibited a similar correlation with CSF p-tau levels, whereas Aβ42 levels were slightly lower in AD with both methods. In the comparison between SIMOA and Lumipulse plasma markers, both techniques exhibited similar diagnostic accuracy for AD for p-tau181 (0.87; 95 %CI 0.81–0.94, vs 0.85; 95 %CI 0.78–0.93), whereas the best performance was reached by p-tau181/ Aβ42 Lumipulse ratio (ROC AUC 0.915, 95 %CI 0.86–0.97). The study thus confirmed the

Aabbreviations: AD, Alzheimer's diseases; Aβ, amyloid beta; CDR, Clinical Dementia Rating Scale; CN, cognitively normal; CSF, Cerebrospinal fluid; CV, coefficient of variation; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; LP, lumbar puncture; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NDD, neurodegenerative disorders; NPI, Neuropsychiatric Inventory; P-tau, phosphorylated tau; RT, room temperature; RUO, research use only; ULTF, ultra-low temperature freezing.

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https://doi.org/10.1016/j.neurobiolaging.2024.08.007

Available online 22 August 2024

construct validity of both Lumipulse and SIMOA techniques for the identification of CSF AD pattern in clinical settings.

1. Introduction

Cerebrospinal fluid (CSF) biomarkers are informative, sensitive, and specific for detection of Alzheimer's diseases (AD) already in early stages (Hansson, 2021). The recent development of plasma biomarkers using Single Molecule Array (SIMOA) technique is changing the era of the diagnosis of AD potentially shifting from CSF to blood assessments (Blennow, 2021; Leuzy et al., 2021a; Zetterberg and Blennow, 2021).

Blood biomarkers may provide significant advantages for clinical practice, as they are less invasive and have the potential for greater suitability in widespread applications (Alawode et al., 2021).

A number of assays have been developed to measure p-tau isoforms in blood, which were recently reviewed in Karikari *et al.* (Karikari et al., 2021). Currently, the most promising blood biomarkers developed by SIMOA technique for detecting AD pathology are phosphorylated tau species (including p-tau181, p-tau217 and p-tau231) (Bayoumy et al., 2021) and amyloid Aβ42/40 ratio (Ashton et al., 2021b; Gonzalez-Ortiz et al., 2023b; Karikari et al., 2020; Mielke et al., 2022; Palmqvist et al., 2019; Schindler et al., 2019; Simrén et al., 2021; Suárez-Calvet et al., 2020). Most published studies focused on p-tau species have employed immunoassays on either the Meso Scale Discovery (MSD) platform (Janelidze et al., 2020; Mielke et al., 2018; Thijssen et al., 2020) or the SIMOA system (Karikari et al., 2020) both with analytical sensitivity below the picomolar range. The recent development of assays by using the chemiluminescent enzyme immunoassay (CLEIA) technology (including the fully-automated Lumipulse platform) represent an attractive further step for their application and wider use in clinical practice (Martínez-Dubarbie et al., 2023). Preliminary data showed a high concordance between Lumipulse plasma p-tau181 levels and amyloid positron emission tomography (PET) and AD diagnosis (Mielke et al., 2021). Moreover, the species identified by the specific antibodies used for Lumipulse automatic detection had proven to be highly effective for the clinical diagnosis of AD compared to SIMOA and MSD assays (Ashton et al., 2023). Despite the growing evidence in the field, there is still a need for validation of Lumipulse plasma biomarkers in real-life scenarios to challenge their technical and biological consistency and validity, in comparison with the more established SIMOA testing.

Specifically, the low fold change (around a 10 % reduction) in the Aβ42/40 ratio in brain amyloidosis positive cases (Janelidze et al., 2021) results in a low robustness of clinical classifications (Rabe et al., 2023) and the performance of this biomarker is also affected by pre-analytical handling of samples (Benedet et al., 2022) and commonly used drugs may result in false positives (Brum et al., 2023b), further supporting the need of real-life studies.

The aim of our study was thus to evaluate the newly developed plasma p-tau181, Aβ42, and Aβ40 biomarkers on LUMIPULSE G600II automated platform and assess their technical validity and correlations with CSF biomarkers in consecutively recruited participants with neurodegenerative disorders. A secondary aim of the research was to evaluate the correlations between biomarkers tested with CLEIA and SIMOA to challenge their biological consistency and explore their diagnostic value in exploratory analyses.

2. Methods

2.1. Study population

The study included participants with mild cognitive impairment (MCI) or mild dementia who underwent CSF assessment at the outpatient Neurodegenerative clinic of the Brescia University Hospital, Italy. Participants who fulfilled a clinical diagnosis of AD according to NIA-AA criteria (Jack et al., 2018), dementia with Lewy bodies (DLB) according to DLB consortium criteria (McKeith et al., 2020, 2017) or frontotemporal dementia (FTD) according to FTD consortium criteria (Gorno-Tempini et al., 2011; Rascovsky et al., 2011) were consecutively enrolled in the study. A standardized full cognitive and behavioural assessment, including Mini-Mental State Examination (MMSE), Neuropsychiatric Inventory (NPI) and Clinical Dementia Rating Scale (CDR) was performed in each participant. Only participants with A+T+N+ biological confirmation of AD were included. The internal cut-off values used in the laboratory were Aβ42 < 650 pg/mL, p-tau > 60 pg/mL, t-tau > 400 pg/mL and p-tau181/Aβ42 ratio > 0.09 (Pilotto et al., 2022). Patients with a clinical diagnosis of FTD and DLB were included in the study only when the p-tau181/Aβ42 ratio resulted negative to (<0.09); participants with MCI with negative CSF amyloid markers were defined as MCI-NDD. A group of neurologically and cognitively normal (CN) individuals were recruited from participants' caregivers and were included as reference group for plasma analyses.

2.2. CSF collection and analysis

Each patient underwent lumbar puncture (LP) in fasting condition according to the standardized protocol of the outpatient Neurodegenerative clinic. The CSF specimens were collected in 15 mL polypropylene sterile tubes, gently mixed to avoid gradient effects, and sent directly to the hospital laboratory for routine assessments. The remaining CSF volume was stored in 2 mL polypropylene tubes at −20°C, and afterwards placed at −80°C. CSF Aβ42, Aβ40, p-tau181, and total tau were measured using the Lumipulse G assays (Fujirebio) on the LUMIPULSE G600II for diagnostic standard analyses. As by the guidelines delivered by the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry, CSF samples were subjected to a maximum of two freeze-thaw cycles (Lewczuk et al., 2018).

2.3. Plasma collection and analysis

Blood samples were collected in 7.5 mL tubes with K2-ethylenediaminetetraacetic acid (K2-EDTA) per each participant. The blood was mixed by gently inverting the tubes 5–10 times. The tubes were thereafter centrifuged at 2500×g for 10 min at room temperature (RT). Plasma aliquots of 0.5 mL were pipetted into polypropylene cryotubes and frozen at ultra-low temperature freezing (ULTF) −80°C for storage.

Plasma samples were brought to RT (21–23 °C) on the day of the analysis. As per manufacturer instructions, plasma samples were centrifuged for 5 min at 2000 g. Afterwards, plasma was transferred to the instrument cuvettes for being tested on Lumipulse by using: Lumipulse G pTAU 181 – Plasma Immunoreaction Cartridges RUO (research use only; cat#81288), Lumipulse G AB1–42 Plasma Immunoreaction Cartridges RUO (cat#81301), and Lumipulse G AB1–40 – Plasma Immunoreaction Cartridges RUO (cat#81298).

SIMOA analyses were performed on SR-X using the same pre-analytical preparation with commercially available p-tau181 Advantage V2 Kits (Quanterix) (Pilotto et al., 2022) and SIMOA Neurology 3-Plex Advantage Kits (Aβ40, Aβ42, Tau).

The Lumipulse technical reliability and the accuracy of plasma Immunoreaction Cartridges RUO were investigated using an intra- and inter-day testing schemes according to Vidali *et al.* and based on CLSI EP15 (Neill Carey et al., 2014; Vidali et al., 2016). Briefly, three different plasma aliquots from the same participant were tested as independent samples for 5 different runs (intra-day testing), to verify the testing

repeatability. Furthermore, to assess the inter-day repeatability of the test, other three plasma aliquots of the same participant were tested in 5 days (inter-day testing), for 15 total runs of the same sample.

2.4. Statistical analyses

Normality distribution was evaluated using the Shapiro-Wilk test and Q-Q plots. Clinical and demographic characteristics as well as cognitive assessments and CSF and plasma markers comparisons between diagnostic groups (AD, NDD and CN) were performed using the Kruskal-Wallis test and the ANOVA test, as appropriate. The comparison between AD and NDD/CN was performed by the Mann-Whitney U test. For biomarkers comparison, the significance levels were set to p=0.01 as multiple comparison correction. The comparability between the two analytical platforms was assessed using Passing-Bablok regression, while their imprecision was assessed by calculating the laboratory's coefficient of variation (CV). The relationship between plasma and CSF biomarkers was calculated with Spearman's test in a correlation matrix. The accuracy of plasma biomarkers in terms of specificity and sensibility to discriminate between AD and non-AD participants was analysed by receiver-operating characteristic (ROC) approach. We computed areas under the curve (AUCs) and their 95 % confidence intervals (CI). Comparison between AUC ROC performance was evaluated using DeLong non-parametric Method (DeLong et al., 1988) All analyses were performed with R Statistical Software (https://www.r-project.org/). Statistical significance was set as α=0.05, and all tests were two-tailed.

