

Validation of Plasma Amyloid- β 42/40 for Detecting Alzheimer Disease Amyloid Plaques

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Abstract

Background and Objectives

To determine the diagnostic accuracy of a plasma A β 42/A β 40 assay in classifying amyloid PET status across global research studies using samples collected by multiple centers that utilize different blood collection and processing protocols.

Methods

Plasma samples (n = 465) were obtained from 3 large Alzheimer disease (AD) research cohorts in the United States (n = 182), Australia (n = 183), and Sweden (n = 100). Plasma A β 42/A β 40 was measured by a high precision immunoprecipitation mass spectrometry (IPMS) assay and compared to the reference standards of amyloid PET and CSF A β 42/A β 40.

Results

In the combined cohort of 465 participants, plasma A β 42/A β 40 had good concordance with amyloid PET status (receiver operating characteristic area under the curve [AUC] 0.84, 95% confidence interval [CI] 0.80–0.87); concordance improved with the inclusion of APOE ϵ 4 carrier status (AUC 0.88, 95% CI 0.85–0.91). The AUC of plasma A β 42/A β 40 with CSF amyloid status was 0.85 (95% CI 0.78–0.91) and improved to 0.93 (95% CI 0.89–0.97) with APOE ϵ 4 status. These findings were consistent across the 3 cohorts, despite differences in protocols. The assay performed similarly in both cognitively unimpaired and impaired individuals.

Discussion

Plasma A β 42/A β 40 is a robust measure for detecting amyloid plaques and can be utilized to aid in the diagnosis of AD, identify those at risk for future dementia due to AD, and improve the diversity of populations enrolled in AD research and clinical trials.

Classification of Evidence

This study provides Class II evidence that plasma A β 42/A β 40, as measured by a high precision IPMS assay, accurately diagnoses brain amyloidosis in both cognitively unimpaired and impaired research participants.

MORE ONLINE

Class of Evidence

Criteria for rating therapeutic and diagnostic studies

[NPub.org/coe](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8888888/)

Infographic

[links.lww.com/WNL/B764](https://www.links.lww.com/WNL/B764)

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Glossary

A4 = Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease; **ADNI** = Alzheimer's Disease Neuroimaging Initiative; **AIBL** = Australian Imaging, Biomarkers and Lifestyle Study; **AD** = Alzheimer disease; **AUC** = area under the curve; **CI** = confidence interval; **CN** = cognitively normal; **IPMS** = immunoprecipitation mass spectrometry; **LME** = linear mixed effects; **MCI** = mild cognitive impairment; **NPA** = negative percent agreement; **NPV** = negative predictive value; **p-tau** = phosphorylated tau; **PPA** = positive percent agreement; **PPV** = positive predictive value; **ROC** = receiver operating characteristic; **SCD** = subjective cognitive decline; **SMC** = significant memory concern; **SUVr** = standardized uptake value ratio.

Alzheimer disease (AD) brain pathology, which is characterized by amyloid plaques and neurofibrillary tangles, accumulates in the brain for 1 to 2 decades before most individuals develop dementia symptoms.¹⁻³ In cognitively unimpaired individuals, biomarkers of AD pathology are used to identify persons at risk of AD dementia for enrollment in prevention trials designed to delay or prevent the development of AD dementia symptoms.⁴ In cognitively impaired individuals, AD biomarkers are used to evaluate whether AD is a potential cause of dementia symptoms.^{5,6} With the Food and Drug Administration approval of the anti-amyloid monoclonal antibody Aduhelm, the first putative disease-modifying treatment for AD, one of the major challenges is efficiently identifying patients with amyloid plaques. Although both amyloid PET and CSF biomarkers of AD accurately detect amyloid plaques, the high cost, limited availability, and perceived invasiveness of these methods limit their use and may extend the time until biomarker-confirmed AD diagnosis.^{7,8}

The recent rapid development of blood-based AD biomarkers promises to accelerate clinical trials and broaden the availability of biomarker testing in the clinic. Plasma A β 42 and A β 40 as measured by conventional techniques like ELISA have relatively high variance and typically perform poorly in distinguishing amyloid-positive and amyloid-negative individuals.^{9,10} However, several groups have reported that high-precision assays of plasma A β 42/A β 40 accurately detect brain amyloidosis.¹¹⁻¹⁵ Some longitudinal studies found that plasma A β 42/A β 40 became abnormal even before amyloid PET.^{13,16} The performance reported for mass spectrometry-based assays has been superior to that of other assays, with receiver operating characteristic area under the curve (ROC AUC) for amyloid PET status of 0.80–0.89 compared to around 0.68 for ELISA and 0.40–0.77 for Simoa assays (eTable 1, [links.lww.com/WNL/B715](https://www.lww.com/WNL/B715)).¹⁷⁻¹⁹ Recent independent studies comparing the performance of different mass spectrometry and immunologic assays found that our immunoprecipitation mass spectrometry (IPMS) assay had the highest concordance with amyloid PET status, while several other assays performed no better than a model with age and APOE ϵ 4 carrier status.^{20,21} The accuracy of the IPMS plasma A β 42/A β 40 assay as a biomarker of brain amyloidosis needs to be validated in multiple cohorts to establish its accuracy and robustness. Furthermore, differences in studies, such as preanalytical factors, may affect measurement of A β , and it is unknown whether plasma A β 42/A β 40 can be measured consistently at different

centers and cohorts with different collection methods. It also remains unclear whether plasma A β 42/A β 40 is a reliable measure of brain amyloidosis in both cognitively unimpaired and impaired individuals.

We sought to determine the performance of the IPMS plasma A β 42/A β 40 assay in 3 different cohorts in the United States, Europe, and Australia. Using samples from 3 major AD studies with different blood collection and processing protocols, we examined whether plasma A β 42/A β 40 is an accurate measure of brain amyloidosis in cognitively unimpaired and cognitively impaired individuals. Because this assay has been developed further commercially (PrecivityAD by C2N Diagnostics) and is now being used by physicians in the clinic to detect amyloid plaques and assist in diagnosis of AD dementia, these studies help inform about the properties of a clinically relevant blood test for amyloid plaques.

Methods

Participants

The study participants represent a convenience sample. Study participants were selected from the Alzheimer's Disease Neuroimaging Initiative (ADNI), the Australian Imaging, Biomarkers and Lifestyle Study (AIBL), and the Swedish BioFINDER study. Participants with at least one stored plasma sample and an amyloid PET scan within 1 year of the plasma sample collection were considered for inclusion regardless of their cognitive status. Plasma samples with relatively large volume available as determined by the biorepository core leader of each study were selected. To maximize the statistical power for precisely estimating of the diagnostic accuracy of plasma A β 42/A β 40 while limiting the sample number, a similar number of amyloid PET positive and negative participants were selected from each study.

