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RESEARCH ARTICLE

Plasma biomarkers of Alzheimer's disease and related dementias in American Indians: The Strong Heart Study

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Abstract

INTRODUCTION: Identification of Alzheimer's disease (AD) needs inexpensive, non-invasive biomarkers, with validation in all populations.

METHODS: We collected plasma markers in older American Indian individuals: phosphorylated-tau181 (pTau181); amyloid-beta (Aβ) 40,42; glial fibrillary acidic protein (GFAP); and neurofilament light chain (NfL). Plasma markers were analyzed for discriminant properties with cognitive status and etiology using receiver operating characteristic (ROC) analysis.

RESULTS: PTau181, GFAP, NfL plasma values were significantly associated with cognition, but Aβ were not. Discriminant performance was moderate for individual markers, with pTau181, GFAP, NfL performing best, but an empirically selected panel of markers (age, sex, education, pTau181, GFAP, NfL, Aβ4240 ratio) had excellent discriminant performance (AUC > 0.8).

DISCUSSION: In American Indian individuals, pTau181 and Aβ values suggested more common pathology than in majority populations. Aβ was less informative than in other populations; however, all four markers were needed for a best-performing dementia diagnostic model. These data validate utility of AD plasma markers, while suggesting population-specific diagnostic characteristics.

KEYWORDS
Alzheimer's disease, ATN biomarkers, cognition, imaging, memory, plasma markers

1 | BACKGROUND

Early identification of Alzheimer's disease (AD) is critical to advancements in prevention and treatment efforts. The 2018 joint statement by National Institute on Aging and Alzheimer's Association recommends a research framework for defining AD using objective, quantifiable features reflecting presence and degree of amyloid deposition (A), pathologic tau (T), and neuronal injury (N).[1] This "AT(N) framework" aims to distinguish AD pathology from the resulting clinical syndrome, as a more sensitive, more specific approach to case identification. In proof of concept, cerebrospinal fluid (CSF) AT(N) biomarkers have high sensitivity and specificity for AD pathology, even in advance of detectable changes on cranial imaging or pathology, and long before detectable changes in cognitive symptoms.[2]

However, collection of CSF requires specific training, may be regarded as invasive by many patients, entails varying costs across healthcare systems, and is considered unacceptable in many communities.[3] The current alternative, positron emission tomography (PET) imaging, is highly expensive, involves exposure to radiation, and is unrealistic in settings with poor access to socioeconomic resources or specialty care. Even structural MRI, which reasonably reflects only (N) markers in the AT(N) framework, can be unattainably expensive, and still does not provide insight into A or T markers. Alternative AT(N) markers that are low or noninvasive, lower cost, and more widely acceptable are needed, especially for characterization of presymptomatic AD in populations heavily affected by health and socioeconomic disparities.

Circulating biomarkers for A, T, and N pathology may offer a viable low-cost, widely acceptable, widely available measure,[4] providing an especially pragmatic innovation for the community clinic setting. Candidate circulating markers of interest in the AT(N) framework include amyloid beta 40 and 42 ($A\beta_{40}$, $A\beta_{42}$; A marker), phosphorylated tau 181 ($pTau_{181;}$ T marker), glial fibrillary protein (GFAP; N marker), and neurofilament light chain (NfL; N marker). Each of these plasma markers may contribute insights into one of the A,T,N components in the AT(N) framework, and collectively have the potential to categorize presymptomatic individuals into those with or without (presymptomatic) AD pathology.

No prior study has measured or evaluated these plasma markers in American Indian individuals or other Indigenous populations, who are both heavily burdened with AD risk but also critically underrepresented in AD research. Therefore, this study aims to describe AT(N) plasma markers, as well as associations with clinical, MRI, and cognitive features of AD in a large cohort of community-based American Indian individuals. This work has the potential to provide insights for researchers and clinicians on AD characteristics, diagnostics, and risk in American Indian elderly and their communities, with the ultimate goal to support future research and public health programs to improve detection, prevention, and treatment opportunities all peoples.

