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FEATURED ARTICLE

An accurate fully automated panel of plasma biomarkers for Alzheimer's disease

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Abstract

Introduction: There is a great need for fully automated plasma assays that can measure amyloid beta (Aβ) pathology and predict future Alzheimer's disease (AD) dementia.

Methods: Two cohorts (*n* = 920) were examined: Panel A+ (*n* = 32 cognitively unimpaired [CU], *n* = 106 mild cognitive impairment [MCI], and *n* = 89 AD) and BioFINDER-1 (*n* = 461 CU, *n* = 232 MCI). Plasma Aβ42/Aβ40, phosphorylated tau (p-tau)181, two p-tau217 variants, ApoE4 protein, neurofilament light, and GFAP were measured using Elecsys prototype immunoassays.

Results: The best biomarker for discriminating Aβ-positive versus Aβ-negative participants was Aβ42/Aβ40 (are under the curve [AUC] 0.83–0.87). Combining Aβ42/Aβ40, p-tau181, and ApoE4 improved the AUCs significantly (0.90 to 0.93; $P < 0.01$). Adding additional biomarkers had marginal effects (ΔAUC $\leq$0.01). In BioFINDER, p-tau181, p-tau217, and ApoE4 predicted AD dementia within 6 years in CU (AUC 0.88) and p-tau181, p-tau217, and Aβ42/Aβ40 in MCI (AUC 0.87).

Discussion: The high accuracies for Aβ pathology and future AD dementia using fully automated instruments are promising for implementing plasma biomarkers in clinical trials and clinical routine.

KEYWORDS

Alzheimer's disease, amyloid beta, apolipoprotein E, area under the curve, blood, cerebrospinal fluid, clinical practice, cognitively unimpaired, diagnostics, Elecsys, fully automated instruments, glial fibrillary acidic protein, immunoassays, implementation, mild cognitive impairment, neurofilament light, phosphorylated tau, plasma, prediction, prognostics

1 | INTRODUCTION

Even though there has been great progress in the development of accurate blood tests for Alzheimer's disease (AD), they have not yet been widely implemented in clinical practice.[1] Validation of blood biomarkers mainly focuses on clinical AD diagnosis[2–5] and detecting amyloid beta (Aβ) pathology in the brain.[6–12] The accumulation of Aβ is considered the primary pathology of AD.[13] The removal or reduction of cerebral Aβ has therefore been the aim of many clinical trials[14,15] and the first anti-Aβ drug (aducanumab) was approved by the Food and Drug Administration in 2021.[16] Having tests that accurately estimate the presence of abnormal Aβ accumulation ("Aβ positivity") is therefore crucial for identifying suitable persons to enroll in drug trials, for ensuring an accurate AD diagnosis,[17–19] and before starting anti-Aβ treatment.[20] Implementing blood tests for Aβ pathology would have great benefits because today's methods for identifying Aβ are either invasive, costly, or only available in specialized centers in some parts of the world (these include Aβ positron emission tomography [PET] and cerebrospinal fluid [CSF] analysis). Blood tests for Aβ may become even more important if ongoing prevention trials in cognitively unimpaired (CU) persons are successful.[21] In that case, Aβ positivity would have to be identified before cognitive impairment starts and it would also be important to predict who will progress to AD dementia (because those that remain stable for many years probably will not benefit the most from the drug). In such screenings of CU individuals, the test would have to be as easily accessible, cost effective, and minimally invasive as possible.

Promising plasma biomarkers that specifically capture key AD processes are the Aβ42/Aβ40 ratio,[7–12,22] tau phosphorylated at threonine 181 (p-tau181)[3,5,23–25] and p-tau217.[2,4,22] Other promising plasma biomarkers that are altered in AD, but not specific to AD pathology, include glial fibrillary acidic protein (GFAP; a marker of glial activation in response to e.g., Aβ accumulation)[6,7,26] and neurofilament light (NfL; a marker of axonal injury).[27,28] To improve the accuracy, the biomarkers have often been combined with apolipoprotein E (*APOE*) genotyping,[8–10,29,30] where the presence of an ε4 allele (encoding the E4 isoform of the apolipoprotein E protein) is associated with higher probability of Aβ positivity.[31] One important obstacle for implementing these biomarkers in clinical practice is that most studies have used research-grade assays suitable for single-batch analysis of all plasma samples (to reduce variability in test results between batches) or required intensive manual labor. A crucial step forward toward clinical implementation would thus be to establish the biomarkers on automated instruments that can run samples whenever they arrive at the laboratory with minimal manual steps and stable results over time (i.e., a fully automated approach). This would allow for setting up the tests in ordinary clinical chemistry laboratories worldwide. Such instruments have been implemented for CSF analyses[32–34] but are not yet readily available for plasma AD biomarkers, although pilot versions have been published (such as the Elecsys plasma prototype assays for Aβ42 and Aβ40[9]).

The primary aim of this study was to examine the clinical performance and diagnostic utility of a novel fully automated plasma biomarker panel for identifying Aβ positivity. The secondary aim was to examine its ability to predict development of future AD dementia. The panel included plasma Aβ42/Aβ40, p-tau181, two variants of p-tau217, NfL, GFAP, and the *APOE* ε4-encoded E4 isoform of ApoE (ApoE4; i.e., quantification of ApoE4 protein in plasma, not *APOE* genotyping). This was examined in cognitively unimpaired and cognitively impaired participants using two independent cohorts (N = 920). In addition, the association between the plasma biomarkers and corresponding CSF biomarkers was examined. For plasma ApoE4, a comparison to the

APOE genotype was performed. Finally, the present plasma Aβ42 and Aβ40 prototype assays were compared in a head-to-head analysis with previous versions on the same fully automated platform.[9]

2 | METHODS

2.1 | Participants and clinical assessments

2.1.1 | The Panel A+ study

Participant enrollment and sample collection were performed between 2001 and 2006 at two clinical sites in Germany (the Geriatric and Rehabilitation Clinic of the Henriettenstift in Hannover and at the Memory Clinic, Department of Neurology, Ulm University) as part of a prospective validation study of new biomarkers. All participants with available plasma and CSF samples were selected for the present study. All patients gave their written informed consent, and the study was approved by the regional ethics committees in Ulm and Hannover, Germany. The study participants were classified as (1) CU (*n* = 32), (2) mild cognitive impairment (MCI; *n* = 106),[35] or (3) mild AD dementia[36] (Mini-Mental State Examination > 17; *n* = 89). Inclusion/exclusion criteria are available in the supporting information and in previous publications.[9,37,38]

