Title: Independent validation of the PrecivityAD2™ blood test to identify presence or absence of brain amyloid pathology in individuals with cognitive impairment

Running Head: PrecivityAD2 reliably identifies AD pathology in cognitively impaired

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Abstract

Objective. The diagnostic performance of the Amyloid Probability Score 2 (APS2) - the algorithmic result of the PrecivityAD2™ blood test - was originally trained and validated in two cohorts of cognitively impaired (CI) individuals. Using an independent cohort to evaluate blood test reliability, we conducted an external diagnostic accuracy assessment of the validated APS2 cut point as it is currently applied in clinical practice.

Methods. Plasma biomarker ratios Aβ42/40 and p-tau217/np-tau217 (expressed as %p-tau217) were quantified and incorporated into the APS2 algorithm in samples obtained from 192 Alzheimer's Disease Neuroimaging Initiative participants with CI (70% mild cognitive impairment

/ 30% dementia). APS2 diagnostic performance was determined using amyloid positron emission tomography (PET) as the reference standard. Plasma biomarkers were quantified in a CLIA-certified, CAP-accredited laboratory (C2N Diagnostics, St. Louis, MO) using liquid chromatography-tandem mass spectrometry.

Results. APS2 values were significantly higher in the 56% of CI participants with a positive amyloid PET scan. Concordance with amyloid PET was high (AUC-ROC 0.95 (95%CI): 0.93–0.98); 54% of participants had a positive APS2. The previously validated APS2 cut point yielded an overall accuracy of 91% (95%CI: 86-94%), sensitivity 90% (95%CI: 83-94%) and specificity 92% (95%CI: 84-96%).

Interpretation. The PrecivityAD2 blood test's APS2 identified brain amyloid pathology with accuracy, sensitivity, and specificity ≥ 90% in this intended use population. This external validation reaffirms the diagnostic robustness of this blood biomarker test and supports its use as a confirmatory test, consistent with published expert recommendations, for assessment of presence or absence of brain amyloid pathology in symptomatic patients.

Keywords. Alzheimer's disease, blood biomarkers, diagnostic performance, amyloid probability score 2, p-tau217, amyloid beta

Introduction

Globally, Alzheimer's disease (AD) affects an estimated 25-30 million people and is a leading cause of disability and death[1–3]. Identifying the presence of brain amyloid plaques and neurofibrillary tau tangles early in the pathophysiological processes that characterize AD is crucial for establishing an accurate and early diagnosis as well as determining timely eligibility for therapeutic interventions that slow disease progression and cognitive decline.

Novel blood biomarker (BBM) tests for diagnosis of AD pathology are currently being developed, validated, and compared[4–22]. Recently, AD BBM key opinion leaders and expert panels convened by the World Health Organization (WHO), Global CEO Initiative on AD (CEOi), and the Alzheimer's Association, outlined diagnostic performance characteristics that should be considered with fluid biomarkers targeted for use in clinical settings[8,12,23–26]. Blood biomarker tests that demonstrate high performance compared to conventional brain amyloid diagnostic tools such as amyloid positron emission tomography (PET) or cerebrospinal fluid (CSF) biomarkers can complement cognitive testing, rapidly aid in ruling in or ruling out an AD diagnosis in symptomatic patients, and accelerate the diagnostic process for more timely initiation of a disease-modifying treatment[12,25]. Further, given the complexities of biomarker variability and the implications of diagnostic thresholds, repeated validation studies and reliability testing are necessary for evaluating robustness, accuracy, and generalizability.

C2N Diagnostics' PrecivityAD2™ blood test is a multianalyte assay with algorithmic analysis that uses immunoprecipitation, liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify plasma concentrations of amyloid beta 42 (Aβ42) and Aβ40 isoforms, and phosphorylated-tau217 (p-tau217) and non-phosphorylated-tau217 (np-tau217) peptides. Plasma Aβ42/40 and p-tau217/np-tau217 (%p-tau217) concentration ratios are calculated and incorporated into an algorithm that generates the Amyloid Probability Score 2 (APS2) - a value that ranges from 0 to 100 and represents the likelihood that brain amyloid pathology is present or absent in individuals with CI[9,15,27]. Prior studies have analytically and clinically validated PrecivityAD2 analytes and APS2 cut points against amyloid PET imaging and CSF biomarkers of brain amyloid pathology[15,16,28–32].

In a recent head-to-head round-robin comparison of BBM tests for AD pathology in cognitively impaired and unimpaired participants, a study developed and launched by the Foundation for the NIH (FNIH) Biomarkers Consortium, plasma samples from the Alzheimer's Disease

Neuroimaging Initiative (ADNI) biorepository were analyzed using the leading amyloid and p-tau tests, each employing different analytical platforms. A prespecified analytic plan was developed for a head-to-head comparison of these plasma biomarkers against amyloid PET as the primary outcome[16]. The FNIH study analyzed the performance of the C2N analytes individually and when combined (%p-tau217, p-tau217, Aβ42/40) into a *de novo* prediction model, and found that the model outperformed the other BBM models for identifying brain amyloid pathology status. However, the diagnostic accuracy of the APS2 algorithm in the intended use population was not determined in that study for two reasons: the algorithm validation was not a prespecified study objective; and the ADNI cohort included cognitively unimpaired participants, which falls outside of the intended use population for the PrecivityAD2 test.