RESEARCH IN CONTEXT

1. **Systematic review**: AT(N) plasma marker distributions and their associations with brain imaging, cognitive, and memory features have yet to be established for many high-risk but underserved populations, including American Indian individuals.
2. **Interpretation**: Our study reported both cognitive normal and impaired ranges for multiple plasma markers associated with Alzheimer's disease (AD) and related dementias in other populations, including phosphorylated tau181 (pTau181), amyloid-beta ($A\beta$) 40,42, and 42/40, glial fibrillary protein (GFAP), and neurofilament light chain (NfL). Associations are reported for these plasma markers with conventional AD risk features such as *apolipoprotein E* (*APOE*) ε4, with brain imaging features of atrophy and vascular injury, with adjudicated cognitive status and etiology, and with categorization of memory status.
3. **Future directions**: Future research is needed to validate plasma markers with gold standard pathology, such as positron emission tomography (PET) imaging; to continue to establish cross-study and cross-population assay calibrations; and to collect these markers over time and in other populations.

2 | METHODS

2.1 | Setting

The Strong Heart Study (SHS) recruited American Indian adults from communities and tribes in the U.S. Northern Plains, Southern Plains, and Southwest starting in 1989–1991. Survivors from the initial SHS study cohort were recruited for examinations related to cognitive aging and AD in 2010–2013 (N = 818), and then invited back for a repeat cognitive and imaging visit in 2017–2019 (N = 403). Full recruitment and examination protocols, including CONSORT diagrams, ethical approvals, and registrations have been previously described.[5,6] All participating institutional, Indian Health Service, and tribal review boards approved study protocols. All participants provided written, informed consent.

2.2 | Plasma assays

Five AT(N) plasma biomarkers ($pTau_{181}$, $A\beta_{40}$, $A\beta_{42}$, GFAP, NfL) were measured in all available, stored samples from the second cognitive

aging visit (2017–2019, $N = 401$). Standard sample handling for ethylene diamine tetraacetic acid (EDTA) plasma samples included: multiple inversion; centrifugation at 1000*G* at 4°C; immediate separation from buffy coat and aliquot into 2 mL cryovial tubes; and −80°C storage. Assays were done by the Clinical Neurochemistry Laboratory at the University of Gothenburg, according to standard protocols evaluated by the Standardization of Alzheimer's Blood Biomarkers workgroup of the Global Biomarker Standardization Consortium of the Alzheimer's Association.[7] Assays were Simoa Neurology 4-Plex E kit (Quanterix) for $A\beta_{42\,\&\,40}$, GFAP, and NfL, which has good test-retest performance metrics.[8] Separately, $pTau_{181}$ was measured using the Simoa prototype two-step assay, as previously described in detail.[9] The preparation protocol included thaw at room temperature, vortex, centrifuge 5 min at 10,000G (4-plex kit) or centrifuge 10 min (two-step assay). The resulting data concentrations are in pg/mL, except for $A\beta_{42/40}$ which is calculated as a ratio. All measurements were performed in one round of experiments, using one batch of reagents, by board-certified laboratory technicians, who were blinded to clinical data. Intra-assay coefficients of variation were below 10% for all of the markers.

2.3 | Cognitive tests

Neuropyschological tests at both visits included the California Verbal Learning Test II Short Form (CVLT II-SF; primary domains assessed: verbal learning, memory),[10] Modified Mini-Mental Status Examination (3MSE; general cognition),[11] Controlled Oral Word Association FAS (COWA-FAS; phonemic fluency, executive function),[12] WAIS digit symbol coding test (processing speed). Tests added at the second visit included the National Alzheimer's Coordinating Center Uniform Data Set (UDS) C2 forms,[13] which are comprised of Montreal Cognitive Assessment (MoCA; general function),[14] Number Span Test forward and backward (auditory attention, working memory), Benson Complex Figure copy and recall (visuospatial),[15] animal and vegetable naming tests (semantic fluency), Trail Making Test A and B (simple and divided attention, executive function),[16] Craft Story immediate and delayed recall (conextual verbal memory),[17] and Multilingual Naming Test (MINT; semantic naming).[18] Functional status was also assessed for instrumental activities of daily living using the Functional Activities Questionnaire.

