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RESEARCH ARTICLE

Crosswalk study on blood collection-tube types for Alzheimer's disease biomarkers

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Abstract

Introduction: Blood-based Alzheimer's disease (AD) biomarkers show promise, but pre-analytical protocol differences may pose problems. We examined seven AD blood biomarkers (amyloid beta [A$_{_}$]$_{42}$, Aβ_{40}, phosphorylated tau [p – tau$_{181}$, total tau [t-tau], neurofilament light chain [NfL], A$\beta_{\frac{42}{40}}$, and $\frac{\text{p-tau}_{181}}{\text{A}\beta_{42}}$) in three collection tube types (ethylenediaminetetraacetic acid [EDTA] plasma, heparin plasma, serum).

Methods: Plasma and serum were obtained from cerebrospinal fluid or amyloid positron emission tomography-positive and -negative participants (N = 38) in the Wisconsin Registry for Alzheimer's Prevention. We modeled AD biomarker values observed in EDTA plasma versus heparin plasma and serum, and assessed correspondence with brain amyloidosis.

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Results: Results suggested bias due to tube type, but crosswalks are possible for some analytes, with excellent model fit for NfL ($R^2 = 0.94$), adequate for amyloid ($R^2 = 0.40$-0.69), and weaker for t-tau ($R^2 = 0.04$-0.42) and $p-tau_{181}$ ($R^2 = 0.22$-0.29). Brain amyloidosis differentiated several measures, especially EDTA plasma $\frac{pTau_{181}}{A\beta_{42}}$ ($d = 1.29$).
Discussion: AD biomarker concentrations vary by tube type. However, correlations for some biomarkers support harmonization across types, suggesting cautious optimism for use in banked blood.

KEYWORDS
Alzheimer's disease, amyloid beta, cognitively unimpaired, neurofilament light, plasma, p-tau181, t-tau

1 | INTRODUCTION

Biomarkers for Alzheimer's disease (AD) can illuminate modifiable risk factors, accelerate clinical trials, and enable accurate and early diagnosis and treatment. While positron emission tomography (PET) and cerebrospinal fluid (CSF) methods have high accuracy,[1,2] they are impractical for broad use in clinical care. Blood-based markers with high validity and reliability are urgently needed, and some have met key aims of blood biomarker validation,[3] with several demonstrations of discrimination between stages of AD pathophysiology, or between AD and other neurodegenerative conditions.[4–9]

Blood repositories from large cohorts of at-risk individuals and patients may facilitate longitudinal evaluation of blood-based AD biomarkers.[10–12] However, heterogeneity in pre-analytic protocols for the collection, processing, and storage of blood products, including the whole blood anticoagulant used for plasma isolation, may pose problems. Two recent studies in healthy, presumptively biomarker-negative participants demonstrated effects of pre-analytic factors on the quantification of total tau (t-tau) and amyloid beta isoforms 42 ($A\beta_{42}$) and 40 ($A\beta_{40}$).[13,14] Such heterogeneity could complicate comparisons between studies and across time.

We examined levels of AD biomarker assay measures in three types of blood collection tubes for participants known to be AD biomarker-negative via CSF or amyloid PET. We assessed correspondence between measures in plasma obtained from whole blood treated with ethylenediaminetetraacetic acid (EDTA plasma) or Na-heparin (heparin plasma), and in serum. The markers considered were $A\beta_{42}$, $A\beta_{40}$, phosphorylated tau-181 ($p-tau_{181}$), t-tau, neurofilament light chain (NfL), $A\beta_{\frac{42}{40}}$, and $\frac{p-tau_{181}}{A\beta_{42}}$.[15,16] We also evaluated the correspondence between analyte levels in each blood medium and central nervous system (CNS)-based AD biomarker measurement (CSF or PET).

2 | METHODS

2.1 | Participants

Blood samples were obtained from 38 participants in the Wisconsin Registry for Alzheimer's Prevention (WRAP)[10] from September 2011 to April 2013 who also had a CNS biomarker. Nineteen participants were determined to be $A\beta^+$, via CSF samples within 2 years of plasma collection (N = 15), and/or via Pittsburgh compound B (PiB)-PET imaging at any time after the blood draw (N = 17). Nineteen participants who were $A\beta^-$ by both CSF and PiB-PET were matched to these on age. None of the 32 participants having both CSF and PET markers were discordant for A$_{\cdot}$. Participant characteristics are summarized in Table 1. Most were free of clinically significant cognitive

impairment by consensus diagnosis,[10] except one participant with mild cognitive impairment (MCI; Aβ^- by CSF and PET) and one with dementia (Aβ^+ by PET). Procedures conformed to ethical standards for human subjects research, and all participants provided informed consent.

2.2 | Blood sample collection and preparation methods

2.2.1 | General considerations

Blood was collected at two study sites according to identical pre-analytic protocols, mostly in the morning (N = 2 midday), after participants fasted for at least 11 hours. Samples were to be centrifuged within 1 hour and frozen at −80°C within 90 minutes. Detailed data on realized sample handling parameters was available for one site (N = 27 [71%]; 15 [56%] Aβ^+). At this site, respective delays in centrifuging and freezing happened with three (median [range] t_{cent}, in minutes: EDTA plasma, 16 [5–84]; heparin plasma, 21 [9–90]; serum, 22 [9–90]) and four participants (median [range] t_{freeze}, in minutes: EDTA plasma, 77 [23–106]; heparin plasma, 81 [27–111]; serum, 81 [27–111]). No samples were thawed more than once at the time of present assays.

2.2.2 | EDTA plasma

Thirty milliliters was drawn from each participant into 3 × 10 mL lavender-top EDTA tubes (BD 366643). Samples were mixed gently by inverting 10 to 12 times and were centrifuged 15 minutes at 2000 *g* at room temperature. Plasma samples were aliquoted by 1 mL increments into 12 × 2 mL cryovials (Wheaton Cryoelite W985863) and excess was discarded.

