Received: 10 September 2024 | Revised: 20 December 2024 | Accepted: 23 December 2024

DOI: 10.1002/alz.14570

Alzheimer's & Dementia®
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

RESEARCH ARTICLE

Accuracy of plasma biomarkers to detect Alzheimer's disease proteinopathy prior to dementia

Karly A. Cody[1,2,3] | Lianlian Du[1,2] | Rachel L. Studer[2,4] | Erin M. Jonaitis[1,2,4] | Sanjay Asthana[1,2] | Bradley T. Christian[1,5,6] | Nathaniel A. Chin[1,2] | Kris M. Kirmess[7] | Matthew R. Meyer[7] | Kevin E. Yarasheski[7] | Tim West[7] | Philip B. Verghese[7] | Joel B. Braunstein[7] | Tobey J. Betthauser[1,2] | Rebecca E. Langhough[1,2,4] | Sterling C. Johnson[1,2,4]

[1]Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

[2]Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

[3]Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California, USA

[4]Wisconsin Alzheimer's Institute, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA

[5]Waisman Laboratory for Brain Imaging and Behavior, University of Wisconsin–Madison, Madison, Wisconsin, USA

[6]Department of Medical Physics, University of Wisconsin–Madison, Madison, Wisconsin, USA

[7]C_2N Diagnostics, St. Louis, Missouri, USA

Correspondence
Sterling C. Johnson, University of Wisconsin-Madison, School of Medicine and Public Health, CSC K6/438, 600 Highland Ave, Madison, WI 53792, USA.
Email: scj@medicine.wisc.edu

Funding information
NIH, Grant/Award Numbers: R01AG027161, R01AG021155; AARF, Grant/Award Number: 19614533; NICHD, Grant/Award Numbers: P50HD105353, S10 OD025245-01; University of Wisconsin Institute for Clinical and Translational Research, Grant/Award

Abstract

INTRODUCTION: Plasma biomarkers sensitive to Alzheimer's disease (AD) proteinopathy prior to the onset of dementia have significant implications for early detection.

METHODS: In 304 individuals without dementia, we investigated whether C_2N Diagnostics' mass spectrometry (MS)-based plasma biomarkers (amyloid beta 42/40, %phosphorylated tau [p-tau]181, and %p-tau217) and amyloid probability scores (APS, PrecivityAD and APS2, PrecivityAD2) are associated with brain amyloid, brain tau, or preclinical cognitive decline.

RESULTS: In this cohort study, %p-tau217 and the APS2 had high discriminative accuracy (area under the curve > 0.93) for identifying elevated brain amyloid and tau and were associated with faster preclinical cognitive decline. Using %p-tau217 or the APS2 in a theoretical AD trial screening scenario reduced amyloid positron emission tomography imaging costs up to 41% or 45%, respectively.

DISCUSSION: These findings suggest that C_2N Diagnostics' MS-based plasma biomarkers can detect brain amyloid and tau with high accuracy prior to dementia and could aid in identifying candidates for clinical trials or therapeutic intervention.

KEYWORDS
biomarkers, mass spectrometry, plasma, positron emission tomography, preclinical Alzheimer's disease

Highlights

- C_2N plasma biomarkers differentiated Alzheimer's disease proteinopathy status prior to dementia.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2025 The Author(s). *Alzheimer's & Dementia* published by Wiley Periodicals LLC on behalf of Alzheimer's Association.

Numbers: UL1TR002375, R44 AG059489; BrightFocus, Grant/Award Number: CA2016636; Gerald and Henrietta Rauenhorst Foundation; Alzheimer's Drug Discovery Foundation, Grant/Award Number: GC-201711-2013978

- Plasma %phosphorylated tau (p-tau)217 and the C_2N Diagnostics PrecivityAD2 (APS2) were concordant with amyloid and tau positron emission tomography status.
- Plasma %p-tau217 and the APS2 were associated with preclinical cognitive decline.

1 | BACKGROUND

Three disease-modifying therapies[1–3] targeting amyloid plaques have recently received US Food and Drug Administration (FDA) approval for use in symptomatic Alzheimer's disease (AD). Emerging evidence[2,3] from clinical trials suggest that these anti-amyloid therapeutics may be more effective when administered at the earliest stages of AD. Correspondingly, future clinical trials are likely to shift toward recruiting individuals with preclinical or prodromal AD based on their levels of brain amyloid and brain tau. While positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) biomarkers are the current gold standards[4–6] for detecting AD proteinopathy, more accessible and less resource-intensive methods, like blood-based biomarkers, are needed for broad scalability and widespread implementation in clinical trials and routine clinical practice.[7]

Advances in mass spectrometry (MS) and immunodetection assays have allowed for the reliable assessment of soluble amyloid beta (Aβ) and phosphorylated tau (p-tau) and have provided a promising alternative for detecting AD proteinopathy. Numerous studies[8–13] have demonstrated that plasma biomarkers can detect brain amyloid in presymptomatic and symptomatic AD. Head-to-head plasma biomarker comparisons[14,15] have indicated that high-precision MS assays of plasma Aβ42, Aβ40, and p-tau217 are superior to other candidate immunoassays for distinguishing brain amyloid in preclinical and prodromal AD and for predicting progression to dementia in individuals with mild cognitive impairment (MCI). Further, recent studies[13,16–19] have demonstrated that the combination of MS-based plasma Aβ42, Aβ40, and p-tau217 as well as the integration of these biomarkers into diagnostic algorithms (e.g., C_2N Diagnostics PrecivityAD and PrecivityAD2) improves diagnostic accuracy for detecting brain amyloid in presymptomatic and symptomatic AD.

Beyond brain amyloid, there is a growing recognition of the importance of brain tau for AD diagnosis and prognosis.[6] Several studies[9,12,20–22] have demonstrated that immunodetection-based assays can accurately identify brain tau across the AD clinical continuum; however, considerably less is known about the utility of MS-based plasma biomarkers, individually or in combination, for detecting brain tau. Given that recently approved anti-amyloid therapeutics have been shown[2,3] to be most effective in individuals with low-to-moderate brain tau, future clinical trial screening and enrollment will depend on inexpensive and scalable plasma biomarkers that can accurately detect brain tau in addition to brain amyloid. Thus, it is critical to investigate the accuracy of MS-based plasma biomarkers for identifying brain amyloid and brain tau in the predementia timeframe.

Here, we describe the utility of AD plasma biomarkers measured using C_2N Diagnostics' high-throughput MS-based assays in a sample of predominantly cognitively unimpaired (CU) individuals spanning middle and late age (48–85 years). We assessed the accuracy of amyloid and tau plasma biomarkers, individually and in combination, to detect elevated levels of brain amyloid and brain tau ascertained using 11C-Pittsburgh compound B (PiB) and 18F-MK-6240 PET imaging, respectively. Findings from this analysis were used to determine how a plasma biomarker screening approach would affect PET imaging requirements in a theoretical anti-amyloid therapeutic trial. Finally, we investigated the associations of these AD plasma biomarkers with retrospective preclinical cognitive trajectories.

2 | METHODS

2.1 | Participants

Participants were drawn from the Wisconsin Registry for Alzheimer's Prevention (WRAP)[23] and Wisconsin Alzheimer's Disease Research Center (WADRC), two longitudinal observational cohorts in which participants undergo serial neuropsychological and health assessments (cohort details are provided in eMethods in supporting information). For both cohorts, participant cognitive status (e.g., CU, MCI, or dementia) was determined at each study visit by consensus diagnosis in accordance with the National Institute on Aging–Alzheimer's Association criteria, without reference to biomarkers.[24,25] For the current study, participants were required to be without dementia and have an amyloid PET scan within 1 year of their plasma assessment (n = 304; complete inclusion criteria are detailed in Figure S1 in supporting information). All subjects provided informed consent and study procedures were approved by the University of Wisconsin–Madison Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

2.2 | Plasma biomarkers

Participants in WADRC and WRAP undergo the same venipuncture procedure and sample processing (detailed in eMethods). A total of 342 participants' plasma samples, selected based on having recently completed or being scheduled to complete an amyloid PET scan, were sent to C_2N Diagnostics (who were blinded to participant metadata) for plasma biomarker quantification in Spring 2022. Plasma

concentrations for Aβ42, Aβ40, p-tau181, non–p-tau (np-tau)181, p-tau217, and np-tau217 (all pg/mL) were quantified by C_2N Diagnostics using a novel liquid chromatography-tandem MS analytical platform.[13,16–18,26–28] After quantification, plasma data were transferred to the WADRC for statistical analysis. Of the 342 plasma samples processed by C_2N Diagnostics, a total of 304 individuals without dementia with an amyloid scan within 1 year of their plasma assessment were included in the current study.

