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FEATURED ARTICLE

Comparative analytical performance of multiple plasma Aβ42 and Aβ40 assays and their ability to predict positron emission tomography amyloid positivity

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Abstract

Introduction: This report details the approach taken to providing a dataset allowing for analyses on the performance of recently developed assays of amyloid beta (Aβ) peptides in plasma and the extent to which they improve the prediction of amyloid positivity.

*Alzheimer's Disease Neuroimaging Initiative (ADNI): Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

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Methods: Alzheimer's Disease Neuroimaging Initiative plasma samples with corresponding amyloid positron emission tomography (PET) data were run on six plasma Aβ assays. Statistical tests were performed to determine whether the plasma Aβ measures significantly improved the area under the receiver operating characteristic curve for predicting amyloid PET status compared to age and apolipoprotein E (*APOE*) genotype.

Results: The age and *APOE* genotype model predicted amyloid status with an area under the curve (AUC) of 0.75. Three assays improved AUCs to 0.81, 0.81, and 0.84 ($P < .05$, uncorrected for multiple comparisons).

Discussion: Measurement of Aβ in plasma contributes to addressing the amyloid component of the ATN (amyloid/tau/neurodegeneration) framework and could be a first step before or in place of a PET or cerebrospinal fluid screening study.

KEYWORDS
Alzheimer's disease, Alzheimer's Disease Neuroimaging Initiative, amyloid, amyloid beta 40, amyloid beta 42, amyloid positron emission tomography, amyloid prediction, biomarkers, plasma

Highlights

- The Foundation of the National Institutes of Health Biomarkers Consortium evaluated six plasma amyloid beta (Aβ) assays using Alzheimer's Disease Neuroimaging Initiative samples.
- Three assays improved prediction of amyloid status over age and apolipoprotein E (*APOE*) genotype.
- Plasma Aβ42/40 predicted amyloid positron emission tomography status better than Aβ42 or Aβ40 alone.

1 | BACKGROUND

The introduction of the ATN (amyloid/tau/neurodegeneration) classification of Alzheimer's disease (AD), which requires positron emission tomography (PET) imaging or cerebrospinal fluid (CSF) analyte measures, has major implications for how one characterizes individuals who are enrolled in clinical trials. It now appears that a new generation of assays of plasma amyloid beta (Aβ) 42 and 40 (Aβ42 and Aβ40) will, at a minimum, provide measures that can serve as a screen for more costly and burdensome tests. To make informed decisions on the potential and limitations of plasma measures as an index of brain state, stakeholders from academia, industry, and advocacy groups have worked together under the aegis of the Biomarkers Consortium of the Foundation of the National Institutes of Health (FNIH) to design and implement a study which will serve that purpose. Others have made significant contributions to our understanding of how different blood assays perform by having various repository samples analyzed across a range of available assays leading, in one case, to a conclusion that mass spectrometric assays consistently outperform immunoassays.[1] Previous experience in the immunoassay field indicates that precision and reproducibility often improve over time when automation, consistency of reagents, and other variables are addressed to generate assays that perform with the robustness needed for making the best decisions with regard to characterizing individuals either to enter into a trial or to be treated with a known therapeutic agent.

The purpose of the present study developed by the FNIH Biomarkers Consortium project team was to evaluate the performance of the most promising current plasma Aβ assays available in increasing the prediction of whether an individual is amyloid PET positive beyond the level of prediction possible using age and apolipoprotein E (*APOE*) genotype. The study involved a formal assay selection process to identify three mass spectrometric and three immunoassays that best met criteria set by scientists responsible for assay selection for clinical trials. Plasma samples were provided by the Alzheimer's Disease Neuroimaging Initiative (ADNI) repository of biofluids from participants who had been characterized in terms of ATN status. Furthermore, open access to all data generated is required as soon as unblinded and uploaded in all ADNI studies. A balanced sample size of those that were amyloid PET positive and amyloid PET negative was prospectively identified as well as a prespecified analytic plan of the type typically required in industry to support decisions about whether to rely on a method. The results of this first study will be used to prioritize assays for inclusion in a larger, more extensive study.

RESEARCH IN CONTEXT

1. **Systematic Review**: The accuracy of different plasma amyloid beta (Aβ)42/40 assays in predicting amyloid positron emission tomography (PET) status was examined. Papers reporting the predictive power of plasma Aβ42/40 ratios for brain amyloid "positivity" vary, leaving open the question of the extent to which one can be confident in the prediction.
2. **Interpretation**: This study examined the performance of six different plasma Aβ42/40 assays using samples collected from individuals who had undergone amyloid PET with florbetapir as part of the Alzheimer's Disease Neuroimaging Initiative (ADNI). Although none of the six plasma Aβ42/40 assays reached the prespecified goal, three of the assays performed significantly better than age and apolipoprotein E (*APOE*) genotype. This suggests that some plasma Aβ42/40 assays predict amyloid status, but not as accurately as may be required for some applications.
3. **Future Directions**: A follow-up study is underway that will add measures of plasma tau to determine whether combining plasma biomarkers may improve prediction of amyloid PET status. If methods are found that enable highly accurate prediction of amyloid PET status, plasma biomarkers may become a standard part of most future clinical trials.

