Hansson *et al*., CSF biomarkers of Alzheimer's disease concord with amyloid-β PET and predict clinical progression: A study of fully-automated immunoassays in BioFINDER and ADNI cohorts

Supplementary materials

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Supplementary methods

Ethics approval

Part 1: The prospective and longitudinal Swedish BioFINDER cohort (www.biofinder.se[1]) study was approved by the Regional Ethics Committee in Lund, Sweden, and all study participants gave their informed consent to participate in the study. The study participants that had undergone [^{18}F]flutemetamol PET imaging had also signed additional informed consent forms agreeing to this procedure, and PET was evaluated and approved by the Swedish Medical Products Agency as well as the radiation committee of Skåne University Hospital, Sweden.

Part 2: Participants signed written informed consent forms. This study was approved by the local ethics committee in Lund, Sweden.

Part 3: Clinical data used in the preparation of this manuscript were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu; downloaded on November 21, 2016). The ADNI was launched in 2004 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD. Written informed consent was obtained for participation in these studies, as approved by the institutional review board at each participating center. For up-to-date information, see www.adni-info.org.

CSF measurement

CSF samples were measured by using the Elecsys β-Amyloid(1-42) CSF, and the Elecsys Phospho-Tau (181P) CSF, and Elecsys Total-Tau CSF immunoassays. CSF samples were excluded from analysis if visibly hemolyzed. In BioFINDER, Aβ(1–42), pTau, and tTau were measured individually from the same aliquot, but pTau and tTau were measured on a separate run 7 months after Aβ(1–42); this was due to a delay in the Tau assays' availability. In ADNI, Aβ(1–42), pTau, and tTau were measured individually from the same aliquot in the same run. For Part 2, the pre-analytical adjustment step, samples from one patient (but prepared with different pre-analytical procedures) were measured within one run where possible; more than one patient were measured on each run. For each approved run, the measurements of quality controls had to be within the predefined quality control limits (e.g., Westgard rules).

Part 2: CSF collection pre-analytical handling

A lumbar fluid manometer (LFM) was used for the collection of the CSF, due to the high volume of the CSF collected in the NPH patients. However, the first 2 mL of CSF, which internal analyses indicated is most affected by the LFM (data not shown), was discarded as per the study protocol. The same LFM type was used for the collection of all CSF samples. CSF samples designated to this study were collected at the end of the procedure; the first 8 mL of CSF from each LP was used for routine clinical analysis.

The samples were processed using 2 different pre-analytical procedures based on the ADNI and BioFINDER protocols (Supplementary Table 2).

Amyloid PET visual read analysis

Readers were trained in, and employed, the FDA-approved visual read algorithm developed for each tracer; these algorithms were vetted against histopathology truth standards in separate Phase 3 registration trials.[2,3] The visual read methods were validated against a histopathology cohort in end-of-life patients receiving scans prior to *post mortem*. Three independent board-certified nuclear medicine physicians with experience in neuro-PET imaging performed the visual read. Per MNI standard procedure, each reader was assigned a proctor for each day of the read. Readers were blinded to any clinical information, including the patient's clinical status, diagnosis, and CSF biomarker measurements.

Prior to the blinded read, each reader, proctor and staff member assigned to perform quality control (QC), underwent training. Proctors were trained on how to launch the PMOD software and the electronic case report form (eCRF). Those performing QC were trained to bring up a screen capture of each read image and verify that the PET code on the printed eCRF matched the image that was displayed in PMOD and eCRF on the screen capture. In addition, they were trained to verify that both the proctor and reader signed each printed CRF. The readers also received training on how to view the images in PMOD, reorient the images, adjust the thresholding and windowing; additionally, they were shown how to bring up the eCRF and complete the form.

The readers were required to complete the FDA-approved GE Healthcare Vizamyl Electronic Reader Training before reading the images of blinded read. For the blinded read, the readers were presented with 10% "repeat images"—images chosen at random from the complete list of scans using the random code generator. These repeat images were used to assess the intra-rater consistency of the blinded readers. The 'majority vote' was defined as two out of three readers in agreement as to whether the scan was positive or negative.