As previously described,[13,16–18,26–28] the following concentration ratios were calculated and used for analyses: Aβ42/40; %p-tau181 (p-tau181/np-tau181, [%]); and %p-tau217 (p-tau217/np-tau217, [%]). Two proprietary amyloid probability scores (APS) generated by C_2N were also investigated: (1) APS (C_2N Diagnostics PrecivityAD), an algorithmic score accounting for plasma Aβ42/40, apolipoprotein E (*APOE*) genotype, and age (where *APOE* genotype was determined using isoform-specific peptides by C_2N Diagnostics[26]) and (2) APS2 (C_2N Diagnostics PrecivityAD2), an algorithmic score accounting for plasma Aβ42/40 and %p-tau217.[13,18] Additional details regarding plasma biomarkers and algorithmic scores are provided in eMethods.

2.3 | PET biomarkers

Participants underwent magnetic resonance imaging (MRI) and amyloid ([^{11}C]-PiB) and tau ([^{18}F]-MK-6240) PET imaging at the University of Wisconsin–Madison. PET and MRI acquisition, processing, and quantification were identical across cohorts and implemented as previously reported.[29–31] Amyloid and tau PET scans within 1 year of the plasma assessment were used for this study. Brain amyloid burden was assessed as a global average [^{11}C]-PiB distribution volume ratio (DVR; cerebellum gray matter reference region), taken across eight bilateral cortical regions of interest;[32] elevated brain amyloid was defined using a previously established[33] global DVR threshold ([^{11}C]-PiB DVR > 1.19; i.e., PiB ±). For translatability, in plots, DVR values were linearly translated to Centiloids (CLs) as previously described.[34] [^{18}F]-MK-6240 standardized uptake value ratio (SUVR; 70–90 minutes; cerebellum gray matter reference region) was used to assess tau burden in a temporal meta region of interest (ROI) encompassing the entorhinal cortex, amygdala, parahippocampal gyrus, fusiform gyrus, inferior and middle temporal gyri.[35] [^{18}F]-MK-6240 positivity (i.e., MK ±) was ascertained using a previously defined threshold (temporal meta ROI MK-6240 SUVR > 1.30; MK ±).[31]

2.4 | Neuropsychological assessment

WRAP and WADRC participants undergo overlapping neuropsychological batteries at each study visit. Given the selection and analysis of plasma samples from the most recent study visit, most neuropsychological assessments occurred before or concurrently with the plasma visit. To examine preclinical cognitive decline, we assessed retrospective longitudinal (> 1 cognitive assessment concurrent with or prior to the plasma assessment) neuropsychological data in the subset of individuals with available plasma data and who were CU at their baseline cognitive assessment (n = 300). Longitudinal cognitive performance was assessed using a modified three-test Preclinical Alzheimer's Cognitive Composite (PACC-3), created as the average of memory (Rey Auditory Verbal Learning Test[36] [sum of learning trials] and Logical Memory IIA[37]), and executive functioning (Trail Making Test Trial B[38]) z scored tests. The delayed story recall measures differed between cohorts (WADRC-Craft Story and WRAP-Logical Memory II-A) and were harmonized using a previously validated crosswalk.[39–41]

RESEARCH IN CONTEXT

1. **Systematic review**: The authors used PubMed to conduct a literature search. Studies indicate that individual mass spectrometry (MS)-based plasma biomarkers accurately predict amyloid positron emission tomography (PET) status. The utility of MS-based plasma biomarkers individually and in combination to predict both amyloid and tau PET status in the preclinical and predementia timeframe remain lacking.
2. **Interpretation**: Our cohort study of 304 mostly unimpaired individuals found that plasma %phosphorylated tau (p-tau)217 (p-tau217/np-tau217) and the C_2N Diagnostics PrecivityAD2, an algorithmic score accounting for plasma amyloid beta 42/40 and %p-tau217, had high discriminative accuracy for predicting amyloid and tau PET status in the predementia and preclinical timeframe and were associated with preclinical cognitive decline. These data complement previous MS-based plasma biomarker studies.
3. **Future directions**: These findings highlight the potential utility of MS-based plasma biomarkers for early detection, research, and clinical trial applications. Future studies are needed to understand longitudinal changes in plasma biomarker accumulation.

2.5 | Statistical analyses

For descriptive purposes, correlations between plasma biomarkers and PET imaging biomarkers were examined with a Spearman correlation test. All correlations were adjusted for multiple comparisons using the Bonferroni–Holm method. Group differences in plasma levels between PET positive (e.g., PiB ± or MK ±) were tested using independent samples t tests.

Diagnostic accuracy of plasma biomarkers, individually and in combination, for predicting elevated brain amyloid (i.e., PiB positivity) and elevated brain tau (i.e., MK positivity) was assessed using area under the curve (AUC) from receiver operating characteristic (ROC) curve analyses and compared using Akaike information criterion (AIC), Bayesian information criterion (BIC), and likelihood ratio tests (c^2). To

optimize correspondence between plasma biomarker positivity with PiB and MK positivity, plasma biomarker cutoffs were selected to maximize Youden index.[42] Differences in AUCs were evaluated using the DeLong test.[43] All plasma biomarker models were fit in the non-demented samples with PiB ($n = 304$) and MK ($n = 286$) imaging, and secondarily in the CU only subsamples (PiB, $n = 281$; MK, $n = 264$). In sensitivity analyses, we examined model performance when adjusting for age and *APOE* ε4 status (except for the APS, which incorporates age and *APOE*). Additionally, in the subset with available health factor data, we examined whether plasma biomarker accuracy for predicting PET positivity was improved when adjusting for body mass index (BMI) and kidney function (i.e., estimated glomerular filtration rate [PiB, $N = 283$; MK, $n = 253$]).

Findings from the AUC-ROC analyses were used to calculate the reduction in amyloid PET scans needed to obtain 500 amyloid PET positive (A ±) individuals in an AD clinical trial screening scenario for the best performing individual plasma biomarker (e.g., plasma %p-tau217) and algorithmic score (e.g., APS2). We estimated the prevalence of amyloid PET positivity to be that observed in this sample. Using the positive predictive value and negative predictive value at the Youden-based threshold and hypothetical costs[44] of $6500 per PET scan and $1450 per plasma assay, we estimated potential cost savings associated with screening with plasma %p-tau217 or APS2 prior to imaging to reduce the number of amyloid PET scans needed to enroll 500 amyloid PET-positive individuals.

In the subset of individuals with C_2N plasma data who were CU at their baseline cognitive assessment ($n = 300$), we assessed longitudinal PACC-3 performance over 8.1 (2.5) (mean [standard deviation(SD)]) years of retrospective cognitive follow-up. Linear mixed-effects (LME) models (random intercept and slope; unstructured covariance) were used to investigate whether plasma biomarkers were associated with preclinical PACC-3 trajectories. Five models were compared: a base model, including age (mean-centered), age^2, sex, education, cohort, and number of prior cognitive battery exposures (i.e., practice); and the base model plus one of each of the plasma biomarkers (e.g., Aβ42/40, %p-tau181, %p-tau217, or APS2) and its interactions with age and age^2. The APS incorporates age and was excluded from these analyses. LME models (maximum likelihood estimation) were compared using corrected AIC (AICc) and likelihood ratio tests. In post hoc analyses, for each LME model, we extracted and compared the age at which PACC-3 trajectories diverged (i.e., when the confidence intervals of estimated PACC-3 trajectories no longer overlapped) between the average plasma biomarker positive and negative participant, with plasma positivity for each plasma biomarker determined using the Youden-based cutoff for amyloid PET positivity. All analyses were performed using R v4.3.1. Statistical significance was set at $p < 0.05$.

3 | RESULTS

Demographic, clinical, and neuroimaging characteristics are detailed in Table 1. Among 304 included participants, mean (SD) age was 67.69 (7.01) years, 206 (68%) were female, and 281 (92%) were CU at their plasma assessment.

