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RESEARCH ARTICLE

Clinical validation of the PrecivityAD2 blood test: A mass spectrometry-based test with algorithm combining %p-tau217 and Aβ42/40 ratio to identify presence of brain amyloid

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Abstract

BACKGROUND: With the availability of disease-modifying therapies for Alzheimer's disease (AD), it is important for clinicians to have tests to aid in AD diagnosis, especially when the presence of amyloid pathology is a criterion for receiving treatment.

METHODS: High-throughput, mass spectrometry-based assays were used to measure %p-tau217 and amyloid beta (Aβ)42/40 ratio in blood samples from 583 individuals with suspected AD (53% positron emission tomography [PET] positive by Centiloid > 25). An algorithm (PrecivityAD2 test) was developed using these plasma biomarkers to identify brain amyloidosis by PET.

RESULTS: The area under the receiver operating characteristic curve (AUC-ROC) for %p-tau217 (0.94) was statistically significantly higher than that for p-tau217 concentration (0.91). The AUC-ROC for the PrecivityAD2 test output, the Amyloid Probability

Matthew R. Meyer and Kristopher M. Kirmess contributed equally to this study.

Score 2, was 0.94, yielding 88% agreement with amyloid PET. Diagnostic performance of the APS2 was similar by ethnicity, sex, age, and apoE4 status.

DISCUSSION: The PrecivityAD2 blood test showed strong clinical validity, with excellent agreement with brain amyloidosis by PET.

KEYWORDS
Alzheimer's, amyloid beta, blood biomarker, clinical validity, diagnostic, p-tau217

1 | BACKGROUND

Alzheimer's disease (AD) accounts for the vast majority (60% to 80%) of dementia cases, and by 2060 AD cases in the United States are expected to reach greater than 13 million.[1,2] As disease-modifying drugs that can specifically treat the underlying causes of AD become available, a great need will exist for accurate, cost-effective, and widely accessible diagnostic tools that can aid in the differential diagnosis of AD and AD-related dementias. In July 2023, lecanemab (LEQEMBI), an anti-beta amyloid monoclonal antibody (mAb), received traditional approval by the US Food and Drug Administration (FDA) for the treatment of patients with mild cognitive impairment (MCI) or mild dementia with confirmed presence of amyloid beta (Aβ) pathology prior to initiating treatment.[3] Another anti-beta amyloid mAb, aducanumab (ADUHELM), received accelerated approval in 2021,[4] and other potentially disease-modifying therapies are on the horizon.[5] As diagnostic tools become available, they can help not only to identify patients suitable to receive approved therapies but also to expedite enrollment of participants with confirmed amyloid pathology in clinical trials of novel, experimental AD therapeutics.[6]

The presence of amyloid pathology is an essential criterion to determine treatment eligibility for novel AD-modifying drugs. Amyloid positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) analysis are US Food and Drug Administration (FDA)-approved strategies for the detection of AD pathological features. However, these diagnostic methods are suboptimal for screening the large number of patients that need to be evaluated for treatment eligibility. Amyloid PET is expensive (PET costs for research use are currently estimated at around $6500), exposes patients to radiation, and is not easily accessible outside of urban centers.[7,8] Compared to CSF biomarkers, which require technical expertise, blood sampling is more convenient, provides greater access, is less costly, and has lower risk of adverse procedural complications. Blood biomarkers that quantify the Aβ42/40 ratio and/or phosphorylated tau (p-tau) species have the potential to address this unmet need.[9] An extensive body of data demonstrates that these biomarkers correlate with amyloid PET and discriminate AD from non-AD clinical phenotypes, which supports their clinical use in the evaluation of amyloid pathology and AD treatment eligibility.[10–12]

The commercially available diagnostic blood test for AD pathology, PrecivityAD (Aβ42/40, apolipoprotein E [apoE] proteotype, age), has strong clinical performance for identifying brain amyloid status when compared to amyloid PET.[6] Herein, we describe a liquid chromatography-mass spectrometry (LC-MS/MS) analytical method for quantifying plasma tau peptides that are phosphorylated at threonine 217 (p-tau217), not phosphorylated at threonine 217 (np-tau217), and the percentage of p-tau217 relative to np-tau217 (the %p-tau217). Using this analytical method and platform, we report analysis of %p-tau217 and Aβ42/40 ratio in 583 study participant samples from the PARIS study, a substudy of the Imaging Dementia—Evidence for Amyloid Scanning (IDEAS) study[13] and the MissionAD study.[14] We found that the plasma %p-tau217 identified brain amyloid pathology with higher sensitivity and specificity than the plasma p-tau217 concentration and that combining the plasma %p-tau217 with the Aβ42/40 ratio into an algorithm to produce the Amyloid Probability Score 2 (APS2) improved the overall robustness of the model predictions.

2 | METHODS

2.1 | Sample collection and analysis

For this cohort study, the PARIS and MissionAD studies were reviewed and approved by central or local ethics and safety review committees or boards. All participants (or their legally authorized representative) reviewed and signed an approved informed consent document. This study is reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline.

2.2 | PARIS study

Participants were prospectively enrolled in the PARIS study, a C_2N-sponsored substudy of the IDEAS study (ClinicalTrials.gov Identifier: NCT02420756).[6] IDEAS evaluated the clinical utility of amyloid PET in Medicare beneficiaries with MCI or dementia meeting Appropriate Use Criteria for amyloid PET.[13] Participants enrolled between October 1, 2018, and January 4, 2019, were included in the PARIS Discovery cohort analysis presented here. Sample collection protocol for PARIS study participants has been described elsewhere.[6] Briefly, participants from the IDEAS study who were within 18 months from time of amyloid PET and who had indicated willingness to participate in future studies were invited to join the PARIS study. Participants provided a blood sample that was processed immediately and shipped to C_2N for frozen storage. While Aβ concentrations had already been

analyzed for the PARIS individuals as part of a previous study, for this study we committed to measuring both Aβ and p-tau217 at the same time, so we re-analyzed the samples for Aβ.[6] Of the 249 participants in the discovery phase of the PARIS study who had PET image analysis, 224 had sufficient sample volume to allow for a second analysis of Aβ concentrations as well as tau measurements by LC-MS/MS. The correlation was strong between the two analyses of the Aβ samples, Pearson's correlation coefficients were 0.87, 0.86, and 0.62 for Aβ40, 42, and the Aβ42/40 ratio, respectively.

2.3 | MissionAD study

The MissionAD program consisted of two global phase 3 studies (ClinicalTrials.gov Identifier: NCT02956486) that tested the safety and efficacy of elenbecestat (BACE inhibitor) in participants with MCI due to AD or mild AD dementia with amyloid positivity confirmed by PET visual read or CSF test.[14] A total of 359 individual baseline screening plasma samples were selected which resulted in approximately 50% positive prevalence of amyloid PET results defined by quantitative amyloid PET. While there was no planned overlap with the study participants from the clinical validation of the PrecivityAD test, 90 of the 359 study participants (25%) were also part of the previous analysis.[6] For analyses of MissionAD samples, investigators remained blinded to all demographic data and PET results until plasma analysis was completed and data were shared. Race and ethnicity were self-reported at time of enrollment into the studies according to the US Census Bureau race and ethnicity categories for use as demographic variable in the analysis (PARIS) and per FDA guidance (MissionAD). The eligibility criteria for the PARIS and MissionAD cohorts have been described.[6]