2 | METHODS

The experimental design compared the ability of plasma assays to predict binary amyloid PET status and also compared statistical measures of assay reliability. Each assay measured plasma biomarkers in the same set of samples. Three immunoassays and three mass spectrometry assays were evaluated and compared for diagnostic accuracy and technical performance (e.g., reproducibility and bias).

2.1 | Selection of assays

The project team was agnostic on the technology used to quantify plasma Aβ42 and Aβ40 peptides and based selection decisions on assay characteristics such as sample volume, analytical throughput, assay range (limits of quantification), dilution linearity, accuracy, intermediate precision, testing controls, and parallelism. Fifteen assay developers submitted validation criteria that were rated by members of the project team. A mean score was then generated to rank the assays and six were selected. Ultimately, the team narrowed the focus to three ligand binding and three mass spectrometry-based methods that were judged to be the most promising for further evaluation.

2.2 | Blinding process

All project team members were blinded to the participant IDs in ADNI. Thus, experimental data were not linked to demographic, clinical, or other biomarker data for the participants until after the experimental data were uploaded to the ADNI website.

2.3 | Sample selection

Plasma samples from the ADNI cohort, equally split between amyloid positive and negative status as determined by PET (positivity defined as standardized uptake value ratio [SUVR] >1.1,[2–4]) were used to measure amyloid using three ligand binding and three mass spectrometry-based assays. The 130 participants were block randomized from a pool of ADNI subjects that met the following criteria:

- PET scans using florbetapir (FBP) tracer within 90 days of blood draw
- Diagnosis at time of blood draw: cognitively normal, mild cognitive impairment, and AD dementia
- Eleven or more available aliquots
- Plasma samples drawn after January 1, 2016
- Plasma samples that were prepared and frozen within 90 minutes of collection

2.4 | Sample size

Sample size was determined using a receiver operating characteristic (ROC) power analysis[5] with the following assumptions:

- Reference model (age and *APOE* genotype): area under the receiver operating characteristic (AUROC) curve for predicting amyloid PET status taken to have an area under the curve (AUC) = 0.75.[6]
- Assay model (Age, *APOE* genotype and assay readout): AUC = 0.90, which constitutes an improvement of 0.15 compared to the reference model.
- Correlation between model classifications across case and control samples of 0.2.
- One-sided alpha of 0.05.

With the above parameters, and 64 samples from each of the case and control groups, the study was powered at 82% to detect an improvement in AUROC from the reference model. Based on these calculations, 130 samples were selected for plasma Aβ42 and Aβ40 in the round-robin study.

After unblinding of the dataset, it was found that the sample set included nine duplicate ADNI participants (i.e., nine pairs of repeated measures) and six instances in which the differences between blood draw and amyloid PET scan were greater than 90 days (98–349 days elapsed between blood draw and FBP scan). Sensitivity analysis showed that excluding the duplicate participants and the participants

whose blood was collected outside the 90-day window did not result in a significant change in the AUROC curve for predicting amyloid PET status. Consequently, one time point for each duplicate subject was removed from the analysis, resulting in a dataset of 121 samples from unique participants.

2.5 | Project sample preanalytical processing

Plasma samples were collected in 10 mL K2-EDTA (purple top) tubes and centrifuged within 1 hour of collection at room temperature in a clinical centrifuge at 1300 g for 10 minutes. Plasma samples were transferred to 13 mL Sarstedt polypropylene transfer tubes and then frozen on dry ice at each ADNI center followed by overnight shipment on dry ice to the ADNI Biobank at the University of Pennsylvania. Aliquoting was performed, after thawing at room temperature, into 0.5 mL polypropylene tubes, and samples were frozen and stored at −80°C. The project samples were shipped overnight on dry ice to the assay providers. Post-shipping processing involved no more than two freeze–thaw cycles and no additives. This procedure follows the ADNI procedure manual for plasma sample preparation and is consistent with best practice guidelines.[7]

2.6 | Plasma endogenous quality control samples

Pooled AD-confirmed control and healthy control plasma samples are used in this project as endogenous quality controls (eQC). AD-confirmed samples were sourced from the University of Pennsylvania/ADNI biorepository and healthy control plasma eQC samples were provided by BioIVT. AD-confirmed eQC samples were selected from individuals whose corresponding CSF values for phosphorylated tau (p-tau)181 and Aβ42 were abnormal (>24 pg/mL for p-tau181 and <980 pg/mL for Aβ42). Healthy control samples were prepared from young individuals. Healthy control aliquots and separately the p-tau and Aβ positive confirmed plasma aliquots were thawed at room temperature, transferred to a Nalgene 2006-0002 (60 mL), narrow-mouth polypropylene (PP) bottle, and gently mixed. From each of these two pools, aliquots were prepared according to the assay volume requirements (250–500 μL). Aliquots were stored at −80°C in micro packaging skirted vials with conical bottom (sterile PPCO, 0.5 mL, Nalgene Cat: 342800-0005) used with MPV closures (low profile, blue sterile PPCO, 11 mm, Nalgene Cat: 342821-0116). Additional eQC data are included in the supporting information.

