

Head-to-Head Comparison of 8 Plasma Amyloid- β 42/40 Assays in Alzheimer Disease

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 Supplemental content

IMPORTANCE Blood-based tests for brain amyloid- β ($A\beta$) pathology are needed for widespread implementation of Alzheimer disease (AD) biomarkers in clinical care and to facilitate patient screening and monitoring of treatment responses in clinical trials.

OBJECTIVE To compare the performance of plasma $A\beta$ 42/40 measured using 8 different $A\beta$ assays when detecting abnormal brain $A\beta$ status in patients with early AD.

DESIGN, SETTING, AND PARTICIPANTS This study included 182 cognitively unimpaired participants and 104 patients with mild cognitive impairment from the BioFINDER cohort who were enrolled at 3 different hospitals in Sweden and underwent $A\beta$ positron emission tomography (PET) imaging and cerebrospinal fluid (CSF) and plasma collection from 2010 to 2014. Plasma $A\beta$ 42/40 was measured using an immunoprecipitation-coupled mass spectrometry developed at Washington University (IP-MS-WashU), antibody-free liquid chromatography MS developed by Araclon (LC-MS-Arc), and immunoassays from Roche Diagnostics (IA-Elc); Euroimmun (IA-EI); and Amsterdam University Medical Center, ADx Neurosciences, and Quanterix (IA-N4PE). Plasma $A\beta$ 42/40 was also measured using an IP-MS-based method from Shimadzu in 200 participants (IP-MS-Shim) and an IP-MS-based method from the University of Gothenburg (IP-MS-UGOT) and another immunoassay from Quanterix (IA-Quan) among 227 participants. For validation, 122 participants (51 cognitively normal, 51 with mild cognitive impairment, and 20 with AD dementia) were included from the Alzheimer Disease Neuroimaging Initiative who underwent $A\beta$ -PET and plasma $A\beta$ assessments using IP-MS-WashU, IP-MS-Shim, IP-MS-UGOT, IA-Elc, IA-N4PE, and IA-Quan assays.

MAIN OUTCOMES AND MEASURES Discriminative accuracy of plasma $A\beta$ 42/40 quantified using 8 different assays for abnormal CSF $A\beta$ 42/40 and $A\beta$ -PET status.

RESULTS A total of 408 participants were included in this study. In the BioFINDER cohort, the mean (SD) age was 71.6 (5.6) years and 49.3% of the cohort were women. When identifying participants with abnormal CSF $A\beta$ 42/40 in the whole cohort, plasma IP-MS-WashU $A\beta$ 42/40 showed significantly higher accuracy (area under the receiver operating characteristic curve [AUC], 0.86; 95% CI, 0.81-0.90) than LC-MS-Arc $A\beta$ 42/40, IA-Elc $A\beta$ 42/40, IA-EI $A\beta$ 42/40, and IA-N4PE $A\beta$ 42/40 (AUC range, 0.69-0.78; $P < .05$). Plasma IP-MS-WashU $A\beta$ 42/40 performed significantly better than IP-MS-UGOT $A\beta$ 42/40 and IA-Quan $A\beta$ 42/40 (AUC, 0.84 vs 0.68 and 0.64, respectively; $P < .001$), while there was no difference in the AUCs between IP-MS-WashU $A\beta$ 42/40 and IP-MS-Shim $A\beta$ 42/40 (0.87 vs 0.83; $P = .16$) in the 2 subcohorts where these biomarkers were available. The results were similar when using $A\beta$ -PET as outcome. Plasma IPMS-WashU $A\beta$ 42/40 and IPMS-Shim $A\beta$ 42/40 showed highest coefficients for correlations with CSF $A\beta$ 42/40 (r range, 0.56-0.65). The BioFINDER results were replicated in the Alzheimer Disease Neuroimaging Initiative cohort (mean [SD] age, 72.4 [5.4] years; 43.4% women), where the IP-MS-WashU assay performed significantly better than the IP-MS-UGOT, IA-Elc, IA-N4PE, and IA-Quan assays but not the IP-MS-Shim assay.

CONCLUSIONS AND RELEVANCE The results from 2 independent cohorts indicate that certain MS-based methods performed better than most of the immunoassays for plasma $A\beta$ 42/40 when detecting brain $A\beta$ pathology.