3.1 | Associations between plasma and PET biomarkers

Lower levels of plasma Aβ42/40 and higher levels of %p-tau217, %p-tau181, APS, and APS2 were positively ($p < 0.05$) correlated with global brain amyloid burden and meta-temporal tau burden (Figure S2 in supporting information). The PiB+ group had significantly lower levels of plasma Aβ42/40 and higher levels of %p-tau181 and %p-tau217 compared to the PiB− group (Table 1; Figure S3A-E in supporting information). Similarly, the PiB+ group had significantly higher APS and APS2 than the PiB− group (Table 1). In the subset with tau PET ($n = 286$), 22 (7.7%) had elevated brain tau (MK+; Table 1). Significantly lower levels of plasma Aβ42/40, higher levels of %p-tau181 and %p-tau217, and higher APS and APS2 were observed in the MK+ group compared to the MK− group (Figure S3F-J).

3.2 | Accuracy of plasma biomarkers for predicting amyloid PET positivity

Among individual plasma biomarkers and algorithmic scores, %p-tau217 (AUC [95% confidence interval (CI)]: 0.95 [0.93–0.98]) and the APS2 (AUC [95% CI]: 0.96 [0.94–0.98]) showed the highest discriminative accuracy for predicting PiB+ (Figure 1A, Table 2). Adding plasma Aβ42/40 to the %p-tau217 model marginally increased the AUC ($\Delta AUC = 0.011$, $p = 0.08$) and significantly improved model fit ($c^2 = 12.0$, $p < 0.001$; $\Delta AIC = -10.0$; Table S1 in supporting information). Adding %p-tau181 to the %p-tau217 model or to the combined Aβ42/40 and %p-tau217 model did not improve model fit ($\Delta AICs < 2$) or accuracy ($\Delta AUCs < 0.001$). AUCs for models incorporating %p-tau217, including the APS2, were significantly higher than models without %p-tau217 but were not significantly different from one another (Table 2).

The APS2 had the highest overall accuracy such that 92% of participants ($n = 279$) had concordant APS2 and PiB statuses, as either APS2− and PiB− (i.e., true negative, $n = 215$) or APS2+ and PiB+ (i.e., true positive, $n = 64$; Figure 2A-E). Similar concordance and overall accuracy (89%) were observed with %p-tau217. While plasma %p-tau217 and the APS2 demonstrated equivalent sensitivity (≈ 88%; Table 2), the APS2 demonstrated higher specificity for brain amyloid and fewer false positive cases (i.e., plasma+PiB−; $n = 16$) compared to %p-tau217 ($n = 25$) at the optimal threshold. ROC analyses in the CU subset (CU, $n = 281$; PiB+ CU, $n = 56$) demonstrated that %p-tau217 and the APS2 also had high diagnostic accuracy for amyloid PET in the preclinical timeframe (AUC [95% CI]$_{\%p\text{-}tau217}$: 0.94 [0.91–0.97]; AUC [95% CI]$_{APS2}$: 0.96 [0.93–0.98]; Table S2 in supporting information).

TABLE 1 Participant characteristics by amyloid PET status.

Characteristic	Overall (*N* = 304)	Amyloid PET positivity[a]		*p*[b]
		PiB− (*n* = 231)	PiB+ (*n* = 73)	
Age at plasma, y, median [IQR], Range	67.6 [62.7, 72.6] Range: 48.3–84.8	66.9 [61.6, 71.6] Range: 48.3–81.8	71.2 [66.9, 76.0] Range: 54.4–84.8	<0.001
Age at amyloid PiB PET, y, Median [IQR], Range	67.6 [62.7, 72.5] Range: 48.1–85.4	67.0 [61.8, 71.5] Range: 48.1–81.9	71.0 [67.2, 76.0] Range: 54.4–85.4	<0.001
Age at tau MK-6240 PET, y[c], Median [IQR], Range	67.63 [63.2, 72.6] Range: 48.1–85.4	67.0 [61.9, 71.6] Range: 48.1–81.9	69.9 [67.1, 75.5] Range: 54.4–85.4	<0.001
Female	206 (68%)	160 (69%)	46 (63%)	0.3
Education, y	16.3 (2.6)	16.1 (2.2)	16.3 (2.2)	0.5
Race				0.7
Black	13 (4%)	8 (4%)	5 (7%)	
American Indian	9 (3%)	7 (3%)	2 (3%)	
Asian	1 (< 1%)	1 (< 1%)	0 (0%)	
White	278 (91%)	212 (92%)	66 (90%)	
Other	3 (1%)	3 (1%)	0 (0%)	
Ethnicity				0.5
Hispanic	5 (2%)	5 (2%)	0 (0%)	
Non-Hispanic	298 (98%)	225 (97%)	73 (100%)	
Unknown	1 (< 1%)	1 (< 1%)	0 (0%)	
APOE ε4 carriers	125 (41%)	75 (32%)	50 (68%)	<0.001
Clinical diagnosis				<0.001
Cognitively unimpaired	281 (92%)	225 (97%)	56 (77%)	
MCI	23 (8%)	6 (2.6%)	17 (23%)	
Global ^{11}C-PiB DVR, median [IQR]	1.08 [1.04, 1.18]	1.06 [1.03, 1.09]	1.54 [1.31, 1.72]	<0.001
Meta-temporal ^{18}F-MK-6240 SUVR, median [IQR][c]	1.07 [0.99, 1.16]	1.06 [0.99, 1.13]	1.14 [1.03, 1.34]	<0.001
MK+[c]	22 (8%)	2 (0.9%)	20 (29%)	<0.001
Plasma biomarkers				
Aβ42/40, median [IQR]	0.098 [0.091, 0.105]	0.101 [0.095, 0.107]	0.089 [0.085, 0.092]	<0.001
%p-tau181, median [IQR]	16.71 [14.13, 19.30]	15.90 [13.86, 18.15]	19.30 [17.45, 23.67]	<0.001
%p-tau217, median [IQR]	1.62 [0.61, 3.05]	1.30 [0.36, 2.00]	5.64 [3.57, 8.04]	<0.001
APS, median [IQR]	11.50 [3.00, 44.00]	6.00 [2.00, 19.50]	60.00 [43.00, 82.00]	<0.001
APS2, median [IQR]	10.00 [5.00, 25.00]	7.00 [5.00, 11.50]	59.00 [31.00, 83.00]	<0.001

Note: Values are present as mean (SD) or No. (%) unless otherwise indicated.

Abbreviations: *APOE*, apolipoprotein E; APS, amyloid probability score; Aβ, amyloid beta; DVR, distribution volume ratio; IQR, interquartile range; MCI, mild cognitive impairment; PET, positron emission tomography; PiB, Pittsburgh compound B; p-tau, phosphorylated tau; ROI, region of interest; SD, standard deviation; SUVR, standardized uptake value ratio.

[a]PiB positivity was determined as a global PiB DVR > 1.19 (equivalent Centiloids > 21.6).

[b]Statistical tests: $\chi 2$ for categorical variables, independent samples *t* test for continuous variables; *p* value for difference between PiB− and PiB+ groups.

[c]A total of 286 individuals had available PiB and ^{18}F-MK-6240 PET imaging. Tau PET positivity (MK+/−) was determined as temporal meta-ROI MK-6240 SUVR > 1.30.

3.3 | Accuracy of plasma biomarkers for predicting tau PET positivity

The APS2 (AUC [95% CI]): 0.94 [0.89–0.99]) and %p-tau217 (AUC [95% CI]: 0.93 [0.87–1.00]) had the highest AUCs for predicting MK positivity and were not significantly different from one another (ΔAUCs < 0.01, $p = 0.30$; Figure 1B; Table 2). Models investigating combinations of plasma biomarkers for predicting tau PET indicated that no other biomarker or combination of biomarkers were significantly better than %p-tau217 or the APS2 (Table 2; Table S3 in supporting information).

