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RESEARCH ARTICLE

Blood biomarkers differentiate AD-related versus non-AD-related cognitive deficits

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Abstract

INTRODUCTION: The utility of blood-based biomarkers for discriminating Alzheimer's disease (AD)-related versus non-AD-related cognitive deficits in preclinical populations remains poorly understood. Here, we tested the capability of blood markers to detect and discriminate variation in performance across multiple cognitive domains in a cognitively unimpaired sample.

METHODS: Participants (n = 648, aged 69.9 ± 3.8, 71% female) underwent a comprehensive cognitive assessment and assays for plasma-based biomarkers amyloid

beta (Aβ)1-42/1-40 by mass spectrometry, phosphorylated tau (p-tau) 181 and 217, p-tau217/Aβ1-42, glial fibrillary acidic protein (GFAP), and neurofilament light (NfL).
RESULTS: Greater p-tau217 was exclusively associated with poorer episodic memory performance ($\beta = -0.11$, SE = 0.04, $p = .003$), and remained so after covarying for NfL. Higher NfL was non-specifically associated with poorer performance across a range of cognitive domains and remained so after covarying for p-tau217.
DISCUSSION: Blood-based biomarkers may differentiate non-AD-related versus AD-related cognitive deficits. This characterization will be important for early intervention and disease monitoring for AD.

KEYWORDS
Alzheimer's disease, biomarkers, blood-based biomarkers, cognition, cognitive function

Highlights

- There is heterogeneity in the causes of cognitive decline in aging.
- AD-related blood biomarkers may help characterize these causes.
- Elevated p-tau217 was exclusively associated with poorer episodic memory.
- Elevated NfL was associated with poorer cognition in a broad range of domains.
- Blood biomarkers may help differentiate AD- and non-AD-related cognitive deficits.

1 | BACKGROUND

Early detection of factors that precipitate the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative conditions that lead to dementia will likely maximize preventive and treatment success.[1] In this regard, blood-based biomarkers have shown significant promise: they are more accessible, cost-effective, and non-invasive than traditional measures, for example, positron-emission tomography (PET) and cerebrospinal fluid methods. Specifically, the amyloid beta (Aβ)1-42/1-40 ratio measured by immunoprecipitation-mass spectrometry (IPMS) reflects levels of brain Aβ,[2–5] while phosphorylated tau (p-tau) 217 and p-tau181 reflect the tau phosphorylation state and are highly associated with brain amyloidosis pathology.[6,7] Glial fibrillary acidic protein (GFAP) is an indicator of astrogliosis, and neurofilament light (NfL) is a marker of neuroaxonal injury and neurodegeneration.[8,9] Levels of these biomarkers are altered in AD; however, their prognostic and diagnostic capabilities remain a matter of debate.[10–12]

Lower plasma Aβ1-42/1-40 and higher levels of p-tau217, p-tau181, NfL, and GFAP are associated with poorer Mini-Mental State Examination (MMSE) performance cross-sectionally[13,14] and predict faster rates of decline[11,12,15,16] in both cognitively unimpaired individuals and in those with AD. However, the ability for these biomarkers to discriminate between multiple cognitive domains remains unknown,[17] which could be critical for differentiating early stages of AD- and non-AD-related cognitive deficits. For example, NfL increases during the course of normal aging and is associated with age-related brain atrophy[18]; however, AD-specific markers such as p-tau are elevated with AD pathology.[10] Thus, NfL may reflect a more general marker of brain atrophy that occurs during normal aging as well as a later-stage outcome from elevated levels of pathology.[18] As such, NfL could be a marker reflecting decline across multiple cognitive domains that are not specific to AD. In contrast, p-tau may be related to cognitive domains most consistently affected in AD (eg, episodic memory). Indeed, one recent study reported this general pattern of results, where Aβ42/40 and p-tau181 were most strongly associated with memory performance[19]; however, that study did not have data on p-tau217 or the p-tau217/Aβ1-42 ratio. Other studies did not investigate these associations because they either lacked comprehensive, domain-specific neuropsychological testing, that is, they only used the MMSE or a preclinical AD cognitive composite (PACC),[11,12,15,16] or did not include a panel of both AD-specific (IPMS Aβ1-42/1-40, p-tau) and non-specific (NfL and GFAP) blood biomarkers.[20] Thus, the specificity of using blood-based biomarkers for detecting domain-specific cognitive decline remains an unresolved, but important, issue, to better understand the heterogeneity of causes of late-life cognitive change.