2.1.2 | The Swedish BioFINDER study

The Swedish BioFINDER-1 study consecutively included participants between 2010 and 2015 from the southern part of Sweden (clinical trial ID: NCT01208675). The participants were classified as CU[13] (*n* = 461, of which 287 were cognitively healthy controls and 174 had subjective cognitive decline [SCD][39]) or as having MCI (*n* = 232), as previously described.[39] Study design and inclusion/exclusion criteria are described at http://biofinder.se and in the supporting information. The participants were followed longitudinally at participating memory clinics with yearly (bi-annually for controls) follow-ups including cognitive testing, informant-based activities of daily living (ADL) questionnaires, and detailed assessments by physicians experienced in neurocognitive disorders. All patients gave their written informed consent to participate, and the study was approved by the regional ethics committee in Lund, Sweden.

2.2 | Plasma and CSF procedures

Plasma and CSF procedures have been published previously for BioFINDER and Panel A+.[9,40] The details are described in the supporting information.

Prototype immunoassays on cobas e 601 analyzers were used to analyze plasma Aβ42, Aβ40, p-tau181 using an antibody pair recognizing a mid-domain tau epitope, p-tau217 using an antibody pair recognizing an N-terminal tau epitope (p-tau217 N-terminal; Roche Diagnostics), p-tau217 using an antibody pair recognizing a mid-domain tau

RESEARCH IN CONTEXT

1. **Systematic Review**: PubMed was searched for articles on plasma biomarkers of Alzheimer's disease (AD) pathology. No article described fully automated assays implemented in clinical practice. Very few articles were found on fully automated plasma assays, and none described a comparison of all key AD biomarkers (amyloid beta [Aβ]42/Aβ40, phosphorylated tau (p-tau)181, p-tau217, glial fibrillary acidic protein, neurofilament light, and apolipoprotein E [*APOE*] ε4), which was performed in the present study.

2. **Interpretation**: In two independent cohorts (*n* = 920), plasma Aβ42/Aβ40 identified Aβ pathology accurately (area under the curve [AUC] 0.83 to 0.87). Combining Aβ42/Aβ40 with p-tau181 and ApoE4 improved the AUCs (0.90 to 0.93). A combination of three biomarkers predicted AD dementia within 6 years in cognitively unimpaired (AUC 0.88) and mild cognitively impaired individuals (AUC 0.87). These results, obtained using Elecsys prototype immunoassays on fully automated instruments, are promising for implementing plasma biomarkers in clinical practice and clinical trials.

3. **Future Directions**: Future studies should examine the assays' accuracy and robustness when analyzing samples over longer time periods using predefined cut-offs.

epitope (p-tau217 mid-domain; Lilly Research Laboratory[2,4]), ApoE4, NfL, and GFAP (see Tables S1–S4 and Figure S1 in supporting information for assay specifications and comparison with the Meso Scale Discovery platform). In BioFINDER, cobas e 411 analyzers were used to analyze ApoE4, NfL, and GFAP for practical reasons to maximize throughput and the same aliquot was used on both instruments. Note that the assays are designed to run on the same cobas instrument using one aliquot. In the statistical analyses, plasma ApoE4 was empirically binarized at 0.9 μg/ml to provide a perfect separation between *APOE* ε4 carriers and non-carriers.

In addition to the above-described plasma Aβ42 and Aβ40 assays, data from the previously published[9] Elecsys Aβ42 and Aβ40 prototype assays were included for a head-to-head comparison. The old assays were updated for the present plasma analysis in Panel A+ and then further modified for the analyses in BioFINDER (see Tables S1–S3).

CSF Aβ42, Aβ40, and p-tau181, were analyzed on cobas e 601 and NfL, and GFAP cobas e 411 analyzers using the Roche NeuroToolKit as previously described.[41]

2.3 | Outcomes

The primary outcome was Aβ status (abnormal/normal) determined using the CSF Aβ42/Aβ40 ratio. The cutoff for Aβ positivity was established in an unbiased way using mixture modeling

TABLE 1 Characteristics of the Panel A+ and BioFINDER cohorts

Variables	Panel A+			BioFINDER		
	Aβ negative n = 117	Aβ positive n = 110	P-value	Aβ negative n = 403	Aβ positive n = 290	P-value
Clinical diagnosis, n	28 CU 68 MCI 21 mild dementia	4 CU 38 MCI 68 mild dementia	<0.001	315 CU* 88 MCI	146 CU** 144 MCI	<0.001
Age, years	63.6 (10.8)	69.5 (7.9)	<0.001	71.9 (5.6)	73.0 (5.3)	0.004
Female, n (%)	52 (44)	62 (56)	0.085	216 (54)	153 (53)	0.83
MMSE, points	27.0 (2.5)	25.3 (2.3)	<0.001	28.6 (1.5)	27.6 (1.8)	<0.001
Plasma Aβ42, pg/ml***	25.0 (6.4)	20.3 (5.3)	<0.001	32.0 (6.2)	27.9 (5.2)	<0.001
Plasma Aβ40, ng/ml	266 (58.2)	258 (61.1)	0.665	263 (42.4)	260 (40.1)	0.34
Plasma Aβ42/Aβ40***	0.0936 (0.0103)	0.0785 (0.00986)	<0.001	0.122 (0.014)	0.108 (0.012)	<0.001
Plasma p-tau181, pg/ml***	0.353 (0.155)	0.538 (0.216)	<0.001	0.90 (0.47)	1.36 (0.67)	<0.001
Plasma p-tau217 N-terminal, pg/ml***	0.127 (0.120)	0.300 (0.154)	<0.001	0.042 (0.10)	0.112 (0.16)	<0.001
Plasma p-tau217 mid-domain, pg/ml***	0.292 (0.934)	0.492 (1.19)	<0.001	0.217 (0.44)	0.316 (0.32)	<0.001
Plasma GFAP, ng/ml	0.0989 (0.0863)	0.155 (0.0940)	<0.001	0.0896 (0.059)	0.130 (0.067)	<0.001
Plasma ApoE4 positivity, n (%)	23 (20)	79 (72)	<0.001	72 (18)	188 (65)	<0.001
Plasma NfL, pg/ml	3.94 (13.6)	3.33 (2.30)	<0.001	2.69 (2.0)	3.13 (2.2)	<0.001
CSF Aβ42/Aβ40	0.0688 (0.00958)	0.0307 (0.00747)	<0.001	0.094 (0.053)	0.043 (0.01)	<0.001
CSF P-tau181, pg/ml	16.1 (5.24)	38.8 (18.7)	<0.001	17.3 (5.36)	29.6 (13.6)	<0.001
CSF GFAP ng/ml	N/A	N/A		13.6 (11.9)	15.7 (6.5)	<0.001
CSF NfL pg/ml	N/A	N/A		162 (128)	210 (151)	<0.001

Abbreviations: Aβ, amyloid beta; APOE, apolipoprotein E; CSF, cerebrospinal fluid; CU, cognitively unimpaired; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NfL, neurofilament light; p-tau, phosphorylated tau.

