Plasma biomarkers identify brain ATN abnormalities in a dementia-free population-based cohort

Menayit Tamrat Dresse[1], Pamela C. L. Ferreira[2], Akshay Prasadan[3], Jihui L. Diaz[2], Xuemei Zeng[2], Bruna Bellaver[2], Guilherme Povala[2], Victor L. Villemagne[2,4], M. Ilyas Kamboh[4,5], Ann D. Cohen[2,4], Tharick A. Pascoal[2,6], Mary Ganguli[1,2,6], Beth E. Snitz [4,6], C. Elizabeth Shaaban[1,4†], Thomas K. Karikari[2,4*†]

[1]Department of Epidemiology, School of Public Health, University of Pittsburgh, Pittsburgh, PA 15213, USA

[2]Department of Psychiatry, School of Medicine, University of Pittsburgh, 3811 O'Hara Street, Pittsburgh, PA 15213, USA

[3]Department of Statistics & Data Science, Dietrich College of Humanities and Social Sciences, Carnegie Mellon University, Pittsburgh, PA, 15213, USA

[4]Alzheimer's Disease Research Center, University of Pittsburgh, Pittsburgh, PA, USA

[5]Department of Human Genetics, School of Public Health, University of Pittsburgh, Pittsburgh, PA 15213, USA

[6]Department of Neurology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15213, USA

*Corresponding author: Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh 15213, PA, USA. Email: karikaritk@upmc.edu

[†]Joint senior authors

Abstract

INTRODUCTION: Using the ATN framework, we evaluated the potential of plasma biomarkers to identify abnormal brain amyloid-beta (Aβ) positron emission tomography (PET), tau-PET and neurodegeneration in a socioeconomically disadvantaged population-based cohort.

METHODS: Community-dwelling dementia-free (n=113, including 102 (91%) cognitively normal) participants underwent ATN neuroimaging and plasma biomarker assessments.

RESULTS: Plasma Aβ42/Aβ40, p-tau181, and p-tau217 showed significant associations with Aβ-PET status (adjusted odds ratio [AOR] of $1.74*10^{-24}$, 1.47, and $3.43*10^{3}$ respectively [p-values<0.05]), with p-tau217 demonstrating the highest classification accuracy for Aβ-PET status (AUC=0.94). Plasma p-tau181 and p-tau217 showed significant associations with tau-PET status (AOR: 1.50 and 22.24, respectively, p-values<0.05), with comparable classification accuracies for tau-PET status (AUC=0.74 and 0.70, respectively). Only plasma NfL showed significant association with neurodegeneration based on cortical thickness (AOR=1.09, p-value<0.05).

CONCLUSION: Our findings highlight potential of plasma p-tau217 as a biomarker for brain Aβ and tau pathophysiology, p-tau181 for tau abnormalities, and NfL for neurodegeneration in the community.

1. Background

Currently, there is no known cure for Alzheimer's disease (AD) [1]. However, disease-modifying treatments approved by the US Food and Drug Administration (FDA) that can help remove brain amyloid-beta (Aβ) aggregates and slow disease progression have recently become available [1–3]. Given this, accurate biomarker-supported assessment during the early stages of the AD continuum is essential for timely and effective intervention [4].

AD has long been clinically diagnosed at a relatively late stage of the biological disease, once overt symptoms of dementia have developed, hindering early detection [5]. The 2018 National Institute of Aging-Alzheimer's Association (NIA-AA) AD research framework, updated and published in June 2024 [6], greatly shifted this syndromic approach to a biological (etiological) diagnosis [7]. This framework groups the main pathological hallmarks of AD under amyloidosis (A), tau pathology (T) and neurodegeneration (N), using validated cerebrospinal fluid (CSF), positron emission tomography (PET) and structural magnetic resonance imaging (MRI) biomarkers [7]. Despite their proven diagnostic importance, the somewhat invasive nature of CSF collection and the high cost and limited accessibility of PET imaging restrict their use on a large scale in routine clinical settings and applicability for repeated population-wide evaluation.

In recent years, more accessible and cost-effective diagnostic approaches, such as plasma biomarkers, have been widely developed and evaluated as promising surrogate markers and predictors of Aβ and tau pathologies, and neurodegeneration [6]. Many of these are included in the 2024 revised criteria of AD diagnosis and staging by the Alzheimer's Association (AA) workgroup [6]. Plasma Aβ42/Aβ40, one of the core Aβ proteinopathy biomarkers, shows a strong negative correlation with brain Aβ pathology measured by PET, especially in the pre-

clinical stage of AD [8,9]. Plasma glial fibrillary acidic protein (GFAP), an intermediate filament protein and a marker of astrocytosis [10], significantly associates with brain Aβ signals and predict Aβ-PET abnormalities [11,12]. In the 2024 revised criteria, GFAP is categorized under "inflammation" representing a biomarker of a non-specific process in AD pathophysiology [6]. Several plasma phosphorylated-tau (p-tau) variants have been developed and validated as candidate biomarkers of both Aβ and tau pathologies of which p-tau181, p-tau217 and p-tau231 are the most studied [12–27]. For neurodegeneration, plasma neurofilament light chain (NfL), a neuronal cytoplasmic protein, has been identified as a reliable, albeit non-specific, marker of brain atrophy [28,29]. Plasma NfL associates well with neuroimaging measures of neurodegeneration, although it lacks specificity for underlying AD-related pathology [28].

However, several of these studies reported individual plasma biomarkers as predictors of Aβ, tau or neurodegeneration abnormalities, often lacking head-to-head evaluation. Additionally, participants involved in such studies were usually recruited from urban academic medical settings and tended to have high socioeconomic status (SES) [30]. This recruitment approach overlooks populations living in medically underserved areas and with low SES, who face significant dementia-related disparities [31–33] resulting in limited external validity. The emerging interdisciplinary population neuroscience approach addresses this challenge by emphasizing the need for studying biofluid and neuroimaging biomarkers within population-based cohorts [34]. This approach enables the identification of biomarker trajectories at the population level while accounting for factors, including SES, that may influence expression and predictive values of the biomarkers [34]. Thus, such population-based studies of AD plasma biomarkers ensure external validity (generalizability) of findings, thereby enriching our understanding of AD in the broader community.

Therefore, in this study our aims were: (i) to evaluate associations in parallel between six plasma biomarkers (Aβ42/Aβ40, GFAP, NfL, p-tau181, p-tau217 and p-tau231) and brain Aβ-PET, tau-PET and magnetic resonance imaging (MRI)-based neurodegeneration measures; and (ii) to assess the accuracy of each plasma biomarker in classifying brain Aβ-PET, tau-PET and neurodegeneration status, in the Monongahela Youghiogheny Healthy Aging Team-Neuroimaging (MYHAT-NI) study. The MYHAT-NI study collected neuroimaging data from a subset of participants enrolled in the large population-based prospective cohort study, MYHAT [35]. Participants in the parent study were recruited from a small-town area in southwestern Pennsylvania, with some areas medically underserved. This region, once a hub of steel manufacturing, experienced regional economic decline following the collapse of the industry in the late 1970s and 1980s [35,36].

2. Methods

2.1. Study participants

We included 113 participants from the MYHAT-NI study, a neuroimaging study which enrolled a subset of participants from the parent MYHAT cohort. The MYHAT study is an ongoing population-based study of dementia-free older adults drawn from a Rust Belt region of southwestern Pennsylvania, United States of America (USA) [36]. Participants aged 65 years or older and not living in a long-term care institution were randomly selected for recruitment from voter registration lists of selected towns in a geographically defined region. Participants were excluded if they were too ill to participate, had severe cognitive impairment, significant vision or hearing impairment, or were decisionally incapacitated. Inclusion criteria for the MYHAT-NI study were participation in the parent MYHAT study and having a Clinical Dementia Rating (CDR) sum-of-boxes score, the sum of ratings in each six cognitive domains assessed in the CDR evaluation [37], <1.0. The only exclusion criteria were contraindication for MRI or PET

neuroimaging. All participants gave written informed consent, and all study procedures were approved by the Institutional Review Board of the University of Pittsburgh. Further details on recruitment and assessment procedures have been described in detail previously [35,36].

2.2. Neuroimaging methods

Neuroimaging was conducted at the time of blood collection at the University of Pittsburgh PET Center. T1-weighted magnetization prepared rapid gradient echo (MPRAGE) structural MRI series were obtained for each participant using a 3T Siemens PRISMA scanner before the PET imaging session. All PET images were acquired using a Siemens Biograph mCT Flow 64-4R PET/CT scanner.

[^{11}C]Pittsburgh Compound B (PiB) (15 mCi) for Aβ pathology imaging or [^{18}F]AV-1451 (7–10 mCi) for tau pathology imaging were administered as slow bolus injections via the antecubital vein. PET emission data were collected in a series of 5-minute frames spanning 50–70 min post-injection for [^{11}C]PiB and 80–100 min for [^{18}F]AV-1451. [^{11}C]PiB and [^{18}F]AV-1451 acquisition frames were summed into a single 20-minute frame and registered to the participant's T1 MR image using the normalized mutual information algorithm [35].

Participants' neuroimaging-based "A", "T", and "N" statuses were classified based on [^{11}C]PiB, [^{18}F]AV-1451 and MRI scans. Neocortical Aβ load was calculated as the mean ^{11}C]PiB Standard Uptake Value Ratio (SUVR) of nine composite regions: anterior cingulate, posterior cingulate, ventral striatum, superior frontal cortex, orbitofrontal cortex, insula, lateral temporal cortex, parietal, and precuneus [38]. For tau load, a composite [^{18}F]AV-1451 SUVR was computed by normalizing the meta temporal composite region (comprising amygdala, entorhinal, fusiform, parahippocampal, inferior temporal and middle temporal) to FreeSurfer cerebellar gray matter

as a reference [39]. MRI-based cortical thickness composite measure was derived from a surface-area weighted average of the mean cortical thickness of four FreeSurfer regions of interest (ROIs) that are most predictive of AD pathology: entorhinal, inferior temporal, middle temporal, and fusiform [39,40].