Details of the 3 studies and the diagnostic criteria can be found in previous publications.²²⁻²⁴ The ADNI cohort included 3 diagnostic categories: cognitively unimpaired (CN), significant memory concern (SMC), and mild cognitive impairment (MCI). The AIBL cohort included 3 diagnostic categories: CN, MCI, and AD dementia. The BioFINDER cohort included 3 diagnostic categories: CN, subjective cognitive decline (SCD), and MCI. To combine these different diagnostic classification systems for this study, cognitive

Table 1 Participant Characteristics, Amyloid PET Tracer, Plasma Collection, and Processing Protocols by Study

	ADNI (n = 182)	AIBL (n = 183)	BioFINDER (n = 100)	Combined (n = 465)	p Value
Age, y	72.5 ± 6.9	74.2 ± 7.9	71.1 ± 5.1	72.9 ± 7.1	0.0009
Amyloid PET status, positive	89 (49)	105 (57)	50 (50)	244 (52)	0.23
APOE ε4 status, carrier	69 (38)	83 (46)	48 (48)	200 (43)	0.18
Sex, female	95 (52)	91 (50)	31 (31)	217 (47)	0.002
Diagnosis (CU/SMC or SCD/MCI/AD)	71/25/86/0	71/0/66/46	28/21/51/0	170/46/203/46	<0.0001
Cognitive status, cognitively impaired	86 (47)	112 (61)	51 (51)	249 (54)	0.02
Amyloid PET tracer (threshold for amyloid PET positivity)	Florbetapir (SUVR ≥1.11)	PiB, flutemetamol, florbetapir (Centiloid >25)	Flutemetamol (SUVR ≥1.42)		
Blood plasma collection and processing protocols					
Fasted blood	Yes	Yes	No		
Anticoagulant	EDTA	EDTA and PGE1	EDTA		
Aliquot volume	500 µL	250 or 500 µL	1,000 µL		
Spin speed	1,000 × G	200 then 800 × G	2000 × G		
Spin temperature	Room temperature	20°C	4°C		
Spin time, min	15	10 or 15	10		
Method of initial freezing	Dry ice	Dry ice	-80°C		
Number of freeze-thaws	2	1	1		

Abbreviations: AD = Alzheimer disease; ADNI = Alzheimer's Disease Neuroimaging Initiative; AIBL = Australian Imaging, Biomarkers and Lifestyle Study; CU = cognitively unimpaired; MCI = mild cognitive impairment; PiB = Pittsburgh compound B; SCD = subjective cognitive decline, only in BioFINDER cohort; SMC = significant memory concern, only in ADNI cohort; SUVR = standardized uptake value ratio. Continuous measures are represented as mean ± SD; p values for comparing the 3 cohorts were estimated with 1-way analysis of variance F tests. Categorical measures are presented as n (%); p values for comparing the 3 cohorts were estimated with χ^2 tests or Fisher exact tests (as appropriate).

impairment was defined by objective evidence of cognitive impairment, rather than by subjective symptoms. This approach is in alignment with National Institute on Aging–Alzheimer's Association criteria.²⁵ Individuals with SMC and SCD were included in the cognitively unimpaired category. The cognitively impaired category included MCI and AD dementia.

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent was obtained from all participants or legally authorized representatives. For ADNI, the protocol and informed consent forms were approved by the institutional review board at each participating site. For AIBL, all procedures were approved by the institutional human research ethics committees of Austin Health, St Vincent's Health, Hollywood Private Hospital, and Edith Cowan University. For BioFINDER, ethics approval was given by the Regional Ethical Committee of Lund University. PET imaging approval was obtained from the Swedish Medical Products Agency and the local Radiation Safety Committee at Skåne University Hospital.

Amyloid PET Imaging

Amyloid PET was used as a primary reference standard for amyloidosis because it is a well-established biomarker that is

widely used in clinical trials and observational studies to determine brain amyloid plaque burden.

In the ADNI cohort, amyloid PET was performed with [¹⁸F]-florbetapir according to standardized protocols.²⁶ A global standardized uptake value ratio (SUVR) was computed using a composite of middle frontal, anterior cingulate, posterior cingulate, inferior parietal, precuneus, supramarginal, middle temporal, and superior temporal. An established threshold of SUVR ≥1.11 was used to determine the amyloid PET positivity.²⁷ Centiloid was calculated based on the standardized ADNI PET protocol.²⁸

In the AIBL cohort, amyloid PET was performed with 3 different radiotracers: ¹¹C-Pittsburgh compound B, [¹⁸F]-flutemetamol, and [¹⁸F]-florbetapir. Methods for all 3 tracers can be found in previous publications.²⁹⁻³¹ A global SUVR was computed using the mean SUVR of the frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions. Method for Centiloid was as previously described.³² Amyloid PET positivity was defined a priori with the established cut-off of Centiloid >25.

In the BioFINDER cohort, amyloid PET was performed with [¹⁸F]-flutemetamol as described previously.³³ A composite

Table 2 Participant Characteristics by Amyloid PET Status

Study	PET negative	PET positive	p Value
ADNI	n = 93	n = 89	
Age, y	71.3 ± 6.9	73.7 ± 6.7	0.02
APOE ε4 status, carrier	21 (23)	48 (54)	<0.0001
Sex, female	42 (45)	53 (60)	0.05
Diagnosis (CU/SMC/MCI)	42/11/40	29/14/46	0.2
Cognitive status, cognitively impaired	40 (43)	46 (52)	0.2
MMSE	29.0 ± 1.3	28.4 ± 1.7	0.01
Centiloid	4.6 ± 9.4	67.0 ± 32.8	<0.0001
Plasma Aβ42/Aβ40	0.133 ± 0.010	0.121 ± 0.010	<0.0001
AIBL	n = 78	n = 105	
Age, y	74.1 ± 8.3	74.4 ± 7.6	0.8
APOE ε4 status, carrier	13 (17)	70 (67)	<0.0001
Sex, female	37 (47)	54 (51)	0.6
Diagnosis (CU/MCI/AD)	38/33/7	33/33/39	<0.0001
Cognitive status, cognitively impaired	40 (51)	72 (69)	0.02
MMSE	26.8 ± 3.2	25.2 ± 3.4	0.001
Centiloid	-3.3 ± 11.8	94.0 ± 35.9	<0.0001
Plasma Aβ42/Aβ40	0.129 ± 0.013	0.115 ± 0.007	<0.0001
BioFINDER	n = 50	n = 50	
Age, y	70.0 ± 5.1	72.1 ± 4.9	0.04
APOE ε4 status, carrier	10 (20)	38 (76)	<0.0001
Sex, female	16 (32)	15 (30)	0.8
Diagnosis (CU/SCD/MCI)	15/10/25	13/11/26	0.9
Cognitive status, cognitively impaired	25 (50)	26 (52)	0.8
MMSE	28.2 ± 1.8	27.7 ± 1.7	0.15
Centiloid ^a	-2.5 ± 8.2	83.0 ± 32.2	<0.0001
Plasma Aβ42/Aβ40	0.130 ± 0.008	0.121 ± 0.005	<0.0001
Combined	n = 221	n = 244	
Age, y	72.0 ± 7.2	73.7 ± 6.8	0.01
APOE ε4 status, carrier	44 (20)	156 (64)	<0.0001
Sex, female	95 (43)	122 (50)	0.13
Diagnosis (CU/SMC or SCD/MCI/AD)	95/21/98/7	75/25/105/39	<0.0001
Cognitive status, cognitively impaired	105 (48)	144 (59)	0.01
MMSE	28.0 ± 2.4	26.9 ± 2.9	<0.0001