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Blood tests for detecting amyloid- β (A β) pathology in Alzheimer disease (AD) would be a major advancement for biomarker implementation in clinical care and highly useful in drug trials.¹ Reliable measurements of A β in blood proved challenging² until the development of advanced mass spectrometry and immunodetection methods. In 2016, plasma A β 42/40 assessed using an ultrasensitive Simoa immunoassay was shown to detect abnormal cerebrospinal fluid (CSF) A β or A β -positron emission tomography (PET) status with moderate accuracy.³ Plasma A β 42/40 determined with high-precision immunoprecipitation-coupled mass spectrometry (IP-MS) was later reported to correlate with A β -PET and identify with high precision individuals with abnormal brain A β burden or those at high risk of future conversion to A β -PET positivity.⁴⁻⁶ More recent articles have suggested that A β 42/40 quantified using ultrasensitive and fully automated immunoassay platforms could predict A β -PET status (especially when combined with *APOE* genotype) with accuracy approaching that of MS-based A β 42/40 measures.^{7,8} However, the varying performance of the different A β assays and platforms across the studies could be at least in part owing to the differences in the cohort characteristics (eg, sample size, included diagnostic groups, and outcome measures) and preanalytical sample handling. To minimize these biases, we performed a head-to-head comparison of 8 A β assays in the same cohort of individuals with early AD from the Swedish BioFINDER study. We assessed how well plasma A β 42/40 measured using different assays could discriminate abnormal from normal CSF A β 42/40 or A β -PET status. Finally, we replicated findings from BioFINDER using data from the Alzheimer Disease Neuroimaging Initiative (ADNI).

Methods

Participants

The study included 286 individuals from the prospective Swedish BioFINDER-1 (NCT03174938) cohort recruited between 2010 and 2014. Among the BioFINDER participants, 182 were cognitively unimpaired elderly individuals and 104 had mild cognitive impairment (MCI). For study design and recruitment procedures, see the eMethods in the Supplement. The BioFINDER study was approved by the Regional Ethics Committee in Lund, Sweden. All participants provided written informed consent. Data were analyzed from March 2021 to July 2021.

For validation, we selected 120 participants (51 cognitively unimpaired, 51 with MCI, and 20 with AD dementia) recruited between 2005 and 2013 from ADNI who had plasma A β assessments. Data were obtained from the ADNI database.⁹ ADNI was launched in 2003 as a public-private partnership led by Principal Investigator Michael W. Weiner, MD. Ethical approval was given by the local ethical committees of all involved sites. Data were analyzed from June 2021 to July 2021.

Plasma and CSF Analysis

All BioFINDER study participants underwent measurements of plasma concentrations of A β 42 and A β 40 using the

Key Points

Question How well does plasma amyloid- β 42/40 (A β 42/40), measured using 8 different assays, detect brain A β pathology in the early stages of Alzheimer disease?

Findings In this study, including 408 participants from 2 independent cohorts (BioFINDER and Alzheimer Disease Neuroimaging Initiative), plasma A β 42/40 quantified using certain mass spectrometry-based methods showed better discriminative accuracy than immunoassays when identifying individuals with abnormal intracerebral A β status according to cerebrospinal fluid A β 42/40 levels and A β positron emission tomography.

Meaning Certain mass spectrometry-based plasma tests might have sufficient performance to detect brain A β pathology in Alzheimer disease.

IP-MS-based method developed at Washington University, St Louis, Missouri (IP-MS-WashU), the antibody-free liquid chromatography-MS developed by Araclon Biotech, Zaragoza, Spain (LC-MS-Arc), Elecsys immunoassays from Roche Diagnostics, Penzberg, Germany (IA-Elc), immunoassays from Euroimmun, Lübeck, Germany (IA-EI), and N4PE Simoa immunoassays (IA-N4PE) developed by Amsterdam University Medical Center, Amsterdam, the Netherlands, and ADx Neurosciences, Ghent, Belgium, and commercially available from Quanterix, Billerica, Massachusetts, in the specific laboratories.^{4-8,10-12} In subcohorts of study participants, plasma samples were analyzed using the IP-MS-based method developed by Shimadzu, Kyoto, Japan (IP-MS-Shim; n = 200; subcohort 1), as well as the IP-MS-based methods developed at the University of Gothenburg, Gothenburg, Sweden (IP-MS-UGOT), and another Simoa immunoassay from Quanterix (IA-Quan; n = 227; subcohort 2).^{3,4,11} A β 42 and A β 40 levels in CSF were determined with Elecsys CSF immunoassays. We included all participants from BioFINDER who underwent [¹⁸F]flutemetamol PET imaging (n = 416) with plasma samples available at the time of analysis except that the samples were randomly selected for the IP-MS-Shim, IP-MS-UGOT, and IA-Quan assays. In ADNI, plasma concentrations of A β 42 and A β 40 were quantified using IP-MS-WashU, IP-MS-Shim, IP-MS-UGOT, IA-Elc, IA-N4PE, and IA-Quan. All participants in ADNI who had plasma A β and A β -PET assessments were included. Further details of blood and CSF collection and analysis are described in the eMethods and eTables 1 and 2 in the Supplement.

