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Preanalytical stability of plasma/serum brain-derived tau

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Abstract

INTRODUCTION: We investigated the effects of matrix type and reagent batch changes on diagnostic performances and longitudinal trajectories of brain-derived tau (BD-tau).

METHODS: We evaluated (i) Cohort 1: paired EDTA plasma and serum from Alzheimer biomarker-positive older adults versus controls ($n = 26$); and (ii) Cohort 2: $n = 79$ acute ischemic stroke patients with 265 longitudinal samples across four time points.

RESULTS: In Cohort 1, plasma and serum BD-tau were strongly correlated (rho = 0.96, $p < 0.0001$) with similar diagnostic performances (AUCs >99%) and correlations with CSF total-tau (rho = 0.93–0.94, $p < 0.0001$). However, absolute concentrations were ~40% higher in plasma versus serum. In Cohort 2, first and repeated BD-tau measurements showed a near-perfect correlation (rho = 0.96, $p < 0.0001$), with no significant between-batch concentration differences. In longitudinal analyses, substituting ~10% of the first-run concentrations for the remeasured values showed overlapping estimated trajectories without significant differences at any time point.

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; AIS, acute ischemic stroke; AUC, area under the curve; BD-tau, brain-derived tau; CI, confidence interval; CNS, central nervous system; CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; p-tau, phosphorylated tau; t-tau, total tau.

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DISCUSSION: BD-tau has equivalent diagnostic accuracies, but non-interchangeable absolute concentrations, in plasma versus serum. Furthermore, the analytical robustness is unaffected by batch-to-batch reagent variations.

KEYWORDS

acute ischemic stroke, Alzheimer's disease, brain-derived tau, plasma, preanalytical handling, serum, total tau

Highlights

- Brain-derived tau (BD-tau) is a novel blood-based biomarker that quantifies tau protein of CNS origin.
- Effects of preanalytical handling procedures on the quality and reproducibility of BD-tau measures are unknown.
- In two cohorts of $n = 105$ participants, we compared BD-tau concentrations and diagnostic performances in paired plasma and serum samples, and evaluated impacts of batch-to-batch reagent variations.
- Paired plasma and serum showed equivalent diagnostic performances to separate amyloid-positive AD from amyloid-negative controls, indicating both can be used independently.
- Repeated measurements and longitudinal trajectories of plasma BD-tau were unaffected by batch-to-batch reagent variation.

1 | BACKGROUND

Existing plasma total-tau (t-tau) assays have poor diagnostic performance[1–4] and either do not correlate or do so weakly with paired cerebrospinal fluid (CSF) t-tau measures, which rather reliably reflects neural injury and degeneration.[5–7] The high expression of tau in multiple peripheral sources is thought to underlie this observation, with an estimated 80% of plasma t-tau signal originating from non–central nervous system (CNS) sources.[1] In effect, the remaining 20% brain-derived signal is masked by the highly abundant peripheral tau. We recently developed a novel blood-based assay that is selective for tau of CNS origin.[8] This assay, referred to as brain-derived tau (BD-tau), uses an antibody that binds a defined peptide sequence at the contiguous exon 4–5 junction of the *MAPT* gene, avoiding peripherally expressed tau, which has the exon 4a insert between exons 4 and 5.[9,10] We have described the technical validation of the assay and its clinical performance to identify autopsy-verified Alzheimer's disease (AD) pathophysiology.[8] Importantly, plasma/serum BD-tau correlated strongly with CSF t-tau, whereas plasma/serum t-tau did not, which, together with biochemical and mass spectrometric data, further supports its selectively and specificity to CNS tau. Besides AD, acute neurological disorders such as acute ischemic stroke (AIS) are associated with significant increases in CSF and plasma t-tau at admission,[11] and thus it is anticipated that BD-tau levels will have prognostic utility in this condition.