3. Results

3.1. Precision and repeatability of Lumipulse G600II testing

A set of K2EDTA plasma samples collected from one AD CSF-confirmed patient (CSF t-tau=809 pg/mL, p-tau181=132.6 pg/mL, Aβ42=446 pg/mL and Aβ40=9128 pg/mL) were aliquoted and tested as fifteen independent samples. The CVs of p-tau181, Aβ42 and Aβ40 were 2.4 %, 3.1 % and 2.8 %, respectively, for 15 repeated measures (see Suppl. Table 1). The inter-day reproducibility of the Lumipulse platform was verified using the ready-to-use controls Level 1 (L1) and Level 2 (L2) given by the manufacturer tested in 5 different days. The mean inter-assay CV was less than 5 % for all three analytes. Specifically, for L1, p-tau181 had a CV of 3.58 %, Aβ42 had a CV of 2.64 %, and Aβ40 had a CV of 0.89 %. For L2, p-tau181 had a CV of 1.89 %, Aβ42 had a CV of 3.19 %, and Aβ40 had a CV of 2.15 % (see Suppl. Table 2). In addition, 15 independent plasma samples, aliquoted from the same patient, were first collected from ULTF −80°C and then stored at −20°C for testing along different consecutive days. P-tau181 levels exhibited good stability, with a CV around 20 % for all three aliquots along the 5 days (aliquot-1 CV=20.75 %; aliquot-2 CV=19.99 %; aliquot-3 CV=18.82 %; Suppl. Table 2). Aβ42 and Aβ40 samples showed lower stability starting with the third day of assessment. Specifically, all three aliquots exhibited a fair stability during the first two days ($A\beta42_{day1}$=15.65 pg/mL at day1, $A\beta42_{day2}$=11.43 pg/mL; $A\beta40_{day1}$=237.68 pg/mL; $A\beta40_{day2}$=183.10 pg/mL), with a similar trend of decrease in total levels between 75 % and 85 % from day 1 to day 3 for Aβ42 and 78–86 % for Aβ40 (see supplementary materials for detailed day per day levels, Suppl. Table 2).

3.2. Cerebrospinal fluid and plasma biomarkers analyses

The clinical study validation comprised 133 participants, namely 83 patients and 50 controls. The clinical assessment and CSF AD markers allowed the classification of patients in 55 AD (40 with MCI and 15 having mild dementia) and 28 other neurodegenerative disorders (including 8 DLB and 5 FTD and 15 MCI with negative AD markers, MCI-NDD). Clinical and demographics data and CSF core biomarkers are indicated in Table 1; age and sex were used as covariates given the slight

Table 1
Participants' characteristics.

	CN (N=50)	AD(N=55)	NDD(N=28)	p-value
Age	65.28 (7.37)	72.37 (7.93)	71.71 (5.86)	<0.001[a]
Sex (F:M)	28:22	36:19	8:20	NS
MMSE	29.20 (1.2)	22.10 (5.01)	25.25 (3.91)	0.001[a]
NPI	-	10.71 (9.08)	12.95 (8.97)	0.285
APOE ε4, ε3-ε4 carriers, n (%)	-	34 (62.15 %)	2 (11.11 %)	0.637
AD CSF core biomarkers – LUMIPULSE G600II				
p-tau181 (pg/mL)	-	107.12 (58.96)	44.67 (20.90)	<0.001
T-tau (pg/mL)	-	686.01 (385.09)	468.29 (414.02)	0.002
Aβ42 (pg/mL)	-	537.06 (146.95)	1025.85 (543.30)	<0.001
Aβ40 (pg/mL)	-	11075.20 (3922.06)	10217.00 (5062.81)	0.308
p-tau181/Aβ42	-	1.94 (0.98)	0.54 (0.38)	<0.001
Aβ42/Aβ40	-	0.05 (0.01)	0.10 (0.05)	<0.001

Data are expressed as mean (M) and standard deviation (SD)
p-values showed the difference between AD CSF core biomarkers profile groups and were computed with a Mann-Whitney U test (age, MMSE, NPI, AD CSF core biomarkers), or a chi-square (sex, *APOE* ε4 carrier status).
NPI was not available in 15 (11.28 %) individuals; *APOE* was not available in 14 (10.52 %) participants.
Aβ42, amyloid beta 1–42; AD, Alzheimer's disease; CN, cognitively normal; MMSE, Mini-Mental State Examination; mL, millilitres; NDD, non-Alzheimer neurodegenerative disorders; p-tau181, p-tau 181 isoform; pg, picograms. The 181/42 ratio is increased of a 10 factor.
[a] = significant comparison CN versus AD

Table 2
Plasma biomarkers assessed by Lumipulse and SIMOA platforms.

	CN(N=50)	AD(N=55)	NDD(N=28)	p-value
Plasma biomarkers – LUMIPULSE				
Plasma p-tau181 (pg/mL)	1.61 (0.56)	3.33 (1.45)	2.08 (1.07)	<0.001[a, b]
Plasma Aβ42 (pg/mL)	23.57 (4.91)	16.06 (4.61)	20.08 (8.21)	<0.001[a, b*]
Plasma Aβ40 (pg/mL)	273.18 (57.15)	243.23 (57.146)	270.51 (110.97)	NS[¤]
Plasma Aβ42/40	0.09 (0.009)	0.07 (0.012)	0.08 (0.02)	<0.001[a, b]
Plasma p-tau181/Aβ42	0.07 (0.03)	0.23 (0.13)	0.13 (0.11)	<0.001[a, b]
Plasma biomarkers – SIMOA				
Plasma p-tau181 (pg/mL)	2.23 (3.56)	3.37 (1.70)	2.20 (1.56)	<0.001[a, b]
Plasma Aβ42 (pg/mL)	8.43 (2.38)	5.41 (1.81)	6.23 (3.77)	0.021[a]
Plasma Aβ40 (pg/mL)	193.85 (56.38)	151.79 (52.06)	149.42 (98.50)	0.006[a]
Plasma Aβ42/40	0.04 (0.007)	0.03 (0.009)	0.05 (0.03)	<0.001[a]
Plasma p-tau181/Aβ42	0.22 (0.21)	0.66 (0.43)	0.43 (0.31)	<0.001[a, b]

Kruskal-Wallis (3 groups) and Mann-Whitney U test
¤ANOVA test
a = significant comparison CN versus AD
b = significant comparison AD versus NDD
*p = 0.006 for AD versus NDD (W = 1037.00)

but significant distribution between-groups. Table 2 and Fig. 1 showed the between-groups distribution of plasma biomarkers assessed by Lumipulse and SIMOA. Plasma levels of p-tau181 measured by Lumipulse were around two-fold higher in patients with AD compared to NDD and CN ($p<0.001$, Table 2 and Fig. 1, Panel A). Aβ42 levels were lower in patients with AD compared to both NDD patients ($p=0.006$) and CN ($p<0.001$), whereas no differences were detected for Aβ40 plasma between groups. The ratio of p-tau181/Aβ42 was significantly higher in AD compared to both NDD and CN groups ($p<0.001$, Table 2 and Fig. 1, panel B); Aβ42/40 ratio was lower in AD compared to NDD and CN ($p<0.001$, Table 2 and Fig. 1, panel C).

Plasma biomarkers measured by SIMOA showed very similar between-group differences for each biomarker compared to Lumipulse. Specifically, p-tau181 levels and p-tau181/Aβ42 ratio were higher in AD compared to NDD and CN (Fig. 2). Plasma Aβ42 and Aβ40 levels were lower in AD compared to CN but similar compared to NDD. Plasma Aβ42/40 ratio was lower in AD compared to CN (Fig. 2).

The correlation matrix evaluating the correlations between Lumipulse and SIMOA and CSF core biomarkers is presented in Fig. 3. The Passing-Bablok regression was applied to compare the two methods for plasma levels of Aβ40, Aβ42 and p-tau181.

Aβ40 and Aβ42 concentrations are underestimated by SIMOA (high bias, which is seen in both the intercept of Passing Bablok and the absolute or percentage difference in Bland Altman), whereas for the p-tau181 levels there is no significant difference between SIMOA and Lumipulse as by the slope and intercept of Passing Bablok and the minimal difference in Bland Altman analysis (Results and graphs of Passing-Bablok regression and Bland-Altman plots are showed in supplementary materials; Suppl. Table 3, Suppl. Table 4, Suppl. Figure 1). The correlation matrix (Fig. 3), as well the density plots and distribution graph presented in Fig. 4 (Panel A) demonstrate the positive correlation of p-tau181 in CSF and plasma analysed using the Lumipulse platform. A similar positive correlation was found for plasma and CSF p-tau181 levels tested by SIMOA (Lumipulse, Fig. 4, Panel B; $\varrho=0.561$, $p<0.001$). Likewise, p-tau181 Lumipulse/SIMOA levels in plasma strongly correlated ($\varrho=0.680$, $p<0.001$, Fig. 4, Panel E). Plasma levels of Aβ40 and Aβ42 did not correlate with CSF markers neither for Lumipulse ($\varrho=0.018$, $p=0.899$; $\varrho=0.039$, $p=0.746$, respectively) nor for the SIMOA analysis ($\varrho=0.163$, $p=0.268$; $\varrho=0.169$, $p=0.166$, respectively). Conversely, the levels of both Aβ40 and Aβ42 in plasma exhibited a robust relationship between the two detection techniques (Aβ40, $\varrho=0.585$, $p<0.001$; Aβ42, $\varrho=0.711$, $p<0.001$; see Fig. 4, Panels C and D). CSF and plasma Aβ42/40 ratios correlated positively (Lumipulse $\varrho=0.491$, $p<0.001$; SIMOA $\varrho=0.363$, $p=0.013$), and there was a significant correlation between Lumipulse and SIMOA ($\varrho=0.487$, $p<0.001$; see Fig. 4, Panel F) on plasma. The plasma p-tau181/Aβ42 ratios exhibited a strong association between the two techniques ($\varrho=0.637$, $p<0.001$), but no correlation with CSF ratios (Fig. 3 and Fig. 4). Additional correlation analysis has been carried out separately for AD and NDD. In both groups, p-tau181 in CSF and plasma using either Lumipulse or SIMOA resulted weak and not significant (Suppl. Figures 2 and 3). p-tau181/Aβ42 ratio in AD group using Lumipulse showed a low positive and significant correlation ($\varrho=0.325$, $p=0.031$) between CSF and plasma, while a negative and not significant correlation ($\varrho=-0.011$, $p=0.944$) using SIMOA. The two testing platforms for p-tau181 showed a high correlation for both groups (AD: $\varrho=0.888$, $p<0.001$; NDD: $\varrho=0.826$, $p<0.001$). Conversely, p-tau181/Aβ42 ratio resulted positively correlated between Lumipulse and SIMOA for AD ($\varrho=0.464$, $p<0.001$), though not for NDD ($\varrho=0.363$, $p=0.088$).

