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RESEARCH ARTICLE

Independent study demonstrates amyloid probability score accurately indicates amyloid pathology

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Abstract

Background: The amyloid probability score (APS) is the model read-out of the analytically validated mass spectrometry-based PrecivityAD® blood test that incorporates the plasma Aβ42/40 ratio, ApoE proteotype, and age to identify the likelihood of brain amyloid plaques among cognitively impaired individuals being evaluated for Alzheimer's disease. **Purpose**: This study aimed to provide additional independent evidence that the pre-established APS algorithm, along with its cutoff values, discriminates between amyloid positive and negative individuals. **Methods**: The diagnostic performance of the PrecivityAD test was analyzed in a cohort of 200 nonrandomly selected Australian Imaging, Biomarker & Lifestyle Flagship Study of Aging (AIBL) study participants, who were either cognitively impaired or healthy controls, and for whom a blood sample and amyloid PET imaging were available. **Results**: In a subset of the dataset aligned with the Intended Use population (patients aged 60 and older with CDR ≥0.5), the pre-established APS algorithm predicted amyloid PET with a sensitivity of 84.9% (CI: 72.9–92.1%) and specificity of 96% (CI: 80.5–99.3%), exclusive of 13 individuals for whom the test was inconclusive. **Interpretation**: The study shows individuals with a high APS are more likely than those with a low APS to have abnormal amounts of amyloid plaques and be on an amyloid accumulation trajectory, a dynamic and evolving process characteristic of progressive AD pathology. Exploratory data suggest APS retains its diagnostic performance in healthy individuals, supporting further screening studies in the cognitively unimpaired.

Introduction

The global number of persons with Alzheimer's disease (AD) dementia and prodromal AD are estimated at 32 and 69 million, respectively.[1] In the United States, an estimated 6.5 million Americans aged 65 and older are living with AD dementia in 2022.[2] The number of people with mild cognitive impairment due to AD – a condition that can progress to dementia due to AD – is estimated at two-fold the number of those with clinical AD[3]. Driven by population aging, the number of Americans with AD

dementia is expected to increase significantly to 13.85 million by 2060,[3] placing major demands on healthcare services and payers. Despite the staggering projections, AD and other dementias are underdiagnosed by clinicians and underreported by patients and families. In a nationally representative cohort, only 41% of older adults with probable dementia were both diagnosed and aware of the diagnosis.[4] The diagnosis of AD based on clinical criteria has insufficient sensitivity (70.9–87.3%) and specificity (44.3–70.8%), leading to high misdiagnosis rates.[5] When a diagnosis does occur, it is typically at a relatively late

stage when cognitive impairment and disability are prominent and less amenable to treatment.

Substantial progress over the last several decades in the development of amyloid PET imaging and cerebrospinal fluid (CSF) biomarkers has enabled the ante-mortem detection of brain amyloid changes, which are necessary for an AD diagnosis. While these technologies have reduced misdiagnosis rates, many barriers to their broad clinical implementation exist due to high cost, the need for specialist care, limited access, and lack of reimbursement.[6] In addition, evolving AD therapeutic and prevention strategies are primarily aimed at slowing or halting disease progression among patients in early and presymptomatic stages – large populations for whom the radiation of amyloid PET scans or invasiveness of lumbar punctures has a less favorable risk/benefit profile, as well as high cost and challenges in implementation. With recent disease-modifying treatments that successfully remove amyloid plaques,[7–10] there is a clinical need to identify individuals who have amyloid plaques to determine treatment eligibility. Thus, a significant unmet need exists for a simple, radiation-free, less-invasive, and less resource- and time-intensive diagnostic method to reduce the underdiagnosis and misdiagnosis of AD and related dementias in individuals with signs or symptoms of cognitive impairment. Encouragingly, plasma-based biomarkers of AD pathology that may be able to reduce this unmet medical need are now available.

C$_2$N Diagnostics' (C$_2$N) PrecivityAD® blood test is a validated laboratory-developed test (LDT) to aid in the diagnosis of AD. The test was developed, trained and its performance evaluated on a dataset generated by combining two independent studies with a total of 686 cognitively impaired individuals 60 years and older being evaluated for AD[11]. Those studies supported the PrecivityAD test's certification under the Clinical Laboratory Improvement Amendments (CLIA) as a test to identify the likelihood of amyloid pathology as measured by amyloid PET imaging in individuals 60 years and older with signs or symptoms of cognitive impairment.

The PrecivityAD test quantifies an individual's plasma Aβ42/40 ratio and identifies apolipoprotein E isoform-specific peptides (ApoE proteotype) to infer *APOE* genotype using high resolution liquid chromatography mass spectrometry (LC–MS/MS).[12] An algorithm combines Aβ42/40 ratio, ApoE proteotype, and age to derive the amyloid probability score (APS), the test read-out. APS results are categorized as Low (0–35), Intermediate (36–57), and High (58–100) – corresponding to the likelihood of amyloid positivity by amyloid PET scan. The Intermediate APS category corresponds to a small subset (~14% in the original CLIA study) for whom the test is inconclusive, requiring additional diagnostic testing.

This study provides additional data on the performance of the PrecivityAD test and the APS read-out in an independent cohort of cognitively impaired as well as healthy control individuals who have participated in the longitudinal Australian Imaging, Biomarker & Lifestyle Flagship Study of Aging (AIBL).

Methods

AIBL study

AIBL is a well-characterized ongoing prospective longitudinal study designed to improve the understanding of AD pathogenesis, focusing on its early diagnosis and the identification of factors that eventually may delay AD onset. The AIBL methodology was previously described.[13,14] AIBL enrolled an inception cohort of 1,112 individuals from 2006 to 2008 and a subsequent enrichment cohort of another 1,247 participants to compensate for attrition. The study has collected an extensive battery of cognitive data, lifestyle information and blood samples from participants every 18 months until month 126. A subset of individuals has also undergone regular amyloid PET imaging. At baseline, all cognitive and clinical data were considered by a review panel to classify participants as probable or possible AD by NINCDS-ADRDA criteria,[15] mild cognitive impairment (MCI) by Winblad *et al.* criteria,[16] cognitively normal healthy controls with or without subjective memory complaints, or as other (dementia other than AD or cognitive impairment not suspected to be due to AD).

Cohort selection and cognitive group assignment

A cohort of 200 nonrandomly selected AIBL study participants, for whom a blood sample and amyloid PET imaging were available, were included in the present analysis. The cohort was intentionally selected by AIBL study staff to meet the study investigators' request for a cohort with approximately 50% healthy controls and 50% amyloid positive individuals (targeting >60% amyloid positivity in cognitively impaired patients) as determined by an amyloid PET scan. None of the study investigators had any involvement in the selection of which AIBL study participants would be included in this analysis.