2.2.3 | Heparin plasma

Ten milliliters was drawn from each participant into 1 × 10 mL green-top sodium heparin tubes (BD 367874). Samples were centrifuged for 10 minutes at 2000 *g* at 4°C. Plasma samples were aliquoted by 1 mL increments into 4 × 2 mL cryovials (Wheaton Cryoelite W985867) and excess was discarded.

2.2.4 | Serum

Nine milliliters was drawn from each participant into 1 × 9 mL unanticoagulated red-top tubes (Greiner 455092) and allowed to clot for no more than 30 minutes. Samples were centrifuged for 10 minutes at 2000 g at 4°C. Serum samples were aliquoted by 1 mL increments into 4 × 2 mL cryovials (Wheaton Cryoelite W985864) as possible, and excess was discarded.

RESEARCH IN CONTEXT

1. Systematic review: We searched PubMed for related works and have cited as appropriate. Ashton et al. recently reviewed the literature on fluid Alzheimer's disease (AD) biomarkers,[3] and in this framework, this paper provides some converging evidence relevant to optimization of operating procedures and early detection of AD pathophysiology.
2. Interpretation: We found excellent correspondence for neurofilament light chain and fair to good correspondence for amyloid biomarkers in ethylenediaminetetraacetic acid plasma, heparin plasma, and serum, though the correspondence across blood collection tube types is weaker for tau biomarkers. We also found preliminary evidence of criterion validity for $\frac{\text{p-tau}_{181}}{\text{A}\beta_{42}}$ and $\text{A}\beta\frac{42}{40}$ in plasma, compared to brain or cerebrospinal fluid amyloid positivity, in a mostly unimpaired group.
3. Future directions: Future analyses with larger samples should explore the robustness of crosswalked blood biomarker values for identifying amyloid positivity and predicting cognitive endpoints such as progression to dementia.

2.3 | Methods for blood biomarker analyses

Samples were analyzed in August 2020, after a median freezer storage time of 7.87 years (range: 7.27–8.9), using the Neurology 3-plex A panel for t-tau, Aβ_{40}, and Aβ_{42}, and the NF-Light singleplex kit for NfL on a single-molecule array (Simoa) HD-X Analyzer according to manufacturer's instructions (Quanterix). For p − tau$_{181}$, an in-house Simoa method was used, described previously.[4] Calibrators were run in duplicate and obvious outlier calibrator replicates were masked before curve fitting. Samples were diluted 4-fold and run as single measurements. Two quality control (QC) levels were run in duplicate in the beginning and the end of each run.

Aβ_{40}: The assay dynamic range is 3.2–580 pg/mL. QC samples had concentrations of 21.2 pg/mL (repeatability: 2.6%; intermediate precision: 6.0%) and 272 pg/mL (repeatability: 3.8%; intermediate precision: 3.8%).

Aβ_{42}: The assay dynamic range is 0.6–215 pg/mL. QC samples had concentrations of 1.7 pg/mL (repeatability: 10.3%; intermediate precision: 21.1%) and 18.6 pg/mL (repeatability: 13.4%; intermediate precision: 13.4%).

t − tau: The assay dynamic range is 0.6–348 pg/mL. QC samples had concentrations of 2.3 pg/mL (repeatability: 5.3%; intermediate precision: 9.5%) and 9.3 pg/mL (repeatability: 4.7%; intermediate precision: 4.7%).

NfL: The assay dynamic range is 1.9–1800 pg/mL in plasma, and 2.1–2244 pg/mL in serum. QC samples in plasma had concentrations of

TABLE 1 Participant characteristics of age-matched groups defined by binarized amyloid status derived from CNS biomarkers

Variable	$A\beta^-$	$A\beta^+$
N	19	19
Sex, female, N (%)	12 (63%)	12 (63%)
Race, White, N (%)	18 (95%)	18 (95%)
Race, Black, N (%)	–	1 (5%)
Race, Asian, N (%)	1 (5%)	–
Age at blood draw, years, mean (SD)	65.22 (3.94)	65.40 (4.03)
Age at lumbar puncture, years, mean (SD)	65.15 (4.05)	65.60 (4.50)
Age at PiB-PET, years, mean (SD)	65.44 (4.51)	65.95 (3.37)
Δ_{age} for blood and LP, mean (SD, min-max)	0.59 (0.51, 0-1.81)	0.50 (0.62, 0.01-1.98)
Δ_{age} for blood and PiB-PET, mean (SD, min-max)	0.88 (1.49, 0-6.66)	1.53 (2.43, 0-7.53)
CSF $\frac{pTau_{181}}{A\beta_{42}}$	0.018 (0.0045)	0.058 (0.032)
CSF $A\beta\frac{42}{40}$	0.071 (0.010)	0.036 (0.0082)
CSF $A\beta_{42}$, pg/mL	1119 (390.6)	456.8 (191.0)
CSF $A\beta_{40}$, pg/mL	15465 (4345)	12815 (3822)
CSF $pTau_{181}$, pg/mL	18.64 (5.18)	23.06 (8.30)
CSF t-tau, pg/mL	216.2 (60.97)	238.1 (76.56)
CSF NfL, pg/mL	99.23 (41.51)	92.00 (37.27)
PiB mDVR	1.05 (0.032)	1.49 (0.22)

Abbreviations: $A_{\cdot}$, amyloid beta; CNS, central nervous system; CSF, cerebrospinal fluid; LP, lumbar puncture; mDVR, modeled distribution volume ratio; NfL, neurofilament light chain; PET, positron emission tomography; PiB, Pittsburgh compound B; $p-tau_{181}$, phosphorylated tau-181; SD, standard deviation; t-tau, total tau.
Note: CSF and PET data were available for N = 34 and 36 participants, respectively; other biomarkers were available on all participants in this sample.

6.4 pg/mL (repeatability: 3.8%; intermediate precision: 4.6%) and 52 pg/mL (repeatability: 2.7%; intermediate precision: 5.1%), and those in serum had concentrations of 15.0 pg/mL (repeatability: 4.4%; intermediate precision: 4.4%) and 45 pg/mL (repeatability: 3.9%; intermediate precision: 6.8%).