3.3. Exploratory discriminant analyses for AD diagnosis

The discrimination accuracy of plasma biomarkers analyzed with Lumipulse and SIMOA techniques for the diagnosis of A+T+N+ AD was separately evaluated using AUC-ROC analysis; AD samples were compared to non-AD samples, including CN (Fig. 5). Plasma p-tau181 and the Aβ42/40 ratio, analyzed on the Lumipulse system, showed AUCs of 0.875 (95 %CI 0.813 – 0.938) and 0.897 (95 %CI 0.842 – 0.952), respectively. P-tau181/Aβ42 ratio was found to be the most reliable marker presenting an AUC of 0.915 (95 %CI 0.863 – 0.967; see Suppl.

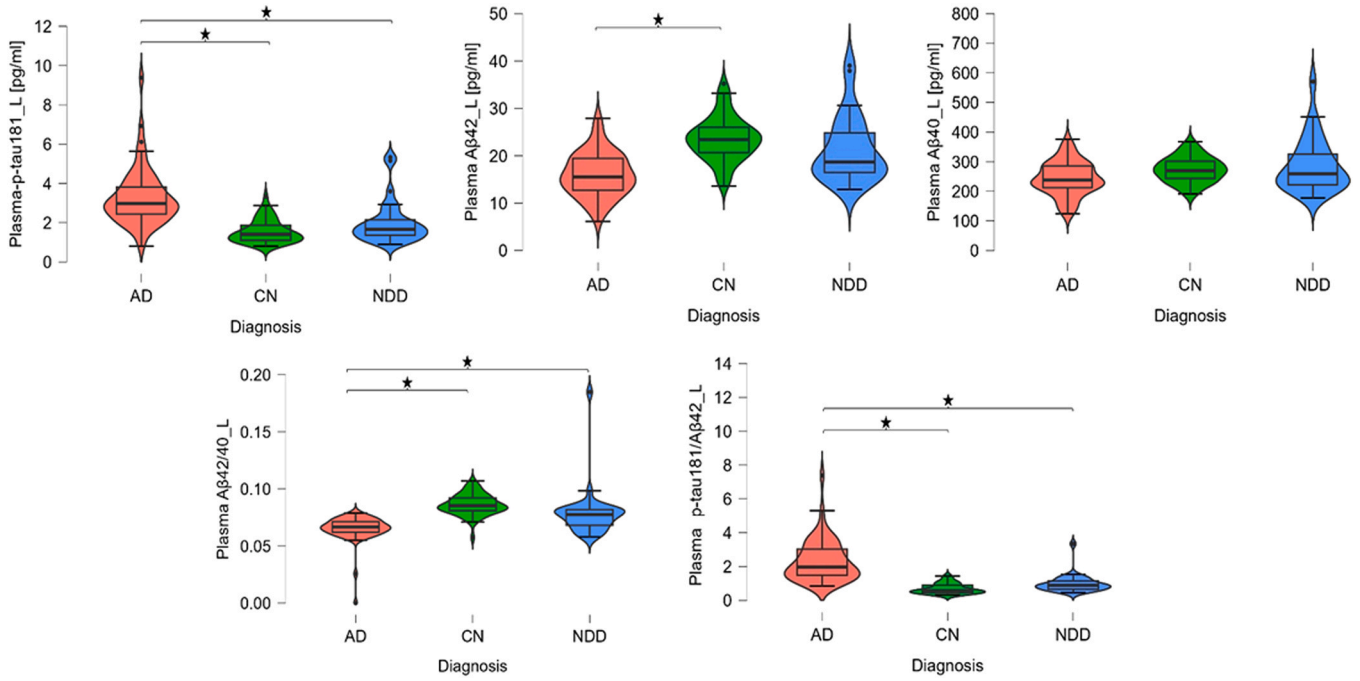

Fig. 1. Plasma p-tau181, Aβ42, Aβ40, Aβ42/40 and p-tau181/Aβ42 levels in AD, CN, and NDD groups measured using Lumipulse. p-tau181 is significantly higher in AD compared to both CN and NDD. Conversely, Aβ42 is significantly lower in AD in respect to CN; Aβ40 did not show any significant difference among the three tested groups. Noteworthy, both ratios Aβ42/40 and p-tau181/Aβ42 can effectively discriminate AD towards CN and NDD. AD, Alzheimer's disease; CN, cognitively normal; NDD, non-Alzheimer neurodegenerative disorders.; p-tau181_L, phosphorylated tau 181 tested on Lumipulse (L); Aβ42_L, β Amyloid Protein Fragment 1–42 tested on Lumipulse (L); Aβ40_L, Amyloid β Protein Fragment 1–40 tested on Lumipulse (L).

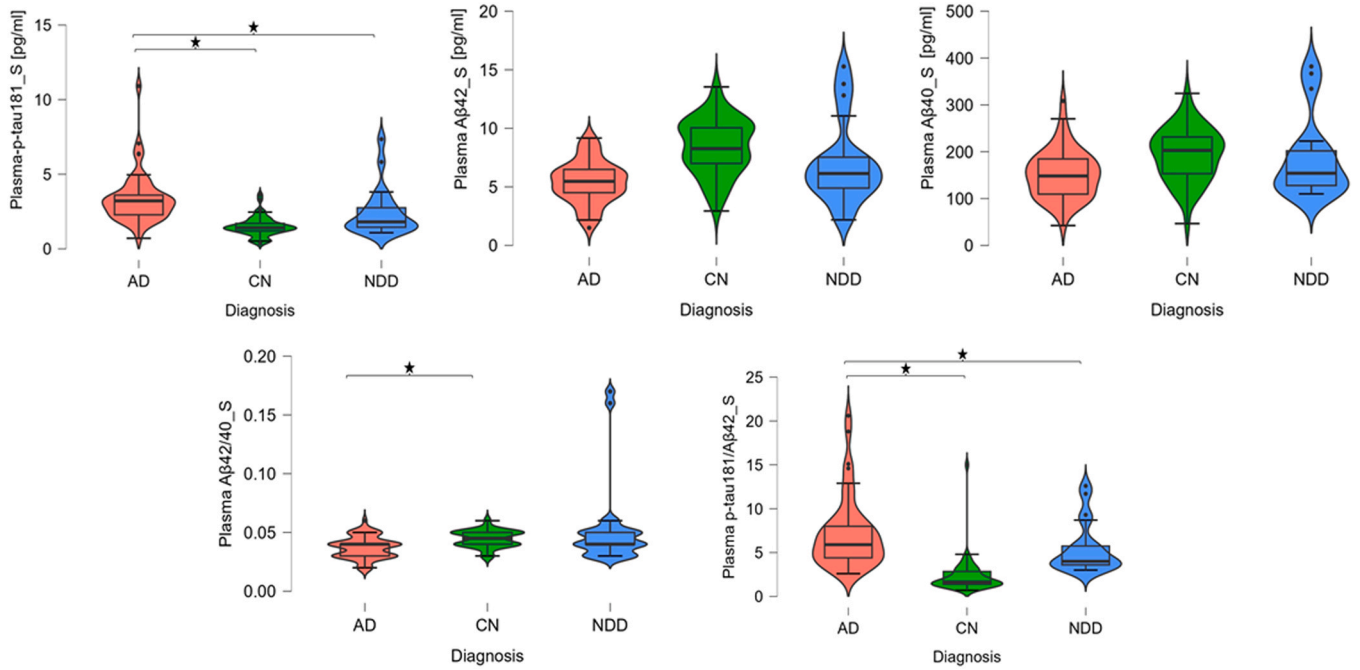

Fig. 2. Plasma p-tau181, Aβ42, Aβ40, Aβ42/40 and p-tau181/Aβ42 levels in AD, CN, and NDD groups measured using Simoa. p-tau181 is significantly higher in AD compared to both CN and NDD. Aβ42 and Aβ40 is significantly lower in AD in respect to CN; Aβ420 did not show any significant difference among the three tested groups. Noteworthy, both ratios Aβ42/40 and p-tau181/Aβ42 can effectively discriminate AD towards CN and NDD. AD, Alzheimer's disease; CN, cognitively normal; NDD, non-Alzheimer neurodegenerative disorders.; p-tau181_S, phosphorylated tau 181 tested on SIMOA (S); Aβ42_S, β Amyloid Protein Fragment 1–42 tested on SIMOA (S); Aβ40_S, Amyloid β Protein Fragment 1–40 tested on SIMOA (S).

Table 5, Suppl. Table 6). On top of that, both plasma p-tau181/Aβ42 ratio and p-tau181 showed a higher fold-change (FC) than Aβ42/40 ratio ($FC_{p\text{-}tau181/A\beta42}$=3.29; $FC_{p\text{-}tau181}$=2.05; $FC_{A\beta42/40}$=0.76; Suppl. Table 7).

Plasma p-tau181 tested on SIMOA and the resulting p-tau181/Aβ42 ratio yielded similar diagnostic accuracy, AUC-ROC of 0.854 (95 %CI 0.782 – 0.925) and 0.867 (95 %CI 0.800 – 0.933), respectively. Aβ42/40 ratio assessed by SIMOA reached an AUC of 0.700 (95 %CI 0.603 – 0.797). As for Lumipulse, the FC of plasma p-tau181/Aβ42 ratio and p-tau181 were 2.97 and 1.51, respectively, while $FC_{A\beta42/40}$ was 0.75. The calculated best cutoffs for Lumipulse and SIMOA were respectively: for p-tau181, 1.93 pg/mL and 1.86 pg/mL; for the ratio p-tau181/Aβ42, 0.11 and 0.37; for Aβ42/40 0.08 and 0.04 (Youden optimal cut-off method). The direct comparison between AUC-ROC curves did not show any statistically significant difference between Lumipulse and SIMOA in plasma tests for p-tau181, Aβ42/40 and p-tau181/Aβ42 ratios.

4. Discussion

This study demonstrated the excellent stability and clinical validity of p-tau181, and amyloid species detected in plasma with Lumipulse assays in comparison with the SIMOA technique. The clinical application indicated a high diagnostic performance of p-tau181 and its ratio in diagnosing AD, with very similar discriminative values of both techniques, extending previous findings with similar techniques in different settings (Bellomo et al., 2024; Gonzalez-Ortiz et al., 2023a).