2.4 | Cognitive status

An expert panel adjudicated cognitive status and possible etiology by consensus from detailed case review of cognitive and functional data from both examinations. Cognitive case status was assigned as cognitive intact; mild cognitive impairment (MCI) for those with cognitive loss or significant impairment in > 1 test for a given cognitive domain but not significant loss in functional status or multi domain involvement; dementia for those with significant loss in functional ability in activities of daily living and/or significant, multi domain cognitive impairment; and impaired not MCI (InMCI) for those who are not intact but who do not fall into typical MCI and dementia patterns of impairment. Primary and secondary etiologies were assigned as one of several possible underlying causes of cognitive impairment, including AD, vascular brain injury (VBI), traumatic brain injury (TBI), or other. Etiologic assignments were based on patterns of cognitive domain loss and additionally informed by clinical and imaging data.

2.5 | Other data

Field center staff collected self-reported age (years), sex (male, female), years of formal education. Apolipoprotein E ε4 (*APOE* ε4) carrier status was measured by standard genotyping procedures[19,20] using blood samples collected at the baseline SHS visit. Estimated glomerular filtration rate (eGFR) was calculated using serum creatinine via the Modification of Diet in Renal Disease (MDRD) equation.[21]

2.6 | Statistical analyses

We summarized participant characteristics for the study population overall and by *APOE* ε4 carrier status using mean and standard deviation (normal, continuous), median and interquartile range (skewed, continuous), or count and percent (dichotomous). Percent difference in median, mean, or count for biomarkers was calculated by comparing *APOE* ε4 carriers to noncarriers, in order to examine potential differences by endogenous or baseline risk. Graphical plots visually summarized distribution and range of plasma marker measures. Values of plasma marker measures and select participant characteristics were summarized by adjudicated cognitive status (cognitive intact, MCI, dementia, In-MCI) or by probable underlying primary or secondary etiology (AD, VBI, TBI, AD). Receiver operating chracteristic (ROC) analysis was conducted to evaluate diagnostic performance, as estimated by area under the curve (AUC), for plasma markers for dementia (compared to cognitive intact) or AD etiology (compared to cognitive intact), with empirical estimation of optimal cutoff for each marker using Liu product maximization method. Lasso regression with bootstrap errors estimation was used to empirically identify the best performing discriminant panel of plasma markers, in combination with age, sex, and education. All statistics were conducted using Stata v17 (College Station, TX) and R v. 4 (R Foundation for Statistical Computing, Vienna Austria).

3 | RESULTS

Our analysis included 401 (of 403, > 99%) participants from the 2017–2019 examination visit (Table 1). This study population was generally elderly (mean age 78, range 70–94), with 20.9% *APOE* ε4 allele carriers. Most AT(N) plasma markers had wide range of variance, with heavy right-skew. Mean $pTau_{181}$ was 8.6 pg/mL (median 5.0), $A\beta_{40}$ 144.5 pg/mL (med 128), $A\beta_{42}$ 8.4 pg/mL (med 8.2), $A\beta_{42/40}$ ratio 0.06 (med 0.1), GFAP 178.2 pg/mL (med 150.0), and NfL 41.1 pg/mL (med 31.6).

TABLE 1 Selected characteristics from American Indian participants of the Strong Heart Study (2017–2019).