At the Youden-based plasma cutoffs, plasma %p-tau217 had the highest overall accuracy (91%) followed by the APS2 (85%; Figure 2F-J). While %p-tau217 and APS2 demonstrated similar

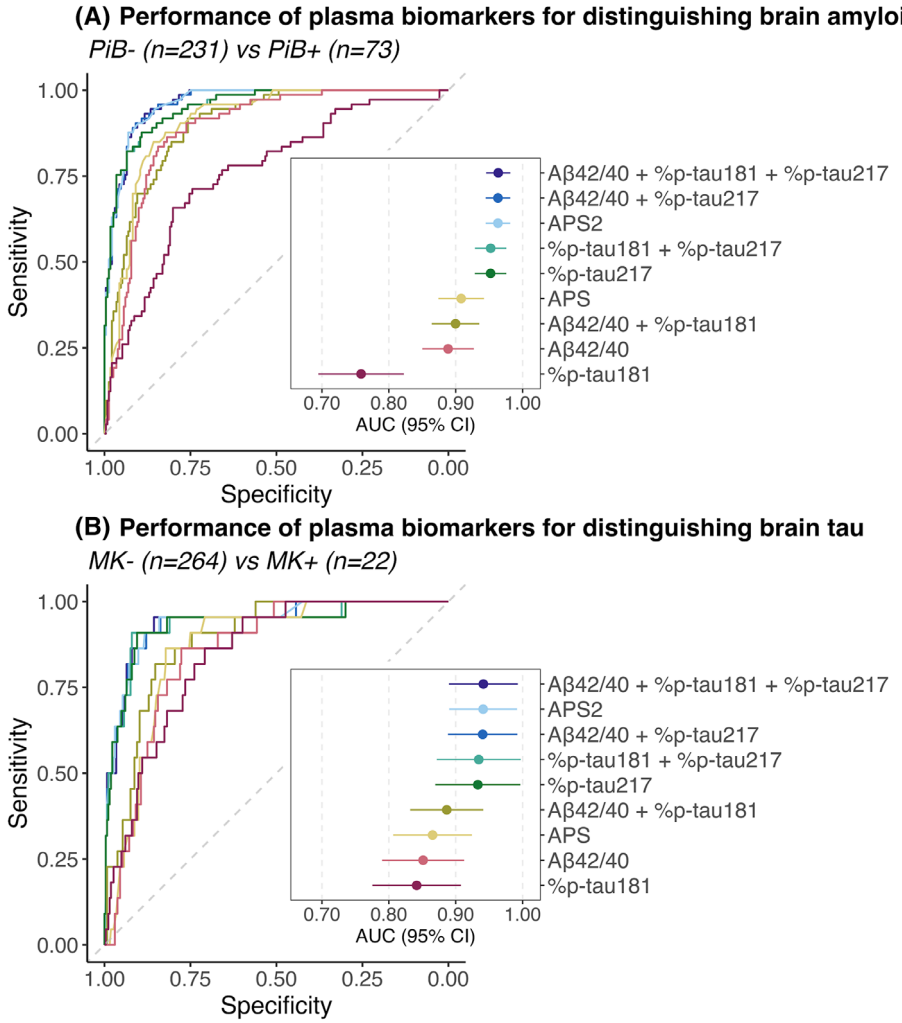

FIGURE 1 ROC analysis of plasma biomarkers for distinguishing brain amyloid and brain tau. The diagnostic performance for predicting amyloid PiB PET positivity (A) and tau MK-6240 PET positivity (B) is shown as ROC curves for plasma biomarkers, individually and in combination, with the inset showing the corresponding AUC and 95% confidence intervals for each plasma biomarker. Aβ, amyloid beta; APS, amyloid probability score; AUC, area under the curve; CI, confidence interval; MCI, mild cognitive impairment; PET, positron emission tomography; PiB, Pittsburgh compound B, p-tau, phosphorylated tau; ROC, receiver operating characteristic; SUVR, standardized uptake value ratio; TMR, temporal meta-ROI.

sensitivity at the Youden-based cutoff, %p-tau217 demonstrated higher specificity for brain tau and classified fewer false positive cases (i.e., plasma+MK−; n = 25) compared to APS2 (n = 29) at the optimal cutoff. Notably, the false positive quadrants for APS2 and %p-tau217 were each largely comprised of PiB+ individuals (e.g., APS2+PiB+ = 81%; %p-tau217+PiB+ = 91%), indicating that optimal plasma biomarker thresholds for tau PET also capture amyloid PET positive individuals. In the CU subset (CU, n = 264; CU MK+, n = 12) %p-tau217 and the APS2 also showed high diagnostic accuracy for predicting preclinical tau PET accumulation (AUC [95% CI]$_{\%p\text{-}tau217}$: 0.91 [0.79–1.00]; AUC [95% CI]$_{APS2}$: 0.92 [0.83–1.00]; Table S4 in supporting information).

3.4 | Sensitivity analyses

In sensitivity analyses, adding age and *APOE* ε4 status to the plasma biomarker models significantly improved the accuracy and fit of all models for predicting PiB positivity (ΔAUC, 0.01–0.07; Table S5 in supporting information) and marginally improved models for predicting MK positivity (ΔAUC, 0.00–0.04; Table S6 in supporting information). Finally, sensitivity analyses adjusting for BMI and kidney function did not significantly improve model accuracy or fit for predicting amyloid or tau PET positivity (Tables S7,S8 in supporting information).

3.5 | Clinical trial implications

We next assessed how pre-screening with the best-performing individual plasma biomarker (e.g., %p-tau217) or algorithmic score (e.g., APS2) might reduce the screening sample size and cost of a theoretical clinical trial aimed at enrolling 500 non-demented amyloid PET-positive individuals. Using the observed PiB+ prevalence (24%) in the current study, we found that pre-screening with %p-tau217 or the APS2 reduced the number of PET scans by ≈ 67% or 70%, respectively. With hypothetical costs of $6500 per PET scan and $1450 per plasma assay,

TABLE 2 Performance of plasma biomarker models for detecting brain amyloid and brain tau.

Plasma biomarkers	Cutoff[a]	AUC (95% CI)[b]	SPEC	SENS	PPV	NPV	ACC	AIC	BIC
Models predicting amyloid PET status									
APS	32.5	0.91 (0.87–0.94)*,**	0.86	0.85	0.65	0.95	0.86	214	221
APS2	22.5	0.96 (0.94–0.98)	0.93	0.88	0.80	0.96	0.92	157	165
Aβ42/40	0.093	0.89 (0.85–0.93)*,**	0.82	0.86	0.60	0.95	0.83	232	240
%p-tau181	18.68	0.76 (0.69–0.82)*,**	0.80	0.66	0.51	0.88	0.77	299	306
%p-tau217	2.66	0.95 (0.93–0.98)	0.89	0.88	0.72	0.96	0.89	154	161
%p-tau181 + %p-tau217	0.19	0.95 (0.93–0.98)	0.89	0.88	0.72	0.96	0.89	156	167
Aβ42/40 + %p-tau181	0.20	0.90 (0.86–0.94)*,**	0.76	0.92	0.54	0.97	0.80	222	234
Aβ42/40 + %p-tau217	0.21	0.96 (0.94–0.98)	0.91	0.90	0.76	0.97	0.91	144	155
Aβ42/40 + %p-tau181+ %p-tau217	0.17	0.96 (0.95–0.98)	0.88	0.93	0.72	0.98	0.89	146	160
Models predicting tau PET status									
APS	47.5	0.87 (0.81–0.92)**	0.82	0.86	0.29	0.99	0.83	122	130
APS2	31.5	0.94 (0.89–0.99)	0.84	0.95	0.33	1.00	0.85	86	94
Aβ42/40	0.092	0.85 (0.79–0.91)*,**	0.78	0.86	0.24	0.99	0.78	134	141
%p-tau181	18.36	0.84 (0.78–0.91) *,**	0.71	0.86	0.20	0.98	0.72	136	143
%p-tau217	4.90	0.93 (0.87–1.00)	0.91	0.91	0.44	0.99	0.91	90	97
%p-tau181 + %p-tau217	0.09	0.93 (0.87–1.00)	0.92	0.91	0.49	0.99	0.92	92	103
Aβ42/40 + %p-tau181	0.11	0.89 (0.83–0.94)**	0.85	0.82	0.32	0.98	0.85	126	137
Aβ42/40 + %p-tau217	0.05	0.94 (0.89–0.99)	0.84	0.95	0.33	1.00	0.85	90	101
Aβ42/40 + %p-tau181+ %p-tau217	0.06	0.94 (0.89–0.99)	0.86	0.95	0.36	1.00	0.86	91	106

Abbreviations: Aβ, amyloid beta; ACC, accuracy; AIC, Akaike information criterion; APS, amyloid probability score; AUC, area under the curve; Aβ, amyloid beta; BIC, Bayesian information criterion; CI, confidence interval; NPV, negative predictive value; PET, positron emission tomography; PPV, positive predictive value; p-tau, phosphorylated tau; SENS, sensitivity; SPEC, specificity.