Despite its high diagnostic and prognostic performances, the potential effects of preanalytical handling procedures on BD-tau levels and repeatability are unknown. Here, we examined matrix effects by comparing BD-tau concentrations and diagnostic performances in paired plasma and serum samples. We additionally evaluated the effects of reagent batch changes on cross-sectional and longitudinal measurements.

2 | METHODS

2.1 | Study participants and sample collection

The neurochemistry cohort (Cohort 1; $n = 26$), Sahlgrenska University Hospital, Gothenburg, Sweden, included paired ethylenediaminetetracetic acid (EDTA) plasma and serum from neurochemically defined biomarker-positive AD participants ($n = 15$) and biomarker-negative controls ($n = 11$) selected based on their CSF biomarker profile.[6,12]

The stroke cohort (Cohort 2) included $n = 79$ AIS patients from the Biostroke study, Centro Hospitalar Universitário do Porto (CHUPorto), Porto, Portugal.[11] These patients were prospectively evaluated with longitudinal blood collection for biomarker assessments. EDTA plasma samples were collected at four time points: on admission ($n = 77$), upon treatment ($n = 75$), and at 24 h ($n = 73$) and 72 h ($n = 40$) after treatment.

The studies were approved by the local ethics review boards at Gothenburg (EPN140811) and Porto (CHUPorto 123-DEFI/122-CES).

Whole blood was obtained by venipuncture into Vacuette tubes (Greiner Bio-One) and processed concurrently into EDTA plasma or serum (plasma only for Cohort 2) following published protocols.[13,14] In brief, the tubes were stored in cold conditions and centrifuged within 2 h of collection at $2000 \times g$ for 10-15 min and stored at $-80°C$ until use.

2.2 | Biomarker measurements

Blood was collected and processed following recommended procedures.[13–15] For BD-tau, the sheep monoclonal antibody TauJ.5H3 (Bioventix Plc, Surrey, UK) was used for capture and an N-terminal-tau mouse monoclonal antibody for detection. Recombinant full-length tau-441 (TO8-50FN, SignalChem) was used as calibrator. Blood samples and calibrators were diluted with Homebrew buffer (101556, Quanterix, MA, USA). Plasma/serum samples were diluted four times with the Homebrew buffer before measurement. Stated concentration values have been adjusted for the pre-measurement dilution. Assay development and validation procedures, including dilution linearity and spike recovery, were described recently.[8] Plasma p-tau181 was measured with a validated in-house method on the Simoa HD-X,[12] while CSF $A\beta42$, p-tau181, and t-tau were analyzed with commercial INNOTEST immunoassays,[16] all at the University of Gothenburg, Mölndal, Sweden. Intra- and inter-run repeatability results were <10%.

2.3 | Statistical analyses

GraphPad Prism version 9.3.1, IBM SPSS Statistics 28.0.0, and Rstudio 4.2.1 with the packages ggstatsplot[17] 0.9.5, ggplot2 3.4.0, beeswarm 0.4.0, and mcr 1.3.1 were used for plot generation and statistical analyses. Non-parametric tests were used for non-normally distributed data. Spearman correlation and the chi-squared test were used for continuous and categorical variables, respectively. Diagnostic performances were evaluated with receiver operating curves (ROC) and the respective area under the curve (AUC) assessments. Fold changes were examined by comparing biomarker values with the mean or median of the control group. Group differences were examined using two-tailed Mann–Whitney or Kruskal–Wallis tests with Dunn's multiple comparison (for two vs. more groups, respectively) unless otherwise stated. The batch effects on cross-sectional data were studied using Passing–Bablok regression and Bland–Altman plots. We studied the trajectories over time of the natural logarithm of plasma BD-tau concentration by fitting a generalized linear mixed-effects model using restricted maximum likelihood estimation. Significance was set at $p < 0.05$.

3 | RESULTS

The cohort demographic and biomarker details are presented in Tables S1 and S2.

