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FEATURED ARTICLE

Detecting amyloid positivity in early Alzheimer's disease using combinations of plasma Aβ42/Aβ40 and p-tau

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Abstract

Introduction: We studied usefulness of combining blood amyloid beta (Aβ)42/Aβ40, phosphorylated tau (p-tau)217, and neurofilament light (NfL) to detect abnormal brain Aβ deposition in different stages of early Alzheimer's disease (AD).

Methods: Plasma biomarkers were measured using mass spectrometry (Aβ42/Aβ40) and immunoassays (p-tau217 and NfL) in cognitively unimpaired individuals (CU, N = 591) and patients with mild cognitive impairment (MCI, N = 304) from two independent cohorts (BioFINDER-1, BioFINDER-2).

Results: In CU, a combination of plasma Aβ42/Aβ40 and p-tau217 detected abnormal brain Aβ status with area under the curve (AUC) of 0.83 to 0.86. In MCI, the models including p-tau217 alone or Aβ42/Aβ40 and p-tau217 had similar AUCs (0.86–0.88); however, the latter showed improved model fit. The models were implemented

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in an online application providing individualized risk assessments (https://brainapps.shinyapps.io/PredictABplasma/).

Discussion: A combination of plasma Aβ42/Aβ40 and p-tau217 discriminated Aβ status with relatively high accuracy, whereas p-tau217 showed strongest associations with Aβ pathology in MCI but not in CU.

KEYWORDS
Alzheimer's disease, amyloid, Aβ42/Aβ40, blood biomarkers, neurofilament light, p-tau217

1 | BACKGROUND

In Alzheimer's disease (AD), the underlying brain amyloid beta (Aβ) and tau pathologies and ensuing neurodegeneration can be reliably detected and monitored using cerebrospinal fluid (CSF) and imaging biomarkers. CSF concentrations of Aβ42 (alone or as a ratio with Aβ40) and phosphorylated tau (p-tau) reflect AD-related changes in Aβ and tau metabolism in the brain.[1] Aβ and tau positron emission tomography (PET) are used to measure Aβ plaque load and insoluble paired helical filament (PHF) tau aggregates.[2,3] Biomarkers of neurodegeneration (due to AD or other causes) include fluorodeoxyglucose (FDG) PET, magnetic resonance imaging (MRI), and CSF neurofilament light (NfL).[4–7] The CSF and imaging biomarkers have been successfully used in research settings and in specialized clinics in some countries and were recently incorporated into an ATN (amyloid, tau, neurodegeneration) classification system, a research framework proposed by the National Institute on Aging for the diagnosis of AD.[8] More accessible and inexpensive methods, like blood tests, however, are needed for widespread applicability in clinical trials as well as for future implementation in routine clinical care. AD biomarker concentrations in blood are low, making their quantification in the presence of other high abundance proteins challenging. However, recent technological advances in mass spectrometry and immunodetection have led to the development of novel methods that have allowed for reliable assessment of Aβ42/Aβ40, p-tau, and NfL in blood.[9]

Blood Aβ42/Aβ40 correlates with CSF Aβ42/Aβ40 and Aβ-PET and can identify with relatively high precision individuals with abnormal brain Aβ burden or those at high risk of future conversion to Aβ-PET positivity.[10–15] When measured in plasma, tau phosphorylated at threonine 217 and 181 (p-tau217 and p-tau181) accurately detect amyloid and tau pathology assessed by PET, differentiate AD from non-AD neurodegenerative disorders, and predict future progression to AD dementia.[16–21] Plasma levels of p-tau217 have been shown to increase in very early preclinical stages of AD and continue to increase over time in patients with preclinical and prodromal AD.[22,23] Elevated levels of blood NfL have also been reported in mild cognitive impairment (MCI) and AD dementia stages in sporadic disease and already in presymptomatic phases in familial AD,[24–26] increasing over time in parallel with other signs of neurodegeneration.[27] However, NfL is not specific to neurodegeneration in AD but is increased (in both CSF and blood) in many other disorders of the central nervous system, including, for example, frontotemporal dementia, progressive supranuclear palsy, corticobasal syndrome, amyotrophic lateral sclerosis, and Creutzfeldt-Jakob disease.[28–32]

Plasma biomarkers have the potential to greatly accelerate the development of effective disease-modifying treatments in AD by facilitating the identification of individuals at the earliest disease stages (i.e., subjects with preclinical or prodromal AD), when the treatments are most likely to be successful. Although plasma AD biomarkers have shown relatively fair accuracy for detecting Aβ pathology, it remains to be established if a blood test combining these biomarkers could offer improved performance. For example, decrease in plasma Aβ levels in AD is very modest (15%–20% at most), while plasma p-tau is a more dynamic biomarker that could better mirror progressive increases in brain Aβ burden. At the same time, because AD biomarkers follow different trajectories with Aβ42/Aβ40 starting to change first followed next by p-tau and then by NfL,[33,34] it is very likely that blood Aβ42/Aβ40 would be the most accurate in the very early disease stages. In the present study, we measured plasma Aβ42/Aβ40 (Araclon mass spectrometry assay), plasma p-tau217 (Lilly-developed immunoassay), and plasma NfL (Simoa-based immunoassay) in two independent cohorts of cognitively unimpaired (CU) participants (n = 591) and patients with MCI (n = 304). We first assessed the accuracy of individual biomarkers and then different combinations of biomarkers to detect abnormal brain Aβ status (defined using either CSF Aβ42/Aβ40 or Aβ-PET) in the CU subjects and patients with MCI, separately. Finally, we tested whether the accuracy was improved by including information on apolipoprotein E (*APOE*) ε4 status of the examined individuals.

