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RESEARCH ARTICLE

Plasma Alzheimer's disease biomarker relationships with incident abnormal amyloid PET

Petrice M. Cogswell[1] | **Heather J. Wiste**[2] | **Stephen D. Weigand**[2] | **Terry M. Therneau**[2] | **Michael E. Griswold**[3] | **Joel B. Braunstein**[4] | **Tim West**[4] | **Philip B. Verghese**[4] | **Jonathan Graff-Radford**[5] | **Alicia Algeciras-Schimnich**[6] | **Val J. Lowe**[1] | **Christopher G. Schwarz**[1] | **Matthew L. Senjem**[1,7] | **Jeffrey L. Gunter**[1] | **David S. Knopman**[5] | **Prashanthi Vemuri**[1] | **Ronald C. Petersen**[2,5] | **Clifford R. Jack Jr.**[1]

[1]Department of Radiology, Mayo Clinic, Rochester, Minnesota, USA

[2]Department of Quantitative Health Sciences, Mayo Clinic, Rochester, Minnesota, USA

[3]Department of Data Science, University of Mississippi Medical Center, Jackson, Mississippi, USA

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

[5]Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA

[6]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

[7]Department of Information Technology, Mayo Clinic, Rochester, Minnesota, USA

Correspondence
Petrice M Cogswell, Mayo Clinic, 200 First St SW, Rochester, MN 55905, USA.
Email: Cogswell.petrice@mayo.edu

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Abstract

INTRODUCTION: Limited data exist on the utility of plasma biomarkers to predict incident abnormal amyloid positron emission tomography (PET). In this study we evaluate the association of plasma Alzheimer's disease (AD) biomarkers with amyloid PET progression among initially amyloid PET negative (A–) individuals.

METHODS: We included 290 A–, cognitively unimpaired Mayo Clinic Study of Aging participants. We estimated the association of each baseline plasma biomarker with progression from A– to A+ and with rate of amyloid PET change.

RESULTS: Interquartile range differences in amyloid beta 42/40, percent phosphorylated tau 217 (%p-tau217), and Amyloid Probability Score 2 were associated with 1.29 ($P = 0.09$), 1.38 ($P < 0.001$), and 1.20 ($P = 0.05$) increases, respectively, in the hazard of progression from A– to A+ and 0.27 ($P = 0.16$), 0.50 ($P = 0.007$), and 0.28 ($P = 0.15$) Centiloid/year increases, respectively, in annual rate of amyloid PET change.

DISCUSSION: Plasma %p-tau217 may be a useful screening tool to enrich for participants with increased likelihood of progressing from normal to abnormal amyloid PET in a primary prevention trial.

KEYWORDS
Alzheimer's disease, Alzheimer's disease biomarkers, amyloid positron emission tomography, Amyloid Probability Score 2, plasma amyloid beta 42/40, plasma phosphorylated tau 217

Highlights

- Plasma phosphorylated tau 217 was associated with amyloid positron emission tomography progression, negative to positive.
- The associations were weaker for amyloid beta 42/40 and Amyloid Probability Score 2.

- Age and apolipoprotein E ε4 carriership were also important predictors.
- These markers may be useful for enrichment of a primary prevention trial.

1 | BACKGROUND

Recent studies of Alzheimer's disease (AD) biomarkers have largely focused on the validation of plasma biomarkers for the detection of prevalent AD pathological changes—amyloid beta (Aβ) and neurofibrillary tangle deposition in the brain.[1] Cross-sectional analyses have shown that Aβ42/40 and phosphorylated tau (p-tau) analytes can detect amyloid positivity and are associated with amyloid load as measured via positron emission tomography (PET) and cerebrospinal fluid.[2–6] Fewer longitudinal studies have shown that baseline plasma Aβ42/40, p-tau181, and p-tau217 are each predictors of amyloid PET change.[7] Based on a growing body of literature supporting the correlation of plasma biomarkers with validated metrics of amyloid deposition in the brain, it has been proposed that plasma biomarkers may be used to screen for current amyloid pathology in clinical practice and clinical trials.[8,9]

Most of these prior studies have focused on the ability of plasma biomarkers to detect abnormal amyloid levels or continuous associations of biomarkers in which correlations are driven by those with abnormal biomarker levels. With the availability of anti-amyloid immunotherapies and evidence that clinical benefit may be greatest with early intervention,[10] it is of high interest to detect early AD-related changes and predict disease progression, including biomarker changes in cognitively unimpaired (CU) individuals and when biomarker values remain below defined positivity thresholds. Plasma Aβ42/40 and p-tau have shown good performance in detecting abnormal amyloid PET in CU individuals,[9,11,12] though performance in CU individuals was slightly lower than in cognitively impaired individuals.[11,13] Limited data on plasma biomarkers in CU individuals with normal amyloid PET suggest that abnormal plasma Aβ42/40 and p-tau217 are predictors of conversion from normal to abnormal amyloid PET.[14,15] Further work is needed to understand to what extent plasma biomarkers may detect early AD-related changes and if they have utility in prediction of disease progression in individuals with normal amyloid PET.

Plasma analyte performance depends on the assay, and in studying early disease changes in which signal is low relative to baseline variability, use of a high-performing assay is of particular value. Mass spectrometry assays of Aβ42/40 and p-tau217 (implemented here as the ratio of phosphorylated to non-phosphorylated p-tau multiplied by 100, %p-tau217) have performed well in head-to-head comparison of AD plasma biomarkers for determination of amyloid status and clinical progression.[4,9,16] However, limited data exist in community-based samples.