For the visual read amyloid-β PET analysis, inter-reader agreements were calculated for each reader pair as the PPA, NPA, and OPA for each reader pair (each reader was used once as the reference reader and once as the comparison reader in the calculations) (Supplementary Table 3).

CSF biomarker values beyond the technical limit of the assay

The measuring range of the Elecsys β-Amyloid (1-42) CSF immunoassay assay is 200 pg/mL (lower technical limit) to 1700 pg/mL (upper technical limit). The performance of the assay beyond the upper technical limit has not been formally established. Therefore, use of values above the upper technical limit, which are provided based on an extrapolation of the calibration curve, is restricted to exploratory research purposes and is excluded from clinical decision-making or for the derivation of medical decision points.

Some of the measured Aβ(1–42) values in the primary analysis populations in this study were above the measuring range (BioFINDER: 25%, pre-analytical comparison: 15%, ADNI: 16%). Accordingly, Aβ(1–42) values > 1700 pg/mL were handled as follows. For figures, concentrations values for the affected samples were estimated from the original signals based on the extrapolated calibration curves (using the calibration signals and target values of the calibrators). For the descriptive tables, Aβ(1–42) values > 1700 pg/mL were set to 1700 pg/mL and included in the analysis; median and median absolute deviation (MAD: median(|Xi – median(X)|) x 1·4826, a robust estimator of the population standard deviation) are reported instead of mean and standard deviation. For the concordance analysis, in the case of the single marker analyses, values outside the measuring range were set to the respective technical limit and included in the analysis. In the case of the biomarker ratios pTau/Aβ(1–42) and tTau/Aβ(1–42), Aβ(1–42) concentrations below the lower technical limit as well as pTau and tTau concentrations above the upper and below the lower technical limit were set to the respective technical limit (lower and upper limits for pTau are 8 and 120 pg/mL, for tTau 80 and 1300 pg/mL, respectively). In total, 6 pTau values and 1 tTau value were below the technical limit in BioFINDER; no values were outside the technical limit in ADNI. The handling of Aβ(1–42) concentrations above the upper technical limit is part of the biomarker rule: *If Elecsys Aβ(1–42) > 1700 pg/mL, test result: negative. Else, compute Tau/Aβ(1–42) ratio and define test result based on the biomarker ratio: If ratio Tau/Aβ(1–42) > cutoff, define test result as positive.*

Else, define test result as negative. For the pre-analytical method comparison, three Aβ(1–42) values outside measuring range (according to the "BioFINDER" protocol) were excluded from the analysis.

ADNI sensitivity analysis

As a sensitivity analysis, new cutoffs were derived in ADNI in a similar way to BioFINDER, as described in the main methods section (fixing the PPA value that optimized the cutoff in BioFINDER).

Additional statistical methods

Data analysis was performed using R, version 3.2.2 and R packages *mcr*, version 1.2.1, *mixtools*, version 1.0.4, *ADNIMERGE*, version 0.0.1, *ggplot2*, version 2.2.1 and SAS version 9.4.

In Part 1, cutoffs for the CSF biomarkers Aβ(1–42), pTau/Aβ(1–42) and tTau/Aβ(1–42) were determined to optimize concordance with PET visual read in BioFINDER based on two criteria: 1) performance and 2) robustness. That is, 1) a trade-off between positive percent agreement (PPA, "sensitivity") and negative percent agreement (NPA, "specificity"), and 2) the stability of PPA and NPA at the chosen cutoff when varying the cutoffs slightly. Besides PPA and NPA, the overall percent agreement (OPA, the proportion of patients classified the same by CSF and PET) was calculated as well as negative (NPV) and positive predictive values (PPV). Agreement measures (PPA, "sensitivity", NPA, "specificity", as well as OPA, overall percent agreement) were determined with exact binomial 95% confidence intervals (CIs). Moreover, the area-under the receiver-operator curve (AUC) with 95% CIs for the CSF biomarkers and visual-read or SUVR-based PET classification was calculated.