RESEARCH IN CONTEXT

1. **Systematic Review**: We found one publication on plasma/serum brain-derived tau (BD-tau) on PubMed that is, Gonzalez-Ortiz et al. (2023) Brain. This study reported analytical validation, including linearity of sample signal proportional to dilution fold, recoverability of BD-tau signal from exogenously added samples, and day-to-day stability of the assay when appropriately frozen aliquots of the same sample are measured using identical reagent batches. In this study, we investigated the impacts of matrix type and reagent batch switches on BD-tau.

2. **Interpretation**: Equivalence in BD-tau diagnostic performances when measured in paired EDTA plasma versus serum samples indicates that both matrix sources can be clinically used to measure this marker, one of the first demonstrations for a new blood-based non-phosphorylated tau assay. However, higher concentrations of BD-tau in plasma highlight a lack of interchangeability with serum. Moreover, nearly identical plasma BD-tau concentrations from two measurement rounds that also had overlapping longitudinal trajectories shows that signals are reproducible across reagent batches.

3. **Future Directions**: BD-tau can be measured in cohorts with plasma or serum samples and using different reagent batches.

3.1 | Cross-sectional performance in plasma versus serum

In the neurochemistry cohort (Cohort-1), where all participants had paired EDTA plasma and serum, BD-tau was increased in $A\beta$-positive AD dementia versus $A\beta$-negative controls when measured in either matrix processed from the same blood draw ($p < 0.0001$; Figure 1A,B).