Available sociodemographics, clinical data	***N* = 401**
Age (years)	78.1 (4.7)
Male sex, *n* (%)	118 (29.4%)
Years education	13.0 (2.5)
APOEε4 status, *n* (%)	83 (20.9%)
Available plasma biomarkers data	***N* = 401**
$pTau_{181}$ pg/mL, mean (SD); median; range	8.6 (25.6); 5.0; 1.5-442.0
$A\beta_{40}$ pg/mL, mean (SD); median; range	144.5 (48.4); 128; 6-518.0
$A\beta_{42}$ pg/mL, mean (SD); median; range	8.4 (2.8); 8.2; 2.2-21.1
$A\beta_{42/40}$ ratio, mean (SD); median; range	0.06 (0.01); 0.1; 0.01-0.18
GFAP pg/mL, mean (SD); median; range	178.7 (95.8); 150.0; 43.0-651.0
NfL pg/mL, mean (SD); median; range	41.1 (30.5); 30.5; 9.7-343.0
Available case review consensus data	***N* = 396**
Adjudicated cognitive intact, *n* (%)	181 (45.7%)
Adjudicated MCI, *n* (%)	139 (35.1%)
Adjudicated dementia, *n* (%)	41 (10.4%)
Adjudicated Impaired not MCI, *n* (%)	35 (8.8%)
Available case review consensus data	***N* = 396**
Any AD etiology, *n* (%)[a]	92 (33.7%)
Any VBI etiology, *n* (%)[a]	110 (37.8%)
Any TBI etiology, *n* (%)[a]	28 (13.4%)

Note: Values provided as mean (SD) unless otherwise specified.
Abbreviations: APOE, apoprotein E; AD, Alzheimer's disease;Aβ, amyloid-beta; GFAP, glial fibrillary protein; NfL, neurofilament light chain; pTau, phosphorylated tau;TBI, traumatic brain injury; VBI, vascular brain injury.
[a]*Adjudicated etiologies include primary and secondary assessments and thus not mutually exclusive; percentages calculated by comparison to no cognitive impairment (cognitive intact, n = 181).*

With respect to outliers, $pTau_{181}$ had 6 measured values substantially higher than the rest (108, 120, 131, 161, and 442 pg/mL); NfL also had 1 outlier (343 pg/mL). Bar and scatter plots of plasma marker measures (Figure S1) illustrate a heavy distribution skew for each of the plasma marker measures, with long right tails corresponding to high concentration of plasma proteins. Adjudicated cognitive status estimated that 54.3% of participants were impaired, with approximately 34% attributed to AD and 38% to VBI (not mutually exclusive).

Because of distribution skews, median values for plasma markers were used in comparisons between *APOE* ε4 allele carriers, a major risk factor for AD dementia (Table 2). Comparing *APOE* ε4 carriers to noncarriers, $pTau_{181}$ and GFAP were substantively higher (12% and 16% higher, respectively) and $A\beta_{42}$ and $A\beta_{42/40}$ ratio substantively lower (8.4%, 16.7% lower, respectively); however, only the difference for $A\beta_{42/40}$ was statistically significant after correction for multiple testing. There was no remarkable difference for $A\beta_{40}$ or for NfL.

Quantitative comparison of plasma marker measures across adjudicated cognitive impairment categories (intact, MCI, dementia, In-MCI) identified significant differences comparing participants who were cognitive-intact versus impaired (Table 3). Participants with impairment had significantly higher $pTau_{181}$ than cognitive intact ($p < 0.001$); those with dementia had highest values and MCI, InMCI had intermediate values. Similarly, both GFAP and NfL were highest among dementia patients ($p = 0.006$, $p < 0.001$, respectively) and intermediate in MCI, InMCI groups. However, $A\beta_{40}$, $A\beta_{42}$, and $A\beta_{42/40}$ were not significantly or markedly different across cognitive categories. ROC analysis with empirical estimation of optimal cutoff for each plasma marker, comparing participants with dementia to those who were cognitive intact, identified GFAP and NfL with best performance (AUC ~ 0.7), $pTau_{181}$, $A\beta_{42}$, $A\beta_{40}$ with moderate performance (AUC > 0.6), and $A\beta_{4240}$ ratio with poor performance (AUC < 0.5). Furthermore, $pTau_{181}$, $A\beta_{42}$, and NfL were highly sensitive for discriminating dementia (sensitivity > 0.7), whereas GFAP was highly specific (> 0.7).