Table 2 Participant Characteristics by Amyloid PET Status
(continued)

Study	PET negative	PET positive	p Value
Centiloid^a	0.2 ± 10.7	81.9 ± 36.0	<0.0001
Plasma Aβ42/Aβ40	0.131 ± 0.011	0.118 ± 0.009	<0.0001

Abbreviations: AD = Alzheimer disease; ADNI = Alzheimer's Disease Neuroimaging Initiative; AIBL = Australian Imaging, Biomarkers and Lifestyle Study; CU = cognitively unimpaired; MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; SCD = subjective cognitive decline; SMC = significant memory concern.

Continuous measures are represented as mean ± SD; p values for comparing group differences were estimated with Student t tests for continuous measures and χ^2 tests for categorical measures. Categorical measures are presented as n (%).

^a Centiloid data were not available for 4 participants in BioFINDER at the time of analysis.

SUVR was computed using the prefrontal, parietal, temporal lateral, anterior cingulate, posterior cingulate, and precuneus region.³³ An established threshold of SUVR ≥ 1.42 was used to determine the amyloid PET positivity.³³ [¹⁸F]flutemetamol SUVR values were converted to Centiloid using the Computational Analysis of PET from AIBL (CapAIBL) pipeline.³² Centiloid data were available for 96 BioFINDER participants at the time of analysis.

CSF Collection and Immunoassay

In ADNI, lumbar puncture was performed as described in the ADNI procedures manual.²⁸ In AIBL and BioFINDER, CSF was collected as previously described.^{34,35} CSF Aβ42 and Aβ40 were measured with the corresponding Elecsys immunoassays on a Roche cobas e601 analyzer. CSF Aβ42/Aβ40 positivity was defined a priori with the established cut-off of ≤ 0.064 for ADNI³⁶ and ≤ 0.066 for BioFINDER. CSF was not evaluated in the AIBL cohort because only 9 participants had available data for CSF Aβ42/Aβ40.

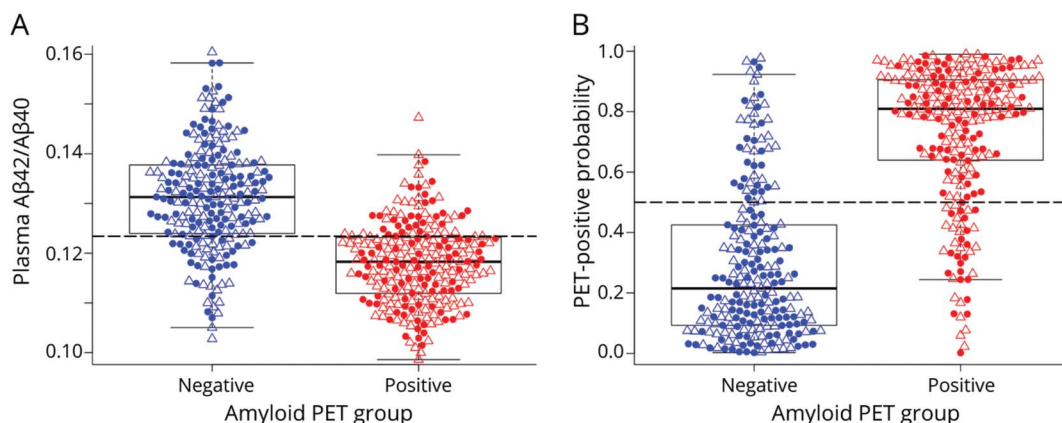
Plasma Collection and Processing

In ADNI, plasma was collected according to the ADNI procedures manual.²⁸ In AIBL and BioFINDER, plasma was collected based on their standardized protocols as described before.^{23,37,38} Table 1 summarizes the plasma collection and processing protocols for the 3 studies, including the participant fasting state, anticoagulant used, centrifuge conditions, and number of freeze-thaw cycles.

Liquid Chromatography–Mass Spectrometry Measurement of Plasma Aβ42/Aβ40

The measurement laboratory remained blinded to all sample information until after lockdown and submission of measurement results to the clinical study team. The simultaneous immunoprecipitation of Aβ38, Aβ40, and Aβ42 from human plasma was performed as previously described¹³ with minor modifications. Briefly, Aβ isoforms were immunoprecipitated from 0.45 mL of plasma on a KingFisher sample processing deck (Thermo Fisher) via a monoclonal anti-Aβ

Figure 1 Plasma A β 42/A β 40 and PET-Positive Probability by Amyloid PET Status for the Combined Cohort



(A and B) The probability of amyloid PET positivity was estimated from a logistic regression model with amyloid PET status (positive or negative) as the outcome and plasma A β 42/A β 40 and *APOE* ϵ 4 status as the predictors (see eTable 4, links.lww.com/WNL/B715, for parameter estimates). Filled circles represent cognitively unimpaired participants; open triangles represent cognitively impaired participants. Dashed gray lines represent the cut-off values shown in eTable 2, links.lww.com/WNL/B715.

mid-domain antibody (HJ5.1, anti-A β 13-28) that was conjugated to M-270 Epoxy Dynabeads (Invitrogen). Prior to immunoprecipitation, samples were spiked with a known amount of 15N-A β 38, 15N-A β 40, and 15N-A β 42 for use as analytical reference standards. Enriched proteins were subsequently digested into peptides using LysN endoprotease (Pierce). Liquid chromatography–tandem mass spectrometry was performed as previously described.¹¹ All analyses were performed on an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher) operated in-line with an M-class nanoAcquity chromatography system (Waters). The precursor and product ion pairs utilized for parallel reaction monitoring analysis of A β species were chosen as previously described.^{11,39} Finally, the derived integrated peak areas were analyzed using the Skyline software package.⁴⁰ Analysis of mass spectrometry data and quality control were performed prior to data unblinding and were as previously described.¹³ Values that failed quality control were not used for further data analysis. Of note, the immunoprecipitation–mass spectrometry assay allows analysis of A β 42 and A β 40 on the same sample simultaneously with stable isotope internal standards, which enables greater precision in measuring the ratio of A β 42 to A β 40 (average 2% coefficient of variation) compared to methods in which A β 42 and A β 40 are measured with different assays or antibodies.