To determine the "natural cutoff" for quantitative PET SUVR-based classification, a two-component Gaussian-mixture-modeling approach was applied to the bimodal univariate distribution of the SUVRs.[4] The crossing point of the equal weighted fitted Gaussian curves was taken as a "natural cutoff". Patients with SUVR values above and below the natural cutoff (BioFINDER=1·24; ADNI=1·16) were classified as SUVR-positive and -negative, respectively.

A linear mixed-effects model (with random intercept) of CDR-SB change over 2 years was used to analyze the predictive properties of CSF biomarkers. Specifically, CDR-SB was used as outcome, and biomarker status (binary variable based on respective cutoff), visit timepoint (baseline, 6, 12, and 24 months, as categorical variable), and the interaction between both were used as independent variables. Additional covariates included age, sex, education, baseline CDR-SB score, and interaction term baseline CDR-SB score and visit timepoint. The CDR-SB change from baseline to 2 years was extracted from the least squares means of the model for the biomarker-negative and biomarker-positive subjects. The difference between CDR-SB change in both biomarker groups was also evaluated (as estimated by the interaction term between biomarker status and visit timepoint).

Supplementary results

Visual read amyloid-β PET analysis; intra- and inter-rater agreement

Three readers independently evaluated the amyloid-β PET images. For BioFINDER, the overall agreement between any pair of readers averaged 90·1%. Reader 2 vs 3 had an overall agreement of 94·8%, whereas reader 1 vs 2 and reader 1 vs 3 had a lower overall agreement. For ADNI, inter-reader agreement OPA, PPA, NPA were slightly higher (93–94%; Supplementary Table 3).

Part 2: Pre-analytical protocol comparison

Results from 'ADNI' and 'BioFINDER' protocols showed a linear relationship and very high correlation (Pearson's correlation coefficient for Aβ(1–42)=0·9664, pTau=0·9988, tTau=0·9982) for all CSF biomarkers (Supplementary Figure 5 D–F), suggesting that the proportional dependency between 'ADNI' and 'BioFINDER' levels was highly consistent. Method comparison analysis yielded intercept estimates that were not significantly different from zero for all three biomarkers (Supplementary Figure 5 A–C). This observation supported the decision to take proportional dependency without offset as an assumption about dependency between biomarker measurements in 'BioFINDER' and 'ADNI' samples.

We observed significant systematic differences between 'BioFINDER' and 'ADNI' pre-analytical procedures for Aβ(1–42) levels (mean percentage difference ADNI vs. BioFINDER (95% CI): -23·7% (-27·4%, -19·9%), p-value of paired t-test < 0·001). No meaningful differences were observed for pTau (0·7% (-0·2%, 1·6%), p=0·135) and tTau (0·6% (-0·5%, 1·8%), p=0·285). Considerable systematic differences between 'ADNI' and 'BioFINDER' procedures obtained for Aβ(1–42) levels suggested necessity to adjust the Aβ(1–42) cutoff defined in BioFINDER for ADNI. While trying to replicate the investigated CSF collection protocols as accurately as possible, the ADNI pre-analytical procedure includes a large number of steps, which may not have been exactly followed in our study. Furthermore, as described in the results of Part 1, the performance of Aβ(1–42), in terms of concordance with amyloid PET, was more severely affected if the cutoff is too low than if the cutoff is too high. Based on these considerations, we decided to use a conservative approach and use the upper 95% confidence limit of the estimated percentage difference between 'ADNI' and 'BioFINDER' that we observed in our study (20%) for the calculation of the adjustment factor for Aβ(1–42) cutoff. As a result, the proposed cutoff adjustment factor was 0·8: Aβ(1–42) (ADNI)= 0·8*Aβ(1–42) (BioFINDER). The BioFINDER cutoffs for the ratios pTau/Aβ(1–42) and tTau/Aβ(1–42) were transferred for the ADNI pre-analytical procedure using the inverse adjustment factor $0{\cdot}8^{-1}$. This resulted in CSF biomarker cutoff values to be evaluated in ADNI: Aβ(1–42): 880 pg/mL, pTau/Aβ(1–42): 0·028, Tau/Aβ(1–42): 0·33, with the rule for Aβ(1–42) > 1700 pg/mL as specified in the supplementary methods.

