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RESEARCH ARTICLE

Cross-sectional and longitudinal evaluation of plasma glial fibrillary acidic protein to detect and predict clinical syndromes of Alzheimer's disease

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Abstract

Introduction: This study examined plasma glial fibrillary acidic protein (GFAP) as a biomarker of cognitive impairment due to Alzheimer's disease (AD) with and against plasma neurofilament light chain (NfL), and phosphorylated tau (p-tau)$_{181+231}$.

Methods: Plasma samples were analyzed using Simoa platform for 567 participants spanning the AD continuum. Cognitive diagnosis, neuropsychological testing, and dementia severity were examined for cross-sectional and longitudinal outcomes.

Results: Plasma GFAP discriminated AD dementia from normal cognition (adjusted mean difference = 0.90 standard deviation [SD]) and mild cognitive impairment (adjusted mean difference = 0.72 SD), and demonstrated superior discrimination compared to alternative plasma biomarkers. Higher GFAP was associated with worse dementia severity and worse performance on 11 of 12 neuropsychological tests. Longitudinally, GFAP predicted decline in memory, but did not predict conversion to mild cognitive impairment or dementia.

Discussion: Plasma GFAP was associated with clinical outcomes related to suspected AD and could be of assistance in a plasma biomarker panel to detect in vivo AD.

KEYWORDS
Alzheimer's disease, glial fibrillary acidic protein, neuropsychology, neurofilament light chain, phosphorylated tau, plasma biomarkers

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1 | BACKGROUND

While amyloid beta (Aβ) and tau biomarkers have been the focus in the detection of Alzheimer's disease (AD), recent research highlights additional mechanisms of neurodegeneration, namely neuroinflammation.[1] Reactive astrocytosis is an inflammatory response of astrocytes to injury and disease and can be identified by glial fibrillary acidic protein (GFAP).[2] These astrocytes might contribute to the formation of Aβ plaques as neuropathological investigations have revealed GFAP-positive astrocytes associate with underpinnings of AD.[3] Therefore, assessing GFAP levels could provide an alternative proxy of in vivo AD neuropathology.

Using traditional means of measurement, namely cerebrospinal fluid (CSF), GFAP was increased in those with AD dementia compared to cognitively unimpaired individuals[4] and reflects correlates of AD/AD-related dementias pathology,[5,6] including Aβ positivity on positron emission tomography (PET) using the ^{18}F-AZD4694A tracer,[7,8] decreased cortical thickness, and cerebral vascular insult.[9] Recent efforts have focused on more scalable approaches. Blood-based GFAP has accurately discriminated individuals with normal cognition (NC) from AD dementia and is correlated with CSF measurements of Aβ.[10–15] Plasma GFAP detected AD dementia more accurately than CSF GFAP suggesting that brain release of GFAP into the bloodstream is an early event in AD and posits an opportunity for timelier in vivo detection.[13,16,17] Longitudinal studies support plasma GFAP to monitor the progression of AD dementia.[18] GFAP accurately predicted conversion from mild cognitive impairment (MCI) to AD dementia[19] and has been found to increase in Aβ-positive individuals with NC.[20] However it is important to note that both CSF and blood-based GFAP are not specific to AD processes.[4,5,21,22]

There have been mixed results comparing the diagnostic performance of plasma GFAP to other plasma biomarkers, such as neurofilament light chain (NfL), phosphorylated tau (p-tau)$_{181}$, and total tau (t-tau). Plasma GFAP has been shown to perform comparably or better than other measures of neurodegeneration, namely NfL and t-tau,[20,23–26] while performing comparably or less optimally than measures of p-tau$_{181}$.[20,22,25] In each study, a combination of these biomarkers showed greatest predictive ability in detecting AD neurodegeneration.

Research on plasma GFAP is nascent and few studies have examined a cohort along the clinical continuum (i.e., NC, MCI, and dementia) both cross-sectionally and longitudinally.[16] There is limited research examining the utility of plasma GFAP compared to a panel of other plasma biomarkers. The objective of this study was to investigate the association between plasma GFAP and cognitive status (i.e., NC, MCI, and AD dementia) as a single biomarker and in conjunction with plasma biomarkers of p-tau$_{181}$, p-tau$_{231}$, and NfL within a large, longitudinal sample. We have reported on plasma p-tau$_{181}$ and NfL in this sample in past publications[27,28] and the focus of the present study is GFAP. The relationship between these plasma biomarkers and neuropsychological test performance was assessed cross-sectionally and longitudinally. We hypothesized that plasma GFAP would accurately discriminate NC participants from those with MCI and AD dementia, and a panel of plasma biomarkers would have optimal discriminatory accuracy. Further, we hypothesized that plasma GFAP levels would predict diagnostic conversion to MCI and AD dementia and higher baseline plasma GFAP would correspond to worse cross-sectional and longitudinal neuropsychological test performance.

RESEARCH IN CONTEXT

1. **Systematic Review**: We reviewed literature on Alzheimer's disease (AD) plasma biomarkers using traditional sources (e.g., PubMed). While AD-specific markers have been the focus of extant research, fewer studies have examined neuroinflammation in AD. Examinations of plasma glial fibrillary acidic protein (GFAP) have been promising; however, minimal research has compared it to more established plasma markers of AD, including neurofilament light chain (NfL) and phosphorylated tau (p-tau).
2. **Interpretation**: Plasma GFAP was associated with cognitive diagnosis, dementia severity, and neuropsychological outcomes in a large cohort spanning the clinical continuum (i.e., normal cognition, mild cognitive impairment, and AD dementia). It performed comparably or better than other plasma biomarkers including NfL, p-tau$_{181}$, and p-tau$_{231}$, both in isolation and in conjunction.
3. **Future Directions**: Replication and further determination of the clinical viability of the biomarker for diagnosis and disease monitoring is indicated, along with examination of GFAP in conjunction with other AD neuropathic changes at autopsy.

2 | METHODS

2.1 | Participants and design

The sample included 567 participants from the Boston University (BU) Alzheimer's Disease Research Center (ADRC) Clinical Core, one of ≈ 33 Centers funded by the National Institute on Aging (NIA). The BU ADRC provides standardized data to the National Alzheimer's Coordinating Center (NACC) to promote research on AD.[29–32] Recruitment of individuals for the BU ADRC Clinical Core is accomplished through partnerships with local organizations, advertisements, and community events. Additional information regarding recruitment and eligibility criteria can be found in other publications.[27,28,33,34] The BU ADRC Clinical Core longitudinally follows older adults (age 50+) that span the diagnostic continuum (i.e., NC, MCI, dementia) from the greater Boston community. Participants are fluent in English with adequate hearing and visual acuity. Exclusion criteria include severe mental illness

(e.g., psychotic spectrum conditions), non-neurodegenerative neurological conditions (e.g., tumor, multiple sclerosis), or unstable medical conditions that would interfere with study participation and/or limit accurate diagnoses.