2.7 | Statistical methods

For each assay readout, logistic regression was used to model FBP PET-derived amyloid status as a function of plasma Aβ 42/40, age, and *APOE* genotype. Amyloid positivity for the reference model was defined by a cut-off of SUVR ≥1.11 for FBP PET scans.[2–4] In addition, a reduced (reference) model was created using age and *APOE* genotype, alone. For each assay, the AUROC for plasma Aβ 42/40, age, and *APOE* genotype for predicting amyloid PET status was compared to the AUROC of the reference model. The difference between AUCs with 95% Wald confidence intervals were calculated for each assay's full model (plasma Aβ 42/40, age, *APOE* genotype) versus the reference model. AUC and 95% confidence interval (CI) were generated using the programming language R's pROC package[8] and nominal significance for the improvement in AUC between reduced and full model was assessed using the method described by DeLong et al.[9] Notably, all significance levels reported in this article are nominal, with no correction for multiple comparisons.

Spearman's rank was used to assess pairwise correlations between assay analytes and ratios and FBP PET SUVR to lessen the impact of outliers. Correlations and their nominal significance are reported to provide a more complete picture of the results. Statistical comparisons of correlation coefficients were not performed as the study was not powered for detecting such differences.

3 | RESULTS

3.1 | Ability of plasma Aβ 42/40 assays to predict FBP PET-based amyloid status

The cohort included cognitively normal individuals (mean age 77.23 + 7.57 years, $n = 49$), 54 individuals with mild cognitive impairment (mean age 78.0 years), and 18 individuals with AD dementia (mean age 79.9 years). Patients were of similar age between amyloid PET positive ($77.2 \pm 7.3, n = 60$) and amyloid PET negative ($78.7 \pm 6.9, n = 61$) groups (Table 1).

Plasma Aβ42 and Aβ40 were measured in the same 121 samples from each unique participant by all six assays. A model of amyloid PET status as predicted by age and *APOE* genotype was used for reference and had an AUC of 0.75 (95% CIs of 0.663 and 0.836). For each assay, the AUROC for plasma Aβ42/40, age, and *APOE* genotype for predicting amyloid PET status was compared to the reference model (Table 2). The prespecified goal was for the plasma Aβ42/40 assays to increase the AUC for predicting amyloid PET status by 0.15 or more compared to age and *APOE* genotype alone. None of the assays improved the AUC by the 0.15 threshold, which was the amount for which this study was powered. Nonetheless, three of the assays improved on the reference model AUC, and these increases were nominally significant ($P < .05$, uncorrected for multiple comparisons). Washington University had an AUC of 0.842 (0.770, 0.913), $P = .007$; Roche had an AUC of 0.811 (0.735, 0.888), $P = .024$; Shimadzu had an AUC of 0.810 (0.734, 0.886), $P = .033$ (Table 2). The forest plot (Figure 1) shows AUC results across all models and assays in the entire cohort by diagnosis groups.

3.2 | Correlations between assay and FBP SUVR

Inter-assay correlations for Aβ40 analytes (Figure S1A in supporting information) were high, with overall pairwise Spearman rank

TABLE 1 Demographic and clinical characteristics of ADNI participants

Characteristics	PET negative ($n = 61$)		PET positive ($n = 60$)
Age (years)	77.2 ± 7.3		78.7 ± 6.9
Sex (*n*, % female)	26 (42.6%)		25 (41.7%)
APOE genotype			
Carrier	15 (24.6%)		34 (56.7%)
Non-carrier	46 (75.4%)		26 (43.3%)
Alternative: *APOE* genotype			
2/3	8 (13.1%)		4 (6.7%)
2/4	0		1 (1.7%)
3/3	38 (62.3%)		22 (36.7%)
3/4	14 (23.0%)		22 (36.7%)
4/4	1 (1.6%)		11 (18.3%)
Diagnosis			
Cognitively normal	31 (50.8%)		18 (30.0%)
Mild cognitive impairment	28 (45.9%)		26 (43.3%)
Dementia	2 (3.3%)		16 (26.7%)
CDR 0/0.5/1/2/3			
Missing CDR data	2		1
0	36		21
0.5	21		21
1	2		16
2	0		1
CDR sum of boxes	0.75 ± 1.38		2.44 ± 2.70
Race			
White	56 (91.8%)		58 (96.7%)
Black	2 (3.3%)		1 (1.7%)
Other	3 (4.9%)		1 (1.7%)
Years of education	16.6 ± 2.6		16.1 ± 2.9
Florbetapir PET SUVR	1.001 ± 0.063		1.347 ± 0.152
MMSE, median (IQR)	29 (28, 30)		27.5 (24, 29.5)
ADAS-Cog 13, median (IQR)	8.00 (5.33, 13.67)		15.84 (8.33, 25.67)
Plasma biomarkers			
Washington University ($n = 120$)	Aβ42 (pg/ml)	38.0 ± 5.3	35.4 ± 5.6
	Aβ40 (pg/ml)	286.0 ± 39.6	289.2 ± 45.4
	Aβ42/40	0.133 ± 0.010	0.123 ± 0.008
Shimadzu ($n = 121$)***	Aβ42 (arbitrary units)	0.336 ± 0.070	0.297 ± 0.059
	Aβ40 (arbitrary units)	8.118 ± 1.727	8.110 ± 1.661
	Aβ42/40	0.042 ± 0.007	0.037 ± 0.005
Roche ($n = 121$)	Aβ42 (pg/ml)	53.2 ± 10.8	47.4 ± 8.7
	Aβ40 (pg/ml)	316.8 ± 49.7	311.4 ± 54.7
	Aβ42/40	0.168 ± 0.022	0.153 ± 0.022
University of Gothenburg ($n = 116$)	Aβ42 (pg/ml)	20.7 ± 6.5	17.9 ± 6.8
	Aβ40 (pg/ml)	286.0 ± 55.3	278.7 ± 60.5
	Aβ42/40	0.072 ± 0.017	0.064 ± 0.023