ADNI sensitivity analysis

A cutoff determination analogous to Part 1 was performed for the ADNI study population as a sensitivity analysis. The resulting optimized CSF biomarker cutoffs were 977 pg/mL, 0·0251, and 0·27 for Aβ(1–42), pTau/Aβ(1–42), and tTau/Aβ(1–42), respectively. At these cutoffs, the overall agreement with visual read amyloid-β PET classification was high (Supplementary Table 8).

Supplementary tables

Supplementary Table 1: Demographics and characteristics of Elecsys CSF measurements for the BioFINDER and ADNI cohorts. *MAD=median absolute deviation; NA=not applicable.*

	BioFINDER					ADNI					
	All (N=728) *	**NC (N=237)**	**SCD (N=191)**	**MCI (N=233)**	**AD (N=60)**	**All (N=918) †**	**CN (N=188)**	**SMC (N=106)**	**EMCI (N=310)**	**LMCI (N=164)**	**AD (N=150)**
Study Phase											
ADNI-GO, n (%)						129 (14·1%)	0 (0·0%)	0 (0·0%)	129 (41·6%)	0 (0·0%)	N=0 (0·0%)
ADNI-2, n (%)						789 (85·9%)	188 (100·0%)	106 (100·0%)	181 (58·4%)	164 (100·0%)	150 (100·0%)
Age, mean years (SD)	72·0 (5·67)	74·0 (4·79)	69·9 (5·67)	70·7 (5·50)	75·9 (5·25)	72·5 (7·28)	73·4 (6·25)	72·2 (5·56)	71·2 (7·50)	72·2 (7·50)	74·7 (8·21)
Sex, n male (%)	352 (48·4%)	93 (39·2%)	89 (46·6%)	142 (60·9%)	26 (43·3%)	480 (52·3%)	90 (47·9%)	44 (41·5%)	171 (55·2%)	87 (53·0%)	88 (58·7%)
Sex, n female (%)	376 (51·6%)	144 (60·8%)	102 (53·4%)	91 (39·1%)	34 (56·7%)	438 (47·7%)	98 (52·1%)	62 (58·5%)	139 (44·8%)	77 (47·0%)	62 (41·3%)
Education, n	711	237	191	231	49	918	188	106	310	164	150
Mean years (SD)	11·7 (3·48)	12·11 (3·46)	12·55 (3·56)	11·19 (3·28)	9·43 (2·60)	16·2 (2·63)	16·5 (2·56)	16·8 (2·52)	16·0 (2·66)	16·5 (2·61)	15·8 (2·68)
***ApoE4* risk alleles, n**	720	234	189	233	57	906	186	106	306	162	146
0 e4, n (%)	425 (59·0%)	170 (72·6%)	114 (60·3%)	116 (49·8%)	21 (36·8%)	497 (54·9%)	133 (71·5%)	71 (67·0%)	175 (57·2%)	70 (43·2%)	48 (32·9%)
1 e4, n (%)	230 (31·9%)	56 (23·9%)	62 (32·8%)	86 (36·9%)	23 (40·4%)	326 (36·0%)	47 (25·3%)	34 (32·1%)	110 (36·0%)	66 (40·7%)	69 (47·3%)
2 e4, n (%)	65 (9·0%)	8 (3·4%)	13 (6·9%)	31 (13·3%)	13 (22·8%)	83 (9·2%)	6 (3·2%)	1 (0·9%)	21 (6·9%)	26 (16·1%)	29 (19·9%)
MMSE mean (SD)	27·6 (2·78)	29·0 (0·96)	28·5 (1·40)	27·1 (1·84)	21·3 (4·41)	27·6 (2·60)	29·0 (1·26)	29·0 (1·19)	28·3 (1·56)	27·6 (1·82)	23·1 (2·08)
Visual PET, n	398	121	120	153	0	888	182	103	301	157	145
Negative, n (%)	275 (69·1%)	108 (89·3%)	91 (75·8%)	74 (48·4%)	0 (0%)	480 (54·1%)	149 (81·9%)	78 (75·7%)	183 (60·8%)	53 (33·8%)	17 (11·7%)
Positive, n (%)	123 (30·9%)	13 (10·7%)	29 (24·2%)	79 (51·6%)	0 (0%)	408 (46·0%)	33 (18·1%)	25 (24·3%)	118 (39·2%)	104 (66·2%)	128 (88·3%)
SUVR, n	352	119	108	123	0	888	182	103	302	157	144
Mean (SD)	1·29 (0·317)	1·17 (0·202)	1·26 (0·294)	1·44 (0·365)	NA	1·26 (0·264)	1·15 (0·196)	1·15 (0·202)	1·21 (0·233)	1·35 (0·271)	1·50 (0·248)
Elecsys CSF biomarker, n	728	237	191	233	60	819	160	95	277	155	132
Aβ(1–42), median pg/mL (MAD)	1194 (696)	1442 (383)	1386 (466)	963 (505)	667 (258)	925 (523)	1285 (615)	1328 (552)	1052 (566)	787 (291)	617 (220)
pTau, median pg/mL (MAD)	19·9 (8·41)	18·5 (5·78)	18·2 (6·82)	21·2 (10·24)	33·7 (13·68)	23·0 (11·0)	19·3 (7·14)	19·0 (7·93)	20·7 (8·94)	27·7 (13·12)	33·2 (12·30)
tTau, median pg/mL (MAD)	232 (89·0)	217 (68·5)	217 (78·6)	248 (101·1)	389 (118·1)	248 (105)	211 (74·0)	218 (82·0)	234 (92·1)	287 (130·3)	335 (129·9)
Ratio pTau/Aβ(1–42), median (MAD)	0·015 (0·009)	0·013 (0·004)	0·013 (0·006)	0·026 (0·022)	0·045 (0·034)	0·024 (0·019)	0·015 (0·007)	0·015 (0·007)	0·017 (0·011)	0·037 (0·030)	0·058 (0·035)
Ratio tTau/Aβ(1–42), median (MAD)	0·184 (0·107)	0·153 (0·051)	0·153 (0·064)	0·279 (0·205)	0·489 (0·254)	0·263 (0·197)	0·172 (0·080)	0·165 (0·076)	0·202 (0·125)	0·389 (0·273)	0·569 (0·256)