Each year, participants are administered a neurological examination, neuropsychological testing, neuropsychiatric questionnaires, measures of functional independence, and other procedures.[30] A voluntary blood draw was added in 2008 for all existing and new participants. This started as a single blood draw for each participant and became annual beginning in 2015. The study visits in the current analyses occurred between 2008 and 2018. Only ≈ one third of participants in the sample had repeated biomarker measurements. "Baseline" visit was designated as the first study visit where a plasma sample was available, with subsequent visits being designated as follow-ups. This sample has been examined in other publications from our research group.[28,35] We included the new biomarkers of GFAP and p-tau$_{231}$. Inclusion criteria for these analyses included participants with at least one blood draw and a diagnosis of NC, MCI due to AD, or AD dementia at the respective study visit. All data collection procedures were approved by the BU Medical Center Institutional Review Board, with written informed consent from the participant or their legally authorized representative.

2.2 | Plasma biomarker analyses

Non-fasting blood samples were collected into plastic dipotassium ethylenediaminetetraacetic acid tubes, processed according to standard procedures, and the plasma was aliquoted and frozen at −80°C. The frozen aliquots were shipped on dry ice to the University of Gothenburg (Sweden) for batch analysis. Plasma GFAP, p-tau$_{181}$, p-tau$_{231}$, and NfL concentrations were measured using Single molecule array (Simoa) methods on an HD-X analyzer (Quanterix), as previously described in detail.[36] For each biomarker, measurements were performed in one round of experiments, using one batch of reagents. Intra-assay coefficients of variation were <10% for all.

2.3 | Neuropsychological testing

Neuropsychological tests administered at each study visit were consistent with NACC Uniform Data Set (UDS) protocol versions 1.0 and 2.0.[29,32] These tests include the Mini-Mental State Examination (MMSE); 30-item short form Boston Naming Test (BNT); Trail Making Test Parts A and B; Animals and Vegetables Fluency; Digit Span (DS); and Wechsler Memory Scale, Revised Logical Memory Immediate Recall (LM-IA) and Delayed Recall (LM-IIA). The Neuropsychologist Assessment Battery (NAB) List Learning Test is also included.

2.4 | Cognitive diagnostic procedures

A cognitive diagnosis (NC, MCI, dementia) was determined for each study visit via multidisciplinary diagnostic consensus conference. Information presented included relevant history and all examination and test findings, if available. MCI and AD dementia diagnoses were determined following the NACC UDS diagnostic criteria for cognitive syndromes and suspected etiologies.[37–40] Diagnostic criteria have changed over time with different versions of the UDS. This criterion included the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA),[38] as well as the 2011 NIA-Alzheimer's Association criteria.[37,40] Plasma biomarkers were not used to inform diagnostic status. Fifty-nine participants with MCI or dementia at the baseline blood draw with a suspected etiology other than AD were excluded from analyses to restrict the sample to the examination of AD-related biomarkers. This included 14 participants with missing diagnoses in the registry, 17 with impairment due to non-AD disorders (including frontotemporal lobar degeneration, possible chronic traumatic encephalopathy, cerebrovascular disease, progressive supranuclear palsy, and Lewy body disease), and 28 with a diagnosis of "cognitively impaired, not MCI."[29] Participants were classified as NC if they performed within the normal range on all neuropsychological tests (i.e., no scores lower than 1.5 standard deviation [SD] below age-normative means).

2.5 | Dementia severity

Dementia severity was evaluated at each visit using the Clinical Dementia Rating (CDR) Dementia Staging Instrument©.[41,42] A global severity rating was determined and used in analyses.

2.6 | Statistical analyses

Descriptive analyses compared the three diagnostic groups (NC, MCI, and AD dementia) at the baseline study visit on demographic variables and biomarker levels. Additional analyses determined cross-sectional and longitudinal relationships between the biomarkers of interest (i.e., GFAP, NfL, p-tau$_{181}$, p-tau$_{231}$) and relevant outcomes including baseline diagnostic status, diagnostic conversion (NC to MCI or AD dementia, MCI to AD dementia), CDR global score, and neuropsychological test performance. Analyses included each biomarker separately along with models containing all biomarkers to determine the incremental validity of the combination compared to each biomarker alone. Specific models conducted included: diagnostic status and conversion, global CDR score, neuropsychological test scores, and covariates.

For diagnostic status and conversion, analysis of covariance (ANCOVA) compared the four biomarkers across diagnostic groups at baseline, accounting for relevant covariates known to be associated with diagnostic status (see discussion of covariates below). Discrimination between diagnostic groups was examined using receiver operating characteristic (ROC) curves and logistic regression using the area under the curve (AUC) statistic. ROC models were conducted using a two-step process: (1) creating a baseline model using only demographic covariates to determine the level of discrimination that could occur using clinical variables and (2) adding the biomarkers of interest to the

baseline model. This approach isolates the additional predictive power of the novel biomarker.

Cox proportional-hazards regression models examined whether baseline biomarker levels predicted increased odds for diagnostic conversion at a subsequent study visit (either from NC to MCI/AD or from MCI to AD). For these analyses, the outcome was dichotomized into participants who changed diagnoses versus remaining diagnostically stable, accounting for the time to conversion based on the study visit at which the diagnosis changed. For the subset of participants with multiple blood draws and plasma biomarker measurements, binary logistic regression examined whether changes in biomarker measurements across study visits (i.e., the difference in value between the two measurements) corresponded with an associated change in clinical diagnosis.

To measure global CDR score, ordinal logistic regression models examined to what extent the plasma biomarkers could distinguish between dementia severity levels, using the global CDR score. These analyses were restricted to participants with dementia at baseline. For individuals with AD dementia at baseline who had follow-up study visits, ordinal logistic regression models examined whether baseline plasma biomarker levels could predict the CDR rating at their final study visit. Likewise, models examined whether changes in biomarker measurements (for participants with multiple blood draws) were associated with corresponding changes in CDR rating.

For neuropsychological test scores, the relationship between performance on neuropsychological test raw scores at baseline and plasma biomarker levels was evaluated using partial Pearson correlations across the entire sample, accounting for relevant covariates described below. For longitudinal analyses, generalized linear models estimated via generalized estimating equations (GEEs) tested whether GFAP and other biomarker levels at baseline could predict subsequent changes in neuropsychological test performance. This was determined by the interaction effect between the biomarker level and time since baseline. The GEE models included the baseline visit and all available subsequent study visits and used an autoregressive (AR1) correlation structure. Only individuals with NC or MCI at baseline were included in these analyses to avoid restricted range (i.e., floor effects) among individuals with AD dementia.