Participants were classified as Aβ-PET positive (A+) or Aβ-PET negative (A-) based on a pre-defined [^{11}C]PiB global SUVR cutoff of >1.346 as A+, and [^{11}C]PiB global SUVR <=1.346 as A- [41–43]. Participants with [^{18}F]AV-1451 meta temporal SUVR >1.18 were considered tau-PET positive (T+), while those with a [^{18}F]AV-1451 meta temporal SUVR <= 1.18 were considered tau-PET negative (T−) [39]. Participants with cortical thickness composite measure <2.7 were classified as neurodegeneration positive (N+) while those with cortical thickness composite measure >= 2.7 were classified as neurodegeneration negative (N-) [40]. Full neuroimaging details were described previously [35].

2.3. Plasma biomarker measurements

All plasma biomarkers in this study were measured using Single molecule array (SIMOA) methods on the HD-X instrument (Quanterix, Billerica, MA, USA) at the Department of Psychiatry, School of Medicine, University of Pittsburgh, USA, [44], except for p-tau231 which was measured at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. All frozen samples were thawed at room temperature and centrifuged at 4000xg for 10 min to remove particulates prior to the measurements. Plasma Aβ42, Aβ40, GFAP and NfL were measured with the Neurology 4-Plex E (#103670). P-tau181 was measured with V2 Advantage kit (#103714); p-tau217 with the ALZpath Simoa® p-tau217 V2 Assay Kit (#104371); and p-tau231 using *in-house* SIMOA methods, described previously [21].

For each assay, two or three quality control (QC) samples of different concentrations were analyzed in duplicate both at the start and end of each technical run to assess reproducibility of each assay. The average within-run coefficients of variation (CVs) of the QC samples were 8.9% for Aβ42, 9.5% for Aβ40, 9.9% for GFAP, 14.3% for NfL 6.6% for p-tau181 and 3.7% for p-tau217. The average between-run CVs were 13.0% for Aβ42, 14.6% for Aβ40, 17.8% for GFAP, 18.3% for NfL, 11.7% for p-tau181, and 11.4% for p-tau217 [44].

2.4. Apolipoprotein E genotyping

Apolipoprotein E (*APOE*) genotyping was carried out using blood or saliva samples as described previously [45]. For this analysis, individuals with one or two *APOE4* allele were grouped into *APOE4* carriers and individuals without an *E4* allele were grouped as non- *APOE4* carriers.

2.5. Statistical analysis

Continuous variables were summarized using mean and standard deviation (SD) and categorical variables and complete neuroimaging-based ATN profile distribution of the study participants were reported using count and percentage. The difference in demographics and plasma biomarkers between neuroimaging-based ATN statuses was tested using Wilcoxon rank-sum test and Fisher's exact test for continuous and categorical variables, respectively. Plasma biomarker concentrations (pg/mL) were not normally distributed, and thus they were log transformed. Box plots of the distributions of the log-transformed plasma biomarkers by neuroimaging-based ATN statuses were inspected.

The classification accuracies of the AD plasma biomarkers were tested, with neuroimaging-based ATN biomarkers serving as the ground truth. A series of logistic regression models using

complete data were fitted to examine this, and both unadjusted and adjusted odds ratios (OR) and their 95% confidence intervals (CI) are reported. Adjusted logistic regression models were controlled for age, sex, education level, *APOE4* carrier status and CDR score. A two-sided p-value less than 0.05 was considered statistically significant. Multiple comparisons correction was not applied in the logistic regression models. Receiver operating characteristics (ROC) curves and their respective areas under the curve (AUC) were estimated using values predicted by the logistic regression models to investigate the performance of these plasma biomarkers to predict ATN status.

The associations of Aβ-PET and tau-PET with plasma biomarkers were also tested using voxel-wise linear regressions. Models were adjusted for age, sex, education level, *APOE4* carrier status and CDR score. In the voxel-wise analyses, multiple comparisons correction was performed using random field theory (RFT), with a voxel threshold of $P < 0.001$. All analyses were conducted in R 4.1.1 (R foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org/), and imaging analyses were carried out using the RMINC package.

Sensitivity analysis was also conducted to evaluate the impact of Body Mass Index (BMI) and Area Deprivation Index (ADI) national rank, a measure of neighborhood deprivation (higher is worse deprivation), on the associations of the plasma biomarkers with neuroimaging-based ATN biomarkers and their classification accuracy of neuroimaging-based ATN status. To evaluate this a series of logistic regression models were fitted by additionally controlled for BMI and ADI in the adjusted models.

3. Results

3.1. Characteristics of study participants

As shown in **Table 1**, n=113 dementia-free participants were included in the study. Based on neuroimaging biomarkers of ATN, 28 (25%) individuals were A+, 39 (36%) were T+ and 33 (29%) were N+. Distribution of the neuroimaging-based ATN profiles among the study participants is shown in **Supplementary Table 1**. The most prevalent profile was A-T-N- (40%) followed by A-T-N+ (17.7%), A+T+N- (13.3%) and A-T+N- (12.4%). The mean (standard deviation (SD)) age of the participants was 77 (6) years, a slight majority were female (54%), most identified as non-Hispanic White (95%), had education attainment of high school or less (57%), had high ADI (57%) (while the ADI of the study cohort range from 32–99) and 16% were *APOE*4 carriers. This study cohort comprised mostly cognitively normal individuals (91% with CDR=0) with a mean (SD) BMI of 28.0 (4.4).

Between A+ and A- groups, significant differences were found for *APOE4* carrier status ($p<0.001$), [^{11}C]PiB global SUVR measure ($p<0.001$) and [^{18}F]AV-1451 meta temporal SUVR measure (p-value<0.001). The A+ group had a higher percentage of *APOE4* carriers than the A- group (43% in A+ vs 7.1% in the A-). Similarly, between T+ and T- groups a significant difference was found in terms of [^{11}C]PiB global SUVR measure ($p<0.001$) and [^{18}F]AV-1451 meta temporal SUVR measure (p-value<0.001). Whereas, between T+ and T- groups the expected difference was seen in terms of *APOE4* carrier status, however the difference was not statistically significant (23% in T+ vs 12% in the T-, $p=0.13$). No significant difference was found across the “A” and “T” groups in terms of age, sex, race/ethnicity, education level, ADI, mini-mental status examination (MMSE) score, BMI and cortical thickness composite measure. However, a significant difference was seen in terms of age (mean (SD): 80 (7) vs. 75 (5),

p=0.002) and cortical thickness composite measure (mean (SD): 2.58 (0.15) vs 2.83 (0.08), p<0.001) between the N+ and N- groups. See **Table 1** for detailed characteristics of the study participants and comparisons across the different neuroimaging-based ATN status groups.

3.2. Plasma biomarker concentrations across neuroimaging-based ATN groups

As shown in **Figure 1,** group comparison of log transformed plasma biomarker concentration between the A+ and A- groups showed a statistically significant higher levels of GFAP (p<0.001), p-tau181 (p=0.007), p-tau217 (p<0.001) and p-tau231 (p=0.034) and lower levels of Aβ42/Aβ40 (p<0.001) in the A+ group. Statistically significant higher levels of p-tau181 (p=0.021), p-tau217 (p=0.002) and p-tau231 (p=0.037), and lower level of Aβ42/Aβ40 (p=0.03) was seen in the T+ group compared to the T- group. Only NfL was shown to be significantly higher in the N+ compared with the N- group (p=0.002).

3.3. Association of plasma biomarkers with neuroimaging-based ATN biomarkers

Of the six plasma biomarkers evaluated, Aβ42/Aβ40 and p-tau217 showed significant associations with Aβ-PET status (positive vs negative) in both the unadjusted and adjusted models for AD commonly known risk factors: age, sex, education level, *APOE4* carrier status, and cognitive measure, CDR **(Table 2).** The associations were stronger in the adjusted models: Aβ42/Aβ40 (adjusted odds ratio (AOR)= $1.74*10^{-24}$,95% confidence interval (CI) [$2.71*10^{-46}$–$1.12*10^{-6}$]); p-tau217 (AOR= $3.43*10^{3}$, 95% CI [126.79–$233.13*10^{3}$]). For plasma p-tau181, the association with Aβ-PET status were statistically significant when adjusted (AOR=1.47, 95% CI [1.02–2.21]). However, no significant association was found between Aβ-PET status and plasma GFAP, NfL, and p-tau231 in either the unadjusted or adjusted models.

With regards to tau-PET status, plasma p-tau181 and p-tau217 showed significant association while adjusted. Similarly, plasma p-tau217 showed the strongest association with tau-PET status (AOR= 22.24, 95% CI 3.64–177.85) followed by plasma p-tau181 (AOR=1.50, 95% CI 1.01–2.15).