[a]Cutoffs were determined based on the highest Youden index for amyloid PET positivity (Global PIB DVR > 1.19; Equivalent Centiloids > 22) or tau PET positivity (Temporal meta-ROI MK-6240 SUVR > 1.30). SPEC, SENS, PPV, NPV, and ACC at the Youden-based cutoff are shown. For individual biomarkers (e.g., Aβ42/40, %p-tau181, %p-tau217) and algorithmic scores (APS and APS2), cutoffs constitute the actual ratio of the biomarker or score levels. For combined biomarker models, cutoffs are from the probabilities from the corresponding logistic regression models.

[b]AUCs were compared with Delong tests and corrected for multiple comparisons using the Benjamini–Hochberg method.

*$p < 0.05$ compared to %p-tau217.

**$p < 0.05$ compared to APS2.

pre-screening with %p-tau217 or the APS2 lowered the amyloid PET imaging costs by ≈ $5.6 to $6.0 million, respectively, from a total cost of $13.5 million (Table 3).

3.6 | Plasma biomarkers and preclinical cognitive decline

Finally, we examined whether AD plasma biomarkers were associated with preclinical cognitive decline among participants unimpaired at their baseline cognitive assessment (n = 300). Participants were on average (SD) 58.7 (6.5) years of age at their baseline cognitive assessment with an average of four assessments over 8.9 (6.6–10.2) (median [interquartile range]) years of cognitive follow-up prior to their plasma assessment. All plasma biomarker models significantly improved prediction of longitudinal PACC-3 compared to the base model (AICc = 2599). In terms of AICc, %p-tau217 predicted longitudinal PACC-3 decline best (AICcΔ = −68 vs. base model) followed by the APS2 (AICcΔ = −44 vs. base model; Table S9 in supporting information). Higher levels of plasma %p-tau217, APS2, and %p-tau181 were each significantly associated with faster PACC-3 decline. Modeled PACC-3 trajectories of the average plasma biomarker-positive and biomarker-negative participant diverged at an estimated age of 71.3 for APS2, 72.6 for %p-tau217, 77.1 for %p-tau181, and 75.2 for Aβ42/40 (Figure 3).

4 | DISCUSSION

In middle- and late-aged individuals without dementia, we assessed the accuracy of MS-based plasma biomarkers and algorithmic scores to detect elevated brain amyloid and brain tau and examined the differential associations of plasma biomarkers with preclinical cognitive decline. We showed that in the predementia and preclinical timeframe, p-tau217-centric plasma measures (%p-tau217 and APS2) were each highly concordant with amyloid and tau PET positivity and significantly

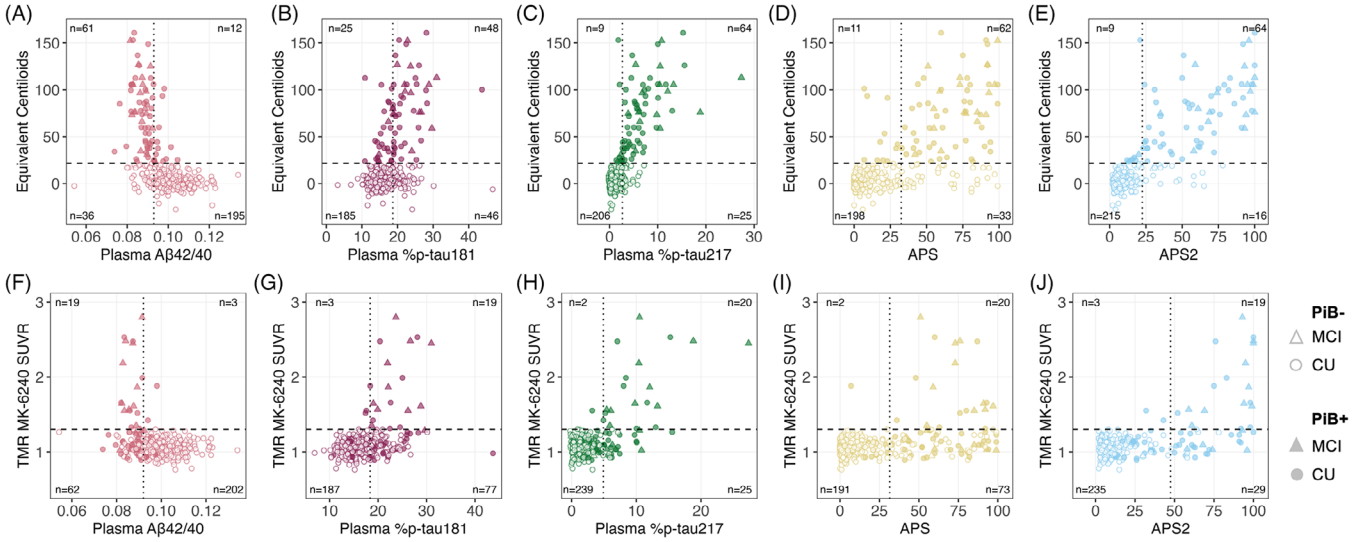

FIGURE 2 Concordance between plasma biomarkers and PET biomarkers of amyloid and tau. Quadrant plots illustrating the association between plasma biomarkers and brain amyloid as measured by equivalent Centiloids (CL; A-E) and meta-temporal tau as measured by MK-6240 SUVR (F-J) with the Youden-based thresholds for each plasma marker shown as vertical dotted lines and the thresholds for amyloid PET positivity (PiB DVR > 1.19, Equivalent CL > 21.6) and tau PET positivity (TMR MK-6240 SUVR > 1.30) shown as horizontal dashed lines. Individual scatter points are shaped by the diagnosis at the plasma assessment (cognitively unimpaired = circles; MCI = triangles) and shaded by amyloid PET positivity (PiB+ filled shape; PiB− empty shape). Youden-based thresholds for each plasma biomarker predicting amyloid PET positivity (Aβ42/40 > 0.093, p-tau181 ratio > 18.68, p-tau217 ratio > 2.66, APS > 32.5, APS2 > 22.5; vertical dotted lines, A-E) and tau PET positivity (Aβ42/40 > 0.092, p-tau181 ratio > 18.36, p-tau217 ratio > 4.90, APS > 47.5, APS2 > 31.5; vertical dotted lines, F-J). Aβ, amyloid beta; APS, amyloid probability score; CU, cognitively unimpaired; DVR, distribution volume ratio; MCI, mild cognitive impairment; PET, positron emission tomography; PiB, Pittsburgh compound B; p-tau, phosphorylated tau; SUVR, standardized uptake value ratio; TMR, temporal meta-ROI.

TABLE 3 Implementation of plasma biomarkers for enriching amyloid PET positivity in an AD clinical trial screening scenario.

	Enrollment target: *N* = 500 who are pre-dementia and have elevated amyloid PET		
	Amyloid PET only	Pre-PET %p-tau217 screening	Pre-PET APS2 screening
Prevalence of biomarker positivity	73/304 = 24% A+	89/304 = 29.3% %p-tau217+	80/304 = 26.3% APS2+
Positive predictive value for A+	–	0.72	0.80
No. of PET scans needed to identify 500 A+	2083	695	625
No. of plasma screens	0	2372	2376
Theoretical cost ($6500 per PET scan; $1450 per plasma assay)[a]	$13,539,500	$7,956,900	$7,507,700

Abbreviations: *APOE*, apolipoprotein E; APS, amyloid probability score; PET, positron emission tomography; p-tau, phosphorylated tau.
[a]These are simplified calculations to illustrate cost savings using one set of parameters; in a real-world trial application, additional factors such as ranges of prevalence estimates, refusal rate estimates, age at screening, and *APOE* genotype may be included in design planning.

associated with retrospective cognitive decline. Moreover, we demonstrated that the use of %p-tau217 or the APS2 as a screening measure for brain amyloid greatly reduced the number of screening PET scans as well as lowered overall screening costs in a preclinical AD trial scenario. Together, these results support the utility of MS-based plasma biomarkers for early detection of brain amyloid and brain tau in AD prior to dementia.