This study examined the specificity of blood-based biomarkers across multiple cognitive domains using a comprehensive neuropsychological assessment battery. We hypothesized that AD-specific blood biomarkers, namely, IPMS Aβ1-42/1-40, p-tau181, p-tau217, and p-tau217/Aβ1-42, would specifically be associated with episodic memory performance, the domain most strongly related to AD. In contrast, we predicted that NfL and GFAP would be associated with a broad range of cognitive domains, reflecting general age-related cognitive decline that is not specific to early stages of AD pathology.

2 | METHOD

2.1 | Participants

Participants ($n = 648$) were enrolled in the Investigating Gains in Neurocognition in an Intervention Trial of Exercise (IGNITE) study, a multicenter randomized clinical trial (ClinicalTrials.gov: NCT02875301) assessing the influence of exercise on cognition and brain health.[21] Participants were community-dwelling men and women aged 65 to 80 years at baseline, free of neurological disease diagnoses (eg, multiple sclerosis, Parkinson's disease, dementia, or stroke), considered inactive (engaged in <20 min of moderate-intensity physical activity three times per week). Consensus adjudication was reached after a comprehensive cognitive assessment to exclude individuals with probable mild cognitive impairment (MCI) or dementia but given known limitations of neuropsychological testing and the variable definitions of MCI, it is possible that some included participants were near the MCI range.[22] Full exclusion criteria are detailed in Erickson et al.[21] The study was approved by the Institutional Review Board at each site, and all participants provided written informed consent before data collection.

2.2 | Blood-based biomarkers

Approximately 47 cc of fasted blood was collected primarily in the morning between 8 and 10 a.m. Isolated plasma was immediately stored at −80°C. The levels of Aβ1-42 and Aβ1-40 (used to calculate the Aβ1-42/1-40 ratio) were measured using IPMS as previously described[23,24] (Supplementary Methods). P-tau181, p-tau217, NfL, and GFAP were measured using the Simoa platform (Quanterix, Billerica, MA, USA). Specifically, NfL and GFAP were analyzed using the N2 PB assay (No. 103520) on a SIMOA-HD X at the University of Kansas Medical Center. P-tau181 was measured using the V2 Advantage kit (No. 103714), and p-tau217 using the ALZpath assay kit (No. 104371) at the Department of Psychiatry, University of Pittsburgh. Quality control samples were assessed at the beginning and the end of each run to evaluate reproducibility. The average within-run coefficients of variation (CVs) were p-tau181 = 6.3%, p-tau217 = 11.0%, NfL = 6.3%, and GFAP = 9.7%. The mean between-run CVs were p-tau181 = 10.3%, p-tau217 = 11.4%, NfL = 8%, and GFAP = 14.8%. P-tau217 and Aβ1-42 measured as described above were used to calculate the p-tau217/Aβ1-42 ratio. For more information on blood biomarker collection and processing, see Supplementary Methods.

2.3 | Cognitive assessment

Cognition was assessed at baseline using a comprehensive neuropsychological battery of verbal, paper-pencil, and computerized assessments. Testing was administered by certified psychometricians and completed across 2 days. Raw scores were normalized and combined using a confirmatory factor analysis approach[25] to determine five latent factors for cognitive domains, including episodic memory (Brief Visuospatial Memory Test, Picture Sequencing Test, Hopkins Verbal Learning Test, Logical Memory Task, Verbal Paired Associates), processing speed (Letter Comparison Test, Digit Symbol Substitution Test, Trail Making Test, Part A), working memory (N-Back Working Memory Task, Spatial Working Memory Task, List Sorting Working Memory Task), visuospatial processing (Matrix Reasoning, Spatial Relations, Clock Draw), and executive function/attentional control (Flanker Task, Stroop Task [incongruent trial], Dimensional Change Card Sort task, Trail Making Test, Part B).

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using traditional database searches. Previous studies examined the association between blood-based biomarkers and cognition primarily in the context of highly specific samples (eg, from memory clinics) or using limited assays and cognitive assessments.
2. **Interpretation**: Our findings demonstrate that blood-based biomarkers can differentiate non-AD-related (NfL and GFAP; broad range of cognitive domains) versus pathology-related (p-tau217 and p-tau217/Aβ1-42; episodic memory specific) differences in cognitive performance in cognitively unimpaired older adults. Further, our findings suggest that individual biomarkers may reflect different mechanistic pathways through which cognition may decline in aging.
3. **Future directions**: Blood-based biomarkers may be a useful, non-invasive strategy for characterizing potential causes of cognitive decline in aging. Longitudinal studies are required to test the accuracy of utilizing biomarkers to differentiate age-related versus pathology-related cognitive change, which will aid in early intervention and disease monitoring.