Statistical Analyses

Differences in participant characteristics among the ADNI, AIBL, and BioFINDER cohorts were compared using 1-way analysis of variance F tests for continuous variables and χ^2 tests or Fisher exact test (as appropriate) for categorical variables. Participant characteristics by amyloid PET status were compared using 2-sample *t* tests for continuous variables and χ^2 tests or Fisher exact test (as appropriate) for categorical variables. The performance of plasma A β 42/A β 40 in

predicting amyloid PET status was evaluated using ROC curve analysis. The relationships between plasma A β 42/A β 40, Centiloid, and age, *APOE* ϵ 4 status (categorized as ϵ 4 carriers or noncarriers), and cognitive status (cognitively unimpaired, cognitively impaired) were assessed using linear regressions. The effect of relevant biological variables, age, sex, *APOE* ϵ 4 status, and cognitive status were evaluated using logistic regression for each study and generalized (logistic) linear mixed effects (LME) models for the pooled cohort. The generalized LME models included a random intercept for each study. The AUC and the PET-positive probability were estimated from the logistic regressions and generalized LME models. AUCs were compared using the DeLong test and *p* values were Bonferroni-corrected if multiple comparisons were performed within each cohort. Positive percent agreement (PPA) was defined as the percentage of amyloid PET-positive participants who were plasma A β 42/A β 40 positive. Negative percent agreement (NPA) was defined as the percentage of amyloid PET-negative participants who were plasma A β 42/A β 40 negative. Cut-offs for plasma A β 42/A β 40 and PET-positive probability were derived based on the maximum of Youden index (PPA + NPA – 1). Statistical analyses were performed using SAS 9.4. Plots were created using R 3.6.1. All tests were 2-sided and *p* < 0.05 was considered statistically significant.

Data Availability

For ADNI and AIBL, data are available upon approved request. For BioFINDER, anonymized data will be shared by request from a qualified academic investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with EU legislation on the general data protection regulation and decisions by the ethical review board of Sweden and Region Skåne, which should be regulated in a material transfer agreement.

Table 3 Models of Amyloid PET Status

Predictors	ADNI (n = 182)	AIBL (n = 183)	BioFINDER (n = 100)	Combined (n = 465)
Plasma A β 42/A β 40	0.82 (0.76–0.89)	0.84 (0.78–0.90)	0.83 (0.75–0.91)	0.84 (0.80–0.87)
Plasma A β 42/A β 40 and APOE ϵ 4 status	0.84 (0.78–0.90)	0.91 (0.87–0.96)	0.90 (0.85–0.96)	0.88 (0.85–0.91)
Plasma A β 42/A β 40, APOE ϵ 4 status, and age	0.85 (0.79–0.91)	0.91 (0.87–0.96)	0.91 (0.86–0.97)	0.88 (0.85–0.91)
Plasma A β 42/A β 40, APOE ϵ 4 status, and sex	0.86 (0.80–0.91)	0.91 (0.87–0.96)	0.91 (0.86–0.96)	0.89 (0.85–0.92)
Plasma A β 42/A β 40, APOE ϵ 4 status, and cognitive status	0.85 (0.79–0.91)	0.91 (0.87–0.96)	0.91 (0.85–0.96)	0.88 (0.85–0.91)
Plasma A β 42/A β 40, APOE ϵ 4 status, age, sex, and cognitive status	0.86 (0.81–0.92)	0.91 (0.87–0.96)	0.91 (0.86–0.97)	0.89 (0.85–0.92)
APOE ϵ 4 status, age, sex, and cognitive status	0.74 (0.67–0.81)	0.79 (0.72–0.86)	0.84 (0.77–0.92)	0.78 (0.74–0.82)

Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; AIBL = Australian Imaging, Biomarkers and Lifestyle Study. Receiver operating characteristic area under the curve for discriminating amyloid PET status for each predictor and combination of predictors (95% confidence intervals).

Results

Participants

The characteristics of the 3 cohorts and combined cohort are shown in Table 1. Participants in the AIBL cohort (74.2 \pm 7.9 years [mean \pm SD]) were slightly older than those in the ADNI (72.5 \pm 6.9 years) or BioFINDER cohorts (71.1 \pm 5.1 years) (p = 0.0009). There were fewer women in the BioFINDER cohort (31%) than in the ADNI (52%) or AIBL cohorts (50%) (p = 0.002). There were more cognitively impaired participants in the AIBL cohort (61%) than in the BioFINDER (51%) or ADNI (47%) cohorts (p = 0.02). The proportion of individuals who were APOE ϵ 4 carriers or amyloid PET positive were not significantly different among the 3 cohorts. Participant characteristics by amyloid PET status are shown in Table 2. In the combined cohort, amyloid PET-positive participants were older (73.7 \pm 6.8 vs 72.0 \pm 7.2 years, p = 0.01), were more likely to carry an APOE ϵ 4 allele (64% vs 20%, p < 0.0001), and were more likely to be cognitively impaired (59% vs 48%, p = 0.01) compared to amyloid PET-negative individuals.

Relationship Between Plasma A β 42/A β 40 and Amyloid PET Status

In each of the cohorts (eFigure 1, upper panels, links.lww.com/WNL/B715), as well as the combined cohort (Figure 1A), the amyloid PET-positive group had lower plasma A β 42/A β 40 compared to the amyloid PET-negative group (p all < 0.0001) (Table 2). ROC analyses demonstrated that plasma A β 42/A β 40 alone predicted amyloid PET status with an AUC ranging from 0.82 in ADNI to 0.84 in AIBL; the AUC in the combined cohort was 0.84 (95% confidence interval [CI] 0.80–0.87) (Table 3 and Figure 2). The optimal plasma A β 42/A β 40 cutoff for distinguishing amyloid PET status based on the Youden index was similar across the 3 cohorts, ranging from 0.123 for AIBL and BioFINDER to 0.125 for ADNI (eTable 2). In the combined cohort, a cut-off of 0.123 had a PPA of 0.77 (95% CI 0.71–0.82), an NPA of 0.78 (95% CI 0.72–0.83), and accuracy of 0.80 (95% CI 0.76–0.84) with amyloid PET status.