The study analyzed plasma and CSF biomarkers at the same timepoint in a clinical setting scenario, thus including participants with different diseases and ages and not defined by *a priori* selection; therefore, confirming the wide applicability of such techniques in real-life settings. The technical validity of Lumipulse platform was assessed by using repeated runs to challenge the intraday and inter-day variability and stability of analytes. The technical comparison between Lumipulse G600II and SIMOA SR-X platforms demonstrated a good correlation between analyses using the Passing-Bablok regression, in line with previous data evaluating several techniques for the diagnosis of AD in clinical settings (Karikari et al., 2022; Suárez-Calvet et al., 2020). The intraday laboratory validation for Lumipulse indicated a CV under 4 % for all the analytes, evidencing the robustness and stability of such technique in standard conditions even in unselected old participants. P-tau species also appeared to be stable through inter-day testing, at least for the first two days of testing. Of note, while p-tau181 levels remained stable for multiple days testing, an important decrease of both amyloid species concentrations was observed after the third day of testing. A possible explanation might be related to the high sensitivity of amyloid species to storage conditions and temperature changes, as the shift from −80°C to −20°C storage could have affected protein concentration. It has been demonstrated that for samples stored at −20 °C, the observed variations in concentration (bias%) are greater than those observed for other storage temperatures (Musso et al., 2023). These findings highlighted once again the higher instability of amyloid species compared to p-tau species also in plasma, in line with few validations on plasma (Rózga et al., 2019; Chang et al., 2020; Walter et al., 2020; Verberk et al., 2022; Mansilla et al., 2023; Sunde et al., 2023) and large data of CSF (Lewczuk et al., 2018). Further technical validations for the specific techniques are thus warranted to challenge the stability of biomarkers *in vivo* in different settings, as the testing performed directly after −80°C storage is not always possible in different settings.

When applied in a clinical setting, plasma assessment confirmed a high biological validity, with a high discrimination accuracy for the diagnosis of AD. Both techniques showed a significantly increased levels of p-tau181 in plasma compared to both controls and participants with other neurodegenerative conditions. Plasma Aβ42/40 ratio appeared significantly reduced in AD compared to CN and NDD only using the Lumipulse technique. The effect size of tau and amyloid markers observed is similar to those described for large MESO Scale and SIMOA based validations. The findings indeed showed an increase of 2–4-fold of p-tau181 already in the early stage of the disease, with a very strong correlation with CSF p-tau values. Of note, the p-tau CSF-plasma

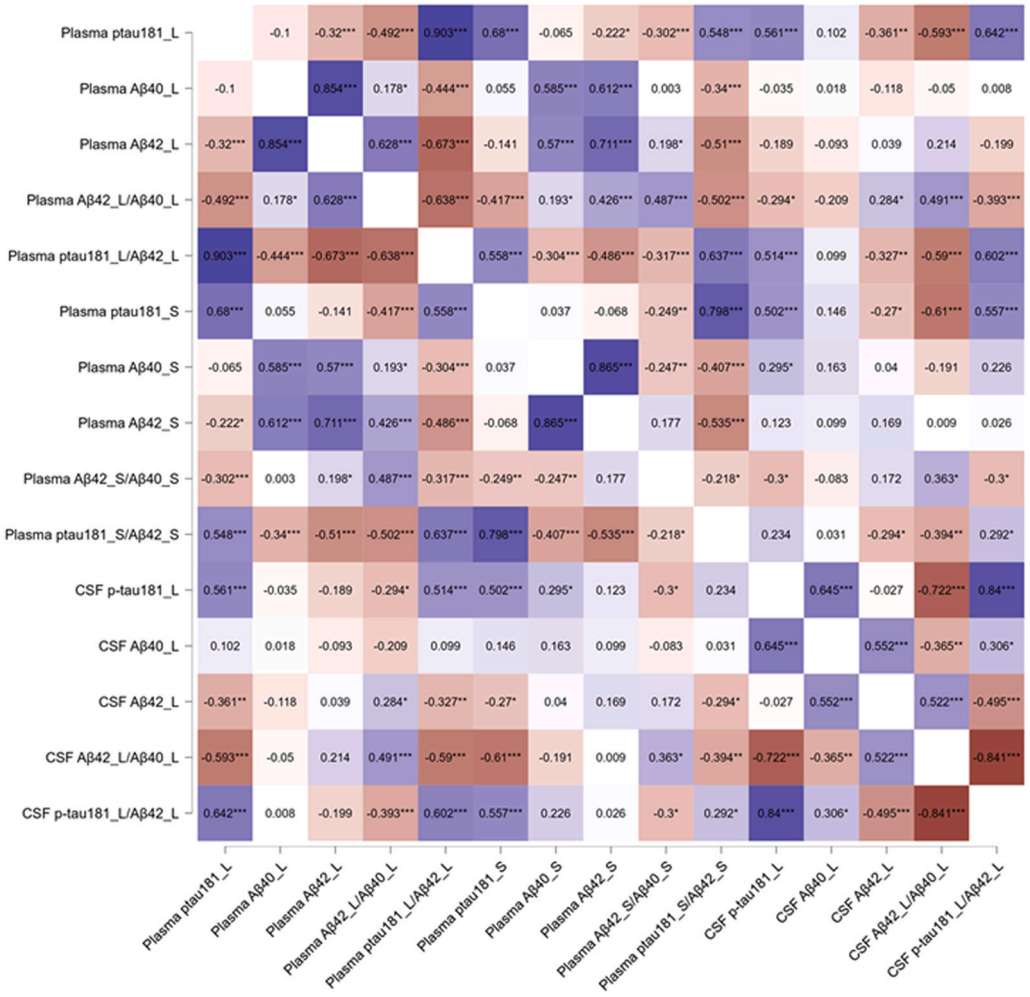

Fig. 3. Correlation matrix based on Spearman's test showing the most robust relations. Biomarkers are differentiated with "L" as for Lumipulse, instead of "S" which stands for SIMOA. Different biological samples are indicated as plasma and CSF, Cerebral Spinal Fluid.

correlations were opening the discussion about the source of plasma p-tau once AD pathology is deposited. In contrast, Amyloid Aβ42/40 exhibited only a 10 %–12 % average difference in plasma of AD patients, with no correlation with CSF amyloid species levels, a finding in line with the literature, which limit the use of Aβ42/40 ratio alone for AD diagnosis. Of note, amyloid species evaluated in plasma by Lumipulse and SIMOA techniques appeared to correlate with each other thus confirming that the detection of Aβ42 and Aβ40 is biologically valid, despite it definitively does not linearly reflect the CSF amyloid-related pathology (Benedet et al., 2022; Brum et al., 2023b). This is likely due to the fact that a major portion of Aβ in plasma does not come from the brain but from peripheral tissues (Bustamante and Zaidi, 2023; Colvin et al., 2015), which question the real value of this biomarker in plasma. Also, the low fold change of Aβ42/40, compared with CSF (and other plasma markers) limit the clinical application of amyloid ratio in clinical practice. The findings also showed that the combination of amyloid and p-tau species using the p-tau181/Aβ42 ratio had the highest diagnostic accuracy using both techniques, with slightly higher value compared to p-tau181 only and very similar values in term of sensitivity and specificity for the different techniques. This highlights, again, the general validity of Lumipulse as a promising alternative technique to SIMOA and Mesoscale, especially when considering the shift from research to clinical use (Ashton et al., 2021a; Karikari et al., 2020; O'Connor et al., 2021; Pilotto et al., 2022). Despite the small sample size, the study confirmed the diagnostic value of plasma markers using both Lumipulse and SIMOA, with similar accuracy compared to previous studies also in a non-selected population (Martínez-Dubarbie et al., 2023; Verde et al., 2023). Further on-going validation studies will explore the diagnostic accuracy, clinical impact, and cost-effectiveness impact of a wider application of plasma biomarkers in the daily routine practice for diagnosing neurodegenerative conditions (Leuzy et al., 2022; O'Connor et al., 2021; Pereira et al., 2021).

One limitation of our study is that a more comprehensive methodological assessment of storage temperature could have not been performed. The validation of new detection methods for p-tau217, which has been demonstrated to have a higher diagnostic accuracy in research settings compared to p-tau181 (Leuzy et al., 2021b), will be also an important step forward for a wider use of such biomarkers in clinical practice. The use of plasma biomarkers within clinical assessment has been now supported by large amount of literature and will definitively change the management of patients, especially when considering the development of disease-modifying treatments (Brum et al., 2023a; Mattsson-Carlgren et al., 2023).

CRediT authorship contribution statement

Alessandro Padovani: Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Diego Bertoli:** Writing – review & editing. **Cristina Mordenti:** Investigation. **Alice Galli:** Formal analysis, Data curation. **Andrea Rizzardi:** Investigation. **Salvatore Caratozzolo:** Writing – review & editing. **Alberto Benussi:** Writing – review & editing. **Virginia Quaresima:** Writing –