Several studies[15–18,26,45,46] have demonstrated that plasma Aβ42/40, p-tau217, the APS, and APS2 can each be used to detect amyloid PET positivity in symptomatic individuals; however, few studies have examined the utility of plasma biomarkers in presymptomatic individuals,[11,12,20,46–48] and fewer yet have examined the accuracy of MS-based plasma biomarkers in the predementia timeframe,[13,49] particularly for predicting brain tau.[19] Here, we build on these previous studies and demonstrate the utility of C_2N Diagnostics' MS-based plasma biomarkers for detecting amyloid and tau PET positivity in predominantly presymptomatic individuals. We found that %p-tau217 had high discriminative accuracy (AUC = 0.95) for predicting brain

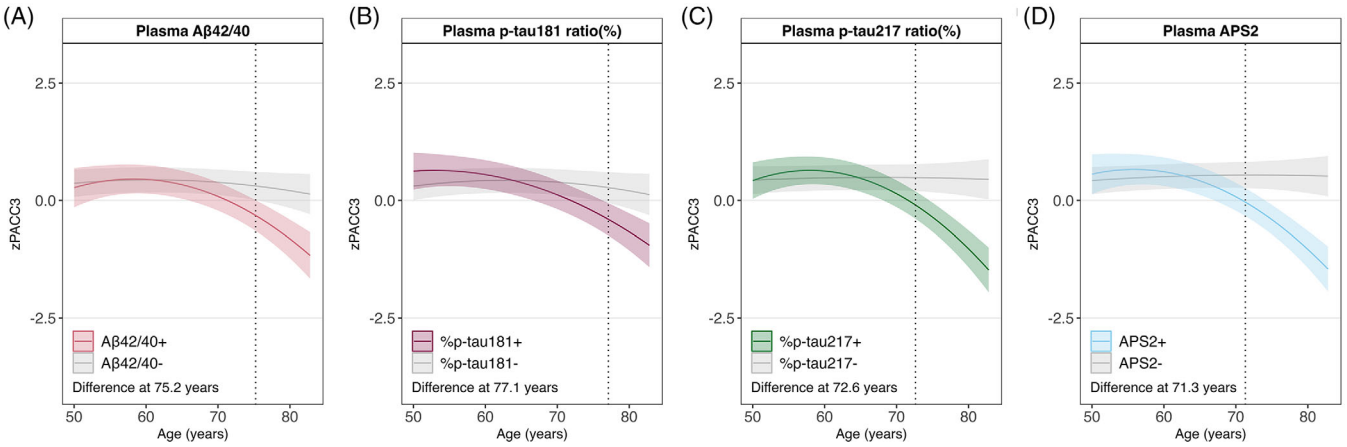

FIGURE 3 Associations of plasma biomarkers with longitudinal cognitive decline. This figure shows retrospective longitudinal PACC-3 trajectories derived from linear mixed-effects models with longitudinal PACC-3 as the outcome and age, age^2, sex, education, cohort, practice, plus each plasma biomarker and it's interactions with age and age^2 included separately from each other as predictors. Each plot shows the estimated PACC-3 trajectory for a plasma biomarker-negative (gray) or biomarker-positive (pink/purple/green/blue) initially cognitively unimpaired participant with average education, average practice, and female sex. Vertical dotted lines indicate the estimated age when PACC-3 trajectories diverged between the average plasma biomarker-negative and biomarker-positive individual for each plasma biomarker. Plasma biomarker positivity was determined using the optimal Youden-based cutoffs for predicting amyloid PET positivity. Shaded areas represent 95% confidence intervals of the regression lines. Aβ, amyloid beta; APS, amyloid probability score; PACC-3, three test Preclinical Alzheimer's Cognitive Composite, PET, positron emission tomography; p-tau, phosphorylated tau.

amyloid in the predementia timeframe. In agreement with previous studies,[13,18,46,50] the combination of Aβ42/40 and %p-tau217 provided a slightly higher AUC (0.96) and a significantly better model fit (ΔAIC = −10) with no added value of plasma %p-tau181. The APS2, an algorithmic combination of plasma Aβ42/40 and %p-tau217, detected amyloid PET status with comparable accuracy (AUC = 0.96). Importantly, similar results and accuracy across plasma biomarkers were observed in the preclinical CU subsample. These findings agree with previous studies[13,46,49,50] of optimal biomarker combinations to predict preclinical amyloid PET positivity and add to a growing literature[50–56] of biofluid biomarker dynamics suggesting that MS-based plasma Aβ42/40 may capture individuals in the earliest stages of disease and p-tau217 may capture individuals throughout the disease course by showing that the combination of the two improves detection of brain amyloid in the preclinical and predementia phase of AD.

In addition to detecting brain amyloid, the APS2 and %p-tau217 each showed high discriminative accuracy for predicting brain tau in the predementia ($AUC_{APS2} = 0.94$; $AUC_{\%p\text{-}tau217} = 0.93$) and preclinical ($AUC_{APS2} = 0.92$; $AUC_{\%p\text{-}tau217} = 0.91$) timeframe. The optimal cutoffs at which the APS2 and %p-tau217 were congruent with tau PET were higher than those for detecting amyloid PET, suggesting that increasing plasma biomarker burden is associated with increasing pathological disease severity. Accordingly, these findings agree with studies indicating that cerebral amyloid accumulation occurs first[57–59] and temporal tau deposition[60] represents a more advanced stage of the disease spectrum. Critically, we demonstrated that a single plasma biomarker (e.g., %p-tau217) can accurately detect both brain amyloid and brain tau, underscoring the efficiency and scalability of this plasma biomarker in clinical and research settings. Further, similar accuracy of plasma %p-tau217 for predicting brain amyloid and brain tau was recently reported[19] in the BioFINDER-2 and Knight ADRC cohorts, where the performance of plasma %p-tau217 was shown to be clinically equivalent or superior to FDA-approved CSF measures, highlighting the consistency and generalizability of the diagnostic accuracy of plasma %p-tau217 across cohorts.

More, we showed that pre-screening with %p-tau217 or the APS2 greatly reduced the number of amyloid PET scans needed to enroll non-demented amyloid-positive individuals into a theoretical clinical trial, highlighting the potential of plasma %p-tau217 or the APS2 to accelerate participant enrollment, lower costs, and improve timelines for anti-amyloid therapeutic interventions. Notably, the combined model of plasma Aβ42/40 and %p-tau217 and the APS2 had similar performance for predicting amyloid PET positivity (ΔAUC < 0.001) and tau PET positivity (ΔAUC < 0.001), indicating that the two models were ostensibly testing the same construct. This is expected as the APS2 was derived by C_2N Diagnostics using a combined model of Aβ42/40 and %p-tau217 in the MissionAD and PARIS cohorts.[18] Here, we show that the APS2 is robust to cohort differences and has utility for predicting amyloid and tau PET positivity in the predementia timeframe.

Overall, we observed substantial agreement between plasma biomarker positivity (at the optimal threshold) and PET positivity, with %ptau217 and APS2 showing the highest agreement (amyloid PET agreement: %ptau217 = 89%, APS2 = 92%; tau PET agreement: %ptau217 = 91%, APS2 = 85%). However, we also observed a consistent pattern of discordance: across all plasma biomarkers, there were more plasma+PET− (e.g., false positive) participants than plasma−PET+ (e.g., false negative) participants (Figure 2A-J). This pattern of discordance aligns with findings[8,16] from previous MS-based plasma biomarker studies. A study by Schindler et al. showed that individuals classified as plasma amyloid+ but PET amyloid− were more

likely to convert to PET amyloid+ over a 4-year follow-up compared to biomarker-negative individuals, suggesting that MS-based plasma amyloid may become abnormal earlier than detectable changes in amyloid PET imaging.[8] While plasma+PET− classification may indicate early changes in plasma biomarkers, it also remains possible that some plasma+PET− participants may have abnormal brain amyloid or tau but are below the threshold for PET positivity. Thus, additional longitudinal plasma and PET follow-up will be necessary to further elucidate the timing of AD-related changes in plasma relative to PET imaging.

