Supplementary Online Content

Janelidze S, Pannee J, Mikulskis A, et al. Concordance between different amyloid immunoassays and visual amyloid positron emission tomographic assessment. *JAMA Neurology*. Published online November 6, 2017. doi:10.1001/jamaneurol.2017.2814

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Participants and [^{18}F]flutemetamol PET

Participants

The study population included 262 patients with mild cognitive complaints from the prospective and longitudinal Swedish BioFINDER cohort (www.biofinder.se) who had undergone [^{18}F]flutemetamol PET evaluation. The patients were referred for assessment of their cognitive complaints and were recruited between 2010 and 2014. They were thoroughly assessed for their cognitive complaints by physicians with special interest in dementia disorders. The inclusion criteria were: 1) cognitive symptoms; 2) not fulfilling the criteria for dementia; 3) a Mini-Mental State Examination (MMSE) score of 24 – 30 points; 4) age 60 – 80 years; and 5) fluent in Swedish. The exclusion criteria were: 1) cognitive impairment that without doubt could be explained by another condition (other than prodromal dementias); 2) severe somatic disease; and 3) refusing lumbar puncture or neuropsychological investigation. These criteria resulted in a clinically relevant population where 47% were classified as subjective cognitive decline (SCD), 40% as amnestic MCI, and 11% as non-amnestic MCI. The classification was based on a neuropsychological battery assessing the cognitive domains of verbal ability, visuospatial construction, episodic memory, and executive functions and the clinical assessment by a senior neuropsychologist. The characteristics of the study participants are given in eTable 1.

[^{18}F]flutemetamol PET

Cerebral Aβ deposition was visualized with the PET tracer [^{18}F]flutemetamol (approved by the Food and Drug Administration, and the European Medical Agency). [^{18}F]flutemetamol was manufactured at the radiopharmaceutical production site in Risø, Denmark, using a FASTlab synthesizer module (GE Healthcare, Cleveland, OH). Subjects received a single dose of [^{18}F]flutemetamol according to a method described previously.[1] PET/CT scanning of the brain was conducted at two sites using the same type of scanner (Gemini, Philips Healthcare, Best, the Netherlands). [^{18}F]flutemetamol scans were rated by a board-certified neuroradiologist who had successfully completed a training programme provided by GE. Images were designated as PET positive or negative. The rater was blinded to all clinical characteristics of the study participants.
In addition, sum images (from 90-110 min post injection) were analyzed using the software NeuroMarQ (GE Healthcare, Cleveland, OH, USA). [^{18}F]flutemetamol activity was quantified with a previously described fully automated PET-only method that uses an adaptive template for handling different uptake patterns in negative and positive [18F]flutemetamol images.[2] [^{18}F]flutemetamol images were spatially normalized to Montreal Neurological Institute template space using the adaptive template method. A volume of interest (VOI) template was applied for the following 9 bilateral regions: prefrontal, parietal, lateral temporal, medial temporal, sensorimotor, occipital, anterior cingulate, posterior cingulate/precuneus, and a global neocortical composite region.[2] The standardized uptake value ratio (SUVR) was defined as the uptake in a VOI normalized for the cerebellar cortex uptake. We used [18F]flutemetamol SUVR cutoff >1.42 for abnormally increased Aβ deposition. This cutoff was established in our previous study based on the same [^{18}F]flutemetamol procedure.[3]

eResults. Visual Aβ PET vs CSF AD Biomarker Measured Using Antibody-Independent MS-based RMP

Both Aβ42 ^{MS} and Aβ42/Aβ40^{MS} accurately predicted visual [^{18}F]flutemetamol PET assessment with area under the curve (AUC) of 0.83 (95% CI 0.75 - 0.92) and 0.93 (0.86 - 0.99), respectively. However, the Aβ42/Aβ40^{MS} ratio performed significantly better than Aβ42 (p=0.004 when comparing AUCs of the two ROC curves using DeLong test). The optimal cutoff for Aβ42 ^{MS} was 741 pg/ml (sensitivity 87%, specificity 75%, Youden's index 0.62) and for Aβ42/Aβ40^{MS} 0.07 (sensitivity 97%, specificity 90%, Youden's index 0.87). The number of cases with discordant CSF Aβ status compared to visual PET assessments was higher for Aβ42^{MS} (n=20, 20%) than for Aβ42/Aβ40^{MS} (n=7, 7%) and consisted mainly of CSF Aβ42-positive and visual PET-negative cases (eTable 2).