Age, education (in years), race (White versus non-White), sex, and apolipoprotein E (*APOE*) $\varepsilon 4$ allele status (carriers versus non-carriers) were included as covariates in all models. These variables were selected a priori based on known associations with AD dementia. The inclusion of these variables allows for the effects of the biomarker in statistical models to be isolated without the confound of other variables associated with diagnostic status. For longitudinal models, additional covariates included the follow-up interval (i.e., years between the baseline and final study visit) and the baseline value of the outcome variable. *P* values, Wald *Z*, and 95% confidence intervals were all adjusted for false discovery rate (FDR) using the Benjamini–Hochberg procedure[43] to reduce the risk of Type I error and maintain the overall false positive rate at 5%. This procedure accounted for the number of comparisons for each type of analysis (specified in the table legends).

3 | RESULTS

3.1 | Participants

Table 1 displays demographic and clinical characteristics of the sample ($N = 567$). The sample size represents participants who had complete data on all primary study variables at baseline. Of the 567 participants, 234 had NC at the baseline, 180 were diagnosed with MCI, and 153 were diagnosed with AD dementia. The groups did not differ on diabetes, hypertension, and hypercholesterolemia. Follow-up clinical data were available for 458 participants (80.8% of the sample), with a mean follow-up interval of 4.69 years (SD = 2.60). Forty-nine participants with NC at baseline experienced a change in cognitive diagnosis by their most recent study visit (39 with MCI, 10 with AD dementia). Twenty-four participants with MCI at baseline later progressed to AD dementia.

Repeat blood draws from multiple study visits were available for 194 participants with a mean follow-up of 5.10 years (SD = 2.65); diagnoses at baseline were NC = 114, MCI = 55, AD dementia = 25. Of the 114 participants with NC at baseline, 19 had MCI at a follow-up biomarker measurement and only 4 were diagnosed with AD dementia. For participants with MCI at baseline, eight had AD dementia at a follow-up blood draw.

The range of values fell within the dynamic range for the assays (GFAP: 40-3096pg/mL; NfL: 1.0–179.2pg/mL; p-tau$_{181}$: 0.2–124.1pg/mL; p-tau$_{231}$: 2.1–235.5pg/mL). A highly positive skew was observed for all four biomarkers and they were log-transformed (natural log). These log values were standardized into *z* scores to facilitate interpretation of coefficients from regression models.

3.2 | Diagnostic status and conversion

There was not a significant difference in means between NC and MCI at baseline for GFAP or the other three biomarkers (Table 2). All biomarkers were higher in those with AD dementia compared to NC. The largest effect size was observed for GFAP (mean adjusted difference [mean diff.] = 0.90). Moderate effect sizes were observed for NfL (mean diff. = 0.65) and p-tau$_{181}$ (mean diff. = 0.52), with a smaller but still significant effect size for p-tau$_{231}$ (mean diff. = 0.29). All biomarkers except for p-tau$_{231}$ were higher in AD dementia compared to MCI. GFAP had the largest effect size (mean diff. = 0.72), with smaller effect sizes for NfL (mean diff. = 0.50) and p-tau$_{181}$ (mean diff. = 0.35). Given the inferiority of p-tau$_{231}$ compared to the other three biomarkers and the minimal validity in discriminating between different severities of cognitive impairment, this biomarker was excluded from further analyses.

ROC modeling examined the ability of the biomarkers to discriminate between diagnostic groups at baseline based on logistic regression models (see Figure 1). This included a baseline model including only covariates and then additional models including the biomarkers to determine the incremental validity in discriminating between groups. GFAP did not significantly improve upon prediction in the comparison

TABLE 1 Baseline demographic and biomarker characteristics by diagnostic group.

	NC($n = 234$)	MCI ($n = 180$)	AD dementia ($n = 153$)	Between-group differences
Age (SD)	72.4 (7.7)	74.8 (7.2)	76.8 (8.1)	NC < MCI < AD
Sex (female)	62.8%	57.8%	44.4%	(NC = MCI) > AD
Race				(NC = AD) > MCI*
White	90.2%	75.0%	91.5%	
Black	9.0%	23.9%	7.2%	
Asian	0.9%	1.1%	1.3%	
Education (SD)	16.6 (2.6)	15.5 (2.8)	14.9 (3.0)	NC > (MCI = AD)
APOE ε4 carrier status	32.4%	32.8%	57.5%	(NC = MCI) < AD
Diabetes	11.5%	11.7%	12.4%	ns
Hypertension	50.0%	55.6%	49.7%	ns
Hypercholesterolemia	58.5%	62.2%	57.5%	ns
MMSE (SD)	29.4 (0.9)	28.2 (1.7)	21.1 (6.2)	NC > MCI > AD
GFAP (pg/mL)	198 (119)	236 (245)	355 (207)	(NC = MCI) < AD
NfL (pg/mL)	15.4 (10.5)	17.6 (9.9)	26.6 (17.4)	(NC = MCI) < AD
p-tau$_{181}$ (pg/mL)	16.1 (11.1)	18.1 (10.0)	25.9 (15.6)	(NC = MCI) < AD
p-tau$_{231}$ (pg/mL)	22.1 (14.6)	24.6 (15.9)	28.5 (16.5)	NC < AD#
# with clinical follow-up	198 (84.6%)	145 (80.6%)	115 (75.2%)	NC > AD#
Years follow-up (SD)	5.35 (2.49)	4.95 (2.64)	3.22 (2.14)	(NC = MCI) > AD
# with repeat biomarkers	114 (48.7%)	55 (30.6%)	25 (16.3%)	NC > MCI > AD
Follow-up interval (SD)	5.50 (2.56)	5.10 (2.78)	3.25 (2.07)	(NC = MCI) > AD

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NC, normal cognition; NfL, neurofilament light chain; p-tau, phosphorylated tau; SD, standard deviation.