(Continues)

TABLE 1 (Continued)

Characteristics	PET negative ($n = 61$)		PET positive ($n = 60$)
ADx NeuroSciences ($n = 121$)	Aβ42 (pg/ml)	7.886 ± 1.812	7.175 ± 1.383
	Aβ40 (pg/ml)	167.6 ± 46.8	169.1 ± 48.9
	Aβ42/40	0.049 ± 0.010	0.044 ± 0.007
Quanterix ($n = 121$)	Aβ42 (pg/ml)	3.425 ± 0.919	3.276 ± 0.680
	Aβ40 (pg/ml)	84.8 ± 18.5	87.1 ± 17.3
	Aβ42/40	0.040 ± 0.006	0.038 ± 0.004

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; ADAS-Cog 13, Alzheimer's Disease Assessment Scale cognitive subscale 13; *APOE*, apolipoprotein E; CDR, Clinical Dementia Rating; CN, cognitively normal; IQR, interquartile range; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, positron emission tomography; SD, standard deviation; SUVR, standardized uptake value ratio.
*MMSE scores were not available for 8 CN, 10 MCI, and 4 AD participants.
**ADAS-Cog 13 scores were not available for 8 CN, 10 MCI, and 4 AD participants.
***Results from the Shimadzu assay are reported as arbitrary units rather than concentrations in pg/mL.

TABLE 2 ROC analyses to discriminate amyloid PET positive from amyloid PET negative individuals and comparison of AUCs between the full model (Aβ42/40, age, *APOE* genotype) and the reference model (age, *APOE* genotype)

Assay provider	Assay	Model Reference: age, *APOE* genotype	AUROC [95% CI] 0.750 [0.663, 0.836]	Estimate of improvement on ref. AUROC	*P*-value vs. ref. model (one-sided)
Washington University at St. Louis	IP-MS	Plasma Aβ42/40, age, *APOE* genotype	0.842 [0.770, 0.913]	0.096	0.0067
		Plasma Aβ42/40	0.814 [0.736, 0.892]	0.069	0.10
Roche	Elecsys Cobas e601	Plasma Aβ42/40, age, *APOE* genotype	0.811 [0.735, 0.888]	0.061	0.024
		Plasma Aβ42/40	0.710 [0.617, 0.803]	−0.040	0.73
Shimadzu	IP MALDI-TOF-MS	Plasma Aβ42/40, age, *APOE* genotype	0.810 [0.734, 0.886]	0.060	0.033
		Plasma Aβ42/40	0.715 [0.625, 0.805]	−0.035	0.73
U. of Gothenburg	IP-MS	Plasma Aβ42/40, age, *APOE* genotype	0.781 [0.696, 0.867]	0.028	0.16
		Plasma Aβ42/40	0.643 [0.542, 0.743]	−0.111	0.95
ADx NeuroSciences	Simoa Neuro 4-plex E Kit (Amyblood)	Plasma Aβ42/40, age, *APOE* genotype	0.770 [0.686, 0.853]	0.02	0.21
		Plasma Aβ42/40	0.661 [0.563, 0.760]	−0.088	0.91
Quanterix	Simoa Aβ40 and Aβ42 Advantage Kit	Plasma Aβ 42/40, age, APOE genotype	0.766 [0.683, 0.849]	0.017	0.24
		Plasma Aβ 42/40	0.645 [0.545, 0.745]	−0.105	0.94

Note: Amyloid positivity was defined by a cut-off of standardized uptake value ratio (SUVR) ≥1.11 for Florbetapir PET scans.[2–4] The difference between AUCs and 95% Wald confidence intervals were calculated for each assay's full model (plasma Aβ 42/40, age, *APOE* genotype) versus the reference model (age, *APOE* genotype). *P*-values are for comparisons of AUCs (using DeLong's test[9] between the full model [Aβ 42/40, age, *APOE* genotype] and the reference model [Age, *APOE* genotype]).
Abbreviations: Aβ, amyloid beta; *APOE*, apolipoprotein E; AUC, area under the curve; AUROC, area under the receiver operating characteristic; CI, confidence interval; IP, immuno-precipitation; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MS, mass spectrometry; PET, positron emission tomography; ref, reference; ROC, receiver operating characteristic.

correlations between 0.74 and 0.93. Average correlation between each assay and other assays (row means of correlation matrix) were uniformly elevated between 0.82 and 0.90. Pairwise correlations for Aβ42 were moderate, ranging between 0.51 and 0.88 (Figure S1B). In contrast, inter-assay correlations of Aβ42/40 were markedly lower, with correlations ranging between −0.04 and 0.58 (Figure 2). Washington University and Shimadzu assays had the highest correlation (0.58, 0.45–0.69 95% CI) and the Roche and ADx NeuroSciences assays had the second highest correlation (0.56, 0.42–0.67 95% CI). The average correlation between each Aβ42/40 assay and other assays ranged between 0.36 and 0.52.