Similar to recently published studies,[8,13,16,17,46,61] we found that adjusting for age and *APOE* ε4 status significantly improved model accuracy and fit when predicting amyloid PET positivity, while only marginally improving model performance for predicting tau PET positivity. As *APOE* isoforms are mechanistically involved in beta-amyloid metabolism, aggregation, and deposition, it is unsurprising that *APOE* ε4 status more strongly improved plasma biomarker performance for predicting amyloid versus tau PET. By contrast, no significant improvements in model fit or accuracy for predicting amyloid or tau PET were observed when additionally adjusting for kidney function and BMI, although the sample was slightly smaller given inconsistent reporting. While these findings are inconsistent with previous plasma biomarker studies[62,63] using Quanterix Simoa and Lilly MSD platforms demonstrating an effect of kidney disease and BMI on assay performance, they align with a recent study using MS-based plasma which showed a minimal effect of creatinine or BMI on the association between plasma Aβ42/40 and CSF Aβ42/40. The lack of impact of these comorbidities on the performance of plasma biomarkers in the current study could be due to cohort composition (e.g., limited number of participants with kidney disease in this sample) or plasma assay differences.

While several of the plasma biomarkers reported in this study were associated with cognitive decline, plasma %p-tau217 and the APS2 exhibited the strongest associations with preclinical decline during the ≈ 8 years of retrospective cognitive follow-up. Across those unimpaired at their baseline cognitive assessment, the cognitive trajectories of the APS2 positive and negative biomarker groupings diverged earliest, around age 71, followed by plasma %p-tau217, where %p-tau217 positive and negative groupings began to diverge closer to age 73. These results build on our plasma to amyloid PET findings and suggest that the combination of plasma Aβ42/40 and plasma %p-tau217 as used in the APS2 is not only most sensitive to elevated brain amyloid, but also sensitive to subtle preclinical cognitive decline. These cognitive effects, using longitudinal "run-in" data in this otherwise cross-sectional study, demonstrate that AD-associated cognitive change is slow and continuous but also observably different from non-AD change in the preclinical timeframe. Together, our findings help elucidate the early cognitive time course in AD and demonstrate the broad applicability of MS-based plasma biomarkers to both identify AD pathology and detect AD-related cognitive decline before the onset of dementia.

Several factors limit the generalizability of our findings. The plasma measures were cross-sectional and the cohort, though well characterized with cognitive, genetic, and imaging data, was highly educated and predominantly non-Hispanic White. Therefore, it is important that these plasma assays continue to be examined in more diverse cohorts. Ongoing work will need to evaluate how these plasma biomarkers change over time. Additionally, given the retrospective nature of the cognitive follow-up, our data do not lend themselves to inference about future cognitive decline. However, due to the longitudinal nature of the source studies the samples and data were drawn from, our retrospective cognitive analyses provide unique insight on cognitive trajectories spanning back to late midlife. Finally, to further characterize the specificity for AD, plasma analysis from individuals with other neurodegenerative age-related pathologies (i.e., Lewy bodies, TAR DNA-binding protein 43, vascular disease, etc.) should be conducted.

5 | CONCLUSION

We showed that plasma %p-tau217 and APS2 detected brain amyloid and tau with high accuracy and were each strongly associated with cognitive decline in the predementia timeframe. Together, our findings demonstrate the utility of MS-based plasma biomarkers for detecting AD brain pathology, for predicting preclinical cognitive trajectories, and for their potential to improve the screening process for future AD clinical trials. Ongoing work is needed to evaluate how these plasma biomarkers perform longitudinally and in individuals from more diverse populations.

ACKNOWLEDGMENTS

The authors extend their deepest thanks to the WRAP participants and staff for their invaluable contributions to the study. This publication was supported by NIH R01AG027161 (Johnson), NIH R01AG021155 (Johnson), AARF 19614533 (Betthauser), NICHD P50HD105353 (Qiang) and S10 OD025245-01 (Christian), and the University of Wisconsin Institute for Clinical and Translational Research NIH UL1TR002375 (Cody). This work was also supported by the NIH (R44 AG059489), BrightFocus (CA2016636), the Gerald and Henrietta Rauenhorst Foundation, and the Alzheimer's Drug Discovery Foundation (GC-201711-2013978). All subjects provided informed consent and study procedures were approved by the University of Wisconsin–Madison Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

CONFLICT OF INTEREST STATEMENT

Dr. Johnson has served as an advisor to Roche Diagnostics, AlzPath, and Enigma Biosciences and has received research support (sponsoring of an unrelated observational study) from Cerveau Technologies. Drs. Kirmess, Meyer, Yarasheski, West, Verghese, and Braunstein are paid employees of C_2N Diagnostics. No other disclosures were reported. Author disclosures are available in the supporting information.

ORCID

Karly A. Cody https://orcid.org/0000-0001-5996-2560
Sterling C. Johnson https://orcid.org/0000-0002-8501-545X

REFERENCES

1. Budd Haeberlein S, Aisen PS, Barkhof F, et al. Two randomized phase 3 studies of aducanumab in early Alzheimer's disease. *J Prev Alzheimer Dis*. 2022;9(2):197-210.
2. van Dyck CH, Swanson CJ, Aisen P, et al. Lecanemab in early Alzheimer's disease. *N Engl J Med*. 2022;388(1):9-21.
3. Sims JR, Zimmer JA, Evans CD, et al. Donanemab in early symptomatic Alzheimer disease: the TRAILBLAZER-ALZ 2 randomized clinical trial. *JAMA*. 2023;330(6):512-527.
4. Blennow K, Mattsson N, Scholl M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. *Trends Pharmacol Sci*. 2015;36(5):297-309.
5. Ossenkoppele R, van der Kant R, Hansson O. Tau biomarkers in Alzheimer's disease: towards implementation in clinical practice and trials. *Lancet Neurol*. 2022;21(8):726-734.
6. Jack CR Jr, Andrews JS, Beach TG, et al. Revised criteria for diagnosis and staging of Alzheimer's disease: alzheimer's Association Workgroup. *Alzheimers Dement*. 2024;20(8):5143-5169.
7. Hansson O, Blennow K, Zetterberg H, Dage J. Blood biomarkers for Alzheimer's disease in clinical practice and trials. *Nat Aging*. 2023;3(5):506-519.
8. Schindler SE, Bollinger JG, Ovod V, et al. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology*. 2019;93(17):e1647-e1659.
9. 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. Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med*. 2020;26(3):379-386.
11. Ashton NJ, Janelidze S, Mattsson-Carlgren N, et al. Differential roles of Abeta42/40, p-tau231 and p-tau217 for Alzheimer's trial selection and disease monitoring. *Nat Med*. 2022;28(12):2555-2562.
12. Jonaitis EM, Janelidze S, Cody KA, et al. Plasma phosphorylated tau 217 in preclinical Alzheimer's disease. *Brain Commun*. 2023;5(2):fcad057.
13. Rissman RA, Langford O, Raman R, et al. Plasma Abeta42/Abeta40 and phospho-tau217 concentration ratios increase the accuracy of amyloid PET classification in preclinical Alzheimer's disease. *Alzheimers Dement*. 2023.
14. Janelidze S, Teunissen CE, Zetterberg H, et al. Head-to-head comparison of 8 plasma amyloid-beta 42/40 assays in Alzheimer disease. *JAMA Neurol*. 2021;78(11):1375-1382.
15. Janelidze S, Bali D, Ashton NJ, et al. Head-to-head comparison of 10 plasma phospho-tau assays in prodromal Alzheimer's disease. *Brain*. 2023;146(4):1592-1601.
16. West T, Kirmess KM, Meyer MR, et al. A blood-based diagnostic test incorporating plasma Abeta42/40 ratio, ApoE proteotype, and age accurately identifies brain amyloid status: findings from a multi cohort validity analysis. *Mol Neurodegener*. 2021;16(1):30.
17. Hu Y, Kirmess KM, Meyer MR, et al. Assessment of a plasma amyloid probability score to estimate amyloid positron emission tomography findings among adults with cognitive impairment. *JAMA Netw Open*. 2022;5(4):e228392.
18. Meyer MR, Kirmess KM, Eastwood S, et al. Clinical validation of the PrecivityAD2 blood test: a mass spectrometry-based test with algorithm combining %p-tau217 and Abeta42/40 ratio to identify presence of brain amyloid. *Alzheimers Dement*. 2024;20(5):3179-3192.
19. Barthelemy NR, Salvado G, Schindler SE, et al. Highly accurate blood test for Alzheimer's disease is similar or superior to clinical cerebrospinal fluid tests. *Nat Med*. 2024;30(4):1085-1095.
20. Janelidze S, Berron D, Smith R, et al. Associations of plasma phospho-Tau217 levels with tau positron emission tomography in early Alzheimer disease. *JAMA Neurol*. 2021;78(2):149-156.
21. Tissot C, Therriault J, Kunach P, et al. Comparing tau status determined via plasma pTau181, pTau231 and [(18)F]MK6240 tau-PET. *EBioMedicine*. 2022;76:103837.
22. Salvado G, Ossenkoppele R, Ashton NJ, et al. Specific associations between plasma biomarkers and postmortem amyloid plaque and tau tangle loads. *EMBO Mol Med*. 2023;15(5):e17123.
23. Johnson SC, Koscik RL, Jonaitis EM, et al. The Wisconsin Registry for Alzheimer's Prevention: a review of findings and current directions. *Alzheimers Dement (Amst)*. 2018;10:130-142.
24. 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*. 2011;7(3):270-279.
25. 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*. 2011;7(3):263-269.
26. Kirmess KM, Meyer MR, Holubasch MS, et al. The PrecivityAD test: accurate and reliable LC-MS/MS assays for quantifying plasma amyloid beta 40 and 42 and apolipoprotein E proteotype for the assessment of brain amyloidosis. *Clin Chim Acta*. 2021;519:267-275.
27. Fogelman I, West T, Braunstein JB, et al. Independent study demonstrates amyloid probability score accurately indicates amyloid pathology. *Ann Clin Transl Neurol*. 2023;10(5):765-778.
28. Monane M, Johnson KG, Snider BJ, et al. A blood biomarker test for brain amyloid impacts the clinical evaluation of cognitive impairment. *Ann Clin Transl Neurol*. 2023;10(10):1738-1748.
29. Betthauser TJ, Cody KA, Zammit MD, et al. In vivo characterization and quantification of neurofibrillary tau PET radioligand. *J Nucl Med*. 2019;60(1):93-99.
30. Betthauser TJ, Koscik RL, Jonaitis EM, et al. Amyloid and tau imaging biomarkers explain cognitive decline from late middle-age. *Brain*. 2020;143(1):320-335.
31. Cody K, Langhough Koscik R, Zammit MD, et al. Characterizing brain tau and cognitive decline along the amyloid timeline in Alzheimer's disease. *Brain*. 2024;147(6):2144-2157.
32. Sprecher KE, Bendlin BB, Racine AM, et al. Amyloid burden is associated with self-reported sleep in nondemented late middle-aged adults. *Neurobiol Aging*. 2015;36(9):2568-2576.
33. Racine AM, Koscik RL, Nicholas CR, et al. Cerebrospinal fluid ratios with Aβ42 predict preclinical brain β-amyloid accumulation. *Alzheimers Dement (Amst)*. 2016;2:27-38.
34. Betthauser TJ, Bilgel M, Koscik RL, et al. Multi-method investigation of factors influencing amyloid onset and impairment in three cohorts. *Brain*. 2022.
35. Jack CR Jr, Wiste HJ, Schwarz CG, et al. Longitudinal tau PET in ageing and Alzheimer's disease. *Brain*. 2018;141(5):1517-1528.
36. Schmidt M. *Rey Auditory Verbal Learning Test: A Handbook*. Western Psychological Services; 1996.
37. Wechsler D. *WMS-R: Wechsler Memory Scale-Revised*. Psychological Corporation; 1987.
38. Reitan R. Validity of the Trail Making Test as an indicator of organic brain damage. *Perceptual Motor Skills*. 1958;8:271-276.
39. Van Hulle C, Jonaitis EM, Betthauser TJ, et al. An examination of a novel multipanel of CSF biomarkers in the Alzheimer's disease clinical and pathological continuum. *Alzheimers Dement*. 2021;17:431-445.
40. Monsell SE, Dodge HH, Zhou XH, et al. Results from the NACC Uniform Data Set Neuropsychological Battery Crosswalk Study. *Alzheimer Dis Assoc Disord*. 2016;30(2):134-139.