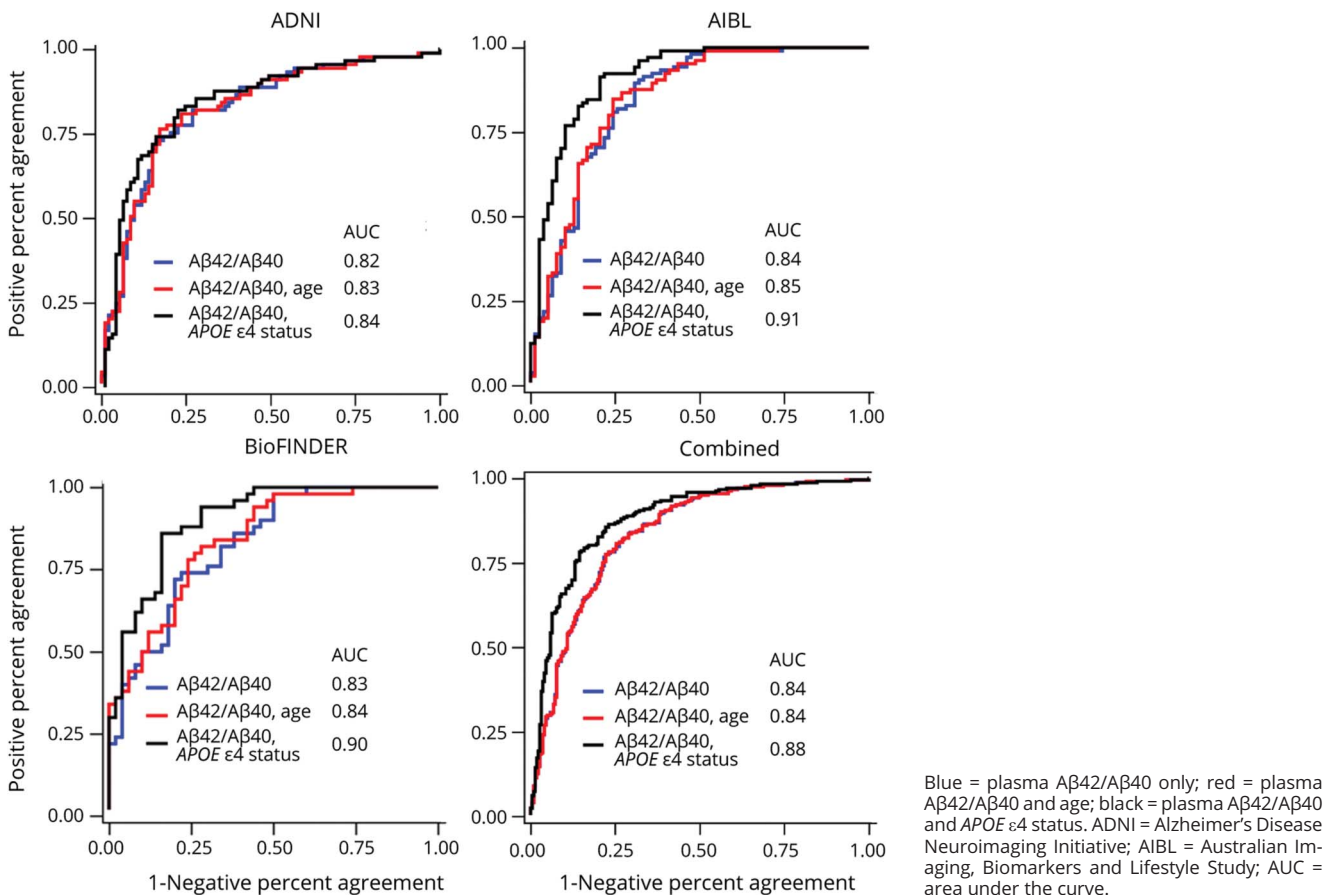
Assuming a rate of amyloid positivity of 37.5% in a population, the estimated positive predictive value (PPV) and negative predictive value (NPV) were 0.68 (95% CI 0.62–0.74) and 0.85 (95% CI 0.82–0.88), respectively.

Influence of Relevant Biological Variables (APOE ϵ 4 Status, Age, Sex, and Cognitive Status)

For the combined cohort, older age, carrying an APOE ϵ 4 allele, and male sex were associated with lower plasma A β 42/A β 40 (p < 0.0001, p < 0.0001, and p = 0.003, respectively). Conditional on age, APOE ϵ 4 status, and sex, plasma A β 42/A β 40 was not associated with cognitive status (p = 0.11) (eTable 3, links.lww.com/WNL/B715). After consideration of age and APOE ϵ 4 status, amyloid PET Centiloid did not differ by sex (p = 0.21), but did differ by cognitive status (p < 0.0001) (eTable 3).

A combination of plasma A β 42/A β 40 and APOE ϵ 4 status improved prediction of amyloid PET status in the 3 cohorts compared to plasma A β 42/A β 40 alone (AUC 0.88 vs 0.84, respectively, for the combined cohort, p < 0.0001) (Table 3). After accounting for plasma A β 42/A β 40 and APOE ϵ 4 status, the addition of age, sex, or cognitive status (separately or in combination) did not significantly improve prediction of amyloid PET status. There was no significant interaction between plasma A β 42/A β 40 and APOE ϵ 4 status (p = 0.5) and the AUCs of plasma A β 42/A β 40 did not differ between APOE ϵ 4 status and noncarriers (0.83 vs 0.81, p = 0.7). Therefore, models including only plasma A β 42/A β 40 and APOE ϵ 4 status were used to predict amyloid PET status (eFigure 1, lower panels, links.lww.com/WNL/B715; Figure 1B; eTable 4). In the combined cohort, a predicted probability of 0.5 for amyloid PET positivity had a maximum Youden index, with a PPA of 0.83 (95% CI 0.77–0.87), NPA of 0.79 (95% CI 0.73–0.84), and accuracy of 0.81 (95% CI 0.77–0.85) with amyloid PET status (eTable 2). The corresponding PPV and NPV in a population with a 37.5% rate of amyloid positivity was 0.70 (95% CI 0.65–0.75) and 0.89 (95% CI 0.86–0.92).