$p-tau_{181}$: The validated measurement interval was 2–128 pg/mL. QC samples had concentrations of 23.1 pg/mL (repeatability: 2.4%; intermediate precision: 3.8%) and 46.9 pg/mL (repeatability: 1.5%; intermediate precision: 6.1%).

In addition to the five measured values, the $A\beta\frac{42}{40}$ and $\frac{pTau_{181}}{A\beta_{42}}$ ratios were calculated based on earlier work indicating their relevance.[15,16]

2.4 | CSF collection methods

Lumbar punctures (LPs) were completed after an 8- to 12-hour fast. Participants provided approximately 22 mL of CSF via gentle extraction with a Sprotte 24- or 25-gauge atraumatic spinal needle into a series of six polypropylene syringes (Pajunk). Median extraction time was 4 minutes (range 2–15). Samples were then combined into a single 30 mL polypropylene tube (Evergreen Labware Products), gently mixed, and centrifuged for 10 minutes at 2000 *g* at at 4°C. CSF was aliquoted by 0.5 mL into 1.5-mL polypropylene tubes (Fisher 05-408-132) and frozen at −80°C within 30 minutes. $A_{\cdot}(1-42)$, $A_{\cdot}(1-40)$, p-tau, t-tau, and NfL were measured as part of the Roche NeuroToolKit. Elecsys β-Amyloid(1-42) CSF, Total-Tau CSF, Phospho-Tau(181) CSF, and β-Amyloid(1-40) CSF immunoassays were measured on a cobas e 601 analyzer; the NfL immunoassay was measured on a cobas e 411 analyzer (Roche Diagnostics International Ltd). CSF $A_{\cdot}$ positivity was determined using an $A\beta\frac{42}{40}$ threshold of .046.[15]

2.5 | PET methods

Participants underwent 70-minute dynamic [11C] (PiB) PET imaging acquired on either a Siemens Biograph Horizon mCT or a Siemens EXACT HR+ tomograph. Amyloid burden was quantified as the mean distribution volume ratio (DVR) across eight bilateral regions of interest defined from T1-weighted magnetic resonance imagin using Logan graphical analysis with the cerebellum gray matter as a reference region.[17] Because PiB-PET scans and blood samples were not temporally aligned, we used group-based trajectory modeling (GBTM) and piecewise regression on a larger dataset of 179 participants with longitudinal PiB data to produce model-based estimates of global PiB DVR (mDVR) at the time of blood sample collection.[18] In this method, the age of PiB^+ was first estimated for individual cases referencing their last available PiB scan as previously described. Piecewise regression was then applied to model the relationship between PiB DVR versus PiB^+ chronicity (age at PiB scan – age PiB^+. Finally, PiB mDVR was estimated for each participant by calculating PiB^+ chronicity at the

time of blood sampling (age at last PiB scan − age at blood sampling + PiB^+ chronicity at last PiB scan) and entering this value into the piecewise equation. For categorical analyses, the threshold for PET A_{β} positivity was observed (i.e., not modeled) DVR > 1.19 at the time of measurement.

2.6 | Statistical methods

Group-based trajectory modeling was performed with SAS v. 9.4;[19,20] other statistical analysis was performed with R.[21] Age-matching for sample selection was achieved using the R package MatchIt.[22] Influence was estimated as Cook's *d*. Linear models relating measured analyte values in EDTA plasma, heparin plasma, and serum were fit with and without influential values. The remaining primary analyses were performed with one influential datapoint removed. Blood analyte levels were compared using unequal-variance *t* tests in groups defined by CNS-based A_{β} status, and receiver-operator characteristic (ROC) curves were constructed to quantify separability. Using ROC-derived thresholds, we computed binary positivity values for each analyte in each medium and calculated percent agreement (PA), positive percent agreement (PPA), and negative percent agreement (NPA) as a secondary measure of concordance between tube types. Spearman correlations were calculated among blood measures and between blood measures and corresponding markers in CSF, as well as with PiB mDVR. Within each family of tests, *P*-values were adjusted for multiplicity using the Benjamini-Hochberg procedure.[23] For all criterion-related analyses, sensitivity analyses were performed, first including influential datapoints, and then excluding two individuals with clinical cognitive impairment.

3 | RESULTS

Descriptive statistics on analyte levels obtained from EDTA plasma, heparin plasma, and serum are shown in Table 2. Levels tended to be highest in heparin plasma, lower in EDTA plasma, and lower still in serum, except for NfL.

3.1 | Linear crosswalks for stored blood products

3.1.1 | EDTA plasma and heparin plasma

The relationship between each analyte as measured in EDTA plasma (y-axis) and heparin plasma (x-axis) plasma samples is illustrated in Figure 1A. Observations tended to be larger in heparin plasma than in EDTA plasma, lying below the line $y = x$ in Figure 1A, except for $A\beta_{\frac{42}{40}}$ and $\frac{p-tau_{181}}{A\beta_{42}}$. Figure S1A in supporting information illustrates a similar comparison by site. One participant (filled circle) was far from the center of mass of the points four of six panels, with unusually low values for $A\beta_{40}$, $A\beta_{42}$, and t-tau in heparin plasma and unusually high values in EDTA plasma (Cook's *d* values: $A\beta_{40}$, 7.54; $A\beta_{42}$, 4.84; t-tau, 0.57; NfL, 0.72). For $p-tau_{181}$ and $\frac{p-tau_{181}}{A\beta_{42}}$, a different datapoint was highly influential (Figure 1A, crossed circle; Cook's *d* values: $p-tau_{181}$, 1.78; $\frac{p-tau_{181}}{A\beta_{42}}$, 1.37). The two participants with clinical impairment were not influential points and were retained.