Plasma Aβ40 levels were not significantly correlated with FBP SUVR (Figure S1A). For Aβ42, weak negative correlations with FBP SUVR ranged between −0.16 and −0.29 across assays (Figure S1B). Weak to moderate Spearman correlations were found between Aβ42/40 and FBP SUVR, ranging from −0.24 to −0.53 for the Washington University

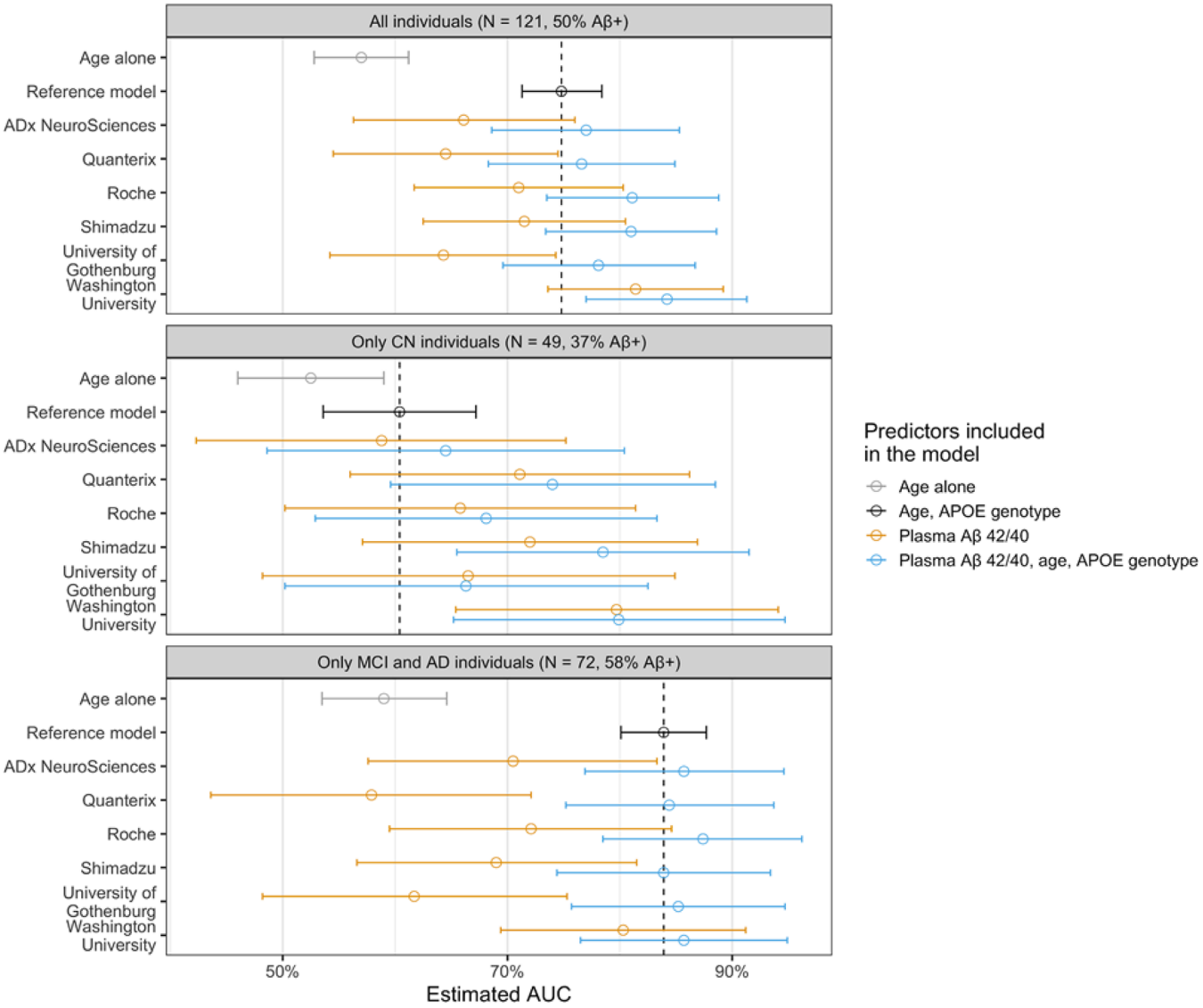

FIGURE 1 Prediction of amyloid positivity based on age alone, age and *APOE* genotype, and plasma Aβ42/40, and all predictors. Three datasets were analyzed: the full dataset of 121 individuals, data from CN individuals only, and data from MCI and AD individuals only. For each dataset, two logistic regression models were fit; one included plasma Aβ 42/40, age and *APOE* genotype as predictors, while the other model only included plasma Aβ 42/40. For each model, we estimated the AUC and 95% confidence interval using the method developed by DeLong et al.[9] Aβ, amyloid beta; AD, Alzheimer's disease; *APOE*, apolipoprotein E; AUC, area under the curve; CN, cognitively normal; MCI, mild cognitive impairment.

assay. With the exception of the Quanterix assay, these associations were weaker when assessed within either the amyloid positive or amyloid negative groups separately (Figure 2).