Figure 2 Receiver Operating Characteristic Curves for Distinguishing Amyloid PET Status



The explanation for improved prediction of amyloid PET status by plasma Aβ42/Aβ40 and APOE ε4 status, relative to plasma Aβ42/Aβ40 alone, was examined. In the combined cohort, plasma Aβ42/Aβ40 levels were lower in APOE ε4 carriers compared to noncarriers ($p < 0.0001$), even after adjusting for age and sex. However, after adjusting for amyloid PET status, plasma Aβ42/Aβ40 was not different between APOE ε4 carriers and noncarriers ($p = 0.21$). Among amyloid PET negative participants who had a positive blood test (false positive) and amyloid PET negative participants who had a positive blood test (false negative), APOE ε4 carriers were more likely to be amyloid PET positive than noncarriers (75% vs 38%, $p = 0.0002$). That is, for those participants who were misclassified by blood test, APOE ε4 status can still provide extra information to improve the classification accuracy. Notably, at the same plasma Aβ42/Aβ40 value, APOE ε4 carriers were more likely to have a positive amyloid PET scan (eTable 4, links.lww.com/WNL/B715); this explains why inclusion of APOE ε4 carrier status improves prediction of amyloid PET status.

Association Between Plasma Aβ42/Aβ40 and APOE ε4 Status With Amyloid PET by Cognitive Status

Plasma Aβ42/Aβ40 and APOE ε4 status distinguished amyloid PET status consistently in both cognitively unimpaired and

impaired individuals. In cognitively unimpaired individuals, plasma Aβ42/Aβ40 and APOE ε4 status predicted amyloid PET status with an AUC ranging from 0.84 in ADNI to 0.95 in AIBL (eTable 5, links.lww.com/WNL/B715); the AUC in the combined cohort was 0.89 (95% CI 0.84–0.93) (Table 4). In cognitively impaired individuals, plasma Aβ42/Aβ40 and APOE ε4 status predicted amyloid PET status with an AUC ranging from 0.85 in ADNI to 0.91 in BioFINDER (eTable 5); the AUC in the combined cohort was 0.87 (95% CI 0.82–0.92) (Table 4). Addition of age and sex did not significantly improve prediction of amyloid PET status in either cognitively unimpaired individuals or cognitively impaired individuals. There are no significant differences in the AUCs between cognitively unimpaired and cognitively impaired subcohorts. PPA, NPA, accuracy, PPV, and NPV based on the cut-offs from the combined cohort (0.123 for plasma Aβ42/Aβ40, 0.5 for the predicted probability of amyloid PET positivity) for cognitively unimpaired and cognitively impaired subcohorts are shown in eTable 6. PPV and NPV were calculated based on amyloid positivity rates of 25% in cognitively unimpaired participants and 75% in cognitively impaired participants.

Correlations Between Plasma Aβ42/Aβ40, Amyloid PET Centiloid, and CSF Aβ42/Aβ40

Spearman correlations between plasma Aβ42/Aβ40 and amyloid PET Centiloid ranged from -0.52 (95% CI -0.65 to

Table 4 Models of Amyloid PET Status in Cognitively Unimpaired and Cognitively Impaired Individuals

	Cognitively unimpaired (n = 216), PET-negative vs PET-positive	Cognitively impaired (n = 249), PET-negative vs PET-positive
Plasma A β 42/A β 40	0.85 (0.79–0.90)	0.82 (0.76–0.88)
Plasma A β 42/A β 40 and APOE ϵ 4 status	0.89 (0.84–0.93)	0.87 (0.82–0.92)
Plasma A β 42/A β 40, APOE ϵ 4 status, and age	0.89 (0.85–0.94)	0.87 (0.82–0.92)
Plasma A β 42/A β 40, APOE ϵ 4 status, and sex	0.90 (0.85–0.94)	0.87 (0.83–0.92)
Plasma A β 42/A β 40, APOE ϵ 4 status, age, and sex	0.90 (0.86–0.94)	0.87 (0.83–0.92)
APOE ϵ 4 status, age, and sex	0.79 (0.73–0.85)	0.77 (0.70–0.83)

Receiver operating characteristic area under the curve for discriminating amyloid PET status for each predictor and combination of predictors (95% confidence intervals).

–0.35) in BioFINDER to –0.57 (95% CI –0.66 to –0.46) in ADNI (eFigure 2, left panel, links.lww.com/WNL/B715). Spearman correlations between plasma A β 42/A β 40 and CSF A β 42/A β 40 were 0.60 (95% CI 0.31–0.79) in ADNI and 0.59 (95% CI 0.45–0.71) in BioFINDER (eFigure 2, right panel). The correlation between plasma and CSF A β 42/A β 40 were not evaluated in the AIBL cohort because only 9 participants had CSF A β 42/A β 40 data available. The AUCs of plasma A β 42/A β 40 in classifying CSF amyloid status (positive or negative) were 0.92 (95% CI 0.84–1) for ADNI and 0.81 (95% CI 0.73–0.89) for BioFINDER. Including the effect of APOE ϵ 4 status in the logistic regressions improved AUC to 1.00 for ADNI and 0.90 (95% CI 0.84–0.96) for BioFINDER. In the combined cohort, the AUC of plasma A β 42/A β 40 with CSF amyloid status was 0.85 (95% CI 0.78–0.91) and improved to 0.93 (95% CI 0.89–0.97) with APOE ϵ 4 status. As in the models for amyloid PET status, age, sex, and cognitive status were not significant predictors of CSF amyloid status in the combined cohort.

Utility of Plasma A β 42/A β 40 as a Screening Test for Brain Amyloidosis

The number of participants and amyloid PET scans needed to identify 1,000 amyloid PET positive participants, with and without prescreening with a blood test, were estimated (Figure 3A). For cognitively unimpaired individuals, prescreening with a blood test can reduce the number of amyloid PET scans required by 59% (prescreen with plasma A β 42/A β 40 alone) or 62% (prescreen with plasma A β 42/A β 40 and APOE ϵ 4 status). For cognitively impaired individuals, prescreening reduces the number of amyloid PET scans required by approximately 19%. The amyloid PET positive rate was assumed to be 25% for the cognitively unimpaired group and 75% for the cognitively impaired group. Data provided in eTable 6, links.lww.com/WNL/B715, were used as the sensitivity and specificity of plasma A β 42/40 test.