The purpose of the present study was to externally validate the prespecified APS2 algorithm and cut point used in clinical care in the FNIH-ADNI cohort of individuals with CI (i.e., the intended use population) (**Fig. 1**).
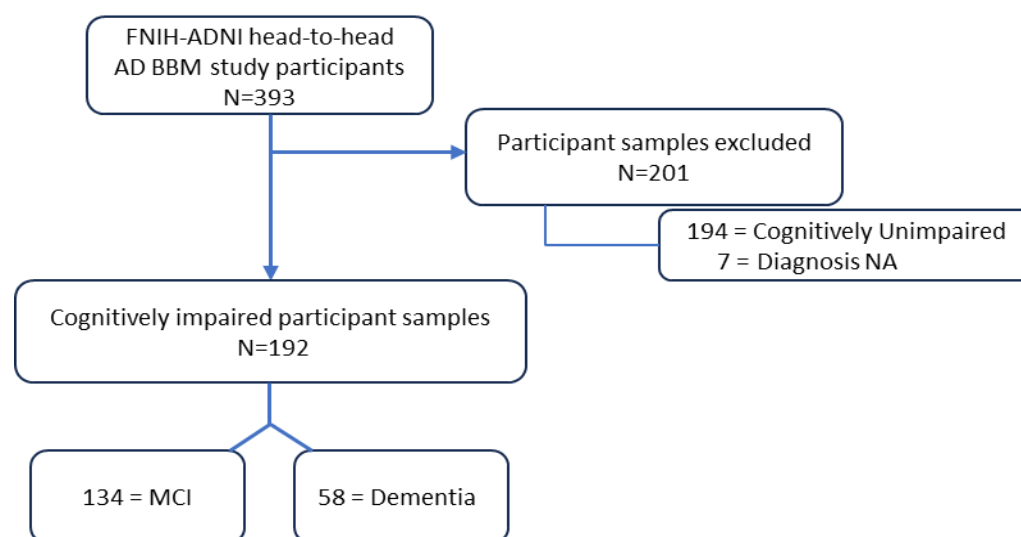

Figure 1. CONSORT diagram showing participant selection. From a total of 393 FNIH-ADNI participant samples[16], 201 with diagnosis assigned as "cognitively unimpaired" or "without diagnosis" data, were excluded. The remaining 192 participants with an "MCI" or "Dementia" diagnosis were included in the current analysis.

Methods and Participants

Participants. All ADNI volunteers received an explanation of study purpose, risks, and benefits, and all participants or their legally authorized representatives signed an IRB-approved consent document for their deidentified study related data to be shared with qualified researchers.

ADNI Cohort selection: The FNIH project evaluated several candidate plasma biomarkers and compared their ability to identify amyloid PET status in cognitively impaired and unimpaired participants. The current analysis focused on the PrecivityAD2 test's ability to identify amyloid PET status in 192 CI ADNI participants - 134 with mild cognitive impairment (MCI) and 58 with dementia - who had K_2 EDTA plasma samples collected within six months of their most recent amyloid PET scan (**Fig 1**). All data analyzed herein, including demographic information (self-reported race, ethnicity, education, CDR, and sex), was downloaded from the ADNI database (*https://adni.loni.usc.edu*). ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The original goal of ADNI was to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of MCI and early AD. The current goals include validating biomarkers for clinical trials, improving the generalizability of ADNI data by increasing diversity in the participant cohort, and providing data concerning the diagnosis and progression of AD to the scientific community.

Amyloid PET status assignment. Standardized protocols for ^{18}Florbetapir or ^{18}Florbetaben amyloid PET imaging were used at ADNI sites. FreeSurfer v7.1 was used to identify the frontal, cingulate, parietal, and lateral temporal cortices, and to quantify the global standardized uptake value ratio (SUVR). SUVR was normalized to the whole cerebellum. The ADNI PET Core applied standardized data transformations to the ^{18}Florbetapir and ^{18}Florbetaben images and estimated the standardized global cortical Aβ burden in Centiloid (CL) units. For the current analysis, the presence of brain amyloid PET pathology was defined as CL > 25.

PrecivityAD2 methods and APS2 algorithm. The analytical methods for PrecivityAD2 biomarkers and the clinical validation of the APS2 algorithm have been described previously[15,28–32]. Briefly, blood was collected into K_2 EDTA vacutainers®, plasma was isolated and stored according to ADNI protocols. Frozen plasma aliquots were shipped on dry ice from the ADNI biorepository to the C2N Diagnostics laboratory (St. Louis, MO USA) for processing and LC-MS/MS analysis of the PrecivityAD2 biomarkers. These biomarkers were incorporated into the prespecified APS2 algorithm. All C2N lab personnel involved in sample analysis remained blinded to all participant meta-data.

Statistical analysis

All data analysis was performed using R version 4.3.1 (The R Foundation for Statistical Computing). Participant characteristics were summarized across the groups using means, medians, and standard deviations for continuous variables and using counts and percentages for categorical variables. Fisher's exact test was used to compare accuracy observed for the different biomarkers. Receiver operating characteristic (ROC) analyses, including the calculation of the area under the ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were conducted using the pROC package for R[33]. The 95% confidence intervals on accuracy, sensitivity, specificity, PPV and NPV were calculated using Wilson's method[34].