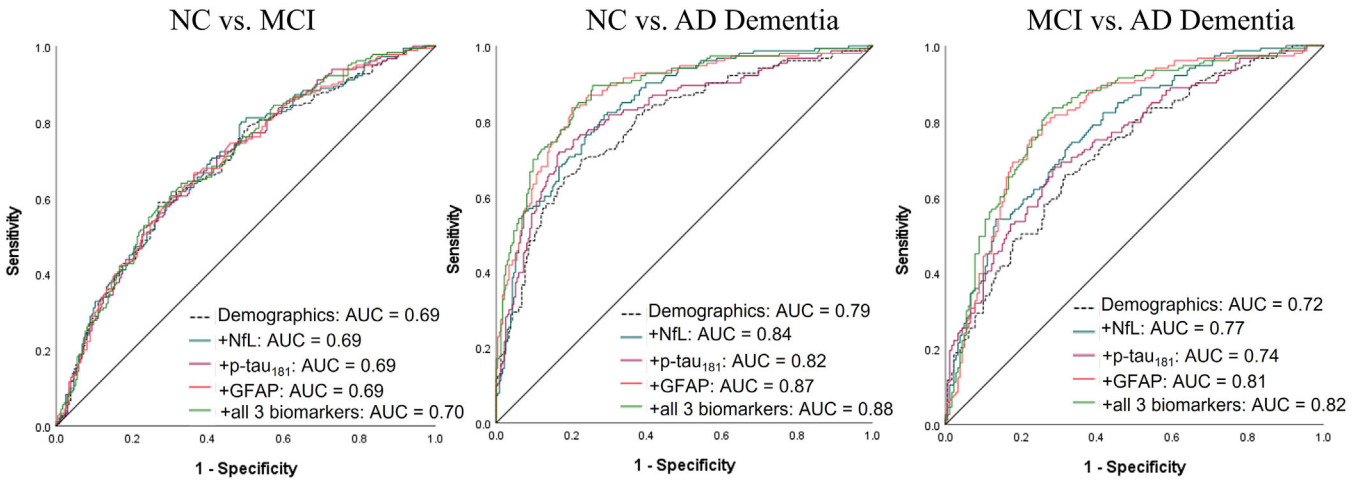

FIGURE 1 Receiver operating characteristic (ROC) curve comparisons between diagnostic groups. ROC curves for plasma GFAP, NfL, and p-tau$_{181}$ comparing the area under the curve (AUC) for diagnostic comparisons at baseline, including models for plasma GFAP, NfL, p-tau$_{181}$, and all three biomarkers combined. Each graph displays a baseline model including only demographic covariates (age, sex, *APOE* ε4 carrier status, education, and race) and models adding plasma GFAP, NfL, and p-tau$_{181}$ simultaneously including all three biomarkers. AD, Alzheimer's disease dementia; *APOE*, apolipoprotein E; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; NC, normal cognition; NfL, neurofilament light chain; p-tau$_{181}$, phosphorylated tau (181)

TABLE 2 Baseline biomarker comparisons between diagnostic groups.

	Adjusted mean difference	95% CI	FDR *P*
NC ($n = 234$) vs. MCI ($n = 180$)			
Baseline GFAP	0.17	[−0.01, 0.33]	0.066
Baseline NfL	0.14	[−0.02, 0.30]	0.093
Baseline p-tau$_{181}$	0.17	[−0.02, 0.36]	0.080
Baseline p-tau$_{231}$	0.07	[−0.12, 0.26]	0.467
NC ($n = 234$) vs. AD ($n = 153$)			
Baseline GFAP	0.90	[0.70, 1.10]	<0.001*
Baseline NfL	0.65	[0.46, 0.84]	<0.001*
Baseline p-tau$_{181}$	0.52	[0.30, 0.74]	<0.001*
Baseline p-tau$_{231}$	0.29	[0.05, 0.53]	0.017*
MCI ($n = 180$) vs. AD ($n = 153$)			
Baseline GFAP	0.72	[0.52, 0.92]	<0.001*
Baseline NfL	0.50	[0.31, 0.69]	<0.001*
Baseline p-tau$_{181}$	0.35	[0.11, 0.59]	0.004*
Baseline p-tau$_{231}$	0.21	[−0.02, 0.44]	0.079

Note: Post hoc comparisons between participant groups at baseline based on log transformed GFAP, NfL, p-tau$_{181}$, and p-tau$_{231}$ levels (converted to *z* scores to facilitate interpretation). All analyses are based on ANCOVA models controlling for age, sex, education, race, and *APOE* ε4 carrier status. Omnibus group effects were significant for all three biomarkers. *$P < 0.05$ after correction for false discovery rate based on 12 analyses.
Abbreviations: AD, Alzheimer's disease dementia; ANCOVA, analysis of covariance; *APOE*, apolipoprotein E; CI, confidence interval; FDR, false discovery rate; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; NC, normal cognition; NfL, neurofilament light chain; p-tau$_{181}$, phosphorylated tau (181), p-tau$_{231}$, phosphorylated tau (231).

between NC and MCI ($P = 0.090$), but neither did NfL ($P = 0.193$) or p-tau$_{181}$ ($P = 0.102$). The AUC remained similar for each biomarker (0.69) compared to a baseline model only containing demographic and *APOE* covariates. The AUC for a combined model with all three biomarkers was essentially unchanged at 0.70, with a maximum Youden *J* statistic of 0.31.

For the comparison between NC and AD dementia, all three biomarkers improved upon the baseline model in separate logistic regression models. GFAP demonstrated the best discrimination between diagnostic groups, with an AUC of 0.87 falling in the range of "excellent discrimination," according to interpretation guidelines from Hosmer and Lemeshow.[44] GFAP also improved predictive accuracy in the corresponding logistic regression model ($P < 0.001$). AUC values for NfL (0.84) and p-tau$_{181}$ (0.82) were lower than GFAP, but still in the range of excellent discrimination (*P*s < 0.001). A model combining all three biomarkers had only minimally better AUC (0.88) compared to GFAP alone, and only GFAP ($P < 0.001$) and NfL ($P = 0.003$) were significant predictors in the corresponding logistic regression model. The maximum Youden *J* statistic was 0.64 for the combined model.

Comparing MCI and AD dementia, all three biomarkers significantly (*P*s ≤ 0.002) improved prediction beyond the baseline demographic model, with GFAP again having the highest AUC (0.81). The AUC values for NfL (0.77) and p-tau$_{181}$ (0.74) were both in the range of "acceptable discrimination." The combined model with all three biomarkers had a minimally better AUC (0.82) compared to GFAP alone, and again only GFAP ($P < 0.001$) and NfL ($P = 0.006$) were significant predictors in the corresponding logistic regression model. The maximum Youden *J* statistic was 0.55 for the combined model.

TABLE 3 Cox proportional hazards models predicting cognitive change.

	OR	95% CI	Wald *Z*	*P*
3.1 Conversion from NC to MCI/AD ($n = 193$)				
Baseline GFAP	1.56	[0.91, 2.68]	1.61	0.108
Baseline NfL	1.37	[0.93, 2.02]	1.58	0.114
Baseline p-tau$_{181}$	1.10	[0.71, 1.72]	0.42	0.673
3.2 Progression from MCI to AD ($n = 95$)				
Baseline GFAP	1.61	[0.88, 2.95]	1.54	0.123
Baseline NfL	0.71	[0.31, 1.61]	0.82	0.413
Baseline p-tau$_{181}$	1.01	[0.57, 1.79]	0.03	0.977

Note: Cox proportional hazards models demonstrating the effect of log-transformed plasma GFAP, NfL, and p-tau$_{181}$ in predicting risk for subsequent cognitive change. Odds ratios are based on the standardized values of the biomarkers at baseline and represent the odds of changing diagnosis at a subsequent study visit. Age, sex, education, race, *APOE* ε4 carrier status, and the time from baseline to diagnostic conversion are included as covariates in each model. For participants who were diagnostically stable, the total duration of study participation is included in place of time to conversion. *P* values are adjusted based on a false discovery rate correction for six comparisons. Sample sizes indicate the total number of participants in each model.
Abbreviations: AD, Alzheimer's disease dementia; *APOE*, apolipoprotein E; CI, confidence interval; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; NC, normal cognition; NfL, neurofilament light chain; OR, odds ratio; p-tau$_{181}$, phosphorylated tau (181).