The cognitive group to which participants were assigned by AIBL at study inception was available for 87% of individuals in this analysis. However, for many individuals such assignment took place many years ago (upon enrollment into AIBL). Therefore, for purposes of this analysis, cognitive status was defined by the CDR value at the visit when the blood sample used for

PrecivityAD testing was collected. Individuals with CDR = 0 were deemed healthy controls, while those with CDR = 0.5 or greater were considered cognitively impaired and within the Intended Use population for the PrecivityAD test.

Timing of blood sample collection and sample age

A single blood sample per participant was provided for PrecivityAD test analysis. For 193 individuals, amyloid PET imaging results were available at the same study visit (*n* = 160), although not necessarily on the same day, or one 18-month visit apart (*n* = 33) from when blood samples were collected. For four individuals, amyloid PET imaging and blood sampling were done two visits apart (i.e., a 36-month lag), four visits apart for two individuals (a 72-month lag), and five visits apart (a 90-month lag) for one individual (Fig. 1). The seven individuals with a time lag of 36 months or more were excluded from the analysis of longitudinal PET data.

Sample age at the time of PrecivityAD testing was available for 199 of the 200 participants. Age was defined as the time gap between blood collection and PrecivityAD

testing. Samples with a time gap shorter than 18 months were deemed fresh, while those with a time gap of 18 months or older were deemed old samples.

PrecivityAD® testing and amyloid PET scans

Samples were shipped to C$_2$N's laboratory facility in St. Louis, MO and analyzed with the PrecivityAD test as previously described[12] in a blinded manner to all corresponding clinical, demographic, imaging, and other personal data. Statistical analyses were performed by C$_2$N after unblinding using amyloid PET scan with Centiloid >25 as the reference standard for amyloid positivity. Centiloid >25 is the cutoff for amyloid positivity C$_2$N Diagnostics prospectively defined for purposes of its CLIA certification.[11] Several amyloid tracers were used in AIBL: ^{18}F-NAV4694 (64%), ^{11}C-PIB (62%), ^{18}F-flutemetamol (48%) and ^{18}F-florbetapir (24%).

Statistical analyses

For the statistical analyses, variables were summarized for the entire cohort and by APS categories and amyloid PET status. Continuous data are summarized by the number
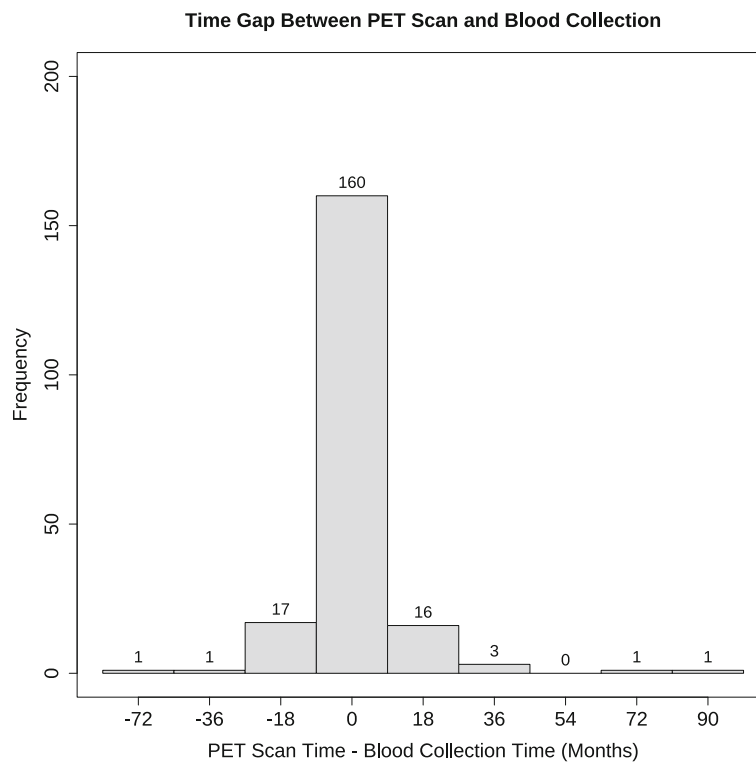
Figure 1. Time gap between amyloid PET scan and blood collection visits. AIBL study visits normally took place 18 months apart. Time zero represents the blood collection time that is closest (same AIBL study visit, but not necessarily the same day) to the PET scan time for a given subject. A negative time means that the blood sample was collected at a visit that preceded the PET scan visit, a positive time means that the blood sample was collected at a visit that followed the PET scan visit.

of observations, mean, and standard deviation. Categorical data are summarized by the number of observations, percentages, and frequency counts. Percentages from longitudinal assessments were compared by Chi-square test. Levene's test was used to evaluate the homogeneity of variances among the groups. Normal QQ plot and Shapiro–Wilk test was used to evaluate normality. Kruskal-Wallis test was used to compare group means when data did not follow a normal distribution.

Boxplots, histograms, and scatterplots were used to visualize the data. Longitudinal PET data was visualized by line plot for each individual showing Centiloid (Y axis) versus Time (X axis).

The concordance between clinical diagnostic results (amyloid PET status) and assay results (APS categories) was evaluated by Sensitivity, Specificity, positive predictive value (PPV) and negative predictive value (NPV) after excluding individuals with an intermediate APS score, and after including intermediate APS scores as either amyloid positive or negative. Wilson score method[17] was used to calculate the 95% confidence interval for each parameter. The area under the receiver operating characteristic curve (AUC) is commonly reported for diagnostic tests in development because the AUC is a summary measure across all possible cutoffs. However, the APS algorithm and cutoffs were previously established and prespecified for this analysis. Therefore, the AUC is not reported because this study's objective was to assess the performance of the test, given the prespecified parameters.

Diagnostic performance was assessed for the PrecivityAD test's Intended Use population defined under CLIA as individuals 60 years and older with signs or symptoms of cognitive impairment. Exploratory analyses examined the PrecivityAD test in patients with longitudinal amyloid PET scans, as well as in a population of healthy controls for whom the PrecivityAD test is not currently clinically indicated.

Results

Demographic characteristics

Among the 200 AIBL individuals analyzed in this study, 103 (51.5%) were healthy controls as defined by a CDR = 0 at the blood sampling visit. The remaining 97 (48.5%) had CDR = 0.5 or greater and were thus deemed cognitively impaired. Except for 6 (3%) cognitively impaired individuals aged between 55 and 59; all others were 60 and older.

By design, the cohort's prevalence of amyloid positivity was 50.5%, and Table 1 provides summary statistics for amyloid negative and positive groups. The amyloid

Table 1. Summary of study participant characteristics across PET centiloid categories (amyloid PET positivity defined by Centiloid >25).