Results

This study included ADNI participants representing the intended use population for the PrecivityAD2 test: men and women above 55 years old with MCI or dementia (**Table 1**). The prevalence of amyloid PET positivity in this cohort was 56%, and 54% of the participants had a positive APS2. On average (**Supplementary Table 1 & Fig. 2A**), plasma Aβ42/40 was significantly lower in the amyloid PET positive group (0.0883 ± 0.0093) compared to the amyloid negative group (0.0983 ± 0.0107; p = 1.8e^{-10}) and the area under the receiver operating characteristic curve (AUC-ROC) was 0.78 (95%CI: 0.71-0.85). Using the test's prespecified plasma Aβ42/40 ratio cut point (0.095) gave an accuracy of 74% (95%CI: 68-80%), sensitivity 81% (95%CI: 73-88%), specificity 65% (95%CI: 55-75%), PPV 75% (95%CI: 66–82%), and NPV 73% (95%CI: 62–82%), values consistent with previous reports [30,31].

Table 1. Demographics for 192 ADNI participants with either MCI or dementia.

	Diagnosis		Centiloid		APS2		
	Dementia	**MCI**	**<25**	**>25**	**Neg**	**Pos**	**Total**
N =	**(58)**	**(134)**	**(84)**	**(108)**	**(88)**	**(103)**	**(192)**
Sex							
Male	27	57	38	46	36	47	84
	(47%)	(42%)	(45%)	(43%)	(41%)	(46%)	(44%)
Female	31	77	46	62	52	56	108
	(53%)	(58%)	(55%)	(57%)	(59%)	(54%)	(56%)
Age							
Mean(sd)	79 (8)	78 (7)	77 (8)	79 (7)	76 (8)	80 (7)	78 (8)
Median	81	77	76	81	76	81	78
Range	58 – 96	59 – 97	62 – 97	58 – 96	59 – 97	58 – 96	58 – 97

Education (yr)

Mean	15.8	15.9	15.9	15.9	15.9	15.9	15.9
(sd)	(2.7)	(2.8)	(2.9)	(2.7)	(2.8)	(2.7)	(2.8)
Median	16	16	16	16	16	16	16
Range	12 - 20	8 – 20	8 - 20	12 – 20	8 – 20	12 – 20	8 – 20

CDR

CDR = 0	2	26	19	9	19	9	28
	(3.4%)	(20%)	(23%)	(8.5%)	(22%)	(8.9%)	(15%)
CDR = 0.5	12	102	59	55	65	49	114
	(21%)	(78%)	(72%)	(52%)	(76%)	(48%)	(61%)
CDR = 1+	44	2	4	42	2	43	46
	(76%)	(1.5%)	(4.9%)	(40%)	(2.3%)	(43%)	(24%)
Missing data	0	4	2	2	2	2	4

Race

White	56	126	78	104	82	99	182
	(94%)	(97%)	(93%)	(96%)	(93%)	(96%)	(95%)
Asian	0	2	1	1	1	1	2
	(0)	(1.5%)	(1.2%)	(0.9%)	(1.1%)	(1.0%)	(1.0%)
Black/African American	1	5	4	2	4	2	6
	(1.7%)	(3.7%)	(4.8%)	(1.9%)	(4.5%)	(1.9%)	(3.1%)

Mean(sd), median and range of values are indicated. Other values represent the number of participants (the percentage of participants) in that column with the specified characteristic. APS2 = Amyloid Probability Score 2; CDR = Clinical Dementia Rating; CL = centiloid; Dx = Diagnosis; MCI = mild cognitive impairment, Neg = negative, Pos = positive. An APS2 value is not reported for one male participant whose plasma was not available for analysis.
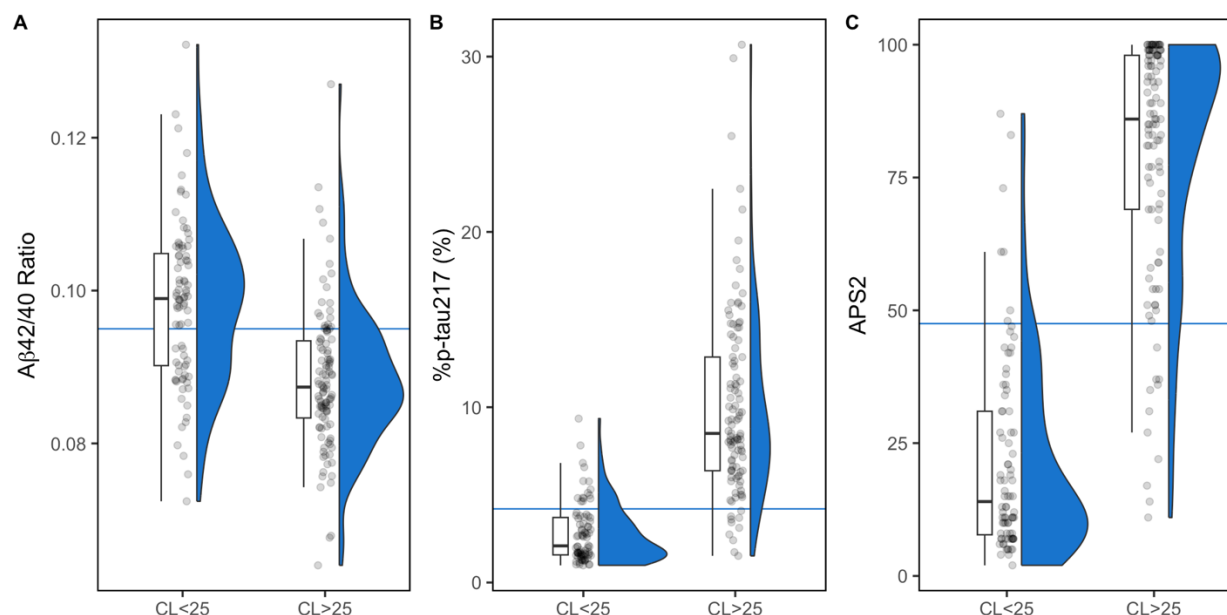
Figure 2. Combined box/whisker plots and distribution density plots for individual plasma **(A)** Aβ42/40, **(B)** %p-tau217, and **(C)** APS2 values. Boxes represent the upper and lower quartiles, the horizontal line in the boxes represents the median value, and the whiskers define the range of measured values. Plasma Aβ42/40 was significantly lower **(A)** and plasma %p-tau217 was significantly higher **(B)** in the CI participants with amyloid PET CL > 25 (i.e., presence of amyloid pathology). In **(A),** the blue line represents the test's prespecified Aβ42/40 cut point = 0.095. **(B)** The blue line represents the test's prespecified %p-tau217 cut point = 4.2%. **(C)** APS2 values were higher for ADNI participants with amyloid PET CL > 25. The blue line represents the test's prespecified APS2 cut point = 47.5.