Table 3 displays the results of longitudinal models examining the relationship between plasma biomarkers and diagnostic change using Cox proportional-hazards regression modeling. The effect for GFAP in predicting conversion from NC to MCI/AD dementia was initially significant at $P = 0.018$ and an odds ratio (OR) of 1.56, but this became non-significant ($P = 0.108$) after applying the Benjamini–Hochberg false discovery error correction. Likewise, GFAP was also initially a significant predictor of progression from MCI to AD dementia ($P = 0.041$; OR = 1.61) but this also became non-significant after a correction for multiple comparisons ($P = 0.123$). Neither of the other biomarkers were significant in either analysis. There were no significant relationships between changes in biomarker levels over time and corresponding changes in cognitive diagnoses for GFAP or the other two biomarkers (*P*s ≥ 0.755).

3.3 | Global CDR

The relationships between the three plasma biomarkers and global CDR were examined both at baseline and longitudinally (Table 4).

TABLE 4 Regression model of discrimination and predictive ability.

	OR	95% CI	Wald *Z*	FDR *P*
4.1 AD dementia: baseline CDR ($n = 153$)				
Baseline GFAP	1.59	[1.04, 2.44]	2.13	0.033*
Baseline NfL	1.94	[1.27, 2.95]	3.09	0.002*
Baseline p-tau$_{181}$	1.23	[0.81, 1.88]	0.96	0.338
4.2 CDR rating at final visit ($n = 110$)				
Baseline GFAP	1.50	[0.85, 2.64]	1.41	0.158
Baseline NfL	1.43	[0.79, 2.59]	1.18	0.239
Baseline p-tau$_{181}$	2.55	[1.44, 4.50]	3.23	0.001*
4.3 Biomarker change: CDR ratings ($n = 23$)				
GFAP change score	0.60	[0.04, 8.98]	0.37	0.712
NfL change score	1.48	[0.14, 15.19]	0.33	0.740
p-tau$_{181}$ change score	0.68	[0.13, 3.65]	0.45	0.651

Note: Ordinal logistic regression models demonstrating the effect of plasma GFAP, NfL, and p-tau$_{181}$ in discrimination between dementia severity levels at baseline (as measured by global CDR rating; 4.1), predicting subsequent changes in CDR among individuals with dementia based on baseline NfL and t-tau (4.2), and examining the correspondence between biomarker change scores and CDR level over time (4.3). Odds ratios are based on the standardized values of log-transformed biomarkers at baseline (i.e., *z* scores) and represent the odds of having a more severe cognitive diagnosis rating for each SD increase in the biomarker at baseline (4.1 and 4.2) or increase in biomarker levels between visits (4.3). Age, sex, education, race, and *APOE* ε4 carrier status are included as covariates in models 4.1 and 4.2. Only age, time since baseline, and baseline CDR values were used as covariates in 4.3 due to limited sample size. *P* values are adjusted based on a false discovery rate correction for nine comparisons. Sample sizes indicate the total number of participants in each model.

Abbreviations: AD, Alzheimer's disease dementia; *APOE*, apolipoprotein E; CDR, Clinical Dementia Rating; CI, confidence interval; FDR, false discovery rate; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; NC, normal cognition; OR, odds ratio; NfL, neurofilament light chain; p-tau$_{181}$, phosphorylated tau (181); SD, standard deviation.

Among the 153 individuals diagnosed with AD dementia at baseline, 42 had a CDR score of 0.5, 73 had a CDR of 1, 26 had a CDR of 2, and 12 had a CDR score of 3. Higher plasma GFAP and NfL were associated with increased odds for a more severe CDR score at baseline. Longitudinally, only higher p-tau$_{181}$ at baseline predicted increased odds for worsening in CDR over time.

3.4 | Neuropsychological test performance

Higher concentrations of GFAP were correlated with raw score performance on all tests (other than Forward Digit Span; Table 5), indicating worse performance with higher levels of the biomarker (inverse correlations on all tests except Trail Making Test, for which higher scores indicate slower completion time). P-tau$_{181}$ and NfL significantly correlated with most neuropsychological tests as well.

TABLE 5 Partial correlation matrix of neuropsychological measures by plasma biomarker.

Test	GFAP	NfL	p-tau$_{181}$
MMSE	−0.31*	−0.27*	−0.18*
Animal Fluency	−0.32*	−0.30*	−0.14*
Vegetable Fluency	−0.28*	−0.25*	−0.14*
Digit Span Forward	−0.08	−0.06	−0.07
Digit Span Backward	−0.18*	−0.19*	−0.06
Trail Making Test A	0.20*	0.25*	0.03
Trail Making Test B	0.27*	0.19*	0.09*
Boston Naming Test	−0.28*	−0.24*	−0.12*
Logical Memory II	−0.29*	−0.23*	−0.13*
NAB Trials 1-3	−0.24*	−0.19*	−0.12*
NAB Short Delay	−0.24*	−0.21*	−0.12*
NAB Long Delay	−0.24*	−0.20*	−0.12*

Note: Matrix displaying partial correlations between plasma log-transformed GFAP, NfL, p-tau$_{181}$, and neuropsychological test performance at baseline across the entire sample ($N = 567$). Raw scores were used for all tests. Age, sex, education, race, and *APOE* ε4 carrier status are included as covariates. *$P < 0.05$ based on false discovery rate for 12 comparisons for each biomarker.

Abbreviations: *APOE*, apolipoprotein E; GFAP, glial fibrillary acidic protein; MMSE, Mini-Mental State Examination; NAB, Neuropsychological Assessment Battery; NfL, neurofilament light chain; p-tau$_{181}$, phosphorylated tau (181).

Table 6 displays the results for baseline levels of GFAP and the other biomarkers in predicting longitudinal changes in neuropsychological test performance over time among participants with NC or MCI at baseline, using GEE. Baseline GFAP selectively predicted declines in performance on measures of delayed memory recall (i.e., Logical Memory II and NAB List Learning Long Delay) but did not predict declines on the other tests evaluated.

3.5 | Post hoc neuropathological analysis

We leveraged neuropathological data from a subset of the sample, described previously,[35] to provide confidence in diagnostic accuracy. Ninety-one participants with a clinical diagnosis of cognitive impairment had autopsy data. Based on NIA-Reagan criteria, 51 (56%) had high likelihood, 6 (6.6%) had intermediate likelihood, and 16 had low likelihood (19.8%). Accuracy improved when the clinical diagnosis was dementia as opposed to MCI (Table S1 in supporting information).