2.4 | Amyloid PET image analyses

Amyloid PET image analyses of PARIS Discovery and MissionAD have been described elsewhere.[6] Briefly, in the PARIS Discovery cohort, three amyloid PET tracers were used: [^{18}F]florbetapir (Amyvid; Lilly Diagnostics), [^{18}F]florbetaben (Neuraceq; Life Molecular Imaging), or [^{18}F]flutemetamol (Vizamyl; GE Healthcare). In the IDEAS study, amyloid PET scans were interpreted visually by local radiologists and nuclear medicine physicians. However, for the PARIS study, these amyloid PET images were obtained and processed centrally by the American College of Radiology by two board-certified radiologists with amyloid PET tracer-specific training. Images were quantitatively assessed by standardized uptake value ratio (SUVR) and Centiloid (CL). In the MissionAD data set analyzed for this study, two amyloid PET tracers were used, [^{18}F]florbetapir and [^{18}F]florbetaben, and amyloid PET images were processed centrally by Bioclinica. Image analyses, including SUVR to CL conversion, are described in Hu et al.[6] For the purposes of determining eligibility for MissionAD, PET visual read was used. Based on published evidence, we defined a priori amyloid positivity for our analyses as a CL value greater than 25, which is a more sensitive threshold for brain amyloid plaques than visual read.[15,16]

RESEARCH IN CONTEXT

1. **Systematic review**: We reviewed the literature on blood biomarkers for brain amyloid pathology using PubMed. A growing body of evidence from academic centers shows the value of the plasma Aβ42/40 ratio and tau phosphorylated at threonine 217 for identifying brain amyloid pathology.
2. **Interpretation**: We showed that an algorithm (PrecivityAD2 blood test) combining the plasma Aβ42/40 ratio and the percent phosphorylation of tau at threonine 217 can be used to identify brain amyloidosis with outstanding diagnostic performance. Additionally, we observed that measuring the concentration of phosphorylated tau normalized to the amount of tau available to be phosphorylated at the individual level had a diagnostic performance superior to just the concentration of phosphorylated tau at threonine 217.
3. **Future directions**: Additional data from orthogonal cohorts, more diverse participants, and participants at different stages in the disease process will help further establish the performance of the PrecivityAD2 test as a high-accuracy test to identify Alzheimer's disease pathology.

2.5 | Plasma sample analyses

Blood sample collection methods were similar between PARIS and MissionAD studies and reviewed in Hu et al. and West et al.[6,17]

2.5.1 | Plasma Aβ42/40 ratio determination

Mass spectrometry-based plasma Aβ42/40 ratio determination has been described elsewhere.[6,18] Briefly, on the day of sample analysis, participant plasma, quality control (QC) plasma samples, frozen calibrators (United States Pharmacopeia (USP)-traceable amino acid analysis performed), and uniformly labeled ^{15}N full-length Aβ40 and Aβ42 internal standards (USP-traceable amino acid analysis performed) were thawed. All calibrators, QC samples, and participant samples were treated identically throughout sample processing and analysis. To each 450 μL of sample, an immunoprecipitation buffer containing known amounts of ^{15}N-Aβ40 and ^{15}N-Aβ42 was added prior to Aβ immunocapture. After immunocapture, the Aβ bound to magnetic beads was digested using Lys-N metalloendoprotease (Thermo Fisher Scientific, Waltham, MA, USA). The Lys-N digested Aβ species into Aβ peptides Aβ28-40 and Aβ28-42. The Aβ digests were further purified and reconstituted in 10% acetonitrile/10% formic acid prior to injection onto the LC-MS/MS system. The Aβ28-40 and Aβ28-42 were separated, identified, and quantified using LC-MS/MS (Acquity UPLC

M-Class liquid chromatography unit [Waters Corp.] interfaced to a Thermo Fisher Scientific Fusion Lumos Mass Spectrometer).

The total peak area for the endogenous ^{14}N Aβ peptides was divided by the total peak area for the exogenously added, uniformly labeled ^{15}N Aβ peptide internal standards to obtain a peak area ratio (PAR). The PAR for each Aβ peptide indicated the peptide concentration based on an external standard curve that spanned the expected physiological range, and Aβ peptide concentrations (pg/mL) were determined from the standard curve. The measured concentrations were expressed as the plasma Aβ42/40 ratio. The LC-MS/MS data were assembled and assessed using TraceFinder 4.1 General Quan software (Thermo Fisher Scientific).

2.5.2 | Plasma p-tau217 and np-tau217 quantification and %p-tau217 calculation

On the day of use, frozen calibrators, QC samples, test samples, Trypsin endopeptidase (Millipore Sigma, St. Louis, MO, USA), and tryptic C-terminally labeled ^{15}N,^{13}C p-tau internal standard (IS, Biosynth, Gardner, MA, USA) were thawed. Once thawed, buffers required for immunoprecipitation were added to each 2-mL well on a 96-well plate. 900 µL of each sample were placed in their respective well of the 96-well plate (Thermo Fisher Scientific). A slurry of conjugated magnetic beads containing an anti-tau antibody was then added to each well on the plate for immunocapture of tau proteins. After 60 min of immunocapture, the magnetic beads bound to tau were removed from their respective matrix and washed with PBS to reduce non-specifically bound contaminants prior to enzymatic digestion.

Once the tau bound magnetic beads were washed, they were placed in a temperature-controlled buffer containing Trypsin endopeptidase, where tau species were digested into peptides. After digestion, the reaction was quenched with formic acid. The digested samples were further purified using reverse phase solid phase extraction (SPE) to remove contaminants. The 96-well collection plate was dried under vacuum before samples were reconstituted with the addition of 0.5% ACN/0.1% formic acid.

The 96-well collection plate containing the resolubilized tau peptides was placed in a temperature-controlled autosampler within the LC system (Waters Acquity UPLC M-Class). Of the reconstituted tau peptides, 4.5 μL was injected onto the analytical LC column, where it was separated, identified, and quantified using LC-MS/MS (Waters Acquity UPLC M-Class LC unit interfaced to a Thermo Scientific Fusion Lumos Tribrid Mass Spectrometer). Tau concentrations were calculated by the summation of peak areas from monitored product ions that result after fragmentation of their respective precursor ion. Precursor ions derived from endogenous and exogenous (known added amounts) stable isotope labeled IS peptides that correspond to phosphorylated and non-phosphorylated tau peptides that contain amino acids 212 to 221. After summation, the total peak area for the endogenous tau peptides was divided by the total peak area for the corresponding exogenously added IS peptides to obtain a PAR. The PAR of the endogenous peptides to their respective labeled IS peptides was determined in unknown samples, and the concentration of each analyte was calculated from calibration curves. These data were assembled and assessed by TraceFinder 5.1 General Quan software (Thermo Fisher Scientific).

%p-tau217 is calculated using the following equation:

$$\%\mathrm{p}-tau217 = \mathrm{p}-tau217/np-tau217 * 100\%.$$

Each 96-well plate included a six-point calibrator curve (including the matrix blank), six QC samples, and participant plasma samples. Calibrators were prepared by spiking 2% recombinant human serum albumin (rHSA) with known amounts of a chemically synthesized, 51-amino-acid peptides (residues 171 to 221 of the Tau-441 protein) that were phosphorylated or not phosphorylated at threonine-217. Isotope labeled, IS peptides were also prepared that contained [U-^{13}C, ^{15}N] Arg at position 221. The concentrations of the stock solutions used to prepare the calibrators and ISs were value-assigned by USP-traceable amino acid analysis. The analytical measurement range of the calibration curves was designed to span the reference ranges of the p-tau217 and np-tau217 measurands.