The goal of this study was to evaluate the association of baseline plasma Aβ42/40 and %p-tau217, as measured by high-resolution mass spectrometry, with progression from normal (A–) to abnormal (A+) amyloid PET and rates of amyloid PET change among CU individuals with initially normal amyloid levels in the Mayo Clinic Study of Aging (MCSA). This study will inform the ability of plasma biomarkers to detect early AD-related changes and their utility in early diagnosis and prognosis.

2 | METHODS

2.1 | Participants

This study included participants enrolled in the MCSA, a longitudinal population-based study of individuals residing in Olmsted County, Minnesota, USA. Participants in this study were ≥ 50 years of age, had a normal amyloid PET scan (Centiloid ≤ 22, standardized uptake values [SUVR] ≤ 1.48)[17] at baseline, a plasma sample drawn at the same visit, at least one subsequent visit with an amyloid PET scan, and plasma measures evaluated via mass spectrometry (Methods S1 and Figure S1 in supporting information). All participants had a clinical diagnosis of CU at baseline. Clinical diagnoses were determined by an expert panel based on established criteria.[18–20] We excluded 42 participants with missing or incomplete mass spectrometry plasma results.

2.2 | Standard protocol approvals, registrations, and patient consents

The study was approved by the Mayo Clinic and Olmsted Medical Center Institutional Review Boards and was performed in accordance with the ethical standards of the Declaration of Helsinki and its later amendments. All participants provided informed written consent.

2.3 | Plasma

Plasma samples were collected and stored via standard protocols, as previously described.[21] The primary plasma AD biomarkers of interest in this work were plasma Aβ42/40 and phosphorylated (p-tau217) to non-phosphorylated (np-tau217) ratio multiplied by 100 (%p-tau217) measured at C_2N Diagnostics via immune precipitated mass spectrometry, assay V1 (Methods S1).[9,22] We also evaluated the Amyloid Probability Score 2 (APS2), a score generated by C_2N via a statistical algorithm that uses both Aβ42/40 and %p-tau217 to provide a numerical value ranging from 0 to 100 that indicates an individual's likelihood of having abnormal amyloid PET.[22]

2.4 | PET imaging

Aβ PET was performed with Pittsburgh compound B[23] on GE scanners (models Discovery 690XT, Discovery RX, and Discovery MI) or Siemens scanners (Biograph Vision 600). Four 5 minute frames were acquired after a 40 minute uptake period. The frames were coregistered, averaged, and processed using in-house pipelines.[24,25] Harmonization between the PET scanners was performed according to the method of Joshi et al.[26] The Aβ PET meta-region of interest SUVRs were derived via the voxel number weighted average of the median uptake in each of the prefrontal, orbitofrontal, parietal, temporal, anterior and posterior cingulate, and precuneus regions normalized to the cerebellar crus gray matter. SUVR values were converted to Centiloid values.[27,28]

2.5 | Statistical methods

Kaplan–Meier curves were used to illustrate the probability of remaining A− over time by baseline plasma biomarker levels and to calculate the median time to A+ progression by baseline plasma biomarker levels. For these analyses, participants were divided into four groups based on the biomarker quartiles.

Cox proportional hazard models were used to evaluate the association of continuous baseline plasma biomarker levels with time to progression from normal amyloid PET levels (A−) to abnormal (A+) after adjusting for other covariates, and weights were included to account for oversampling of events in this study (Methods S1). Time was defined as years from the first visit with amyloid PET and plasma biomarkers (i.e., baseline or time 0) to the first visit with A+ (event) or the last visit with an A− PET scan (censored). We first fit a basic model including only baseline age, sex, and apolipoprotein E (*APOE*) ε4 carrier status as predictors, and then fit separate models adding each baseline plasma biomarker—Aβ42/40, %p-tau217, and APS2—to this basic model, one at a time. Participants with at least one *APOE* ε4 allele were considered *APOE* ε4 carriers. To aid in interpretation of covariate effects for age, Aβ42/40, %p-tau217, and APS2, which are continuous measures on very different scales, we summarize hazard ratios not in terms of a one-unit increase but in terms of the following contrasts: a 10 year increase in age and an interquartile range (IQR) difference for Aβ42/40 (i.e., 0.019 lower), %p-tau217 (i.e., 0.60 higher), and APS2 (i.e., 12 points higher).

We estimated the concordance (C) statistic for each of the Cox models, which is a measure of predictive discrimination similar to the area under the curve (AUC). C statistic values between 0.7 and 0.8 represent "acceptable," 0.8 to 0.9 "excellent," and > 0.9 "outstanding" discrimination.[29]

To evaluate the association of baseline plasma biomarker levels with continuous change in amyloid PET Centiloid after adjusting for other covariates, we fit linear regression models with generalized estimating equations (GEE) to account for the correlation among longitudinal measurements of amyloid PET within a person. While both linear mixed effects (LME) models and GEE models can be used to model mean values of a continuous outcome over time, we used GEE models for this study as they appropriately handle case weights, which were included to account for our sample selection (Methods S1). Amyloid PET Centiloid at each visit was the outcome. A first-order autoregressive (AR1) correlation structure was used to allow for higher correlations among amyloid PET measurements collected closer in time and semi-robust variance estimates were used. As above, we first fit a basic model including only age at the first visit with plasma and PET (i.e., the index or baseline visit), sex, *APOE* ε4 carrier status, time (years) from baseline, and interactions between each covariate and time as predictors. We then fit separate GEE models adding each baseline plasma biomarker—Aβ42/40, %p-tau217, and APS2—and the interaction with the plasma biomarker and time to this basic model. While these models estimate associations between the covariates and both amyloid PET levels at baseline as well as annual rates of amyloid PET change, we focus on the associations with rates of change in this work. The coefficients were summarized using the same contrasts as were used in the Cox models.