References

1. Koole M, Lewis DM, Buckley C, et al. Whole-body biodistribution and radiation dosimetry of 18F-GE067: a radioligand for in vivo brain amyloid imaging. *J Nucl Med.* 2009;50(5):818-822.
2. Lundqvist R, Lilja J, Thomas BA, et al. Implementation and validation of an adaptive template registration method for 18F-flutemetamol imaging data. *J Nucl Med.* 2013;54(8):1472-1478.

3. Palmqvist S, Zetterberg H, Blennow K, et al. Accuracy of brain amyloid detection in clinical practice using cerebrospinal fluid beta-amyloid 42: a cross-validation study against amyloid positron emission tomography. *JAMA Neurol.* 2014;71(10):1282-1289.
4. Janelidze S, Zetterberg H, Mattsson N, et al. CSF Abeta42/Abeta40 and Abeta42/Abeta38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol.* 2016;3(3):154-165.

eTable 1. Biofinder Cohort Characteristics

	Biofinder cohort
Age	70.9 ± 5.5
Gender (female, %)	41%
Diagnosis	SCD 115 (44%) MCI 143 (55%)
MMSE	27.8 ± 1.7
Composite 18-flutemetamol PET score	1.6 ± 0.5
Innotest	
$A\beta42^{INC}$, pg/ml	578.5 ± 227.1
$A\beta42^{INM}$, pg/ml	1114.9 ± 424.3
Aβ40, pg/ml	9545.1 ± 3230.5
$A\beta42^{INC}$/Aβ40	0.066 ± 0.030
$A\beta42^{INM}$/Aβ38	0.123 ± 0.045
P-tau 181P, pg/mL	61.4 ± 28.2
Euroimmun	
Aβ42, pg/ml	523.3 ± 246.6
Aβ40, pg/ml	4770.5 ± 1754.4
Aβ42/Aβ40	0.115 ± 0.047
T-tau, pg/mL	386.0 ± 173.6
Mesoscale discovery	
Aβ42, pg/ml	531.5 ± 230.9
Aβ40, pg/ml	5898.2 ± 1376.2
Aβ42/Aβ40	0.090 ± 0.032
Mass Spectrometry (n=98)	
Aβ42, pg/ml	841.6 ± 399.5
Aβ40, pg/ml	10744.9 ± 3423.7
Aβ42/Aβ40	0.080 ± 0.028

Data are shown as mean±SD unless otherwise specified.
INC, Innotest classical; INM, Innotest modified; EI, Euroimmun; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; MSD, Mesoscale Discovery; MS, mass spectrometry; SCD, subjective cognitive decline.

Etable 2. Comparisons Of ROC Analysis Of CSF Biomarkers For Distinguishing Abnormal From Normal Visual Reading Assessments Of [^{18}F]Flutemetamol PET