Similar quantitative comparisons of plasma marker diagnostic performance, comparing probable primary or secondary etiologies underlying cognitive impairment (AD, VBI, TBI) with no cognitive impairment, suggested moderate performance for $pTau_{181}$, GFAP in discriminating AD from intact (AUC > 0.6; Table 4). Similarly, GFAP and NfL had moderate performance discriminating VBI from intact (AUC > 0.6), and $pTau_{181}$ in discriminating TBI from intact (AUC > 0.6). Furthermore, $pTau_{181}$ and GFAP were highly specific in discriminating TBI (specificity > 0.7), but none of the markers was highly sensitive or specific for AD or VBI.

Finally, an empirically selected panel of plasma markers, in combination with age, sex, and education, identified $pTau_{181}$, $A\beta_{4240}$ ratio, GFAP, and NfL to comprise the best-performing model for discriminating dementia from cognitive intact participants (AUC 0.83, 95% 0.77, 0.89).

Examination of plasma markers comparing memory impairment categories demonstrated similar patterns (Supplement Table): pTau and NfL were associated with memory impairment, but GFAP and Aβ markers were not. Associations for pTau and NfL were highest among those with encoding type memory impairment, and NfL values were intermediate among those with retrieval type impairment.

Qualitative, visual examination of plasma marker distributions suggests differences by adjudicated etiologies underlying cognitive case status, as well (Figure S2). In general, distribution and mean of pTau was higher among those assessed with possible AD, both as primary or as mixed etiology, as well as those with TBI etiology, but not among those with vascular etiology. In contrast, Aβ40 and 42 were lower for those with TBI etiology, but not different among those with AD or vascular etiologies. Aβ42/40 ratio was not different across any groups. GFAP was slightly higher among those with vascular injury, both primary and mixed, and also somewhat higher (bimodal) among those with possible underlying TBI etiology. For NfL, all distributions had long tails, but means were slightly higher among those with vascular and TBI etiologies.

TABLE 2 Plasma marker measures, overall and by APOE ε4 status, in American Indian participants of the Strong Heart Study (2017–2019).

	Overall $N = 401$	No APOE ε4 allele $n = 314$	APOE ε4 carrier $n = 83$	Percent difference: APOE ε4 versus not	*p-Value*	*FDR Q-value*
$pTau_{181}$ pg/mL	5.0 (3.5, 7.4)	4.9 (3.4, 7.2)	5.5 (3.9, 8.3)	+ 12%	*0.067*	*0.182*
$A\beta_{40}$ pg/mL	128 (116, 175)	128 (116, 175)	127 (113, 177)	-0.8%	*0.780*	*0.780*
$A\beta_{42}$ pg/mL	8.2 (6.7, 9.7)	8.3 (6.8, 9.8)	7.6 (6.2, 9.4)	-8.4%	*0.140*	*0.210*
$A\beta_{42/40}$ ratio	0.06 (0.05, 0.07)	0.06 (0.05, 0.07)	0.05 (0.04, 0.06)	- 16.7%	*0.001*	*0.006*
GFAP pg/mL	150 (114, 218)	149 (112, 215)	173 (121, 240)	+ 16.1%	*0.091*	*0.182*
NfL pg/mL	31.6 (23.0, 48.9)	31.5 (22.9, 48.6)	31.8 (23.4, 49.6)	+ 3.2%	*0.470*	*0.564*

Note: Values provided as med (IQR) unless otherwise indicated. p-Values based on Wilcoxon rank-sum test; FDR (false discovery rate). Q-value based on Benjamini–Hochberg method and assessed for significance at $Q < 0.1$.
Abbreviations: APOE, apoprotein E; Aβ, amyloid-beta; GFAP, glial fibrillary protein; NfL, neurofilament light chain; pTau, phosphorylated tau.

TABLE 3 Plasma marker measures, comparing cognitive impairment categories, among American Indian participants of the Strong Heart Study (2017–2019).