3.3 | Assay precision

In an effort to evaluate assay robustness, a pool of p-tau– and Aβ-positive plasma eQCs were generated from individuals whose corresponding CSF values for p-tau181 and Aβ42 were abnormal and a healthy control plasma eQC was generated from a pool of young, healthy donors. Healthy and p-tau181– and Aβ-positive plasma eQCs were analyzed in each of the analytical runs across each of the six assays and intra-assay precision values were calculated where appropriate. The healthy as well as the p-tau181– and Aβ-positive plasma eQC precision values were <15% for all assays and demonstrated acceptable precision as defined in the 2018 Food and Drug Administration Bioanalytical Method Validation guidance document.

4 | DISCUSSION

This study evaluated the ability of six different assays for plasma Aβ42/40 to improve the prediction of amyloid PET status compared to age and *APOE* genotype. Earlier analyses of ADNI data indicated that age and *APOE* ε4 carrier status yielded an AUROC of

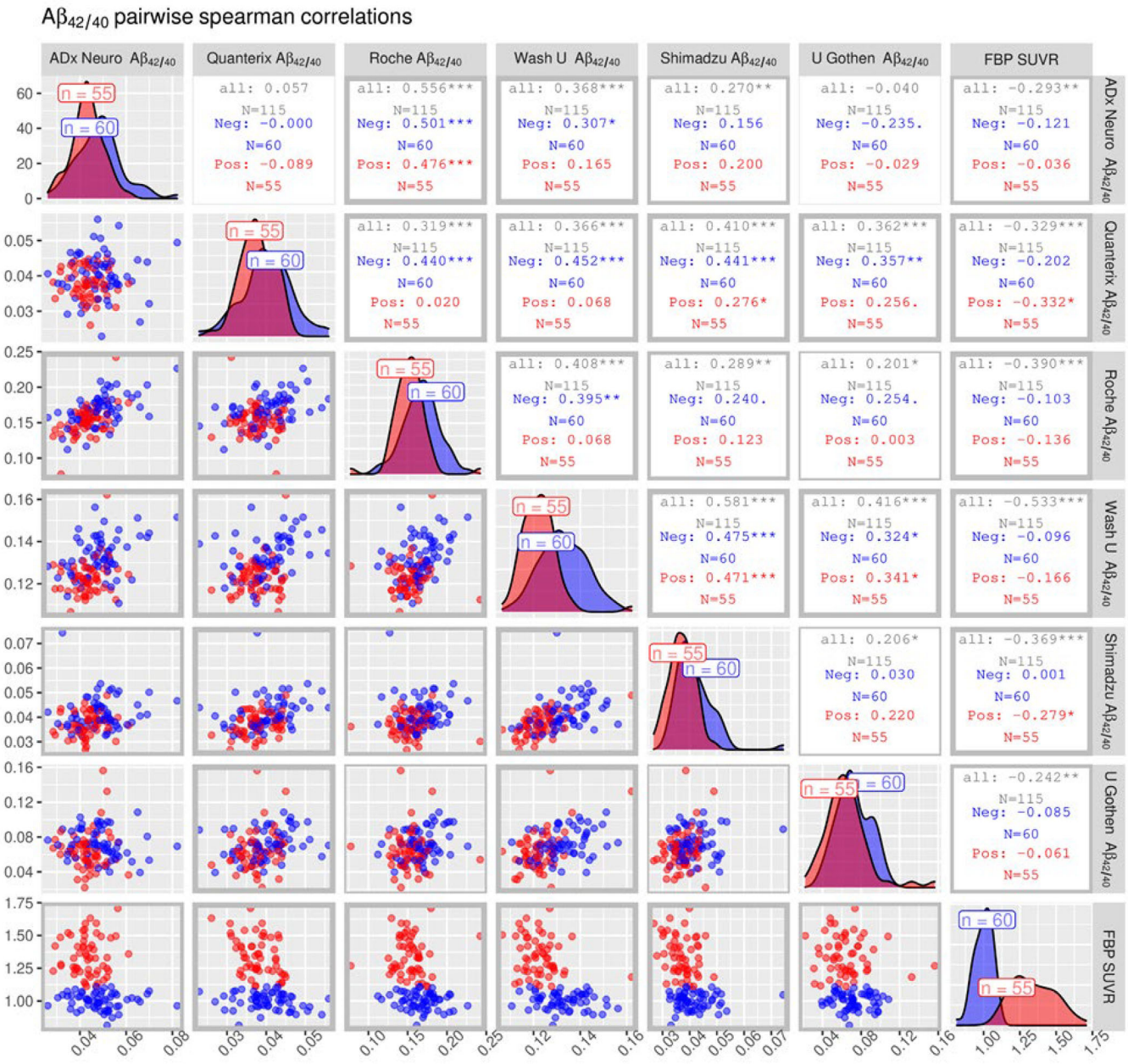

FIGURE 2 Matrix of pairwise scatterplots, distributions, and Spearman rank correlations for all six assays and FBP PET SUVR for Aβ42/40. The lower triangular portion of the matrix displays scatterplots of variable pairs, colored by amyloid status (blue for negative, red for positive). The diagonal contains smoothed analyte distributions, and the upper triangular portion displays corresponding Spearman rank correlations and the number of samples from unique subjects available across the entire cohort and by amyloid status. The small overlap of positive and negative FBP SUVR distributions is an artifact of histogram smoothing by the display function. Off diagonal cells with overall nominally significant ($P < .05$) associations are highlighted with a gray square and correlation values are suffixed with asterisks according to P-values: $^{***}P < .001$, $^{**}P < .01$, $^{*}P < .05$. Six failed measurements reported by assay vendors were excluded from the dataset ($N = 115$). Aβ, amyloid beta; FBP, florbetapir; PET, positron emission tomography; SUVR, standardized uptake value ratio.