On average, plasma %p-tau217 was significantly higher in the amyloid positive group (10.02 ± 5.40) than in the amyloid negative group with an AUC-ROC of 0.94 (95%CI: 0.92–0.98) (2.80 ± 1.69; p = 2.2e^{-16}, **Supplementary Table 1 & Fig. 2B**). Using the test's prespecified plasma %p-tau217 cut point (4.2%), yielded an accuracy of 87% (95%CI: 81-91%), sensitivity 91% (95%CI: 84-95%), specificity 82% (95%CI: 73-89%), PPV 87% (95%CI: 79-92%) and NPV 87% (95%CI: 78-93%), values consistent with previous reports [15,27].

The APS2 algorithm uses both the plasma Aβ42/40 and %p-tau217 values to calculate a likelihood score between 0 - 100. In the original CI validation cohort[15], an APS2 value of 47.5 was chosen as the cut point for distinguishing the presence of brain amyloid pathology from the absence. In the current study, APS2 was significantly higher in the amyloid PET positive group (83.8 ± 16.5) compared to the amyloid negative group (18.6 ± 12.8; p = 2.2e^{-16}) and the AUC-

ROC was 0.95 (95%CI: 0.93–0.98, **Supplementary Table 1 & Fig**. **2C**). Using the test's prespecified APS2 cut point, accuracy was 91% (95%CI: 86–94%), sensitivity 90% (95%CI: 83–94%), specificity 92% (95%CI: 84–96%), PPV 93% (95%CI: 87–97%) and NPV 88% (95%CI: 79–93%) (**Table 2**), values similar to those previously reported[15]. APS2 diagnostic accuracy at the prespecified cut point was better than that for plasma Aβ42/40 (74% vs. 91%, p < 0.0001), and numerically better than that for plasma %p-tau217 (87% vs. 91%, p = 0.33).

Table 2. PrecivityAD2™ APS2 algorithm corresponding positive predictive and negative predictive values by prevalence of disease.

Observed Sensitivity/ Specificity	Prevalence of brain amyloid positivity in patient population	PPV (95% CI)	NPV (95% CI)
	20%	73% (59 – 83%)	97% (93 – 99%)
	30%	82% (71 – 90%)	95% (90 – 98%)
	40%	88% (79 – 93%)	93% (87 – 96%)
	50%	92% (84 – 96%)	90% (82 – 94%)
90%/92%	56% (Observed)	93% (87 – 97%)	88% (79 – 93%)
	60%	94% (88 – 97%)	86% (76 – 92%)
	70%	96% (91 – 98%)	79% (68 – 87%)
	80%	98% (94 – 99%)	69% (55 – 80%)

Based upon the observed sensitivity and specificity of the APS2 algorithm in this study, we also examined the impact of varying prevalence of amyloid pathology on the PrecivityAD2 test's PPV and NPV (**Table 2**). This analysis is impactful in the context of individualized patient care where patient risk of disease influences confidence to rely on a diagnostic test to rule in or rule out disease. The observed prevalence of amyloid pathology in this study was 56%, consistent with an MCI / early dementia population, with an observed PPV and NPV of 93% and 88%, respectively. Among a predominantly asymptomatic, aged adult population (e.g., 20% - 30% prevalence of brain amyloid), the APS2 provides a very high NPV with point estimates of 95%-97%, highlighting its value for ruling out disease. Among a population with dementia (e.g., 70-80% prevalence of brain amyloid), the APS2 provides a very high PPV with point estimates of 96%-98%, highlighting its value for ruling in disease.

Discussion

The findings in this independent cohort confirm the high diagnostic performance of the previously validated and prespecified single APS2 cut point to distinguish CI individuals with brain amyloid pathology from those without amyloid pathology. The current findings add clinically relevant information to the assay performance reported in the head-to-head study of Schindler and colleagues[16] given that APS2 is reported to and used by physicians as a diagnostic aid for their symptomatic patients. The observed PrecivityAD2 test accuracy, sensitivity, and specificity values from the current study, and calculated PPV and NPV percentages at different patient population prevalences are consistent with prior clinical validation data[15]. Further, they meet the current, recommended performance standards for confirmatory diagnostic BBM tests to identify amyloid pathology based on accuracy, sensitivity, and specificity values using a single cut point in the intended use population[12,24–26]. For an MCI / early dementia patient population with assumed 50% AD prevalence the APS2 algorithm and cut point showed $\geq$90% accuracy, with sensitivity and specificity values corresponding to 92% PPV and 90% NPV (**Table 2**).

