Comparative analytical performance of multiple plasma Aβ42 and Aβ40 assays and their relationship to amyloid positron emission tomography (PET)

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BACKGROUND

The National Institute on Aging and the Alzheimer's Association framework for classifying Alzheimer's disease (AD) utilizes measures of pathology for amyloid, tau, and neurodegeneration (ATN) to identify participants for clinical trials [1]. Currently, amyloid pathology is determined by costly amyloid PET or invasive CSF measurements. When applied to participant selection, these measures are associated with high screen failure rates and contribute to the high costs and long duration of recruitment of AD clinical trials. Recent reports indicate that the ratio of amyloid beta peptide 42 to 40 (Aβ 42/40) in plasma is associated with amyloid PET measures [2–10]. This study evaluated six plasma assays for Aβ42 and Aβ40 on their ability to predict amyloid PET positivity compared to age and *APOE* genotype alone.

METHODS

The project team consisted of representatives from pharmaceutical industry, nonprofit/patient advocacy, and academic institutions. Six plasma Aβ assays were selected to be part of the comparison: three liquid chromatography–mass spectrometry (LC-MS/MS) assays and three immunoassays. Plasma samples with corresponding amyloid PET data within 3 months were selected from Alzheimer's Disease Neuroimaging Initiative (ADNI) n=121 (49.6% Aβ+): cognitively normal (CN) n=49 (36.7% Aβ+), mild cognitive impairment (MCI) n=54 (48.2% Aβ+), and AD n=18 (88.9% Aβ+). Each participant's sample was tested in a blinded fashion on all six assays with analytical controls. Statistical tests were performed to identify which assays could significantly improve the Area Under the Receiver Operating Characteristic (ROC) curve for predicting amyloid PET status compared to age and APOE genotype alone (reference model). ADNI participants were categorized as amyloid positive or negative by applying a threshold of florbetapir standardized uptake value ratio (SUVr) ≥ 1.11 to a cortical summary region normalized by the whole cerebellum reference region [11–13]. Comparison between each pair of ROCs and confidence intervals were calculated using the method described by DeLong et al. [14]. Spearman's rank method was used for assessment of pairwise correlation between assays.

RESULTS

Plasma Aβ42 and Aβ40 were measured in the same 121 samples by all six assays and compared to amyloid PET status. The AUC for plasma Aβ 42/40, age, and APOE genotype for predicting amyloid PET status for each assay were compared to the reference model in Table 2. Only one assay performed better than the reference model on Aβ 42/40 ratio alone (ROC 81.4 versus 75.0). Three of the assays significantly improved the ROCs over the reference model of age and APOE genotype (Table 2). Pairwise correlations of the Aβ 42/40 ratio for each assay (Figure 1) were low to moderate (0.04-0.58), but much higher for Aβ42 (0.51-0.88) and Aβ40 (0.74-0.93) separately.

Table 1. Demographic and clinical characteristics of ADNI participants

	CN	MCI	AD
Number	49	54	18
Age in years, Mean (SD)	77.2 (7.6)	78.0 (7.1)	79.9 (6)
Sex, n (%)			
Female	27 (55.1)	19 (35.2)	5 (27.8)
Male	22 (44.9)	35 (64.8)	13 (72.2)
APOE genotype, n (%)			
2/4	0 (0)	0 (0)	1 (5.6)
3/3	23 (46.9)	30 (55.6)	7 (38.9)
3/4	16, (32.7)	13 (24.1)	7 (38.9)
4/4	1 (2.0)	8 (14.8)	3 (16.7)
2/3	9 (18.4)	3 (5.6)	0 (0)
Amyloid PET Positivity, n (%)	18 (36.7)	26 (48.2)	16 (88.9)
MMSE*			
Mean (SD)	29.3 (0.7)	27.6 (2.7)	21.6 (5.6)
Median (Range)	29.0 (2.0)	28.0 (11.0)	23.5 (18.0)
ADAS-Cog 13**			
Mean (SD)	6.8 (3.5)	15.1 (9.1)	32.2 (13.8)
Median (Range)	6.3 (16.0)	15.3 (45.0)	28.7 (49.0)

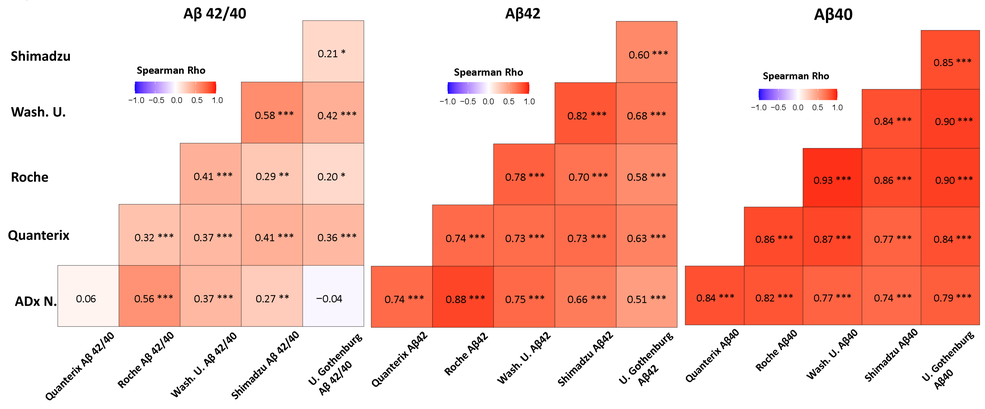
AD, dementia diagnosis; ADAS-Cog 13, Alzheimer's Disease Assessment Scale cognitive subscale 13; *APOE*, gene encoding apolipoprotein E; CN, cognitively normal; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; SD, standard deviation
*MMSE scores were not available for 8 CN, 10 MCI, and 4 AD participants.
** ADAS-Cog 13 scores were not available for 8 CN, 10 MCI, and 4 AD participants.