2.4 | Covariates

Covariates were selected a priori based on associations with variables of interest (ie, cognition[26–29] and biomarkers[30,31]) and included age, sex, race, education (years), body mass index (BMI), and medical conditions. Participants self-reported their race from National Institutes of Health-defined categories. Due to small sample sizes (Table 1), we created a binary variable defined as non-White and White to include in our models, with the aim of accounting for constructs (eg, experiences of discrimination) that might underlie racial differences in our variables of interest.

TABLE 1 Descriptive data for the IGNITE sample.

Variable	Included sample, $n = 632$
Age (years)	69.9 (3.8)
Sex, *n* (%)	
Female	450 (71%)
Male	182 (29%)
Race and ethnicity, *n* (%)	
Non-Hispanic White	474 (75%)
Hispanic White	11 (2%)
Non-Hispanic Non-White	138 (22%)
Hispanic Non-white	9 (1%)
Study site	
Pittsburgh	209 (33%)
Kansas	213 (34%)
Northeastern	210 (33%)
Education (years)	16.3 (2.2)
APOE ε4 carriers, *n* (%)	172 (27%)
Body mass index (kg/m^2)	29.7 (5.7)
Total CIRS score	3.3 (2.4)
MoCA score	25.8 (2.6)
Aβ1-42/1-40	0.10 (0.04)
p-tau217 pg/mL	0.44 (0.33)
p-tau217/Aβ1-42	1.16 (0.97)
p-tau181 pg/mL	2.85 (1.6)
GFAP pg/mL	185.0 (148.2)
NFL pg/mL	17.2 (9.1)

Note: Unless otherwise specified, data are presented as mean (standard deviation). Descriptive data for the "Included sample" contain data from participants that had any biomarker data available. Because not every participant had missing data from the same biomarker, there were slightly different samples in the analysis for each biomarker. This leads to the different sample sizes described in Figure 1 and Table 1.

Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; CIRS-G, Cumulative Illness Rating Scale for Geriatrics; GFAP, glial fibrillary acidic protein; MoCA, Montreal Cognitive Assessment; NfL, neurofilament light; p-tau, phosphorylated tau.

Medical conditions were measured using the Cumulative Illness Rating Scale for Geriatrics (CIRS-G).[32] The CIRS-G measures illness burden in 14 different organ systems, with each category yielding a severity rating from 0 (no problem) to 4 (severe impairment), with a total score ranging from 0 to 56. Higher scores on the CIRS-G are indicative of greater overall illness burden across organ systems. Apolipoprotein E (*APOE*) genotype was determined by two single nucleotide polymorphisms, rs7412 and rs429358, using TaqMan assays, as described previously.[33] BMI was determined by weight (kg)/height^2 (m), which were measured using stadiometers and scales calibrated across sites.

2.5 | Statistical analyses

Analyses were conducted using R statistical computing packages version 4.3.2. Visual inspection and skewness and kurtosis statistics tested normality. Outliers were identified and removed using >12 median absolute deviations above the median,[34] which resulted in the following thresholds and participants removed: for Aβ1-42/1-40 $n = 2 > 0.24$, GFAP $n = 2 > 1010$pg/mL, NfL $n = 2 > 76$pg/mL, p-tau217 $n = 2 > 2.16$pg/mL, and p-tau181 $n = 1; > 15$pg/mL.

Associations between biomarkers were determined via Spearman's correlation coefficient (ρ). Separate linear regression models assessed independent associations between each of the five biomarkers and cognitive outcomes. Fully adjusted models included age, sex, years of education, race, study site, BMI, and CIRS-G total score as covariates. *APOE* genotype was excluded as a covariate to increase clinical translatability and because its inclusion did not improve model fit. The Benjamini-Hochberg method evaluated whether associations remained significant after adjusting the type I error rate for multiple comparisons.[35]