Continued evaluations of test performance in independent populations, where patient heterogeneity and variation in sample collection methods can be substantial, enable healthcare providers to understand a test's robustness, i.e., how the test will perform in their own patient population[35]. When clinical validity is assured, healthcare providers expect that a biomarker test for brain amyloid will have a direct impact on patient care by aiding in the diagnosis of the cause of cognitive decline and by guiding patient management[36].

Clinical utility studies have found that the PrecivityAD2 test improved physicians' certainty in making clinically meaningful changes in AD/non-AD diagnoses, treatment appropriateness decisions including when to prescribe or not prescribe anti-AD therapies, and referral patterns for brain amyloid PET imaging[9,37]. PrecivityAD2 testing also helped physicians rule out AD, allowing for other diagnostic considerations[37–39]. A recent survey by the Alzheimer's Association found that 70% of Americans want to know early if they have AD to allow for earlier interventions[40]. Early and accurate AD diagnosis can lead to positive patient outcomes, such as living independently for longer and maintaining a good quality of life because of access to appropriate services and support[41]. Other potential benefits may include more time for the patient and family to prepare, adjust and set realistic expectations for patient function, mood, and behaviors. For healthcare providers, early diagnosis may allow for more time to manage and treat underlying co-morbidities, address AD risk factors and safety issues, make referrals when

necessary, and develop a comprehensive clinical management plan (e.g., existing or novel drugs, enroll in a clinical trial)[41].

Possible explanations for the reliability and replicability of the performance metrics of the PrecivityAD2 test's APS2 algorithm include the use of a highly specific, high-resolution LC-MS/MS analytical platform for quantifying disease-specific peptides/proteins, and rigorous optimization and validation of plasma sample preparation methods in compliance with CLIA and CAP laboratory standards. In comparison to single-analyte immunoassays, this BBM test leverages the established analyte multiplexing capabilities and analyte specificity of LC-MS/MS to detect and quantify multiple Aβ or tau peptide species in each sample preparation and analytical workflow, which reduces inter-assay variability. This optimizes the quantitation of low abundant, plasma peptide species reflective of AD brain pathology. Variability is also reduced with the implementation of biomarker concentration ratios (rather than single analyte concentration alone) that can normalize for potential non-AD related factors expressed at the individual patient level. Variations in biomarker transport rates, blood volume, and biomarker metabolism have been shown to affect patient-specific AD biomarker levels, as have circadian rhythm, medications and differing lifestyle practices[42–44]. Presence of comorbidities, a common finding in individuals with AD, can also impact blood biomarker concentrations[45,46]. Higher plasma concentrations of Aβ42, Aβ40, p-tau217 and np-tau217 have been observed with chronic kidney disease, a condition that affects 40-50% of adults with AD[47,48]. Both Aβ42/40 and %p-tau217 are less impacted by chronic kidney disease compared to single-analyte biomarkers as concentration measures of both numerator and denominator for each set of biomarkers are thought to be similarly affected by alterations in renal clearance[48–51].

Although this replication cohort demonstrated diversity in patient age and sex (58-97 years, 50% women), the cohort is limited by the high education level (mean 16 years) and low number of under-represented minority individuals. These demographics highlight the cohort selection bias inherent to participants who have volunteered for enrollment into ADNI. Further, given the relatively small sample size, planned future evaluations will include larger, real-world cohorts that also include CI participants from varied social, educational and demographic backgrounds.

Conclusion

The findings indicate that using the clinically validated PrecivityAD2 blood test's APS2 algorithm in an independent population of CI individuals provided >90% accuracy, sensitivity, and specificity,

consistent with prior clinical validation data. These findings provide further evidence of the replicability and reliability of the test's APS2 algorithm and single cut point for identifying brain amyloid status in the intended use population. These results also support the test's use as an alternative confirmatory diagnostic to CSF or amyloid PET biomarker assessments in accordance with established recommendations for clinical implementation.

Author Contributions. All authors reviewed and edited initial manuscript drafts and reviewed and approved the final manuscript version. The study was conceptualized and executed by JC, TW, KMK, IF, SR, SA, PBV, JBB, and KEY. PBV supervised and KMK developed, monitored, and conducted quality control assessments during the plasma sample analysis at C2N Diagnostics. TW downloaded ADNI demographic and imaging data and provided independent statistical analysis.

Availability of data and materials. The datasets used and analyzed can be downloaded from the ADNI database (http://www.adni.loni.usc.edu) and the Parkinson's Progression Markers Initiative (PPMI) database (https://www.ppmi-info.org/access-data-specimens/download-data), RRID:SCR_006431.