Summaries of linear regression models predicting EDTA plasma levels of each analyte from heparin plasma levels are presented in Table 3. Final model fit statistics were excellent for models predicting EDTA plasma levels of NfL from those in heparin plasma ($R^2 = 0.94$), and were fair to good for predicting EDTA plasma levels of amyloid analytes ($A\beta_{42}R^2 = 0.69$, $A\beta_{40}R^2 = 0.4$) and t-tau ($t-tauR^2 = 0.42$) from those in heparin plasma, but weak for predicting EDTA plasma levels of $p-tau_{181}$ ($R^2 = 0.22$) from those in heparin plasma. Residual plots are shown in Figure S2A in supporting information.

3.1.2 | Plasma and serum

The relationship between each analyte as measured in EDTA plasma (y-axis) and serum (x-axis) samples is illustrated in Figure 1B (by site in Figure S1B). Observations tended to be larger in EDTA plasma than in serum, lying above the line $y = x$, except for $A\beta_{\frac{42}{40}}$ and NfL. Participants identified as influential datapoints in the EDTA plasma-heparin plasma models were also influential here (Cook's *d* values: $A\beta_{40}$, 0.093; $A\beta_{42}$, 0.23; t-tau, 0.031; NfL, 1.32; $p-tau_{181}$, 1.64; $\frac{p-tau_{181}}{A\beta_{42}}$, 1.98). The

TABLE 2 Mean (SD) analyte values obtained from EDTA plasma, heparin plasma, and serum samples (N = 38 per collection tube type)

	EDTA		Heparin		Serum	
Biomarker	**$A\beta^-$**	**$A\beta^+$**	**$A\beta^-$**	**$A\beta^+$**	**$A\beta^-$**	**$A\beta^+$**
$\frac{p-tau_{181}}{A\beta_{42}}$	1.11 (0.43)	1.77 (0.56)	1.63 (1.41)	2.03 (1.06)	0.78 (0.80)	1.11 (0.53)
$A\beta_{\frac{42}{40}}$	0.068 (0.011)	0.059 (0.010)	0.068 (0.013)	0.058 (0.0072)	0.092 (0.016)	0.083 (0.014)
$A\beta_{42}$, pg/mL	13.64 (2.29)	11.80 (1.68)	16.16 (3.24)	15.59 (2.62)	12.35 (2.38)	11.01 (2.10)
$A\beta_{40}$, pg/mL	202.5 (24.00)	201.0 (25.10)	242.7 (47.87)	270.7 (40.51)	134.6 (20.44)	135.2 (29.05)
$p-tau_{181}$, pg/mL	14.73 (5.15)	20.62 (6.01)	26.15 (24.53)	30.69 (15.54)	9.10 (8.20)	11.90 (5.59)
t-tau, pg/mL	1.96 (0.55)	2.00 (0.60)	3.77 (1.28)	4.36 (1.73)	0.43 (0.38)	0.35 (0.16)
NfL, pg/mL	12.28 (4.59)	12.82 (4.36)	15.35 (5.82)	16.29 (5.88)	13.67 (4.87)	15.04 (5.10)

Abbreviations: A_{β}, amyloid beta; EDTA, ethylenediaminetetraacetic acid; NfL, neurofilament light chain; $p-tau_{181}$, phosphorylated tau-181; SD, standard deviation; t-tau, total tau.