	Difference between AUCs	95% CI for the difference	P-value
Innotest			
$A\beta42^{INC}$ vs $A\beta42^{INC}/A\beta40$	0.0012	-0.0347 to 0.0370	0.9495
$A\beta42^{INC}$ vs $A\beta42^{INC}$/T-tau	0.0237	-0.0074 to 0.0548	0.1346
$A\beta42^{INC}$ vs $A\beta42^{INC}$/P-tau	**0.0284**	**0.0023 to 0.0545**	**0.0329**
$A\beta42^{INC}/A\beta40$ vs $A\beta42^{INC}$/t-tau	**0.0249**	**0.0068 to 0.0430**	**0.0071**
$A\beta42^{INC}/A\beta40$ vs $A\beta42^{INC}$/P-tau	**0.0295**	**0.0094 to 0.0497**	**0.0040**
$A\beta42^{INM}$ vs. $A\beta42^{INM}/A\beta40$	**0.0597**	**0.0132 to 0.1060**	**0.0119**
$A\beta42^{INM}$ vs. $A\beta42^{INM}$ /T-tau	**0.0687**	**0.0259 to 0.1120**	**0.0017**
$A\beta42^{INM}$ vs. $A\beta42^{INM}$ /P-tau	**0.0750**	**0.0365 to 0.1140**	**0.0001**
$A\beta42^{INM}/A\beta40$ vs. $A\beta42^{INM}$ /T-tau	0.0090	-0.0060 to 0.0240	0.2400
$A\beta42^{INM}/A\beta40$ vs $A\beta42^{INM}$ /P-tau	0.0153	-0.0039 to 0.0346	0.1183
Euroimmun			
$A\beta42^{EI}$ vs. $A\beta42^{EI}/A\beta40$	**0.0529**	**0.0120 to 0.0939**	**0.0112**
$A\beta42^{EI}$ vs. $A\beta42^{EI}$ /T-tau	**0.0582**	**0.0202 to 0.0962**	**0.0027**
$A\beta42^{EI}$ vs. $A\beta42^{EI}$ /p-tau	**0.0650**	**0.0298 to 0.1000**	**0.0003**
$A\beta42^{EI}/A\beta40$ vs. $A\beta42^{EI}$ /T-tau	0.0053	-0.0099 to 0.0204	0.4976
$A\beta42^{EI}/A\beta40$ vs $A\beta42^{EI}$ /P-tau	0.0121	-0.0036 to 0.0277	0.1320
Mesoscale discovery			
$A\beta42^{MSD}$ vs. $A\beta42^{MSD}$ /Aβ40	**0.0594**	**0.0269 to 0.0919**	**0.0003**
$A\beta42^{MSD}$ vs $A\beta42^{MSD}$ /T-tau	**0.0475**	**0.0096 to 0.0853**	**0.0139**
$A\beta42^{MSD}$ vs $A\beta42^{MSD}$ /P-tau	**0.0564**	**0.0230 to 0.0897**	**0.0009**
$A\beta42^{MSD}$ /Aβ40 vs $A\beta42^{MSD}$ /T-tau	0.0119	-0.0074 to 0.0312	0.2257
$A\beta42^{MSD}$ /Aβ40 vs $A\beta42^{MSD}$ /P-tau	0.0030	-0.0094 to 0.0154	0.6353

AUC, area under the curve; INC, Innotest classical; INM, Innotest modified; EI, Euroimmun; MSD, Mesoscale Discovery. Significant results are shown in bold.

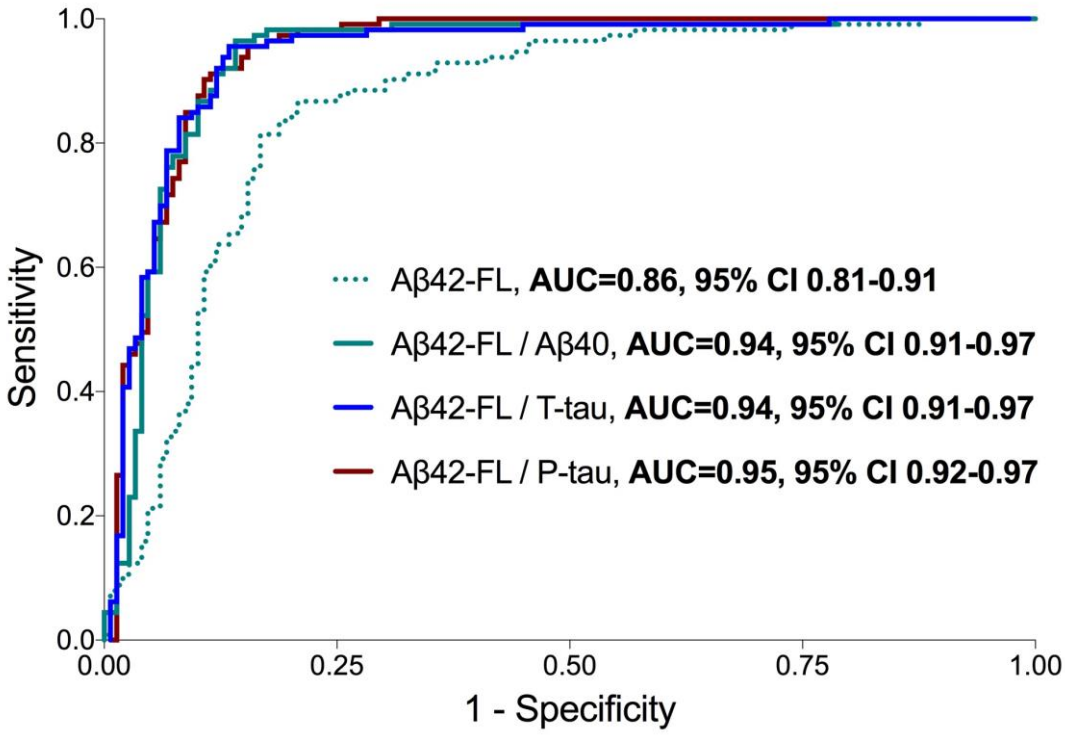
eTable 3. Concordance Between CSF Biomarkers and Visual PET Rating