The time and cost savings associated with prescreening for brain amyloidosis with a blood test were evaluated for an AD prevention trial similar to the Anti-Amyloid Treatment in

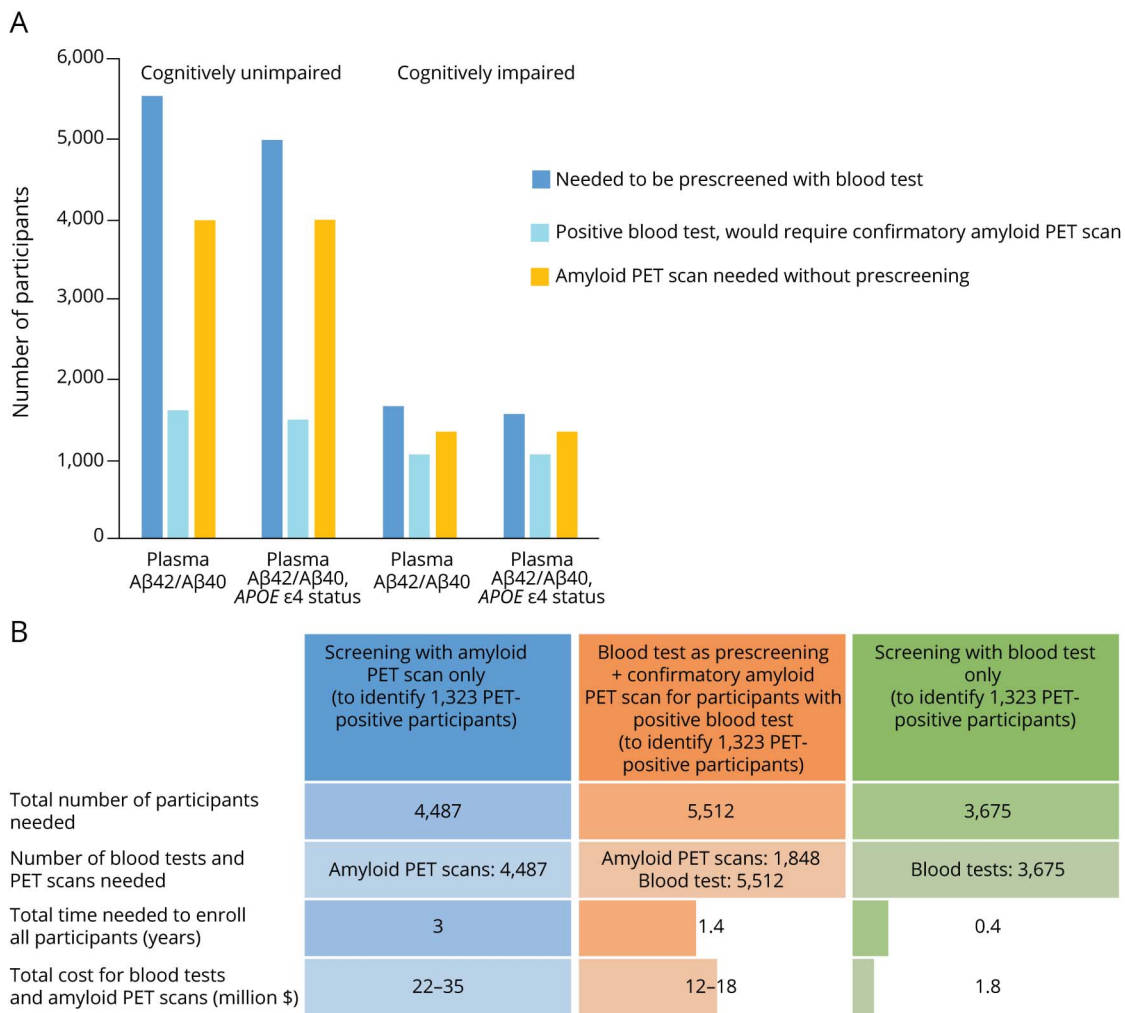
Asymptomatic Alzheimer’s Disease (A4) study (Figure 3B). In the A4 study, amyloid PET scans were performed in 4,487 participants over a 3-year period, and 1,323 of participants (30%) were classified as amyloid PET positive. Prescreening with the blood test reduced the time for enrollment and screening costs (amyloid PET scans + blood test) by half. Screening with blood test alone could be completed in less than 6 months, compared to 3 years with screening with amyloid PET scan alone, and cut costs by 10-fold or more. The blood test was assumed to have a sensitivity of 0.80 and specificity of 0.83 in cognitively unimpaired participants (eTable 6, links.lww.com/WNL/B715). Costs were assumed to be \$5,000–\$8,000 per amyloid PET scan and \$500 per blood test.

Given uncertainty in the amyloid PET positivity rate in the general population, eFigure 3, A and B (links.lww.com/WNL/B715), provide the number of participants and amyloid PET scans needed to identify 1,000 amyloid PET positive participants, with and without prescreening with a blood test, based on a series of amyloid PET positive rate, sensitivity, and specificity values. These figures demonstrated that prescreening with a blood test was more effective when the amyloid PET positivity rate was low in a population (e.g., cognitively unimpaired group), and an assay with high sensitivity and specificity was much more efficient in reducing both the number of participants to be screened and the number of confirmatory PET scans needed.

Discussion

In the 3 independent cohorts that used different protocols for blood collection and processing, as well as multiple amyloid PET radioligands, there was good and consistent correspondence between plasma A β 42/A β 40 and amyloid PET status. The good performance of the test in the BioFINDER cohort, in which blood was collected from nonfasting participants, demonstrates this test may be useful in routine clinical settings, where blood is usually drawn under nonfasting conditions.

Figure 3 Plasma A β 42/A β 40 as a Prescreening Test for Brain Amyloidosis



(A) The number of participants and amyloid PET scans needed to identify 1,000 amyloid PET-positive participants, with and without prescreening with a blood test. The amyloid PET positive rate was assumed to be 25% for the cognitively unimpaired group and 75% for the cognitively impaired group. Data provided in eTable 6, links.lww.com/WNL/B715, were used as the sensitivity and specificity of plasma A β 42/40 test. (B) The time and cost savings associated with prescreening for brain amyloidosis with plasma A β 42/A β 40 were evaluated for an Alzheimer disease prevention trial similar to the Anti-Amyloid Treatment in Asymptomatic Alzheimer’s Disease (A4) Study. In the A4 study, amyloid PET scans were performed in 4,487 participants over a 3-year period, and 1,323 participants (30%) were classified as amyloid PET positive. The sensitivity of 0.80 and specificity of 0.83 based on cognitively unimpaired participants as shown in eTable 6, links.lww.com/WNL/B715, were used for the accuracy of the blood test. Costs were assumed to be \$5,000–\$8,000 per amyloid PET scan and \$500 per blood test.

Similar cut-offs between different cohorts and studies suggest that plasma A β 42/A β 40 measurements are consistent across blood collection and processing protocols that use different anticoagulants and centrifugation conditions. Furthermore, plasma A β 42/A β 40 and APOE ϵ 4 were able to distinguish amyloid PET status similarly in cognitively impaired and cognitively unimpaired individuals. These results suggest that plasma A β 42/A β 40 with APOE ϵ 4 status would be helpful for both screening cognitively unimpaired individuals for potential enrollment in AD secondary prevention clinical trials and testing cognitively impaired individuals in the clinic to determine whether AD is the probable etiology.