4 | DISCUSSION

The present study assessed the validity of plasma GFAP to correlate with and predict cognitive impairment associated with AD

TABLE 6 General estimating equations predicting change in neuropsychological performance by plasma biomarker.

	GFAP			NfL			p-tau$_{181}$		
	β	SE	*P*	*β*	SE	*P*	*β*	SE	*P*
MMSE	−0.05	0.03	0.168	−0.04	0.02	0.024*	−0.03	0.03	0.487
Animal fluency	−0.11	0.05	0.116	−0.07	0.05	0.199	−0.08	0.06	0.581
Vegetable fluency	−0.09	0.05	0.163	−0.14	0.04	0.002*	−0.06	0.06	0.502
DSF	−0.02	0.01	0.324	−0.02	0.01	0.094	−0.01	0.01	0.714
DSB	−0.01	0.02	0.383	−0.00	0.01	0.817	0.00	0.01	0.926
Trails A	0.20	0.22	0.401	0.42	0.16	0.021*	−0.13	0.41	0.901
Trails B	1.08	0.66	0.149	1.67	0.55	0.013*	0.63	0.96	0.679
BNT	−0.09	0.05	0.158	−0.08	0.03	0.026*	−0.07	0.03	0.480
LM-II	−0.20	0.07	0.018*	−0.15	0.05	0.010*	−0.08	0.05	0.480
NAB Trials 1-3	−0.08	0.08	0.373	−0.07	0.06	0.307	0.01	0.06	0.955
NAB SD	−0.07	0.04	0.164	−0.02	0.03	0.631	−0.05	0.03	0.402
NAB LD	−0.14	0.04	0.005*	−0.08	0.03	0.025*	−0.04	0.03	0.381

Note: Results from generalized estimating equations predicting the relationship between baseline log-transformed plasma GFAP, NfL, and p-tau$_{181}$ and subsequent change on neuropsychological testing in individuals diagnosed with NC or MCI at baseline who had longitudinal data available ($n = 343$). Negative coefficients represent declining raw scores with higher levels of the biomarker at baseline. Age at baseline, time since baseline, and the baseline score on the neuropsychological measure were included as covariates in all models in addition to demographic covariates. All study visits were included in these models.
*$P < 0.05$ after correction for false discovery rate based on 12 analyses for each biomarker.
Abbreviations: BNT, Boston Naming Test; DSF and DSB, Digit Span Forward and Backward; GFAP, glial fibrillary acidic protein; LM-II, Logical Memory II; MMSE, Mini-Mental State Examination; NAB SD and LD, Neuropsychological Assessment Battery List Learning Test, Short and Long Delay; NfL, neurofilament light chain; p-tau$_{181}$, phosphorylated tau (181).

against plasma biomarkers of NfL, p-tau$_{181}$, and p-tau$_{231}$. All analyses controlled for age, sex, race, *APOE* ε4 status, and education. At baseline, GFAP did not differentiate participants with NC from those with MCI, but did discriminate participants with NC and MCI from those with AD dementia with superior effect sizes compared to the other biomarkers. Higher GFAP was associated with higher global CDR ratings and demonstrated consistently stronger relationships with neuropsychological test scores at baseline compared to NfL, p-tau$_{181}$, and p-tau$_{231}$. Finally, both plasma GFAP and NfL predicted decline on neuropsychological measures of verbal episodic memory, with NfL also predicting decline on additional measures. The biomarkers assessed in the current study represent two thirds of the AT(N) framework for in vivo biomarker detection,[45,46] namely tau pathology and neurodegeneration, with the absence of plasma Aβ exhibiting a limitation. While GFAP is correlated with AD pathology,[1] it has been proposed as a marker for several neurodegenerative disorders.[21,22] Therefore, GFAP represents an opportunity for successive hurdles to narrow initial prospective biomarker panels, with subsequent assessment containing AD-specific biomarkers.

Our findings are consistent with a recent meta-analysis by Gonzales et al.[18] in which four longitudinal cohorts assessed blood-based GFAP with various cognitive and diagnostic outcomes in non-demented, community-based populations. While only one cohort (Cardiovascular Health Study [CHS]) found that higher GFAP associated with worse cognition, the remaining cohorts found the same direction of effect. Further analysis of the CHS cohort revealed a significant association between serum GFAP and conversion from NC to AD dementia. The present study expands upon these findings by including participants spanning the clinical continuum and using multiple plasma biomarkers. Our lack of association between plasma GFAP levels and longitudinal conversion to dementia could be explained by low conversion rate and subsequent small sample size compared to the CHS sample. However, our ability to detect cognitive changes in neuropsychological measures characteristic of AD dementia[47] is promising.

While GFAP differentiated NC and MCI from those with AD dementia, it did not differentiate NC from MCI. Parvizi et al.[12] assessed the diagnostic discrimination of plasma GFAP and likewise did not observe significant findings when separating cognitively impaired individuals into MCI and AD dementia. There is growing evidence supporting plasma GFAP as one of the first pre-clinical markers,[6,14,19] observable 8 to 16 years before dementia onset.[15,25] Therefore, there might be less of a stark contrast in GFAP levels that separate NC and MCI within a similarly aged population as the pathological load of GFAP could be comparable, with dementia representing the tipping point for further accumulation. Regardless, these remain somewhat arbitrary diagnostic bins and the strong associations between GFAP and continuous measures of function (e.g., neuropsychological test raw scores) are informative.

We have several limitations. Although validated support for plasma biomarkers is strengthening, we lacked alternative biomarkers and neuropathology for all participants. Amyloid has been associated with GFAP;[3] therefore, our lack of amyloid biomarkers limits our understanding of the relationship between GFAP and AD. Amyloid status has been shown to drive the relationship between measures of plasma

p-tau and AD disease status;[20] thus, not separating our sample by amyloid status may be influencing our analysis of p-tau isotopes.

We recognize that there are alternative immunoassays and our results should be confirmed against high-performing versions for verification. While we did include a longitudinal component, our follow-up sample was smaller than our baseline sample. Thus, the negative findings between cognitive decline and corresponding changes in biomarker levels could reflect Type II error. A larger longitudinal sample and longer duration period could allow for better comparison and strength in making predictive assertions. Our sample is representative of a clinic setting and our findings may have limited generalizability to the general population. Our sample is predominantly White (non-Hispanic), college educated participants, further limiting the external validity of our results. However, studies with more diverse samples parallel our findings.[10,15] Further, we excluded all other suspected etiologies other than AD due to small sample sizes of alternative etiologies. While allowing us to examine the relationship between the plasma biomarkers and AD diagnostic status, we recognize that this could present a potential bias and limits our analysis to evaluating the specificity solely for AD syndromes. As we now know that cognitive decline is likely to be a result of mixed pathologies[48] inclusion of alternative etiologies could provide stronger ecological validity.