As exploratory analyses, we examined whether associations between AD-specific biomarkers (Aβ1-42/1-40, p-tau217, p-tau217/Aβ1-42, and p-tau181) and cognition remained significant after controlling for a non-AD-specific marker (NfL) and vice versa: whether non-AD-specific markers (NfL and GFAP) remained significantly associated with cognition after controlling for an AD-specific marker (p-tau217). This approach aimed to illustrate whether biomarkers explained shared or independent variance in cognitive function, providing evidence for potential mechanisms underlying these associations. We also tested whether a p-tau217 clinical cutoff for determining Aβ positivity, which was previously generated within this sample, related to cognitive performance (≥0.46 pg/mL).[36] Finally, we wanted to explore the optimal cutoff value for p-tau217 predicting cognitive performance. To achieve this aim, we fit a threshold regression model, which can be used to identify a threshold value in X (p-tau217), where the association between Y (respective cognitive domain) and X abruptly changes before and after a specific value of X, known as a "change point" or a "threshold value." We used the R package chngpt[37] to implement the threshold regression model to identify the data-driven optimal cut point of the p-tau217 by maximizing the likelihood function of the respective cognitive domain. We estimated 95% confidence intervals (CIs) of the threshold parameter estimator at the significant level of 0.05 using 1000 bootstrap samples.[37–39]

3 | RESULTS

Participants were aged 69.9 ± 3.8 years, 71% were female, and 75% self-identified as non-Hispanic White (Table 1). Descriptive statistics for the sample with available data ($n = 632$) are presented in Table 1, and descriptive statistics for the remaining IGNITE sample not included in the current analyses ($n = 16$) are available in Table S1. Statistical comparisons were not tested between these groups due to the small

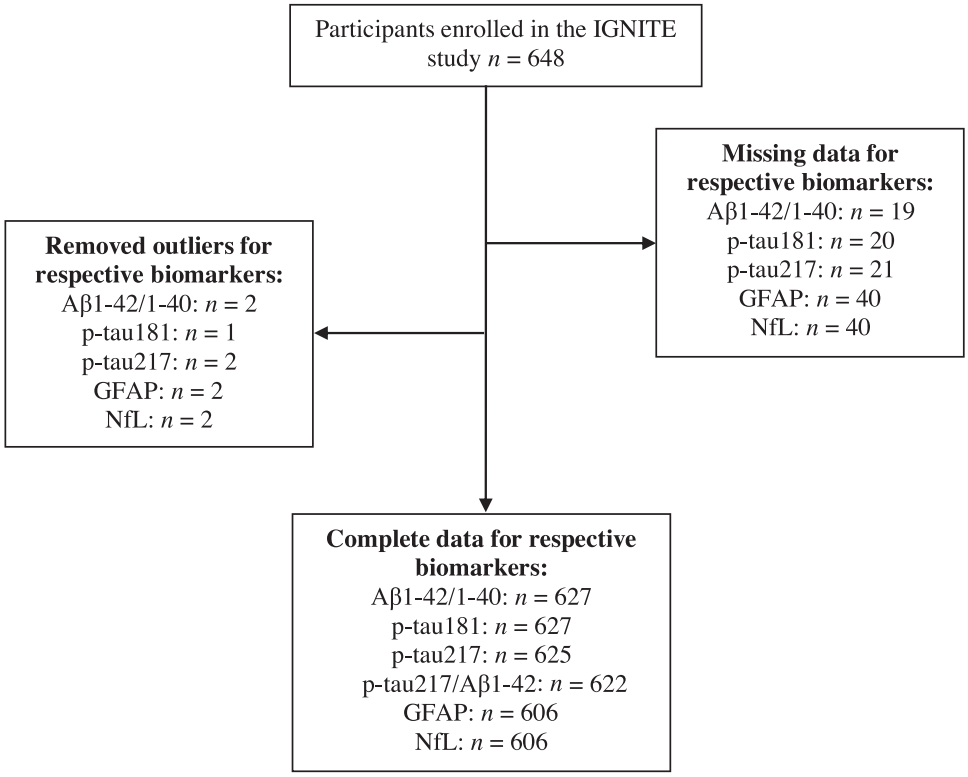

FIGURE 1 Flow diagram depicting sample size for each analysis. Aβ1-42/1-40, plasma amyloid beta 1-42/1-40 ratio; IGNITE, Investigating Gains in Neurocognition in an Intervention Trial of Exercise; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau, phosphorylated tau.

sample size remaining (n = 16, 2.5% of the total sample). The sample size for each analysis varied due to missingness and outlier removal for biomarker data, ranging from n = 606 for NfL and GFAP to n = 627 for p-tau181. Sample size for each biomarker is detailed in Figure 1 (there were no missing cognitive data). Table 1 includes descriptive data and sample size for the participants inclusive of any biomarker analysis. However, there were slightly different amounts of missing data for each biomarker, so the sample size detailed in Table 1 (n = 632), which is inclusive of the whole sample, is slightly larger than the maximum sample size for the separate biomarker analyses (n = 627).