	Cognitive intact $N = 181$ 45.6%	MCI $N = 140$ 35.3%	Dementia $N = 41$ 10.3%	In-MCI $N = 35$ 8.8%	Comparing dementia, versus cognitive intact: Empirical, optimal cut point	Comparing dementia, versus cognitive intact: ROC (AUC, sensitivity, specificity) at cut point
$pTau_{181}$ pg/mL	7.6 (17.3)	6.8 (4.7)	10.2 (18.4)	6.6 (4.3)	>4.5	0.66 (0.78, 0.53)
$A\beta_{40}$ pg/mL	140.1 (43.6)	143.9 (45.3)	155.8 (41.7)	143.7 (49.0)	<135.5	0.63 (0.61, 0.65)
$A\beta_{42}$ pg/mL	8.2 (2.9)	8.5 (2.9)	8.8 (2.3)	8.3 (2.5)	<7.9	0.60 (0.71, 0.49)
$A\beta_{42/40}$ ratio	0.059 (0.014)	0.059 (0.014)	0.057 (0.009)	0.060 (0.013)	>0.06	0.46 (0.51, 0.42)
GFAP pg/mL	162.2 (80.9)	175.9 (88.0)	216.2 (88.6)	186.5 (125.3)	>199	0.73 (0.68, 0.78)
NfL pg/mL	34.9 (21.3)	42.4 (28.7)	53.7 (31.1)	43.7 (30.9)	>32.7	0.69 (0.73, 0.64)

Note: *Values provided as mean (SD) unless otherwise indicated.* Cutpoints and ROC comparisons with major risk factors: age (> 75 years, AUC 0.61), sex (male, AUC 0.54), education (< 13, AUC 0.33), APOE (e4 carrier, AUC 0.59). Statistical tests by Kruskal–Wallis test across cognitive categories, with FDR (false discovery rate) Q-value estimated by Benjamini–Hochberg method, assessed for significance at $Q < 0.1$: pTau181 $Q < 0.001$; Ab40 $Q = 0.192$; Ab42 $Q = 0.420$; Ab4240 $Q = 0.420$; GFAP $Q = 0.006$, NFL $Q < 0.001$.

TABLE 4 Plasma marker measures, comparing cognitive impairment etiologies, among American Indian participants of the Strong Heart Study (2017–2019).

	Plasma marker values among AD etiology $n = 92$ (33.7%)	AUC (sensitivity, specificity) comparing AD versus intact	Plasma marker values among VBI etiology $n = 110$ (37.8%)	AUC (sensitivity, specificity) comparing VBI versus intact	Plasma marker values among TBI etiology $n = 28$ (13.4%)	AUC (sensitivity, specificity) comparing TBI versus intact
$pTau_{181}$ pg/mL	7.9 (12.6)	0.61 (0.61, 0.62)	8.4 (12.4)	0.59 (0.58, 0.60)	6.5 (3.8)	0.63 (0.54, 0.72)
$A\beta_{40}$ pg/mL	143.8 (41.8)	0.55 (0.53, 0.57)	151.2 (47.6)	0.58 (0.68, 0.47)	144.7 (62.3)	0.52 (0.43, 0.61)
$A\beta_{42}$ pg/mL	8.4 (2.6)	0.53 (0.44, 0.61)	8.9 (3.0)	0.56 (0.65, 0.48)	8.0 (2.9)	0.50 (0.54, 0.46)
$A\beta_{42/40}$ ratio	0.06 (0.02)	0.48 (0.43, 0.53)	0.06 (0.01)	0.51 (0.54, 0.47)	0.06 (0.01)	0.50 (0.64, 0.36)
GFAP pg/mL	193.5 (101.2)	0.60 (0.53, 0.68)	187.0 (90.4)	0.61 (0.54, 0.68)	150.9 (74.2)	0.56 (0.36, 0.77)
NfL pg/mL	41.2 (27.9)	0.57 (0.57, 0.58)	51.4 (33.7)	0.65 (0.64, 0.66)	41.5 (32.3)	0.58 (0.61, 0.56)

Note: *Values provided as mean (SD) unless otherwise indicated.*Etiologies assigned as primary or secondary and thus not mutually exclusive. Comparators for AD, VBI, TBI are no cognitive impairment. ROC analysis assessed at empirically defined cutpoint (Liu product maximization method).