	Amyloid PET categories	
	Positive	Negative
N (%)	101 (50.5)	99 (49.5)
Age (mean [SD])	72.38 (6.70)	70.39 (6.53)
Amyloid PET centiloid values (mean [SD])	87.89 (35.37)	0.48 (8.15)
Abeta42/40.PLASMA (mean [SD])	0.088 (0.006)	0.102 (0.007)
APS (mean [SD])	64.35 (24.90)	14.15 (15.18)
MMSE (mean [SD])	25.63 (4.07)	27.97 (3.35)
Neuropsych. CDR Sum of boxes (mean [SD])	2.24 (2.51)	0.45 (1.48)
Sex (%)		
Male	56 (53.3)	49 (46.7)
Female	45 (47.4)	50 (52.6)
APS category (%)		
Low (0–35)	17 (16.3)	87 (83.7)
Intermediate (36–57)	17 (60.7)	11 (39.3)
High (58–100)	67 (98.5)	1 (1.5)
Cognitive group (%)		
Healthy control	32 (31.1)	71 (68.9)
Cognitively impaired	69 (71.1)	28 (28.9)
CDR group (%)		
No dementia (0)	32 (31.1)	71 (68.9)
Questionable dementia (0.5)	50 (65.8)	26 (34.2)
Mild dementia (1)	18 (94.7)	1 (5.3)
Moderate dementia (2)	1 (50.0)	1 (50.0)

APS, amyloid probability score; CDR, clinical dementia rating scale; MMSE, mini mental state examination

positive group was slightly older than the amyloid negative (mean age of 72.4 versus 70.4, respectively; $p = 0.035$), but there was no statistically significant difference in sex between the two groups. The majority (71.1%) of cognitively impaired individuals and 31.1% of healthy controls were amyloid PET positive. Among all amyloid positive individuals, 13% were *APOE4* homozygous and 46% were heterozygous; 14% of amyloid negative individuals were *APOE4* heterozygous, and none were homozygous.

Table 2 compares participant characteristics across APS categories. Among the 200 individuals, 68 (34.0%) were in the High APS category, 104 (52.0%) were in the Low APS category, and – consistent with observations from the PrecivityAD test's clinical validation for CLIA certification – 14.0% were in the Intermediate APS category for whom the PrecivityAD test is indeterminate. There were statistically significant differences in age ($p = 2.5e-05$) and sex ($p = 0.007$) between individuals in the High and Low APS categories. There were no differences in age between individuals in the High and Intermediate APS categories, but differences in sex were statistically significant ($p = 0.00191$).

Table 2. Summary of study participant characteristics across APS subgroups.

	APS categories		
	Low	Intermediate	High
N (%)	104 (52.0)	28 (14.0)	68 (34.0)
Age (mean [SD])	69.57 (6.69)	71.57 (5.38)	74.12 (6.25)
Amyloid PET Centiloid value (mean [SD])	14.60 (36.96)	51.39 (49.38)	87.75 (35.73)
Abeta42/40.PLASMA (mean [SD])	0.102 (0.007)	0.090 (0.003)	0.086 (0.004)
APS (mean [SD])	11.51 (10.39)	47.36 (6.72)	79.07 (11.26)
MMSE (mean [SD])	27.68 (3.46)	27.25 (3.01)	25.24 (4.41)
Neuropsych. CDR Sum of Boxes (mean (SD))	0.73 (1.76)	1.25 (1.72)	2.37 (2.71)
Sex (%)			
Male	48 (45.7)	11 (10.5)	46 (43.8)
Female	56 (58.9)	17 (17.9)	22 (23.2)
Amyloid PET categories (%)			
Positive	17 (16.8)	17 (16.8)	67 (66.3)
Negative	87 (87.9)	11 (11.1)	1 (1.0)
Cognitive group (%)			
Healthy control	67 (65.0)	15 (14.6)	21 (20.4)
Cognitively impaired	37 (38.1)	13 (13.4)	47 (48.5)
CDR group (%)			
No dementia (0)	67 (65.0)	15 (14.6)	21 (20.4)
Questionable dementia (0.5)	32 (42.1)	11 (14.5)	33 (43.4)
Mild dementia (1)	4 (21.1)	2 (10.5)	13 (68.4)
Moderate dementia (2)	1 (50.0)	0 (0.0)	1 (50.0)

Most (65.0%) healthy individuals and 38.1% of cognitively impaired individuals were in the Low APS category, while 48.5% of cognitively impaired and 20.4% of healthy individuals were in the High APS category. The majority (87.9%) of amyloid PET negative individuals were in the Low APS category, while 66.3% of amyloid PET positive individuals were in the High APS category.

The Aβ42/40 ratio

Consistent with prior studies,[11,18–22] the plasma Aβ42/40 ratio discriminated between amyloid positive and negative individuals (Fig. 2A). Ninety six percent of amyloid PET negative individuals and 41.6% of amyloid PET positive individuals, had Aβ42/40 ratios above the PrecivityAD test's previously established CLIA cutoff of 0.089 (Fig. 2B).

Amyloid probability score

APS values calculated with the PrecivityAD test classified each individual as having a Low, Intermediate, or High likelihood of amyloid positivity by amyloid PET scan, using the test cutoff previously established for the PrecivityAD test for purposes of CLIA certification[11] and as currently used in clinical care.

Levene's test was used to evaluate the homogeneity of variance of PET Centiloid across APS categories. The *p*-value was 0.0012, indicating that the variances were different among the 3 APS categories. The residuals from the linear model (Centiloid is the dependent variable; APS category is the independent variable) were used to evaluate the normality of Centiloid. The result showed that the Centiloid scores did not follow a normal distribution. Kruskal-Wallis's test showed that the Centiloid values among the three APS categories were different ($p < 0.001$).

APS differentiated amyloid positive from amyloid negative AIBL participants. Across both cognitive groups combined, all but one individual (98.5%) in the High APS category were amyloid positive by Centiloid >25, while most (83.7%) Low APS individuals had negative amyloid PET scans by Centiloid (Fig. 2C,D). Consistent with prior experience, the Intermediate APS group did not show a clear discrimination between amyloid PET positive (60.7%) and negative (39.3%) individuals.

Performance in the PrecivityAD test's intended use population

The PrecivityAD test's Intended Use population under CLIA consists of individuals 60 years and older with signs or symptoms of cognitive impairment. To compare the PrecivityAD test's performance in this AIBL cohort with the CLIA certification cohort, an analysis was conducted on a subset of the dataset that included only individuals who met the PrecivityAD test's Intended Use: 91 individuals who were 60 and older with CDR ≥0.5.