A β -PET Imaging

In BioFINDER, A β imaging was performed using [¹⁸F]flutemetamol PET 90 to 110 minutes postinjection, as described in the eMethods in the Supplement. Standardized uptake value ratio was defined as the uptake in a global neocortical target region of interest with the cerebellar cortex as reference region.¹³ In ADNI, A β imaging was performed using [¹⁸F]florbetapir PET 50 to 70 minutes postinjection using a global neocortical target region of interest with the whole cerebellum as reference region.^{14,15}

Table 1. Characteristics of Study Participants in BioFINDER

Characteristic	Median (IQR)		P value ^b
	A β negative (n = 168) ^a	A β positive (n = 118) ^a	
Diagnosis, CU/MCI, No.	127/41	55/63	<.001
Age, y	71.0 (67.0-75.0)	74.0 (70.0-77.0)	.001
Female, No. (%)	90 (53.6)	51 (43.2)	.93
Duration of education, y ^c	12.0 (9.0-14.0)	11.0 (9.0-13.0)	.91
MMSE	29.0 (28.0-30.0)	28.0 (26.0-29.0)	<.001
APOE ϵ 4 positivity, No. (%) ^d	35 (21.0)	77 (65.3)	<.001
A β -PET, [¹⁸ F]flutemetamol SUVR	1.19 (1.12-1.28)	1.86 (1.57-2.15)	<.001
CSF A β 42/40	0.093 (0.079-0.102)	0.041 (0.034-0.049)	<.001
Plasma A β 42/40			
IP-MS-WashU	0.132 (0.126-0.139)	0.122 (0.117-0.126)	<.001
LC-MS-Arc	0.322 (0.298-0.346)	0.288 (0.266-0.304)	<.001
IA-Elc	0.068 (0.064-0.072)	0.062 (0.058-0.065)	<.001
IA-EI	0.179 (0.162-0.199)	0.162 (0.146-0.174)	<.001
IA-N4PE	0.135 (0.119-0.147)	0.119 (0.105-0.132)	<.001

Abbreviations: A β , amyloid- β ; CSF, cerebrospinal fluid; CU, cognitively unimpaired; IA-EI, immunoassay from Euroimmun; IA-Elc, Elecsys immunoassay from Roche Diagnostics; IA-N4PE, N4PE Simoa immunoassay from Quanterix; IP-MS-WashU, immunoprecipitation-coupled mass spectrometry method developed at Washington University; IQR, interquartile range; LC-MS-Arc, antibody-free liquid chromatography-mass spectrometry method developed by Araclon; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET positron emission tomography; SUVR, standardized uptake value ratio.

^a A β status was defined using the CSF A β 42/40 cutoff (0.059) derived from mixture modeling as previously described.⁷

^b Differences between the groups were tested using Mann-Whitney *U* test and Fisher exact test (diagnosis, sex, and APOE).

^c Education is missing for 2 study participants.

^d APOE ϵ 4 is missing for 1 study participant.

Statistical Analysis

SPSS version 22 (IBM) was used for statistical analysis. Correlations between biomarkers were assessed with the Spearman test. Differences between the groups were tested using Mann-Whitney *U* test or Fisher exact test. Unadjusted 2-sided *P* values <0.05 were considered statistically significant. Discrimination accuracies of biomarkers were determined with logistic regression models and receiver operating characteristic curve analysis. Area under the receiver operating characteristic curve (AUC) of 2 receiver operating characteristic curves were compared with DeLong test with adjustment for multiple comparisons using a false discovery rate of 5%. In BioFINDER, CSF A β 42/40 was used as the outcome in the main analysis. We also performed a sensitivity analysis with A β -PET and CSF A β 42/40 measured with the Euroimmun assay as outcomes to ensure that the results were not biased by the use of the same antibodies in the CSF and plasma for the Elecsys A β 42/40 assays. In ADNI, CSF A β 42 and A β 40 measures at the time of plasma collection were only available in a small group of participants, and therefore we used A β PET as the outcome. CSF A β 42/40 and A β -PET data were binarized using previously described cutoffs (CSF A β 42/40 Elecsys, 0.059; CSF A β 42/40 Euroimmun, 0.091; A β -PET BioFINDER, 1.42; ADNI, 1.11).^{7,13-16}

Results

Participants in BioFINDER

Of the 286 participants without dementia in BioFINDER, 141 (49.3%) were women, and the mean (SD) age was 71.6