The mean fold differences in the AD versus control groups were 7.36 for plasma and 6.20 for serum, compared with 2.39 for plasma p-tau181. Similarly to CSF t-tau, the plasma/serum BD-tau fold differences reduced to ~2.40 when using median values to calculate the fold changes (Table S1), given the non-normal distributions. In comparisons of BD-tau levels in sample pairs across the entire cohort, the absolute mean values were 10.28 pg/mL for plasma and 5.99 pg/mL for serum, suggesting that for every plasma BD-tau value the corresponding level in serum was approximately 40% lower on average (Figure 1C). Similar results were recorded when using medians instead of means: 4.19 pg/mL for plasma and 2.32 pg/mL for serum. Nonetheless, BD-tau levels in both plasma and serum were above the assay's lower limit of quantification of 0.03 pg/mL (lowest recorded values were 0.48 pg/mL for serum and 0.63 pg/mL for plasma for the same individual).
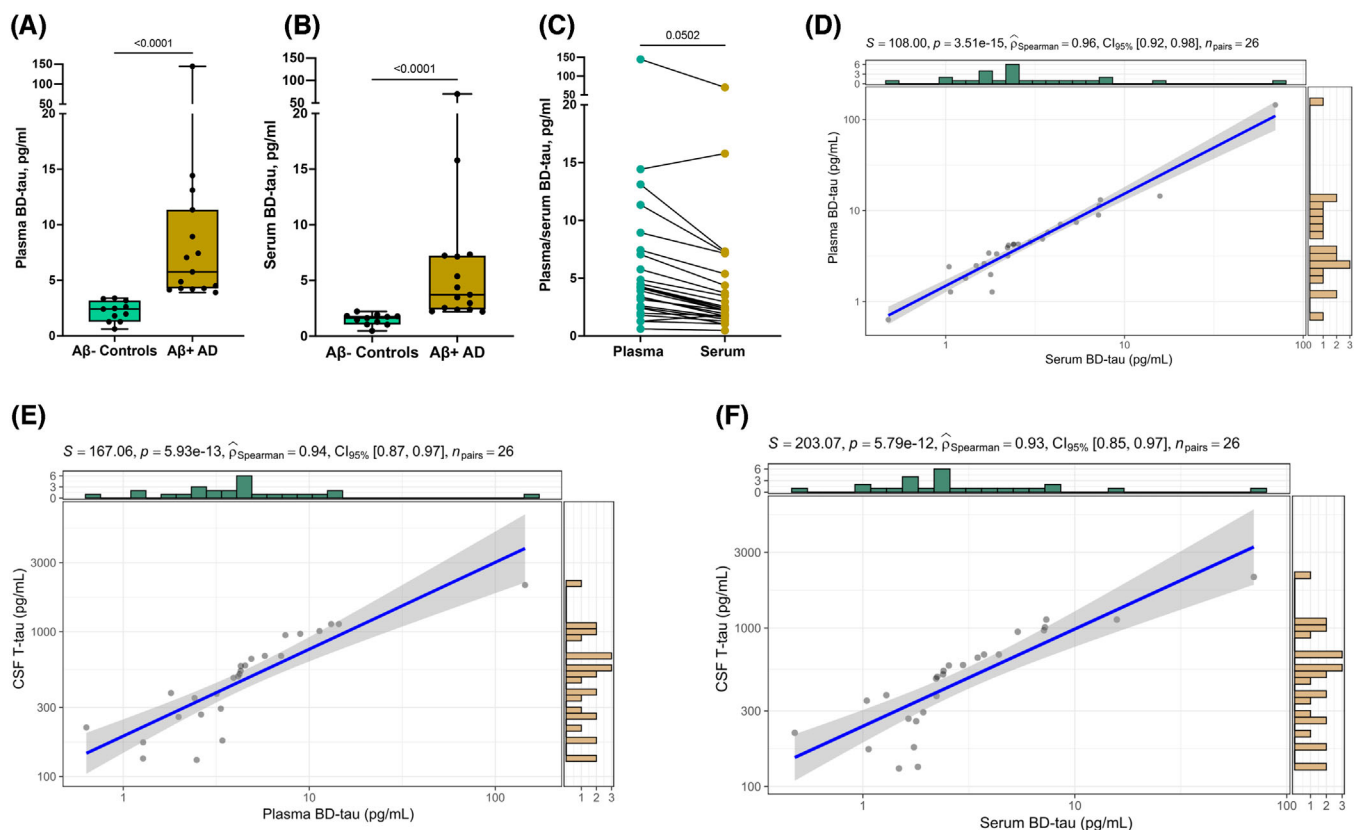
FIGURE 1 Comparison of BD-tau concentrations measured in paired EDTA plasma versus serum collected from the same individuals at identical clinical visits (Cohort 1). (A) BD-tau levels measured in plasma. (B) BD-tau levels measured in serum. (C) Pairs of BD-tau measures in plasma versus serum samples from the same individuals. (D) Scatter plot showing correlation between the paired measures of BD-tau in the plasma versus serum samples. (E and F) Scatter plots and Spearman correlation of CSF t-tau versus each of plasma BD-tau and serum BD-tau. The scales of the scatter plots in (D), (E), and (F) are log10. However, the biomarker concentrations are provided in absolute pg/mL values. The histograms in green and yellow show the marginal distributions of the two variables being compared. The information provided at the top of each scatter plot is as follows. The S value is the sum of the square differences between each variable's rank. p is the p value associated with the hypothesis test carried out to see whether the correlation coefficient was significant. rho (shown as $\hat{\rho}$) is the Spearman correlation coefficient (ρ/rho). n pairs = number of matched plasma-serum observations. BD-tau, brain-derived tau; CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; t-tau, total tau.

Plasma versus serum BD-tau were strongly correlated (Spearman rho = 0.96, $p < 0.0001$; Figure 1D). In agreement with this, plasma and serum BD-tau had similar diagnostic accuracies of 100% and 99.4% (95% confidence interval [CI] = 97.5%–100%), respectively. Furthermore, plasma and serum BD-tau showed similarly strong correlations with CSF t-tau (rho = 0.94 vs. 0.93; $p < 0.0001$; Figure 1E); excluding the high BD-tau value pair >50 pg/mL did not affect the strength of the correlations, which became rho = 0.94 and 0.92 respectively. Plasma and serum BD-tau additionally correlated equivalently with CSF Aβ42 (rho = −0.70 vs. −0.69; $p \leq 0.0001$), CSF p-tau181 (rho = 0.90 vs. 0.89; $p < 0.0001$), and plasma p-tau181 (rho = 0.75 vs. 0.74; $p < 0.0001$).