APS discriminated between amyloid positive and negative individuals (Fig. 3A–C). Using the prespecified CLIA cutoffs and excluding 13 individuals from the Intermediate APS group, the sensitivity of APS in predicting amyloid PET scan results was 84.9% (CI: 72.9–92.1%), specificity was 96% (CI: 80.5–99.3%), with a PPV of 97.8% (CI: 88.7–99.6%) and NPV of 75% (CI: 57.9–86.7%), with an amyloid positivity prevalence of 68%. The diagnostic performance of APS differed when the small number of Intermediate results were counted as either amyloid positive (sensitivity: 88.0% [CI: 77.5–93.6%]; specificity: 92.3% [CI: 75.9–97.9%]) or negative (sensitivity: 69.2% [CI: 57.7–79.1%]; specificity: 96.2% [CI: 81.1–99.3%]). See Tables S1 and S2 for more details. To enable direct comparisons, the PrecivityAD test's performance in both the AIBL and CLIA cohort was reweighted to an amyloid prevalence of 60% (Table 3). The CLIA validation cohort showed a higher
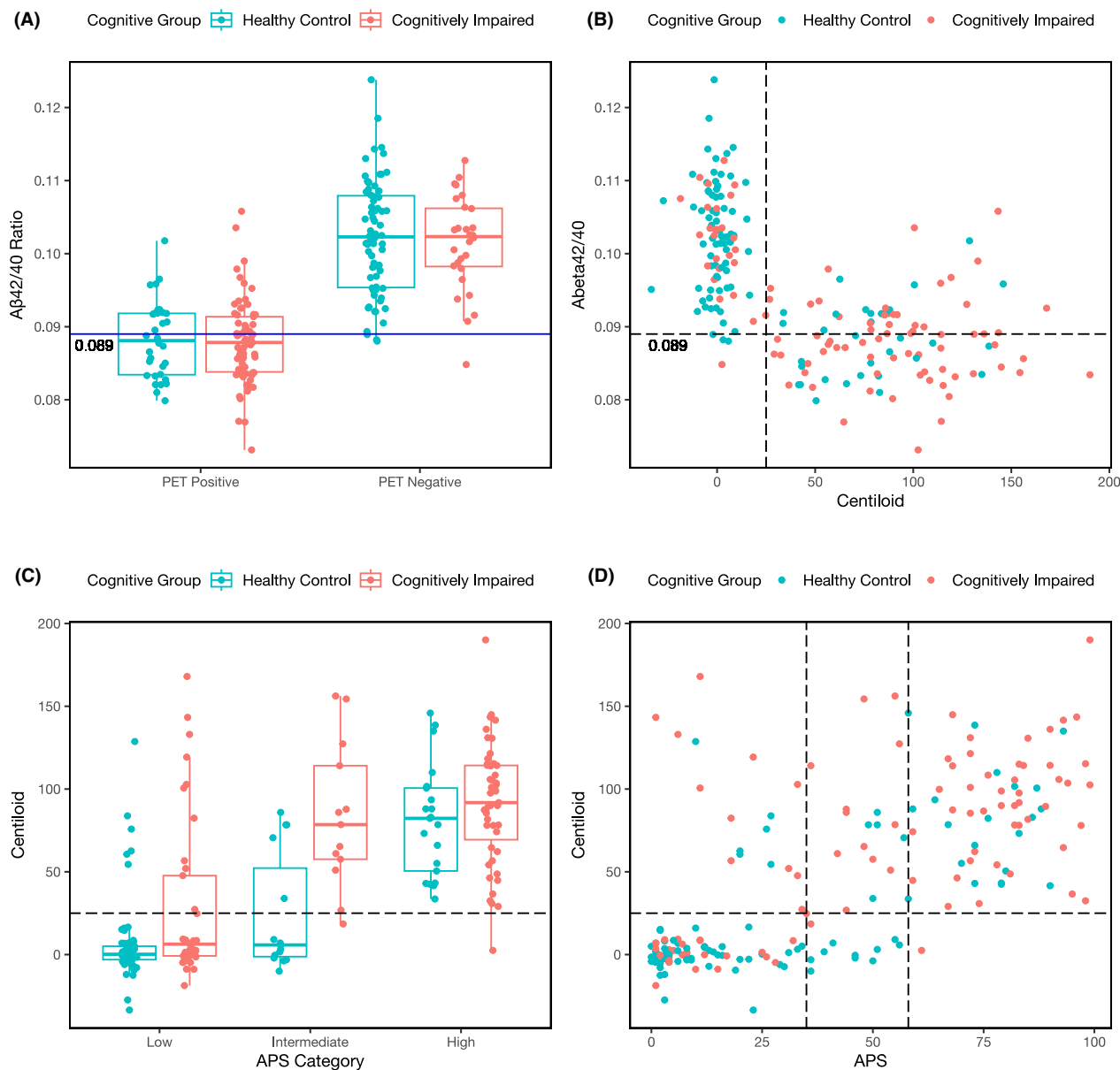
Figure 2. Associations between Aβ42/40 Ratio and APS values with amyloid PET results. Amyloid PET positivity defined as Centiloid greater than 25. APS categories are defined by APS scores as Low (0–35), Intermediate (36–57), and High (58–100). Individuals are colored by cognitive groups (Healthy control and cognitively impaired). (A) Subjects' plasma Aβ42/40 ratio by amyloid status. The previously established CLIA cutoff is 0.089 (horizontal line). (B) Scatter Plots for Aβ42/40 Ratio versus amyloid PET. The vertical dashed line is drawn at Centiloid of 25; the horizontal dashed line is the previously established CLIA cutoff drawn at 0.089. (C) Box and whisker plot of Centiloid values for each of the three APS categories. The horizontal dashed line represents Centiloid of 25. (D) Scatter plot comparing Centiloid and APS. The horizontal dashed line represents Centiloid of 25; the vertical dashed lines represent APS of 35 and 58.

NPV of 86.3% (CI: 85.0–87.5%) versus 80.9% (CI: 65.6–90.4%) for AIBL, while AIBL showed a higher PPV of 97.0% (CI: 86.5–99.4%) as compared to the CLIA cohort's 85.9% (CI: 84.9–86.8%). Table 4 describes NPV and PPV for the AIBL and CLIA cohorts reweighted to other prevalence rates.