We also fit the Cox and GEE models including both baseline plasma Aβ42/40 and %p-tau217 as additive predictors and models with an interaction between baseline plasma Aβ42/40 and %p-tau217. Because the APS2 score incorporates information from both Aβ42/40 and %p-tau217, we did not fit models combining APS2 and another plasma biomarker.

Although the focus of this work is on the associations of baseline plasma biomarkers with change in amyloid PET, for reference, we

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature using traditional (e.g., PubMed) sources. Plasma Alzheimer's disease (AD) biomarkers have performed well in predicting prevalent abnormal amyloid positron emission tomography (PET; i.e., cross-sectional analyses). However, limited data exist on the utility of plasma biomarkers to predict incident abnormal amyloid PET. Prior cross-sectional and limited longitudinal studies have been cited.
2. **Interpretation**: Higher plasma percent phosphorylated tau 217 was associated with an increase in the hazard of progression to abnormal amyloid PET and the continuous rate of amyloid PET change, while the associations for plasma amyloid beta 42/40 and Amyloid Probability Score 2 were weaker. Plasma AD biomarkers may be informative about amyloid PET progression and a useful screening tool to enrich for participants with increased likelihood of progressing from normal to abnormal amyloid PET in a primary prevention trial.
3. **Future directions**: These results should be verified in more diverse populations with a range of medical comorbidities, which may affect plasma biomarker levels.

TABLE 1 Participant characteristics. Biomarker values are summarized for the baseline visit.

	Total (*N* = 290)	Remained A− (*n* = 147)	Progressed to A+ (*n* = 143)
Age, years			
Median (Q1, Q3)	71 (63, 76)	69 (62, 75)	73 (65, 77)
Range	52–92	53–92	52–92
Sex, *n* (%)			
Female	132 (46%)	66 (45%)	66 (46%)
Male	158 (54%)	81 (55%)	77 (54%)
Education, years, median (Q1, Q3)	14 (12, 16)	15 (12, 16)	14 (12, 16)
APOE ε4 genotype, *n* (%)			
ε4 non-carrier	211 (73%)	112 (76%)	99 (69%)
ε4 carrier	79 (27%)	35 (24%)	44 (31%)
Short Test of Mental Status, median (Q1, Q3)	36 (34, 37)	36 (34, 37)	36 (34, 37)
Amyloid PET, Centiloid, median (Q1, Q3)	12 (6, 16)	7 (4, 11)	15 (12, 18)
Aβ42/40, median (Q1, Q3)	0.093 (0.085, 0.104)	0.096 (0.088, 0.106)	0.091 (0.084, 0.102)
%p-tau217, median (Q1, Q3)	0.68 (0.37, 0.97)	0.60 (0.34, 0.91)	0.77 (0.38, 1.13)
Amyloid Probability Score 2, median (Q1, Q3)	10 (6, 18)	9 (5, 14)	13 (8, 24)
Number of amyloid PET scans, *n* (%)			
2	42 (14%)	16 (11%)	26 (18%)
3	126 (43%)	61 (41%)	65 (45%)
4	96 (33%)	60 (41%)	36 (25%)
5+	26 (9%)	10 (7%)	16 (11%)
Time from first amyloid PET + plasma visit to last amyloid PET visit, years, median (Q1, Q3)	7.1 (5.1, 8.9)	6.5 (5.1, 8.9)	7.7 (5.1, 9.1)

Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; PET, positron emission tomography; p-tau, phosphorylated tau.

also fit Cox models with baseline amyloid PET rather than a plasma biomarker as the predictor, along with age, sex, and *APOE* ε4 carrier status as predictors.

Finally, for comparison to existing literature, we also fit a version of all models without *APOE* ε4 carrier status as a predictor.

All analyses were performed using the R Language and Environment for Statistical Computing version 4.2.2. Kaplan–Meier, Cox proportional hazard models, and C statistics were computed using the survival package version 3.6-1. GEE models were computed using the geepack package version 1.3.9. Example code for the model fits is provided in Methods S1.

3 | RESULTS

3.1 | Participants

A total of 290 participants with baseline visits between January 16, 2009 and January 16, 2020 met inclusion criteria with median (IQR) age of 71 (63, 76) years and 158 (54%) male (Table 1). The median (IQR) time from the first amyloid PET and plasma visit to the last amyloid PET visit was 7.1 (5.1, 8.9) years. Table 1 also shows baseline descriptive characteristics of participants by amyloid PET progression status. Although direct comparisons are not appropriate because they don't account for time to progression and censoring, participants who progressed to A+ had higher amyloid PET Centiloid, lower plasma Aβ42/40, and higher %p-tau217 and APS2 at the baseline visit. For reference, the cross-sectional associations of the baseline plasma biomarker levels and baseline amyloid PET and plasma biomarker levels are shown in Figures S2 and S3 in supporting information.