3.2 | Batch effects on cross-sectional and longitudinal data

In the stroke cohort (Cohort-2), we first measured plasma BD-tau in 265 longitudinal samples from $n = 79$ AIS patients. Later, 28 samples selected to reflect low, medium, and high concentrations

were blindly evaluated with a different reagent batch (different lots of bead-coated capture and biotin-conjugated detection antibodies). There was a strong correlation (rho = 0.96, $p < 0.0001$) between the 28 samples independently analyzed twice (Figure 2A,B). In cross-sectional analysis, we observed no difference between batches when comparing the first-run concentrations with another set where the values for the 28 repeated samples had been substituted for those in the rerun experiment (Figure 2A,B,C). In longitudinal analyses, the first-run versus remeasured value comparisons showed overlapping estimated trajectories and no significant difference at any time point (Figure 2C–E). The Passing–Bablok regression equation was as follows: Second method = 0.93 (95% CI 0.77 to 0.99) × First method − 0.55 (95% CI: −0.98 to 0.72; Figure 2F). Bland–Altman analysis showed high agreement between the BD-tau measures, especially at low to average concentrations. The mean bias was 9.14 pg/mL (95% CI: 1.94 to 16.33). There was also a proportional bias between the two measures, which is explained by the fact that 24/28 reanalyzed samples had slightly lower but statistically indifferent values in the second analysis (Figure 2G). This bias was within the expected range of unavoidable
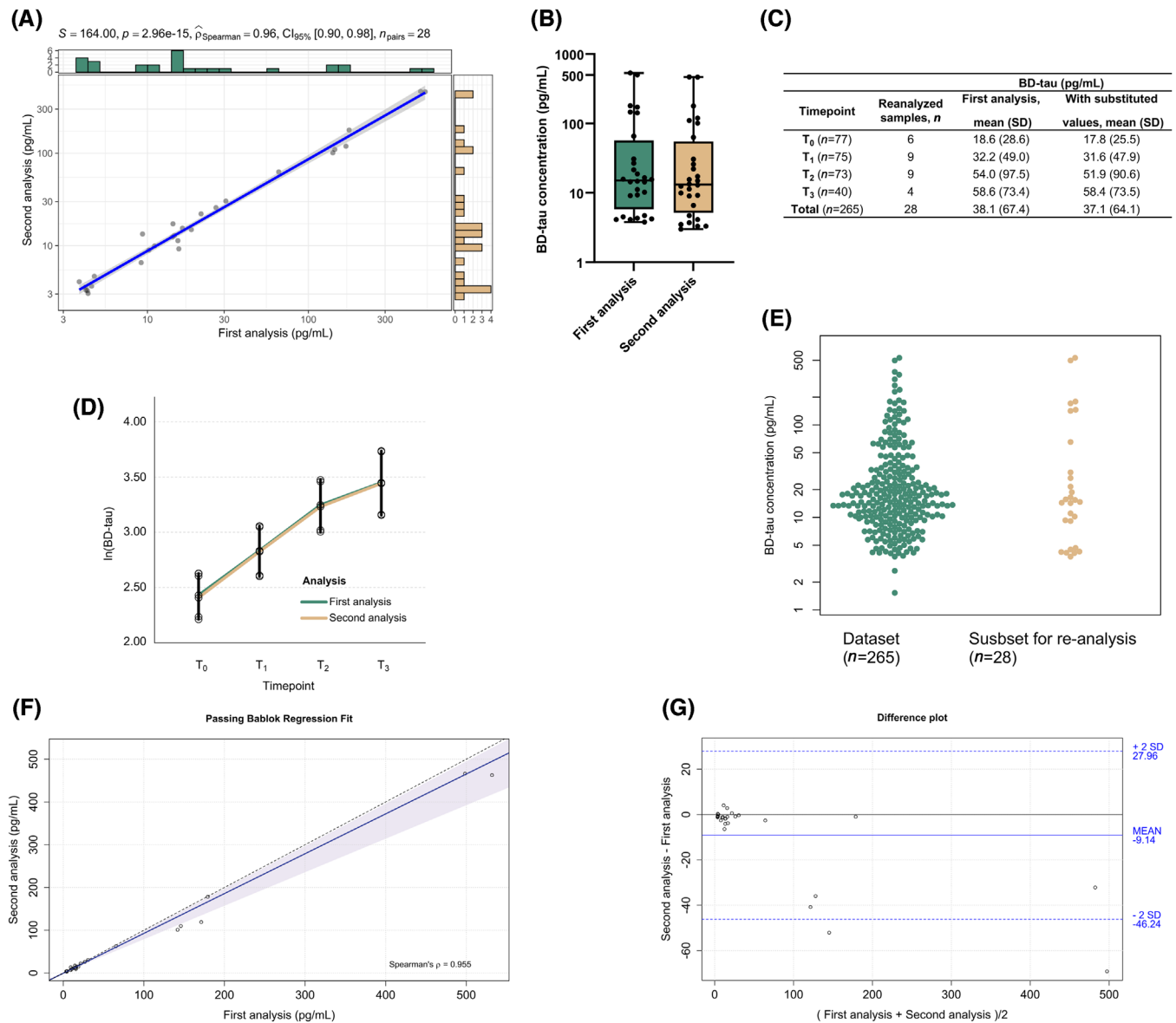
(A) $S = 164.00$, $p = 2.96e{-}15$, $\hat{\rho}_{Spearman} = 0.96$, $CI_{95\%}$ [0.90, 0.98], $n_{pairs} = 28$