2 | METHODS

2.1 | Study participants

The study was approved by the Regional Ethics Committee in Lund, Sweden. All participants provided written informed consent. They were recruited in southern Sweden (Skåne University Hospital and the Hospital of Ängelholm) as previously reported.[16,20] Further details on study design and recruitment procedures are given in the supporting information. From BioFINDER-1 (clinical trial no. NCT01208675), we included 123 cognitively healthy controls, 118 patients with subjective

cognitive decline (SCD), and 140 MCI patients recruited between 2010 and 2015. The BioFINDER-2 cohort (clinical trial no. NCT03174938) comprised 235 cognitively healthy controls, 115 SCD, and 164 MCI patients recruited between 2017 and 2019. In accordance with the research framework by the National Institute on Aging-Alzheimer's Association study patients with SCD and cognitively healthy controls were considered the CU group (BioFINDER-1 N = 241, BioFINDER-2 N = 350).[8] From both cohorts, we included all participants with available plasma Aβ42/Aβ40, plasma p-tau217, plasma NfL, and *APOE* genotype data.

2.2 | Plasma and CSF sampling and analysis

Ethylenediaminetetraacetic acid (EDTA)-plasma and CSF samples were collected and handled as previously described.[12,16] Plasma p-tau217 concentration was measured according to the published protocols using immunoassay on a Mesoscale Discovery platform developed by Lilly Research Laboratories.[20,22] BioFINDER-1 and BioFINDER-2 samples were analyzed at the Clinical Memory Research Unit, Lund University (Sweden) and at Lilly Research Laboratories, respectively. Briefly, biotinylated-IBA493 was used as a capture antibody and SULFO-TAG-4G10-E2 (anti-tau) as the detector and samples were diluted 1:2. In BioFINDER-2, the assay was calibrated with a recombinant tau (4R2N) protein that was phosphorylated in vitro using a reaction with glycogen synthase kinase-3 and characterized by mass spectrometry, while in BioFINDER-1, we used a synthetic p-tau217 peptide.

Given that prior studies have suggested a potentially better performance of blood Aβ42/Aβ40 measured with mass spectrometry compared to immunoassays,[10–12,14,15] we used mass spectrometry to quantify plasma levels of Aβ42 and Aβ40 in the present study. Further details of the assays are described in the supporting information.

In BioFINDER-1, plasma NfL concentration was measured using Simoa N4PE kit (Quanterix) at the Neurochemistry Laboratory of the Amsterdam UMC location VUmc (Netherlands). In BioFINDER-2, plasma NfL concentration was measured at the Clinical Neurochemistry Laboratory in Gothenburg using a Simoa kit (Quanterix).

CSF Aβ42 and Aβ40 concentrations in BioFINDER-1 were quantified at Euroimmun using enzyme-linked immunosorbent assay (ELISA) kits (Euroimmun). CSF Aβ42 and Aβ40 concentrations in BioFINDER-2 were measured with Meso Scale Discovery immunoassays (MSD) at the Clinical Neurochemistry Laboratory in Gothenburg. CSF Aβ42/Aβ40 measured using either Euroimmun or MSD assays perform equally well when predicting Aβ-PET assessment outcome.[35] CSF Aβ42/Aβ40 data were binarized using previously published cutoffs (< 0.091 in BioFINDER-1 and < 0.0752 in BioFINDER-2[20,36]).

2.3 | Aβ-PET imaging

Aβ imaging was performed using [^{18}F]flutemetamol PET, as described in the supporting information. Briefly, standardized uptake value ratio (SUVR) images were created using dynamic (list-mode) 90- to 100-minute post-injection data and the whole cerebellum, pons/brainstem, and eroded cortical white matter as reference region.[37] Aβ-PET status (abnormal/normal) was determined by applying a Gaussian mixture modeling-based cutoff to neocortical SUVR values.

RESEARCH IN CONTEXT

1. **Systematic review**: We searched and reviewed the literature on Alzheimer's disease (AD), blood biomarkers of amyloid and tau pathologies, and neurodegeneration using PubMed. While prior publications suggested that blood amyloid beta (Aβ)42/Aβ40, phosphorylated tau (p-tau), and neurofilament light (NfL) become abnormal very early in the disease course, no studies have investigated which combination of these biomarkers most accurately predicts brain Aβ pathology in early AD.
2. **Interpretation**: The main findings from two independent cohorts were that in preclinical and prodromal AD, a combination of plasma Aβ42/Aβ40 and plasma p-tau217 detected with high precision abnormal brain Aβ status.
3. **Future directions**: Blood biomarkers have the potential to greatly accelerate the development of effective disease-modifying treatments in AD by facilitating identification of individuals at the earliest disease stages when the treatments are most likely to be successful. Replication in other cohorts will be needed before the findings of the present study could be implemented in clinical trials.

HIGHLIGHTS

- Brain Aβ pathology in preclinical and prodromal Alzheimer's disease (AD) was accurately detected by a combination of plasma Aβ42/Aβ40 and P-tau217.
- In prodromal AD, plasma P-tau217 by itself exhibited high predictive value for Aβ status.

2.4 | Statistical analysis

All analyses were performed using SPSS version 26 (IBM). Differences in baseline demographic and clinical data and biomarker levels were tested with Chi-square and Mann-Whitney tests. Plasma biomarker data were transformed to z-scores based on the distribution in the Aβ− CU sample. Discrimination accuracies of biomarkers were determined with logistic regression models and receiver operating characteristic (ROC) curve analysis. *APOE* risk allele status was modeled as one variable coded for the presence of ε4 allele (1 for ε4 carriers and 0 for noncarriers). Improvements in model fit were estimated using Akaike