Six QC samples covered the anticipated high, medium, and low plasma concentrations for p-tau217 and np-tau217 peptides (three QC samples for p-tau217 and three for np-tau217; for concentrations and coefficient of variation of the QC samples please see Table S1). The QC samples were analyzed once per run and randomly and evenly distributed throughout the unknown samples. QC multirules were applied: The run was rejected when one or more QC concentrations fell outside of three standard deviations (SDs), or two or more QC sample concentrations exceeded two SDs, or if the range of two or more QCs exceeded four SDs (1-3S, 2-2S, R-4S rule). Total allowable error for np-tau217 was defined as 30% or 15 pg/mL, whichever was greater. Total allowable error for p-tau217 was set at the greater of 30% or 0.3 pg/mL.

2.6 | Statistical analysis

All data analysis was performed using R version 4.3.1 (The R Foundation for Statistical Computing). A fully formed R script was generated based on dummy data and executed upon final data collection, including the following pre-specified analyses: comparison of %ptau217 to p-tau217 concentration, setup of the PrecivityAD2 model and cross-validation by original cohort, definition of optimal cut points by Youden. Receiver operating characteristic (ROC) analyses, including the calculation of the area under the ROC curve (AUC-ROC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were conducted using the pROC package for R,[19] and optimal cutoff values were determined by the Youden index (maximized sensitivity and specificity of the predictive test). Confidence intervals (95% CI) for AUC and comparisons between ROCs were calculated using the DeLong method.[20] Logistic regression models (LRMs) were used to predict amyloid positivity based on biomarker data using amyloid positivity by PET as the dependent variable. The robustness of LRMs was compared using the Akaike information criterion (AIC), Bayesian information criterion (BIC), and likelihood ratio test (LRT).[21] Comparisons of accuracy and study participant distribution by

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TABLE 1 Demographics.

	CL < 25 (*N* = 276)	CL > 25 (*N* = 307)	All data (*N* = 583)
Age			
Mean (SD)	70.7 (6.8)	74.3 (6.5)	72.6 (6.9)
Range	55–85	55–91	55–91
Sex			
Female	136 (49.3%)	148 (48.2%)	284 (48.7%)
Race			
American Indian or Alaska Native	0 (0%)	1 (0.3%)	1 (0.2%)
Asian	1 (0.4%)	2 (0.7%)	3 (0.5%)
Black or African American	23 (8.3%)	9 (2.9%)	32 (5.5%)
Native Hawaiian or other Pacific Islander	0 (0%)	0 (0%)	0 (0%)
Unknown	3 (1.1%)	6 (2%)	9 (1.5%)
White	245 (88.8%)	287 (93.5%)	532 (91.3%)
Ethnicity			
Hispanic or Latino	132 (47.8%)	45 (14.7%)	177 (30.4%)
Not Hispanic or Latino	138 (50.0%)	254 (82.7%)	392 (67.2%)
Not Reported	1 (0.4%)	2 (0.7%)	3 (0.5%)
Unknown	5 (1.8%)	6 (2%)	11 (1.9%)
Diagnosis			
MCI	248 (89.9%)	228 (74.3%)	476 (81.6%)
Dementia	28 (10.1%)	79 (25.7%)	107 (18.4%)
MMSE			
Percent MMSE = 27 to 30 [*N*]	54.5% [138]	46.6% [122]	50.5% [260]
Mean (SD)	26.4 (2.6)	25.7 (3.6)	26.1 (3.2)
Min	7	1	1
Max	30	30	30
Missing data	23	45	68
ApoE			
ε2/ε2	0 (–)	0 (–)	0 (–)
ε2/ε3	27 (9.8%)	15 (4.9%)	42 (7.2%)
ε2/ε4	6 (2.2%)	10 (3.3%)	16 (2.8%)
ε3/ε3	192 (69.8%)	106 (34.6%)	298 (51.3%)
ε3/ε4	47 (17.1%)	131 (42.8%)	178 (30.6%)
ε4/ε4	3 (1.1%)	44 (14.4%)	47 (8.1%)
ε4 carrier	56 (20.4%)	185 (60.5%)	241 (41.5%)
Missing data	1	1	2
PET Imaging			
Visual read positive	27 (9.8%)	275 (89.6%)	302 (51.8%)
Centiloid positive	0 (–)	307 (100%)	307 (52.7%)
Centiloid mean (SD)	1.0 (11.7)	81.4 (33.3)	43.3 (47.6)
Centiloid min	−29.2	25.2	−29.2
Centiloid max	23.4	177.1	177.1
Plasma %p-tau217			
Mean (SD)	3.02 (3.10)	11.14 (6.42)	7.30 (6.53)
Min	0.63	1.02	0.63
Max	33.47	37.01	37.01

(Continues)

TABLE 1 (Continued)

	CL < 25 (*N* = 276)	CL > 25 (*N* = 307)	All data (*N* = 583)
Plasma p-tau217 concentration (pg/mL)			
Mean (SD)	1.57 (1.90)	6.24 (4.31)	4.03 (4.11)
Min	0.65	0.65	0.65
Max	17.24	36.88	36.88
Below LOD for p-tau217 [*N*]	149	11	160
Plasma np-tau217 concentration (pg/mL)			
Mean (SD)	50.63 (23.83)	55.39 (19.54)	53.14 (21.79)
Min	10.71	20.53	10.71
Max	294.76	149.01	294.76
Plasma Aβ42/40			
Mean (SD)	0.1001 (0.0150)	0.0878 (0.0101)	0.0936 (0.0141)
Min	0.0639	0.0633	0.0633
Max	0.1632	0.1429	0.1632

Note: Demographics of combined cohort as well as by Centiloid > 25 status.
Abbreviations: CL, Centiloid; LOD, limit of detection; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, positron emission tomography.

concordance/discordance class were performed using Fisher's exact test for count data, and the 95% CIs on accuracy, sensitivity, specificity, PPVs, and NPVs were calculated using Wilson's method.

3 | RESULTS

3.1 | Demographic and clinical characteristics

Blood samples from study participants enrolled in PARIS Discovery (*N* = 224) and MissionAD (*N* = 359) were analyzed for both Aβ and tau217. Due to the similarity in sample collection, baseline demographics, and clinical diagnosis (majority MCI) between the two cohorts, we combined the two cohorts into one, similarly to how the PrecivityAD blood test was clinically validated.[6] The demographics of the combined cohort by amyloid status can be found in Table 1, and Table S2 shows the demographics split by the original two cohorts. In the combined cohort, the mean age was 72.6 (SD 6.9) years, with 49% female representation. While the majority of the participants were white (>90%), almost 50% of study participants from MissionAD were of Hispanic or Latino origin, leading to 30% representation of this ethnicity in the combined cohort. All individuals were symptomatic, with a diagnosis of either MCI (81.6%) or dementia (18.4%), 41.5% had one or more copies of apoE4, and 52.7% of the participants were positive by amyloid PET as defined by a CL score greater than 25.

3.2 | Value imputation for samples with p-tau217 below limit of detection

For samples where the p-tau217 concentration fell below the limit of detection (LOD, 1.3 pg/mL), the concentration was imputed as half the LOD (0.65 pg/mL) and the %p-tau217 was calculated based on this imputed p-tau217 concentration. The rigorously set LOD is based on Clinical and Laboratory Standards Institute (CLSI) guidelines. Thus, in instances where the mass spectrometer detects both the presence of the internal standard and the p-tau217 peptide but the peak area of the endogenous peptide falls below the LOD, imputation was used. Since the p-tau217 concentration can be especially low in healthy people, we believe that it is still important to provide an actionable %p-tau217 value in cases where the concentration of p-tau217 falls below the limit of detection but the presence of the peptide is confirmed by the mass spectrometer. While no samples in this dataset were below the LOD for np-tau217 or above limit of quantitation (ALQ) for either of the tau217 measures, we decided a priori not to impute for those cases.