5 | CONCLUSIONS

While alternative plasma markers of NfL and p-tau$_{181}$ contribute to diagnostic ability, plasma GFAP performed superiorly even in models using a combined biomarker panel. Further, plasma GFAP predicted performance on neuropsychological measures that are indicative of underlying AD. Blood-based GFAP could offer a more feasible assessment and screening tool for AD and resulting clinical syndromes in both research and clinical settings.

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CONFLICT OF INTEREST STATEMENT

The authors MA, MAS, TKK, HJA, BF, YT, BM, JNP, EGS, IS, LAF, GRJ, KWT, AEB, MKO, LEG, NWK, RK, TDS, ACM, WQ, and JM have no competing interests to declare. HZ has served on scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, 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). KB has served as a consultant, on advisory boards, or on 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. NJA has given lectures in symposia sponsored by Eli-Lily. AEB has served as a consultant or on advisory boards for Eli Lilly and Roche Pharmaceuticals and has received grant monies from Cumulus Neuroscience, VoxNeuro, Bristol Myers Squibb, and Cyclerion. He receives publishing royalties from Elsevier and Oxford University Press. RA serves on the scientific advisory board of Signant Health, as consultant to Biogen, and has given a lecture in a symposium sponsored by Eisai. RAS has served as a consultant to Biogen and Lundbeck. He receives royalties for published neuropsychological tests from Psychological Assessment Resources, Inc. MLA has received honorarium from the Michael J. Fox Foundation for services unrelated to this study. He receives royalties from Oxford University Press.

CONSENT STATEMENT

All data collection procedures were approved by the Boston University Medical Center Institutional Review Board and were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtainted from the research participant or their legally authorized representative.

REFERENCES

1. Alawode DOT, Fox NC, Zetterberg H, Heslegrave AJ. Alzheimer's disease biomarkers revisited from the amyloid cascade hypothesis standpoint. *Front Neurosci*. 2022;16:837390. 10.3389/fnins.2022.837390
2. Heneka MT, Carson MJ, El Khoury J, et al. Neuroinflammation in Alzheimer's Disease. *Lancet Neurol*. 2015;14(4):388-405. 10.1016/S1474-4422(15)70016-5
3. Serrano-Pozo A, Mielke ML, Gómez-Isla T, et al. Reactive glia not only associates with plaques but also parallels tangles in Alzheimer's disease. *Am J Pathol*. 2011;179(3):1373-1384. 10.1016/j.ajpath.2011.05.047
4. Bellaver B, Ferrari-Souza JP, da RosLU, et al. Astrocyte biomarkers in Alzheimer disease: a systematic review and meta-analysis. *Neurology*. 2021;96(24):e2944-e2955. 10.1212/WNL.0000000000012⟨3:hyphenbreak⟩⟨/3:hyphenbreak⟩109
5. Ganne A, Balasubramaniam M, Griffin WST, Shmookler Reis RJ, Ayyadevara S. Glial fibrillary acidic protein: a biomarker and drug target for Alzheimer's disease. *Pharmaceutics*. 2022;14(7):1354. 10.3390/pharmaceutics14071354
6. Salvadó G, Shekari M, Falcon C, et al. Brain alterations in the early Alzheimer's continuum with amyloid-β, tau, glial and neurodegeneration CSF markers. *Brain Commun*. 2022;4(3):134. 10.1093/braincomms/fcac134
7. Asken BM, Elahi FM, La Joie R, et al. Plasma glial fibrillary acidic protein levels differ along the spectra of amyloid burden and clinical disease stage. *J Alzheimers Dis JAD*. 2020;78(1):265-276. 10.3233/JAD-200755
8. Ferrari-Souza JP, Ferreira PCL, Bellaver B, et al. Astrocyte biomarker signatures of amyloid-β and tau pathologies in Alzheimer's disease. *Mol Psychiatry*. 2022;27:4781-4789. 10.1038/s41380-022-01716-2
9. Shir D, Graff-Radford J, Hofrenning EI, et al. Association of plasma glial fibrillary acidic protein (GFAP) with neuroimaging of Alzheimer's disease and vascular pathology. *Alzheimers Dement Amst Neth*. 2022;14(1):e12291. 10.1002/dad2.12291
10. Gonzales MM, Wang CP, Short MI, et al. Blood biomarkers for cognitive decline and clinical progression in a Mexican American cohort. *Alzheimers Dement Amst Neth*. 2022;14(1):e12298. 10.1002/dad2.12298
11. Oeckl P, Anderl-Straub S, Arnim CAFV, et al. Serum GFAP differentiates Alzheimer's disease from frontotemporal dementia and predicts MCI-to-dementia conversion. *J Neurol Neurosurg Psychiatry*. 2022;93(6):659-667. 10.1136/jnnp-2021-328547
12. Parvizi T, König T, Wurm R, et al. Real-world applicability of glial fibrillary acidic protein and neurofilament light chain in Alzheimer's disease. *Front Aging Neurosci*. 2022;14:887498. 10.3389/fnagi.2022.887498
13. Pereira JB, Janelidze S, Smith R, et al. Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. *Brain*. 2021;144(11):3505-3516. 10.1093/brain/awab223
14. Prins S, de Kam ML, Teunissen CE, Groeneveld GJ. Inflammatory plasma biomarkers in subjects with preclinical Alzheimer's disease. *Alzheimers Res Ther*. 2022;14(1):106. 10.1186/s13195-022-01051-2