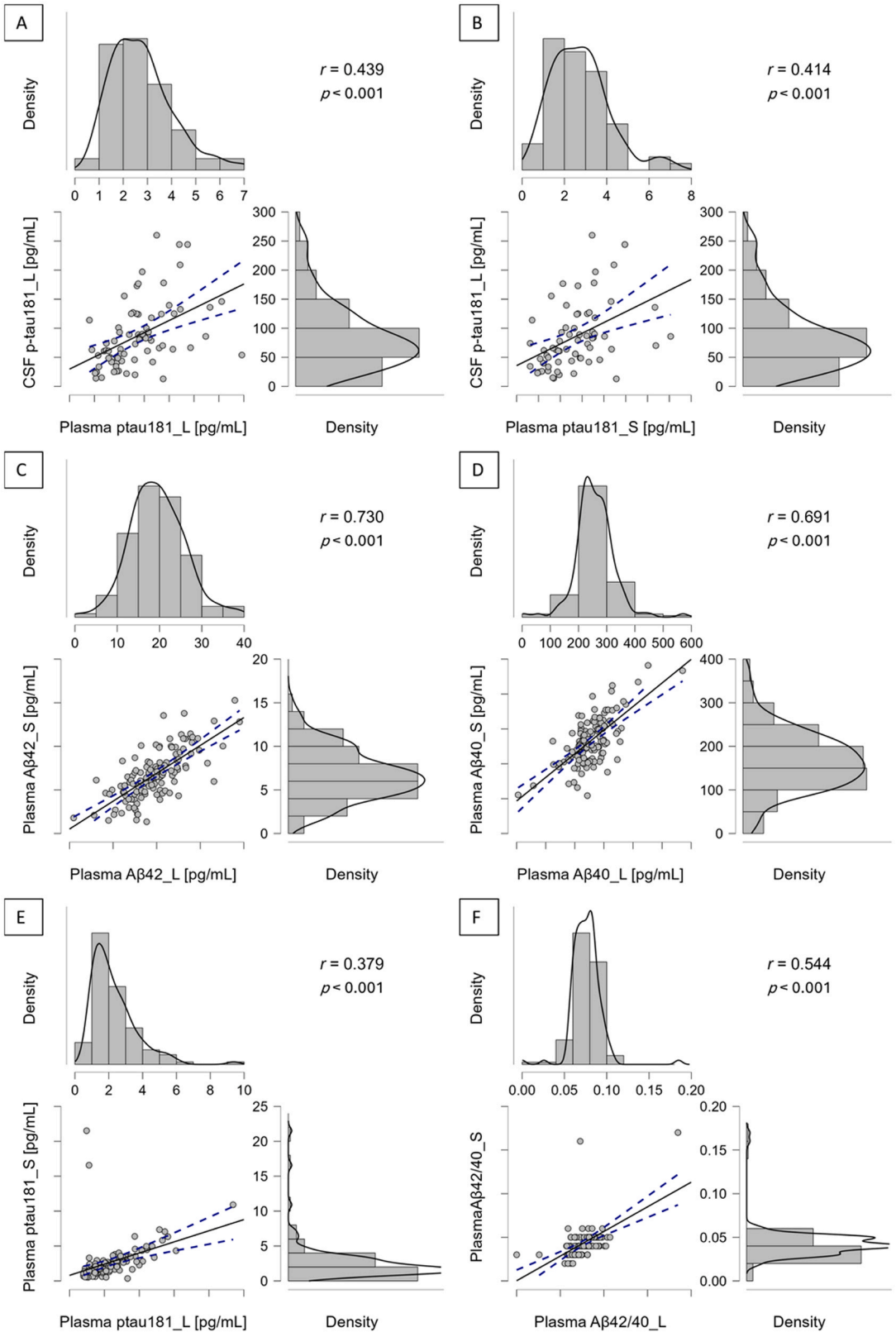

Fig. 4. Scatter plots with density curves for associated variables and 95 % CI (dashed lines). The association of CSF and plasma for p-tau181, is showed in panels A and B, which reflect CSF samples in contrast with plasma tested on Lumipulse G600II and on Simoa SR-X, respectively. Panels E and F shows the robust association of plasma p-tau181 and Aβ42/40 between Lumipulse G600II and Simoa SR-X; while the correlation of plasma Aβ42 and Aβ40 between Lumipulse and Simoa is plotted in panels C and D. L, Lumipulse; S, SIMOA; CSF, Cerebral spinal fluid; p-tau181_S, phosphorylated tau 181; Aβ42_S, β Amyloid Protein Fragment 1–42; Aβ40_S, Amyloid β Protein Fragment 1–40.

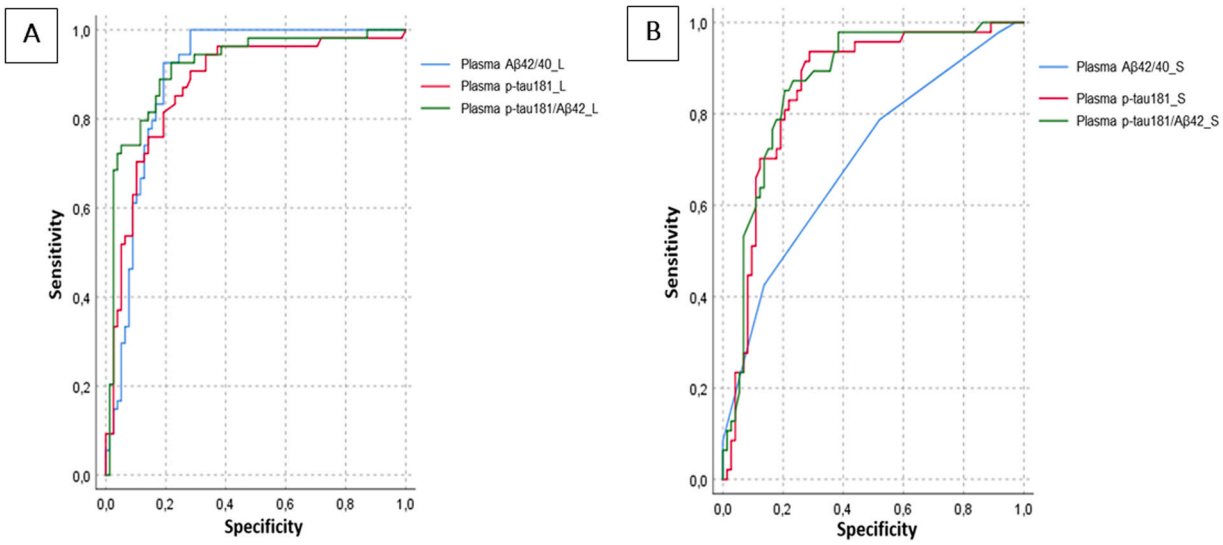

Fig. 5. ROC curves for Lumipulse G600II (L) discrimination in panel A and SIMOA SR-X (S) in panel B.

review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nicholas J Ashton:** Writing – review & editing, Validation, Supervision. **Kaj Blennow:** Writing – review & editing, Validation, Supervision. **Andrea Pilotto:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Henrik Zetterberg:** Writing – review & editing, Validation, Supervision. **Chiara Trasciatti:** Software, Formal analysis, Data curation. **Silvia Clara Giliani:** Writing – review & editing, Resources. **Chiara Tolassi:** Data curation. **Duilio Brugnoni:** Writing – review & editing, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Marta Parigi:** Data curation.

Ethics approval and consent to participate

The study was approved by the local ethics committee (NP 1471, DMA, Brescia) and was performed in conformity with the Helsinki Declaration; informed consent was obtained from each study participant or their legally authorized representative.

Declaration of Competing Interest

APi received travel grants from Bial, Abbvie, Zambon pharma, Roche, Lundbeck pharma; he received grants from Bial, Biomarin, Abbvie, CHiesi, and Zambon pharmaceuticals. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Alzecure, Biogen, Cellectricon, Fujirebio, Lilly, 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 (outside submitted work). APa received grant support from Ministry of Health (MINSAL) and Ministry of Education, Research and University (MIUR), from CARIPLO Foundation; personal compensation as a consultant/scientific advisory board member for Biogen, Lundbeck, Roche, Nutricia, General Healthcare (GE).

Acknowledgements and Funding

The authors wish to thank the study participants who took part in this research. We are very grateful for the assistance received from all the nurses of the Neurology department of the Spedali Civili of Brescia. We want to thank especially the laboratory technicians of the Central Clinical Laboratory for their amazing availability.

VQ is supported by the H2020 IMI IDEA-FAST (ID853981).

APi has been supported by grants of Airalzh Foundation AGYR2021 Life-Bio Grant, The LIMPE-DISMOV Foundation Segala Grant 2021, the Italian Ministry of University and Research PRIN COCOON (2017MYJ5TH) and PRIN 2021 RePlast, the H2020 IMI IDEA-FAST (ID853981), Italian Ministry of Health, Grant/Award Number: RF-2018-12366209 and PNRR-Health PNRR-MAD-2022-12376110.

CTr is supported by the Italian Ministry of University and Research.

CTo is supported by the Ministry of Health PRIN 2021 RePlast.

AG is supported by the Ministry of Health PRIN 2021 RePlast and the H2020 IMI IDEA-FAST (ID853981).

AR is supported by the H2020 IMI IDEA-FAST (ID853981) and the PNRR-Health PNRR-MAD-2022-12376110.

AB was partially supported by Fondazione Cariplo (grant n° 2021-1516), and by the Fondation pour la Recherche sur Alzheimer.

HZ is a Wallenberg Scholar and a Distinguished Professor at the Swedish Research Council supported by grants from the Swedish Research Council (#2023-00356; #2022-01018 and #2019-02397), the European Union's Horizon Europe research and innovation program under grant agreement No 101053962, Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C, and #ADSF-24-1284328-C), the Bluefield Project, Cure Alzheimer's Fund, the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2022-0270), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860197 (MIRIADE), the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre, and the UK Dementia Research Institute at UCL (UKDRI-1003).

APa has been supported by grants of the Italian Ministry of University and Research PRIN COCOON (2017MYJ5TH) and PRIN 2021 RePlast, the H2020 IMI IDEA-FAST (ID853981), Italian Ministry of

Health, Grant/Award Number: RF-2018-12366209 and PNRR-Health PNRR-MAD-2022–12376110.

Authors' contributions

VQ and APi: contributed to design the work, acquisition, analysis, interpretation of data. CTo, MP, DB, CM, AG, AR: data acquisition and analysis. CTr: data analysis and statistics. APa, DBr: have drafted the work and substantively revised it. SC, AB, NJA, KB, HZ, SG: revised and approved the submitted version.

Consent for publication

Not applicable.

Additional material

Supplementary File.docx, Method comparison and Passing-Bablok regression

Supplementary tables**.docx**, 7 additional tables about repeatability tests ROC analysis and Fold-change values

Supplementary Figure 1**.pptx**, Passing-Bablok regression analysis and Bland-Altman graphs for Aβ40 (panel A), Aβ42 (panel B) and p-tau181 (panel C)

A) Panel A shows the scatter diagram with regression line (dashed red line) for Aβ40 measures. Identity line is dashed. Regression line equation: y = −114.5695 + 1.1061x; 95 % CI for intercept −176.02 to −58.64 and for slope 0.92–1.34. Cusum test for linearity indicates no significant deviation from linearity (P > 0.05). The Bland-Altman graph plots the distribution of difference around fitted regression line.