TABLE 1 Demographic and clinical characteristics

	BioFINDER-2			BioFINDER-1		
	CU	MCI	*P*-value	CU	MCI	*P*-value
N	350	164		241	140	
Age, years	64 (53–75)	71 (66–76)	<.0001	72 (68–75)	71 (67–76)	.47
Female no., (%)	183 (52.3)	79 (48.2)	.38	134 (55.6)	50 (35.7)	.0002
Duration of education, years*	12 (10–15)	12 (9–15)	.54	12 (9–14)	11 (9–13)	0.006
MMSE	29 (28–30)	27 (25–29)	<.0001	29 (28–30)	27 (26–29)	<.0001
APOE ε4 positivity No., %	155 (44.3)	87 (53)	.06	90 (37.3)	73 (52.1)	.005
CSF Aβ42/Aβ0†	1.00 (0.78–1.12)	0.69 (0.50–1.04)	<.0001	0.12 (0.08–0.14)	0.07 (0.05–0.13)	<.0001
CSF Aβ42/Aβ0 positivity, No., (%)	81 (23.1)	89 (54.3)	<.0001	78 (32.4)	86 (61.4)	<.0001
Aβ-PET, [^{18}F]Flutemetamol SUVR neocortical meta-ROI*	0.62 (0.59–0.66)	0.72 (0.61–0.72)	<.0001	0.67 (0.64–0.77)	0.93 (0.70–1.09)	<.0001
Aβ-PET positivity No., %	75 (22.0)	83 (52.9)	<.0001	673 (29.1)	86 (66.2)	<.0001
Plasma Aβ42/Aβ0	0.22 (0.20–0.25)	0.21 (0.19–0.24)	.044	0.31 (0.29–0.34)	0.29 (0.27–0.32)	.0002
Plasma p-tau217 pg/mL†	0.94 (0.31–1.78)	1.66 (0.43–3.70)	<.0001	0.15 (0.07–0.24)	0.27 (0.13–0.39)	<.0001
Plasma NfL pg/mL†	12.13 (8.30–16.96)	16.35 (11.60–22.95)	<.0001	27.58 (21.61–36.45)	35.00 (26.87–45.32)	<.0001

Notes: Data are shown median (interquartile range) unless otherwise specified; *P* values are from Chi-square (sex, *APOE* ε4 status, CSF Aβ42/Aβ0 status, Aβ-PET status), Mann-Whitney tests (age, duration of education, MMSE) and univariate analysis of variance adjusted for age and sex (plasma biomarkers, CSF Aβ42/Aβ0, [^{18}F]Flutemetamol SUVR).

*In BioFINDER-2, education was missing for 1 MCI patient and Aβ-PET was not available for 16 participants; in BioFINDER-1, education was missing for 3 MCI patients and Aβ-PET was not available for 21 participants.

†Samples from BioFINDER-1 and BioFINDER-2 were analyzed using different assays (as described in the Materials and Methods section) and biomarker concentrations are therefore not comparable across the cohorts.

Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NfL, neurofilament light; p-tau, phosphorylated tau; ROI, region of interest; SUVR, standardized uptake value ratio

information criterion (AIC) with a decrease of 2 or more in AIC indicating better model fit.[38] The AIC values were transformed to the Akaike weights that can be interpreted as the probability that the respective model is the most correct among the candidate models in the given sample.[39] The performance of plasma biomarkers was assessed separately in BioFINDER-1 and BioFINDER-2. We also performed external validation across cohorts by testing the model estimates derived in BioFINDER-2 on BioFINDER-1 and vice versa. The fitted models were used to create an online application that calculates individualized probability for Aβ positivity. Area under the curve (AUC) of two ROC curves were compared to DeLong test with adjustment for multiple comparisons using the Benjamini-Hochberg method and a false discovery rate (FDR) of 5%. The FDR correction was applied separately for the analysis in the BioFINDER-1 and BioFINDER-2 cohorts. Statistical significance was set at $P < .05$. Out of 895 study participants, 334 had plasma p-tau217 levels below the detection limit of the assay. When it was not possible to interpolate plasma p-tau217 concentrations from the standard curve due to the very low signal, the values were imputed to the lowest measurable value. Out of 334 samples below the detection limit, plasma p-tau217 values were only imputed for 95 cases (10% of the whole study population). Almost all imputed data (97%, N = 92) were in the Aβ– group. Furthermore, the large majority of the samples below the detection limit (87%, N = 289) were also in the Aβ– group. Given that data below the detection limit were confined to the Aβ– group, these values were considered to represent truly very low p-tau217 concentrations and were included in all statistical analyses. Results from the larger BioFINDER-2 cohort are presented first.

3 | RESULTS

3.1 | Participant characteristics

The BioFINDER-2 cohort included 350 CU participants (269 CSF Aβ–; 81 CSF Aβ+) and 164 patients with MCI (75 CSF Aβ–; 89 CSF Aβ+). The BioFINDER-1 cohort included 241 CU participants (163 CSF Aβ–; 78 CSF Aβ+) and 140 patients with MCI (54 CSF Aβ–; 86 CSF Aβ+). The majority of participants in BioFINDER-2 (n = 498) and BioFINDER-1 (n = 360) also underwent Aβ-PET. Baseline demographic and clinical characteristics of both cohorts are summarized in Table 1 and Tables S1 and S2 in supporting information. In BioFINDER-2, CU participants were on average younger than MCI participants. In BioFINDER-1, the CU group included more men than the MCI group. In both cohorts,