Whereas only plasma NfL showed a statistically significant association with neurodegeneration based on cortical thickness measure in both unadjusted and adjusted models with common AD risk factors and cognitive measure (AOR= 1.09, 95% CI 1.03–1.15). No significant association was seen between plasma Aβ42/Aβ40, GFAP, p-tau181, p-tau217, p-tau231 and neurodegeneration based on cortical thickness measure **(Table 2).**

3.4. Accuracy of plasma biomarkers in classifying neuroimaging-based ATN status

Summary results of the ROC curves demonstrating the classification accuracies of the plasma biomarkers are shown in **Figure 2**. Plasma p-tau217 showed the highest AUC in distinguishing A+ from A- (AUC= 0.94, 95% CI (0.90–0.98)), followed by plasma Aβ42/Aβ40 (AUC= 0.88, 95% CI (0.78–0.94)) and p-tau181 (AUC= 0.85, 95% CI (0.76–0.93)), while adjusting for age, sex, education level, *APOE4* carrier status and CDR **(Figure 2A).**

Similarly, plasma p-tau181 and p-tau217 demonstrated the highest AUC in differentiating T+ from T- individuals (AUC= 0.74, 95% CI (0.64–0.84) and AUC=0.70, 95% CI (0.59–0.80)) respectively, while adjusted **(Figure 2B)**. Whereas NfL and plasma p-tau181 showed the best performance in distinguishing N+ from N- status (AUC=0.75, 95% CI (0.65–0.86) and (AUC= 0.77, 95% CI (0.65–0.87)) respectively, while adjusted **(Figure 2C)**.

Lastly, the results of the sensitivity analysis demonstrated that both the association of the plasma biomarkers with neuroimaging-based ATN biomarkers and their classification accuracy of neuroimaging-based ATN status were robust to the adjustment for ADI **(Supplementary Table 2)** and BMI **(Supplementary Tables 3).** Only NfL and plasma p-tau181 classification accuracies in distinguishing N+ from N- status slightly improved when BMI was included in the adjusted model (AUC=0.79 and AUC=0.80, respectively) **(Supplementary Table 3).**

3.5. Voxel-wise associations of Aβ-PET SUVR with plasma biomarkers

We performed voxel-wise linear regression analysis between plasma biomarkers and Aβ-PET SUVR images. We found a significant positive association between Aβ-PET and plasma p-tau217 (**Figure 3A**) in AD-related regions, such as fusiform, inferior temporal and precuneus; in regions such as inferior and middle temporal gyrus with GFAP (**Supplementary Figure 1**), and a small cluster in the inferior temporal region with NfL (**Figure 3A**). Plasma Aβ42/Aβ40 was negatively associated with Aβ-PET in Aβ deposition regions, such as frontal lobe and anterior cingulate (**Figure 3A**).

3.6. Voxel-wise associations of tau-PET SUVR with plasma biomarkers

We performed voxel-wise linear regression analysis between plasma biomarkers and tau-PET SUVR images. We found a significant positive association between tau-PET and plasma p-tau217 (**Figure 3B**) in early Braak regions (Braak I and II); and in small clusters with plasma Aβ42/Aβ40, NfL (**Figure 3B**), p-tau181 and p-tau231 **(Supplementary Figure 1).**

4. Discussion

In this population-based study composed of mostly cognitively normal older adults from a socioeconomically disadvantaged area, we investigated six plasma biomarkers in parallel (i.e.,

Aβ42/Aβ40, GFAP, NfL, p-tau181, p-tau217, and p-tau231), regarding predicting Aβ (A) and tau (T) pathologies and neurodegeneration (N) on neuroimaging. Thus, we evaluated their potential utility as ATN markers of AD.

The major findings were: (i) Of the six plasma biomarkers, both plasma Aβ42/Aβ40 ratio and p-tau217 showed strong associations with Aβ-PET status independent of common risk factors and a cognitive measure: age, sex, education level, *APOE4* carrier status and CDR. Plasma p-tau181 showed a significant association with Aβ-PET status only when combined with the aforementioned AD common risk factors and cognitive measure. (ii) Plasma p-tau181 and p-tau217 were consistently associated with tau-PET status i.e. independent of common risk factors and cognitive measure. (iii) Similarly, on a voxel scale, p-tau217 was strongly associated with neocortical Aβ deposition. (iv) Only plasma NfL was associated with neurodegeneration status as determined by MRI-based cortical thickness composite measure. (v) Notably, plasma p-tau217 demonstrated the highest classification performance for distinguishing individuals with Aβ-PET positive from negative profiles, followed by Aβ42/Aβ40. (vi) Whereas plasma p-tau181 and p-tau217 for tau status, and comparably p-tau181 and NfL for neurodegeneration status showed the best performance. The classification performances of all plasma biomarkers were improved when combined with age, sex, education level, *APOE4* carrier status and CDR.

Recent advancements in plasma biomarker assays have enabled more accessible and non-invasive prediction of brain Aβ deposition. Plasma Aβ42/Aβ40 is one of the biological markers to change during the early preclinical stage of AD [8,9,46]. Unlike the individual levels of Aβ42, the Aβ42/Aβ40 ratio has consistently shown a strong concordance with brain amyloidosis, likely because the ratio may normalize differences related to biological variations, circadian rhythms or sample processing [47,48]. In line with our observation, strong inverse correlation has been widely reported previously among individuals without dementia [8,9,49–53]. In studies done by

Schindler et.al. and Li et.al., similar high classification accuracy results (ROC AUC ranging 0.88-0.94) were found [8,51], despite immunoprecipitation-mass spectrometry methods having been used in those studies, compared to immunoassays used in the current study. These findings highlight the potential of plasma Aβ42/Aβ40 as a reliable surrogate marker of brain amyloidosis enabling efficient population-wide screening.

Among the three p-tau epitopes assessed in our study, plasma p-tau217 was found to be the strongest predictor of brain Aβ deposition with the best classification performance (ROC AUC=0.94), even higher than plasma Aβ42/Aβ40. Similar results have been reported in several studies where plasma p-tau217 has been correlated with brain amyloidosis in early disease stages [14,15,17,18,23,27]. For instance, a study done in the ALFA+ cohort of individuals with pre-clinical AD, showed that plasma p-tau217 had the strongest association with Aβ-PET in early accumulating regions with 89% classification accuracy [14]. Moreover, a study done by Schindler et.al. showed that plasma p-tau217 measures, either individually or in combination, had the strongest association with all AD outcome measures [26]. In our study, compared to plasma p-tau217, p-tau181 showed a weaker association and lower classification accuracy for Aβ-PET status. This is in line with a head-to-head comparative study done by Mendes et.al. [54], and Mielke et.al. [55] revealing that plasma p-tau217 might be more efficient than plasma p-tau181 in identifying Aβ-PET positivity.

Previous studies have extensively explored biologically plausible molecular and cellular mechanisms to understand the underlying relationship between the various isoforms of phosphorylated tau and brain Aβ pathology [56–60]. Evidence indicates that Aβ plays an upstream role in AD progression by promoting tau phosphorylation [56]. Aβ plaques are linked to an increase in the release of axonal hyperphosphorylated tau, which subsequently leads to

the formation and progression of tau neurofibrillary tangle pathology [59]. This apparent Aβ-triggered release of soluble phosphorylated tau forms in blood starts at one of the earliest stages of the AD continuum. Overall, our finding highlights the robustness of plasma p-tau217 to identify early brain amyloidosis in dementia-free community-dwelling individuals and can serve as a stand-alone biomarker for screening of high-risk individuals for early intervention as well as for participation in AD clinical trials and disease-modifying therapies, while reducing the need for the use of invasive (e.g., CSF) or neuroimaging biomarkers.

Tau deposition is another important hallmark of AD neuropathology. Our findings of significant association between both plasma p-tau181 and p-tau217 and tau-PET status are consistent with results from other studies [19,20,23–25]. Interestingly, both biomarkers, however, showed weaker association and lower classification accuracy for tau-PET than for Aβ-PET. This adds to the understanding that these plasma p-tau isoforms, closer to the N-terminus of the tau protein, become more closely related to tau tangles, identified by PET, in later stages of AD [23,61]. For instance, a study by Ferreira et.al. demonstrated stronger correlation between plasma p-tau217 and tau-PET signals in cognitively impaired individuals compared to those without impairment [23]. A possible explanation for this could be that tau-PET imaging may not reliably detect smaller amounts of tau aggregates in the brain making it less sensitive to the earliest and most subtle tau pathology [62]. Another factor to consider is that the soluble biochemical pools of tau, with epitopes closer to the N-terminus of the tau protein, are affected – and are detectable – earlier than the microtubule binding domain (MTB), the aggregating portion of the tau molecule, closer to the C-terminus [63]. Despite these limitations, we propose that plasma p-tau181 and p-tau217 could serve as potential screening tools for brain tau pathology.

Furthermore, for “N” status, NfL was the only biomarker we found to demonstrate significant association with a classification accuracy of 75% for detecting neurodegeneration based on cortical thickness measures in cognitively normal individuals. This supports the growing evidence that plasma NfL reflects neuronal damage and could serve as a proxy for imaging-based neurodegeneration markers in various neurodegenerative conditions, including AD [28,64]. A prior study done by Matsson et al. reported strong associations between plasma NfL and hippocampal atrophy, as well as cortical thickness–both indicators of neurodegeneration in AD [65]. Additionally, studies conducted on individuals with AD-related genetic mutations reported that blood NfL can predict the onset of AD more than a decade before clinical manifestations [65,66]. Although plasma NfL is not AD-specific, given our findings, we propose its potential usefulness as a non-invasive and more accessible screening biomarker for neurodegeneration. Subsequent studies will evaluate AD-type neurodegeneration markers such as brain-derived tau [67–69].