Notes: Data are shown as mean (SD) if not otherwise specified.

*Including 107 with SCD.

**Including 67 with SCD.

***Assays were updated after the analysis in Panel A+ (before the BioFINDER analysis). Values in Panel A+ and BioFINDER are therefore not comparable (see Table S3 for details on the assay modifications).

statistics.[9,40,42–45] The cutoffs to define Aβ positivity in Panel A+ was < 0.047 and in BioFINDER < 0.066 (note the pre-analytical differences between cohorts that may explain differences in cut-offs).[46]

The secondary outcome was progression to AD dementia (only BioFINDER) within 6 years. Additional secondary outcomes were (1) Aβ status (abnormal/normal) determined using the CSF p-tau181/Aβ42 ratio and (2) Aβ PET imaging using 18F-Flutemetamol. Further details of the outcomes are described in the supporting information. The CSF p-tau181/Aβ42 cutoffs for Aβ positivity were > 0.024 in Panel A+ and > 0.018 in BioFINDER (established using mixture modelling statistics in the present populations). For 18F-Flutemetamol PET, a previously published cut-off of > 0.53 standardized uptake value ratio (SUVR) was used to define Aβ positivity.[4]

2.4 | Statistical analysis

Aβ status or progression to AD dementia were used as the dependent variables and the plasma biomarkers were used as independent vari-

ables in logistic regression models. Stepwise additions of biomarkers are shown in Figures 1, 3, and 4, starting with the biomarker with highest area under the receiver operating characteristic (AUC) curve and then adding biomarkers based on the biomarker whose addition resulted in the largest decrease in Akaike information criterion (AIC). Accuracy was measured using the AUC and AUCs were compared using DeLong statistics. R version 4.0 was used for all statistical analyses. See supporting information for further description of the statistical analysis.

3 | RESULTS

3.1 | Participants and assay precision

The Panel A+ cohort included 227 participants of which 32 (14%) were CU, 106 (47%) had MCI, and 89 (39%) mild dementia. The BioFINDER-1 study included 693 participants of which 461 (67%) were CU and 232 (33%) had MCI. Baseline characteristics are shown Table 1 and Tables S5–S6 in supporting information. Inter- and intra-instrument coeffi-
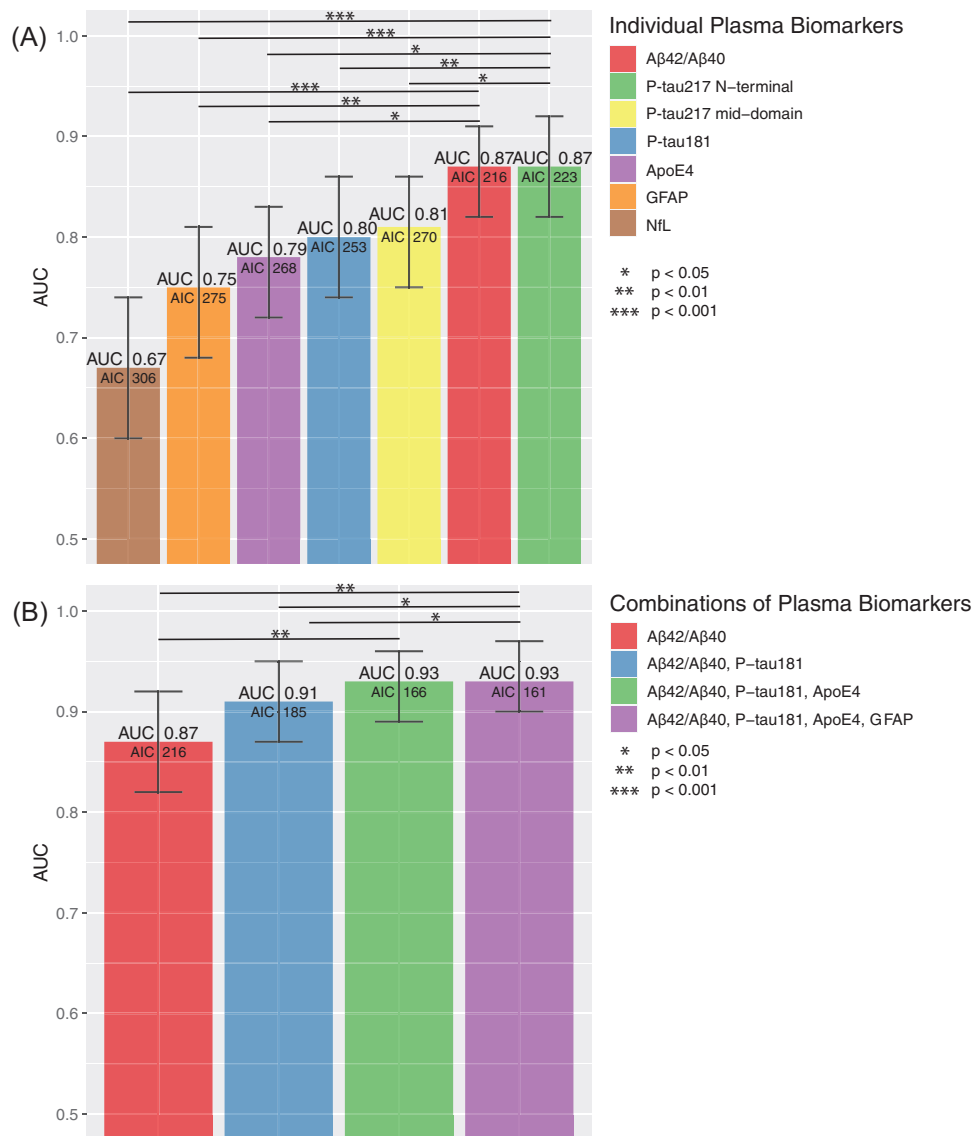
FIGURE 1 Discrimination of Aβ positive (*n* = 117) versus negative (*n* = 110) participants in Panel A+. ROC analysis of single (A) and combinations (B) of plasma biomarkers for discriminating Aβ positive versus Aβ negative participants. Bars show the AUC and whiskers the 95% CI of the AUC. Models (B) were built, starting with the biomarker with the highest AUC. Additional biomarkers were added step-wise based on how much the AIC was reduced. Biomarkers that did not reduce the AIC were not added. Statistical comparisons of AUCs between the two best models and the other models are shown in the figures. Note that using p-tau217 N-terminal instead of p-tau181 provided similar AUCs (ΔAUC < 0.01), see Table S9 in supporting information. Additional models and model comparisons are shown in Tables S7–S9 in supporting information. Aβ, amyloid beta (positivity defined by CSF Aβ42/Aβ40) AIC, Akaike information criterion; ApoE4, the E4 isoform of apolipoprotein E; AUC, area under the receiver operating characteristic curve; CI, confidence interval; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau217 mid-domain, phospho-tau217 using an antibody pair recognizing a mid-domain tau epitope; p-tau217 N-terminal, phospho-tau217 using an antibody pair recognizing an N-terminal tau epitope; ROC, receiver operating characteristic