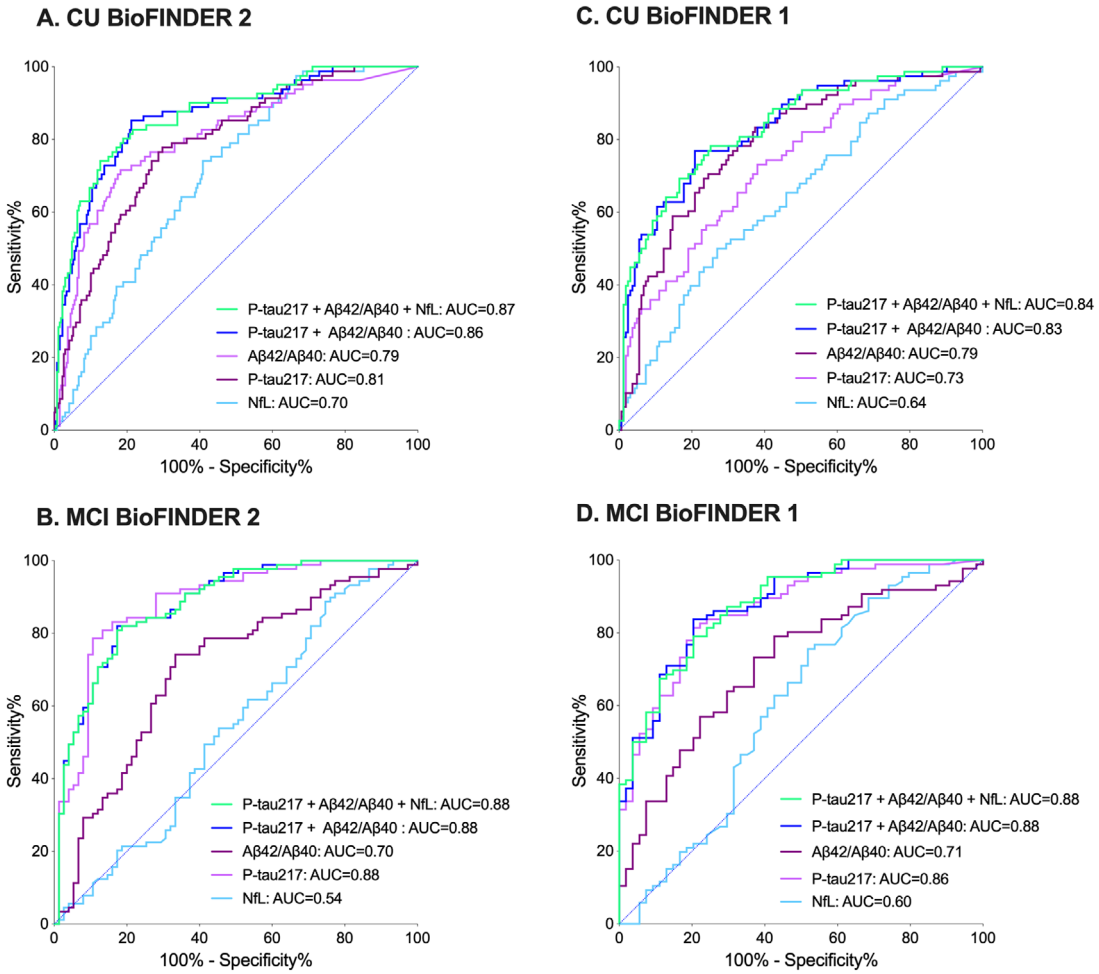

FIGURE 1 Receiver operating characteristic (ROC) curve analyses for discriminating cerebrospinal fluid (CSF) amyloid beta (Aβ)42/Aβ40 status. ROC curves are shown for plasma Aβ42/Aβ40, plasma phosphorylated tau (p-tau)217, plasma neurofilament light (NfL), a combination of plasma Aβ42/Aβ40 and plasma p-tau 217, and the full model including all three plasma biomarkers (plasma Aβ42/Aβ40, plasma P-tau 217, and plasma NfL). AUC, area under the curve; CU, cognitively unimpaired; MCI, mild cognitive impairment

the CSF and plasma biomarkers as well as Aβ-PET measures were more abnormal in MCI patients compared to CU individuals (Table 1). Plasma biomarker concentrations are shown in FigureS1 in supporting information.

3.2 | Detecting abnormal CSF Aβ status in the BioFINDER-2 cohort

3.2.1 | CU participants

In CU participants, univariate associations with abnormal CSF Aβ42/Aβ40 status were stronger for plasma Aβ42/Aβ40 (AUC = 0.79 [confidence interval (CI) 0.73–0.84]; odds ratio [OR] = 0.26, $P < .0001$) and plasma p-tau217 (AUC = 0.81 [CI 0.75–0.86]; OR = 2.49, $P < .0001$) than for plasma NfL (AUC = 0.70 [CI 0.65–0.76], OR = 1.71, $P < .0001$; Table 2). The model combining plasma p-tau217 and plasma Aβ42/Aβ40 showed high discriminative accuracy with an AUC of 0.86 (CI 0.82–0.91) and was not significantly different from the model including all three plasma biomarkers (ΔAUC = 0.005, $P = .61$), although the AIC indicated somewhat better fit for the three-biomarker model (Table 2, Figure 1A).

3.2.2 | MCI patients

In MCI patients, plasma p-tau217 (AUC = 0.88 [CI 0.83–0.94]; OR = 2.29, $P < .0001$) outperformed plasma Aβ42/Aβ40 (AUC = 0.70 [CI 0.62–0.78]; OR = 0.48, $P = .0001$), while plasma NfL was not significant (AUC = 0.54, CI [0.45–0.63]; OR = 0.97, $P = .68$) to detect Aβ positivity (Table 3). The plasma p-tau217 model was noninferior to the model with all three plasma biomarkers (ΔAUC = 0.00, $P = .95$) and to the model including plasma p-tau217 and plasma Aβ42/Aβ40 (ΔAUC = 0.00, $P = .95$), even though the AIC suggested that the model combining plasma p-tau217 and plasma Aβ42/Aβ40 fit the data better than all the other models (Table 3 and Figure 1B).

TABLE 2 Associations with CSF Aβ42/Aβ40 status in CU individuals in BioFINDER-2 and BioFINDER-1

Model	Odds ratio (*P*-value)			AUC (95% CI)	*P*-value vs. full plasma model*	AIC (ΔAIC) vs. full plasma model	wAIC
	Aβ42/Aβ40	p-tau217	NfL				
BioFINDER-2†							
Aβ42/Aβ40, p-tau217, NfL	0.28 (*P* < .0001)	2.23 (*P* < .0001)	1.48 (p = 0.008)	0.868 [0.822, 0.914]	NA	283 (ref)	0.92
Aβ42/Aβ40, p-tau217	0.27 (*P* < .0001)	2.42 (*P* < .0001)	NA	0.863 [0.817, 0.910]	.61	288 (5)	0.08
Aβ42/Aβ40	0.26 (*P* < .0001)	NA	NA	0.786 [0.732, 0.841]	<.0001	314 (31)	1.7e-07
p-tau217	NA	2.49 (*P* < .0001)	NA	0.805 [0.748, 0.862]	.030	343 (60)	8.6e-14
NfL	NA	NA	1.71 (p < 0.0001)	0.704 [0.646, 0.762]	<.0001	363 (80)	3.9e-18
BioFINDER-1‡							
Aβ42/Aβ40, p-tau217, NfL	0.34 (*P* < .0001)	2.11 (*P* < .0001)	1.29 (*P* = .094)	0.837 [0.782, 0.891]	NA	228 (ref)	0.62
Aβ42/Aβ40, p-tau217	0.33 (*P* < .0001)	2.20 (*P* < .0001)	NA	0.833 [0.778, 0.888]	.60	229 (1)	0.38
Aβ42/Aβ40	0.33 (*P* < .0001)	NA	NA	0.790 [0.730, 0.851]	.08	260 (31)	7.0e-08
p-tau217	NA	2.10 (*P* < .0001)	NA	0.731 [0.664, 0.798]	.001	270 (42)	4.7e-10
NfL	NA	NA	1.50 (*P* = .0011)	0.639 [0.565, 0.713]	<.0001	295 (66)	1.8e-15

Notes: Data are from logistic regression models with binarized CSF Aβ42/Aβ40 status as outcome. For plasma biomarkers, odds ratios represent increased risk of CSF Aβ42/Aβ40 positivity for each SD change in biomarker value. ΔAIC, the difference between the AIC values of the reference model and other models; wAIC, the Akaike weight for a given model calculated from ΔAIC.