	PET_{vis}-neg	PET_{vis}-pos	Cohen's κ
INC			
$Aβ42^{INC}$-neg	46.6%	1.5%	0.76
$Aβ42^{INC}$-pos	**10.3%**	41.6%	
$Aβ42^{INC}$/Aβ40-neg	46.9%	4.2%	0.72
$Aβ42^{INC}$/Aβ40-pos	**9.9%**	38.9%	
$Aβ42^{INC}$/P-tau-neg	50.4%	2.7%	0.81
$Aβ42^{INC}$/P-tau-pos	**6.5%**	40.5%	
INM			
$Aβ42^{INM}$-neg	42.4%	3.8%	0.63
$Aβ42^{INC}$-pos	**14.5%**	39.3%	
$Aβ42^{INM}$/Aβ40-neg	48.5%	3.4%	0.76
$Aβ42^{INM}$/Aβ40-pos	**8.4%**	39.7%	
$Aβ42^{INM}$/P-tau-neg	49.6%	2.3%	0.80
$Aβ42^{INM}$/P-tau-pos	**7.3%**	40.8%	
EUROIMMUN			
$Aβ42^{EI}$-neg	45.8%	7.6%	0.62
$Aβ42^{EI}$-pos	**11.1%**	35.5%	
$Aβ42^{EI}$/Aβ40-neg	50.4%	3.4%	0.79
$Aβ42^{EI}$/Aβ40-pos	**6.5%**	39.7%	
$Aβ42^{EI}$/P-tau-neg	49.2%	2.3%	0.80
$Aβ42^{EI}$/P-tau-pos	**7.6%**	40.8%	
Mesoscale discovery			
$Aβ42^{MSD}$-neg	43.5%	2.7%	0.68
$Aβ42^{MSD}$-pos	**13.4%**	40.5%	

Aβ42^{MSD}/Aβ40-neg	51.1%	2.3%	
			0.84
Aβ42^{MSD}/Aβ40-pos	**5.7%**	40.8%	
Aβ42^{MSD}/P-tau-neg	49.2%	1.5%	
			0.82
Aβ42^{MSD}/P-tau-pos	**7.6%**	41.6%	
MS			
Aβ42^{MS}-neg	43.9%	5.1%	
			0.60
Aβ42^{MS}-pos	**15.3%**	35.7%	
Aβ42/Aβ40^{MS} -neg	53.1%	1.0%	
			0.85
Aβ42/Aβ40^{MS} -pos	**6.1%**	39.8%	