3.1 | AD pathophysiological biomarkers and cognitive performance

Zero-order correlations between biomarkers are detailed in Figure S1. Consistent with our hypotheses, higher levels of AD-specific biomarkers including p-tau217 and 181 were exclusively associated with performance in cognitive domains that are specific to early AD, namely, poorer episodic memory (Table 2). The association of p-tau217 with episodic memory remained significant following covariate adjustment and correction for multiple comparisons (Table 2; Figure 2). Similarly, higher p-tau217/Aβ1-42 ratio was associated with poorer episodic memory and poorer processing speed (Table 2). In addition, the association of p-tau217 and p-tau217/Aβ1-42 with episodic memory remained significant after including NfL as a covariate (Table 2), indicating that the associations between episodic memory performance and p-tau217 were not confounded by associations with NfL (see following discussion). Aβ1-42/1-40 was not associated with performance in any cognitive domain (Table 2). We conducted a sensitivity analysis excluding those participants reporting kidney conditions on the CIRS-G (n = 23), and importantly, all the foregoing results remained the same.

For our exploratory analyses, we examined whether a p-tau217 clinical cutoff for determining Aβ positivity, which was previously generated within a subsample of the current participants, related to episodic memory performance (≥0.46 pg/mL).[36] As predicted, p-tau217-positive individuals (concentrations ≥ 0.46 pg/mL) had significantly worse episodic memory performance than p-tau217-negative individuals after covariate adjustment (β = –0.16, SE = 0.08, p = .042; Figure S2), with no significant group differences in any other cognitive domain (data not shown). The cut point for the p-tau217/Aβ1-42 ratio (≥1.2), despite showing slightly better sensitivity to PET amyloid,[36] was not significantly associated with episodic memory (β = –0.14, SE = 0.08, p = .070) or performance in any other cognitive domain.

Based on our threshold regression model approach, the estimated threshold parameter for p-tau217 predicting episodic memory was > 0.79 pg/mL [95% CI 0.18 to 0.93]. Specifically, the negative association between p-tau217 level and episodic memory strengthened at p-tau217 levels > 0.79 pg/mL (β = –0.70, p = .028). There were 63 participants (10.08% of the sample) with p-tau217 levels > 0.79 pg/mL. After adjusting for covariates, those with p-tau217 levels > 0.79 pg/mL

TABLE 2 Standardized betas (standard error) from linear regressions of associations between biomarkers and cognition.

	Episodic memory	Processing speed	Working memory	Attention	Visuospatial
Plasma Aβ1-42/1-40					
Unadjusted	0.02 (0.04)	0.06 (0.04)	0.05 (0.04)	0.06 (0.04)	0.03 (0.04)
Fully adjusted	0.01 (0.04)	0.03 (0.04)	0.04 (0.03)	0.04 (0.04)	0.02 (0.03)
NfL adjusted	0.00 (0.04)	0.02 (0.04)	0.02 (0.04)	0.03 (0.04)	0.01 (0.04)
Plasma p-tau217					
Unadjusted	**−0.13 (0.04)*****	**−0.10 (0.04)***	−0.07 (0.04)	−0.05 (0.04)	0.01 (0.04)
Fully adjusted	**−0.11 (0.04)****,[a]	−0.06 (0.04)	−0.05 (0.04)	−0.02 (0.04)	0.02 (0.04)
NfL adjusted	**−0.09 (0.04)***	−0.03 (0.04)	−0.01 (0.04)	0.02 (0.04)	0.05 (0.04)
Plasma p-tau217/Aβ1-42					
Unadjusted	**−0.13 (0.04)*****	**−0.11 (0.04)****	**−0.08 (0.04)***	−0.06 (0.04)	−0.01 (0.04)
Fully adjusted	**−0.10 (0.04)****,[a]	**−0.08 (0.04)***,[a]	−0.06 (0.04)	−0.03 (0.04)	0.01 (0.04)
NfL adjusted	**−0.09 (0.04)***	−0.06 (0.04)	−0.03 (0.04)	−0.01 (0.04)	0.02 (0.04)
Plasma p-tau181					
Unadjusted	**−0.08 (0.04)***	−0.03 (0.04)	−0.01 (0.04)	0.01 (0.04)	0.03 (0.04)
Fully adjusted	−0.06 (0.04)	−0.01 (0.04)	−0.01 (0.04)	0.01 (0.04)	0.01 (0.04)
NfL adjusted	−0.04 (0.04)	0.02 (0.04)	0.02 (0.04)	0.04 (0.04)	0.03 (0.04)
Plasma GFAP					
Unadjusted	−0.03 (0.04)	**−0.09 (0.04)***	**−0.10 (0.04)***	**−0.09 (0.04)***	−0.05 (0.04)
Fully adjusted	−0.07 (0.04)	**−0.09 (0.04)***,[a]	**−0.08 (0.04)***,[a]	−0.07 (0.04)	−0.00 (0.04)
p-tau217 adjusted	−0.03 (0.04)	**−0.08 (0.04)***	**−0.08 (0.04)***	−0.08 (0.04)	−0.02 (0.04)
Plasma NfL					
Unadjusted	**−0.14 (0.04)*****	**−0.18 (0.04)*****	**−0.18 (0.04)*****	**−0.17 (0.04)*****	**−0.12 (0.04)****
Fully adjusted	**−0.11 (0.04)****,[a]	**−0.12 (0.04)****,[a]	**−0.13 (0.04)*****,[a]	**−0.11 (0.04)*****,[a]	−0.07 (0.04)
p-tau217 adjusted	**−0.08 (0.04)***	**−0.11 (0.04)****	**−0.12 (0.04)*****	**−0.11 (0.04)****	**−0.08 (0.04)***