(B)

(C)

Timepoint	Reanalyzed samples, n	BD-tau (pg/mL)	
		First analysis, mean (SD)	With substituted values, mean (SD)
T_0 ($n=77$)	6	18.6 (28.6)	17.8 (25.5)
T_1 ($n=75$)	9	32.2 (49.0)	31.6 (47.9)
T_2 ($n=73$)	9	54.0 (97.5)	51.9 (90.6)
T_3 ($n=40$)	4	58.6 (73.4)	58.4 (73.5)
Total ($n=265$)	28	38.1 (67.4)	37.1 (64.1)

(D)

(E)

(F)

(G)

FIGURE 2 Remeasurement of ~10% plasma samples from acute ischemic stroke patients using new reagent batches and the effect on the full cohort (Cohort 2). (A) Correlation plot of the 28 samples that were measured twice. (B) Boxplot showing the distribution of BD-tau concentrations for the samples in the first and second measurements. (C) Descriptive table of the first-run BD-tau concentrations for the entire 265 samples divided according to time points compared with the second set where the values for the 28 repeated samples had been respectively substituted. (D) Longitudinal profiles of natural log-transformed BD-tau with estimated means and 95% confidence levels based on linear mixed-effects model. (E) Swarm plot depicting BD-tau concentrations for the selected samples ($n = 28$) in the second analysis versus the total dataset ($n = 265$). Agreement analyses of both measurements are shown in (F) Passing–Bablok regression analysis and (G) Bland–Altman plot considering the absolute values of both analyses. For the Passing–Bablok regression, the solid and shadow indicate the regression line and confidence interval for the regression line, respectively. The 95% confidence bounds of the regression fit line were calculated with the bootstrap (quantile) method. In the Bland–Altman plot, the solid line indicates the mean difference between the methods, and the dashed lines the confidence interval calculated by multiplying the standard deviation by 2. The scale of the scatter plots in (A) is log10. However, the biomarker concentrations are provided in absolute pg/mL values. The histograms in green and yellow show the marginal distributions of the two variables being compared. The information provided at the top of each scatter plot are as follows. The S value is the sum of the square differences between each variable's rank. p is the p value associated with the hypothesis test carried out to see whether the correlation coefficient is significant. rho (shown as $\hat{\rho}$) is the Spearman correlation coefficient (ρ/rho). n pairs = number of matched observations in the first versus second rounds of BD-tau measurements. BD-tau, brain-derived tau. Tx, time point X.