Finally, it is worth noting that in our study we found no statistically significant association between plasma GFAP and the “T” and “N” neuroimaging biomarkers, and between p-tau231 and the “A” and “N” neuroimaging biomarkers. This partly aligns with a study by Pereira et.al. on the Swedish BioFINDER-2 cohort, that reported no correlation between plasma GFAP and tau aggregation as measured by PET in cognitively normal individuals [11]. Additionally, plasma GFAP was found to be only weakly associated with hippocampal volume [70]. In contrast, other studies have reported significant associations between both plasma GFAP and p-tau231; specially with Aβ-PET measures in early stages of AD [10,14,21,23]. These inconsistencies highlight the need for further research to clarify the roles of these plasma biomarkers along the AD continuum.

Our study has several strengths. First, the MYHAT-NI cohort recruited participants directly from an active, randomly recruited population-based cohort which reduces the selection bias often present in clinic-based or convenience sampling. This improves the applicability of our findings to a wider population of older adults. However, because the cohort consisted largely of individuals identifying as non-Hispanic white, further validation is needed across diverse racial and ethnic groups. Second, the study was conducted in socioeconomically disadvantaged small-town communities. This ensures our study can shed light on the limited information we have on how AD plasma biomarkers perform in populations living in under-resourced, small-town areas. Third, we studied the performance of several novel AD plasma biomarkers in parallel obtained from the same individuals using well-established neuroimaging reference standards. Fourth, we accounted for several known AD risk factors and cognitive measure, enhancing robustness of our findings. A key limitation of the study is the lack of additional SES and medical variables. Consideration of other structural and social determinants of health and health-related factors, such as chronic kidney disease and creatinine level (that are found to be related to certain plasma biomarkers levels [71]), could provide a more comprehensive understanding of ATN biomarkers associations and performance in diverse populations and health conditions and should be incorporated in future work. Furthermore, the small number of CDR = 0.5 participants in the present study (11 out of 113) limits insights gained into impact of early cognitive decline to the outcomes.

In conclusion, this study has identified promising plasma biomarkers to identify AD ATN pathologies in dementia-free individuals. Plasma p-tau217 emerged as a robust stand-alone predictor of both amyloid and tau statuses, with plasma Aβ42/Aβ40, p-tau181, and NfL also demonstrating strong predictive capabilities for “A”, “T” and “N” statuses, respectively. Our findings underscore the potential of using a combination of plasma biomarkers and known risk factors and cognitive measure to enhance classification accuracy and make them valuable tools

for selecting individuals for clinical trials and disease-modifying therapies. Importantly, our results demonstrate that, in this community-based cohort, cortical thickness was not related to amyloid pathology perhaps due to the latter becoming abnormal earlier. Moreover, the findings including ATN profile distributions agree with multiple published papers showing strong association and classification accuracies of leading plasma biomarkers such as p-tau217 for AD. Together, these results concord with other investigations performed in cohorts recruited from different settings including in clinics and populations of other demographics, supporting the use of plasma biomarkers for AD detection and monitoring in community-based settings.

References

1. Geldmacher DS. Treatment of Alzheimer Disease. CONTINUUM: Lifelong Learning in Neurology. 2024;30:1823.

2. Mintun MA, Lo AC, Evans CD, Wessels AM, Ardayfio PA, Andersen SW, et al. Donanemab in Early Alzheimer's Disease. New England Journal of Medicine. 2021;384:1691–704.

3. Dyck CH van, Swanson CJ, Aisen P, Bateman RJ, Chen C, Gee M, et al. Lecanemab in Early Alzheimer's Disease. New England Journal of Medicine. 2023;388:9–21.

4. Livingston G, Huntley J, Liu KY, Costafreda SG, Selbæk G, Alladi S, et al. Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. The Lancet. 2024;404:572–628.

5. Knopman DS, Petersen RC, Jack CR. A brief history of "Alzheimer disease." Neurology. 2019;92:1053–9.

6. Jack Jr. CR, Andrews JS, Beach TG, Buracchio T, Dunn B, Graf A, et al. Revised criteria for diagnosis and staging of Alzheimer's disease: Alzheimer's Association Workgroup. Alzheimer's & Dementia. 2024;20:5143–69.

7. Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimer's & Dementia. 2018;14:535–62.

8. Schindler SE, Bollinger JG, Ovod V, Mawuenyega KG, Li Y, Gordon BA, et al. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. Neurology. 2019;93:e1647–59.

9. Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V, et al. High performance plasma amyloid-β biomarkers for Alzheimer's disease. Nature. 2018;554:249–54.

10. Colangelo AM, Alberghina L, Papa M. Astrogliosis as a therapeutic target for neurodegenerative diseases. Neuroscience Letters. 2014;565:59–64.

11. Pereira JB, Janelidze S, Smith R, Mattsson-Carlgren N, Palmqvist S, Teunissen CE, et al. Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. Brain. 2021;144:3505–16.

12. Chatterjee P, Pedrini S, Ashton NJ, Tegg M, Goozee K, Singh AK, et al. Diagnostic and prognostic plasma biomarkers for preclinical Alzheimer's disease. Alzheimers Dement. 2022;18:1141–54.

13. Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, 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. The Lancet Neurology. 2020;19:422–33.

14. Milà-Alomà M, Ashton NJ, Shekari M, Salvadó G, Ortiz-Romero P, Montoliu-Gaya L, et al. Plasma p-tau231 and p-tau217 as state markers of amyloid-β pathology in preclinical Alzheimer's disease. Nat Med. 2022;28:1797–801.

15. Jonaitis EM, Janelidze S, Cody KA, Langhough R, Du L, Chin NA, et al. Plasma phosphorylated tau 217 in preclinical Alzheimer's disease. Brain Communications. 2023;5:fcad057.

16. Ferreira PCL, Zhang Y, Snitz B, Chang C-CH, Bellaver B, Jacobsen E, et al. Plasma biomarkers identify older adults at risk of Alzheimer's disease and related dementias in a real-world population-based cohort. Alzheimer's & Dementia. 2023;19:4507–19.

17. Mattsson-Carlgren N, Janelidze S, Bateman RJ, Smith R, Stomrud E, Serrano GE, et al. Soluble P-tau217 reflects amyloid and tau pathology and mediates the association of amyloid with tau. EMBO Molecular Medicine. 2021;13:e14022.

18. Xiao Z, Wu W, Ma X, Wu J, Liang X, Zhou X, et al. Plasma p-tau217, p-tau181, and NfL as early indicators of dementia risk in a community cohort: The Shanghai Aging Study. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring. 2023;15:e12514.

19. Karikari TK, Benedet AL, Ashton NJ, Lantero Rodriguez J, Snellman A, Suárez-Calvet M, et al. Diagnostic performance and prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's Disease Neuroimaging Initiative. Mol Psychiatry. 2021;26:429–42.

20. Shen X-N, Huang Y-Y, Chen S-D, Guo Y, Tan L, Dong Q, et al. Plasma phosphorylated-tau181 as a predictive biomarker for Alzheimer's amyloid, tau and FDG PET status. Transl Psychiatry. 2021;11:1–10.

21. Ashton NJ, Pascoal TA, Karikari TK, Benedet AL, Lantero-Rodriguez J, Brinkmalm G, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. Acta Neuropathol. 2021;141:709–24.

22. Karikari TK, Ashton NJ, Brinkmalm G, Brum WS, Benedet AL, Montoliu-Gaya L, et al. Blood phospho-tau in Alzheimer disease: analysis, interpretation, and clinical utility. Nat Rev Neurol. 2022;18:400–18.

528 23. Ferreira PCL, Therriault J, Tissot C, Ferrari-Souza JP, Benedet AL, Povala G, et al. Plasma
529 p-tau231 and p-tau217 inform on tau tangles aggregation in cognitively impaired individuals.
530 Alzheimer's & Dementia. 2023;19:4463–74.

531 24. Janelidze S, Berron D, Smith R, Strandberg O, Proctor NK, Dage JL, et al. Associations of
532 Plasma Phospho-Tau217 Levels With Tau Positron Emission Tomography in Early Alzheimer
533 Disease. JAMA Neurology. 2021;78:149–56.

534 25. Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, et al. Plasma phospho-tau181
535 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-
536 positron emission tomography. Alzheimer's & Dementia. 2018;14:989–97.

537 26. Schindler SE, Petersen KK, Saef B, Tosun D, Shaw LM, Zetterberg H, et al. Head-to-head
538 comparison of leading blood tests for Alzheimer's disease pathology. Alzheimer's & Dementia.
539 2024;20:8074–96.

540 27. Janelidze S, Barthélemy NR, Salvadó G, Schindler SE, Palmqvist S, Mattsson-Carlgren N,
541 et al. Plasma Phosphorylated Tau 217 and Aβ42/40 to Predict Early Brain Aβ Accumulation in
542 People Without Cognitive Impairment. JAMA Neurology. 2024;81:947–57.

543 28. Mattsson N, Andreasson U, Zetterberg H, Blennow K, for the Alzheimer's Disease
544 Neuroimaging Initiative. Association of Plasma Neurofilament Light With Neurodegeneration in
545 Patients With Alzheimer Disease. JAMA Neurology. 2017;74:557–66.

546 29. Gaetani L, Blennow K, Calabresi P, Filippo MD, Parnetti L, Zetterberg H. Neurofilament light
547 chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019;90:870–
548 81.

549 30. Karikari TK. Blood Tests for Alzheimer's Disease: Increasing Efforts to Expand and Diversify
550 Research Participation Is Critical for Widespread Validation and Acceptance. Journal of
551 Alzheimer's Disease. 2022;90:967–74.

552 31. Livingston G, Huntley J, Liu KY, Costafreda SG, Selbæk G, Alladi S, et al. Dementia
553 prevention, intervention, and care: 2024 report of the Lancet standing Commission. The Lancet.
554 2024;404:572–628.