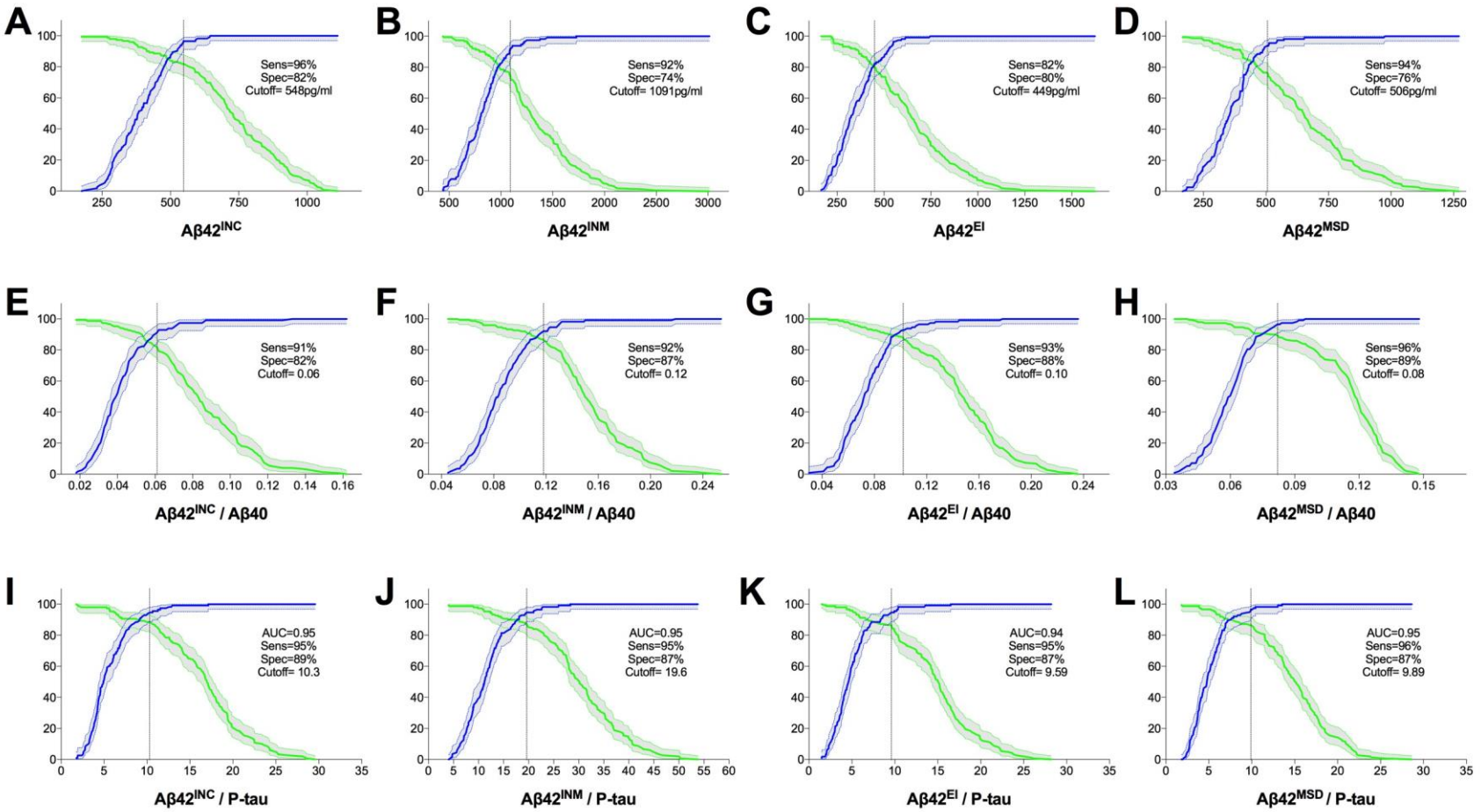
CSF Aβ42 positive and visual PET negative discordant group, where the strongest effect of the ratios was observed, is shown in bold.
INC, Innotest classical; INM, Innotest modified; EI, Euroimmun; MS, mass spectrometry; MSD, Mesoscale Discovery.

eFigure 1. CSF AD Biomarkers as Predictors of Amyloid PET Status According to Visual Analysis