Note: Unadjusted models include no covariates, fully adjusted models include age, sex, education, race, body mass index, study site, and total CIRS-G score. Reported as standardized β (standard error).

Abbreviations: Aβ, amyloid beta; CIRS-G, Cumulative Illness Rating Scale for Geriatrics; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; p-tau217, phosphorylated tau 217; p-tau181, phosphorylated tau 181.

Bold font indicates significance at $p < 0.05$.

[a]Significant after correction for multiple comparisons using the Benjamini–Hochberg method applied to fully adjusted models within each biomarker.

*Uncorrected $p < 0.05$.

**Uncorrected $p < 0.01$.

***Uncorrected $p \leq 0.001$.

had significantly worse episodic memory performance compared to those with lower levels ($\beta = -0.20$, SE = 0.07, $p = .006$). The estimated threshold value was not statistically significant for any other cognitive domain or using the p-tau217/Aβ1-42 ratio for all five cognitive domains.

3.2 | NfL and GFAP associations with cognitive performance

Consistent with our predictions, higher levels of NfL were associated with worse performance across all cognitive domains except visuospatial function (Table 2; Figure 2), suggesting a broad and non-specific pattern of worse cognition with higher NfL. Importantly, these associations remained significant even when including p-tau217 as a covariate in the models (Table 2). In contrast, higher levels of GFAP were only associated with poorer processing speed and working memory performance (Table 2). When controlling for p-tau217, associations of GFAP with processing speed and working memory remained significant (Table 2). Similar to the AD pathology-specific biomarkers, when excluding those reporting kidney conditions on the CIRS-G ($n = 23$), the results were largely retained except the association between NfL and visuospatial performance became significant ($\beta = -0.08$, SE = 0.04, $p = .025$).