3.3 | Analyte measurement in blood samples

The concentrations of Aβ peptides (Aβ42 and 40, one sample aliquot) and p-tau217 peptides (another sample aliquot) were measured in separate aliquots from the same study participants. Aβ40 concentrations were not significantly different by amyloid status (CL < 25: 483 pg/mL, SD 101; CL > 25: 487 pg/mL, SD: 93.2, $p = 0.59$), but Aβ42 concentrations were significantly lower in the amyloid-positive participants (CL < 25: 48 pg/mL, SD 11; CL > 25: 43 pg/mL, SD: 8.9, $p < 0.0001$). However, as has been observed previously, the diagnostic differentiation of Aβ42 is improved when it is combined with Aβ40 to create the Aβ42/40 ratio, with the Aβ42/40 ratio being 0.12-fold lower in amyloid-positive compared to amyloid-negative individuals (Figure 1A); AUC-ROC Aβ42 was 0.64 (95% CI: 0.60 to 0.69) and AUC-ROC Aβ42/40 was 0.75 (Figure 2B, 95% CI: 0.71 to 0.79, $p < 0.001$ by DeLong comparison of ROC curves). The improved performance of the Aβ42/40 ratio likely derived from the normalization of the Aβ42 concentration to the overall amount of Aβ (Aβ40) in a sample. The

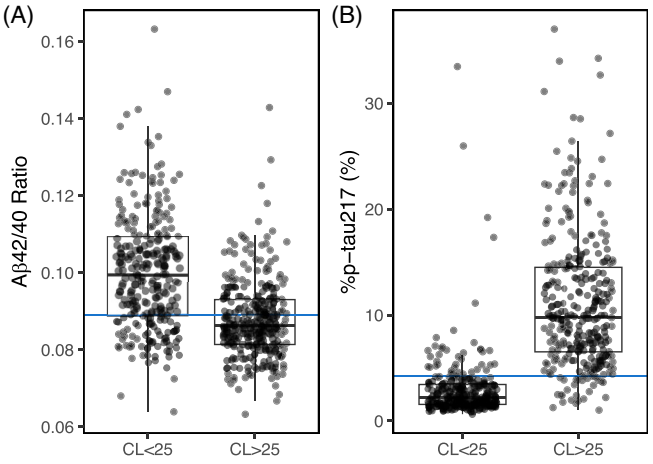

FIGURE 1 Differences in Aβ42/40 ratio and %p-tau217 by CL status. Aβ42/40 ratio was significantly lower ($p < 0.001$) in CL > 25 participants (A). Blue line shows cut point of 0.089, which was defined for Aβ42/40 ratio in previous studies. Conversely, %p-tau217 was statistically significantly higher ($p < 0.001$) in CL > 25 participants (B). Blue line shows optimal cut point (by Youden) of 4.2%. Aβ, amyloid beta; CL, Centiloid.

optimal cut point (by Youden) for the Aβ42/40 ratio in the combined cohort was 0.094, an approximately 5% higher ratio than the optimal cut point previously reported (0.089).[6]

The plasma tau217 mass spectrometry assay simultaneously quantifies the concentration of both p-tau217 and np-tau217 peptides. The concentration of np-tau217 was slightly higher in amyloid positive compared to amyloid negative participants (CL < 25: 51 pg/mL, SD 24; CL > 25: 55 pg/mL, SD: 20, $p < 0.01$), but there was considerable overlap, so the diagnostic performance of np-tau217 was low (AUC-ROC: 0.60, 95% CI: 0.55 to 0.64). However, the average concentration of p-tau217 was 3.9-fold higher in amyloid-positive study participants (CL < 25: 1.6 pg/mL, SD 1.9; CL > 25: 6.2 pg/mL, SD: 4.3, $p < 0.0001$) and the differentiation between amyloid-positive and amyloid-negative study participants was much greater, allowing for an AUC-ROC of 0.91 (95% CI: 0.89 to 0.94, Figure 2B). Similar to Aβ, where Aβ42 is normalized to the concentration of Aβ40 by calculating a ratio, p-tau217 can be normalized to the levels of np-tau217 for greater diagnostic accuracy. The %p-tau217 was significantly higher (3.6-fold) in amyloid-positive study participants (Figure 1B, CL < 25: 3.0%, SD 3.1; CL > 25: 11%, SD: 6.4, $p < 0.0001$), yielding an AUC-ROC of 0.94 (95% CI: 0.92 to 0.96), a statistically significant ($p < 0.001$ by DeLong) improvement over the p-tau217 concentration, Figure 2B.

3.4 | Development of a multi-analyte assay with algorithm (MAAA, PrecivityAD2) for predicting amyloid status

Logistic regression (LR) makes it possible to combine the diagnostic ability of multiple biomarkers and risk factors into one output for predicting a binary outcome, in this case presence/absence of brain amyloidosis (CL > 25 indicates presence of brain amyloid).[6,17,22] We first constructed a baseline LRM that included only %p-tau217 as input. In this model, %p-tau217 was highly significant ($p < 0.0001$), and a 3% point (approximately 1 SD in the CL < = 25 group) higher %p-tau217 was associated with an odds ratio of 5.4 (95% CI: 4.1 to 7.2) for identifying brain amyloidosis. The AIC for the %p-tau217-alone model was 435. To create the PrecivityAD2 model and generate an APS2 value, a new LRM was trained on both p-tau217 and Aβ42/40 ratios. In the PrecivityAD2 model, both analyte ratios were highly significant contributors ($p < 0.0001$). Similarly to the %p-tau217 alone model, in the PrecivityAD2 model, the odds ratio for a 3% point increase in %p-tau217 was 4.6 (95% CI: 3.5 to 6.0) and a 0.015 decrease (approximately 1 SD in the CL < 25 group) in Aβ42/40 ratio was associated with an odds ratio of 2.1 (95% CI: 1.5 to 2.9). The AIC for the PrecivityAD2 model was 412, significantly lower than the AIC for the model that relied on only %p-tau217.[21,23]

The output of the PrecivityAD2 LRM is a likelihood score between 0 and 1. To produce more easily read output, the model score was multiplied by 100 and rounded to the nearest whole number, producing the APS2 (Figure 2A), similar to the APS output of the PrecivityAD algorithm.[6] Figure 3A shows the distribution of Aβ42/40 and %p-tau217 in the full sample set overlaid on a heatmaps illustrating the APS2 values associated with each combination of %p-tau217 and Aβ42/40 ratio. The shape of the points reflects the amyloid PET status for each of the 583 study participants. The colors of the APS2 heatmap are anchored at green for 0, red for 100, and yellow for APS2 = 47.5. This makes it easy to visualize how the APS2 cut point intersects with the cut points for both Aβ42/40 ratio and %p-tau217 and how changes in the two components of the test influence the test output. Compared to the Aβ42/40 ratio (interquartile range 0.018 pg/mL, 0.084 to 0.102), the %p-tau217 has a wider dynamic range (interquartile range 8.16%, 2.21 to 10.4), with many more individuals having %p-tau217 values that are far from the optimal cut point for %p-tau217 (4.2% by Youden index).

The distribution of APS2 results for the 583 study participants is heavily skewed toward the low and high ends of the APS2 scale, enabling a binary classification with a single cut point to distinguish between amyloid presence and absence (Figure 2A). At the optimal cut point for APS2 (47.5), the accuracy was 88% (95% CI: 85 to 91), sensitivity (positive percentage agreement with PET) was 88% (95% CI: 84 to 91), and specificity (negative percent agreement with PET) was 89% (95% CI: 84 to 92). In the tested population, with a prevalence of 53%, the PPV was 90% (95% CI: 86 to 93) and the NPV was 87% (95% CI: 82 to 90). Table 2 lists the PPV and NPV calculated for populations where background disease prevalence is different from 53%. Of note, in populations with low prevalence rates, such as patients presenting with subjective cognitive decline with an estimated prevalence of 30%, the APS2 had a very high NPV, 95% (95% CI: 92 to 96). In populations with an estimated high prevalence, such as patients with dementia, the APS2 had a very high PPV, 94% (95% CI: 91 to 96).