cients of variation (CV) were generally below < 4.0% for all biomarkers (Tables S1 and S4).

3.2 | Identification of Aβ positivity in Panel A+

One hundred ten (48%) participants were Aβ positive in the whole sample. Boxplots and data points of the biomarkers are shown in Figures

S2–S3 in supporting information. Figure 1A,B shows the accuracy for estimating Aβ positivity and Tables S7–S9 in supporting information additional biomarker combinations as well as model comparisons. Plasma Aβ42/Aβ40 and plasma p-tau217 N-terminal, respectively, had the highest AUCs (both 0.87, 95% confidence interval [CI] 0.82 to 0.91 and 0.82 to 0.92, respectively). Adding plasma ApoE4 to another biomarker significantly improved its accuracy and these two biomarker combinations had AUCs of 0.86 to 0.91 except for the combination of
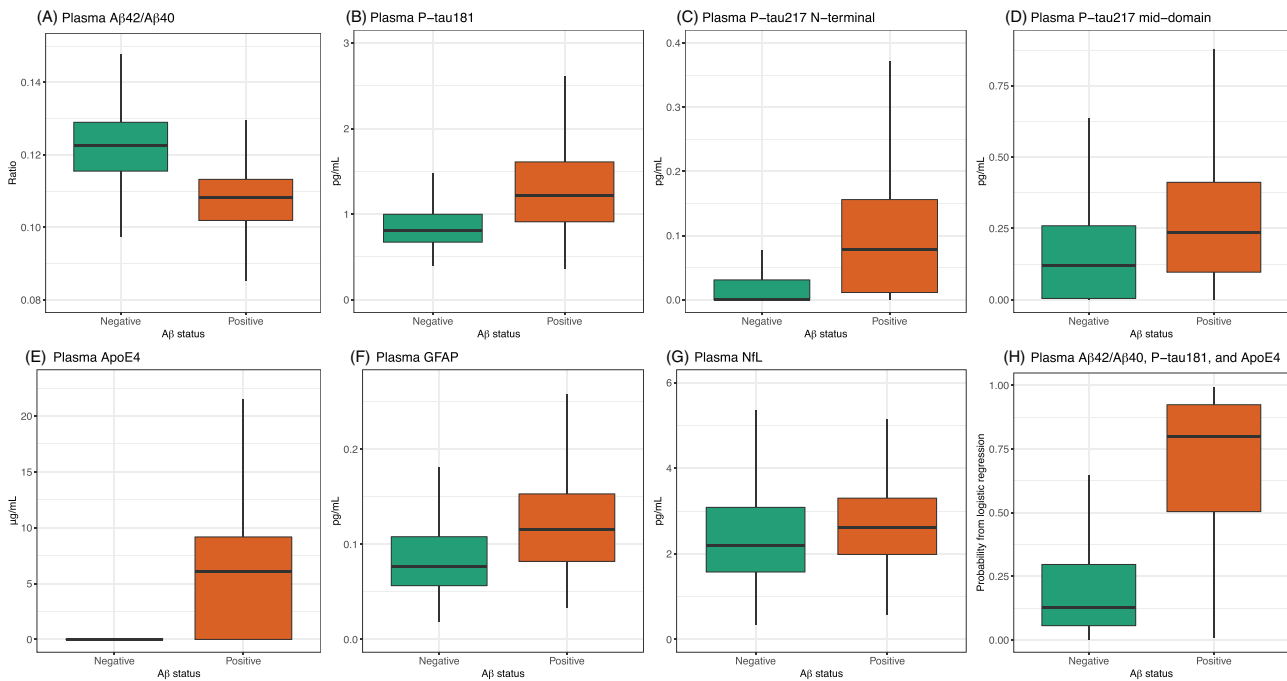
FIGURE 2 Boxplots of the plasma biomarker concentrations in BioFINDER grouped by Aβ status. (A), Aβ42/40, (B) p-tau181, (C) p-tau217 N-terminal, (D) p-tau217 mid-domain, (E) ApoE4, (F) GFAP, (G) NfL probability from a logistic regression model cerebrospinal fluid Aβ42/40, p-tau181, and ApoE4. Individual data points are shown in Figure S4. Corresponding data for Panel A+ are shown in Figure S2–S3 in supporting information. Aβ, amyloid beta (positivity defined by CSF Aβ42/Aβ40) ApoE4, the E4 isoform of apolipoprotein E; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; p-tau217 mid-domain, phospho-tau217 using an antibody pair recognizing a mid-domain tau epitope; p-tau217 N-terminal, phospho-tau217 using an antibody pair recognizing an N-terminal tau epitope

plasma ApoE4 and NfL (0.83; Table S8). Combining different biomarkers, the highest AUC was achieved using Aβ42/Aβ40, p-tau181, and ApoE (0.93, 95% CI 0.89 to 0.96; Figure 1B). Equally high AUC was achieved = using p-tau217 N-terminal instead of p-tau181 (Table S9). Adding GFAP to the model improved the AIC slightly (ΔAIC –5) but with similar AUC (0.93). Using plasma Aβ42 and Aβ40 as separate variables in the models instead of the Aβ42/Aβ40 ratio provided similar AUCs (data not shown). Adding age and/or sex to the models did not result in significantly higher AUCs (data not shown).