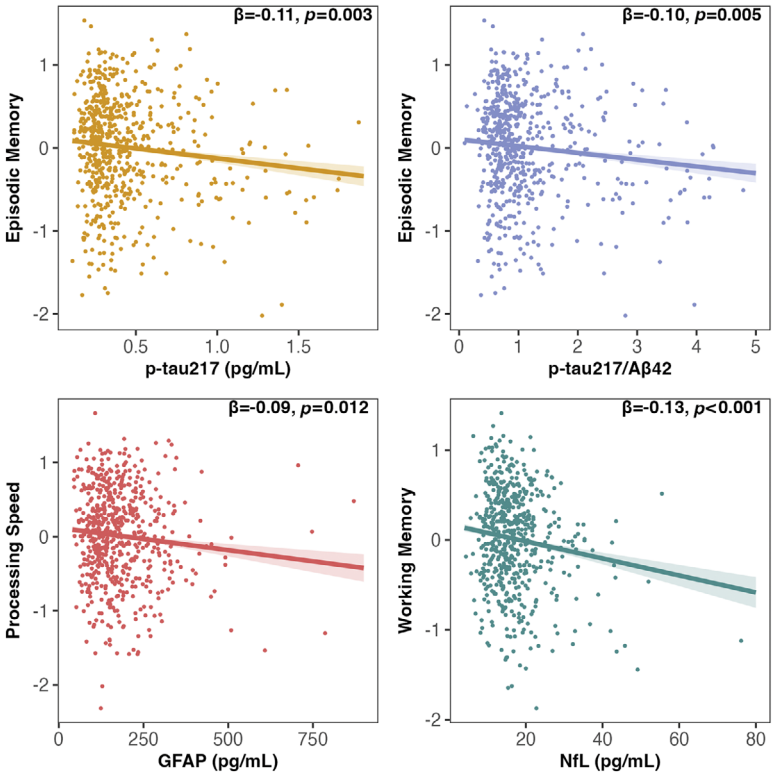

FIGURE 2 Linear regression between biomarkers and cognitive outcomes controlling for age, sex, years of education, race, study site, BMI, and CIRS-G total score. Only the cognitive domain with the largest effect size for each biomarker is illustrated here; others can be found in Figure S3. BMI, body mass index; CIRS-G, Cumulative Illness Rating Scale for Geriatrics; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau, phosphorylated tau.

3.3 | Clinical significance of associations

To further quantify the magnitude of association between blood biomarkers and cognitive function, we used a minimum clinically meaningful threshold of −0.08SD within-person cognitive decline per year. This threshold was based on a previous longitudinal study demonstrating a −0.08SD rate of decline in cognitively unimpaired individuals with high brain Aβ (51 to 100 Centiloids), 28% of whom developed mild cognitive impairment or dementia over the subsequent 5 years.[40] We quantified the increase in blood biomarker level to reach this −0.08SD threshold. Using the cognitive domain with the largest effect size for each biomarker, a 0.36 pg/mL per year increase in p-tau217 would reflect a clinically meaningful 0.08SD decrease in episodic memory performance. For GFAP, a 134.39 pg/mL per year increase would reflect a clinically meaningful 0.08SD decrease in processing speed. For NfL, an 8.50 pg/mL per year increase would reflect a clinically meaningful 0.08SD decrease in working memory.

4 | DISCUSSION

We evaluated whether associations between blood-based biomarkers and cognitive function in older adults were domain specific. Consistent with our predictions, higher levels of p-tau217 and higher p-tau217/Aβ1-42 showed a distinct association with poorer episodic memory performance. In contrast, higher NfL, a general marker of neurodegeneration, was associated with poorer cognitive performance across most cognitive domains, namely, episodic memory, processing speed, working memory, and attentional control. Similarly, higher GFAP was associated with poorer processing speed and working memory.

Our results demonstrate that blood-based biomarkers can potentially differentiate non-AD (NfL and GFAP) from AD pathophysiology-related (p-tau217 and p-tau217/Aβ1-42) cognitive decline, reflecting the heterogeneity of causes of late-life cognitive change. The specificity of p-tau217 with episodic memory performance, the cognitive domain most impacted in early AD, indicates that this biomarker may reflect early, pathology-related amnestic deficits in cognitively unimpaired older adults. Confirming our current findings, we previously showed that in a subset of the current sample (n = 357), p-tau217 and p-tau217/Aβ1-42, but not NfL, were associated with levels of brain Aβ, and both p-tau217 and p-tau217/Aβ1-42 demonstrated good diagnostic accuracy for predicting brain Aβ positivity.[36] Further, in the current study, we found that both p-tau217 and NfL were independently associated with episodic memory performance when included in the same statistical model. This demonstrates that p-tau217 and NfL explain unique variance in episodic memory, likely reflecting different mechanistic pathways through which cognition may decline in aging. For example, NfL is a general marker of neurodegeneration[9] and may reflect a portion of episodic memory decline associated with normative cognitive aging, whereas p-tau217, which is strongly associated with brain Aβ, likely reflects pathology-related deficits in episodic memory associated with early AD.

It is notable that the Aβ1-42/1-40 ratio was not associated with cognitive performance in our sample. This finding is consistent with the notion that tau pathology, as opposed to Aβ, is more closely associated

with cognitive performance in preclinical AD.[41] Additionally, we and others have demonstrated that Aβ1-42/1-40 is less strongly associated with brain Aβ compared to other biomarkers (ie, p-tau217).[36,42,43] Thus, our results are in line with these prior data and indicate that the Aβ1-42/1-40 blood biomarker is less sensitive to AD pathophysiology-related cognitive deficits than other blood biomarkers of AD and neurodegeneration.