While the APS2 result can be dichotomized using the optimal cut point of 47.5, there is intrinsic value to the numerical nature of the

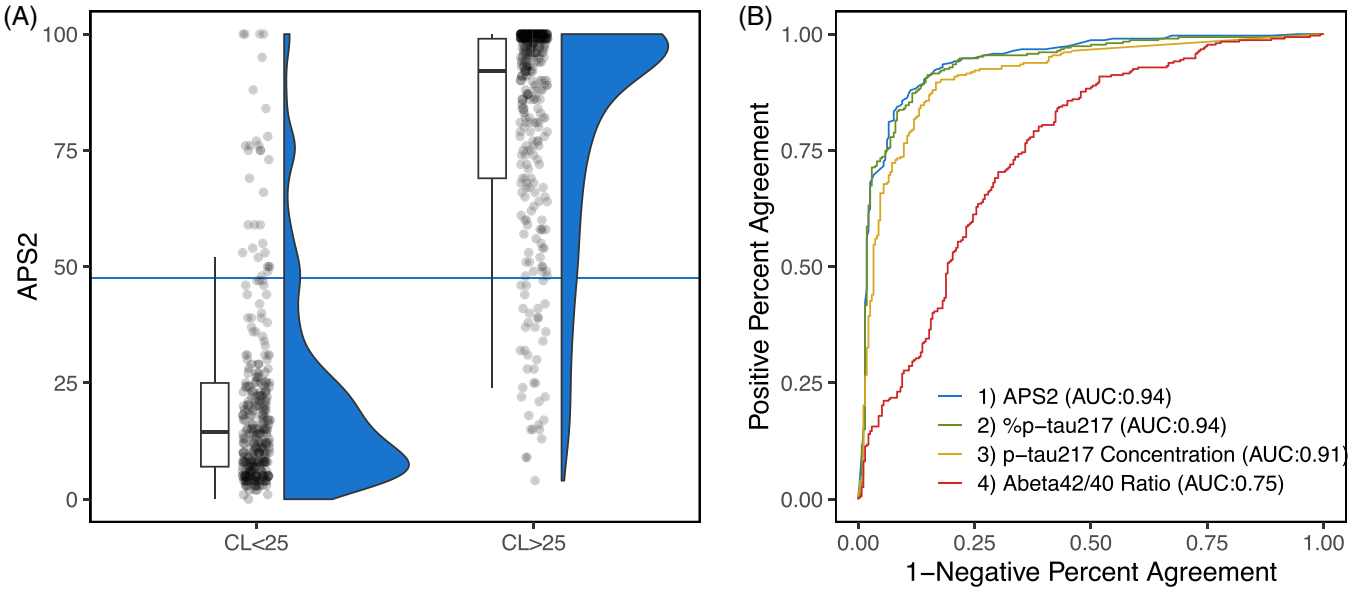

FIGURE 2 Diagnostic performance of APS2. APS2 was statistically significantly higher ($p < 0.001$) in CL > 25 participants (A). Blue line shows optimal cut point (by Youden) of 47.5. AUC-ROC for individual analytes as well as APS2 result (B). The AUC-ROC for p-tau217 concentration (0.91, 95% CI: 0.89 to 0.94) was significantly lower than the AUC-ROC for %p-tau217 (0.94, 95% CI: 0.92 to 0.96, $p < 0.0001$, DeLong comparison). The AUC-ROC for APS2 (0.94, 95% CI: 0.92 to 0.96) was not significantly different from the AUC-ROC for %p-tau217 ($p = 0.27$, DeLong comparison). APS2, Amyloid Probability Score 2; AUC-ROC, area under receiver operating characteristic curve; CI, confidence interval; CL, Centiloid.

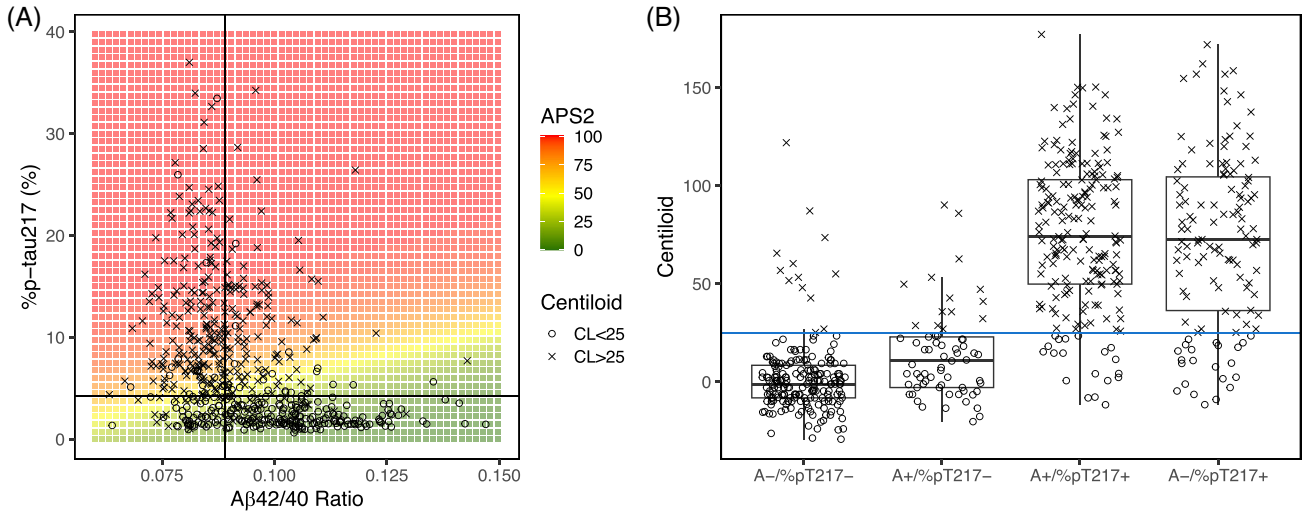

FIGURE 3 Concordance and discordance of Aβ42/40 ratio and %p-tau217. APS2 heatmap and distribution of Aβ42/40 ratio and %p-tau217 in combined cohort (A). APS2 values were calculated for all combinations of Aβ42/40 ratio and %p-tau217 and color coded starting at green for 0 and ending in red for 100, with yellow anchored at optimal APS2 cut point of 47.5. Thus, the yellow diagonal line demonstrates where the Aβ42/40 ratio and %p-tau217 merge to create a cut point that incorporates both amyloid (A) and tau (%pT217) biomarkers. The shape of the points represents the CL status for each of the study participants, with a circle (o) representing CL < 25 and a cross (x) representing CL > 25. Partitioning study participants into Aβ42/40 ratio positive/negative (A+/−) and %p-tau217 positive/negative (%pT217+/−) shows significantly higher CL values in %pT217+ versus %pT217− participants (B). In the %pT217− participants, being A+ was associated with higher CL values, though the interquartile range of these CL values did not exceed the CL cut point of 25. Aβ, amyloid beta; APS2, Amyloid Probability Score 2; CL, Centiloid.

APS2 result, as results that fall farther from the cut point are associated with higher diagnostic certainty. Table 3 shows the PPV/NPV for various ranges of APS2 cut points in a population with a disease prevalence of 50%. The table also shows the value of providing the APS2 as a numerical result: Lower APS2 results are clearly associated with increases in NPV while higher APS2 results are associated with increases in PPV. For example, as a whole, APS2 results greater than or equal to 80 were associated with a combined PPV of 96%, and APS2

TABLE 2 APS2 PPV/NPV by prevalence.