Abbreviations

Aβ42	amyloid beta-42 (pg/mL)
Aβ40	amyloid beta-40 (pg/mL)
Aβ42/40	amyloid beta-42/amyloid beta-40 concentration ratio
AD	Alzheimer's disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
APS2	Amyloid Probability Score 2
AUC	area under the curve
BBM	blood biomarker
CAP	College of American Pathologists
CDR	Clinical Dementia Rating
CEOi	Global CEO Initiative on Alzheimer's disease
CI	cognitive impairment
CL	centiloid

CLIA	Clinical Laboratory Improvement Amendments
CSF	cerebrospinal fluid
K_2 EDTA	dipotassium ethylenediamine tetraacetic acid
FNIH	Foundation for the National Institutes of Health
IVD	*in vitro* diagnostics
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LDT	laboratory developed tests
MCI	mild cognitive impairment
MRI	magnetic resonance imaging
NA	no value available
NaN	value cannot be calculated
np-tau217	non-phosphorylated tau at threonine 217 (pg/mL)
NPV	negative predictive value
p-tau217	phosphorylated tau at threonine 217 (pg/mL)
%p-tau217	phosphorylated tau217/non-phosphorylated tau217 concentration ratio
PARIS	**P**lasma Test for **A**myloidosis **RI**sk **S**creening study
PET	positron emission tomography
PPV	positive predictive value
ROC	receiver operating characteristic curve
sd	standard deviation
SUVR	global standardized uptake value ratio
95%CI	95% confidence interval
WHO	World Health Organization

References

1   Steinmetz JD, Seeher KM, Schiess N, *et al.* Global, regional, and national burden of disorders affecting the nervous system, 1990–2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet Neurol*. 2024;23:344–81. doi: 10.1016/S1474-4422(24)00038-3

2   Alzheimer's Association. 2024 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*. 2024;20:3708–821. doi: 10.1002/alz.13809

3   Gustavsson A, Norton N, Fast T, *et al.* Global estimates on the number of persons across the Alzheimer's disease continuum. *Alzheimer's & Dementia*. 2023;19:658–70. doi: 10.1002/alz.12694

4   Warmenhoven N, Salvadó G, Janelidze S, *et al.* A comprehensive head-to-head comparison of key plasma phosphorylated tau 217 biomarker tests. *Brain*. 2025;148:416–31. doi: 10.1093/brain/awae346

5   Janelidze S, Bali D, Ashton NJ, *et al.* Head-to-head comparison of 10 plasma phospho-tau assays in prodromal Alzheimer's disease. *Brain*. 2023;146:1592–601. doi: 10.1093/brain/awac333

6   Janelidze S, Teunissen CE, Zetterberg H, *et al.* Head-to-Head Comparison of 8 Plasma Amyloid-β 42/40 Assays in Alzheimer Disease. *JAMA Neurol*. 2021;78:1375. doi: 10.1001/jamaneurol.2021.3180

7   Lu J, Ma X, Zhang H, *et al.* Head-to-head comparison of plasma and PET imaging ATN markers in subjects with cognitive complaints. *Transl Neurodegener*. 2023;12:34. doi: 10.1186/s40035-023-00365-x

8   Hampel H, Hu Y, Cummings J, *et al.* Blood-based biomarkers for Alzheimer's disease: Current state and future use in a transformed global healthcare landscape. *Neuron*. 2023;111:2781–99. doi: 10.1016/j.neuron.2023.05.017

9   Palmqvist S, Tideman P, Mattsson-Carlgren N, *et al.* Blood Biomarkers to Detect Alzheimer Disease in Primary Care and Secondary Care. *JAMA*. 2024;332:1245. doi: 10.1001/jama.2024.13855

10   Therriault J, Schindler SE, Salvadó G, *et al.* Biomarker-based staging of Alzheimer disease: rationale and clinical applications. *Nat Rev Neurol*. 2024;20:232–44. doi: 10.1038/s41582-024-00942-2

11   Zicha S, Bateman RJ, Shaw LM, *et al.* Comparative analytical performance of multiple plasma Aβ42 and Aβ40 assays and their ability to predict positron emission tomography amyloid positivity. *Alzheimer's & Dementia*. 2023;19:956–66. doi: 10.1002/alz.12697

12   Schindler SE, Galasko D, Pereira AC, *et al.* Acceptable performance of blood biomarker tests of amyloid pathology — recommendations from the Global CEO Initiative on Alzheimer's Disease. *Nat Rev Neurol*. 2024;20:426–39. doi: 10.1038/s41582-024-00977-5

13   Gonzalez-Ortiz F, Kirsebom B-E, Contador J, *et al.* Plasma brain-derived tau is an amyloid-associated neurodegeneration biomarker in Alzheimer's disease. *Nat Commun*. 2024;15:2908. doi: 10.1038/s41467-024-47286-5

14   Li Y, Schindler SE, Bollinger JG, *et al.* Validation of Plasma Amyloid-β 42/40 for Detecting Alzheimer Disease Amyloid Plaques. *Neurology*. 2022;98:e688–99. doi: 10.1212/WNL.0000000000013211

15   Meyer MR, Kirmess KM, Eastwood S, *et al.* Clinical validation of the PrecivityAD2 blood test: A mass spectrometry-based test with algorithm combining %p-tau217 and Aβ42/40 ratio to identify presence of brain amyloid. *Alzheimer's & Dementia*. 2024;20:3179–92. doi: 10.1002/alz.13764

16   Schindler SE, Petersen KK, Saef B, *et al.* Head-to-head comparison of leading blood tests for Alzheimer's disease pathology. *Alzheimer's & Dementia*. 2024;20:8074–96. doi: 10.1002/alz.14315

17   Schöll M, Vrillon A, Ikeuchi T, *et al.* Cutting through the noise: A narrative review of Alzheimer's disease plasma biomarkers for routine clinical use. *J Prev Alzheimers Dis*. 2025;100056. doi: 10.1016/j.tjpad.2024.100056

18   Janelidze S, Palmqvist S, Leuzy A, *et al.* Detecting amyloid positivity in early Alzheimer's disease using combinations of plasma Aβ42/Aβ40 and p-tau. *Alzheimer's & Dementia*. 2022;18:283–93. doi: 10.1002/alz.12395