555 32. Abner E, Jicha G, Christian W, Schreurs B. Rural-Urban differences in Alzheimer's disease
556 and related disorders diagnostic prevalence in Kentucky and West Virginia. J Rural Health.
557 2016;32:314–20.

558 33. Wiese LAK, Gibson A, Guest MA, Nelson AR, Weaver R, Gupta A, et al. Global rural health
559 disparities in Alzheimer's disease and related dementias: State of the science. Alzheimer's &
560 Dementia. 2023;19:4204–25.

561 34. Ganguli M, Albanese E, Seshadri S, Bennett DA, Lyketsos C, Kukull WA, et al. Population
562 Neuroscience: Dementia Epidemiology Serving Precision Medicine and Population Health.
563 Alzheimer Disease & Associated Disorders. 2018;32:1.

564 35. Sullivan KJ, Liu A, Chang C-CH, Cohen AD, Lopresti BJ, Minhas DS, et al. Alzheimer's
565 Disease Pathology in a Community-Based Sample of Older Adults Without Dementia: The
566 MYHAT Neuroimaging Study. Brain Imaging Behav. 2021;15:1355–63.

36. Ganguli M, Snitz B, Bilt JV, Chang C-CH. How much do depressive symptoms affect cognition at the population level? The monongahela–youghiogheny healthy aging team (MYHAT) study. International Journal of Geriatric Psychiatry. 2009;24:1277–84.

37. Morris JC. The Clinical Dementia Rating (CDR). Neurology. 1993;43:2412-2412-a.

38. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, et al. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Annals of Neurology. 2004;55:306–19.

39. Gogola A, Lopresti BJ, Tudorascu D, Snitz B, Minhas D, Doré V, et al. Biostatistical Estimation of Tau Threshold Hallmarks (BETTH) Algorithm for Human Tau PET Imaging Studies. Journal of Nuclear Medicine. 2023;64:1798–805.

40. Jack CR, Wiste HJ, Weigand SD, Therneau TM, Lowe VJ, Knopman DS, et al. Defining imaging biomarker cut points for brain aging and Alzheimer's disease. Alzheimer's & Dementia. 2017;13:205–16.

41. Snitz BE, Tudorascu DL, Yu Z, Campbell E, Lopresti BJ, Laymon CM, et al. Associations between NIH Toolbox Cognition Battery and in vivo brain amyloid and tau pathology in non-demented older adults. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring. 2020;12:e12018.

42. Lopez OL, Becker JT, Chang Y, Klunk WE, Mathis C, Price J, et al. Amyloid deposition and brain structure as long-term predictors of MCI, dementia, and mortality. Neurology. 2018;90:e1920–8.

43. Cohen AD, Mowrey W, Weissfeld LA, Aizenstein HJ, McDade E, Mountz JM, et al. Classification of amyloid-positivity in controls: Comparison of visual read and quantitative approaches. NeuroImage. 2013;71:207–15.

44. Zeng X, Lafferty TK, Sehrawat A, Chen Y, Ferreira PCL, Bellaver B, et al. Multi-analyte proteomic analysis identifies blood-based neuroinflammation, cerebrovascular and synaptic biomarkers in preclinical Alzheimer's disease. Molecular Neurodegeneration. 2024;19:68.

45. Fan K, Francis L, Aslam MM, Bedison MA, Lawrence E, Acharya V, et al. Investigation of the independent role of a rare *APOE* variant (L28P; *APOE*4Pittsburgh*) in late-onset Alzheimer disease. Neurobiology of Aging. 2023;122:107–11.

46. Keshavan A, Pannee J, Karikari TK, Rodriguez JL, Ashton NJ, Nicholas JM, et al. Population-based blood screening for preclinical Alzheimer's disease in a British birth cohort at age 70. Brain. 2021;144:434–49.

47. Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-β levels. Neurology. 2007;68:666–9.

48. Willemse E, van Uffelen K, Brix B, Engelborghs S, Vanderstichele H, Teunissen C. How to handle adsorption of cerebrospinal fluid amyloid β (1–42) in laboratory practice? Identifying problematic handlings and resolving the issue by use of the Aβ42/Aβ40 ratio. Alzheimer's & Dementia. 2017;13:885–92.

605 49. Fandos N, Pérez-Grijalba V, Pesini P, Olmos S, Bossa M, Villemagne VL, et al. Plasma
606 amyloid β 42/40 ratios as biomarkers for amyloid β cerebral deposition in cognitively normal
607 individuals. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring.
608 2017;8:179–87.

609 50. Pérez-Grijalba V, Arbizu J, Romero J, Prieto E, Pesini P, Sarasa L, et al. Plasma Aβ42/40
610 ratio alone or combined with FDG-PET can accurately predict amyloid-PET positivity: a cross-
611 sectional analysis from the AB255 Study. Alz Res Therapy. 2019;11:96.

612 51. Li Y, Schindler SE, Bollinger JG, Ovod V, Mawuenyega KG, Weiner MW, et al. Validation of
613 Plasma Amyloid-β 42/40 for Detecting Alzheimer Disease Amyloid Plaques. Neurology.
614 2022;98:e688–99.

615 52. Risacher SL, Fandos N, Romero J, Sherriff I, Pesini P, Saykin AJ, et al. Plasma amyloid
616 beta levels are associated with cerebral amyloid and tau deposition. Alzheimer's & Dementia:
617 Diagnosis, Assessment & Disease Monitoring. 2019;11:510–9.

618 53. Wisch JK, Gordon BA, Boerwinkle AH, Luckett PH, Bollinger JG, Ovod V, et al. Predicting
619 continuous amyloid PET values with CSF and plasma Aβ42/Aβ40. Alzheimer's & Dementia:
620 Diagnosis, Assessment & Disease Monitoring. 2023;15:e12405.

621 54. Mendes AJ, Ribaldi F, Lathuiliere A, Ashton NJ, Janelidze S, Zetterberg H, et al. Head-to-
622 head study of diagnostic accuracy of plasma and cerebrospinal fluid p-tau217 versus p-tau181
623 and p-tau231 in a memory clinic cohort. J Neurol. 2024;271:2053–66.

624 55. Mielke MM, Frank RD, Dage JL, Jeromin A, Ashton NJ, Blennow K, et al. Comparison of
625 Plasma Phosphorylated Tau Species With Amyloid and Tau Positron Emission Tomography,
626 Neurodegeneration, Vascular Pathology, and Cognitive Outcomes. JAMA Neurol.
627 2021;78:1108–17.

628 56. Bloom GS. Amyloid-β and Tau: The Trigger and Bullet in Alzheimer Disease Pathogenesis.
629 JAMA Neurology. 2014;71:505–8.

630 57. Kwak SS, Washicosky KJ, Brand E, von Maydell D, Aronson J, Kim S, et al. Amyloid-β42/40
631 ratio drives tau pathology in 3D human neural cell culture models of Alzheimer's disease. Nat
632 Commun. 2020;11:1377.

633 58. Venkatramani A, Panda D. Regulation of neuronal microtubule dynamics by tau:
634 Implications for tauopathies. International Journal of Biological Macromolecules. 2019;133:473–
635 83.

636 59. He Z, Guo JL, McBride JD, Narasimhan S, Kim H, Changolkar L, et al. Amyloid-β plaques
637 enhance Alzheimer's brain tau-seeded pathologies by facilitating neuritic plaque tau
638 aggregation. Nat Med. 2018;24:29–38.

639 60. Mattsson-Carlgren N, Andersson E, Janelidze S, Ossenkoppele R, Insel P, Strandberg O, et
640 al. Aβ deposition is associated with increases in soluble and phosphorylated tau that precede a
641 positive Tau PET in Alzheimer's disease. Science Advances. 2020;6:eaaz2387.

642 61. Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al.
643 Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other
644 Neurodegenerative Disorders. JAMA. 2020;324:772–81.

645 62. Fleisher AS, Pontecorvo MJ, Devous MD Sr, Lu M, Arora AK, Truocchio SP, et al. Positron
646 Emission Tomography Imaging With [18F]flortaucipir and Postmortem Assessment of Alzheimer
647 Disease Neuropathologic Changes. JAMA Neurology. 2020;77:829–39.

648 63. Robbins M, Clayton E, Kaminski Schierle GS. Synaptic tau: A pathological or physiological
649 phenomenon? Acta Neuropathol Commun. 2021;9:149.

650 64. Gaetani L, Blennow K, Calabresi P, Filippo MD, Parnetti L, Zetterberg H. Neurofilament light
651 chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry. 2019;90:870–
652 81.

653 65. Ingannato A, Bagnoli S, Mazzeo S, Giacomucci G, Bessi V, Ferrari C, et al. Plasma GFAP,
654 NfL and pTau 181 detect preclinical stages of dementia. Front Endocrinol [Internet]. 2024 [cited
655 2024 Sep 23];15. Available from:
656 https://www.frontiersin.org/journals/endocrinology/articles/10.3389/fendo.2024.1375302/full

657 66. Weston PSJ, Poole T, Ryan NS, Nair A, Liang Y, Macpherson K, et al. Serum neurofilament
658 light in familial Alzheimer disease. Neurology. 2017;89:2167–75.

659 67. Gonzalez-Ortiz F, Turton M, Kac PR, Smirnov D, Premi E, Ghidoni R, et al. Brain-derived
660 tau: a novel blood-based biomarker for Alzheimer's disease-type neurodegeneration. Brain.
661 2023;146:1152–65.

662 68. Gonzalez-Ortiz F, Kirsebom B-E, Contador J, Tanley JE, Selnes P, Gísladóttir B, et al.
663 Plasma brain-derived tau is an amyloid-associated neurodegeneration biomarker in Alzheimer's
664 disease. Nat Commun. 2024;15:2908.