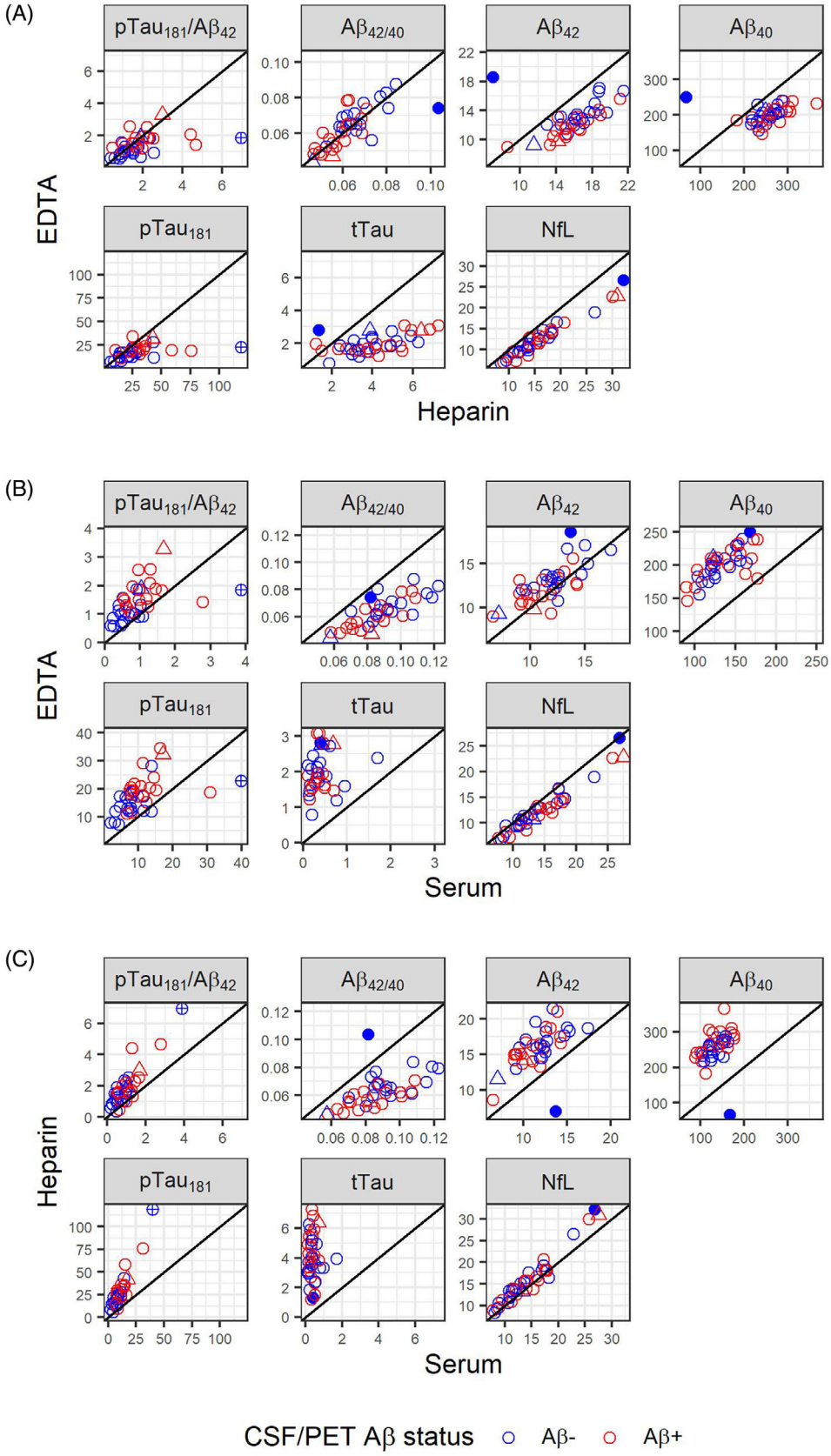

FIGURE 1 Analyte values measured in EDTA plasma against those measured in (A) heparin plasma and (B) serum, as well as (C) values measured in heparin plasma against those measured in serum. All analyte values were measured in pg/mL (ratios are unitless). Shaded/crossed circles denote influential datapoints; triangles denote clinical cognitive impairment. Solid line represents equivalence ($y = x$). $A_{..}$, amyloid beta; EDTA, ethylenediaminetetraacetic acid; NfL, neurofilament light chain; $p-tau_{181}$, phosphorylated tau-181; t-tau, total tau

TABLE 3 Univariate linear regression models predicting analyte values in EDTA plasma samples from those in heparin plasma and serum samples

Biomarker	Model	R^2	P	R^2_{sens}	ρ_S	PA_{obs}	PPA_{obs}	NPA_{obs}	PA_{mod}	PPA_{mod}	NPA_{mod}
$\frac{p-tau_{181}}{A\beta_{42}}$	$\hat{y}_{EDTA} = 0.87 + 0.64 \times y_{SERUM}$	0.28	.00070	0.21	0.64	0.76	0.83	0.64	0.76	0.96	0.43
	$\hat{y}_{EDTA} = 0.88 + 0.33 \times y_{HEP}$	0.25	.0015	0.20	0.60	0.79	0.75	0.86	0.68	0.92	0.29
	$\hat{y}_{HEP} = 0.42 + 1.46 \times y_{SERUM}$	0.62	.0000000091	0.79	0.73	0.76	0.90	0.61	0.82	0.85	0.78
$A\beta\frac{42}{40}$	$\hat{y}_{EDTA} = 0.0052 + 0.94 \times y_{HEP}$	0.66	.0000000011	0.56	0.81	0.71	0.88	0.59	0.87	0.81	0.91
	$\hat{y}_{EDTA} = 0.015 + 0.54 \times y_{SERUM}$	0.63	.0000000039	0.60	0.75	0.74	0.94	0.59	0.82	0.75	0.86
	$\hat{y}_{HEP} = 0.024 + 0.43 \times y_{SERUM}$	0.53	.00000031	0.31	0.65	0.66	0.74	0.53	0.63	0.70	0.53
$A\beta_{42}$	$\hat{y}_{EDTA} = 2.14 + 0.65 \times y_{HEP}$	0.69	.00000000024	0.17	0.67	0.68	1.00	0.52	0.68	0.46	0.80
	$\hat{y}_{EDTA} = 5.00 + 0.65 \times y_{SERUM}$	0.58	.000000052	0.54	0.73	0.79	0.77	0.80	0.79	0.69	0.84
	$\hat{y}_{HEP} = 6.70 + 0.81 \times y_{SERUM}$	0.54	.00000020	0.31	0.65	0.68	0.56	0.92	0.87	0.88	0.85
$A\beta_{40}$	$\hat{y}_{EDTA} = 111.3 + 0.67 \times y_{SERUM}$	0.49	.0000012	0.52	0.69	0.63	0.95	0.19	0.76	0.82	0.69
	$\hat{y}_{EDTA} = 86.89 + 0.43 \times y_{HEP}$	0.40	.000025	0.043	0.57	0.76	1.00	0.44	0.76	0.91	0.56
	$\hat{y}_{HEP} = 150.5 + 0.83 \times y_{SERUM}$	0.36	.000084	0.074	0.55	0.87	0.97	0.43	0.84	0.94	0.43
$p-tau_{181}$	$\hat{y}_{EDTA} = 11.18 + 0.65 \times y_{SERUM}$	0.29	.00062	0.23	0.64	0.76	0.83	0.70	0.68	0.61	0.75
	$\hat{y}_{EDTA} = 11.96 + 0.21 \times y_{HEP}$	0.22	.0031	0.17	0.64	0.84	0.94	0.75	0.74	0.67	0.80
	$\hat{y}_{HEP} = 3.31 + 2.33 \times y_{SERUM}$	0.75	.0000000000037	0.87	0.80	0.82	0.82	0.81	0.82	0.77	0.88
t-tau	$\hat{y}_{EDTA} = 0.94 + 0.25 \times y_{HEP}$	0.42	.000013	0.28	0.51	0.66	0.60	0.70	0.66	0.60	0.70
	$\hat{y}_{EDTA} = 1.81 + 0.37 \times y_{SERUM}$	0.039	.24	0.037	0.28	0.58	0.067	0.91	0.55	0.33	0.70
	$\hat{y}_{HEP} = 4.17 \pm 0.0890 \times y_{SERUM}$	0.00031	.92	0.00033	-0.0290	0.50	0	0.86	0.58	0.25	0.82
NfL	$\hat{y}_{EDTA} = 0.89 + 0.81 \times y_{SERUM}$	0.94	0	0.94	0.96	0.89	0.82	1.00	0.92	0.91	0.94
	$\hat{y}_{EDTA} = 1.23 + 0.71 \times y_{HEP}$	0.94	0	0.95	0.96	0.89	0.95	0.81	0.89	0.95	0.81
	$\hat{y}_{HEP} = 0.031 + 1.09 \times y_{SERUM}$	0.94	0	0.95	0.95	0.84	0.75	1.00	0.87	0.83	0.93

Abbreviations: R^2_{sens}: R^2 in full set including one influential outlier; ρ_S, Spearman correlation between model-predicted and observed values; $A_{\cdot}$, amyloid beta; EDTA, ethylenediaminetetraacetic acid; NfL, neurofilament light chain; NPA, negative percentage agreement between binarized observations (obs) or model predictions (mod); PA, percentage agreement; PPA, positive percentage agreement; $p-tau_{181}$, phosphorylated tau-181; t-tau, total tau.