Our finding that markers of AD pathology, namely, p-tau217 and p-tau217/Aβ1-42, are associated with episodic memory performance contrasts with previous evidence showing a lack of an association between brain Aβ and cognitive function[44] and with the notion that AD pathology accumulates earlier than cognitive deficits appear (ie, asymptomatic AD). We showed that even slightly elevated levels of blood biomarkers are reflected in subtle, domain-specific differences in cognitive performance. Importantly, we measured cognitive performance at a more granular and comprehensive level than previous studies in cognitively unimpaired individuals. Our results indicate that those with increased AD pathology (in the absence of cognitive impairment) are not entirely asymptomatic but that the cognitive assessments in prior studies lacked the comprehensiveness and sensitivity to detect AD-related cognitive deficits.

The current results and results from Olvera-Rojas et al.[36] indicate that elevated levels of p-tau217 (≥0.46 pg/mL) reflect both Aβ positivity and differences in episodic memory performance (>0.79 pg/mL) in cognitively unimpaired individuals. Taken together, these results suggest that a p-tau217 threshold (≥0.46 pg/mL) could be used as a marker of clinically elevated AD pathology, while a higher threshold (>0.79 pg/mL) could be used as a reference for a greater likelihood of early manifestation of memory decline. These thresholds are consistent with biomarker temporality and staging of AD such that Aβ accumulation occurs prior to clinical and cognitive decline and only once Aβ accumulation reaches a higher level (>0.79) would it reflect episodic memory deficits.[45] Further, we indicate that an increase of 0.36 pg/mL per year for p-tau217 could reflect a clinically meaningful decline of −0.08SD in episodic memory performance, but this must be confirmed by longitudinal studies. Conversely, elevated levels of NfL with an absence of AD pathology might be more strongly associated with non-AD-related cognitive deficits. Although further research in independent cohorts is required, these cut points contribute to furthering the field in terms of early diagnosis of brain Aβ positivity requiring a single blood draw and may be used in conjunction with cognitive assessments to monitor magnitude of change in both brain Aβ and cognitive performance.

We have expanded previous research[12,16] by utilizing a confirmatory factor analysis to generate latent cognitive factors across 15+ instruments, reducing methodological error.[25] This approach allowed us to investigate cognitive domain-specific associations with precision, a challenge in previous research that used global cognitive measures (eg, MMSE) or PACC scores composed of few individual outcomes (eg, delayed recall, animal fluency, MMSE, and trail making test[16]). Our results suggest that in cognitively unimpaired individuals, it is likely that the episodic memory portion(s) of PACC scores drive the association with p-tau217, an association which may be AD-specific. NfL and GFAP have previously been related to cognitive function[14,30]; however, the current results allow us to determine that at least in the case of NfL, these associations do not appear to be domain-specific but reflect widespread associations across multiple cognitive domains susceptible to age-related decline.

We were able to control for factors such as BMI and medical conditions that influence blood biomarker levels[31,46] and are prevalent within the older adult community. It is compelling that even after controlling for these factors, blood biomarkers were able to provide important information about cognitive performance and its likely origin (pathological vs non-pathological) in a community-based sample. However, important limitations of our study are its cross-sectional nature, which impeded our ability to draw conclusions about how biomarker–cognition associations vary longitudinally, and our inability to directly test mechanisms underlying the association between blood biomarkers and cognitive outcomes (eg, brain amyloid and neurodegeneration). Further, the effect sizes for associations between blood biomarkers and cognitive function in this study were relatively small and replication is required. However, these associations may still assist clinicians in their interpretation of cognitive data, particularly in clinical settings where non-specific, brief cognitive measures are often used. For example, a combination of biomarker data and cognitive assessments would provide greater specificity to the cognitive domains affected and potential underlying causes of subtle cognitive deficits that may otherwise go undetected.

Our results support the hypothesis that non-specific blood biomarkers may reflect age-related cognitive changes, whereas AD-related biomarkers reflect AD pathology-specific cognitive change. Further, we show that the same p-tau217 cut point used for defining brain Aβ positivity (≥0.46 pg/mL) is associated with poorer episodic memory performance. Additional longitudinal studies are required to confirm our findings, which will contribute to informing early intervention and disease monitoring for AD and detecting whether the type and level of cognitive change is resulting from AD-related pathological processes. In sum, the current results help to further characterize how plasma biomarkers reflect clinical outcomes and may improve their utility for AD diagnosis within the community.

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All participants provided written informed consent prior to any study-related procedures.

CONFLICT OF INTEREST STATEMENT

Thomas K. Karikari, Yijun Chen, and Xuemei Zeng are inventors on a University of Pittsburgh patent regarding the IPMS assay for Aβ

peptides. Thomas K. Karikari serves a consultant for Quanterix, outside the submitted work. All other authors have no conflicts to disclose. Author disclosures are available in the supporting information.

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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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