665 69. Gonzalez-Ortiz F, Dias A, Turton M, Magalhães R, Kac PR, Correia M, et al. Preanalytical
666 stability of plasma/serum brain-derived tau. Alzheimer's & Dementia. 2023;19:4764–70.

667 70. Yang Z, Sreenivasan K, Toledano Strom EN, Osse AML, Pasia LG, Cosme CG, et al.
668 Clinical and biological relevance of glial fibrillary acidic protein in Alzheimer's disease.
669 Alzheimer's Research & Therapy. 2023;15:190.

670 71. Pichet Binette A, Janelidze S, Cullen N, Dage JL, Bateman RJ, Zetterberg H, et al.
671 Confounding factors of Alzheimer's disease plasma biomarkers and their impact on clinical
672 performance. Alzheimer's & Dementia. 2023;19:1403–14.

673

674 **Acknowledgments**

675 We are grateful for our study participants, their families, caregivers, staff of the MYHAT-NI and

676 MYHAT studies.

677

Funding

PCLF is supported by the Alzheimer's Association (AARFD-22-923814). BB is supported by the Alzheimer's Association (AARFD-22-974627) and the National Institute on Aging (NIA) (5 P01 AG025204-17). GP is supported by the Alzheimer's Association (24AARFD-1243899). MIK and genetic studies on AD and AD-associated biomarkers in Dr. Kamboh's laboratory are partly supported by the R01 AG064877, R37 AG023651, P30 AG066468 and U19 AG068054. BES and the MYHAT-NI study are supported by the NIA (R01 AG052521 and R01 AG023651). CES is supported by the NIA (K01 AG071849). TKK and the Karikari Laboratory were supported by NIH/NIA (R01 AG083874, U24AG082930, P30 AG066468, RF1 AG077474, R01 AG083156, R37 AG023651, R01 AG025516, R01 AG073267, R01 AG075336, R01 AG072641, P01 AG025204), NIH/NINDS (U01 NS131740, U01 NS141777), NIH/NIMH (R01 MH108509), Aging Mind Foundation (DAF2255207), DoD (HT94252320064), the Cañizares Anderson Fund, and a professorial endowment from the Department of Psychiatry, University of Pittsburgh. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the funders.

Author contribution

MTD, XZ, BES, CES, and TKK contributed to the study's conception and design. BES and MG led the MYHAT-NI cohort study establishment including participant recruitment, cognitive evaluation, neuroimaging measures, among others. AP, JLD, PCLF and TAP led statistical analyses and produced the figures. PCLF, BB, GP, VLV, ADC and TAP contributed to neuroimaging data collection and analysis. MIK led *APOE* genotyping. MTD, AP, PCLF, BES, CES, XZ and TKK were major contributors in writing the initial manuscript draft, which was subsequently critically reviewed by all authors. All authors contributed to and approved the final version of the manuscript.

Ethics declarations

Ethics approval and consent to participate

The MYHAT-NI study was performed under written informed consent and approved by the University of Pittsburgh Institutional Review Board (STUDY19020264). This study was performed in accordance with the Declaration of Helsinki.

Consent for publication

All participants gave written informed consent, and all study procedures were approved by the Institutional Review Board of the University of Pittsburgh.

Competing interests

XZ is a listed inventor on the University of Pittsburgh provisional patent *#63/672,952.* CES is the Co-Chair of the ISTAART Sex and Gender Interest Group, Diversity and Disparities Professional Interest Area and a member of the ISTAART Advisory Council. TKK has consulted for Quanterix Corporation, SpearBio Inc., and Neurogen Biomarking LLC., has served on advisory board for Neurogen Biomarking LLC. (which comes with minority stock equity interest), and has received in-kind research support from Janssen Research Laboratories and Alamar Biosciences, outside the submitted work. He has received honoraria for speaker/grant review engagements from the NIH, UPENN, UW-Madison, Advent Health, Brain Health conference, Barcelona-Pittsburgh conference, the International Neuropsychological Society, the Icahn School of Medicine at Mount Sinai and CQDM Canada, all outside of the submitted work. TKK is an inventor on several patents and provisional patents regarding biofluid biomarker methods, targets and reagents/compositions. He and his laboratory program stand to potentially benefit should his employer(s) transfer and/or licensing any of these resources to other organizations. The other authors report no conflict of interest.

Keywords

Amyloid beta, tau pathology, neurodegeneration, neuroimaging, ATNframework, p-tau217, p-tau181, p-tau231, GFAP, neurofilament light chain

Abbreviations

Aβ- Amyloid-beta

AD- Alzheimer's disease

APOE4: Apolipoprotein E4

ATN- Amyloid-beta, tau, neurodegeneration

AUC- Area under the curve

CDR- Clinical dementia rating

CSF- Cerebrospinal fluid

CV- Coefficient of variation

GFAP- Glial fibrillary acidic protein

MCI- Mild cognitive impairment

MRI- Magnetic resonance imaging

MYHAT- Monongahela Youghiogheny Healthy Aging Team

MYHAT NI- Monongahela Youghiogheny Healthy Aging Team-Neuroimaging

NfL- Neurofilament light chain

NIA-AA- National Institute on Aging-Alzheimer's Association

PET- Positron emission tomography

PiB- Pittsburgh compound-B

p-tau- Phosphorylated-tau

Simoa- Single-molecule array

SUVR- Standardized uptake value ratio

Table titles

Table 1. Study participants' characteristics and biomarker profiles by neuroimaging-based ATN status groups