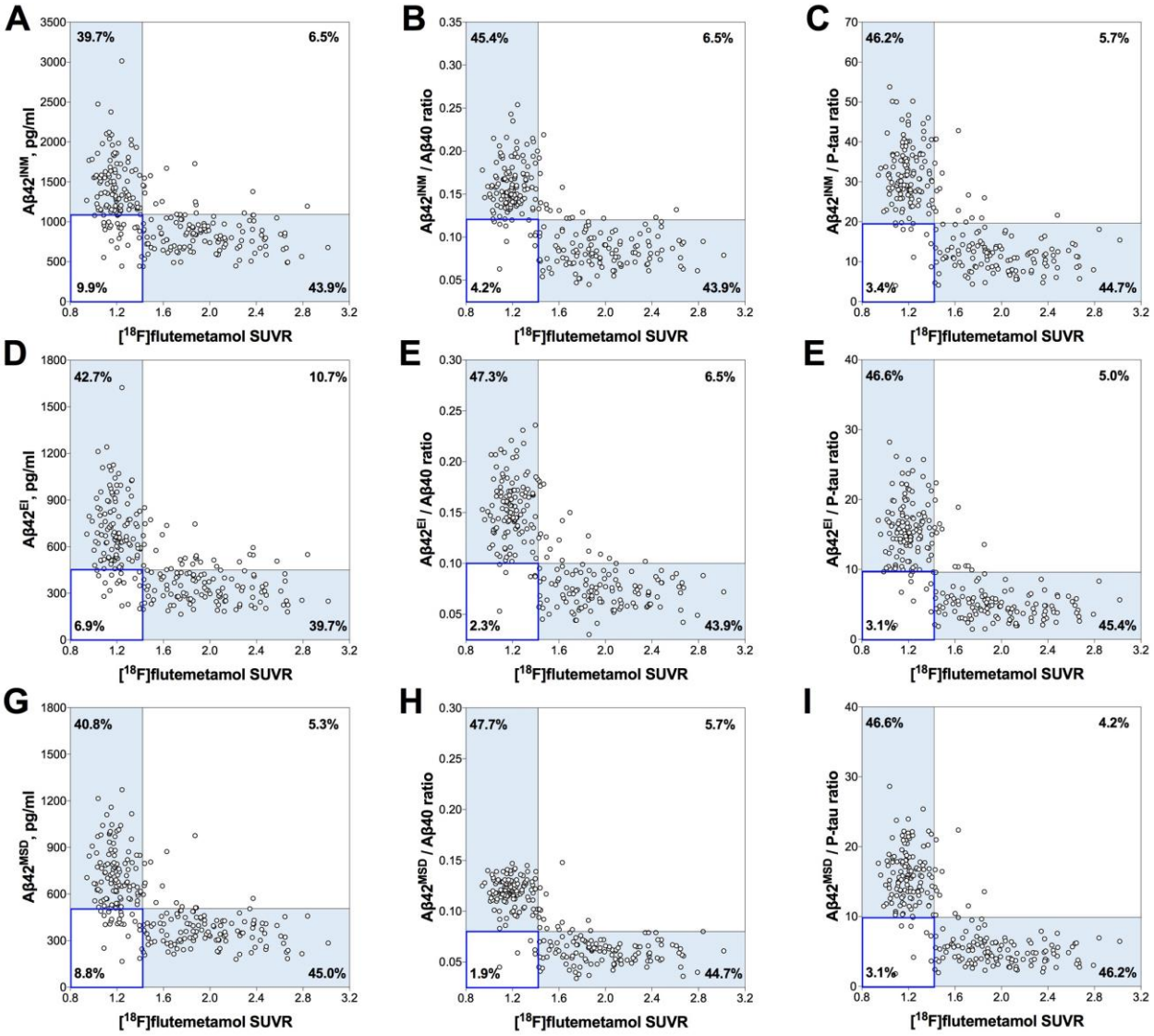
CSF Aβ42 was analyzed with fully automated Lumipulse assay from Fujirebio. CSF Aβ40, T-tau, and P-tau were measured as described in the materials and methods. ROC curves were generated for Aβ42, the Aβ42/Aβ40, the Aβ42/T-tau, and the Aβ42/P-tau ratios to determine their accuracy in differentiating PET PET Aβ-negative (n=149) and Aβ-positive (n=113) visual readings. AUC, area under the curve; FL, Fujirebio Lumipulse.

eFigure 2. Sensitivities and Specificities of CSF Aβ42 and the Aβ42/Aβ40 and Aβ42/P-tau Ratios at Different Cutoffs For Predicting Visual Amyloid PET Assessment