3.3 | Identification of Aβ positivity in BioFINDER

Two hundred ninety (42%) participants were Aβ positive in the whole sample, 146 (32%) in the CU group, and 144 (62%) in the MCI group. Boxplots of the biomarkers are shown in Figure 2A-H with individual data points in Figure S4A-H in supporting information. Figure 3 shows biomarker performance and the main combinations, while Tables S10–S16 in supporting information show additional models. Plasma Aβ42/Aβ40 had the highest AUC in the whole sample (0.83, 95% CI 0.80 to 0.86) and in separate CU (0.84, 95% CI 0.80 to 0.87) and MCI groups (0.81, 95% CI 0.75 to 0.87). Adding ApoE4 to another biomarker significantly improved its accuracy (Table S11). Similar to Panel A+, the combination of plasma Aβ42/Aβ40, p-tau181, and ApoE4 had a high AUC (0.90, 95% CI 0.88 to 0.93; Figure 3A). Adding p-tau217 N-terminal improved the model fit (ΔAIC –22), but with similar AUC (0.91,

95% CI 0.89 to 0.93; Figure 3D, Table S12). Results were similar in separate CU and MCI groups (Figures 3B, 3C, 2E, 2F, and Tables S13–S16). Adding age or sex to the models did not result in significantly higher AUCs (data not shown).

3.4 | Prediction of future AD dementia in BioFINDER

Of 342 CU participants who either converted to dementia within 6 years or were followed for ≥ 6 years, 29 (8%) progressed to AD dementia, 22 (6%) progressed to other dementias, and 292 (85%) did not progress to dementia. Plasma p-tau181 had the highest AUC for predicting progression to AD dementia (0.84, 95%CI 0.77 to 0.92; Figure 4A, Table S17 in supporting information). Combining p-tau181, p-tau217 mid-domain, and ApoE provided a non-significantly higher AUC (0.88, 95% CI 0.82 to 0.95; Figure 4C, Table S18 in supporting information).

Of 194 participants with MCI who either converted to dementia within 6 years or were followed for ≥ 6 years, 99 (51%) progressed to AD dementia, 66 (34%) progressed to other dementias, and 30 (15%) did not progress to dementia. Single biomarkers are presented in Table S19 in supporting information and Figure 4B and biomarker combinations in Table S20 in supporting information and Figure 4D. The combination of p-tau181, p-tau217 N-terminal, and Aβ42/Aβ40 had an AUC of 0.87 (0.82 to 0.92; Figure 4D). Combining five biomarkers pro-
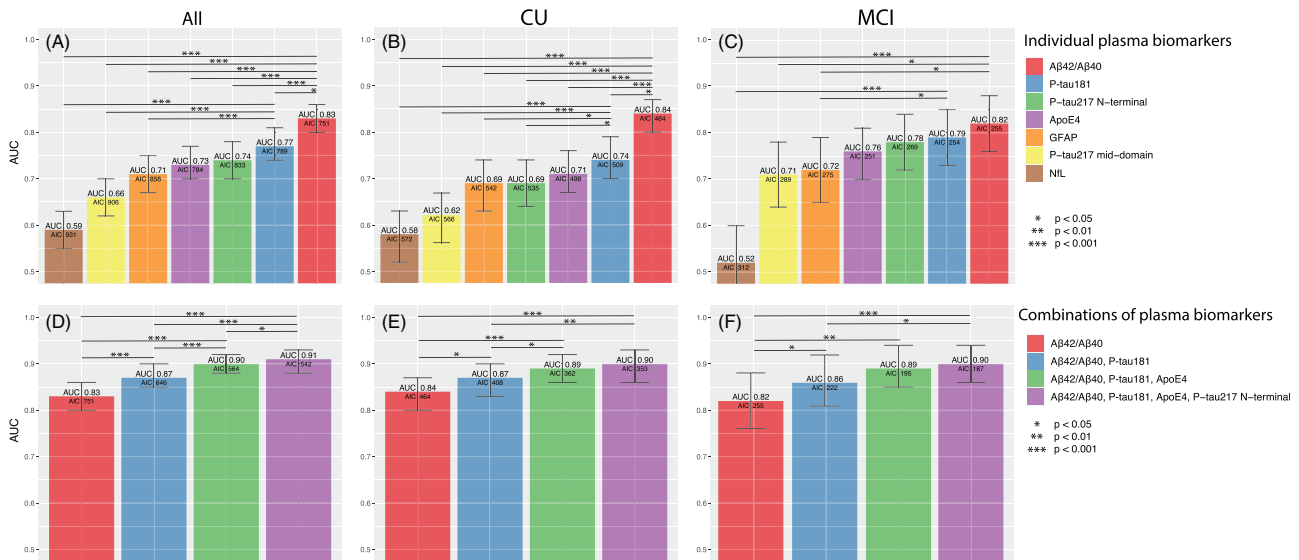
FIGURE 3 Discrimination of Aβ positive ($n = 403$) versus negative ($n = 290$) participants in BioFINDER ($n = 693$). ROC analysis from all participants (A, D), CU (B, E) and MCI (C, F) using single (A-C) and combinations (D-F) of plasma biomarkers. Bars show the AUC and whiskers the 95% CI of the AUC. Models were built, starting with the biomarker with the highest AUC. Additional biomarkers were added step-wise based on how much the AIC was reduced. Biomarkers that did not reduce the AIC were not added. Statistical comparisons of AUCs between the two best models and the other models are shown in the figures. Additional models and model comparisons are shown in Tables S10–S16 in supporting information. Aβ, amyloid beta (positivity defined by cerebrospinal fluid Aβ42/Aβ40) AIC, Akaike information criterion; ApoE4, the E4 isoform of apolipoprotein E; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CU, cognitively unimpaired; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; NfL, neurofilament light; p-tau217 mid-domain, phospho-tau217 using an antibody pair recognizing a mid-domain tau epitope; p-tau217 N-terminal, phospho-tau217 using an antibody pair recognizing an N-terminal tau epitope

vided the lowest AIC, but did not significantly improve the AUC (0.89, 95% CI 0.85 to 0.93; $P = 0.10$; Figure 4D, Table S20).