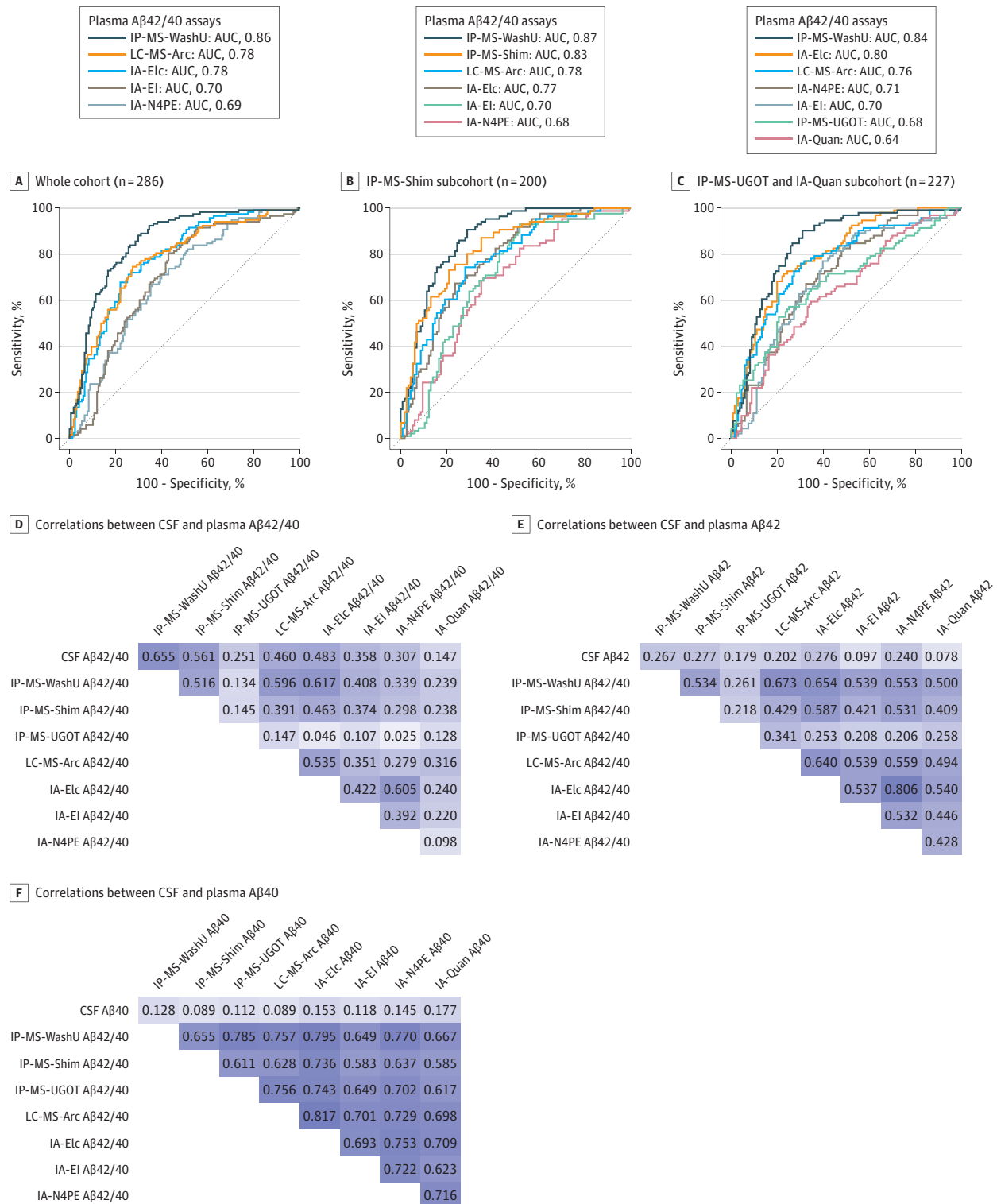
(5.6) years. The baseline demographic and clinical characteristics of the whole cohort as well as the 2 subcohorts with IP-MS-Shim A β 42/40 or IA-Quan A β 42/40 data available are summarized in Table 1 and eTables 3 and 4 in the Supplement, respectively. For all tested assays, plasma A β 42 and A β 42/40 were lower in individuals who were A β positive compared with those who were A β negative whereas there were no differences in the levels of A β 40 (Table 1; eTables 3, 4, and 5 in the Supplement).

Prediction of CSF A β Status Using Different Plasma A β Assays in BioFINDER

When identifying individuals with abnormal CSF A β 42/40 in the whole cohort (Figure, A; Table 2), plasma IP-MS-WashU A β 42/40 had significantly better discriminative accuracy (AUC, 0.86; 95% CI, 0.81-0.90) than plasma LC-MS-Arc A β 42/40 (AUC, 0.78; 95% CI, 0.72-0.83; *P* < .01), IA-Elc A β 42/40 (AUC, 0.78; 95% CI, 0.73-0.83; *P* < .01), IA-EI A β 42/40 (AUC, 0.70; 95% CI, 0.64-0.76; *P* < .001), and IA-N4PE A β 42/40 (AUC, 0.69; 95% CI, 0.63-0.75; *P* < .001).

In the 2 subcohorts of participants where IP-MS-Shim A β 42/40 or IP-MS-UGOT A β 42/40 and IA-Quan A β 42/40 were also available, IP-MS-WashU A β 42/40 showed higher discriminative accuracy for CSF A β 42/40 status than IP-MS-UGOT A β 42/40 (AUC, 0.84; 95% CI, 0.79-0.89 vs AUC, 0.68; 95% CI, 0.61-0.75; *P* < .001) and IA-Quan A β 42/40 (AUC, 0.84; 95% CI, 0.79-0.89 vs AUC, 0.64; 95% CI, 0.56-0.71; *P* < .001), while the difference in AUCs between IP-MS-WashU A β 42/40 and IP-MS-Shim A β 42/40 was not significant (AUC, 0.87; 95% CI, 0.82-0.92 vs AUC, 0.83; 95% CI, 0.77-0.88; *P* = .16) (Figure, B and C, Table 2).