3.2 | Probability of progression from normal to abnormal amyloid PET by baseline plasma biomarker quartile

Figure 1 shows the probability of remaining A− over time by baseline plasma biomarker quartile (see Table 1 and Figure S3 for distributions of the plasma biomarker data and quartile values). For each biomarker, the probability of remaining A− was lower for more abnormal biomarker levels and higher for more normal biomarker levels. The median time to A+ progression was 6.4 years for Aβ42/40 Q1 and 6.5 years for %p-tau217 Q4 and APS2 Q4, while the median time to A+

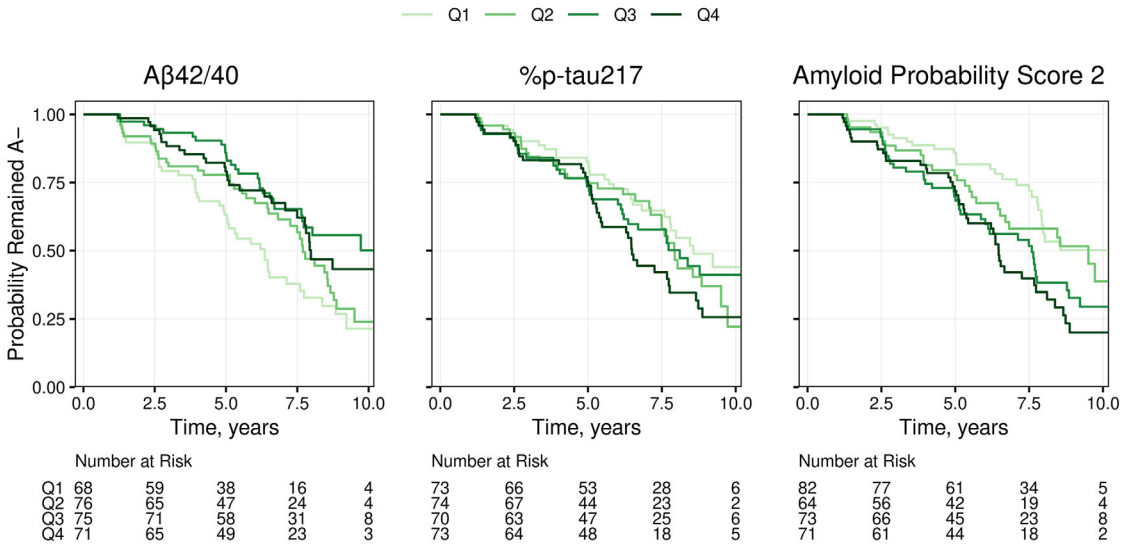

FIGURE 1 Kaplan–Meier estimates for probability remaining A− by baseline plasma biomarker quartile. Participants were divided into four groups for each plasma biomarker using the 25th, 50th, and 75th percentiles as cut points. For Aβ42/40, the quartiles were defined as Q1, < 0.085; Q2, 0.085 to < 0.093; Q3 0.093 to < 0.104; and Q4, ≥ 0.104. For %p-tau217, the quartiles were defined as Q1, < 0.37; Q2, 0.37 to < 0.68; Q3, 0.68 to < 0.97; Q4, ≥ 0.97. For the Amyloid Probability Score 2, the quartiles were defined as Q1, < 7; Q2, 7 to 10; Q3, 11 to 18; and Q4, ≥ 19. Note that lower values are more abnormal for Aβ42/40 while higher values are more abnormal for %p-tau217 and Amyloid Probability Score 2. %p-tau217, percent phosphorylated tau 217; Aβ, amyloid beta.

progression was 8.0 years for Aβ42/40 Q4, 8.6 years for %p-tau217 Q1, and > 10 years for APS2 Q1.

3.3 | Within-participant change in amyloid PET by baseline plasma biomarker quartile

Figure 2 shows individual trajectories for amyloid PET Centiloid over time for all participants grouped by baseline plasma biomarker quartile. Estimated mean (95%) amyloid PET Centiloid over time is shown with the black lines (gray-shaded regions) within each plasma biomarker quartile. Figure S4 in supporting information shows the four estimated mean lines for each plasma biomarker quartile on the same plot to better compare across quartile groups. While on average the amyloid PET Centiloid values increased more for the more abnormal quartiles, there was a broad range of change in amyloid PET across individuals within each plasma biomarker quartile.

3.4 | Associations with continuous baseline plasma biomarkers and progression from normal to abnormal amyloid PET

The left panel in Figure 3 summarizes the hazard ratios from the three Cox models for progression from A− to A+, each with a single baseline plasma biomarker predictor, based on the contrasts described above. After adjusting for age, sex, and *APOE* ε4 carriership, IQR differences in Aβ42/40, %p-tau217, and APS2 showed relative hazard estimates of 1.29 (95% confidence interval [CI]: 0.96–1.73, $P = 0.09$), 1.38 (95% CI: 1.16–1.65, $P < 0.001$), and 1.20 (95% CI: 1.00–1.43, $P = 0.05$), respectively (Table S1 in supporting information). A 10 year older age was associated with a 54% to 59% higher rate of progression to A+ ($P < 0.001$) while *APOE* ε4 carriership hazard ratios ranged from 1.41 (95% CI: 0.89–2.25, $P = 0.14$) and 1.37 (95% CI: 0.87–2.15, $P = 0.17$) in the Aβ42/40 and APS2 models, respectively, to 1.55 (95% CI: 1.01–2.38, $P = 0.04$) in the %p-tau217 model. The effect of sex was non-significant in all models ($P \geq 0.53$). The C statistics were very similar among the three plasma biomarker Cox models as well as the basic model, ranging from 0.67 to 0.69.