*P-values (adjusted for multiple comparisons) are for comparisons of AUCs (using DeLong test) between the full model (Aβ42/Aβ40, p-tau217, NfL) and other models.

†Out of 350 CU participants, 269 were classified as CSF Aβ42/Aβ40 negative and 81 were classified as CSF Aβ42/Aβ40 positive.

‡Out of 241 CU participants, 163 were classified as CSF Aβ42/Aβ40 negative and 78 were classified as CSF Aβ42/Aβ40 positive.

Abbreviations: Aβ, amyloid beta; AIC, Akaike information criterion; *APOE*, apolipoprotein E; AUC, area under the curve; CI, confidence interval; CSF, cerebrospinal fluid; CU, cognitively unimpaired; NfL, neurofilament light; p-tau, phosphorylated tau; SD, standard deviation

3.3 | Validation in the BioFINDER-1 cohort and across the cohorts

3.3.1 | CU participants in BioFINDER-1

In CU participants, plasma Aβ42/Aβ40 (AUC = 0.79 [CI 0. 0.73–0.85], OR = 0.33, *P* < .0001) and plasma p-tau217 (AUC = 0.73 [CI 0.66–0.80]; OR = 2.10, *P* < .0001) were more strongly associated with abnormal CSF Aβ42/Aβ40 status than plasma NfL (AUC = 0.64 [CI 0.56–0.71], OR = 1.50, *P* = .001; Table 2). Just as in BioFINDER-2, the model combining plasma Aβ42/Aβ40 and plasma p-tau217 showed high discriminative accuracy (AUC = 0.83 [CI 0.78–0.89]), which was non-inferior to the model including all three plasma biomarkers in terms of both AUC or AIC (ΔAUC = 0.004, *P* = .60; ΔAIC = 1; Table 2 and Figure 1C).

3.3.2 | MCI patients in BioFINDER-1

In MCI patients, plasma p-tau217 (AUC 0.86 [CI 0.80–0.92]; OR = 3.28, *P* < .0001) outperformed plasma Aβ42/Aβ40 (AUC 0.71 [CI 0.63–0.80]; OR = 0.44, *P* = .0003), while plasma NfL was not significant (AUC 0.60, CI [0.50–0.71]; OR = 1.00, *P* = .98; Table 3). Similar to what was seen in BioFINDER-2, the AUC for the model including plasma p-tau217 was noninferior to the models including all three plasma biomarkers (ΔAUC = 0.014, *P* = .31) or plasma Aβ42/Aβ40 and plasma p-tau217 (ΔAUC = 0.014, *P* = .31) with somewhat better model fit for the last two models (Table 3 and Figure 1D).

3.3.3 | Validation across cohorts

To assess the generalizability of the findings of the present study to wider samples we performed external cross-validation by testing the model fit from BioFINDER-2 in BioFINDER-1 and vice versa. Using this approach, we again observed that a combination of plasma Aβ42/Aβ40 and plasma p-tau217 could discriminate CSF Aβ42/Aβ40 status in CU with AUCs of 0.83 to 0.87 and in MCI with AUCs of 0.87 to 0.89 and no further improvement was seen when adding plasma NfL to the models (Table 4).

3.4 | Added value of *APOE* in the BioFINDER-1 and 2 cohorts

Adding *APOE* ε4 status improved model fit as determined using AIC for all tested plasma biomarkers and biomarker combinations (Tables S3-6 in supporting information). However, in both cohorts, there were no significant differences in AUCs when adding *APOE* ε4 to the combinations of plasma Aβ42/Aβ40 and plasma p-tau217 in CU participants and in MCI patients (Tables S3-6).

TABLE 3 Associations with CSF Aβ42/Aβ40 status in MCI patients in BioFINDER-2 and BioFINDER-1

Model	Odds ratio (*P*-value)			AUC (95% CI)	*P*-value vs. full plasma model*	AIC (ΔAIC) vs. full plasma model	wAIC
	Aβ42/Aβ40	p-tau217	NfL				
BioFINDER-2†							
Aβ42/Aβ40, p-tau217, NfL	0.42 (*P* = .0001)	2.32 (*P* < .0001)	0.99 (*P* = .89)	0.879 [0.827, 0.931]	NA	182 (ref)	0.27
Aβ42/Aβ40, p-tau217	0.42 (*P* = .0001)	2.32 (*P* < .0001)	NA	0.880 [0.828, 0.932]	.61	180 (-2)	0.73
Aβ42/Aβ40	0.48 (*P* = .0001)	NA	NA	0.703 [0.621, 0.784]	<.0001	213 (31)	5.0e-08
p-tau217	NA	2.29 (*P* < .0001)	NA	0.882 [0.828, 0.936]	.95	195 (13)	0.0004
NfL	NA	NA	0.97 (*P* = .68)	0.538 [0.448, 0.628]	<.0001	230 (48)	1.0e-11
BioFINDER-1‡							
Aβ42/Aβ40, p-tau217, NfL	0.48 (*P* = .0072)	3.20 (*P* < .0001)	0.88 (*P* = .45)	0.877 [0.821, 0.934]	NA	124 (ref)	0.36
Aβ42/Aβ40, p-tau217	0.50 (*P* = .0096)	3.19 (*P* < .0001)	NA	0.877 [0.820, 0.934]	.86	123 (−1)	0.59
Aβ42/Aβ40	0.44 (*P* = .0003)	NA	NA	0.714 [0.628, 0.801]	.0005	176 (51)	1.8e-12
p-tau217	NA	3.28 (*P* < .0001)	NA	0.863 [0.803, 0.924]	.31	128 (4)	0.05
NfL	NA	NA	1.00 (*P* = .98)	0.603 [0.500, 0.705]	<.0001	191 (67)	1.0e-15

Notes: Data are from logistic regression models with binarized CSF Aβ42/Aβ40 status as outcome. For plasma biomarkers, odds ratios represent increased risk of CSF Aβ42/Aβ40 positivity for each SD change in biomarker value. ΔAIC, the difference between the AIC values of the reference model and other models; wAIC, the Akaike weight for a given model calculated from ΔAIC.