Figure. Receiver Operating Characteristic (ROC) Analysis for Abnormal Cerebrospinal Fluid (CSF) Amyloid-β42/40 (Aβ42/40) and Correlations Between CSF and Plasma Aβ



A, ROC curve analysis for differentiating participants with abnormal CSF Aβ42/40 from those with normal CSF Aβ42/40 (cutoff, 0.0597) in the whole cohort. B, ROC curve analysis in the subcohorts where IPMS-Shim Aβ42/40 was available. C, ROC curve analysis in the subcohorts where IPMS-UGOT and IA-Quan Aβ42/40 were available. D, Spearman correlations between plasma and CSF Aβ42/40 in a subcohort (n = 155) individuals where all plasma samples were analyzed using all 8 assays. E, Spearman correlations between plasma and CSF Aβ42 in a subcohort (n = 155) where all plasma samples were analyzed using all 8 assays. F, Spearman correlations between plasma and CSF Aβ40 in a subcohort (n = 155) where all plasma samples were analyzed using all 8 assays.

For comparison, one of the most promising plasma biomarkers of AD, p-tau217,^{10,17} distinguished 117 individuals with abnormal CSF A β 42/40 from 168 individuals with normal CSF A β 42/40 with an AUC of 0.79 (95% CI, 0.74-0.84), which was numerically lower but not significantly different from the AUC of IP-MS-WashU A β 42/40 (0.86; 95% CI, 0.81-0.90; unadjusted $P = .06$).

Sensitivity Analyses in BioFINDER

The results were similar when using CSF A β 42/40 analyzed with the Euroimmun immunoassay instead of the Elecsys immunoassay as the reference standard (eTable 6 in the Supplement). Further, the overall results were very similar when using A β -PET as the outcome, with most assays showing numerically lower AUCs compared with AUCs for CSF A β 42/40 as the outcome (Table 2).

Correlations Between Plasma and CSF A β in BioFINDER

Spearman coefficients were highest for correlations of CSF A β 42/40 with plasma IP-MS-WashU A β 42/40 (r , 0.65; $P < .001$), followed by IP-MS-Shim A β 42/40 (r , 0.56; $P < .001$), IA-Elc A β 42/40 (r , 0.48; $P < .001$), LC-MS-Arc A β 42/40 (r , 0.46; $P < .001$), IA-EI A β 42/40 (r , 0.36; $P < .001$), IA-N4PE A β 42/40 (r , 0.31; $P < .001$), IP-MS-UGOT A β 42/40 (r , 0.25; $P = .002$) and IA-Quan A β 42/40 (r , 0.15; $P = .06$) (Figure, D). Further, there were correlations between plasma A β measured using different assays for both A β 42 (r range; 0.21-0.81) and A β 40 (r range, 0.58-0.82), but the coefficients were lower for correlations between plasma and CSF A β 42 (r range, 0.08-0.28) and plasma and CSF A β 40 (r range, 0.09-0.18) (Figure, E and F for all assays used in a subcohort of 155 participants; eFigure in the Supplement for the 5 assays used in the whole cohort).

Combining Plasma A β With APOE ϵ 4 in BioFINDER

Adding APOE ϵ 4 status improved the accuracy of all A β 42/40 measures (Δ AUC, 0.027-0.140; eTable 7 in the Supplement) with the AUCs of the 3 MS-based methods and IA-Elc A β 42/40 consistently above 0.82 in the whole cohort and the 2 subcohorts in which AUCs differences between the 3 MS-based methods lost statistical significance.

Validation in ADNI

Of 122 participants in ADNI, 53 (43.4%) were women, and the mean (SD) age was 72.4 (5.4) years. The baseline demographic and clinical characteristics are summarized in Table 3. For all 6 tested assays, plasma A β 42/40 was lower in individuals who were A β positive compared with individuals who were A β negative whereas there were no differences in the levels of A β 40 (Table 3; eTable 8 in the Supplement). Plasma A β 42 concentrations were also lower in the A β -positive group than in A β -negative group for all assays except IA-Quan, which did not show significant differences between the groups (eTable 8 in the Supplement). In ADNI, for IP-MS-Shim, we used a previously described composite biomarker score because it identified abnormal A β -PET more accurately than A β 42/40 in this cohort. Similar to the results in BioFINDER, we found that plasma IP-MS-WashU A β 42/40 showed better performance (AUC, 0.85; 95% CI, 0.77-0.92) than plasma IP-MS-UGOT

Table 2. Receiver Operating Curve (ROC) Analysis for Abnormal Cerebrospinal Fluid (CSF) Amyloid- β 42/40 (A β 42/40) and A β -Positron Emission Tomography (PET) Status in BioFINDER^a