Prevalence	PPV (95% CI)	NPV (95% CI)
30%, as expected in SCD population	77% (71 to 82)	95% (92 to 96)
50%, as expected in MCI population	89% (85 to 92)	88% (84 to 91)
53%, observed prevalence	90% (86 to 93)	87% (82 to 90)
65%, as expected in dementia population	94% (91 to 96)	80% (74 to 85)

Note: PPV and NPV change with population prevalence. This table shows the calculated PPV and NPV for population prevalence rates commonly observed in different patient populations.
Abbreviations: APS2, Amyloid Probability Score 2; CI, confidence interval; MCI, mild cognitive impairment; NPV, negative predictive value; PPV, positive predictive value; SCD, subjective cognitive decline.

results lower than 20 had a combined NPV of 95% or greater. From this table we can also see that 87% of the APS2 results (APS2 0 to 30 & APS2 60 to 100) fell in a range where the combined percentage agreement was at least 93% (240+267 out of 583 individuals).

The PrecivityAD test relied on two different cut points to define an intermediate category of individuals for whom the test could not predict the outcome with high enough certainty to provide clinical benefit.[6] To assess the effect of having an intermediate category for the PrecivityAD2 test output (APS2), we performed a sensitivity analysis using the same boundaries as used for the PrecivityAD test (intermediate category set to $35.5 < \text{APS} < 57.5$). This intermediate category would be assigned to 56 (9.6%) patients, and the prevalence of amyloid positivity in this category was 53.6% (not significantly different from overall amyloid PET prevalence observed in the study). Excluding individuals in this intermediate category yielded a sensitivity of 92% (95% CI: 89% to 95%), a specificity of 92% (95% CI: 89% to 95%), and an overall agreement of 92% (95% CI: 89% to 94%). While the sensitivity and specificity were numerically improved by applying this intermediate category, neither was significantly improved from the sensitivity ($p = 0.17$) and specificity ($p = 0.31$) of the full dataset using the 47.5 cut point, nor was the overall accuracy improved ($p = 0.07$). Of interest, when plotting the CL values for the individuals that fall in the three different APS categories, the mean CL values for individuals in the intermediate category were close to the CL 25 cut point and significantly different from the mean CL values of the other two categories (Figure S1). This suggests that individuals with APS2 results in the 35.5 to 57.5 range have CL values that are close to the CL cut point.

There was no significant difference ($p = 0.27$ by DeLong) in the AUC-ROC between the APS2 (0.94, 95% CI: 0.92 to 0.96) and the %p-tau217-alone model (0.94, 95% CI: 0.92 to 0.96) (Figure 2B). However, by AIC, PrecivityAD2 provided a significantly more robust and better fitting model (AIC difference between models is 23, much greater than the difference of 2 generally accepted as an improvement in AIC measures[23]) as this model produces output that more closely matches the expected outcomes. Like the AIC, the BIC, which incorporates a greater penalty for more complex models, also showed that the PrecivityAD2 model (BIC: 425) was more robust than the %p-tau217-alone model (BIC: 444). Since the %p-tau217 and PrecivityAD2 models are nested (i.e., %p-tau217 is a part of the PrecivityAD2 model), the LRT was used as another objective measure to compare how well the two models predicted the outcome. The LRT showed that the PrecivityAD2 model was significantly better ($p < 0.0001$) than the %p-tau217-alone model.

For the PrecivityAD test, we found that adding the apoE proteotype to the model led to a significant increase in model performance.[6] For the PrecivityAD2 model we similarly explored adding the apoE proteotype to the model to determine whether this improved model performance. The apoE proteotype was added to the model as the presence/absence of E2 and using dummy variables to represent homozygosity or heterozygosity for the E4 proteotype. In the model that included the Aβ42/40 ratio, %p-tau217, and apoE, the odds ratio for a 3% point increase in %p-tau217 was 4.3 (95% CI: 3.3 to 5.8), very similar to the odds ratio in the PrecivityAD2 model. Both homozygosity and heterozygosity for E4 were significant contributors to the model ($p < 0.001$) but E2 presence was not ($p = 0.4$). The AUC-ROC for the model including the apoE proteotype was 0.95 (95% CI: 0.94 to 0.97), which is a significant improvement over the AUC for the PrecivityAD2 algorithm ($p = 0.02$ by DeLong). However, at the optimal cut point for the apoE including model, the overall accuracy (90%, 95% CI: 87% to 92%, $p = 0.58$), sensitivity (90%, 95% CI: 86% to 93%, $p = 0.43$), and specificity (89%, 95% CI: 85% to 92%, $p = 1$) were not significantly improved over the performance of the PrecivityAD2 algorithm. Similar results were obtained in comparisons of a model that included %p-tau217 and apoE to %p-tau217 alone. In this comparison of models, we observed a small but significant increase in AUC-ROC; again, however, this did not result in a significant improvement in performance at the optimal cut point.

3.5 | Concordance of blood-based biomarkers

The optimal cut point by Youden for %p-tau217 in blood for predicting brain amyloidosis was 4.2%. For the Aβ ratio, we previously established 0.089 as the optimal cut point for predicting amyloid positivity by the same criteria.[6] Using these two cut points it is possible to classify study participants as Aβ ratio positive/negative (abbreviated to A+/A− in the classification scheme) as well as %p-tau217 positive/negative (abbreviated to %pT217+/%pT217− in the classification scheme) and investigate the concordance and discordance between the two biomarkers. It is important to acknowledge that the A+/A− and %pT217+/%pT217− in this analysis refers specifically to the blood biomarkers and not the neuropathology of the patients. Both the Aβ and the %p-tau217 cut points used for this classification are designed to identify amyloid plaques and not tau tangles. The number and percentage of total study participants in each of the four Aβ ratio (A+/A−) and %p-tau217 (%pT217+/%pT217−) quadrants are listed in Table S3. There were 189 study participants (32%) where the two ratios were discordant, and in the discordant cases the overall accuracy was significantly lower (81%, 95% CI: 75% to 86%, $p < 0.001$) than in the concordant cases (92%, 95% CI: 88% to 94%). In the discordant cases, the PET amyloid status agreed with %p-tau217 for the majority of

TABLE 3 PPV and NPV by APS2 cut point.

APS2 Range	APS2 Classification	APS2 Positive, *n* (%)	APS2 Negative, *n* (%)	PPV	NPV
0 to 10	Negative		111 (19%)		98% (93 to 99)
0 to 20	Negative		189 (32%)		95% (91 to 97)
0 to 30	Negative		240 (41%)		93% (89 to 96)
0 to 40	Negative		267 (46%)		90% (86 to 93)
47.5	**Cut point**	**301 (52%)**	**282 (48%)**	**89% (85 to 92)**	**88% (84 to 91)**
50 to 100	Positive	294 (50%)		89% (85 to 92)	
60 to 100	Positive	267 (46%)		93% (89 to 95)	
70 to 100	Positive	244 (42%)		93% (89 to 95)	
80 to 100	Positive	213 (37%)		96% (93 to 98)	
90 to 100	Positive	173 (30%)		97% (93 to 99)	

Note: With population prevalence set to 50% (MCI population), the PPV and NPV (95% CI) were calculated at APS2 cut points in intervals of 10, as well as at the APS2 optimal cut point (47.5, bold font). The number and percentage of patients who fall within each of the APS2 ranges are shown along with the PPV for APS2-positive patients and the NPV for APS2-negative patients.
Abbreviations: APS2, Amyloid Probability Score 2; CI, confidence intervals; MCI, mild cognitive impairment; NPV, negative predictive value; PPV, positive predictive value.

the cases, 82% for %p-tau217 positive and 78% for %p-tau217 negative (Table S3). For study participants with a positive %p-tau217, there was no difference in CL values by Aβ ratio status ($p = 1$, Bonferroni corrected *t* test), suggesting that a positive %p-tau217 was a stronger indicator of brain amyloidosis than the Aβ42/40 ratio. The accuracy of the APS2 in the discordant and concordant categories was independent of which cohort the samples came from (Table S3).