Exploratory longitudinal PET analysis

For the entire cohort, the average number of PET scans per individual was 2.5 (ranging from 1 to 7 scans). Centiloid values for repeat amyloid PET scans were available for 133 (68.9%) individuals, allowing a longitudinal
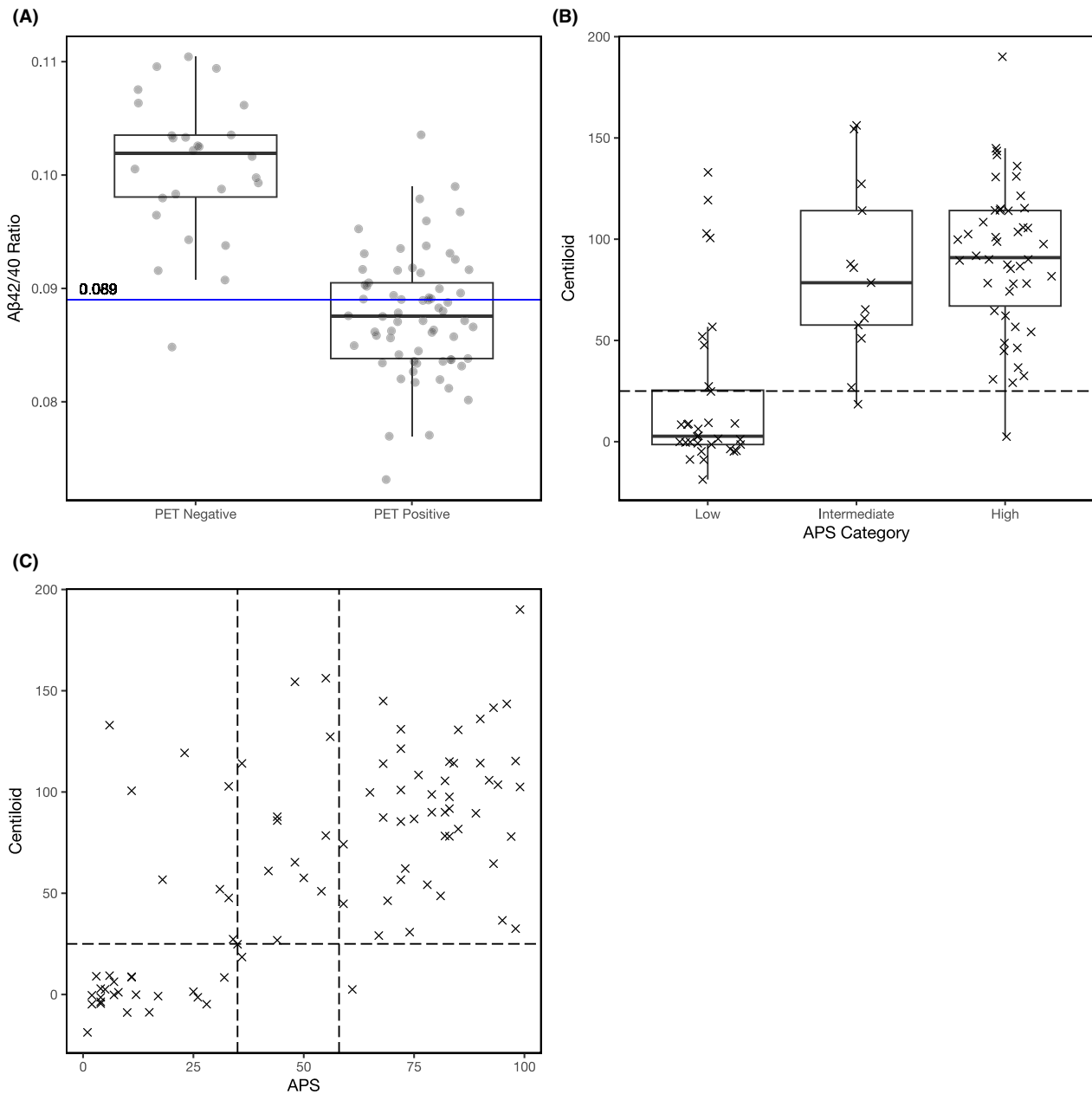
Figure 3. Associations between assay results (Aβ42/40 Ratio, APS) and PET Centiloid in PrecivityAD test's Intended Use population (*N* = 91). PET positivity was defined by Centiloid greater than 25. APS categories were defined by APS scores as Low (0–35), Intermediate (36–57), and High (58–100). (A) Subjects' plasma Aβ42/40 ratio by amyloid PET status. The previously established CLIA cutoff is 0.089. (B) Box and whisker plot of Centiloid values for each of the three APS categories. The horizontal dashed line represents Centiloid of 25. (C) Scatter plot comparing Centiloid and APS. The horizontal dashed line represents Centiloid of 25. The vertical dashed lines represent APS of 35 and 58.

amyloid accumulation subset analysis. The amyloid PET visit that corresponded to the draw date for the blood samples utilized in this analysis was assigned time zero to facilitate visualization of amyloid progression over time relative to PrecivityAD testing date. Figure 4 displays longitudinal Centiloid values for healthy and cognitively impaired individuals with at least two completed scans by APS category.

Two amyloid PET patterns emerged: (i) individuals with stable and low Centiloid values; (ii) individuals with increasing Centiloid values indicative of a progressive amyloid accumulation trajectory. Most individuals in the

Table 3. NPV and PPV based on comparing Low and High APS to previously reported CLIA validation data.[11]

	CLIA cohort n = 686	AIBL cohort n = 91	CLIA cohort n = 686	AIBL cohort n = 91
	Observed PET amyloid prevalence		Reweighted PET amyloid prevalence	
Amyloid positivity prevalence	378/686 = 55.1%	53/78 = 68%	60%	60%
APS performance				
NPV of low score (0–35)[1]	88.1% (83.2–91.7)	75.0% (57.9–86.7)	86.3% (85.0–87.5)	80.9% (65.6–90.4)
PPV of high score (58–100)[1]	83.0% (78.8–86.5)	97.8% (88.7–99.6)	85.9% (84.9–86.8)	97.0% (86.5–99.4)

[1]Number (%) of individuals excluded from NPV/PPV analysis due to intermediate score was 95/686 (13.8%) for CLIA dataset and 28/200 (14.0%) for AIBL cohort.

Table 4. NPV and PPV under different amyloid positivity prevalence values.

Amyloid positivity prevalence (%)	PPV of high score (58–100) (%)		NPV of low score (0–35) (%)	
	CLIA cohort (%)	AIBL cohort (%)	CLIA cohort (%)	AIBL cohort (%)
40	73.0	93.4	93.4	90.5
45	76.8	94.6	92.0	88.6
50	80.2	95.5	90.4	86.4
55	83.2	96.3	88.5	83.9
60	85.9	97.0	86.3	80.9
65	88.3	97.5	83.6	77.4
70	90.4	98.0	80.2	73.2
75	92.4	98.5	75.9	67.9
80	94.2	98.8	70.2	61.4

Low APS category had a stable Centiloid pattern indicative of no amyloid accumulation, while those in the High APS category had a progressive Centiloid pattern. The Intermediate APS category included a mix of individuals with stable and progressive Centiloid values.

Exploratory performance in healthy controls

The PrecivityAD test is not yet indicated for healthy individuals, but as an exploratory analysis, the performance of the APS was evaluated among the 103 healthy individuals with CDR = 0 who were 60 and older (Fig. 5A–C). When excluding 15 individuals from the Intermediate group (for whom there is still uncertainty regarding their amyloid status, that is, require follow-up testing), APS discriminated between amyloid positive and negative individuals, with a sensitivity of 77.8% (CI 59.2–89.4%), specificity of 100% (CI 94.1–100%), PPV of 100% (CI 84.5–100%), and NPV of 91% (CI 81.8–95.8%).