3.5 | Associations with continuous baseline plasma biomarker and continuous change in amyloid PET

The right panel in Figure 3 summarizes covariate-by-time regression coefficients from three linear regression models with GEEs, each with a single plasma predictor. The plot shows point and interval estimates for the mean difference in rates of amyloid PET accumulation. After adjusting for age, sex, and *APOE* ε4 carriership, IQR differences in Aβ42/40, %p-tau217, and APS2 showed estimated associations with rates of amyloid PET changes of 0.27 Centiloid/year (95% CI: −0.11 to 0.65, $P = 0.16$), 0.50 Centiloid/year (95% CI: 0.14–0.86, $P = 0.007$), 0.28 Centiloid/year (95% CI: −0.10 to 0.65, $P = 0.15$), respectively. Regression coefficients for these and other terms in the models are summarized in Table S2 in supporting information.

APOE ε4 carriership was associated with a 0.71 (95% CI: 0.04–1.38, $P = 0.04$) Centiloid/year greater increase in the annual rate of amyloid PET accumulation in the %p-tau217 model; the estimates were

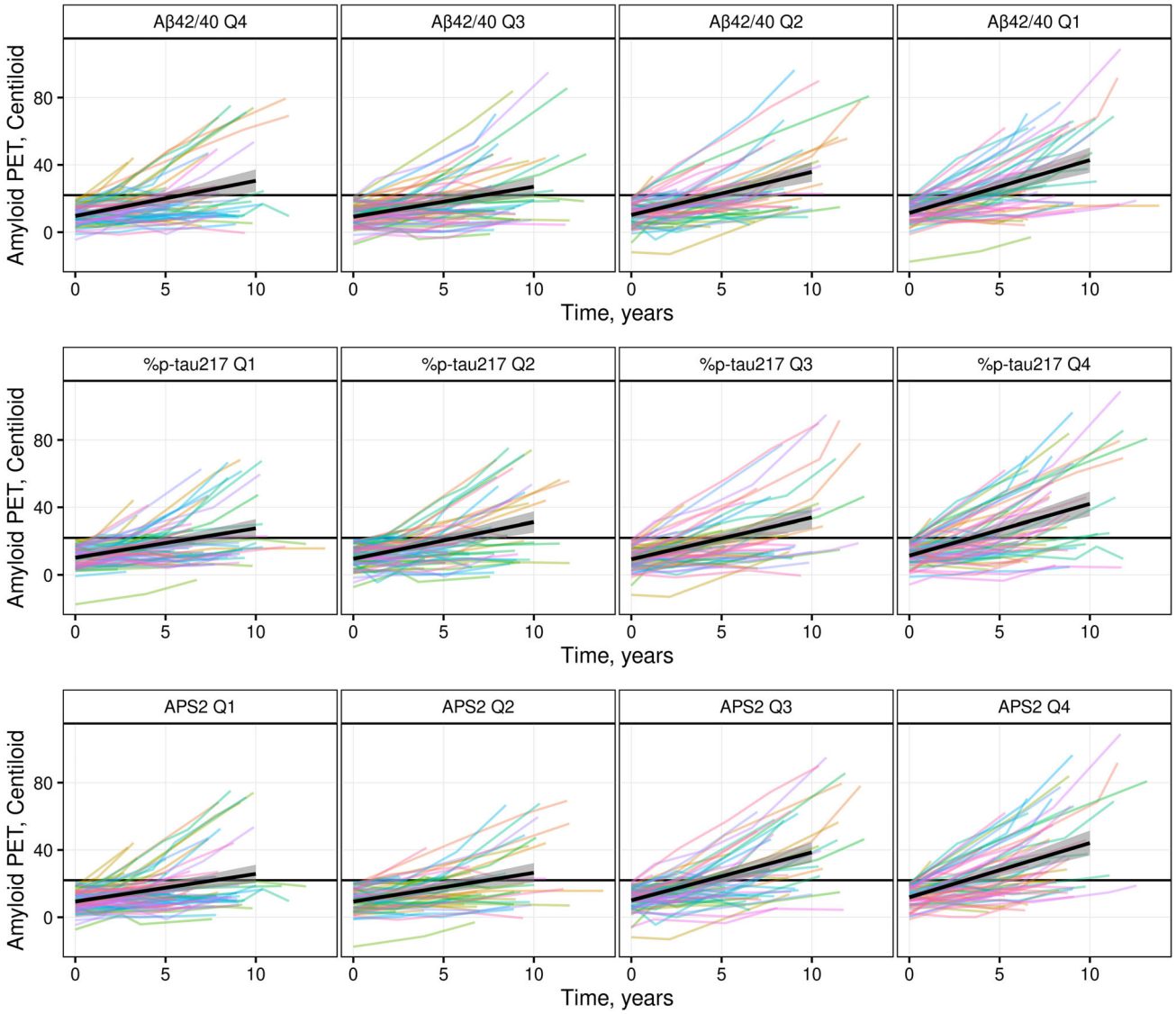

FIGURE 2 Line plots of individual amyloid PET Centiloid trajectories over time by baseline plasma biomarker quartile. Multiple colors were used to help differentiate trajectories of individual participants. Participants were divided into four groups for each plasma biomarker using the 25th, 50th, and 75th percentiles as cut points. For Aβ42/40, the quartiles were defined as Q1, < 0.085; Q2, 0.085 to < 0.093; Q3 0.093 to < 0.104; and Q4, ≥ 0.104. For %p-tau217, the quartiles were defined as Q1, < 0.37; Q2, 0.37 to < 0.68; Q3, 0.68 to < 0.97; Q4, ≥ 0.97. For the Amyloid Probability Score 2, the quartiles were defined as Q1, < 7; Q2, 7 to 10; Q3, 11 to 18; and Q4, ≥ 19. The solid black lines with gray-shaded regions represent the estimated mean (95% confidence interval) amyloid PET by time within each plasma biomarker quartile estimated from a linear regression model with generalized estimating equations. A separate model was fit for each plasma biomarker with amyloid PET Centiloid at each visit as the outcome and time, baseline plasma biomarker quartile, and the interaction of time and biomarker quartile as predictors. Figure S3 in supporting information shows the estimated mean curves for each biomarker on the same panel to facilitate comparison. %p-tau217, percent phosphorylated tau 217; Aβ, amyloid beta; APS2, Amyloid Probability Score 2; PET, positron emission tomography.