For study participants with a negative %p-tau217, those who were positive by Aβ42/40 ratio had higher mean CL values compared to participants who were negative by Aβ42/40, showing that being positive by Aβ42/40 is associated with increased CL values (Figure 3B). While the increase in CL values did not reach statistical significance when corrected for multiple comparisons of the four different A/%pT217 classes ($p = 0.087$, Bonferroni corrected *t* test, six total comparisons), if the two %pT217+ classes were combined into one, reducing the number of comparisons to three, CL values were statistically significantly higher in the A+/%pT217− versus A−/%pT217− subgroup ($p < 0.05$). The interquartile range for CL values in the A+/%pT217− study participants did not reach the CL 25 cut point for amyloid status; thus, the elevated CL values in participants in the A+/%pT217− group were not an indication that these participants were amyloid positive at the time of the blood draw but rather that they were, on average, in the early stages of amyloid accumulation.

3.6 | Performance of APS2 in subgroups

Demographics for the combined cohort can be found in Table 1. To test APS2 performance in different demographic subgroups, subsets of the combined dataset were created and the accuracy of the 47.5 APS2 cut point for predicting brain amyloidosis was calculated (Figure 4). The accuracy did not vary by the underlying cohort (PARIS vs MissionAD,

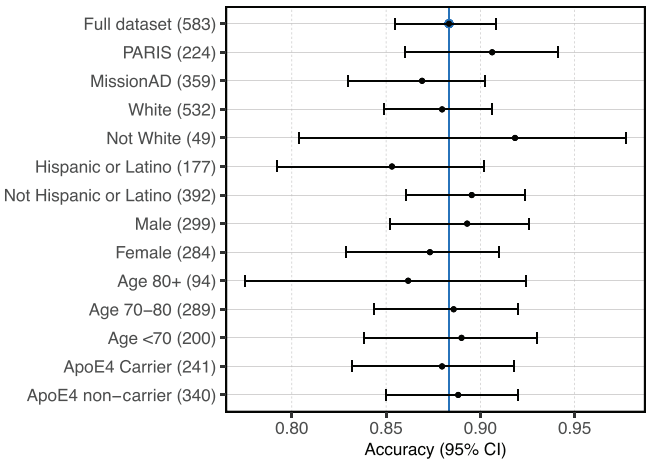

FIGURE 4 Accuracy of APS2 in subgroups. APS2 accuracy and the associated 95% confidence intervals were measured in the full data set as well as in subgroups based on race, ethnicity, sex, age, and apoE4 carrier status. Blue line shows the accuracy measured in the full dataset. Numbers in parentheses are the number of study participants enrolled in each of the subgroups. APS2, Amyloid Probability Score 2.

$p = 0.19$). Since the racial diversity was low, race was compared as white participants ($n = 532$) versus participants who reported a race other than white ($n = 49$). While the accuracy was numerically higher in the participants who reported a race other than white, this was not statistically significant due to the low number of non-White participants ($p = 0.64$). For ethnicity, the study had much greater diversity, with 30% reporting as Hispanic or Latino, and again, here, there was no significant difference in APS2 accuracy of the test by ethnicity ($p = 0.16$). When stratifying by age range and sex, there was also no significant difference in APS2 accuracy ($p = 0.75$ for age range and $p = 0.51$ for sex). Finally, there was no difference in APS2 accuracy in participants who

had one or more copies of apoE4 (apoE4 carriers) compared to apoE4 non-carriers ($p = 0.79$).

4 | DISCUSSION

The results presented herein show that LC-MS/MS provides accurate and reliable %p-tau217 and Aβ42/40 ratio quantitation in plasma. Combining those analytes into an algorithm to produce the APS2 output yields a test (PrecivityAD2) with outstanding diagnostic performance characteristics[24] for predicting brain amyloidosis among individuals with cognitive impairment undergoing evaluation for AD. This finding is aligned with the 2022 EU/US CTAD Task Force Report recommendation on the use of blood biomarkers including p-tau measures and Aβ42/40 ratio alone or in combination to aid in AD diagnosis.[10] The %p-tau217 measurement by LC-MS/MS reported in this study also showed excellent diagnostic performance; however, the APS2 value provided for a more robust and better fitting prediction output (by AIC, BIC, LRT).[21] The PrecivityAD2 model was developed on the combined data from PARIS and MissionAD, but when the same model was developed on either of the two cohorts and cross validated on the other cohort, the cohort-specific models behaved very similarly with respect to performance to the PrecivityAD2 model (data not shown), suggesting that the PrecivityAD2 model will perform comparably in new samples that are not part of a training set. We used the same method for validating the PrecivityAD test, and that algorithm has since shown external validation in an orthogonal set of samples.[25] More studies are under way that seek to further demonstrate the robust clinical performance and generalizability of the APS2 model.

A notable strength of the findings from this validation study relates to the fact that subjects included in the analysis originated exclusively from the intended-use population of patients who would be appropriately indicated for using the PrecivityAD2 test in clinical care. Individuals from one of the two evaluated cohorts (PARIS, as a substudy of IDEAS) represented a real-world, diagnostically challenging population of US Medicare beneficiaries with cognitive impairment or dementia. These were all individuals prospectively meeting the appropriate use criteria for amyloid assessment, where the etiology of cognitive impairment was unknown, AD was a diagnostic consideration, and knowledge of amyloid status (as determined by PET) was expected to change diagnosis and management.[13]

While the overall study population was majority white (91%), there was good representation (30%) of Hispanic or Latino ethnicity. We found no significant difference in diagnostic performance of the PrecivityAD2 test by ethnicity, sex, age, or apoE4 carrier status. This suggests that the results of the PrecivityAD2 test can be similarly interpreted across different ethnicities, ages, sexes, and apoE4 genotypes. Further research is ongoing across many different cohorts evaluating the diagnostic performance of the PrecivityAD2 test in different patient populations.

The findings herein also show that the PrecivityAD2 algorithm can identify brain amyloid status with sensitivity, specificity, PPVs, and NPVs that approximate those of amyloid PET. Studies comparing *ante mortem* PET with ^{11}C-PiB or ^{18}F-amyloid tracers with *post mortem* neuropathology/neuritic plaque analyses have established that the sensitivity for qualitative (visual read) PET analysis was 88% to 98%, and specificity was 80% to 95%.[16,26–29] Interpretation of amyloid PET using quantitative methods (eg, SUVR, Centiloid) against *post mortem* neuropathology can increase the diagnostic performance, with sensitivity (94% to 97%) and specificity (90% to 95%) ranging higher than what is found for amyloid PET visual read.[30,31] In the current study of symptomatic adults, we found that the APS2 result attained an overall sensitivity of 88% (95% CI: 84% to 91%) and specificity of 89% (95% CI: 84% to 92%), similar to or better than the ranges of sensitivities and specificities reported for the gold standard *ante mortem* qualitative PET analyses when compared to *post mortem* neuropathology. This increase in performance was even more pronounced as values approached the upper (100) and lower (0) ends of the APS2 quantitative scale. For example, individuals with APS2 scores above 80 or below 20 (69% of individuals) had combined accuracies of their APS2 result of 95% or greater.