Table 2: Association between plasma and neuroimaging-based ATN biomarkers

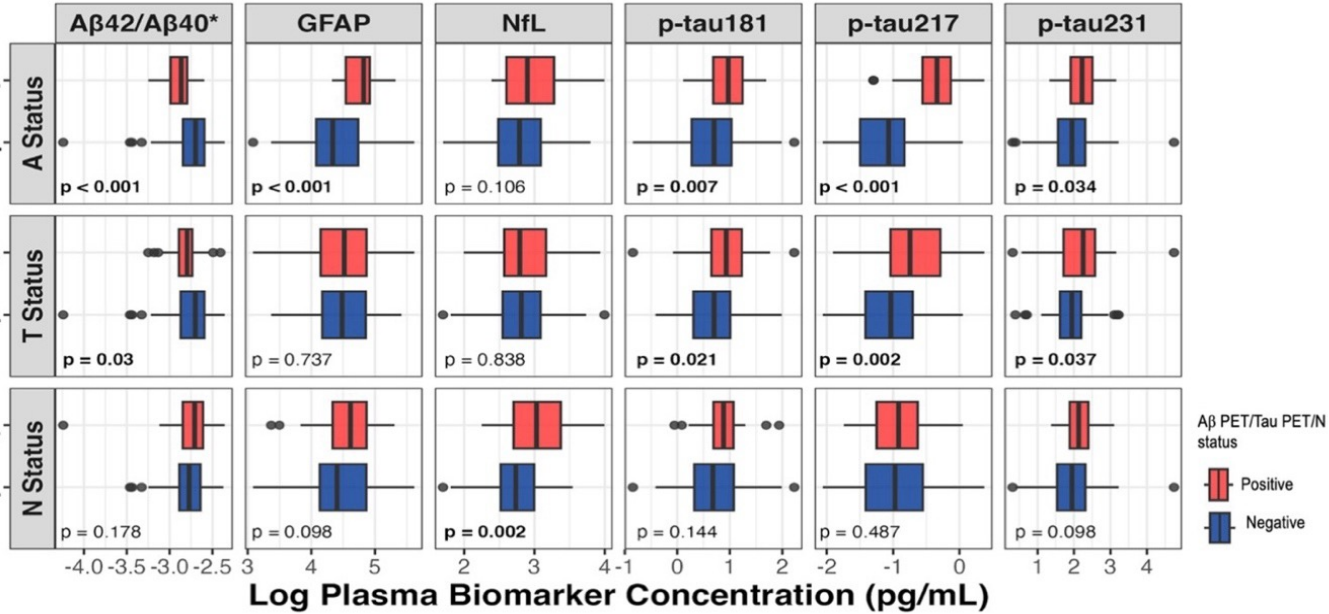
Figure captions

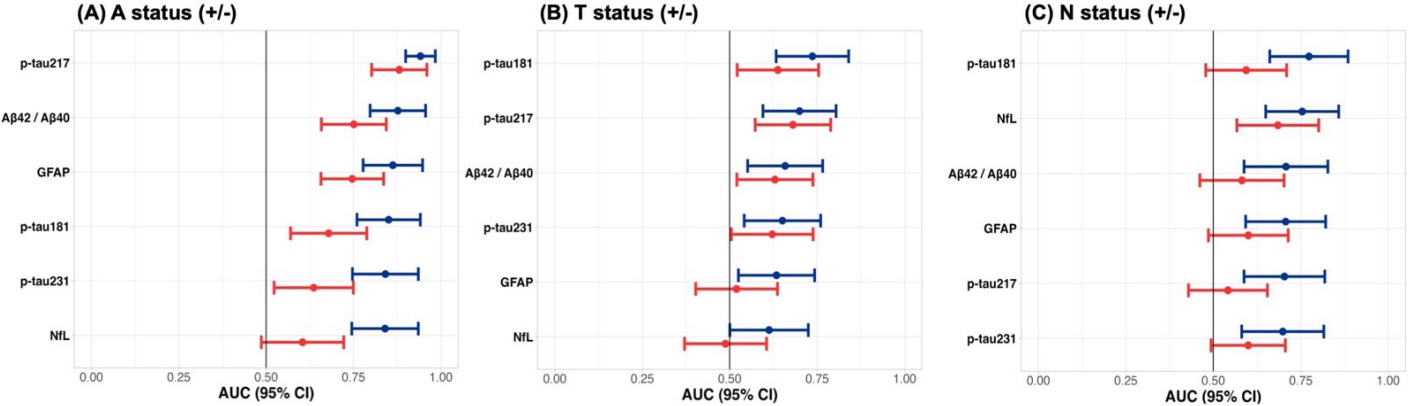
Figure 1: Boxplot comparisons of unadjusted log transformed plasma biomarker concentrations across neuroimaging-based ATN groups. Box ends represent the 25th and 75th percentiles and the horizontal line within each box indicates the median. Whiskers extend to the upper and lower adjacent values. P-values from two-sided student's t-test are shown. Abbreviations: ATN: amyloid-beta, tau, neurodegeneration: Aβ42/Aβ40- amyloid-beta42/amyloid-beta40; GFAP- glial fibrillary acidic protein; NfL- neurofilament light chain; p-tau181- phosphorylated-tau181; p-tau217- phosphorylated-tau217; p-tau231- phosphorylated-tau231; pg/mL: picogram per milli liter; SUVR: standard uptake value ratio. A+: [^{11}C]PiB global SUVR >1.346; T+: [^{18}F]AV-1451 meta temporal SUVR >1.18; N+: cortical thickness composite measure <2.7
* Aβ42/Aβ40 measured in units of logarithm of concentration ratio

Figure 2: Classification accuracies of plasma biomarkers to identify neuroimaging-based ATN status. Forest plot of area under the receiver operating characteristics curve (ROC AUC) values summary with their corresponding 95% confidence intervals (represented by the error bars and arranged in descending order of the ROC AUC values) illustrating the predictive accuracies of plasma Aβ42/Aβ40, GFAP, NfL, p-tau181, p-tau217, p-tau231 for: **(A)** Aβ-PET, **(B)** tau-PET, **(C)** neurodegeneration, statuses.
Abbreviations: Aβ42/Aβ40: amyloid-beta42/amyloid-beta40; GFAP: glial fibrillary acidic protein; NfL: neurofilament light chain; p-tau181: phosphorylated-tau181, p-tau217: phosphorylated-tau217; p-tau231: phosphorylated-tau231; AUC: area under the curve; CI: confidence interval. A status (+/-): Aβ -PET status (positive/negative); T status (+/-): tau-PET status (positive/negative); N status (+/-): Neurodegeneration status (positive/negative); A status positive: [^{11}C]PiB global SUVR >1.346; T status positive: [^{18}F]AV-1451 meta temporal SUVR >1.18; N status positive: cortical thickness composite measure <2.7.

Figure 3: Voxel-wise associations between plasma biomarkers and Aβ-PET (Panel A) and tau-PET (Panel B); after adjusting for age, sex, education level, *APOE4* carrier status, and CDR. All results were adjusted for multiple comparisons using random field theory with a voxel

804 threshold of p<0.001. Abbreviations: PET-positron emission tomography; *APOE4*:
805 Apolipoprotein E4; CDR- clinical dementia rating; SUVR-standard uptake value ratio;
806 Aβ42/Aβ40-amyloid-beta42/amyloid-beta 40; NfL- neurofilament light chain; p-tau217-
807 phosphorylated-tau217.
808
809
810


Aβ42/Aβ40*
GFAP
NfL
p-tau181
p-tau217
p-tau231
A Status
T Status
N Status
Pos.
Neg.
p < 0.001
p < 0.001
p = 0.106
p = 0.007
p < 0.001
p = 0.034
p = 0.03
p = 0.737
p = 0.838
p = 0.021
p = 0.002
p = 0.037
p = 0.178
p = 0.098
p = 0.002
p = 0.144
p = 0.487
p = 0.098
-4.0 -3.5 -3.0 -2.5
3 4 5
2 3 4
-1 0 1 2
-2 -1 0
1 2 3 4
Log Plasma Biomarker Concentration (pg/mL)
Aβ PET/Tau PET/N status
Positive
Negative


(A) A status (+/-)
p-tau217
Aβ42 / Aβ40
GFAP
p-tau181
p-tau231
NfL
0.00
0.25
0.50
0.75
1.00
AUC (95% CI)
(B) T status (+/-)
p-tau181
p-tau217
Aβ42 / Aβ40
p-tau231
GFAP
NfL
0.00
0.25
0.50
0.75
1.00
AUC (95% CI)
(C) N status (+/-)
p-tau181
NfL
Aβ42 / Aβ40
GFAP
p-tau217
p-tau231
0.00
0.25
0.50
0.75
1.00
AUC (95% CI)
Adjusted for age, sex, education, APOE ε4, CDR
Yes
No

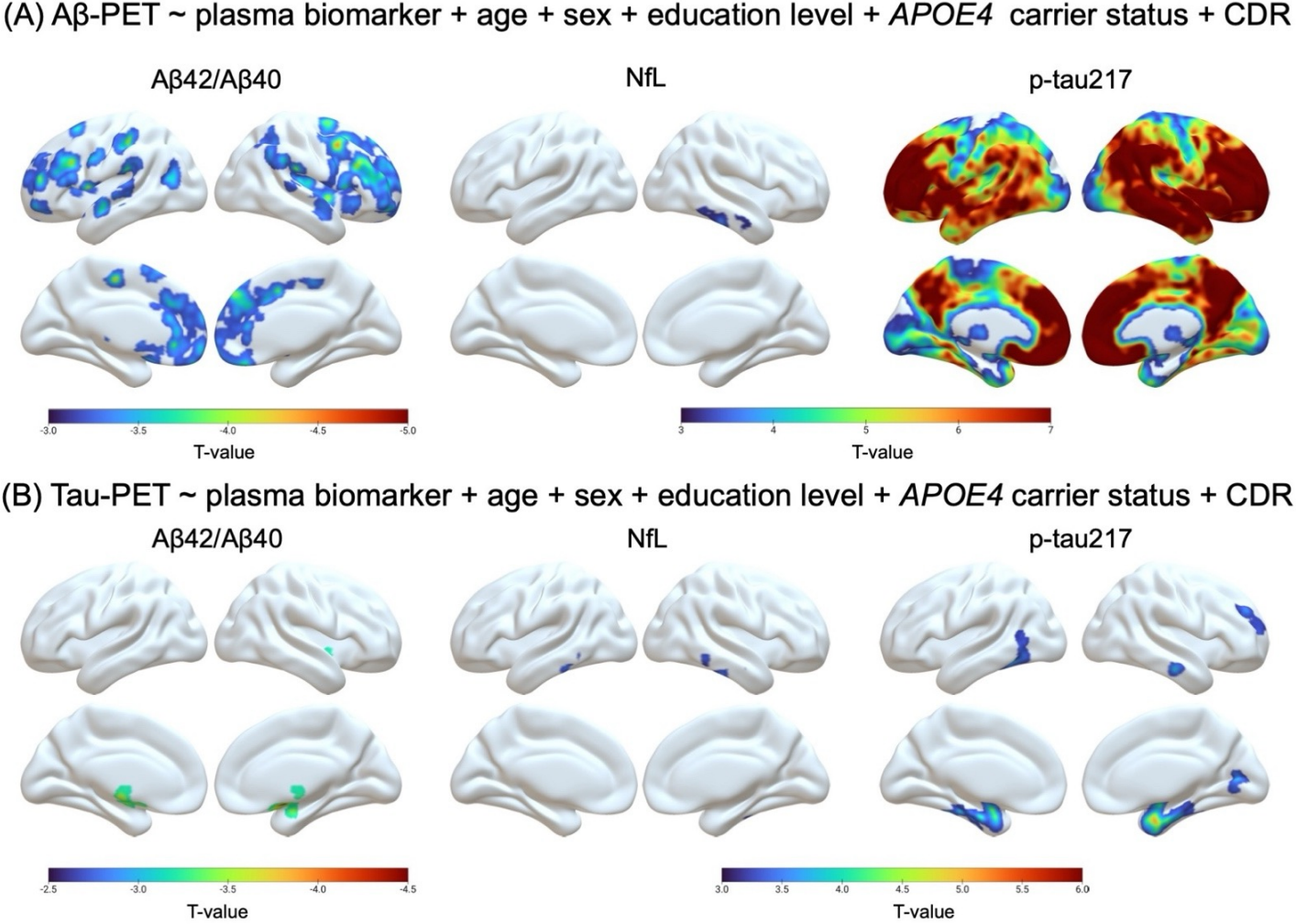

(A) Aβ-PET ~ plasma biomarker + age + sex + education level + APOE4 carrier status + CDR
Aβ42/Aβ40
NfL
p-tau217
-3.0
-3.5
-4.0
-4.5
-5.0
T-value
3
4
5
6
7
T-value
(B) Tau-PET ~ plasma biomarker + age + sex + education level + APOE4 carrier status + CDR
Aβ42/Aβ40
NfL
p-tau217
-2.5
-3.0
-3.5
-4.0
-4.5
T-value
3.0
3.5
4.0
4.5
5.0
5.5
6.0
T-value

Table 1. Demographic, cognitive and biomarker characteristics of the MYHAT-NI cohort.