*BioFINDER: All patients with CSF measurements available, including patients with missing visual PET, 7 patients of the MCS cohort did not have the subclassification for SCD or MCI; †ADNI: All patients, including patients with missing visual PET or CSF values are shown.

Supplementary Table 2: (Part 2) Overview of the pre-analytical CSF handling protocols of BioFINDER and ADNI cohort

ADNI	BioFINDER
Collect 9 mL of CSF using a gravity drip in each of two x 14 mL primary collection tubes (BD Falcon 14-959-10B). Total volume: 18 mL	Collect 10 mL of CSF in each of two 13 mL primary collection tubes (Sarstedt 60·540·012). Total volume: 20 mL
Do not mix tubes	
Do not centrifuge	Centrifuge for 10 min at 2000g at 4°C
Transfer 9 mL of CSF from each of the two 14 mL primary collection tubes into 2 x 13 mL secondary tubes (Sarstedt 60·541) each with a sterile disposable polyethylene transfer pipets (Beral#137115AMEND). Repeat to obtain two 13 mL secondary tubes with 9 mL of CSF in each secondary tube	Transfer CSF from both 13 mL primary collection tubes into one Sarstedt (62·547·254, 50 mL) PP secondary tube using disposable transfer pipets (PP-plastic, VWR, Cat# 612-4494)
Mix by soft rotations 3-4 times	Mix by rotating 3-4 times
Immediately freeze secondary tubes on dry ice for 20 min	Prepare approximately 18 x 1·0 mL aliquots in 2 mL tertiary tubes (Sarstedt Cat# 72·694·006) with a pipet (Pipette tips, 1·25mL, Sarstedt 70·11·86)
Store secondary tubes frozen at -60° C or below for ≥ 24h and subsequently send them to ADNI Biomarker Core Lab on dry ice	Do not mix aliquots
	Freeze aliquots (-60° C or below)
After ≥ 24 hours at ADNI Biomarker Core Lab: • Thaw secondary tubes on Rollermixer for 30 min, RT • Transfer CSF from both 13 mL secondary tubes into one 30 mL tertiary tube (Sarstedt 62·543·001) without any pipet • Mix 30 mL tertiary tube for 5 min at RT on Rollermixer • Prepare 30 x 0·5 mL aliquots in 0·5 mL quaternary tubes (Nalgene #967-21613) with a pipet using PP tips (Sarstedt 70·11·86) • Do not mix aliquots • Freeze aliquots (-60° C or below)	
After freezing: ship aliquots on dry ice to testing site	