19   Montoliu-Gaya L, Benedet AL, Tissot C, *et al.* Mass spectrometric simultaneous quantification of tau species in plasma shows differential associations with amyloid and tau pathologies. *Nat Aging*. 2023;3:661–9. doi: 10.1038/s43587-023-00405-1

20   Ashton NJ, Brum WS, Di Molfetta G, *et al.* Diagnostic Accuracy of a Plasma Phosphorylated Tau 217 Immunoassay for Alzheimer Disease Pathology. *JAMA Neurol*. 2024;81:255. doi: 10.1001/jamaneurol.2023.5319

21   Figdore DJ, Wiste HJ, Bornhorst JA, *et al.* Performance of the Lumipulse plasma Aβ42/40 and pTau181 immunoassays in the detection of amyloid pathology. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*. 2024;16:e12545. doi: 10.1002/dad2.12545

22   Pilotto A, Quaresima V, Trasciatti C, *et al.* Plasma p-tau217 in Alzheimer's disease: Lumipulse and ALZpath SIMOA head-to-head comparison. *Brain*. 2025;148:408–15. doi: 10.1093/brain/awae368

23   Dubois B, Villain N, Frisoni GB, *et al.* Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. *Lancet Neurol*. 2021;20:484–96. doi: 10.1016/S1474-4422(21)00066-1

24   Jack CR, Andrews JS, Beach TG, *et al.* Revised criteria for diagnosis and staging of

       Alzheimer's disease: Alzheimer's Association Workgroup. *Alzheimer's & Dementia*.

       2024;20:5143–69. doi: 10.1002/alz.13859

25   Mielke MM, Anderson M, Ashford JW, *et al.* Recommendations for clinical implementation

       of blood-based biomarkers for Alzheimer's disease. *Alzheimer's & Dementia*.

       2024;20:8216–24. doi: 10.1002/alz.14184

26   World Health Organization. Preferred product characteristics of blood-based biomarker

       diagnostics for Alzheimer disease. Geneva 2024.

27   Rissman RA, Langford O, Raman R, *et al.* Plasma Aβ42/Aβ40 and phospho-tau217

       concentration ratios increase the accuracy of amyloid PET classification in preclinical

       Alzheimer's disease. *Alzheimer's & Dementia*. 2024;20:1214–24. doi: 10.1002/alz.13542

28   Eastwood SM, Meyer MR, Kirmess KM, *et al.* PrecivityAD2™ Blood Test: Analytical

       Validation of an LC-MS/MS Assay for Quantifying Plasma Phospho-tau217 and Non-

       Phospho-tau217 Peptide Concentrations That Are Used with Plasma Amyloid-β42/40 in a

       Multianalyte Assay with Algorithmic Analysis for Detecting Brain Amyloid Pathology.

       Diagnostics. 2024;14:1739. doi: 10.3390/diagnostics14161739

29   Kirmess KM, Meyer MR, Holubasch MS, *et al.* The PrecivityAD™ test: Accurate and

       reliable LC-MS/MS assays for quantifying plasma amyloid beta 40 and 42 and

       apolipoprotein E proteotype for the assessment of brain amyloidosis. *Clinica Chimica

       Acta*. 2021;519:267–75. doi: 10.1016/j.cca.2021.05.011

30   West T, Kirmess KM, Meyer MR, *et al.* A blood-based diagnostic test incorporating

       plasma Aβ42/40 ratio, ApoE proteotype, and age accurately identifies brain amyloid

       status: findings from a multi cohort validity analysis. *Mol Neurodegener*. 2021;16:30. doi:

       10.1186/s13024-021-00451-6

31   Hu Y, Kirmess KM, Meyer MR, *et al.* Assessment of a Plasma Amyloid Probability Score to Estimate Amyloid Positron Emission Tomography Findings Among Adults With Cognitive Impairment. *JAMA Netw Open*. 2022;5:e228392. doi: 10.1001/jamanetworkopen.2022.8392

32   Fogelman I, West T, Braunstein JB, *et al.* Independent study demonstrates amyloid probability score accurately indicates amyloid pathology. *Ann Clin Transl Neurol*. 2023;10:765–78. doi: 10.1002/acn3.51763

33   Robin X, Turck N, Hainard A, *et al.* pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics*. 2011;12:77. doi: 10.1186/1471-2105-12-77

34   DeVore J. *Probability and Statistics for Engineering and the Sciences*. 7th Edition. Brooks/Cole Cengage Learning 2009.

35   Angioni D, Delrieu J, Hansson O, *et al.* Blood Biomarkers from Research Use to Clinical Practice: What Must Be Done? A Report from the EU/US CTAD Task Force. *J Prev Alzheimers Dis*. 2022;9:569–79. doi: 10.14283/jpad.2022.85

36   Rabinovici GD, Knopman DS, Arbizu J, *et al.* Updated appropriate use criteria for amyloid and tau PET: A report from the Alzheimer's Association and Society for Nuclear Medicine and Molecular Imaging Workgroup. *Alzheimer's & Dementia*. 2025;21:e14338. doi: 10.1002/alz.14338

37   Monane M, Maraganore DM, Carlile RM, *et al.* Clinical Utility of an Alzheimer's Disease Blood Test Among Cognitively Impaired Patients: Results from the Quality Improvement PrecivityAD2 (QUIP II) Clinician Survey Study. *Diagnostics*. 2025;15:167. doi: 10.3390/diagnostics15020167