Plasma A β 42/40 Assay	AUC (95% CI) ^b	
	CSF A β 42/40	A β -PET
Entire cohort		
A β +, No.	118	110
A β -, No.	168	176
IP-MS-WashU	0.855 (0.810-0.899)	0.833 (0.787-0.879)
IA-Elc	0.778 (0.725-0.832) ^c	0.727 (0.669-0.784) ^d
LC-MS-Arc	0.776 (0.721-0.830) ^c	0.753 (0.696-0.811) ^c
IA-EI	0.697 (0.635-0.758) ^d	0.672 (0.609-0.735) ^d
IA-N4PE	0.687 (0.626-0.748) ^d	0.655 (0.591-0.719) ^d
Subcohort with IP-MS-Shim A β 42/40 ^e		
A β +, No.	86	86
A β -, No.	114	114
IP-MS-WashU	0.872 (0.824-0.920)	0.872 (0.824-0.920)
IP-MS-Shim	0.825 (0.767-0.882)	0.825 (0.767-0.882)
LC-MS-Arc	0.775 (0.711-0.839) ^c	0.775 (0.711-0.839) ^c
IA-Elc	0.773 (0.709-0.837) ^c	0.773 (0.709-0.837) ^c
IA-EI	0.704 (0.631-0.777) ^d	0.704 (0.631-0.777) ^d
IA-N4PE	0.679 (0.605-0.753) ^d	0.679 (0.605-0.753) ^d
Subcohort with IP-MS-UGOT and IA-Quan A β 42/40		
A β +, No.	91	86
A β -, No.	136	141
IP-MS-WashU	0.838 (0.785-0.891)	0.814 (0.760-0.868)
IA-Elc	0.795 (0.738-0.853)	0.728 (0.663-0.793) ^c
LC-MS-Arc	0.763 (0.700-0.827) ^f	0.742 (0.676-0.809) ^f
IA-N4PE	0.706 (0.639-0.773) ^c	0.649 (0.577-0.721) ^d
IA-EI	0.697 (0.628-0.767) ^d	0.667 (0.596-0.738) ^d
IP-MS-UGOT	0.678 (0.605-0.750) ^d	0.632 (0.557-0.707) ^d
IA-Quan	0.636 (0.563-0.709) ^d	0.600 (0.525-0.675) ^d

Abbreviations: AUC, area under the curve; IA-EI, immunoassay from Euroimmun; IA-Elc, Elecsys immunoassay from Roche Diagnostics; IA-N4PE, N4PE Simoa immunoassay from Quanterix; IA-Quan, Simoa immunoassay from Quanterix; IP-MS-Shim, immunoprecipitation coupled mass spectrometry method developed by Shimadzu; IP-MS-WashU, immunoprecipitation-coupled mass spectrometry method developed at Washington University; IP-MS-UGOT, immunoprecipitation-coupled mass spectrometry method developed at the University of Gothenburg; LC-MS-Arc, antibody-free liquid chromatography-mass spectrometry method developed by Araclon.

^a CSF A β 42/40 and A β -PET data were binarized using previously described cutoffs (0.059 and 1.42, respectively).^{7,13}

^b AUC of 2 ROC curves were compared with DeLong test.

^c $P < .01$, compared with IP-MS-WashU A β 42/40.

^d $P < .001$, compared with IP-MS-WashU A β 42/40.

^e In this subcohort, CSF A β 42/40 and A β -PET concordance was 100%.

^f $P < .05$ compared with A β 42/40IP-MS-WashU.

Table 3. Characteristics of Study Participants in the Alzheimer Disease Neuroimaging Initiative

Characteristic	Median (IQR)		P value ^b
	A β negative (n = 63) ^a	A β positive (n = 59) ^a	
Diagnosis, CN/MCI/AD, No.	35/26//2	16/25/18	<.001
Age, y	70.7 (65.7-76.0)	74.2 (69.9-77.5)	.02
Female, No. (%)	28 (44.4)	25 (42.4)	.86
Duration of education, y	18.0 (15.0-19.0)	16.0 (13.0-18.0)	.24
MMSE	29.0 (28.0-30.0)	27.0 (23.0-29.0)	<.001
APOE ϵ 4 positivity, No. (%)	18 (28.6)	32 (54.2)	.006
A β -PET, [¹⁸ F]florbetapir SUVR	1.006 (0.960-1.037)	1.321 (1.235-1.470)	<.001
CSF A β 42/40	NA	NA	NA
Plasma A β 42/40			
IP-MS-WashU	0.132 (0.128-0.141)	0.122 (0.117-0.127)	<.001
IP-MS-Shim	0.040 (0.037-0.045)	0.037 (0.034-0.039)	<.001
IP-MS-UGOT	0.071 (0.061-0.089)	0.064 (0.052-0.073)	.002
IA-Elc	0.171 (0.154-0.182)	0.152 (0.141-0.164)	<.001
IA-N4PE	0.049 (0.042-0.054)	0.043 (0.039-0.047)	<.001
IA-Quan	0.040 (0.037-0.044)	0.037 (0.034-0.041)	.01

Abbreviations: A β , amyloid- β ; AD, Alzheimer disease dementia; CSF, cerebrospinal fluid; CU, cognitively unimpaired; IA-Elc, Elecsys immunoassay from Roche Diagnostics; IA-N4PE, N4PE Simoa immunoassay from Quanterix; IA-Quan, Simoa immunoassay from Quanterix; IP-MS-Shim, immunoprecipitation coupled mass spectrometry method developed by Shimadzu; IP-MS-UGOT, immunoprecipitation-coupled mass spectrometry method developed at the University of Gothenburg; IP-MS-WashU, immunoprecipitation-coupled mass spectrometry method developed at Washington University; IQR, interquartile range; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; PET, positron emission tomography; SUVR, standardized uptake value ratio.