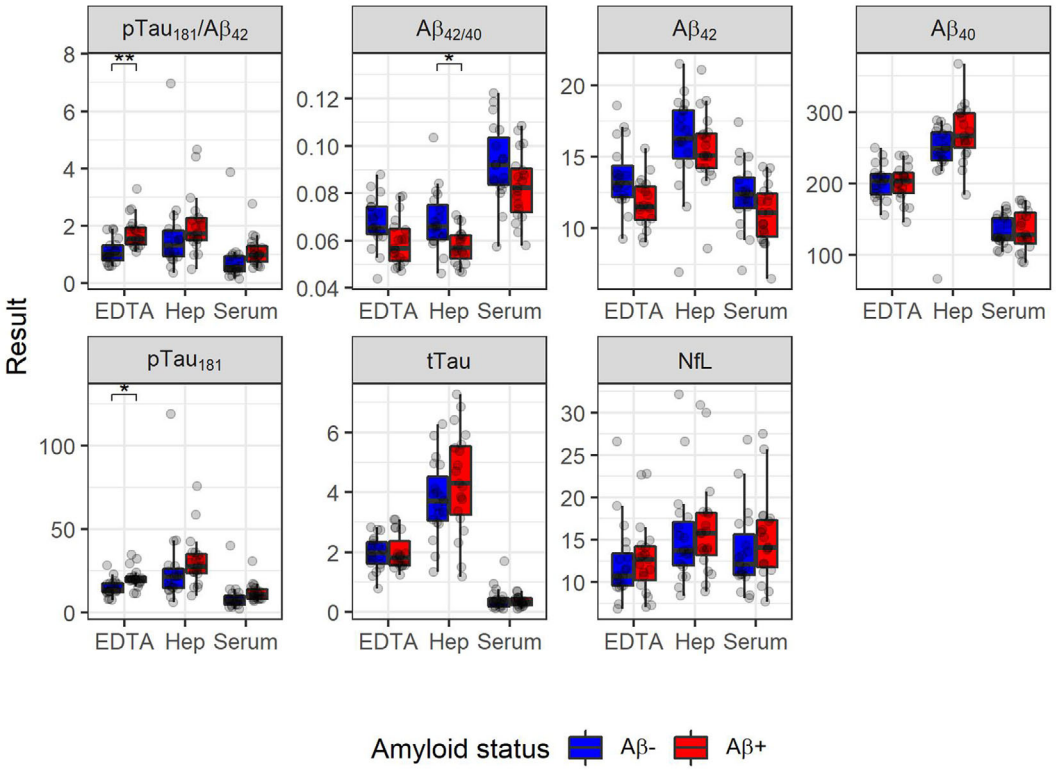

FIGURE 2 Boxplots showing plasma values of each analyte by binary amyloid status from either CSF or PET. All analyte values were measured in pg/mL (ratios are unitless). A$_{\beta}$, amyloid beta; EDTA, ethylenediaminetetraacetic acid; NfL, neurofilament light chain; p − tau$_{181}$, phosphorylated tau-181; t-tau, total tau. *P < .05; **P < .01

two participants with clinical impairment were not influential points and were retained. A similar plot relating heparin plasma to serum values is shown in Figure 1C (by site in Figure S1C).

Summaries of linear regression models predicting EDTA plasma and heparin plasma levels of each analyte from serum levels are presented in Table 3. As with models relating plasma measurements, final model fit statistics were excellent for models predicting plasma levels of NfL from those in serum (EDTA plasma: R^2 == 0.94; heparin plasma: R^2 = 0.94), and were fair to good for predicting plasma levels of amyloid analytes from those in serum (EDTA plasma: $A\beta_{42}R^2$ == (R^2 = 0.58, $A\beta_{40}R^2$ = 0.49; heparin plasma: $A\beta_{42}R^2$ == (R^2 = 0.54, $A\beta_{40}R^2$ = 0.36). For p − tau$_{181}$, serum models were weak for predicting levels in EDTA plasma (R^2 == 0.29), but considerably better for heparin plasma (R^2 = 0.75). With t-tau, low serum levels compromised both models (EDTA plasma: R^2 = 0.039; heparin plasma: R^2 = 0.00031). Residual plots are shown in Figure S2B-C.

3.2 | Congruence with criterion measures

3.2.1 | Plasma measures and binary CSF/PET amyloid status

Boxplots showing the range of plasma values observed by CSF/PET A$_{\beta}$ status are shown in Figure 2. Three fairly strong group differences were observed between $A\beta^-$ and $A\beta^+$ individuals: for $\frac{\text{p-tau}_{181}}{A\beta_{42}}$ and p − tau$_{181}$ in EDTA plasma, and for $A\beta_{\frac{42}{40}}$ in heparin plasma, with effect sizes of approximately 1 (EDTA plasma $\frac{\text{p-tau}_{181}}{A\beta_{42}}$: Cohen's d = 1.29, AUC = 0.84, sensitivity = 0.95, specificity = 0.68; EDTA plasma p − tau$_{181}$: d = 1.02, AUC = 0.80, sensitivity = 0.79, specificity = 0.84; heparin plasma $A\beta_{\frac{42}{40}}$: d = -0.980, AUC = 0.76, sensitivity = 0.84, specificity = 0.63). The full set of comparisons is shown in Table 4. A secondary analysis excluding two with clinical cognitive impairment produced similar effect sizes (EDTA plasma $\frac{\text{p-tau}_{181}}{A\beta_{42}}$: Cohen's d = 1.45, AUC = 0.85, sensitivity = 0.94, specificity = 0.71; EDTA plasma p − tau$_{181}$: d = 0.98, AUC = 0.79, sensitivity = 0.78, specificity = 0.82; heparin plasma $A\beta_{\frac{42}{40}}$: d = -1.17, AUC = 0.78, sensitivity = 0.83, specificity = 0.65).