For this study, amyloid PET status and CSF A β 42/A β 40 status were defined based on the cut-offs provided by each

study. To account for potential methodologic differences between cohorts, for the combined cohort analyses we used mixed effects models with random intercepts for each cohort. Overall, the correlations between plasma A β 42/A β 40, Centiloid, and CSF A β 42/A β 40 observed in this study were similar to those reported by other studies.^{12,13} Although the correlations were strong, they are not close to 1.0, suggesting these 3 measures do not provide identical information. The differences and relationships over time between plasma A β 42/A β 40, CSF A β 42/A β 40, and amyloid PET need to be evaluated further in future studies.

This study had several limitations. More than half of the participants were cognitively impaired (n = 249), including 46 AD cases and 203 MCI cases, providing sufficient numbers to

address whether cognitive status had an effect on plasma amyloid 42/40 detection of amyloid plaques. However, although there were small numbers (8%–10%) of symptomatic participants with AD dementia in ADNI and BioFINDER, they were not selected for this study because the selection criteria for this study were availability of the plasma samples and amyloid PET data within 1 year, not dementia status. Second, in this study, a similar number of plasma samples were selected from amyloid PET positive and negative groups, so the amyloid PET positive rates may not represent those in the ADNI, AIBL, and BioFINDER cohorts, but may be more typical of an early AD or MCI population.⁴¹ As PPV and NPV are influenced by amyloid positive rate, the PPV and NPV provided in this research were calculated using an assumed amyloid positive rate (37.5% overall, 25% for cognitively unimpaired, and 75% for cognitively impaired) in the general population instead of using the amyloid PET positive rate observed in the 3 cohorts. Third, ADNI, AIBL, and BioFINDER cohorts have low diversity; therefore it is unclear whether the performance of plasma A β 42/A β 40 differs by racial and ethnic groups. Several studies have found that African Americans have significantly lower levels of CSF t-tau and p-tau181 compared to non-Hispanic Whites even after adjusting for age, sex, APOE ϵ 4 status, and cognitive status^{42,43}; however, there is very limited information available on racial or ethnic differences in plasma A β 42/A β 40. Further studies to evaluate racial or ethnic differences in plasma A β 42/A β 40 are underway.

In clinical trials, AD biomarker testing is used to screen cognitively unimpaired individuals for potential enrollment in trials that aim to evaluate treatments that may prevent or slow the onset of AD dementia symptoms.⁴ Amyloid PET has been used to screen potential participants for secondary prevention clinical trials,⁴⁴ but because of cost and limited availability, enrollment is costly and slow. Participants in AD research cohorts including ADNI, AIBL, and BioFINDER are more likely to be healthy, well-educated, and White than the overall population with AD.⁴⁵ Recruitment of racial minority groups is more difficult for studies that include testing with unfamiliar techniques like amyloid PET and CSF biomarkers.^{46,47} In contrast, blood tests are relatively well-accepted by most individuals. It seems likely that use of an AD blood test, rather than amyloid PET or CSF biomarkers, may facilitate recruitment of more representative research cohorts and enable development of therapies that are likely to be effective in all groups.

In the clinic, AD biomarker testing is used to determine whether the etiology of cognitive impairment is likely to be AD. Appropriate use criteria for amyloid PET and CSF biomarkers typically recommend testing only for patients with significant diagnostic uncertainty, largely because of the cost, limited availability, and perceived risks or invasiveness of these methods.^{5,6} Appropriate use criteria for blood-based biomarkers have not yet been formulated, but the drawbacks of blood-based biomarkers are reduced. Given the relatively

high rate of AD misdiagnosis even by AD research centers,⁴⁸ broad-based testing of individuals presenting with cognitive concerns to a dementia specialty clinic may be warranted, especially if out-of-pocket costs to patients are reasonable.

Over the past 3 years, there has been rapid development of multiple blood-based biomarkers, with studies reporting that different phosphorylated tau (p-tau) species, particularly p-tau181 and p-tau217, are highly concordant with amyloid PET status as well as cognitive status.^{38,49,50} Levels of these p-tau isoforms may reflect both brain amyloidosis and the neuronal damage that results in cognitive impairment.³⁸ Further studies are needed to explore the correlation between plasma p-tau isoforms and A β 42/A β 40 at different stages of the AD time course. Recent findings indicate that the region of tau that aggregates in tau tangles, the microtubule binding region, can be accurately measured in CSF and correlates well with tau PET.⁵¹ Evaluating combinations of biomarkers that reflect multiple AD-related processes, including amyloidosis, tauopathy, neurodegeneration, and inflammation, will improve our understanding of AD and aid in accurate diagnosis and prediction of AD risk.^{13,52}

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Name	Location	Contribution
Yan Li, PhD	Washington University	Performed statistical analyses and interpreted data, prepared initial draft of manuscript, revised manuscript
Suzanne E. Schindler, MD, PhD	Washington University	Interpreted data, drafted and revised manuscript
James G. Bollinger, PhD	Washington University	Developed mass spectrometry methods, collected and interpreted data, drafted mass spectrometry methods section, revised manuscript
Vitaliy Ovod, MS	Washington University	Developed mass spectrometry methods, collected and interpreted data, revised manuscript
Kwasi G. Mawuenyega, PhD	Washington University	Developed mass spectrometry methods, collected and interpreted data, revised manuscript
Michael W. Weiner, MD	University of California San Francisco	Interpreted data, revised manuscript
Leslie M. Shaw, PhD	University of Pennsylvania	Interpreted data, revised manuscript, provided plasma samples
Colin L. Masters, MD	University of Melbourne	Interpreted data, revised manuscript, provided plasma samples
Christopher J. Fowler, PhD	University of Melbourne	Interpreted data, revised manuscript
John Q. Trojanowski, MD, PhD	University of Pennsylvania	Interpreted data, revised manuscript

Appendix (continued)

Name	Location	Contribution
Magdalena Korecka, PhD	University of Pennsylvania	Interpreted data, revised manuscript
Ralph N. Martins, PhD	Edith Cowan University	Interpreted data, revised manuscript
Shorena Janelidze, PhD	Lund University	Interpreted data, revised manuscript
Oskar Hansson, MD, PhD	Lund University	Interpreted data, revised manuscript, provided plasma samples
Randall J. Bateman, MD	Washington University	Designed study, developed mass spectrometry methods, interpreted data, drafted manuscript, revised manuscript

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