similar but slightly smaller in the Aβ42/40 and APS2 models (0.6 Centiloid/year, $P = 0.08$ for both). A 10 year older age was associated with a greater annual increase of 0.38 to 0.46 Centiloid/year ($P \leq 0.01$). Sex was not significantly associated with annual rate of amyloid PET accumulation in any of the models ($P \geq 0.23$).

The covariate effects for the Cox proportional hazard models and GEEs with and without *APOE* ε4 carriership and with versus without model weights are shown in Figures S5 and S6 in supporting information and are very similar to the main models described above.

3.6 | Combined plasma biomarker models

The effect sizes and *P* values from the Cox proportional hazard models and GEE models were quite similar for Aβ42/40 and %p-tau217 when both were included in the same model compared to including each separately in a model (Tables S3 and S4 in supporting information). Additionally, there was no evidence of an interaction between Aβ42/40 and %p-tau217 for any of the models ($P \geq 0.77$).

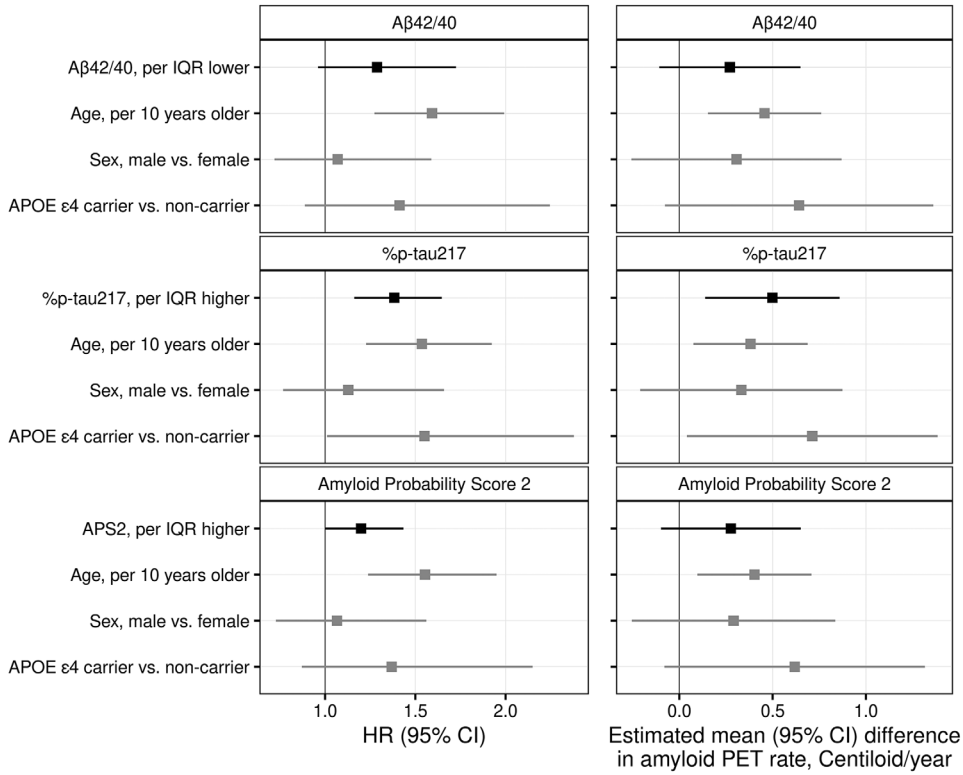

FIGURE 3 Forest plots summarizing baseline plasma biomarker associations with progression from normal amyloid PET (A–) to abnormal (A+) amyloid PET (left) and with annual rate of change in amyloid PET Centiloid (right). Hazard ratios (95% confidence intervals) were estimated from Cox proportional hazard models with time from A– to A+ as the outcome and baseline age, sex, *APOE ε*4 carriership, and baseline plasma biomarker level as predictors. Mean (95% confidence intervals) differences in annual rate of change in amyloid PET Centiloid were estimated from linear regression models with generalized estimating equations with amyloid PET Centiloid at each visit as the outcome and baseline age, sex, *APOE ε*4 carriership, baseline plasma biomarker level, time, and interactions with time and all other covariates as predictors. Separate models were fit for each plasma measure. Age associations are summarized for a 10 year difference. Plasma biomarker associations are summarized for an interquartile range difference: Aβ42/40, 0.019 lower; %p-tau217, 0.60 higher; APS2, 12 higher. %p-tau217, percent phosphorylated tau 217; Aβ, amyloid beta; *APOE*, apolipoprotein E; APS2, Amyloid Probability Score 2; CI, confidence interval; HR, hazard ratio; IQR, interquartile range; PET, positron emission tomography.