Table 2. ROC analyses to discriminate amyloid PET positive from amyloid PET negative individuals and comparison of AUCs between the full model (Aβ 42/40, age, *APOE* genotype) and the reference model (age, *APOE* genotype)

Assay Provider	Assay	Model	ROAUC [95% CI]	Estimate of Improvement on Ref. AUROC	p-value vs. Ref. Model (one-sided)
		Reference: age, *APOE* genotype	75.0 [66.3, 83.6]		
Washington University at St. Louis	IP-MS	Plasma Aβ 42/40, age, *APOE* genotype	84.2 [77.0, 91.3]	9.6	0.0067
		Plasma Aβ 42/40	81.4 [73.6, 89.2]	6.9	0.10
Roche	Elecsys Cobas e601	Plasma Aβ 42/40, age, *APOE* genotype	81.1 [73.5, 88.8]	6.1	0.024
		Plasma Aβ 42/40	71.0 [61.7, 80.3]	-4.0	0.73
Shimadzu	IP MALDI-TOF-MS	Plasma Aβ 42/40, age, *APOE* genotype	81.0 [73.4, 88.6]	6.0	0.033
		Plasma Aβ 42/40	71.5 [62.5, 80.5]	-3.5	0.73
U. of Gothenburg	IP-MS	Plasma Aβ 42/40, age, *APOE* genotype	78.1 [69.6, 86.7]	2.8	0.16
		Plasma Aβ 42/40	64.3 [54.2, 74.3]	-11.1	0.95
ADx NeuroSciences	Simoa Neuro 4-plex E Kit (Amyblood)	Plasma Aβ 42/40, age, *APOE* genotype	77.0 [68.6, 85.3]	2.0	0.21
		Plasma Aβ 42/40	66.1 [56.3, 76.0]	-8.8	0.91
Quanterix	Simoa Aβ40 and Aβ42 Advantage Kit	Plasma Aβ 42/40, age, *APOE* genotype	76.6 [68.3, 84.9]	1.7	0.24
		Plasma Aβ 42/40	64.5 [54.5, 74.5]	-10.5	0.94

Aβ, amyloid beta; *APOE*, gene encoding apolipoprotein E; AUC, area under the curve; CI, confidence interval; IP, immuno-precipitation; MS, mass spectrometry; ref, reference
Amyloid positivity was defined by a cut-off of standardized uptake value ratio (SUVr) ≥ 1.11 for florbetapir PET scans [11-13]. The difference between AUCs and 95% Wald confidence intervals were calculated for each assay's full model (plasma Aβ 42/40, age, *APOE* genotype) vs. the reference model (age, *APOE* genotype). p-values are for comparisons of AUCs (using DeLong's test) between the full model (Aβ 42/40, age, *APOE* genotype) and the reference model (Age, *APOE* genotype).

Figure 1. Pairwise Spearman's Rank Correlation Coefficients Between Assays for Aβ 42/40, Aβ42, and Aβ40



Matrices display the strength of the relationship between amyloid levels between plasma Aβ assays as Spearman correlation coefficients (ρ). Six failed measurements reported by assay vendors were excluded from the dataset (n=115).
*** p < 0.001, ** p < 0.01, * p < 0.05

CONCLUSIONS

Two mass spectrometry-based and one automated ligand-binding assay performed well in predicting PET amyloid status. Peripheral measures of the ratio of Aβ42 to Aβ40 continue to show great promise as a diagnostic tool for AD pathology. Interestingly, the correlations between the assays for Aβ40 and Aβ42 analytes alone were excellent and an improvement on the correlations that had been previously presented out of the GBSC (Global Biomarkers Standardization Consortium) at AAIC in 2019. With the excellent correlation between the available assays and good predictive performance of a subset of assays, we may be approaching a performance ceiling of the diagnostic performance of these biomarkers when measured in blood. Further clarity on the relationship of these plasma measures to pathological and clinical trajectories in patients are necessary to inform on their use as patient selection tools in clinical trials and eventually as a diagnostic in the clinic. Additionally, adding pathologically relevant markers like pTau181, -217, and -231 may improve the signal-to-noise and improve the predictive performance of amyloid PET by plasma measures above a ROC of 0.90.

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