We applied the above ROC thresholds to assess concordance between binary positivity for each blood biomarker as defined both from raw observations and via model predictions (estimated EDTA plasma levels as a function of heparin plasma or serum levels; estimated heparin levels as a function of serum levels). Percentage agreement for each set of values is shown in Table 3.

3.2.2 | Plasma measures and CNS measures

For those with at least one CSF measurement, scatterplots illustrating congruence between analyte levels in plasma and the most proximal

TABLE 4 Comparison of analyte measurements in blood and CSF (N = 34) and PET (N = 36)

Biomarker	Medium	*t*	*df*	$P_{t.adj}$	Cohen's *d*	AUC (95% CI)	ρ_{CSF}	$\rho_{CSF.sens}$	$p_{\rho CSF.adj}$	ρ_{PiB}	$\rho_{PiB.sens}$	$p_{\rho PiB.adj}$
$\frac{p-tau_{181}}{A\beta_{42}}$	EDTA	3.94	33.64	.0082	1.29	0.84 (0.71, 0.97)	0.42	0.36	0.11	0.55	0.58	0.0055
	Heparin	0.99	30.97	.46	0.33	0.69 (0.51, 0.87)	0.23	0.041	0.37	0.32	0.27	0.12
	Serum	1.39	28.76	.37	0.46	0.78 (0.63, 0.93)	0.40	0.10	0.12	0.48	0.50	0.021
$A\beta_{\frac{42}{40}}$	EDTA	−2.35	34.37	.10	−0.770	0.73 (0.57, 0.90)	0.42	0.38	0.11	−0.550	−0.570	0.0055
	Heparin	−2.96	30.99	.041	−0.980	0.76 (0.60, 0.92)	0.44	0.39	0.11	−0.550	−0.590	0.0055
	Serum	−1.97	33.58	.20	−0.650	0.67 (0.49, 0.85)	0.38	0.32	0.12	−0.470	−0.420	0.021
$A\beta_{42}$	EDTA	−2.56	33.22	.080	−0.840	0.75 (0.59, 0.91)	0.24	0.24	0.37	−0.460	−0.500	0.021
	Heparin	−1.29	34.98	.39	−0.430	0.60 (0.41, 0.79)	0.055	0.010	0.80	−0.320	−0.210	0.12
	Serum	−1.69	33.67	.27	−0.560	0.67 (0.50, 0.85)	0.13	0.13	0.64	−0.360	−0.390	0.082
$A\beta_{40}$	EDTA	0.15	34.72	.88	0.050	0.50 (0.31, 0.69)	0.039	0.079	0.83	0.17	0.077	0.45
	Heparin	1.70	28.55	.27	0.55	0.68 (0.50, 0.85)	−0.130	−0.120	0.64	0.30	0.36	0.13
	Serum	0.31	31.46	.80	0.10	0.52 (0.32, 0.71)	−0.0710	−0.0400	0.77	0.066	0.0099	0.74
$p-tau_{181}$	EDTA	3.12	34.82	.038	1.02	0.80 (0.65, 0.95)	0.32	0.38	0.25	0.43	0.44	0.027
	Heparin	0.55	28.13	.68	0.18	0.68 (0.51, 0.86)	0.084	0.033	0.77	0.22	0.28	0.30
	Serum	1.13	29.34	.43	0.37	0.74 (0.58, 0.91)	0.27	0.16	0.33	0.32	0.35	0.12
t-tau	EDTA	0.45	34.84	.72	0.15	0.52 (0.32, 0.72)	0.070	0.096	0.77	0.10	0.036	0.64
	Heparin	0.94	31.72	.47	0.31	0.61 (0.43, 0.80)	0.27	0.28	0.33	0.27	0.32	0.19
	Serum	−0.800	22.67	.53	−0.270	0.50 (0.31, 0.69)	−0.0970	−0.110	0.77	0.021	−0.0110	0.90
NfL	EDTA	1.08	32.52	.43	0.35	0.57 (0.38, 0.76)	0.21	0.32	0.38	0.080	−0.00750	0.72
	Heparin	1.11	32.83	.43	0.36	0.59 (0.40, 0.77)	0.23	0.37	0.37	0.14	0.045	0.53
	Serum	1.43	33.18	.37	0.47	0.61 (0.43, 0.80)	0.22	0.32	0.37	0.17	0.080	0.45

Notes: Correlation coefficient estimates (ρ_{CSF}, ρ_{PiB}) represent Spearman correlations when outliers were excluded (see details in Methods); sensitivity analyses including these observations are also reported ($\rho_{CSF.sens}$, $\rho_{PiB.sens}$). *P*-values for primary *t* tests and correlations were adjusted using the Benjamini-Hochberg correction for multiplicity ($p_{t.adj}$, $p_{\rho CSF.adj}$, $p_{\rho CSF.adj}$).

Abbreviations: R^2_{sens}: R^2 in full set including one influential outlier; AUC, area under the receiver-operator characteristic curve (with 95% confidence interval); $A_{\cdot}$, amyloid beta; EDTA, ethylenediaminetetraacetic acid; NfL, neurofilament light chain; PiB, modeled global Pittsburgh compound B distribution volume ratio (mDVR); $p-tau_{181}$, phosphorylated tau-181; SD, standard deviation; see Methods); t-tau, total tau.