Frozen sample storage stability

The 200 AIBL participant plasma samples analyzed using the PrecivityAD test varied in age—the length of time they were frozen at −80°C before analysis. One sample lacked a blood collection date, but 60% of the remaining 199 samples were analyzed within 18 months of collection (fresh; mean of 7 months, ranging from 0 to 18), while 40% of the samples were analyzed between 19 and 140 months after collection (older; mean of 76 months). An analysis of PrecivityAD test performance showed comparable sensitivity, specificity, PPV, NPV and stability regardless of the duration (up to 140 months) that samples were stored frozen at −80°C before analysis (See Text S1).

Discussion

The PrecivityAD blood test is a clinically available, CLIA-certified, College of American Pathologists (CAP)-accredited, laboratory-developed test that aids in the diagnosis of AD in individuals 60 years and older with cognitive impairment. It incorporates the Aβ42/40 ratio, ApoE proteotype, and age into an algorithm to generate an individual's APS, the test read-out. APS can be classified into Low, Intermediate or High categories reflecting the likelihood of amyloid positivity by amyloid PET scan. The validity of the three-category APS read-out was previously demonstrated.[11,12] The current study, using a nonrandom cohort of AIBL study participants, provides independent additional supporting evidence for the diagnostic accuracy of the APS value generated by the PrecivityAD test as a blood-based biomarker for amyloid pathology as measured by amyloid PET scans. Additional exploratory results presented suggest that APS values: (i) may accurately predict brain amyloid status in cognitively unimpaired individuals; (ii) may inform the potential trajectory of amyloid accumulation among individuals with Low or High APS values; and (iii) were based on plasma biomarker measures that were stable in frozen samples stored at −80°C for up to 140 months before analysis.

Using the model and test cutoff values previously established in the CLIA validation cohort in October 2020, APS discriminated between amyloid positive and negative AIBL participants. An analysis focused on the AIBL cohort subset
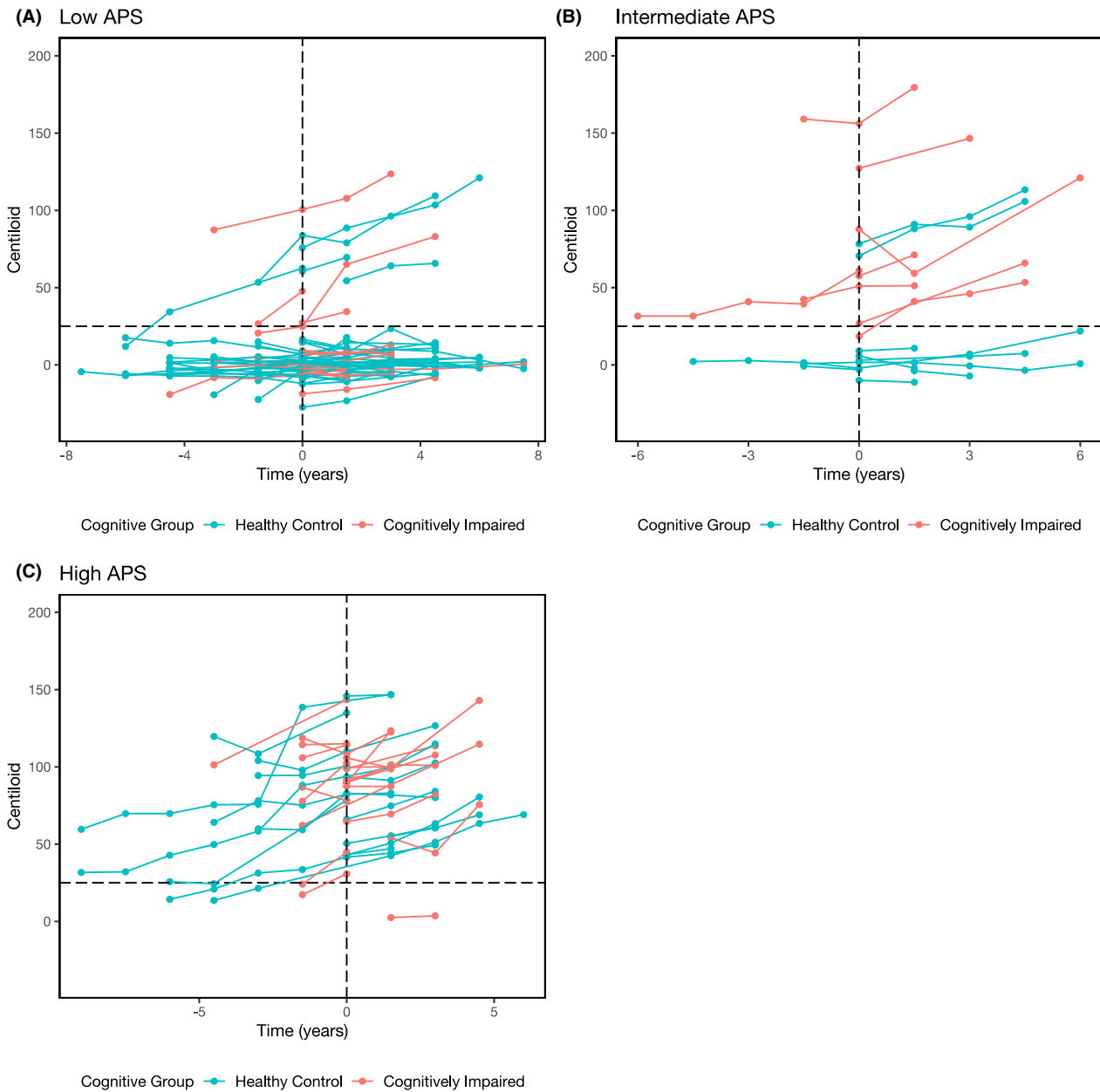
(A) Low APS

(B) Intermediate APS

(C) High APS

Figure 4. Longitudinal amyloid PET measurements (Centiloid) by APS category. Centiloid values over time for individuals who had more than one amyloid PET scan shown for the various APS categories (A: Low, B: Intermediate, C: High). Blood draw visit for APS measurement is represented as time zero.

who met the PrecivityAD test's Intended Use (individuals 60 and older with cognitive impairment) showed high concordance between APS and amyloid PET results (sensitivity 84.9% (CI: 72.9–92.1%), specificity 96% (CI: 80.5–99.3%)) after excluding 14% of the participants with an intermediate APS prediction for whom the test is noninformative.