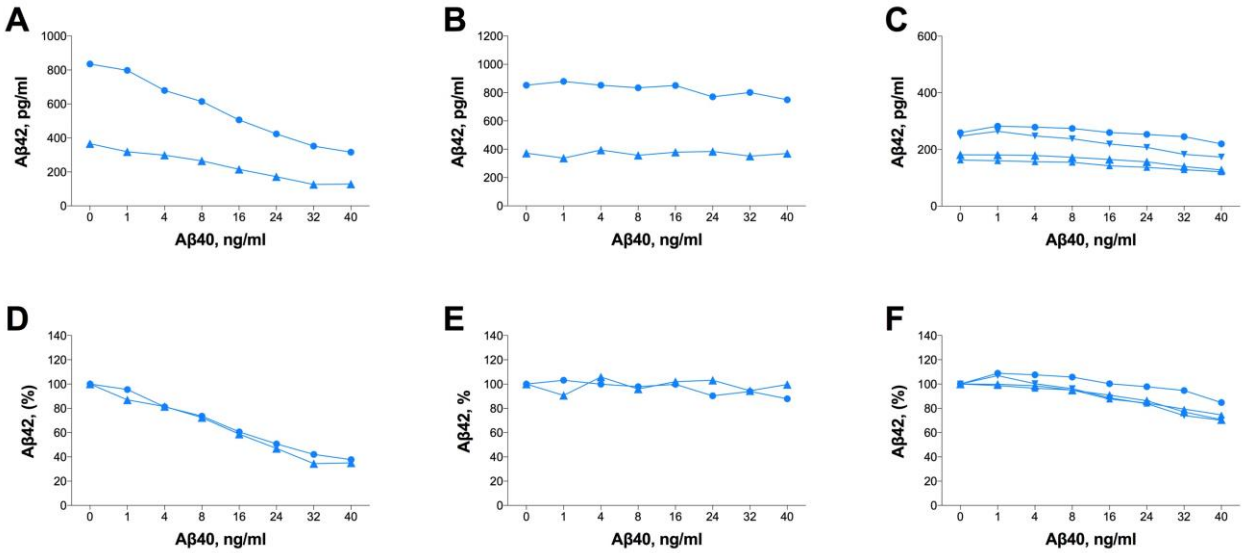
Sensitivity (blue curves) and specificity (green curves) were derived from the receiver-operator characteristic (ROC) curve analysis. The shaded area around the curves represents 95% confidence interval. Dashed lines indicate cutoff points associated with Youden's index. INC, Innotest classical; INM, Innotest modified; EI, Euroimmun; MSD, Mesoscale Discovery; Sens, sensitivity; Spec, specificity.

eFigure 3. Agreement Between CSF Aβ Biomarkers and Amyloid PET SUVR



Scatterplots of [^{18}F]flutemetamol SUVR and CSF Aβ42^{INM} (**A**), Aβ42^{INM}/Aβ40 (**B**), Aβ42^{INM}/P-tau (**C**), Aβ42^{EI} **(D)**, Aβ42^{EI}/Aβ40 (**E**), Aβ42^{EI}/P-tau (**F**), Aβ42^{MSD} (**G**), Aβ42^{MSD}/Aβ40 (**H**) and Aβ42^{MSD}/P-tau (**I**). Percentages of the discordant and concordant cases are shown in the corners of the quadrants. Horizontal lines indicate Youden's index cutoffs for CSF biomarkers. Vertical lines indicate cutoff >1.42 SUVR. Data on quantitative [18F]flutemetamol PET and CSF Aβ measured using EI and MSD assays was previously reported[4] and are shown in Fig.S2 for comparison with other assays. EI, Euroimmun; INM, Innotest modified; MSD, Mesoscale Discovery; PET, positron emission tomography; SUVR, the standardized uptake value ratio.

eFigure 4. Spiking of CSF Samples With Increasing Concentrations of Aβ 1-40



Two CSF samples were spiked with 0, 1, 4, 8, 16, 24, 32, and 40 ng/ml of Aβ 1-40. CSF concentration of Aβ42 was determined using the classical (**A, C**) and modified (**B, D**) Innotest assays as well as MSD (**C, F**) assay as described in the materials and methods.