15. Rajan KB, Aggarwal NT, McAninch EA, et al. Remote blood biomarkers of longitudinal cognitive outcomes in a population study. *Ann Neurol*. 2020;88(6):1065-1076. 10.1002/ana.25874
16. Benedet AL, Milà-Alomà M, Vrillon A, et al. Differences between plasma and cerebrospinal fluid glial fibrillary acidic protein levels across the Alzheimer disease continuum. *JAMA Neurol*. 2021;78(12):1471-1483. 10.1001/jamaneurol.2021.3671
17. Simrén J, Weninger H, Brum WS, et al. Differences between blood and cerebrospinal fluid glial fibrillary acidic protein levels: the effect of sample stability. *Alzheimers Dement*. 10.1002/alz.12806. Published online September 14, 2022:alz.12806.
18. Gonzales MM, Wiedner C, Wang CP, et al. A population-based meta-analysis of circulating GFAP for cognition and dementia risk. *Ann Clin Transl Neurol*. 2022;9:1574-1585. 10.1002/acn3.51652
19. Cicognola C, Janelidze S, Hertze J, et al. Plasma glial fibrillary acidic protein detects Alzheimer pathology and predicts future conversion to Alzheimer dementia in patients with mild cognitive impairment. *Alzheimers Res Ther*. 2021;13(1):68. 10.1186/s13195-021-00804-9
20. Chatterjee P, Pedrini S, Ashton NJ, et al. Diagnostic and prognostic plasma biomarkers for preclinical Alzheimer's disease. *Alzheimers Dement*. 2022;18(6):1141-1154. 10.1002/alz.12447
21. Chouliaras L, Thomas A, Malpetti M, et al. Differential levels of plasma biomarkers of neurodegeneration in Lewy body dementia, Alzheimer's disease, frontotemporal dementia and progressive supranuclear palsy. *J Neurol Neurosurg Psychiatry*. 2022;93(6):651-658. 10.1136/jnnp-2021-327788
22. Baiardi S, Quadalti C, Mammana A, et al. Diagnostic value of plasma p-tau181, NfL, and GFAP in a clinical setting cohort of prevalent neurodegenerative dementias. *Alzheimers Res Ther*. 2022;14(1):153. 10.1186/s13195-022-01093-6
23. Benussi A, Cantoni V, Rivolta J, et al. Classification accuracy of blood-based and neurophysiological markers in the differential diagnosis of Alzheimer's disease and frontotemporal lobar degeneration. *Alzheimers Res Ther*. 2022;14(1):155. 10.1186/s13195-022-01094-5
24. Ebenau JL, Pelkmans W, Verberk IMW, et al. Association of CSF, plasma, and imaging markers of neurodegeneration with clinical progression in people with subjective cognitive decline. *Neurology*. 2022;98(13):e1315-e1326. 10.1212/WNL.0000000000200035
25. Stocker H, Beyer L, Perna L, et al. Association of plasma biomarkers, p-tau181, glial fibrillary acidic protein, and neurofilament light, with intermediate and long-term clinical Alzheimer's disease risk: results from a prospective cohort followed over 17 years. *Alzheimers Dement*. 2022;19(1):25-35. 10.1002/alz.12614. Published online March 2, 2022:alz.12614.
26. Verberk IMW, Laarhuis MB, van den Bosch KA, et al. Serum markers glial fibrillary acidic protein and neurofilament light for prognosis and monitoring in cognitively normal older people: a prospective memory clinic-based cohort study. *Lancet Healthy Longev*. 2021;2(2):e87-e95. 10.1016/S2666-7568(20)30061-1
27. Frank B, Ally M, Brekke B, et al. Plasma p-tau181 shows stronger network association to Alzheimer's disease dementia than neurofilament light and total tau. *Alzheimers Dement*. 2022;18(8):1523-1536. 10.1002/alz.12508
28. Sugarman MA, Zetterberg H, Blennow K, et al. A longitudinal examination of plasma neurofilament light and total tau for the clinical detection and monitoring of Alzheimer's disease. *Neurobiol Aging*. 2020;94:60-70. 10.1016/j.neurobiolaging.2020.05.011
29. Beekly DL, Ramos EM, van Belle G, et al. The National Alzheimer's Coordinating Center (NACC) database: an Alzheimer disease database. *Alzheimer Dis Assoc Disord*. 2004;18(4):270-277.
30. Besser L, Kukull W, Knopman DS, et al. Version 3 of the national Alzheimer's coordinating center's uniform data set. *Alzheimer Dis Assoc Disord*. 2018;32(4):351-358. 10.1097/WAD.0000000000000279
31. Morris JC, Weintraub S, Chui HC, et al. The Uniform Data Set (UDS): clinical and cognitive variables and descriptive data from Alzheimer disease centers. *Alzheimer Dis Assoc Disord*. 2006;20(4):210-216. 10.1097/01.wad.0000213865.09806.92
32. Weintraub S, Salmon D, Mercaldo N, et al. The Alzheimer's Disease Centers' Uniform Data Set (UDS): the neuropsychologic test battery. *Alzheimer Dis Assoc Disord*. 2009;23(2):91-101. 10.1097/WAD.0b013e318191c7dd
33. Gavett BE, Lou KR, Daneshvar DH, Green RC, Jefferson AL, Stern RA. Diagnostic accuracy statistics for seven Neuropsychological Assessment Battery (NAB) test variables in the diagnosis of Alzheimer's disease. *Appl Neuropsychol Adult*. 2012;19(2):108-115. 10.1080/09084282.2011.643947
34. Jefferson AL, Wong S, Gracer TS, Ozonoff A, Green RC, Stern RA. Geriatric performance on an abbreviated version of the boston naming test. *Appl Neuropsychol*. 2007;14(3):215-223. 10.1080/09084280701509166
35. Morrison MS, Aparicio HJ, Blennow K, et al. Ante-mortem plasma phosphorylated tau (181) predicts Alzheimer's disease neuropathology and regional tau at autopsy. *Brain*. 2022;145(10):3546-3557. 10.1093/brain/awac175
36. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol*. 2020;19(5):422-433. 10.1016/S1474-4422(20)30071-5
37. Albert MS, DeKosky ST, Dickson D, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement J Alzheimers Assoc*. 2011;7(3):270-279. 10.1016/j.jalz.2011.03.008
38. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34(7):939-939. 10.1212/WNL.34.7.939
39. Winblad B, Palmer K, Kivipelto M, et al. Mild cognitive impairment – beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J Intern Med*. 2004;256(3):240-246. 10.1111/j.1365-2796.2004.01380.x
40. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement J Alzheimers Assoc*. 2011;7(3):263-269. 10.1016/j.jalz.2011.03.005
41. Hughes CP, Berg L, Danziger W, Coben LA, Martin RL. A new clinical scale for the staging of dementia. *Br J Psychiatry*. 1982;140(6):566-572. 10.1192/bjp.140.6.566
42. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology*. 1993;43(11):2412. 10.1212/WNL.43.11.2412-a
43. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol*. 1995;57(1):289-300. 10.1111/j.2517-6161.1995.tb02031.x
44. Hosmer DW, Lemeshow S. *Applied logistic regression: hosmer/applied logistic regression*. John Wiley & Sons, Inc.; 2000. 10.1002/0471722146
45. Jack CR, Bennett DA, Blennow K, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology*. 2016;87(5):539-547. 10.1212/WNL.0000000000002923
46. Jack jr CR, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement*. 2018;14(4):535-562. 10.1016/j.jalz.2018.02.018
47. Weintraub S, Wicklund AH, Salmon DP. The neuropsychological profile of Alzheimer disease. *Cold Spring Harb Perspect Med*. 2012;2(4):a006171. 10.1101/cshperspect.a006171

48. Boyle PA, Yu L, Wilson RS, Leurgans SE, Schneider JA, Bennett DA. Person-specific contribution of neuropathologies to cognitive loss in old age. *Ann Neurol*. 2018;83(1):74-83. 10.1002/ana.25123

SUPPORTING INFORMATION

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

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