The plasma Aβ42/40 ratio also showed strong diagnostic performance in the present cohort, but the APS had a

marginally better diagnostic performance versus the Aβ42/40 ratio alone. In line with previous reports,[11,23] by including well-known risk/susceptibility markers such as ApoE proteotype (i.e., inferred genotype) and age, APS represents a more robust parameter. West and colleagues[23] used prototype assays for plasma ApoE proteotype and Aβ42/40, and modeled for amyloid likelihood based on CSF and amyloid PET biomarkers. They found
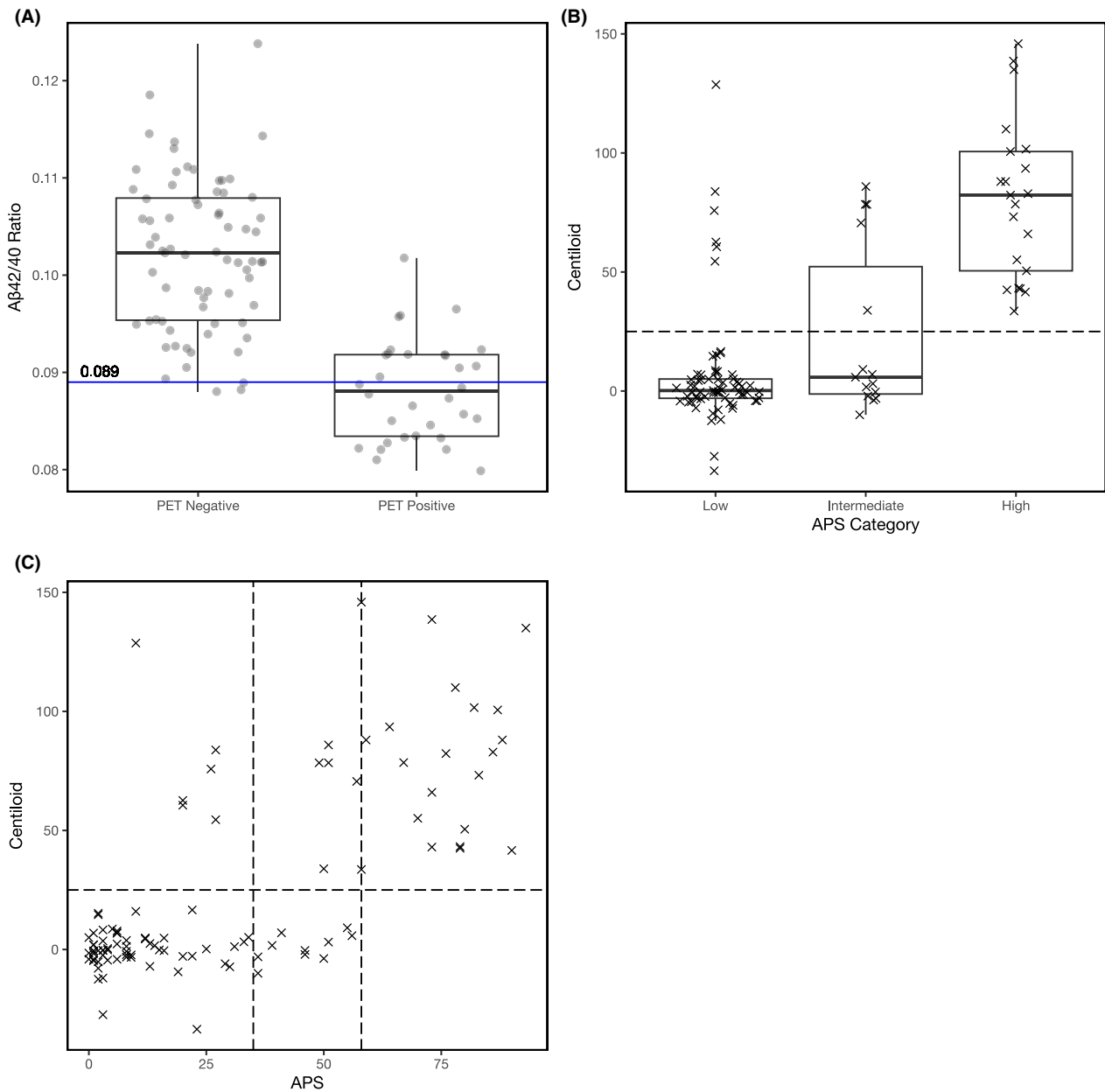
Figure 5. Associations between assay results (Aβ42/40 Ratio, APS) and PET Centiloid in 103 healthy controls. PET positivity was defined by Centiloid greater than 25. APS categories were defined by APS scores as Low (0–35), Intermediate (36–57), and High (58–100). (A) Subjects' plasma Aβ42/40 ratio by amyloid status. The previously established CLIA cutoff is 0.089. (B) Box and whisker plot of Centiloid values for each of the three APS categories. The horizontal dashed line represents Centiloid of 25. (C) Scatter plot comparing Centiloid and APS. The horizontal dashed line represents Centiloid of 25. The vertical dashed lines represent APS of 35 and 58.

that among 414 individuals across six diverse cohorts, plasma Aβ42/40 predicted brain amyloid positivity with an AUC-ROC performance of 0.81 and accuracy of 75% at the optimal Youden index cutoff value. The addition of Aβ42/40 ratio, ApoE, age, and cohort (to control for heterogeneity) to the prediction model significantly increased the AUC-ROC to 0.90 and the overall accuracy

to 86%.[23] Similar improvements to amyloid prediction by the addition of ApoE proteotype status and age were observed in the CLIA validation studies of the PrecivityAD test and APS model used in current clinical care.[11] In addition, recent recommendations by the EU/US CTAD Task Force on the topic of what must be done to progress blood biomarkers from research use to clinical

practice for the purpose of timely and accurate AD diagnosis, further support the use of combined biomarkers and risk factors in prediction models to limit misdiagnoses by healthcare providers.[24]

In the present study, an exploratory analysis in healthy individuals indicated APS CLIA cutoff values established in cognitively impaired individuals continue to perform with high diagnostic accuracy in other populations including those who are cognitively unimpaired. Further studies in healthy individuals are currently underway to establish and expand the PrecivityAD test's diagnostic performance. Until further clinical validity is established, it is premature to use blood biomarkers for predictive evaluation of cognitively unimpaired individuals in the clinic as part of their routine clinical care or wellness visits. Instead, appropriate context of use in this population applies to the clinical research setting where blood biomarkers are proving their utility as (i) prescreening tools to rule-out unnecessary CSF or PET evaluations in individuals who are likely to be amyloid negative, thereby lowering screening costs, accelerating trial enrollment timelines, and reducing inconvenience for otherwise willing study participants[25,26]; (ii) an inclusion criterion (diagnostic tool) without the need for confirmatory CSF or PET testing; and (iii) as theragnostic markers to show biological target engagement and/or disease-modifying effects of investigational therapies.[9,10,24]

Sixty-nine percent of individuals from the current AIBL analysis had at least two amyloid PET scans, which enabled an exploratory evaluation of PrecivityAD test results in the context of each individual's longitudinal amyloid accumulation trajectory. In this preliminary analysis, a single APS measurement discriminated between the presence or absence of amyloid plaques, and it also helped identify cognitively impaired and healthy control individuals on an amyloid accumulation trajectory versus those with stable, low amyloid levels. Low APS category individuals were more likely to have stable amyloid PET scans, suggesting no amyloid accumulation and low AD risk particularly for healthy control individuals. Most High APS category individuals- whether healthy control or cognitively impaired – appeared more likely to be on a cumulative amyloid trajectory consistent with AD progression. Findings also showed that Intermediate APS results were inconclusive as the category contained individuals with both stable and accumulating amyloid in balanced amounts. Further longitudinal analysis of PrecivityAD will expand these exploratory analyses of individual's trajectory for amyloid accumulation.