3.5 | Secondary analyses

3.5.1 | Correlation between plasma and CSF biomarkers

Significant correlations among the biomarkers in BioFINDER are visualized in Figure 5A (correlation coefficients in Figure S5 in supporting information) and for Panel A+ in Figure S6A, S6B in supporting information. In BioFINDER, the Spearman rho between the corresponding plasma and CSF biomarkers were 0.63 for NfL, 0.54 for p-tau181, 0.54 for GFAP, and 0.40 for Aβ42/Aβ40. In Panel A+ it was 0.64 for Aβ42/Aβ40 and 0.52 for p-tau181. Note that Elecsys CSF NfL and GFAP were not available in Panel A+ and CSF p-tau217 was not available in either cohort.

3.5.2 | Plasma ApoE4 concentrations and APOE genotype

As shown in Figure 5B (BioFINDER) and Figure S6C, plasma ApoE4 levels were only increased in APOE ε4 carriers. Dichotomized plasma ApoE4 concentration separated APOE ε4 carriers versus non-carriers perfectly, but with a partial overlap between one and two ε4 alleles. As

predictor of Aβ status in Panel A+, number of ε4 alleles had an AUC of 0.80, which was non-significantly different from binarized plasma ApoE4 (0.78; $P = 0.61$). In the larger BioFINDER cohort, a similar AUC difference was significant (number of ε4 alleles, 0.75; binarized plasma ApoE4, 0.73; $P < 0.001$).

3.5.3 | Head-to-head comparison of the updated and older plasma Aβ42/Aβ40 ratios

In Panel A+, the plasma Aβ42/Aβ40 ratio using the updated Aβ42 immunoassay had a significantly higher AUC (0.87, 95% CI 0.82 to 0.92) compared to the Aβ42/Aβ40 ratio using the older Aβ42 immunoassay (0.80, 95% CI 0.74 to 0.86; $P = 0.0086$) for discriminating Aβ-positive versus -negative participants. In BioFINDER ($n = 668$ in this comparison), the new Aβ42/Aβ40 ratio (which was further improved compared to the updated immunoassays in Panel A+; see Table S3) also had a significantly higher AUC (0.83, 95% 0.80 to 0.86) compared to the earlier Aβ42/Aβ40 ratio (AUC 0.79, 95% CI 0.75 to 0.82; $P = 0.026$).

3.5.4 | Results using CSF p-tau181/Aβ42 and Aβ PET as outcomes

Using CSF p-tau181/Aβ42 as outcome instead of CSF Aβ42/Aβ40 in Panel A+ and BioFINDER provided similar accuracies for all plasma biomarkers (Tables S21–S24 in supporting information). Using Aβ PET
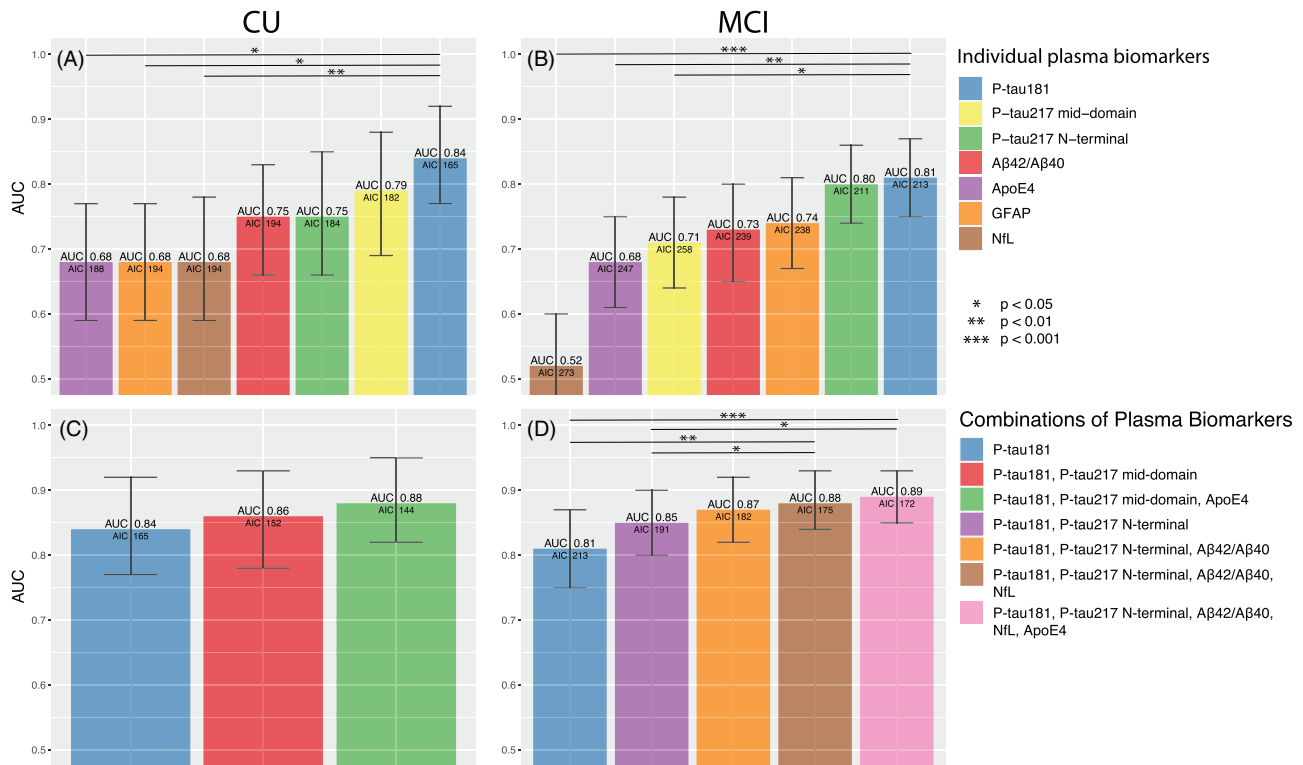
FIGURE 4 Prediction of development of AD dementia within 6 years in BioFINDER. ROC analysis from participants that were CU (A, C) or had MCI (B, D) at baseline using single (A-B) and combinations (C-D) of plasma biomarkers. Bars show the AUC and whiskers the 95% CI of the AUC. Models were built, starting with the one with the highest AUC. Additional biomarkers were added step-wise based on how much the AIC was reduced. Biomarkers that did not reduce the AIC were not added. Statistical comparisons of AUCs between the two best models and the other models are shown in the figures. Additional models and model comparisons are shown in Tables S17–S20 in supporting information (including all steps in panel D). Aβ, amyloid beta (positivity defined by cerebrospinal fluid Aβ42/Aβ40); AIC, Akaike information criterion; ApoE4, the E4 isoform of apolipoprotein E; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CU, cognitively unimpaired; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; NfL, neurofilament light; p-tau217 mid-domain, phospho-tau217 using an antibody pair recognizing a mid-domain tau epitope; p-tau217 N-terminal, phospho-tau217 using an antibody pair recognizing an N-terminal tau epitope