*P-values (adjusted for multiple comparisons) are for comparisons of AUCs (using DeLong test) between the full model (Aβ42/Aβ40, p-tau217, NfL) and other models.

†Out of 164 MCI patients, 75 were classified as CSF Aβ42/Aβ40 negative and 89 were classified as CSF Aβ42/Aβ40 positive.

‡Out of 140 MCI patients, 54 were classified as CSF Aβ42/Aβ40 negative and 86 were classified as CSF Aβ42/Aβ40 positive.

Abbreviations: Aβ, amyloid beta; AIC, Akaike information criterion; *APOE*, apolipoprotein E; AUC, area under the curve; CI, confidence interval; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; NfL, neurofilament light; p-tau, phosphorylated tau; SD, standard deviation.

TABLE 4 Associations with CSF Aβ42/Aβ40 status, external validation across cohorts

Model	BioFINDER-1*		BioFINDER-2†	
	AUC (95% CI)	*P*-value vs. full plasma model‡	AUC (95% CI)	*P*-value vs. full plasma model‡
CU				
Aβ42/Aβ40, p-tau217, NfL	0.837 [0.783, 0.892]	NA	0.870 [0.824, 0.916]	NA
Aβ42/Aβ40, p-tau217	0.834 [0.779, 0.889]	.73	0.866 [0.819, 0.912]	.61
Aβ42/Aβ40	0.790 [0.730, 0.851]	.08	0.786 [0.732, 0.841]	.0001
p-tau217	0.731 [0.664, 0.798]	.018	0.805 [0.748, 0.862]	.026
NfL	0.639 [0.565, 0.713]	<.0001	0.704 [0.646, 0.762]	<.0001
MCI				
Aβ42/Aβ40, p-tau217, NfL	0.872 [0.814, 0.930]	NA	0.890 [0.840, 0.940]	NA
Aβ42/Aβ40, p-tau217	0.872 [0.814, 0.930]	.86	0.895 [0.846, 0.943]	.61
Aβ42/Aβ40	0.714 [0.628, 0.801]	.0001	0.703 [0.621, 0.784]	<.0001
p-tau217	0.863 [0.803, 0.924]	.70	0.882 [0.828, 0.936]	.79
NfL	0.603 [0.500, 0.705]	<.0001	0.538 [0.448, 0.629]	<.0001

Notes: Data are from logistic regression models with binarized CSF Aβ42/Aβ40 status as outcome.

*Regression estimates from the models fit with the data from BioFINDER-2 were tested in BioFINDER-1.

†Regression estimates from the models fit with the data from BioFINDER-1 were tested in BioFINDER-2.

‡*P*-values (adjusted for multiple comparisons) are for comparisons of AUCs (using DeLong test) between the full model (Aβ42/Aβ40, p-tau217, NfL) and other models.

Abbreviations: Aβ, amyloid beta; AIC, Akaike information criterion; *APOE*, apolipoprotein E; AUC, area under the curve; CI, confidence interval; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; NfL, neurofilament light; p-tau, phosphorylated tau.