approximately 0.75 for the prediction of amyloid PET status.[6] As honed by FNIH Biomarkers Consortium governance, the project was required to have a gate staging structure with a target criterion. Thus, an AUROC of 0.90 was prespecified as being sufficient for a prescreening tool that would identify potential participants for clinical trials. A total of 130 plasma samples from the ADNI repository from participants with matching amyloid PET data were selected to provide 82% power to detect an increase of 0.15 in the AUC. After implementing a prespecified analytic plan, we found that Aβ42/40 values from the six assays increased the AUC from 0.75 (based on age and *APOE* status) by 0.02 to 0.10, such that the AUROC for the highest performing assay was 0.84. While none of the assays reached the pre-specified threshold of AUC ≥ 0.90, three of the assays significantly improved the AUC from 0.75, with resulting AUROCs between 0.81 and 0.84. In secondary

analyses, the performance of the six plasma Aβ42/40 assays in predicting amyloid PET status (a dichotomous measure) was similar to the Spearman correlation of plasma Aβ42/40 with amyloid PET SUVR (a continuous measure). Additionally, the AUC of Aβ40 and Aβ42 individually with amyloid PET was evaluated, and for all six assays the ratio of Aβ42/40 performed better than Aβ42 or Aβ40 alone.

This study found lower AUCs of the plasma Aβ42/40 assays for prediction of amyloid status compared to some other studies of the same assays.[1,10–12] It is possible that the lower concordance may be at least partially related to the FBP PET reference standard used for this study. FBP may have higher variability than Pittsburgh compound B (PiB),[13] and previous studies have found higher concordance of plasma Aβ42/40 with PiB than other amyloid PET tracers.[10] Furthermore, a recent method comparison study that considered the current dataset found consistently higher AUCs for the plasma Aβ42/40 assays when CSF Aβ42/40 rather than amyloid PET was used as the reference standard, with the best performing plasma Aβ42/40 assay having an AUC of 0.86 with CSF Aβ42/40 and 0.83 with amyloid PET before consideration of age and *APOE* genotype.[1]

A recent method comparison study in the Swedish BioFINDER cohort, which also analyzed the current dataset, concluded that the mass spectrometry (MS)-based assays consistently outperformed immunoassays in predicting amyloid PET status.[1] However, the BioFINDER study used a different analytic approach than the prespecified one adhered to in the present study. The current study found that the Roche Diagnostics immunoassay also significantly improved on the prespecified analysis of predicting amyloid positivity, along with the Washington University liquid chromatography (LC)-MS/MS assay and the Shimadzu matrix-assisted laser desorption/ionization time-of-flight assay. In fact, the AUC increases and the confidence limits for the Roche and Shimadzu assays were almost identical. The results reported here therefore suggest that some immunoassay approaches may provide comparable performance for predicting PET amyloid positivity.

Assays that accurately measure biological characteristics are expected to have correlated results. An earlier plasma Aβ round-robin performed in 2018 in 10 participating centers using seven immunological assays and four mass-spectrometric methods on 81 plasma samples showed weak to moderate correlations across Aβ40 methods and weak correlations across Aβ42 methods.[14] The study published in 2021 discussed that different pre-analytical sample processing may be one of many potential reasons for discrepant measurements among the samples, underscoring the importance of refinement and development of reference methods and materials. Subsequently, there has been progress on the standardization of blood sample collection and handling,[7] and since the original publications, the assays have been refined by the independent groups who provided data in the study.

Spearman correlations between assays were high to moderate for Aβ42 (ρ = 0.51–0.88) and Aβ40 (ρ = 0.74 and 0.93), a substantial improvement over the earlier round-robin assay comparison[14] and evidence of how immunoassays improve over time. However, in earlier standardization projects, for example, for CSF Aβ40 and Aβ42, correlations above 0.90 were seen prior to formal standardization work.[15] Interestingly, correlations between assays for Aβ42/40 were weak to moderate (ρ = −0.04 and 0.58), and the highest performing assays correlated with each other more than lower performing assays. The reduction in inter-assay correlation for Aβ42/40, despite the improvement in the diagnostic performance of the ratio, is most likely due to the increased noise of the ratio relative to the limited noise in its constituent variables. In other words, the ratio likely reduces biological variation but amplifies analytical variation.