41. Jonaitis EM, Hermann BP, Mueller KD, et al. Longitudinal normative standards for cognitive tests and composites using harmonized data from two Wisconsin AD-risk-enriched cohorts. *Alzheimers Dement*. 2024;20(5):3305-3321.
42. Youden WJ. Index for rating diagnostic tests. *Cancer*. 1950;3(1):32-35.
43. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44(3):837-845.
44. Schindler SE, Li Y, Li M, et al. Using Alzheimer's disease blood tests to accelerate clinical trial enrollment. *Alzheimers Dement*. 2023;19(4):1175-1183.
45. Groot C, Cicognola C, Bali D, et al. Diagnostic and prognostic performance to detect Alzheimer's disease and clinical progression of a novel assay for plasma p-tau217. *Alzheimers Res Ther*. 2022;14(1):67.
46. Janelidze S, Palmqvist S, Leuzy A, et al. Detecting amyloid positivity in early Alzheimer's disease using combinations of plasma Abeta42/Abeta40 and p-tau. *Alzheimers Dement*. 2022;18(2):283-293.
47. Mila-Aloma M, Ashton NJ, Shekari M, et al. Plasma p-tau231 and p-tau217 as state markers of amyloid-beta pathology in preclinical Alzheimer's disease. *Nat Med*. 2022;28(9):1797-1801.
48. Janelidze S, Barthelemy NR, Salvado G, et al. Plasma phosphorylated Tau 217 and Abeta42/40 to predict early brain Abeta accumulation in people without cognitive impairment. *JAMA Neurol*. 2024;81(9):947-957.
49. Niimi Y, Janelidze S, Sato K, et al. Combining plasma Abeta and p-tau217 improves detection of brain amyloid in non-demented elderly. *Alzheimers Res Ther*. 2024;16(1):115.
50. Barthélemy NR, Li Y, Joseph-Mathurin N, et al. A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat Med*. 2020;26(3):398-407.
51. Mattsson-Carlgren N, Janelidze S, Bateman RJ, et al. Soluble P-tau217 reflects amyloid and tau pathology and mediates the association of amyloid with tau. *EMBO Mol Med*. 2021;13(6):e14022.
52. Schindler SE, Li Y, Todd KW, et al. Emerging cerebrospinal fluid biomarkers in autosomal dominant Alzheimer's disease. *Alzheimers Dement*. 2019;15(5):655-665.
53. McDade E, Wang G, Gordon BA, et al. Longitudinal cognitive and biomarker changes in dominantly inherited Alzheimer disease. *Neurology*. 2018;91(14):e1295-e1306.
54. Palmqvist S, Insel PS, Stomrud E, et al. Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease. *EMBO Mol Med*. 2019;11(12):e11170.
55. Mattsson-Carlgren N, Andersson E, Janelidze S, et al. Aβ deposition is associated with increases in soluble and phosphorylated tau that precede a positive Tau PET in Alzheimer's disease. *Sci Adv*. 2020;6(16):eaaz2387.
56. Mattsson-Carlgren N, Janelidze S, Palmqvist S, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain*. 2020;143(11):3234-3241.
57. Jack CR Jr, 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.
58. Jack CR, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *The Lancet Neurology*. 2013;12(2):207-216.
59. Jack CR, Knopman DS, Jagust WJ, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol*. 2010;9(1):119-128.
60. Lu M, Pontecorvo MJ. Aggregated Tau measured by visual interpretation of flortaucipir positron emission tomography and the associated risk of clinical progression of mild cognitive impairment and Alzheimer disease: results from 2 Phase III clinical trials. *JAMA Neurol*. 2021;78(4):445-453.
61. Palmqvist S, Janelidze S, Stomrud E, et al. Performance of fully automated plasma assays as screening tests for Alzheimer disease-related β-Amyloid status. *JAMA Neurol*. 2019;76(9):1060-1069.
62. Mielke MM, Dage JL, Frank RD, et al. Performance of plasma phosphorylated tau 181 and 217 in the community. *Nat Med*. 2022;28(7):1398-1405.
63. Bilgel M, An Y, Walker KA, et al. Longitudinal changes in Alzheimer's-related plasma biomarkers and brain amyloid. *Alzheimers Dement*. 2023;19(10):4335-4345.

SUPPORTING INFORMATION

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

How to cite this article: Cody KA, Du L, Studer RL, et al. Accuracy of plasma biomarkers to detect Alzheimer's disease proteinopathy prior to dementia. *Alzheimer's Dement*. 2025;21:e14570. https://doi.org/10.1002/alz.14570