3.7 | Baseline amyloid PET model

The probability of remaining A– over time by baseline amyloid PET quartile (Figure S7 in supporting information) showed distinct separation between groups. After adjusting for age, sex, and *APOE ε4* carriership, an IQR difference in amyloid PET Centiloid showed a relative hazard estimate of 8.04 (95% CI: 5.24–12.33, $P < 0.001$) for progression from A– to A+ (Table S5 in supporting information). The C statistic from the model was 0.83.

4 | DISCUSSION

In this study, we evaluated the relationship between baseline plasma AD biomarkers, as measured by mass spectrometry assays, and change in amyloid PET among CU individuals with initially normal amyloid PET levels. Of particular interest was assessing associations with incident abnormal amyloid PET. Both the Cox and GEE models showed a similar pattern of associations; higher baseline plasma %p-tau217 was associated with an increase in the hazard of progression to abnormal amyloid PET and the continuous rate of amyloid PET change, while the associations for plasma Aβ42/40 and APS2 were weaker, albeit still showing trends toward association. Age and *APOE ε*4 carriership were also important predictors.

An IQR difference in the plasma markers resulted in effect sizes of 1.2 to 1.4 relative hazard of progression to A+ in the Cox models and estimated differences in rates of amyloid PET changes of 0.27 to 0.50 Centiloid/year in the GEE models. In both sets of models, %p-tau217 had a larger effect size than Aβ42/40 and APS2 and was the only plasma marker that reached statistical significance; %p-tau217 provided a better and more stable association with amyloid PET change than Aβ42/40 and APS2. Prior work by Schindler et al. showed that in individuals with normal amyloid PET levels, abnormal Aβ42/40 was associated with an increased risk of progressing to abnormal amyloid PET.[14] Recently published work by Janelidze et al.[15] showed statistically significant associations of both Aβ42/40 and %p-tau217 with subsequent rates of amyloid PET change among initially A– individuals. Our results support the idea that more abnormal baseline plasma

biomarker levels are associated with increased risk of progression from normal to abnormal amyloid PET. However, our Aβ42/40 results were less conclusive compared to prior work and compared to %p-tau217. The weaker associations and broader confidence intervals for Aβ42/40 and APS2 compared to %p-tau217 may at least in part be related to the high level of variability in Aβ42/40 compared to %p-tau217, as shown in prior work.[13] Variability and noise are challenges among all Aβ42/40 assays, and the C_2N assay used in this study is one of the best-performing assays to date.[2] Differences in our results compared to the similar Janelidze et al. study may be related to differences between the study samples—our population-based sample was on average older and had a broader age range. We defined A− as < 22 Centiloids versus < 40 in the comparison study, and correspondingly our cohort had on average lower %p-tau217 levels; the Janelidze et al. study also fit models based on Centiloid thresholds of 20 and 12 thresholds, and effect sizes decreased with the Centiloid threshold. Finally, we required baseline plasma and PET to be obtained on the same visit, whereas the PET may have been performed up to 1 year prior to the blood draw in the comparison study; a later blood draw would be expected to have a higher predictive power.

When both Aβ42/40 and %p-tau217 were included in the same model, the effect sizes for each remained similar to the individual models, and we did not see any evidence of an interaction between Aβ42/40 and %p-tau217. These findings support prior work suggesting that both Aβ42/40 and %p-tau217 may be useful, and contributory, in predicting amyloid PET progression among A−.[9] Additionally, there was very little correlation between Aβ42/40 and %p-tau217 among the participants in this study (rho = −0.04) indicating that these biomarkers may be providing independent information in predicting amyloid PET levels among participants with normal amyloid PET. The APS2 is one way to combine the information of these two biomarkers. However, in this application the performance of the APS2 was not superior to %p-tau217 alone.

With the clinical focus on early disease detection and prevention, these results would be applicable to a primary prevention trial with the aim to prevent progression from normal to abnormal amyloid PET. The ≈ 20% to 40% increase in hazard of progression for each of the plasma biomarker contrasts in our models suggests that if these plasma biomarkers were applied as a screening test in a primary prevention trial, a 20% to 40% reduction in sample size could be achieved.[30]

Age and *APOE* ε4 carriership were important independent predictors of amyloid PET progression in our study. Although the *APOE* ε4 effect only reached statistical significance in the %p-tau217 models, the effect size was similar and with broad confidence intervals for all plasma biomarker models. Prior work has similarly shown the value of age and *APOE* ε4 carriership for predicting amyloid status.[14,31] However, the additive value of plasma biomarkers in models with age and *APOE* ε4 carriership has been variable. In this work, the Cox and GEE models showed there was an association of baseline plasma biomarkers with amyloid PET progression, after adjusting for age, sex, and *APOE* ε4 carriership. Despite reasonable effect sizes for the plasma biomarkers, the C statistic of these models was similar to that of the base model. In some studies *APOE* ε4 carriership has not significantly changed accuracy for prediction of amyloid positivity beyond that of Aβ42/40 and p-tau217,[11] which may be due to the close association of *APOE* ε4 carriership and amyloid status being better captured in later clinical and biomarker stages than included in this study. Sex did not have a significant effect in any model.