The biological basis for using the Aβ42 to Aβ40 ratio has been explored. One study estimated that 30% to 50% of plasma Aβ was transported from the brain through the blood—brain-barrier.[16] In a stable isotope labeling study of Aβ42 and Aβ40 kinetics reported by Ovod et al., differences in rates of clearance from plasma between Aβ40 and Aβ42 were noted in the presence of amyloid plaques.[11] Based on these blood Aβ kinetic findings, it was hypothesized that the ratio would better identify plaques in the brain compared to either analyte alone. This was confirmed in the report with measures of Aβ42/40 in blood, showing an AUC of 0.88 regardless of time of day. The inter-individual and intra-individual differences in concentration of blood plasma Aβ were controlled for by using the ratio, which mitigated diurnal and other changes in blood plasma concentrations.[17–20] These findings were further confirmed in two additional studies that were presented at the Alzheimer's Association International Conference in 2017.[21] Regardless of the underlying biological processes, the present study confirms that plasma Aβ42/40 consistently outperforms the individual analytes in detecting brain Aβ accumulation.

Although not the goal of this study, the question naturally arises whether amyloid blood measures could serve to make a clinical diagnosis of AD. It is possible that the addition of other blood measures may bring one closer to this goal, such as APP 699-711 provided by the Shimadzu platform.[10] The relevant data are available on the Laboratory of Neuroimaging website to calculate the contribution of secreted amyloid precursor protein to predictions. And, subsequent to the design of this study, there have been many reports on the ability of one or another species of p-tau in plasma or serum to predict brain amyloid positivity.[22] Future studies planned by the FNIH Biomarkers Consortium group in this study will explore how adding the p-tau (181, 217, 231 analytes) measures in plasma can improve the AUROCs of predicting amyloid positivity and increase to values above 0.90. Overall, these data support the idea that blood-based biomarkers may be used to pre-screen individuals for amyloid PET positivity prior to the more expensive, burdensome confirmatory PET procedure.

A major limitation of this study is the lack of diversity among samples selected from the ADNI cohort, and results may not be generalizable to under-represented populations in which little is known about blood biomarker performance. Evaluation of biomarker assays in diverse cohorts is critically needed to ensure that biomarkers perform consistently across racial and ethnic groups.

The main conclusion of this study is that assays that accurately quantify plasma Aβ40/42 can improve prediction of amyloid positivity compared to age and *APOE* alone. The assays differ when comparing the absolute values, suggesting a need for certified reference standards for Aβ42 and Aβ40. Because the goal of the FNIH Biomarkers

Consortium is to provide data that could be used by anyone interested in selecting amyloid positive participants for clinical trials, an accessible dataset has been provided that includes data from six different assays.

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CONFLICTS OF INTEREST

W.A. Horton, Y. Li, R. L. Martone, and E. G. Rosenbaugh have nothing to declare. S. Zicha, M. Baratta, I. Dobler, and D. Raunig receive salary and company stock as compensation for their employment with Takeda Pharmaceutical Company Limited. Washington University and Randall J. Bateman (RJB) have equity ownership interest in C2N Diagnostics and receive income based on technology (blood plasma assay) licensed by Washington University to Diagnostics. RJB receives income from C2N Diagnostics for serving on the scientific advisory board. Washington University, with RJB as co-inventor, has submitted the US nonprovisional patent application "Plasma Based Methods for Determining A-Beta Amyloidosis." RJB has received honoraria as a speaker/consultant/advisory board member from Amgen, Eisai, Hoffman-LaRoche, and Janssen; and reimbursement of travel expenses from Hoffman-La Roche and Janssen. L. M. Shaw receives research support from NIH/NIA U19 AG024904, ADNI3 grant; NIH/NIA P30 AG010124, UPENN ADCC grant; and the Michael J. Fox Foundation for Parkinson's Research. He is a consultant for Biogen and Roche Diagnostics and is on the speaker's bureaus for Biogen and Fujirebio. H. Zetterberg has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave; has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). W.Z. Potter was previously employed by the National Institute of Mental Health, and he is a stockholder in Merck & Co., Inc. He is a Co-Chair Emeritus for the FNIH Biomarkers Consortium

Neuroscience Steering Committee. Currently residing in Philadelphia, PA, he serves on DSMBs for AgeneBio and Regenacy and as a consultant for Karuna, Otsuka, Neurocrine, Eliem, and Emerald Lake Safety. Additionally, he receives grant support from the NIA. T. W. Bannon receives salary and company stock as compensation for his employment with AbbVie Inc. Y. Mordashova is employed by AbbVie Deutschland GmbH & Co. KG and may own AbbVie stock or stock options. Z. S. Saad is employed by Janssen Pharmaceuticals and may hold stock or stock options. S. Schindler has analyzed data from C2N Diagnostics that was provided to Washington University at no cost. Washington University has a financial interest in C2N Diagnostics. S. E. Schindler has not directly received any research or personal compensation from C2N Diagnostics. K. Ferber and C. E. Rubel receive salary and company stock as compensation for their employment with Biogen. J. G. Bollinger has a provisional patent application for "Plasma Based Methods for Detecting CNS Amyloid Deposition (provisional)" 2) US Patent for "Novel reagents for detection of carboxylic acids by mass spectrometry." C. J. Weber, R. M. Edelmayer, and E. A. Meyers are employed by Alzheimer's Association. E. Spanakis was previously employed by AbbVie Deutschland GmbH & Co KG. R. L. Martone was previously employed by Biogen. 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.

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