Similarly to most diagnostic tests, the PrecivityAD2 test has reduced diagnostic accuracy for individuals with APS2 scores close to the cut point. We created an intermediate category to include individuals around the diagnostic cut point, similarly to the method we established with the PrecivityAD test and as has been proposed for other tests using p-tau217.[6,32] We explored an intermediate category for the APS2 that included 9.6% of individuals, a comparable number of individuals to the 7.5% who are assigned a "likely positive" diagnosis with the FDA-approved Lumipulse G CSF test.[33] With the APS2 intermediate category, PPV equaled 92% and NPV equaled 91% at a 53.6% observed disease prevalence. However, statistically, there was no significant difference in the PPV and NPV with or without the intermediate category. The mean CL values for the individuals who fell in the intermediate category was close to the CL cut point of 25, suggesting that the gold standard assignment of PET status for these individuals is also associated with lower certainty. Blood tests for AD should always be interpreted in the context of clinical presentation, and we believe that clinicians understand that test results close to the cut point may warrant additional testing. Additionally, presenting a binary result can help avoid unnecessary test layering, which in a cost-conscious healthcare system represents an important consideration for clinical diagnostic tools.

A recent report compared several blood biomarkers for neurodegeneration and AD pathology: p-tau181 and p-tau217 by Lilly Research Laboratories; p-tau231 by University of Gothenburg; plasma glial fibrilary acidic protein (GFAP) and neurofilament light chain (NfL) by Elecsys; and Aβ42 and Aβ40 immunoassays by Roche Diagnostics.[34] All the biomarkers were quantified in *ante mortem* blood matched with *post mortem* neuropathology measures of amyloid and tau from donor brains with or without significant AD neuropathology. The investigators found modest but significant independent associations (Spearman ρ) between plasma biomarkers and *post mortem* amyloid plaque and tau tangle loads: plasma Aβ42/40 ratio and p-tau231 concentrations were only associated with plaques, GFAP levels only with tangles, and p-tau217 and p-tau181 levels with both plaques and tangles. Of note,

the authors found that a model combining plasma p-tau217 concentration and Aβ42/40 ratio provided the greatest predictive accuracy for the presence of AD neuropathology and correlated with plaque load and that only plasma p-tau217 levels correlated with cerebral tau tangle load. The authors suggested that high-performing plasma p-tau217 and Aβ42/40 analytical platforms might provide the optimal blood-based biomarker combination to assess AD-related pathology in vivo.[34] The current findings from this study extend this assertion and conclude that the combination of plasma %p-tau217 and Aβ42/40 ratio (PrecivityAD2), quantified using the highly accurate LC-MS/MS analytical platform,[35,36] provide high diagnostic performance for detecting brain amyloid status in older, symptomatic individuals.

Of interest, we found that in comparison to plasma p-tau217 concentration, measured in picograms per milliliter, plasma %p-tau217 provided greater diagnostic performance for identifying brain amyloid pathology based on amyloid PET. This is consistent with multiple previous reports for both CSF and plasma showing that %p-tau217 performed better than p-tau217 concentration.[37–39] This improved performance is likely due to the normalization of phosphorylated tau to the amount of tau that is available to be phosphorylated in an individual. A multitude of factors can affect the overall tau concentration in the blood of an individual, including impaired clearance of proteins from blood due to kidney dysfunction or other comorbidities.[40] Our current dataset lacked the necessary variables to carefully assess the effect of kidney disease as well as other comorbidities; however, that work is currently ongoing in other studies. Published data by others[41] using an LC-MS/MS method akin to that used herein for protein quantitation have revealed that kidney disease as measured by lower levels of estimated glomerular filtration rate is associated with higher plasma levels of soluble tau, including p-tau217 and p-tau181, in individuals with and without cognitive impairment. The confounding effect of kidney disease was markedly diminished with the %p-tau217 measurement, suggesting that the p-tau217/np-tau217 ratio is a more reliable measure of brain p-tau pathology in individuals across the AD spectrum and, thus, may be more diagnostically robust for implementation in real-world clinical practice.[42]

This study has the following limitations. While there was good representation of Hispanic ethnicity, the findings need to be qualified by the limited racial distribution enrolled in PARIS (IDEAS) and MissionAD, which enrolled primarily white participants. Some differences in the relative predictive values of plasma Aβ42/40 and p-tau species for brain amyloidosis and neurocognitive decline have been reported in African American cohorts.[43] Likewise, not all comorbidities (eg, hepatic and renal functions, cardiovascular disease, brain trauma) were captured or controlled for in the current cohorts and analysis. These have the potential to affect the plasma biomarker concentrations and, thus, diagnostic performance; however, use of concentration ratios makes it possible to control for these co-factors.[42] Multiple samples analyzed in this study were below the LOD for p-tau217 (37% for MissionAD and 13% for PARIS). While this does not impede cross-sectional diagnostic ability of the assay, this does somewhat limit one's ability to perform correlation analysis. Samples that are below LOD for p-tau217 are especially common in individuals with very low amyloid burden, reflecting the lower mean CL observed in MissionAD compared to PARIS. While the selection of CL > 25 as the amyloid PET cutoff value in the current cohorts was justified previously,[6] a universally agreed upon consensus CL cutoff value has yet to be established. Tau PET tracers and measures were not available at the time of PARIS and MissionAD enrollment. Therefore, the ability of the PrecivityAD2 blood test to identify brain tau pathology was not evaluated in this study, but such work is currently ongoing. Recent evidence suggests that select plasma and CSF p-tau species measurements, and especially their p-tau ratios, can identify brain tau status in addition to cerebral amyloidosis.[37,44–46]

5 | CONCLUSIONS

The PrecivityAD2 blood test has been clinically validated across two independent cohorts of individuals with cognitive impairment. Biomarker measures that are core to AD pathology were incorporated into an algorithm that generated an APS2 with a single cutoff value that has outstanding diagnostic performance[24] for identifying cerebral amyloid PET status and also appears unaffected by certain clinical and demographic characteristics. Future studies will characterize how this diagnostic tool improves disease detection rates and treatment plans across a diverse population of patients undergoing evaluation for AD.

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CONFLICT OF INTEREST STATEMENT

All C_2N co-authors are salaried employees or consultants with equity interests in C_2N Diagnostics. S.I., M.I., D.V., and P.S. are employees of Eisai Co., Ltd. D.M.H. co-founded, has equity in, and is on the scientific advisory board of C2N Diagnostics. D.M.H. is on the scientific advisory board of Denali, Genentech, and Cajal Neuroscience and consults for Asteroid. R.J.B. co-founded, has equity in, and is on the scientific advisory board of C2N Diagnostics. R.J.B. has consulting relationships (12 months) with Roche (unpaid). B.A.S. reported grants and personal fees from the American College of Radiology during the conduct of the study; grants from Blue Earth Diagnostics, Curium Pharma, and Progenics Pharmaceuticals; and personal fees from Curium Pharma, Progenics Pharmaceuticals, Avid Radiopharmaceuticals, Capella Imaging, GE Healthcare, Lantheus Medical Imaging, and Siemens Healthineers outside the submitted work. G.D.R. receives research support from Avid Radiopharmaceuticals, GE Healthcare, Life

Molecular Imaging, and Genentech. He has received consulting fees from Alector, Eli Lilly, Johnson & Johnson, and Merck. He is Associate Editor for *JAMA Neurology*. Ms. Hanna reported receiving grants from ACR during the conduct of the IDEAS study. All other authors declared no competing interests. Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

The PARIS and MissionAD studies were reviewed and approved by central or local ethics and safety review committees or boards. All participants (or their legally authorized representative) reviewed and signed an approved informed consent document.

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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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