Strengths of this study include prespecified cutoff values and prespecified measures to test the performance of the PrecivityAD test in a completely different cohort with different collection strategies from the original CLIA validation cohort.[11] These findings support a diagnostic test that can accurately predict amyloid plaque burden for individuals aged 60 and older with mild cognitive impairment or mild AD in a robust manner using predefined cutoff values. As the first commercially available AD blood test for amyloid plaques, introduced over 2 years ago, this report helps to address one of the key issues identified in the EU/US CTAD Task Force's clinical use recommendations regarding the need for cutoff stability and robustness across cohorts over time.[24] This analysis based on APS, combined with other studies of plasma Aβ42/40 across multiple cohorts,[20–23] indicate plasma APS and Aβ42/40 are robust measures of amyloidosis across independent cohorts. Because the PrecivityAD test has established, prespecified cutoff values, measures traditionally used in biomarker studies that summarize results across all possible cutoffs, such as AUC, are no longer relevant. Instead, sensitivity, specificity, NPV, and PPV are reported to provide more information on the performance of the established test with prespecified cutoffs in the intended use population.

Clinical validation and establishing APS cutoff values for the PrecivityAD test required special PPV and NPV considerations, since these two properties are important functions of the underlying brain amyloid prevalence rates in the Intended Use population. From the CLIA validation cohort, the APS classification system (Low, Intermediate, and High scores) balanced high NPV and high PPV (~86% for each) for patients in either the Low or High APS categories, respectively, while minimizing the number of patients (~14%) with APS values in the noninformative Intermediate score range. The high NPV and PPV values were intended to provide healthcare providers with greater confidence to determine the likely absence or presence of brain amyloid for approximately 86% of the Intended Use population, while referring individuals with Intermediate scores for further diagnostic evaluations. The current findings support that assertion, as the three category APS system reliably classified patients according to their brain amyloid status.

One limitation of this study is that individuals were not a representative random sample from the population of patients clinically recommended for AD biomarker testing. AIBL's healthy control individuals (CDR = 0; 51.5% of AIBL samples) do not meet clinical recommendations[24] for AD biomarker testing due to the absence of signs or symptoms of cognitive impairment. Most (73.3%) of the AIBL healthy control individuals had low Centiloid values that were stable over time reflecting no amyloid accumulation. In contrast, cohorts for whom AD biomarkers are clinically recommended also typically include individuals on an amyloid accumulation trajectory who are considered amyloid negative because their

Centiloid values are below positivity thresholds.[18,27,28] Some subthreshold amyloid PET individuals may present as discordant false positive cases (APS positive, but amyloid PET negative), as there is evidence that the PrecivityAD test may detect amyloid plaques earlier than PET scans.[19] Yet, given the nonrandom nature of this AIBL cohort, which was generated by AIBL staff based on the current investigators' request for a prespecified composition, the number of false positive cases in this cohort was artificially low. This may explain the observed differences in actual PPV and NPV values between this AIBL analysis and the CLIA cohort analysis for the PrecivityAD test. In addition, differences in study geography, cohort disease status, plasma sample collection methods, and different imaging techniques to define amyloid positivity, among other potential unknown confounders, may also account for the differences in NPV and PPV in this AIBL cohort versus the CLIA validation dataset.

Another limitation of the predictive performance estimates in this study, but in line with the CLIA validation of the PrecivityAD test, is that individuals in the Intermediate APS category were excluded from the sensitivity/ specificity analysis. Therefore, the PPV, NPV, sensitivity, and specificity reported reflect the performance based on individuals for whom the PrecivityAD test has the most confidence (86% of individuals tested).

The lack of information on the clinical AD phenotypes and the proportion of cases presenting with atypical features is another limitation. However, other studies that supported the PrecivityAD test's clinical validation included atypical presentations and demonstrated that diagnostic performance was comparable between typical and atypical AD patients.[11] Finally, although the AIBL study included a comprehensive battery of neuropsychological tests, only CDR was used to assign participants to their cognitive group (healthy controls versus cognitive impaired). The use of a single parameter to classify participants by cognitive stage may have resulted in misclassifications.

In conclusion, this AIBL cohort analysis provides additional independent evidence that APS is a reliable brain amyloidosis biomarker and a potential indicator of individuals actively accumulating amyloid, a dynamic and evolving process characteristic of progressive AD pathology. Further, this analysis provides confirmatory evidence that the established APS cutoff values have high diagnostic performance when used across different cohorts. The exploratory analyses demonstrate the APS model retains strong diagnostic performance even among healthy control individuals, supporting further screening studies in cognitively unimpaired individuals (high risk) for whom biomarker testing outside of the clinical research context might be warranted.

Author Contribution

All authors reviewed and edited initial manuscript drafts, and reviewed and approved the final manuscript version. The study was conceived and executed by IF, TW, JBB, PBV, KMK, MRM, JHC, ES, KLF, JG, CER, DG, and KEY. ES, KLF, JG, CER and DG procured the AIBL samples. KMK and MRM conducted, and JHC and KEY supervised the sample analyses. TW, SH, JY, SY provided independent biostatistical analyses.

Acknowledgments

Conflict of Interest

C$_2$N Diagnostics employees receive cash and/or equity compensation and contributed to the development of the PrecivityAD test. Randall J. Bateman (RJB) is a cofounder of C$_2$N Diagnostics. Washington University and RJB have equity ownership interest in C$_2$N Diagnostics and may receive income based on technology (stable isotope labeling kinetics and blood plasma assay) licensed by Washington University to C$_2$N Diagnostics. RJB receives income from C$_2$N Diagnostics for serving on the scientific advisory board. RJB has received honoraria as a speaker, consultant, or advisory board member from Amgen and Roche. David M. Holtzman (DMH) is a cofounder of C$_2$N Diagnostics, has equity interest, and may receive income based on technology (stable isotope labeling kinetics and blood plasma assay) licensed by Washington University to C$_2$N Diagnostics. He reports serving on the scientific advisory board of C$_2$N Diagnostics, Denali, Genentech, and Cajal Neuroscience and consults for Alector. Eli Shobin, Kyle L. Ferber, Jake Gagnon, Carrie E. Rubel, and Danielle Graham are paid employees and shareholders of Biogen.

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Supporting Information

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

Table S1.
Text S1.