38   Canestaro WJ, Bateman RJ, Holtzman DM, *et al.* Use of a Blood Biomarker Test Improves Economic Utility in the Evaluation of Older Patients Presenting with Cognitive Impairment. *Popul Health Manag*. 2024;27:174–84. doi: 10.1089/pop.2023.0309

39   Monane M, Johnson KG, Snider BJ, *et al.* A blood biomarker test for brain amyloid impacts the clinical evaluation of cognitive impairment. *Ann Clin Transl Neurol*. 2023;10:1738–48. doi: 10.1002/acn3.51863

40   2022 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*. 2022;18:700–89. doi: 10.1002/alz.12638

41   Reiss AB, de Levante Raphael D, Chin NA, *et al.* The physician's Alzheimer's disease management guide: Early detection and diagnosis of cognitive impairment, Alzheimer's disease and related dementia. *AIMS Public Health*. 2022;9:661–89. doi: 10.3934/publichealth.2022047

42   Benedict C, Blennow K, Zetterberg H, *et al.* Effects of acute sleep loss on diurnal plasma dynamics of CNS health biomarkers in young men. *Neurology*. 2020;94:e1181–9. doi: 10.1212/WNL.0000000000008866

43   Li P, Gao L, Gaba A, *et al.* Circadian disturbances in Alzheimer's disease progression: a prospective observational cohort study of community-based older adults. *Lancet Healthy Longev*. 2020;1:e96–105. doi: 10.1016/S2666-7568(20)30015-5

44   Weigand AJ, Bondi MW, Thomas KR, *et al.* Association of anticholinergic medications and AD biomarkers with incidence of MCI among cognitively normal older adults. *Neurology*. 2020;95:e2295–304. doi: 10.1212/WNL.0000000000010643

45   Browne J, Edwards DA, Rhodes KM, *et al.* Association of comorbidity and health service usage among patients with dementia in the UK: a population-based study. *BMJ Open*. 2017;7:e012546. doi: 10.1136/bmjopen-2016-012546

46   Ramanan VK, Graff-Radford J, Syrjanen J, *et al.* Association of Plasma Biomarkers of Alzheimer Disease With Cognition and Medical Comorbidities in a Biracial Cohort. *Neurology*. 2023;101:e1402–11. doi: 10.1212/WNL.0000000000207675

47   Kelly DM, Rothwell PM. Disentangling the Relationship Between Chronic Kidney Disease and Cognitive Disorders. *Front Neurol*. 2022;13:830064. doi: 10.3389/fneur.2022.830064

48   Bornhorst JA, Lundgreen CS, Weigand SD, *et al.* Quantitative Assessment of the Effect of Chronic Kidney Disease on Plasma P-Tau217 Concentrations. *Neurology*. 2025;104:e210287. doi: 10.1212/WNL.0000000000210287

49   Pichet Binette A, Janelidze S, Cullen N, *et al.* Confounding factors of Alzheimer's disease plasma biomarkers and their impact on clinical performance. *Alzheimer's & Dementia*. 2023;19:1403–14. doi: 10.1002/alz.12787

50   Janelidze S, Barthélemy NR, He Y, *et al.* Mitigating the Associations of Kidney Dysfunction With Blood Biomarkers of Alzheimer Disease by Using Phosphorylated Tau to Total Tau Ratios. *JAMA Neurol*. 2023;80:516. doi: 10.1001/jamaneurol.2023.0199

51   Lehmann S, Schraen-Maschke S, Vidal J, *et al.* Plasma Aβ42/Aβ40 ratio is independent of renal function. *Alzheimer's & Dementia*. 2023;19:2737–9. doi: 10.1002/alz.12949
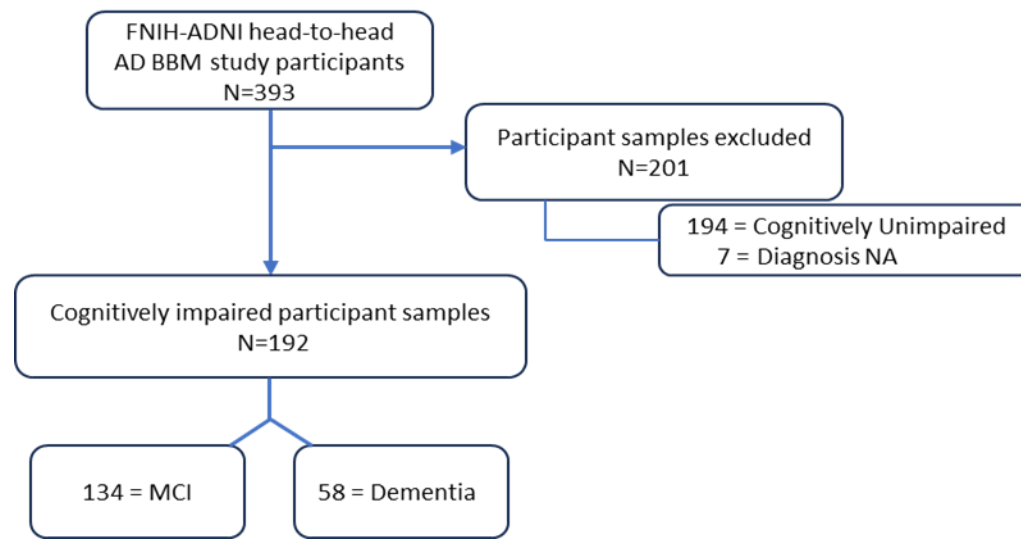
Figure 1.

Figure 2.