B) Panel B shows the scatter diagram with regression line (dashed red line) for Aβ42 values. Identity line is dashed. Regression line equation: y = −1.0417 + 0.4016x; 95 % CI for intercept −2.50 to −1.14 and for slope 0.35–0.47. Cusum test for linearity indicates no significant deviation from linearity (P > 0.05). The Bland-Altman graph plots the distribution of difference around fitted regression line.

C) Panel C shows the scatter diagram with regression line (dashed red line) for p-tau181 data. Identity line is dashed. Regression line equation: y = −0.3398 + 1.2023x; 95 % CI for intercept −0.64 to −0.06 and for slope 1,08 to 134. Cusum test for linearity indicates no significant deviation from linearity (P > 0.05). The Bland-Altman graph plots the distribution of difference around fitted regression line.

Supplementary Figure 2**.pptx**, Correlation matrix of AD patients

Suppl. Figure 2. Correlation matrix based on Spearman's test showing the most robust relations among AD cohort. Biomarkers are differentiated with "L" as for Lumipulse, instead of "S" which stands for SIMOA. Different biological samples are indicated as plasma and CSF, Cerebral Spinal Fluid.

Supplementary Figure 3**.pptx**, Correlation matrix of NDD patients

Suppl. Figure 3. Correlation matrix based on Spearman's test showing the most robust relations among NDD patients. Biomarkers are differentiated with "L" as for Lumipulse, instead of "S" which stands for SIMOA. Different biological samples are indicated as plasma and CSF, Cerebral Spinal Fluid.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neurobiolaging.2024.08.007.

References

Alawode, D.O.T., Heslegrave, A.J., Ashton, N.J., Karikari, T.K., Simrén, J., Montoliu-Gaya, L., Pannee, J., OConnor, A., Weston, P.S.J., Lantero-Rodriguez, J., Keshavan, A., Snellman, A., Gobom, J., Paterson, R.W., Schott, J.M., Blennow, K., Fox, N.C., Zetterberg, H., 2021. Transitioning from cerebrospinal fluid to blood tests to facilitate diagnosis and disease monitoring in Alzheimer's disease. J. Intern. Med. 290, 583–601. https://doi.org/10.1111/JOIM.13332.

Ashton, N.J., Pascoal, T.A., Karikari, T.K., Benedet, A.L., Lantero-Rodriguez, J., Brinkmalm, G., Snellman, A., Schöll, M., Troakes, C., Hye, A., Gauthier, S., Vanmechelen, E., Zetterberg, H., Rosa-Neto, P., Blennow, K., 2021b. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. Acta Neuropathol. 141, 709–724. https://doi.org/10.1007/s00401-021-02275-6.

Ashton, N.J., Janelidze, S., Al Khleifat, A., Leuzy, A., van der Ende, E.L., Karikari, T.K., Benedet, A.L., Pascoal, T.A., Lleó, A., Parnetti, L., Galimberti, D., Bonanni, L., Pilotto, A., Padovani, A., Lycke, J., Novakova, L., Axelsson, M., Velayudhan, L., Rabinovici, G.D., Miller, B., Pariante, C., Nikkheslat, N., Resnick, S.M., Thambisetty, M., Schöll, M., Fernández-Eulate, G., Gil-Bea, F.J., López de Munain, A., Al-Chalabi, A., Rosa-Neto, P., Strydom, A., Svenningsson, P., Stomrud, E., Santillo, A., Aarsland, D., van Swieten, J.C., Palmqvist, S., Zetterberg, H., Blennow, K., Hye, A., Hansson, O., 2021a. A multicentre validation study of the diagnostic value of plasma neurofilament light. Nat. Commun. 12 https://doi.org/10.1038/S41467-021-23620-Z.

Ashton, N.J., Puig-Pijoan, A., Milà-Alomà, M., Fernández-Lebrero, A., García-Escobar, G., González-Ortiz, F., Kac, P.R., Brum, W.S., Benedet, A.L., Lantero-Rodriguez, J., Day, T.A., Vanbrabant, J., Stoops, E., Vanmechelen, E., Triana-Baltzer, G., Moughadam, S., Kolb, H., Ortiz-Romero, P., Karikari, T.K., Minguillon, C., Hernández Sánchez, J.J., Navalpotro-Gómez, I., Grau-Rivera, O., María Manero, R., Puente-Periz, V., de la Torre, R., Roquer, J., Dage, J.L., Zetterberg, H., Blennow, K., Suárez-Calvet, M., 2023. Plasma and CSF biomarkers in a memory clinic: Head-to-head comparison of phosphorylated tau immunoassays. Alzheimer's Dement. 19, 1913–1924. https://doi.org/10.1002/ALZ.12841.

Bayoumy, S., W Verberk, I.M., den Dulk, B., Hussainali, Z., Zwan, M., van der Flier, W. M., Ashton, N.J., Zetterberg, H., Blennow, K., Vanbrabant, J., Stoops, E., Vanmechelen, E., Dage, J.L., Teunissen, C.E., 2021. Clinical and analytical comparison of six Simoa assays for plasma P-tau isoforms P-tau181, P-tau217, and P-tau231. https://doi.org/10.1186/s13195-021-00939-9.

Bellomo, G., Bayoumy, S., Megaro, A., Toja, A., Nardi, G., Gaetani, L., Blujdea, E.R., Paolini Paoletti, F., Wojdała, A.L., Chiasserini, D., van der Flier, W.M., Verberk, I.M. W., Teunissen, C., Parnetti, L., 2024. Fully automated measurement of plasma Aβ42/40 and p-tau181: analytical robustness and concordance with cerebrospinal fluid profile along the Alzheimer's disease continuum in two independent cohorts. Alzheimer's Dement. https://doi.org/10.1002/alz.13687.

Benedet, A.L., Brum, W.S., Hansson, O., Karikari, T.K., Zimmer, E.R., Zetterberg, H., Blennow, K., Ashton, N.J., 2022. The accuracy and robustness of plasma biomarker models for amyloid PET positivity. Alzheimers Res. Ther. 14 https://doi.org/10.1186/s13195-021-00942-0.

Blennow, K., 2021. Phenotyping Alzheimer's disease with blood tests. Science 373 (1979), 626–628. https://doi.org/10.1126/SCIENCE.ABI5208.

Brum, W.S., Docherty, K.F., Ashton, N.J., Zetterberg, H., Hansson, O., McMurray, J.J.V., Blennow, K., 2023b. Effect of neprilysin inhibition on Alzheimer disease plasma biomarkers. JAMA Neurol. https://doi.org/10.1001/jamaneurol.2023.4719.

Brum, W.S., Cullen, N.C., Janelidze, S., Ashton, N.J., Zimmer, E.R., Therriault, J., Benedet, A.L., Rahmouni, N., Tissot, C., Stevenson, J., Servaes, S., Triana-Baltzer, G., Kolb, H.C., Palmqvist, S., Stomrud, E., Rosa-Neto, P., Blennow, K., Hansson, O., 2023a. A two-step workflow based on plasma p-tau217 to screen for amyloid β positivity with further confirmatory testing only in uncertain cases. Nat. Aging 3, 1079–1090. https://doi.org/10.1038/s43587-023-00471-5.

Bustamante, J.G., Zaidi, S.R.H., 2023. Amyloidosis. StatPearls.

Chang, J.F., Liu, H.C., Chen, H.H., Chen, W.P., Juang, J.L., Wang, P.N., Yang, S.Y., 2020. Effect of times to blood processing on the stability of blood proteins associated with dementia. Dement Geriatr. Cogn. Disord. 49, 303–311. https://doi.org/10.1159/000509358.

Colvin, R.B., Chang, A., Farris, A.B., Kambham, N., Cornell, L.D., Meehan, S.M., Liapis, H., Gaut, J.P., Bonsib, S.M., Seshan, S.V., Jain, S., Larsen, C.P., 2015. Diagnostic Pathology: Kidney Diseases: A volume in Diagnostic Pathology, Diagnostic Pathology: Kidney Diseases. Elsevier. https://doi.org/10.1016/B978-0-323-37707-2.50001-X.

DeLong, E., DeLong, D.M., Clarke-Pearson, D.L., 1988. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 44, 837–845.

Gonzalez-Ortiz, F., Turton, M., Kac, P.R., Smirnov, D., Premi, E., Ghidoni, R., Benussi, L., Cantoni, V., Saraceno, C., Rivolta, J., Ashton, N.J., Borroni, B., Galasko, D., Harrison, P., Zetterberg, H., Blennow, K., Karikari, T.K., 2023b. Brain-derived tau: a novel blood-based biomarker for Alzheimer's disease-type neurodegeneration. Brain 146, 1152–1165. https://doi.org/10.1093/brain/awac407.

Gonzalez-Ortiz, F., Kac, P.R., Brum, W.S., Zetterberg, H., Blennow, K., Karikari, T.K., 2023a. Plasma phospho-tau in Alzheimer's disease: towards diagnostic and therapeutic trial applications. Mol. Neurodegener. https://doi.org/10.1186/s13024-023-00605-8.

Gorno-Tempini, M.L., Hillis, A.E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S.F., Ogar, J.M., Rohrer, J.D., Black, S., Boeve, B.F., Manes, F., Dronkers, N.F., Vandenberghe, R., Rascovsky, K., Patterson, K., Miller, B.L., Knopman, D.S., Hodges, J.R., Mesulam, M.M., Grossman, M., 2011. Classification of primary progressive aphasia and its variants. Neurology 76, 1006. https://doi.org/10.1212/WNL.0B013E31821103E6.

Hansson, O., 2021. Biomarkers for neurodegenerative diseases. Nat. Med. https://doi.org/10.1038/s41591-021-01382-x.

Jack, C.R., Bennett, D.A., Blennow, K., Carrillo, M.C., Dunn, B., Haeberlein, S.B., Holtzman, D.M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J.L., Montine, T., Phelps, C., Rankin, K.P., Rowe, C.C., Scheltens, P., Siemers, E., Snyder, H.M., Sperling, R., Elliott, C., Masliah, E., Ryan, L., Silverberg, N., 2018.

NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement. 14, 535–562. https://doi.org/10.1016/J.JALZ.2018.02.018.

Janelidze, S., Stomrud, E., Smith, R., Palmqvist, S., Mattsson, N., Airey, D.C., Proctor, N. K., Chai, X., Shcherbinin, S., Sims, J.R., Triana-Baltzer, G., Theunis, C., Slemmon, R., Mercken, M., Kolb, H., Dage, J.L., Hansson, O., 2020. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. Nat. Commun. 11 https://doi.org/10.1038/s41467-020-15436-0.

Janelidze, S., Teunissen, C.E., Zetterberg, H., José, Allué, A., Sarasa, L., Eichenlaub, U., Bittner, T., Ovod, V., Verberk, I.M.W., Toba, K., Nakamura, Akinori, Bateman, R.J., Blennow, K., Hansson, Oskar, 2021. Head-to-head comparison of 8 plasma amyloid-β 42/40 assays in Alzheimer disease. JAMA Neurol. 78, 1375–1382. https://doi.org/10.1001/jamaneurol.2021.3180.

Karikari, T.K., Pascoal, T.A., Ashton, N.J., Janelidze, S., Benedet, A.L., Rodriguez, J.L., Chamoun, M., Savard, M., Kang, M.S., Therriault, J., Schöll, M., Massarweh, G., Soucy, J.P., Höglund, K., Brinkmalm, G., Mattsson, N., Palmqvist, S., Gauthier, S., Stomrud, E., Zetterberg, H., Hansson, O., Rosa-Neto, P., Blennow, K., 2020. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. Lancet Neurol. 19, 422–433. https://doi.org/10.1016/S1474-4422(20)30071-5.

Karikari, T.K., Benedet, A.L., Ashton, N.J., Lantero Rodriguez, J., Snellman, A., Suárez-Calvet, M., Saha-Chaudhuri, P., Lussier, F., Kvartsberg, H., Rial, A.M., Pascoal, T.A., Andreasson, U., Schöll, M., Weiner, M.W., Rosa-Neto, P., Trojanowski, J.Q., Shaw, L. M., Blennow, K., Zetterberg, H., 2021. Diagnostic performance and prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's disease neuroimaging initiative. Mol. Psychiatry 26, 429–442. https://doi.org/10.1038/s41380-020-00923-z.

Karikari, T.K., Ashton, N.J., Brinkmalm, G., Brum, W.S., Benedet, A.L., Montoliu-Gaya, L., Lantero-Rodriguez, J., Pascoal, T.A., Suárez-Calvet, M., Rosa-Neto, P., Blennow, K., Zetterberg, H., 2022. Blood phospho-tau in Alzheimer disease: analysis, interpretation, and clinical utility. Nat. Rev. Neurol. 18, 400–418. https://doi.org/10.1038/s41582-022-00665-2.

Leuzy, A., Janelidze, S., Mattsson-Carlgren, N., Palmqvist, S., Jacobs, D., Cicognola, C., Stomrud, E., Vanmechelen, E., Dage, J.L., Hansson, O., 2021b. Comparing the clinical utility and diagnostic performance of CSF P-Tau181, P-Tau217, and P-Tau231 assays. Neurology 97, E1681–E1694. https://doi.org/10.1212/WNL.0000000000012727.

Leuzy, A., Cullen, N.C., Mattsson-Carlgren, N., Hansson, O., 2021a. Current advances in plasma and cerebrospinal fluid biomarkers in Alzheimer's disease. Curr. Opin. Neurol. 34, 266–274. https://doi.org/10.1097/WCO.0000000000000904.

Leuzy, A., Mattsson-Carlgren, N., Palmqvist, S., Janelidze, S., Dage, J.L., Hansson, O., 2022. Blood-based biomarkers for Alzheimer's disease. EMBO Mol. Med. 14, e14408 https://doi.org/10.15252/EMMM.202114408.

Lewczuk, P., Riederer, P., O'Bryant, S.E., Verbeek, M.M., Dubois, B., Visser, P.J., Jellinger, K.A., Engelborghs, S., Ramirez, A., Parnetti, L., Jack, C.R., Teunissen, C.E., Hampel, H., Lleó, A., Jessen, F., Glodzik, L., de Leon, M.J., Fagan, A.M., Molinuevo, J.L., Jansen, W.J., Winblad, B., Shaw, L.M., Andreasson, U., Otto, M., Mollenhauer, B., Wiltfang, J., Turner, M.R., Zerr, I., Handels, R., Thompson, A.G., Johansson, G., Ermann, N., Trojanowski, J.Q., Karaca, I., Wagner, H., Oeckl, P., van Waalwijk van Doorn, L., Bjerke, M., Kapogiannis, D., Kuiperij, H.B., Farotti, L., Li, Y., Gordon, B.A., Epelbaum, S., Vos, S.J.B., Klijn, C.J.M., Van Nostrand, W.E., Minguillon, C., Schmitz, M., Gallo, C., Lopez Mato, A., Thibaut, F., Lista, S., Alcolea, D., Zetterberg, H., Blennow, K., Kornhuber, J., 2018. Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: An update of the Consensus of the Task Force on Biological Markers in Psychiatry of the World Federation of Societies of Biological Psychiatry. World J. Biol. Psychiatry. https://doi.org/10.1080/15622975.2017.1375556.

Mansilla, A., Canyelles, M., Ferrer, R., Arranz, J., Rodríguez-Baz, Í., Zhu, N., Rubio-Guerra, S., El Bounasri, S., Sánchez, O., Torres, S., Fortea, J., Lleó, A., Alcolea, D., Tondo, M., 2023. Effects of storage conditions on the stability of blood-based markers for the diagnosis of Alzheimer's disease. Clin. Chem. Lab. Med. https://doi.org/10.1515/cclm-2023-0245.

Martínez-Dubarbie, F., Guerra-Ruiz, A., López-García, S., Lage, C., Fernández-Matarrubia, M., Infante, J., Pozueta-Cantudo, A., García-Martínez, M., Corrales-Pardo, A., Bravo, M., López-Hoyos, M., Irure-Ventura, J., Sánchez-Juan, P., Teresa García-Unzueta, M., Rodríguez-Rodríguez, E., 2023. Accuracy of plasma Aβ40, Aβ42, and p-tau181 to detect CSF Alzheimer's pathological changes in cognitively unimpaired subjects using the Lumipulse automated platform. Alzheimers Res. Ther. 15, 163. https://doi.org/10.1186/s13195-023-01319-1.

Mattsson-Carlgren, N., Collij, L.E., Stomrud, E., Pichet Binette, A., Ossenkoppele, R., Smith, R., Karlsson, L., Lantero-Rodriguez, J., Snellman, A., Strandberg, O., Palmqvist, S., Ashton, N.J., Blennow, K., Janelidze, S., Hansson, O., Authors, C., 2023. Plasma biomarker strategy for selecting patients with Alzheimer disease for antiamyloid immunotherapies. JAMA Neurol. https://doi.org/10.1001/jamaneurol.2023.4596.

McKeith, I.G., Boeve, B.F., DIckson, D.W., Halliday, G., Taylor, J.P., Weintraub, D., Aarsland, D., Galvin, J., Attems, J., Ballard, C.G., Bayston, A., Beach, T.G., Blanc, F., Bohnen, N., Bonanni, L., Bras, J., Brundin, P., Burn, D., Chen-Plotkin, A., Duda, J.E., El-Agnaf, O., Feldman, H., Ferman, T.J., Ffytche, D., Fujishiro, H., Galasko, D., Goldman, J.G., Gomperts, S.N., Graff-Radford, N.R., Honig, L.S., Iranzo, A., Kantarci, K., Kaufer, D., Kukull, W., Lee, V.M.Y., Leverenz, J.B., Lewis, S., Lippa, C., Lunde, A., Masellis, M., Masliah, E., McLean, P., Mollenhauer, B., Montine, T.J., Moreno, E., Mori, E., Murray, M., O'Brien, J.T., Orimo, S., Postuma, R.B., Ramaswamy, S., Ross, O.A., Salmon, D.P., Singleton, A., Taylor, A., Thomas, A., Tiraboschi, P., Toledo, J.B., Trojanowski, J.Q., Tsuang, D., Walker, Z., Yamada, M., Kosaka, K., 2017. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. Neurology 89, 88–100. https://doi.org/10.1212/WNL.0000000000004058.

McKeith, I.G., Ferman, T.J., Thomas, A.J., Blanc, F., Boeve, B.F., Fujishiro, H., Kantarci, K., Muscio, C., O'Brien, J.T., Postuma, R.B., Aarsland, D., Ballard, C., Bonanni, L., Donaghy, P., Emre, M., Galvin, J.E., Galasko, D., Goldman, J.G., Gomperts, S.N., Honig, L.S., Ikeda, M., Leverenz, J.B., Lewis, S.J.G., Marder, K.S., Masellis, M., Salmon, D.P., Taylor, J.P., Tsuang, D.W., Walker, Z., Tiraboschi, P., 2020. Research criteria for the diagnosis of prodromal dementia with Lewy bodies. Neurology 94, 743–755. https://doi.org/10.1212/WNL.0000000000009323.

Mielke, M.M., Hagen, C.E., Xu, J., Chai, X., Vemuri, P., Lowe, V.J., Airey, D.C., Knopman, D.S., Roberts, R.O., Machulda, M.M., Jack, C.R.E., Petersen, R.C., Dage, J. L., 2018. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau-and amyloid-positron emission tomography. Alzheimer's Dement. 14, 989–997. https://doi.org/10.1016/j.jalz.2018.02.013.

Mielke, M.M., Frank, R.D., Dage, J.L., Jeromin, A., Ashton, N.J., Blennow, K., Karikari, T. K., Vanmechelen, E., Zetterberg, H., Algeciras-Schimnich, A., Knopman, D.S., Lowe, V., Bu, G., Vemuri, P., Graff-Radford, J., Jack, C.R., Petersen, R.C., 2021. Comparison of plasma phosphorylated tau species with amyloid and tau positron emission tomography, neurodegeneration, vascular pathology, and cognitive outcomes. JAMA Neurol. https://doi.org/10.1001/jamaneurol.2021.2293.