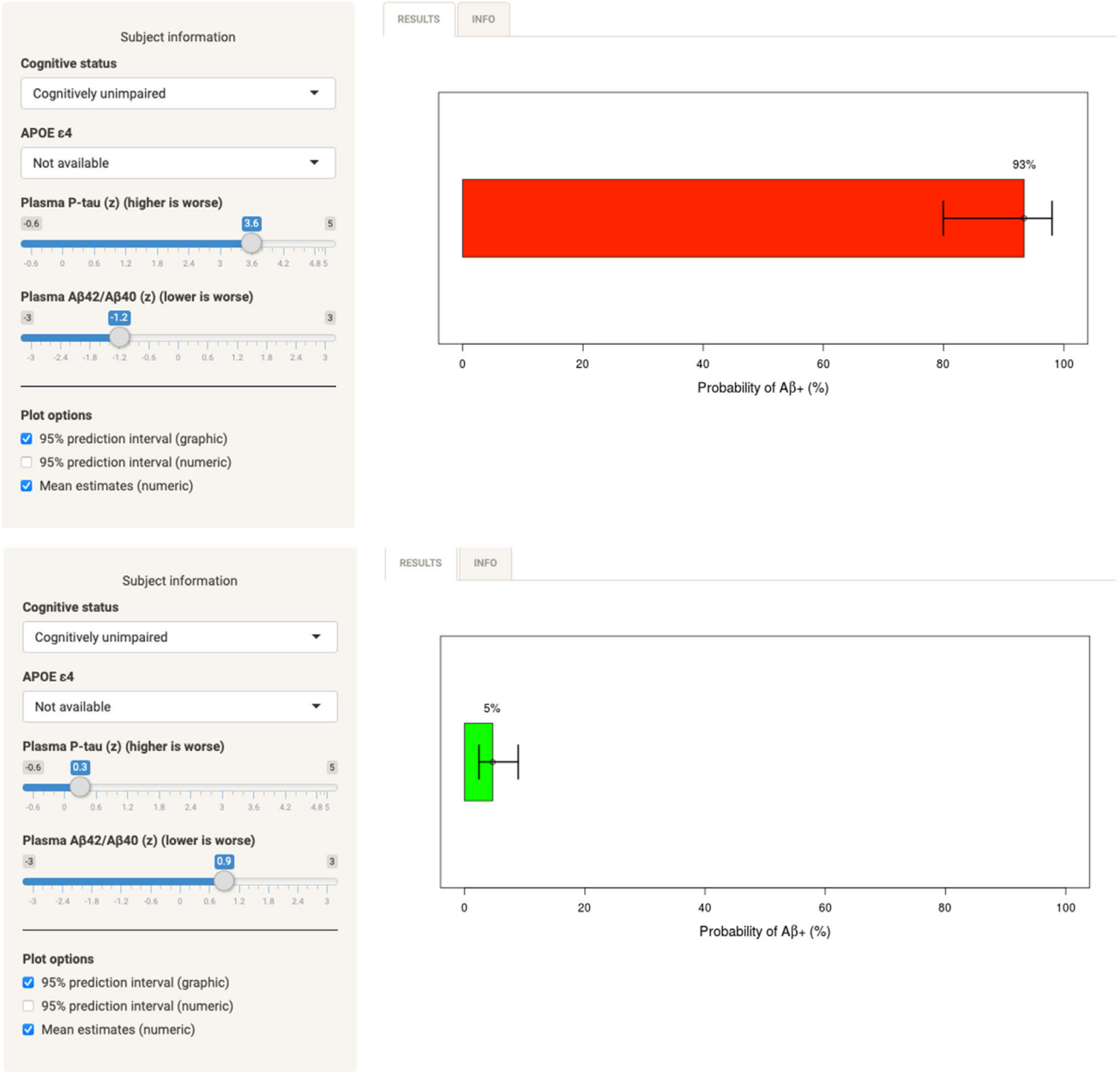

FIGURE 2 Individualized probability for Aβ positivity. Implementation of logistic regression model from BioFINDER-2 at https://brainapps.shinyapps.io/PredictABplasma/. The online application allows user to enter diagnosis (CU, MCI), plasma Aβ42/Aβ40 and p-tau217 values (z-scores) and *APOE* ε4 (1 for ε4 carriers, 0 for noncarriers, NA for not available) and based on these data calculates individualized probability for Aβ positivity. For example, a CU individual with no *APOE* ε4 status available and z-score values of −1.2 and 3.6 for plasma Aβ42/Aβ40 and p-tau217, respectively, has 93% probability of being CSF Aβ42/Aβ40 positive; whereas a CU individual with no *APOE* ε4 status available and z-score values of 0.9 and 0.3 for plasma Aβ42/Aβ40 and p-tau217, respectively, has 5% probability of being CSF Aβ42/Aβ40 positive. Aβ, amyloid beta; *APOE*, apolipoprotein E; CU, cognitively unimpaired; MCI, mild cognitive impairment; NfL, neurofilament light; p-tau, phosphorylated tau

3.5 | Estimating individualized probability for Aβ positivity

Individual predicted probabilities from logistic regression models in BioFINDER-2 and BioFINDER-1 are shown in Figures S2 and S3 in supporting information. Given that a combination of plasma Aβ42/Aβ40 and plasma p-tau217 showed the best performance with respect to AUC and/or AIC, we implemented the two biomarker fitted model (with or without *APOE* ε4 status) from BioFINDER-2 as an online tool (Figure 2, https://brainapps.shinyapps.io/PredictABplasma/). The online application allows the user to enter diagnosis (CU, MCI), plasma Aβ42/Aβ40 and p-tau217 values (z-scores), and *APOE* ε4 (1 for ε4 carriers, 0 for noncarriers, NA for not available) and based on these data calculates individualized probability for CSF Aβ positivity. For example, a CU individual with no *APOE* ε4 status available and z-score values of −1.2 and 3.6 for plasma Aβ42/Aβ40 and p-tau217, respectively, has

93% probability of being CSF Aβ42/Aβ40 positive; whereas a CU individual with no *APOE* ε4 status available and z-score values of 0.9 and 0.3 for plasma Aβ42/Aβ40 and p-tau217, respectively, has 5% probability of being CSF Aβ42/Aβ40 positive (Figure 2). We added an option of entering *APOE* ε4 status because the models including *APOE* ε4 fit the data better compared to the models without *APOE* ε4 and because previous studies have shown better performance of plasma Aβ42/Aβ40 when combined with *APOE* ε4 status.[12,13]

3.6 | Associations with Aβ-PET in the BioFINDER-1 and 2 cohorts

The performances of the plasma biomarkers were very similar using Aβ-PET status as outcome instead of CSF Aβ42/Aβ40. A combination of plasma Aβ42/Aβ40 and plasma p-tau217 could predict Aβ-PET status in CU with AUCs of 0.82 to 0.84 and in MCI with AUCs of 0.86 to 0.91 (Tables S7–S10 in supporting information). The AUCs for these models were not statistically different from the AUCs of the full three-biomarker models in the same study sample (Tables S7–S10). In both cohorts, there were no significant changes in AUCs when adding *APOE* ε4 to the most models including plasma Aβ42/Aβ40 and plasma p-tau217 as predictors (Tables S7–S10).

Voxel-based analysis revealed strong associations of Aβ-PET retention in especially medial frontoparietal regions with the combination of plasma Aβ42/Aβ40 and plasma p-tau217 in CU participants and with plasma p-tau217 in MCI patients (Figure S4 in supporting information).

4 | DISCUSSION

In this study including two independent cohorts, we explored the utility of currently available plasma biomarkers to detect Aβ pathology at different disease stages. We show that in CU individuals, plasma Aβ42/Aβ40 and plasma p-tau217 discriminated Aβ status more accurately than plasma NfL and that the best performing model included plasma Aβ42/Aβ40 and plasma p-tau217 with no added value of plasma NfL. In patients with MCI, plasma p-tau217 was superior to plasma Aβ42/Aβ40 and plasma NfL. Adding plasma Aβ42/Aβ40 and plasma NfL did not significantly improve the performance of p-tau217 in terms of AUC. However, the models combining plasma Aβ42/Aβ40 and p-tau217 fit the data better than the model including plasma p-tau217 by itself or all three biomarkers. Adding *APOE* ε4 status did not result in significantly better discriminative accuracy, even though the model fits were improved. The findings were consistent using either CSF Aβ42/Aβ40 or Aβ-PET status as outcome.