		Aβ-PET status			Tau-PET status			Neurodegeneration status		
	Total N= 113	**A+ N= 28 (25%)**	**A- N= 85 (75%)**	**p-value[a]**	**T+ N= 39 (35%)**	**T- N= 74 (65%)**	**p-value[a]**	**N+ N= 33 (29%)**	**N- N= 80 (71%)**	**p-value[a]**
Demographic factors										
Age, Mean (SD)	77 (6)	79 (7)	76 (6)	0.055	77 (6)	77 (6)	0.9	80 (7)	75 (5)	**0.002**
Sex, female, n (%)	61 (54%)	18 (64%)	43 (51%)	0.2	23 (59%)	38 (51%)	0.4	17 (52%)	44 (55%)	0.7
Race/ethnicity, non-Hispanic White, n (%)	107 (95%)	27 (96%)	80 (94%)	>0.9	38 (97%)	69 (93%)	0.7	32 (97%)	75 (94%)	0.7
Socioeconomic factor										
Education, n (%)				0.14			0.6			0.6
High School or Less	64 (57%)	15 (54%)	49 (58%)		23 (59%)	11 (15%)		20 (61%)	44 (55%)	
Partial/Full College/ Trade	35 (31%)	12 (43%)	23 (27%)		13 (33%)	22 (30%)		8 (24%)	27 (34%)	
Graduate Degree	14 (12%)	1 (3.6%)	13 (15%)		3 (7.7%)	11 (15%)		5 (15%)	9 (11%)	
High Area Deprivation Index[b], n(%)	63 (57%)	14 (52%)	49 (59%)	0.5	20 (53%)	43 (60%)	0.5	18 (56%)	45 (58%)	0.9
Genetic factor										
APOE4 carriers, n (%)	18 (16%)	12 (43%)	6 (7.1%)	**<0.001**	9 (23%)	9 (12%)	0.13	5 (15%)	13 (16%)	0.9
Cognitive function assessment										
CDR=0, n (%) CDR=0 CDR=0.5	102 (91%) 10 (9%)	22 (79%) 6 (21%)	80 (95%) 4 (5%)	**0.015**	35 (90%) 4 (10%)	67 (92%) 6 (8%)	0.7	28 (85%) 5 (15%)	74 (94%) 5 (6%)	0.2
MMSE Score, n (%) >= 24	108 (96%) 4 (4%)	27 (96%)	81 (96%)	0.4	38 (97%)	70 (96%)	0.4	32 (97%)	76 (96%)	**0.025**

19–23		1 (4%)	3 (4%)		1 (3%)	3 (4%)		1 (3%)	3 (4%)	
Health-related factor										
Body Mass Index, Mean (SD)	28.0 (4.4)	27.0 (4.6)	28.4 (4.2)	0.2	27.6 (4.7)	28.3 (4.2)	0.6	28.5 (4.1)	27.9 (4.5)	0.5
Neuroimaging measures, Mean (SD)										
[^{11}C]PiB global SUVR	1.28 (0.31)	1.74 (0.28)	1.12 (0.07)	**<0.001**	1.46 (0.40)	1.18 (0.19)	**<0.001**	1.26 (0.31)	1.29 (0.31)	0.4
[^{18}F]AV-1451 meta temporal SUVR	1.15 (0.08)	1.21 (0.07)	1.13 (0.07	**<0.001**	1.23(0.04)	1.11 (0.06)	**<0.001**	1.13 (0.08)	1.16 (0.08)	0.3
Cortical thickness composite measure	2.75 (0.16)	2.77 (0.15)	2.75 (0.16)	0.6	2.78 (0.14)	2.74 (0.16)	0.2	2.58 (0.15)	2.83 (0.08)	**<0.001**
Plasma biomarker measures, pg/mL, Mean (SD)										
Aβ42	5.13 (1.62)	4.95 (1.21)	5.18 (1.74)	0.5	5.08 (1.39)	5.15 (1.74)	0.7	5.41(1.76)	5.00 (1.56)	0.4
Aβ40	81 (23)	89 (19)	79 (24)	**0.017**	84 (29)	80 (20)	0.5	84 (24)	80 (23)	0.8
Aβ42/Aβ40	0.063 (0.014)	0.056 (0.009)	0.065 (0.015)	**<0.001**	0.060 (0.011)	0.064 (0.015)	**0.030**	0.065 (0.016)	0.062 (0.013)	0.2
GFAP	99 (49)	124 (37)	91 (50)	**<0.001**	102 (54)	97 (46)	0.7	108 (46)	95 (50)	0.10
NfL	19 (9)	22 (11)	18 (8)	0.11	20 (11)	18 (9)	0.8	24 (12)	16 (7)	**0.002**
p-tau181	2.41 (1.47)	2.81 (1.06)	2.28 (1.56)	**0.007**	2.85 (1.69)	2.17(1.29	**0.021**	2.58 (1.25)	2.35 (1.54)	0.14
p-tau217	0.45 (0.26)	0.74 (0.29)	0.35 (0.17)	**<0.001**	0.57 (0.33)	0.38 (0.20)	**0.002**	0.45 (0.22)	0.45 (0.28)	0.5
p-tau231	9.5 (11.3)	10.3 (5.2)	9.3 (12.6)	**0.034**	12.5 (17.8)	8.0 (5.0)	**0.037**	9.2 (4.0)	9.7 (13.2)	0.10

[a]p-values were calculated using Wilcoxon rank-sum test and fisher's exact test for continuous and categorical variables, respectively. Bold p-values indicate statistical significance.

[b]Area Deprivation Index (ADI) national rank is dichotomized as: Low ADI= 0%–80%; High ADI (i.e. the most disadvantaged) = 80%–100%

Abbreviations: ATN: amyloid-beta, tau, neurodegeneration; PET: positron emission tomography; Aβ42/Aβ40: amyloid-beta42/amyloid-beta40; GFAP: glial fibrillary acidic protein; NfL: neurofilament light chain; p-tau181: phosphorylated-tau181; p-tau217: phosphorylated-tau217; p-tau231:

phosphorylated-tau231; *APOE4*: Apolipoprotein E4; CDR: Clinical Dementia Rating; MMSE: mini-mental status examination score; SUVR: standard uptake value ratio; PiB: Pittsburgh Compound B; SD: standard deviation; A+ (Aβ-PET+): [^{11}C]PiB global SUVR >1.346; A- (Aβ-PET -): [^{11}C]PiB global SUVR <=1.346; T+ (tau-PET+): [^{18}F]AV-1451 meta temporal SUVR >1.18; T- (tau-PET -): [^{18}F]AV-1451 meta temporal SUVR <=1.18; N+ (Neurodegeneration +): cortical thickness composite measure <2.7; N- (Neurodegeneration -): cortical thickness composite measure >= 2.7

Table 2. Association of plasma and neuroimaging biomarkers

	Unadjusted OR (95% CI)	Adjusted OR (95% CI) [a]
A status ~ plasma biomarkers*		
Aβ42/Aβ40	**$5.14*10^{-23}$ ($4.01*10^{-39}$ – $1.33*10^{-8}$)**	**$1.74*10^{-24}$ ($2.71*10^{-46}$ – $1.12*10^{-6}$)**
GFAP	1.01 (1.00–1.02)	1.01 (1.00–1.02)
NfL	1.04 (1.00–1.09)	1.05 (0.99–1.12)
P-tau181	1.25 (0.94–1.69)	**1.47 (1.02–2.21)**
p-tau217	**$1.64*10^{3}$ (117–$4.52*10^{4}$)**	**$3.43*10^{3}$ ($126.79–233.13*10^{3}$)**
p-tau231	1.01 (0.96–1.05)	1.01 (0.96–1.05)
T status ~ plasma biomarkers*		
Aβ42/Aβ40	$6.56*10^{-10}$ ($4.75*10^{-23}$–$2.42*10^{3}$)	$2.79*10^{-10}$ ($5.92*10^{-22}$–$5.76*10^{5}$)
GFAP	1.00 (0.99–1.01)	1.00 (0.99–1.01)
NfL	1.02 (0.98–1.06)	1.02 (0.97–1.07)
P-tau181	**1.37 (1.04–1.89)**	**1.50 (1.01– 2.15)**
p-tau217	**19.16 (3.80–121.67)**	**22.24 (3.64–177.85)**
p-tau231	1.07 (1.00–1.15)	1.07 (1.00–1.16)
N status ~ plasma biomarkers*		
Aβ42/Aβ40	$2.29*10^{7}$ ($2.24*10^{-6}$–$1.66*10^{21}$)	$1.00*10^{8}$ ($3.18*10^{-11}$–$4.62*10^{23}$)
GFAP	1.01 (1.00–1.01)	1.00 (0.99–1.01)
NfL	**1.10 (1.05–1.16)**	**1.09 (1.03–1.15)**
P-tau181	1.11 (0.82–1.47)	1.10 (0.76–1.54)
p-tau217	1.13 (0.23–5.07)	0.59 (0.083–3.52)
p-tau231	1.00 (0.94–1.03)	0.99 (0.93–1.03)

*plasma biomarkers measured in picogram per milliliter (pg/mL); [a]Adjusted for age, sex, education level, *APOE4* carrier status, Clinical Dementia Rating (CDR)

Abbreviations: ATN: amyloid-beta, tau, neurodegeneration; Aβ42/Aβ40: amyloid-beta42/amyloid-beta40; GFAP: glial fibrillary acidic protein; NfL: neurofilament light chain; p-tau181: phosphorylated-tau181; p-tau217: phosphorylated-tau217; p-tau231: phosphorylated-tau231; OR: odds ratio; CI: confidence interval. A status: Aβ-PET status (positive/negative); T status: tau-PET status (positive/negative); N status: Neurodegeneration status (positive/negative); A status positive: [^{11}C]PiB global SUVR >1.346; T status positive: [^{18}F]AV-1451 meta temporal SUVR >1.18; N status positive: cortical thickness composite measure <2.7.