4 | DISCUSSION

Overall, this is the first report on plasma markers related to AD and other brain diseases—including $pTau_{181}$, $A\beta_{40}$, $A\beta_{42}$, $A\beta_{42/40}$ ratio, GFAP, and NfL—among older American Indian individuals, describing associations with clinical, imaging, and cognitive findings. Our findings include association of *APOE* ε4 with $A\beta_{42/40}$ ratio only; associations of imaged brain volumes with $A\beta_{40}$, $A\beta_{42}$, GFAP, and NfL but imaged brain infarcts with $pTau_{181}$; and associations of memory impairments with $pTau_{181}$, NfL, age, and sex. We also identified differences in

distribution of $pTau_{181}$ closely associated with possible AD etiology, as well as other markers (GFAP, NfL) with vascular and traumatic injury—all of which are important underlying features for cognitive status in this population.

In this study of American Indian individuals, our marker of $pTau_{181}$ (mean 8.6 pg/mL; range 1.5–442.0), had a lower overall mean but a larger variance, compared with prior studies in non-Hispanic White (mean 16, range 10-23), African American (mean 14, range 9.4–22.9), and Hispanic/Latino (mean 18.0, range 11.3–25.0) individuals. Values > 40-60 are often considerd consistent with AD.[22,23] Thus, our population of American Indian individuals did not appear to have evidence of mean differences in pathologic tau (T), but did have some outliers with much higher values than in prior studies.

In contrast, two of our markers of amyloid (A) pathology—$A\beta_{42}$ and $A\beta_{42/40}$—were much lower than in non-Hispanic White or other populations, also consistent with much greater degree of pathology (3X), especially for the larger, more insoluble, more AD-specific $A\beta_{42}$.[24] In cognitive intact non-Hispanic White individuals, $A\beta_{40}$ is estimated to have mean 95 pg/mL, $A\beta_{42}$ 22 pg/mL, and $A\beta_{42/40}$ ratio 0.23; for cognitive impaired, these numbers are 98 pg/mL, 20 pg/mL, 0.2 ratio (respectively).[25] In our study, mean $A\beta_{40}$ was 125 pg/mL, $A\beta_{42}$ 8.0 pg/mL, and $A\beta_{42/40}$ ratio 0.06 among cognitive-intact participants and $A\beta_{40}$ 143 pg/mL, $A\beta_{42}$ 8.3 pg/mL, and $A\beta_{42/40}$ ratio 0.06 in dementia participants. Thus, compared with prior studies of non-Hispanic White individuals, this study of American Indian individuals had 1.3X (intact) to 1.5X (impaired) $A\beta_{40}$, that is, less abnormal; 0.3X (intact), 0.4X (impaired) $A\beta_{42}$, that is, more abnormal; and 0.26X (intact), 0.3X (impaired) $A\beta_{42/40}$ ratio, that is, more abnormal. Together, these findings suggest that American Indian individuals may have earlier, faster, or more common accumulation of AD pathology than non-Hispanic White individuals,[24] possibly to the point of obscuring within-group comparisons. Furthermore, these data suggest that prior epidemiologic reports of comparable risk between American Indian and non-Hispanic White individuals may be underestimates.

GFAP and NfL, were similar in this study as in other populations, especially in consideration of age-comparable standards.[23,25–29] However, given that plasma biomarker data from these studies have all reported research-grade assays that are not calibrated to a set standard, and thus may not be directly comparable, any comparisons for differences in absolute concentrations should be interpreted with caution.

Prior studies of plasma marker diagnostics similarly detected excellent discriminant capacity in pathological as well as neuropsychological studies; however, in contrast to our data, with best performance from GFAP, NfL, and $pTau_{181}$, prior studies found $A\beta_{42/40}$ ratio and $pTau_{181}$ performed best.[30] In addition, prior studies detected very good performance, with $pTau_{181}$ AUC > 0.9 (cutoff > 2.7 pg/mL),[31] whereas our findings were more moderate, with $pTau_{181}$ AUC = 0.6 (cutoff > 7.9 pg/mL). However, similar to studies identifying that a panel of markers performs better than individual markers,[32] we detected a combination of core markers (age, sex, education, $pTau_{181}$, $A\beta_{42/40}$ ratio, GFAP, NfL) had excellent performance, despite poor to moderate performance of any marker individually.