^a A β status was defined using a previously described A β -PET cutoff (1.11).^{14,15}

^b Differences between the groups were tested using Mann-Whitney *U* test, χ^2 (diagnosis), or Fisher exact test (sex and APOE).

Table 4. Receiver Operating Curve (ROC) Analysis for Abnormal A β -PET in the Alzheimer Disease Neuroimaging Initiative^a

Plasma assay	A β -PET, AUC (95% CI) ^b
A β +, No.	59
A β -, No.	63
A β 42/40 IP-MS-WashU	0.845 (0.772-0.917)
Composite IP-MS-Shim	0.821 (0.747-0.895)
A β 42/40 IA-Elc	0.740 (0.651-0.829) ^c
A β 42/40 IA-N4PE	0.685 (0.590-0.781) ^d
A β 42/40 IP-MS-UGOT	0.662 (0.565-0.758) ^e
A β 42/40 IA-Quan	0.634 (0.534-0.734) ^e

Abbreviations: A β , amyloid- β ; AUC, area under the curve; IA-Elc, Elecsys immunoassay from Roche Diagnostics; IA-N4PE, N4PE Simoa immunoassay from Quanterix; IA-Quan, Simoa immunoassay from Quanterix; IP-MS-Shim, immunoprecipitation coupled mass spectrometry method developed by Shimadzu; IP-MS-UGOT, immunoprecipitation-coupled mass spectrometry method developed at the University of Gothenburg; IP-MS-WashU, immunoprecipitation-coupled mass spectrometry method developed at Washington University; ROC, receiver operating characteristic; PET, positron emission tomography.

^a In ADNI, CSF A β 42 and A β 40 measures at the time of plasma collection were only available in a small group of participants, and therefore we used A β PET as the outcome.

^b AUCs of 2 ROC curves were compared with DeLong test. A β -PET data was binarized using a previously described threshold of 1.11.^{14,15}

^c *P* < .05, compared with IP-MS-WashU A β 42/40.

^d *P* < .01, compared with IP-MS-WashU A β 42/40.

^e *P* < .001, compared with A β 42/40IP-MS-WashU.

A β 42/40 (AUC, 0.66; 95% CI, 0.57-0.76; *P* < .001), IA-Elc A β 42/40 (AUC, 0.74; 95% CI, 0.65-0.83; *P* < .05), IA-N4PE A β 42/40 (AUC, 0.69; 95% CI, 0.59-0.78; *P* < .01), and IA-Quan A β 42/40 (AUC, 0.63; 95% CI, 0.53-0.73; *P* < .001)

but not IP-MS-Shim composite biomarker score (AUC, 0.82; 95% CI, 0.75-0.89; *P* = .54) (Table 4).

Discussion

In this cross-sectional study examining the performance of 8 plasma assays for quantification of A β 42/40, we found that certain MS-based methods offered better precision than immunoassays for identifying individuals with early AD. In 2 independent cohorts, 2 IP-MS methods (IP-MS-WashU and IP-MS-Shim) had the highest discriminative accuracy for determining CSF A β 42/40 and A β -PET status. In BioFINDER, Spearman coefficients were highest for correlations of CSF A β 42/40 with IP-MS-WashU A β 42/40 and IP-MS-Shim A β 42/40 as well.

A β 42/40 measured using IP-MS has previously shown high accuracy in detecting abnormal brain A β status in AD with AUCs ranging from 0.88 to 0.97,⁴⁻⁶ and the IP-MS blood test for A β developed by Washington University can now be used in clinical care in US. In the present study, the AUCs of both IP-MS-based methods were somewhat lower (0.82-0.87) than in other cohorts, highlighting that the impact of differences in cohort characteristics and sample handling is not negligible. Nevertheless, plasma A β 42/40 quantified with the IP-MS-WashU approach showed significantly better performance than the immunoassays. These findings could be explained by high specificity of MS-based technologies in general, which is considered a substantial advantage over immunoassay, but also by differences in the antibody specificities and sample handling procedures. It is also possible that the A β IP-MS methods are less prone to matrix effects

that can be especially pronounced in protein-rich and compositionally complex biological fluids such as blood.¹⁸ However, while MS is a powerful research tool, fully automated immunoassays or MS will probably be needed to provide global access to blood-based biomarkers for routine clinical use in primary care settings. Among the immunoassays, IA-Elc A β 42/40 had the numerically highest AUC, most likely because Elecsys A β immunoassays are performed on a fully automated platform with very high analytical reliability and precision.¹⁹

Limitations

This study has limitations. One limitation is that IP-MS-Shim A β 42/40, IP-MS-UGOT A β 42/40, and IA-Quan A β 42/40 were not available in the whole cohort. Other limitations include

the relatively small size of the A β -negative cognitively unimpaired group and that the assays were performed at different laboratories, possibly introducing some preanalytical variation. Future investigations should examine the performance of different A β methods separately in cognitively unimpaired participants and those with MCI.