analytical error that is observed for any biofluid biomarker and technical platform.[18]

4 | DISCUSSION

An important criterion in evaluating the utility of a blood biomarker is to establish the potential effects of preanalytical factors, including the coagulation method and batch-to-batch reagent variation.[18] We have characterized these preanalytical factors for BD-tau, leading to the finding that the biomarker is reliably measurable in either plasma or serum to reflect the same CNS-derived tau changes to support diagnostic decision-making.

Importantly, the high diagnostic performance for differentiating Aβ-positive AD dementia individuals from Aβ-negative controls was independent of the use of plasma or serum. Moreover, paired plasma and serum BD-tau correlated strongly and equivalently with CSF t-tau, demonstrating that the superior performance of BD-tau over current blood-based t-tau assays regarding association with CSF t-tau, as reported recently,[8] is independent of matrix type. Additionally, comparable plasma versus serum BD-tau correlations with CSF Aβ42 and p-tau181 showed that the new biomarker detects AD pathophysiology in peripheral blood regardless of matrix source. However, concentrations in serum were ∼40% lower than those in paired plasma. This means that absolute concentrations measured in plasma and serum are not identical and, thus, non-interchangeable. To this end, BD-tau evaluation in cohort studies should be restricted to either plasma or serum samples collected with an identical protocol, without switching between matrix types. These results agree with previous reports for blood-based p-tau181, p-tau231, and t-tau[12,14,15] but not p-tau217, which does not work well in serum in an AD context. Furthermore, these results suggest similar diagnostic capabilities between plasma and serum, which has not been reported for any of the other currently available non-phosphorylated-tau assays.

Longitudinal biofluid biomarker analyses can be complicated by the need to remeasure baseline samples with the same reagent batch as the follow-up ones to avoid batch effects.[19] Here, we evaluated a workaround of measuring a small, representative selection of baseline samples along with the follow-up ones (∼10% of the total samples) to improve efficiency and save time and resources. Plasma BD-tau concentrations in the first and repeated runs were nearly identical, with minor analytical errors that were within the range of expectations. These variations are unavoidable in clinical chemistry; however, the extent to which they affect assay performance is a reflection of how robust that biomarker is.[18] In this study, these analytical errors were insignificant to the overall biomarker performance, with the longitudinal trajectories of the whole cohort in the first run equivalent to when the second measures were substituted in.

While BD-tau appears to show specificity to AD pathology versus other neurodegenerative diseases,[8] its increase in AIS suggest release into the bloodstream following acute neurological insults. This indicates that similar to CSF t-tau, plasma BD-tau would be additionally important for predicting clinical outcome after AIS.[20]

The strengths of the study include the (pre)analytical comparison of BD-tau performances in paired plasma versus serum and the evaluation of the impact of reagent batch changes. Its limitations include the small sample sizes, especially in Cohort 1, given the known challenges associated with obtaining both plasma and serum from participants with CSF biomarker characterization.

Altogether, the results indicate that BD-tau is an analytically robust biomarker, supporting its further evaluation as a novel indicator of CNS-tau in peripheral blood.

CONFLICT OF INTEREST STATEMENT

MT and PH are employees of Bioventix Plc. HZ has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave and has given lectures at symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche. K.B. has served as a consultant or on advisory boards for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers. LFM has served on scientific advisory boards and/or as a consultant for Alnylan and Janssen and has given lectures at symposia sponsored by Alnylan. HZ and KB are cofounders of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. The other authors declare no competing interest. Author disclosures are available in the supporting information.

CONSENT STATEMENT

The studies were approved by the local ethics review boards at Porto (CHUPorto 123-DEFI/122-CES) and at Gothenburg (EPN140811). All participants or their next of kin consented to their inclusion in this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.