Mielke, M.M., Aakre, J.A., Algeciras-Schimnich, A., Proctor, N.K., Machulda, M.M., Eichenlaub, U., Knopman, D.S., Vemuri, P., Graff-Radford, J., Jack, C.R., Petersen, R. C., Dage, J.L., 2022. Comparison of CSF phosphorylated tau 181 and 217 for cognitive decline. Alzheimer's Dement. 18, 602–611. https://doi.org/10.1002/alz.12415.

Musso, G., Cosma, C., Zaninotto, M., Gabelli, C., Basso, D., Plebani, M., 2023. Pre-analytical variability of the Lumipulse immunoassay for plasma biomarkers of Alzheimer's disease. Clin. Chem. Lab. Med. 61, E53–E56. ⟨https://doi.org/10.1515/CCLM-2022-0770/MACHINEREADABLECITATION/RIS⟩.

Neill Carey, R., Durham, F.A.P., Hauck, W.W., Kallner, A., Kondratovich, M.V., Middle, J. G., Pierson-Perry, J.F., Smith, M.B., Srinivasan, A., 2014. User Verification of Precision and Estimation of Bias; Approved Guideline-Third Edition 34, 12. ⟨www.clsi.org⟩.

O'Connor, A., Karikari, T.K., Poole, T., Ashton, N.J., Lantero Rodriguez, J., Khatun, A., Swift, I., Heslegrave, A.J., Abel, E., Chung, E., Weston, P.S.J., Pavisic, I.M., Ryan, N. S., Barker, S., Rossor, M.N., Polke, J.M., Frost, C., Mead, S., Blennow, K., Zetterberg, H., Fox, N.C., 2021. Plasma phospho-tau181 in presymptomatic and symptomatic familial Alzheimer's disease: a longitudinal cohort study. Mol. Psychiatry 26, 5967–5976. https://doi.org/10.1038/s41380-020-0838-x.

Palmqvist, S., Insel, P.S., Stomrud, E., Janelidze, S., Zetterberg, H., Brix, B., Eichenlaub, U., Dage, J.L., Chai, X., Blennow, K., Mattsson, N., Hansson, O., 2019. Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease Editor: Céline Carret Transaction Report. EMBO Mol. Med. 11 https://doi.org/10.15252/emmm.201911170.

Pereira, J.B., Janelidze, S., Stomrud, E., Palmqvist, S., Van Westen, D., Dage, J.L., Mattsson-Carlgren, N., Hansson, O., 2021. Plasma markers predict changes in amyloid, tau, atrophy and cognition in non-demented subjects. Brain 144, 2826–2836. https://doi.org/10.1093/BRAIN/AWAB163.

Pilotto, A., Parigi, M., Bonzi, G., Battaglio, B., Ferrari, E., Mensi, L., Benussi, A., Caratozzolo, S., Cosseddu, M., Turrone, R., Archetti, S., Ashton, N.J., Zetterberg, H., Giliani, S., Padovani, A., 2022. Differences between plasma and cerebrospinal fluid p-tau181 and p-tau231 in early Alzheimer's disease. J. Alzheimer's Dis. 87, 991–997. https://doi.org/10.3233/jad-215646.

Rabe, C., Bittner, T., Jethwa, A., Suridjan, I., Manuilova, E., Friesenhahn, M., Stomrud, E., Zetterberg, H., Blennow, K., Hansson, O., 2023. Clinical performance and robustness evaluation of plasma amyloid-β42/40 prescreening. Alzheimer's. Alzheimer's Dement. 19, 1393–1402. https://doi.org/10.1002/ALZ.12801.

Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., Van Swieten, J.C., Seelaar, H., Dopper, E.G.P., Onyike, C.U., Hillis, A.E., Josephs, K.A., Boeve, B.F., Kertesz, A., Seeley, W.W., Rankin, K.P., Johnson, J.K., Gorno-Tempini, M.L., Rosen, H., Prioleau-Latham, C.E., Lee, A., Kipps, C.M., Lillo, P., Piguet, O., Rohrer, J.D., Rossor, M.N., Warren, J.D., Fox, N.C., Galasko, D., Salmon, D.P., Black, S.E., Mesulam, M., Weintraub, S., Dickerson, B.C., Diehl-Schmid, J., Pasquier, F., Deramecourt, V., Lebert, F., Pijnenburg, Y., Chow, T.W., Manes, F., Grafman, J., Cappa, S.F., Freedman, M., Grossman, M., Miller, B.L., 2011. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 134, 2456–2477. https://doi.org/10.1093/BRAIN/AWR179.

Rózga, M., Bittner, T., Batrla, R., Karl, J., 2019. Preanalytical sample handling recommendations for Alzheimer's disease plasma biomarkers. Alzheimer's Dement.: Diagn. Assess. Dis. Monit. 11, 291–300. https://doi.org/10.1016/J.DADM.2019.02.002.

Schindler, S.E., Bollinger, J.G., Ovod, V., Mawuenyega, K.G., Li, Y., Gordon, B.A., Holtzman, D.M., Morris, J.C., Benzinger, T.L.S., Xiong, C., Fagan, A.M., Bateman, R. J., 2019. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. Neurology 93, e1647–e1659. https://doi.org/10.1212/WNL.0000000000008081.

Simrén, J., Leuzy, A., Karikari, T.K., Hye, A., Benedet, A.L., Lantero-Rodriguez, J., Mattsson-Carlgren, N., Schöll, M., Mecocci, P., Vellas, B., Tsolaki, M., Kloszewska, I., Soininen, H., Lovestone, S., Aarsland, D., Hansson, O., Rosa-Neto, P., Westman, E., Blennow, K., Zetterberg, H., Ashton, N.J., 2021. The diagnostic and prognostic capabilities of plasma biomarkers in Alzheimer's disease. Alzheimer's Dement. 17, 1145–1156. ⟨https://doi.org/10.1002/ALZ.12283⟩.

Suárez-Calvet, M., Karikari, T.K., Ashton, N.J., Lantero Rodríguez, J., Milà-Alomà, M., Gispert, J.D., Salvadó, G., Minguillon, C., Fauria, K., Shekari, M., Grau-Rivera, O., Arenaza-Urquijo, E.M., Sala-Vila, A., Sánchez-Benavides, G., González-de-

Echávarri, J.M., Kollmorgen, G., Stoops, E., Vanmechelen, E., Zetterberg, H., Blennow, K., Molinuevo, J.L., Beteta, A., Cacciaglia, R., Cañas, A., Deulofeu, C., Cumplido, I., Dominguez, R., Emilio, M., Falcon, C., Fuentes, S., Hernandez, L., Huesa, G., Huguet, J., Marne, P., Menchón, T., Operto, G., Polo, A., Pradas, S., Soteras, A., Vilanova, M., Vilor-Tejedor, N., 2020. Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in Aβ pathology are detected. EMBO Mol. Med. 12 https://doi.org/10.15252/EMMM.202012921.

Sunde, A.L., Alsnes, I.V., Aarsland, D., Ashton, N.J., Tovar-Rios, D.A., De Santis, G., Blennow, K., Zetterberg, H., Kjosavik, S.R., 2023. Preanalytical stability of plasma biomarkers for Alzheimer's disease pathology. Alzheimers Dement (Amst.) 15, e12439. https://doi.org/10.1002/DAD2.12439.

Thijssen, E.H., Joie, R., La, Wolf, A., Strom, A., Wang, P., Iaccarino, L., Bourakova, V., Cobigo, Y., Heuer, H., Spina, S., Vandevrede, L., Chai, X., Proctor, N.K., Airey, D.C., Shcherbinin, S., Evans, C.D., Sims, J.R., Zetterberg, H., Blennow, K., Karydas, A.M., Teunissen, C.E., Kramer, J.H., Grinberg, L.T., Seeley, W.W., Rosen, H., Boeve, B.F., Miller, B.L., Rabinovici, G.D., Dage, J.L., Rojas, J.C., 2020. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration investigators. Nat. Med. 26, 387–397. https://doi.org/10.1038/s41591-020-0762-2.

Verberk, I.M.W., Misdorp, E.O., Koelewijn, J., Ball, A.J., Blennow, K., Dage, J.L., Fandos, N., Hansson, O., Hirtz, C., Janelidze, S., Kang, S., Kirmess, K., Kindermans, J., Lee, R., Meyer, M.R., Shan, D., Shaw, L.M., Waligorska, T., West, T., Zetterberg, H., Edelmayer, R.M., Teunissen, C.E., 2022. Characterization of pre-analytical sample handling effects on a panel of Alzheimer's disease–related blood-based biomarkers: Results from the Standardization of Alzheimer's Blood Biomarkers (SABB) working group. Alzheimer's Dement. 18, 1484–1497. https://doi.org/10.1002/alz.12510.

Verde, F., Milone, I., Dubini, A., Colombrita, C., Perego, A., Solca, F., Maranzano, A., Ciusani, E., Poletti, B., Ratti, A., Torresani, E., Silani, V., Ticozzi, N., 2023. Influence of kidney function and CSF/serum albumin ratio on plasma Aβ42 and Aβ40 levels measured on a fully automated platform in patients with Alzheimer's disease 44, 3287–3290. https://doi.org/10.1007/s10072-023-06882-x.

Vidali, M., Tronchin, M., Dittadi, R., Gruppo di Studio SIBioC - Medicina di Laboratorio "Statistica per il laboratorio," 2016. SIBioC DOCUMENTS DOCUMENTI SIBioC PREMESSA. Biochim Clin 40. ⟨https://doi.org/10.19186/BC_2016.006⟩.

Walter, M., Wiltfang, J., Vogelgsang, J., 2020. Pre-analytical sampling and storage conditions of amyloid-β peptides in venous and capillary blood. J. Alzheimer's Dis. 78, 529–535. https://doi.org/10.3233/JAD-200777.

Zetterberg, H., Blennow, K., 2021. Moving fluid biomarkers for Alzheimer's disease from research tools to routine clinical diagnostics. Mol. Neurodegener. 16, 1–7. ⟨https://doi.org/10.1186/S13024-021-00430-X/TABLES/1⟩.