Previous research suggested that plasma Aβ42/Aβ40 can be used to detect pathological CSF Aβ42/Aβ40 and Aβ-PET scans across different disease stages, especially when combined with *APOE* genotype.[10–12,14,15] Recent data have also indicated that plasma p-tau can accurately discriminate abnormal versus normal Aβ-PET status.[16,18,20,21] Here we show that when measured in the same cohorts, plasma Aβ42/Aβ40 and plasma p-tau217 identified CU individuals with abnormal Aβ status with similar precision (Aβ42/Aβ40, AUC 0.79; p-tau217, AUC 0.73–0.81). Furthermore, this is the first study to demonstrate in CU participants that the combination of Aβ42/Aβ40 and plasma p-tau217 improved discriminative accuracy with AUCs reaching 0.83 to 0.86 in the two independent cohorts. In contrast, in MCI patients, plasma p-tau217 (AUCs 0.86–0.88) outperformed plasma Aβ42/Aβ40 (AUCs 0.70–0.71) and there was no further increase in AUC when combining the two biomarkers. These findings are not surprising given prior data suggesting differences in the biomarker dynamics in AD. While in both CSF and blood, Aβ42/Aβ40 start to change before p-tau,[34,40,41] p-tau levels continue to increase over the course of AD[23,42] and the magnitude of this increase (especially for p-tau217) is considerably larger compared to the drop in Aβ biomarkers levels.[10,12,20] Thus, it is likely that plasma Aβ42/Aβ40 captures CU individuals in the earliest stages of the disease leading to an improved performance of combined plasma Aβ42/Aβ40 and plasma p-tau217 measures in preclinical AD.

The discriminative accuracy of plasma Aβ42/Aβ40 (quantified using mass spectrometry) was somewhat lower compared to some of the previous mass spectrometry findings,[10,11] but not others.[43] It remains to be seen whether differences in the performance of plasma Aβ42/Aβ40 between the present and other studies and between the CU and MCI groups that we report here are cohort specific. For this, head-to-head comparisons of available blood Aβ assays across different diagnostic groups would be needed.

We did not find any improvements in AUC combining plasma Aβ42/Aβ40 and plasma p-tau217 with plasma NfL. In CU participants in BioFINDER-2, the models including all three biomarkers fit the data better compared to a combination of plasma Aβ42/Aβ40 and plasma p-tau217. However, in the same group in BioFINDER-1, plasma NfL was not a significant predictor of CSF Aβ status in the models including all three biomarkers. These results indicate that while the performance of plasma Aβ42/Aβ40 and plasma p-tau217 was consistent across the cohorts, that was not the case for NfL and that the effects of NfL were small (if any). NfL is a biomarker of axonal injury and neuronal loss and higher plasma levels of this biomarker are associated with faster rates of atrophy on MRI and cognitive deterioration.[25,27] When combined, plasma p-tau and plasma NfL were reported to better predict longitudinal changes in Mini-Mental State Examination and conversion to AD dementia in patients with MCI.[44] Thus, while useful in the evaluation of other neurodegenerative and acute brain disorders, in patients with suspected early AD, plasma NfL might be more suitable to predict disease progression rather than as a biomarker linked to Aβ pathology.

In line with published data,[12,13] we found that the models combining plasma biomarkers and *APOE* ε4 status fit the data better than the corresponding models without *APOE* ε4 status when using AIC. However, adding *APOE* ε4 did not significantly improve the AUCs of the best performing models. While information on *APOE* genotype might potentially improve performance of the plasma AD biomarkers, it is important to consider that *APOE* ε4 does not reflect Aβ status but merely indicates disease risk and that its use for patient screening and selection in clinical trials may lead to biased inclusion of *APOE* ε4 carriers.

This study has several limitations. Although the performance of the plasma biomarkers was validated across independent BioFINDER-1 and BioFINDER-2 cohorts, these are specialized cohorts that could have characteristics distinguishing them from other specialized cohorts. Therefore, it is important that our findings are replicated in a more heterogeneous population-based sample and within the intended population in primary care.[45,46] Plasma levels of p-tau217 were measured using a research grade assay, which could be one explanation for the slight difference in AUCs between the BioFINDER-1 and 2 cohorts. For some cases, p-tau217 concentrations were below the detection limit of the assay and a more sensitive assay on a fully automated platform is needed to reliably measure plasma p-tau217 at low concentration. Nonetheless, as long as individuals with levels below the detection limit represent truly low values (as previously shown for this assay[20]), this insufficient sensitivity will not strongly affect the accuracy for detecting Aβ pathology. Finally, implementation of blood-based biomarkers would require standardization of pre-analytical and analytical procedures and development of the certified reference materials. However, to provide an example of potential clinical utility of plasma biomarkers we built an online application in which the user could obtain individualized probability of Aβ positivity after entering diagnosis, plasma Aβ42/Aβ40 and p-tau217 values, and optionally *APOE* ε4 status.

To conclude, we show that the presence of Aβ pathology in early AD could be effectively detected by combining plasma measurements of Aβ42/Aβ40 and p-tau217. In patients with MCI, plasma p-tau217 exhibited the highest predictive value for Aβ status compared to other biomarkers. These findings will aid the implementation of plasma biomarkers in clinical practice and drug trials.

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CONFLICT OF INTEREST

SJ, SP, AL, NMC, ES, IMWV, NJA report no conflicts of interest. HZ has served on scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, and CogRx; has given lectures in symposia sponsored by Fujirebio, Alzecure, and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KB has served as a consultant, on advisory boards, or on data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. PP, LS, and JAA are full-time employees at Araclon Biotech-Grifols. CET has a collaboration contract with ADx Neurosciences and Quanterix, performed contract research or received grants from AC-Immune, Axon Neurosciences, Biogen, Brainstorm Therapeutics, Celgene, EIP Pharma, Eisai, Roche, Toyama, Vivoryon. JLD is a full-time employee of Eli Lilly and Company. OH has acquired research support (for the institution) from AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, GE Healthcare, Pfizer, and Roche. In the past 2 years, he has received consultancy/speaker fees from AC Immune, Alzpath, Biogen, Cerveau, and Roche.

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SUPPORTING INFORMATION

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

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