The models with baseline amyloid PET as a predictor were included for reference, and as in prior work indicate that amyloid PET values in the normal range are strongly associated with future change in amyloid PET,[24] with very large effect sizes relative to those of the plasma biomarkers. Gradations of subthreshold amyloid PET levels are meaningful; those closer to the positivity threshold are more likely to subsequently progress.

Cross-sectional biomarker associations were not the focus of this study as all participants in the study had normal levels of amyloid PET at baseline. However, correlations between the plasma biomarkers and amyloid PET are shown in Figure S3. The associations between each of the plasma biomarkers and amyloid PET were low, and lower than seen in prior studies that include the full disease spectrum and individuals with abnormal amyloid PET.[5] When evaluating associations in the subthreshold biomarker levels, noise (e.g., analytic and biological variability) competes with the relevant signal.

Strengths of this study include the use of one of the best-performing plasma biomarker assays to date, correlation with serial PET, and a community-based sample. There are also limitations. The cohort is predominantly White; we plan to obtain data in a more diverse sample in future work. The sample is a subset of the population-based MCSA. To adjust for potential biases in sample selection, weights were used in the models, and the weighted and unweighted models showed very similar results (Figure S6).

In conclusion, we evaluated the association of baseline plasma Aβ42/40, %p-tau217, and APS2 with progression from A− to A+ and rates of amyloid PET change among A− CU individuals. The pattern of results of the Cox and GEE models were similar—plasma %p-tau217 showed clear associations with rate of progression from A− to A+ and change in amyloid PET rate among A− individuals, while associations for plasma Aβ42/40 and APS2 showed trends. Age and *APOE* ε4 carriership were also related to rate of progression from A− to A+ and change in amyloid PET rate among A− individuals. These results suggest utility of plasma biomarkers, particularly %p-tau217, as screening tools to enrich for participants with increased likelihood of progressing from normal to abnormal amyloid PET in a primary prevention trial.

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CONFLICT OF INTEREST STATEMENT

P.M.C. has received honoria from Eisai Inc. and Kaplan for medical education presentations. H.J.W., S.D.W., T.M.T., M.E.G., and J.L.G. have no disclosures. J.B.B., T.W., and P.B.V. are paid employees of C_2N Diagnostics. J.G.R. serves as an assistant editor for *Neurology* and receives research support from the NIH. A.A.-S. has participated on

advisory boards for Roche Diagnostics, Fujirebio Diagnostics, and Siemens Healthineers. V.J.L. is a consultant for AVID Radiopharmaceuticals, Eisai Co. Inc., Bayer Schering Pharma, GE Healthcare, Piramal Life Sciences, and Merck Research, and receives research support from GE Healthcare, Siemens Molecular Imaging, AVID Radiopharmaceuticals, and NIH (NIA, NCI). C.G.S. receives research support from the NIH. M.L.S. holds stock in medical related companies, unrelated to the current work: Align Technology, Inc., LHC Group, Inc., Medtronic, Inc., Mesa Laboratories, Inc., Natus Medical Inc., and Varex Imaging Corporation. He has also owned stock in these medical related companies within the past 3 years, unrelated to the current work: CRISPR Therapeutics, Gilead Sciences, Inc., Globus Medical Inc., Inovio Biomedical Corp., Ionis Pharmaceuticals, Johnson & Johnson, Medtronic, Inc., Oncothyreon, Inc., Parexel International Corporation. D.S.K. served on a data safety monitoring board for the DIAN study. He serves on a data safety monitoring board for a tau therapeutic for Biogen but receives no personal compensation. He was a site investigator in the Biogen aducanumab trials. He is an investigator in a clinical trial sponsored by Lilly Pharmaceuticals and the University of Southern California. He serves as a consultant for Samus Therapeutics, Third Rock, Roche, and Alzeca Biosciences but receives no personal compensation. He receives research support from the NIH. P.V. received speaker fees from Miller Medical Communications, Inc. and receives research support from the NIH. R.C.P. serves as a consultant for Roche Inc., Merck Inc., and Biogen, Inc. He serves on the data safety monitoring board for Genentech, Inc and receives royalties from Oxford University Press and UpToDate. C.R.J. receives no personal compensation from any commercial entity. He receives research support from NIH and the Alexander Family Alzheimer's Disease Research Professorship of the Mayo Clinic. Author disclosures are available in the supporting information.

DATA AVAILABILITY STATEMENT

Data from the Mayo Clinic Study of Aging are available to qualified academic and industry researchers by request to the MCSA Executive Committee (https://www.mayo.edu/research/centers-programs/alzheimers-disease-research-center/research-activities/mayo-clinic-study-aging/for-researchers/data-sharing-resources).

CONSENT STATEMENT

The study was approved by the Mayo Clinic and Olmsted Medical Center Institutional Review Boards and was performed in accordance with the ethical standards of the Declaration of Helsinki and its later amendments. All participants provided informed written consent.

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SUPPORTING INFORMATION

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

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