4.1 | Prior null findings for APOE ε4

In the context of prior reports from our research group that *APOE ε4* had no detectable association with imaging, cognitive, or memory features in this population,[33] these current findings are consistent with lack of generalizability of conventional markers to this population, perhaps because of risk saturation throughout the population. However, selective survival as well as latent resilience factors can also contribute to observed null associations. Future research should continue to examine these methodological issues, and to establish population strata for whom conventional AD biomarkers may be differentially accurate.

4.2 | Strengths and limitations

These analyses include data from comprehensive, standardized collection protocols in a well-characterized cohort of an understudied population. The novel biomarker assays have potential to inform better cognitive impairment and dementia case definitions, as well as guide future research for the purposes of evaluating diagnostic, therapeutic, and prevention efforts. Furthermore, these analyses are theory-driven, and not empirical, with the strong potential to provide novel information both about this population as well as about the underlying neurology. As mentioned, because this population represents a survival cohort, differential selection may influence our findings, if likelihood of participation is associated with the outcome. Previous reports in this cohort have found little evidence of selective survival using indirect analysis.[34] However, future research should focus on younger population strata in order to include preclinical groups in the study sampling frame.

In this study, we adjusted for eGFR because renal filtration losses have been reported to increase plasma marker concentrations. However, the association of plasma markers with renal dysfunction may not be mediated by filtration function, as measured by eGFR. Proteins are not cleared by the glomerular basement membrane, where defects result in proteinuria or lower plasma protein concentrations. Therefore, alternative mechanisms accounting for observed renal associations, such as proximal tubular secretion (dys)function or hormone (dys)production, may be needed in future research on plasma biomarkers of brain injury. Furthermore, we did not evaluate associations for endocrine features, which may further serve as mediating or modifying clinical features in these associations, and represent an important avenue for future investigation.

5 | CONCLUSION

In summary, this report contains seminal evaluation of blood biomarkers for brain injury, especially AD, in American Indian elders, using sociodemographic, clinical features, imaging, and cognitive evaluations. Future research to validate these measures using PET or other gold standards, to examine these measures in younger groups, to examine

standardized data for comparison to existing studies, and to examine the predictive and diagnostic utility of these markers are still needed. However, our findings establish these measures, and their coinciding features, as potential markers in determinance of brain injury, including AD, with implications for researchers and clinicians developing understanding of this complex and devastating condition in this unique population.

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CONFLICT OF INTEREST STATEMENT

A.S.D. receives support from several NIH-funded projects. A.S.D. has no COI to report. Longstreth: W.T.L. receives support from several NIH-funded projects. W.T.L. has no COI to report. Rhoads: K.R. receives support from several NIH-funded projects. K.R. provides 2–3 expert witness consultations per year.Umans: J.U. receives support from several NIH-funded projects. J.U. has no COI to report. Buchwald: D.B. receives support from several NIH-funded projects. D.B. has no COI to report. T.G. receives support from several NIH-funded projects. T.G. has no COI to report. K.B. is supported by the Swedish Research Council (#2017-00915 and #2022-00732), the Swedish Alzheimer Foundation (#AF-930351, #AF-939721 and #AF-968270), Hjärnfonden, Sweden (#FO2017-0243 and #ALZ2022-0006), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986 and #ALFGBG-965240), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495), and the Alzheimer's Association 2022-2025 Grant (SG-23-1038904 QC). KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, 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, outside the work presented in this paper. E.R. receives support from several NIH-funded projects. E.R. is co-founder and advisor for ALZPath. H.Z. is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Union's Horizon Europe research and innovation programme 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, and #ADSF-21-831377-C), the Bluefield Project, 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), and the UK Dementia Research Institute at UCL (UKDRI-1003). H.Z. has served at 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, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). Author disclosures are available in the supporting information.

CONSENT STATEMENT

All participating institutional, Indian Health Service, and tribal review boards approved study protocols. All participants provided written, informed consent.

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SUPPORTING INFORMATION

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

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