Conclusions

In conclusion, plasma A β 42/40 determined using certain MS-based methods identified individuals with abnormal brain A β burden more accurately than immunoassay-based A β 42/40 measures. These findings can help inform the future clinical use of blood tests for A β pathology in AD.

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Conflict of Interest Disclosures: Dr Teunissen reports nonfinancial support from Quanterix during the conduct of the study; has a patent pending for glial fibrillary acidic protein as a biomarker; serves as an editorial board member of *Alzheimer Research and Therapy*, Medicaat Neurologie, Springer, and *Neurology: Neuroimmunology & Neuroinflammation*; serves as an editor of *Neuromethods Book Series*, Springer; and reports grants from Roche Diagnostics, nonfinancial support from ADX Neurosciences and Eli Lilly, and compensation from ACImmune, Axon Neurosciences, Biogen, Brainstorm Cell Therapeutics, Celgene, Denali Therapeutics, EIP Pharma, Eisai, PeopleBio, Toyama Pharmaceutical Association, and Vivoryon Therapeutics outside the submitted work. Dr Zetterberg reports personal fees from Alector, AlzeCure Pharma, AZTherapies, Biogen, Biosplice Therapeutics, Celectricon, Cognition Therapeutics, Denali Therapeutics, Eisai, Fujirebio Diagnostics, NervGen, Pinteon Therapeutics, Red Abbey Labs, Roche Diagnostics, Siemens Healthineers, and Wave Life Sciences outside the submitted work; and is a cofounder of Brain Biomarker Solutions in Gothenburg AB. Drs Allué and Sarasa report a patent pending for EP2020382352 and are employed at Araclon Biotech. Dr Eichenlaub is employed at Roche Diagnostics. Dr Bittner is employed at F. Hoffmann-La Roche, has a patent pending for blood-based biomarkers, and owns stock from F. Hoffmann-La Roche. Mr Ovod has submitted the US provisional patent application "Plasma Based

Methods for Detecting CNS Amyloid Deposition" as a coinventor and may receive royalty income based on technology (stable isotope labeling kinetics and blood plasma assay) licensed by Washington University to C2N Diagnostics. Dr Toba reports grants from Amedisys during the conduct of the study. Dr Nakamura reports grants from Amedisys during the conduct of the study. Dr Bateman reports consulting for Eisai and Janssen; personal fees from ACImmune, Amgen, C2N Diagnostics, and Pfizer; grants from AbbVie, Avid Radiopharmaceuticals, Biogen, Centene, Eisai, Eli Lilly, F. Hoffmann-La Roche, Genentech, Janssen Pharmaceuticals, and United Neuroscience outside the submitted work; he is a member of the DIAN-TU Pharma Consortium (Eli Lilly and Company/Avid Radiopharmaceuticals, Hoffmann-La Roche/Genentech, Biogen, Eisai, Janssen, and United Neuroscience), the NFL Consortium (AbbVie, Biogen, Roche, BMS), and Tau SIK Consortium (AbbVie, Avid Radiopharmaceuticals, Biogen, Eli Lilly); has a patent for a plasma amyloid-beta test licensed to C2N Diagnostics; in addition, Washington University and Dr. Bateman have equity ownership interest in C2N Diagnostics and receive royalty income based on blood plasma assay technology licensed by Washington University to C2N Diagnostics; Washington University and Dr Bateman have submitted the US provisional patent application "Plasma Based Methods for Detecting CNS Amyloid Deposition." Dr Blennow reports personal fees from Abcam, Axon, Biogen, the Japanese Organization for Medical Device Development, Julius Clinical, Eli Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, Shimadzu, and Siemens Healthineers and grants from the Swedish Research Council during the conduct of the study as well as research funding from Biogen, Eisai, and Roche Diagnostics and nonfinancial support from AC Immune and Eli Lilly outside the submitted work; and is a cofounder of Brain Biomarker Solutions in Gothenburg AB, which is a part of the GU Ventures Incubator Program. Dr Hansson reports research support paid to his institution from Avid Radiopharmaceuticals, Biogen, Eisai, Eli Lilly, General Electric Healthcare, Pfizer, and Roche Diagnostics and has received personal fees from AC Immune, Alzpath, Biogen, Cerveau, and Roche Diagnostics. No other disclosures were reported.

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