as outcome (available in 461 BioFINDER participants) also provided similar results (Tables S25–S26 in supporting information).

3.5.5 | Comparison of plasma p-tau/total tau ratios in BioFINDER

No significant improvements in AUCs were seen when using plasma p-tau/total tau ratios for identifying Aβ positivity (Table S27 in supporting information).

4 | DISCUSSION

Using the Elecsys prototype immunoassays on fully automated instruments, the combination of plasma Aβ42/Aβ40, p-tau181, and ApoE4 had high accuracy in two independent cohorts for identifying Aβ positivity (AUCs 0.90 to 0.93), with similar results in CU and MCI subgroups and when using alternative outcomes (CSF p-tau181/Aβ42 or Aβ PET). A combination of three biomarkers could predict progression to AD dementia within 6 years with AUCs of 0.88 (among CU) and

0.87 (among MCI; Figure 4). Measuring plasma ApoE4 concentration perfectly separated *APOE* ε4 carriers (identified by genotyping) from non-carriers (Figure 5B, and Figure S6C). Finally, the updated plasma Aβ42/Aβ40 assays had significantly better accuracy for detecting cerebral Aβ compared to the previous versions.

The greatest novelty of the present study lies in the transformation of all key AD biomarkers onto a fully automated platform (Elecsys immunoassays on a cobas e analyzer). This platform has been well validated for CSF AD biomarkers in several large cohorts and has been implemented for use in routine clinical practice worldwide.[33,49–51] When running daily analyses in routine clinical practice, stable measurements over time, and between instruments and laboratories, are important. This was demonstrated by the low CVs (< 4.5%) for all plasma biomarkers (Tables S1 and S4) and in line with previous CSF biomarker results for the Elecsys immunoassays.[33] For plasma analyses, however, there is only one previous study examining Aβ42 and Aβ40 Elecsys assays.[9] In the present study, we showed that the updated Elecsys Aβ42/Aβ40 assays had higher accuracy for Aβ positivity with precise/robust measurements (CVs of 0.4% to 2.9%; see Table S1), and potentially could be classified as a clinical grade assay. The large overlap between the Aβ positive and negative groups
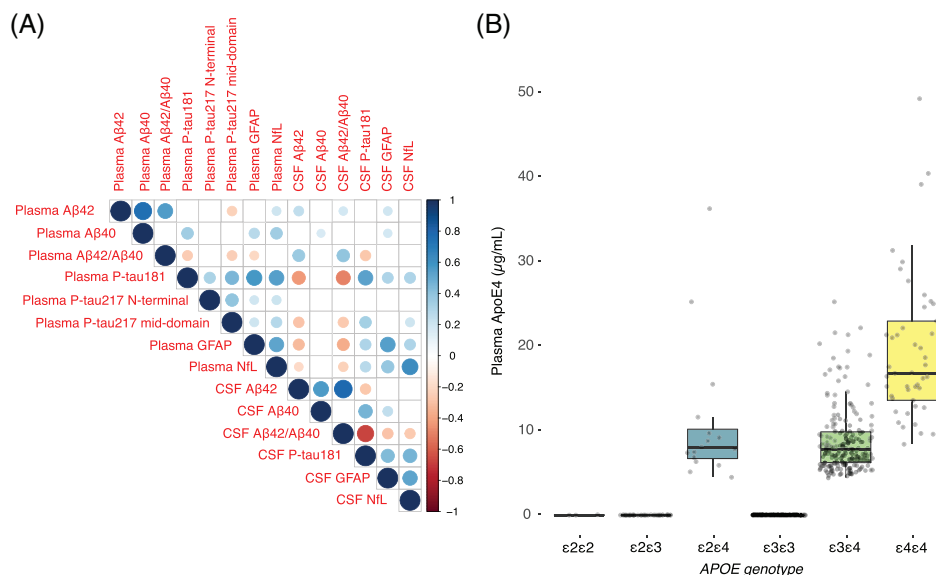
FIGURE 5 Associations between plasma and CSF biomarkers and between plasma ApoE4 and *APOE* genotype in BioFINDER. A, Spearman correlation matrix of the association between plasma and CSF biomarkers. Size and color of the circles indicates the Spearman rho (according to the scale on the right side). Blank boxes are non-significant correlations. Exact Spearman rho values are shown in Figure S5 in supporting information. B, Boxplots of plasma ApoE4 levels by *APOE* genotype. Box ends denotes the 25th and 75th percentile and the horizontal line the median. Whiskers extend to the upper and lower adjacent values within 1.5 × interquartile range of the 25th and 75th percentiles. Black dots indicated values above/below the whiskers. In addition, shaded gray dots show all individual participants. One participant was missing *APOE* genotyping and was excluded. One outlier with *APOE* ε4/ε4 genotype and plasma ApoE4 concentration of 117.7 µg/ml is not shown (to improve visualization), but that participant is included in the calculation of the median, box and whiskers. Corresponding data for Panel A+ is shown in Figure S6 in supporting information. Aβ, amyloid beta (positivity defined by CSF Aβ42/Aβ40) AIC, Akaike information criterion; ApoE4, the E4 isoform of apolipoprotein E; AUC, area under the receiver operating characteristic curve; CI, confidence interval; CSF, cerebrospinal fluid; CU, cognitively unimpaired; GFAP, glial fibrillary acidic protein; MCI, mild cognitive impairment; NfL, neurofilament light; p-tau217 mid-domain, phospho-tau217 using an antibody pair recognizing a mid-domain tau epitope; p-tau217 N-terminal, phospho-tau217 using an antibody pair recognizing an N-terminal tau epitope

(Figure 2A) could, however, become an issue when applying a predefined cutoff and measuring samples in daily/weekly batches over time if, for example, calibration errors occur. One way of overcoming this might be to use a combination of biomarkers that more clearly separate the groups and is less sensitive to errors in a single biomarker assay (Figure 2H).