CSF measurement are shown in Figure S3A-B in supporting information. Observed correlations were all small to medium in size, with none reaching statistical significance after correcting for multiple comparisons (Table 4).

For those participants with amyloid PET, scatterplots illustrating congruence between plasma measurements and estimated concurrent global PiB burden (mDVR) are shown in Figure S4A-B in supporting information. Several moderate correlations were observed that remained significant after adjustment for multiple comparisons; the strongest of these was between PiB mDVR and $\frac{\text{p-tau}_{181}}{\text{A}\beta_{42}}$ as measured in EDTA plasma (Table 4; $r_{PiB} = 0.55$, $p_{r.PiB} = .0055$). Other strong correlations were seen between PiB mDVR and $\text{p} - \text{tau}_{181}$ levels as measured in EDTA plasma ($r_{PiB} = 0.43$, $p_{r.PiB} = .027$), and $\text{A}\beta_{\frac{42}{40}}$ levels as measured in both heparin plasma (Figure S3B, panel 1; $r_{PiB} = -0.550$, $p_{r.PiB} = .0055$) and EDTA plasma ($r_{PiB} = -0.550$, $p_{r.PiB} = .0055$). In a secondary analysis excluding two with clinical cognitive impairment, results for these analytes were similar (EDTA plasma $\frac{\text{p-tau}_{181}}{\text{A}\beta_{42}}$: $r_{PiB} = 0.51$, $p_{r.PiB} = .021$; EDTA plasma $\text{p} - \text{tau}_{181}$: $r_{PiB} = 0.37$, $p_{r.PiB} = .10$; heparin plasma $\text{A}\beta_{\frac{42}{40}}$: $r_{PiB} = -0.550$, $p_{r.PiB} = .0055$; EDTA plasma $\text{A}\beta_{\frac{42}{40}}$: $r_{PiB} = -0.550$, $p_{r.PiB} = .0055$). Full numeric results are listed in Table 4.

4 | DISCUSSION

Reliable measurement of AD biomarkers in blood could be of special relevance for existing studies of preclinical populations with extensive blood biobanks. We wished to understand the robustness of these markers across different types of stored blood samples. To this end, we modeled the relationship between levels of five analytes and two analyte ratios as measured in EDTA plasma, heparin plasma, and serum in WRAP participants with independently obtained CNS amyloid biomarker status. EDTA plasma–heparin plasma relationships were excellent for NfL and acceptable for most analytes after removal of one outlier, though all were weaker than those recently found in a smaller convenience sample.[14] However, correspondence with EDTA plasma levels of $\text{p} - \text{tau}_{181}$ was poor for both heparin plasma and serum, suggesting low interchangeability for this analyte. Further, serum measurements for tTau were unacceptably low, unrelated to plasma measurements, and variable by site (Figure S1), echoing a recent finding from another group that serum is a poor medium for this analyte.[14] Because few in our sample had CSF $\text{p} - \text{tau}_{181}$ values exceeding our previously published cutoff,[15] restriction of range may have been a factor. Importantly, all model results suggested nonequivalence of these measures (Figure 1), indicating that cutpoints developed on one sample type cannot be applied to another type without adjustment. However, percentage agreement between pairs of binarized variables was generally acceptable, both with raw measurements and with model-predicted values (Table 3). As new biomarkers are discovered, the optimal sample type should be determined for each.

Concordance with CNS-based measures was a secondary aim, but results were encouraging. Large group differences by CNS $A_{\cdot}$ positivity were evident in $\text{p} - \text{tau}_{181}$ levels and in the ratios $\frac{\text{p-tau}_{181}}{\text{A}\beta_{42}}$ and $\text{A}\beta_{\frac{42}{40}}$. Further, relationships between these biomarkers and estimated concurrent global PiB burden were stronger. Interestingly, these relationships obtained despite the small number of CSF $\text{p} - \text{tau}^{+}_{181}$ cases noted above, perhaps suggesting that our cutoff is conservative in unimpaired samples.[6] Correspondence between blood and CSF levels of the same biomarkers was lower than correspondence with PET. Several explanations are possible: the aforementioned range restriction; the temporal mismatch between CSF and blood draws, a weakness mitigated with PET data by modeling concurrent amyloid burden (mDVR);[18] and the known "stickiness" of $\text{A}\beta_{42}$, which limits its use as a standalone criterion. To the latter point, correlations between corresponding blood and CSF biomarker ratios were stronger than those between raw analyte levels.

Although this small sample was not designed to assess diagnostic properties of the assays, the reasonable sensitivity of EDTA plasma $\frac{\text{p-tau}_{181}}{\text{A}\beta_{42}}$ and $\text{p} - \text{tau}_{181}$, along with heparin plasma $\text{A}\beta_{\frac{42}{40}}$, to binary CNS $A_{\cdot}$ status suggests these markers may have promise for screening, as has been previously suggested.[4,7,16] AD has many properties favoring a screening program: seriousness, a long preclinical phase with high prevalence, and a consensus that candidate treatments have been administered too late, rendering them ineffective.[24] A sensitive, inexpensive blood test would improve the feasibility of screening as part of the broad strategy for fighting AD, with more specific, expensive measures like amyloid PET used serially to identify true positives with confidence. Future work will explore this in more depth.

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CONFLICTS OF INTEREST

SCJ serves on an advisory board for Roche Diagnostics. KB 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 AB (BBS), which is a part of the GU Ventures Incubator Program, all unrelated to the present study. HZ has served on scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, and CogRx; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, and Biogen; 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). BB has received precursors and tracers from Avid Radiopharmaceuticals. CEG is a member of the Executive Board for the Alzheimer's and Dementia Alliance of Wisconsin, and receives support from the Alzheimer's Association for travel to AAIC, for which she is on the Scientific Program Committee. GK is a full-time employee of Roche Diagnostics GmbH. IS is a full-time employee and shareholder of Roche Diagnostics International Ltd. Authors KH, CE, OO, TB, CVH, RK, LH, and EMJ have no disclosures.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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