A novelty of the plasma biomarker panel was to measure plasma ApoE4 protein concentrations, which perfectly separated *APOE* ε4 carriers from non-carriers (Figure 5B and Figure S6C). In terms of estimating Aβ positivity, ApoE4 provides similar information as *APOE* genotyping, but it may facilitate test logistics and implementation. Instead of sending a separate blood sample for *APOE* genotyping, the present approach allows for an analysis of all essential biomarkers at the same laboratory and on the same instrument from the same sample. In addition to being a risk marker for future and present Aβ pathology, ApoE4 plays an important role in the risk/benefit assessment before starting anti-Aβ treatment.[20] Note, however, that plasma ApoE4 did not perfectly separate carriers of one versus two ε4 alleles and in instances in which that needs to be specified, traditional *APOE* genotyping must still be performed.

Regarding p-tau, we compared one p-tau181 and two p-tau217 assays (a mid-domain antibody and an N-terminal antibody). Compared to using p-tau217 mid-domain on other platforms such as the

Meso Scale Discovery (MSD),[2] the present accuracies were lower and suggest that further optimization is needed. The p-tau217 N-terminal had higher accuracies for Aβ positivity than the mid-domain assay (Figures 1A and 3A–3C; Tables S7, S10, S13, and S15), but on the other hand had a much higher number of participants below the detection limit (discussed in the Limitations section). For plasma p-tau181, there were no participants below detection limit and its performance both as a single biomarker and in combination with Aβ42/Aβ40 and ApoE4 was overall similar to p-tau217. This highlights that although p-tau217 has performed better in previous studies for identifying AD pathology,[2,4,52–55] the type of analysis platform might be just as important[56,57] and for the present platform p-tau181 seems to more suitable than p-tau217.

Combining Aβ42/Aβ40, ApoE4, and p-tau181, high AUCs were achieved in both cohorts (AUC 0.90 to 0.93), and in separate populations of CU (AUC 0.89) and MCI (AUC 0.89) participants (Figures 1 and 3). Although adding additional biomarkers such as GFAP improved model fit (important from the aspect of robust measurements over time and between populations), it did not consistently increase the accuracy for Aβ positivity further (Figure 1B, Figure 3E, 3F; Tables S9, S12, S14, and S16).

For predicting development of future AD dementia in BioFINDER, other biomarker combinations had the best performances (Figure 4C,

4D; Tables S18 and S20). Similar to previous studies,[2,27] plasma p-tau biomarkers performed best as single biomarkers both in the CU and MCI groups (Figure 4A, 4B). When identifying the optimal combination of biomarkers, both p-tau181 and p-tau217 were selected and provided independent information about progression to AD dementia (Figure 4C, 4D).

When using a combination of biomarkers to estimate cerebral Aβ or progression to AD dementia, the individual outcome is a probability (for Aβ positivity or AD) from 0 to 1 (Figure 2H). Instead of defining individual biomarker cutoffs, the cutoff from a combination (i.e., logistic regression model) would naturally be > 0.5 (> 50% probability of Aβ positivity). Alternatively, more information from the biomarker results could be used if results are stratified (low, intermediate, high probability) or even on a continuous scale with the probability of Aβ positivity ranging from 0% to 100% as implemented in recently published biomarker algorithms.[22,29]

The main limitation of the present study was that measured plasma p-tau217 N-terminal and mid-domain concentrations were in many cases below the lower level of detection (Table S4). For p-tau217 N-terminal, 39% had to be assigned the calculation minimum level and for p-tau217 mid-domain, 18% (Table S4). However, > 90% of Aβ positive participants had levels above the calculation limit for p-tau217 mid-domain and > 80% for p-tau217 N-terminal. This indicates that these unmeasurably low levels to a large extent represent true low concentrations and being able to measure lower concentration might not change the accuracy noticeably. Nonetheless, this issue highlights that further assay optimization is needed and this is also why we advocate the use of p-tau181 on this platform instead. As for future novel biomarkers, it would be interesting to examine other both shorter and longer Aβ peptide ratios such as Aβ37/Aβ42, as recently shown to be promising.[58]

In summary, we found that using a fully automated instrument, a combination of three biomarkers identified Aβ positivity in two independent cohorts with high accuracy and predicted development of future AD dementia. The findings could be important in the implementation of blood-based biomarkers for AD diagnostics, recruitment in AD trials, and monitoring anti-Aβ therapies because of the high precision (intra- and inter-instrument CV) in combination with a high accuracy. Important steps in a clinical implementation process are to validate the assays' accuracy and robustness when analyzing samples over longer periods of time using predefined cut-offs and to examine the performance in more diverse populations.

CONFLICTS OF INTEREST

S.P. has served on scientific advisory boards and/or given lectures in symposia sponsored by F. Hoffmann-La Roche, Biogen, and Geras Solutions. C.A.F.vA. has served at scientific advisory boards for Biogen, Roche, and Dr Willmar Schwabe GmbH & Co. KG and has received funding for travel and speaker honoraria from Biogen, Roche Diagnostics AG, and Dr Willmar Schwabe GmbH & Co. KG and has received research support from Roche Diagnostics AG. H.Z. has served on scientific advisory boards and/or as a consultant for Abbvie, Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies, CogRx, and Red Abbey Labs; has given lectures in symposia sponsored by Cellectricon, 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. K.B. has served as a consultant or on advisory boards for Abcam, Axon, Biogen, Lilly, MagQu, Novartis, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. T.B. is a full-time employee of F. Hoffmann-La Roche and owns stock in F. Hoffmann-La Roche. I.S. is a full-time employee of Roche Diagnostics International LtD and owns stock in F. Hoffmann-La Roche. A.J., E.M., G.K., and U.E. are full-time employees of Roche Diagnostics GmbH. The other authors report no conflicts of interest. O.H. 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 Roche, Genentech, Siemens, Biogen, Alzpath, and Cerveau. Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

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