Supplementary Table 3: Inter-reader agreement for visual read PET analysis. *NPA = negative percent agreement; OPA = overall percent agreement; PPA = positive percent agreement.*

	BioFINDER	ADNI
OPA, mean % (range)	90·1 (87·5–94·8)	93·4 (91·1–96·9)
PPA, mean % (range)	86·5 (71·0–98·4)	93·7 (83·3–99·8)
NPA, mean % (range)	93·0 (83·7–99·2)	94·0 (84·3–99·8)
Reader 1 vs 2, % OPA	87·5	96·9
Reader 2 vs 3, % OPA	94·8	91·1
Reader 1 vs 3, % OPA	88·0	92·2

Readers were not the same individuals across BioFINDER and ADNI analyses.

Supplementary Table 4: BioFINDER and ADNI clinical inclusion criteria. *MCI = mild cognitive impairment; MCS = mild cognitive symptoms; NINCDS/ADRDA = National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association; SMC = significant memory concern.*

<table>
<tr><th></th><th colspan="3">BioFINDER cohort[5]</th><th colspan="3">ADNI cohort[6]</th></tr>
<tr><th></th><th>Cognitively healthy elderly</th><th>MCS</th><th>AD dementia</th><th>SMC</th><th>MCI</th><th>AD dementia</th></tr>
<tr><td colspan="7">Inclusion criteria</td></tr>
<tr><td>MMSE score</td><td>27–30</td><td>24 – 30</td><td>-</td><td>24–30</td><td>24–30</td><td>20–26</td></tr>
<tr><td>Age, years</td><td>≥ 60</td><td>60 – 80</td><td>-</td><td>65–90</td><td>55–90</td><td>55–90</td></tr>
<tr><td rowspan="3">Other</td><td>Absence of cognitive symptoms.</td><td>Referred to the memory clinics due to cognitive symptoms experienced by the patient and/or informant.</td><td rowspan="3">Fulfills NINCDS/ADRDA criteria for AD dementia.</td><td rowspan="3">Score within normal range for cognition (or CDR=0) but indicate that they have a concern, and exhibit slight forgetfulness.</td><td rowspan="3">Report a subjective memory concern either autonomously or via an informant or clinician. No significant levels of impairment in other cognitive domains: essentially preserved activities of daily living and no signs of dementia.</td><td rowspan="3">Meets the NINCDS/ADRDA criteria for probable AD.</td></tr>
<tr><td>Do not fulfill criteria for MCI or any dementia disorder.</td><td>Do not fulfill the criteria for any dementia disorder.
Essentially preserved activities of daily living.</td></tr>
<tr><td colspan="2">Fluent in Swedish.</td></tr>
<tr><td colspan="7">Exclusion criteria</td></tr>
<tr><td rowspan="2"></td><td colspan="2">Significant neurologic or psychiatric illness; refusing lumbar puncture.</td><td>-</td><td colspan="3" rowspan="2">Significant neurologic disease, major depression or history or schizophrenia, history of alcohol or substance abuse or dependence within the past 2 years. Participation in clinical studies involving neuropsychological measures being collected more than one time per year.</td></tr>
<tr><td colspan="3">Significant unstable systemic illness or organ failure, such as terminal cancer, that makes it difficult to participate in the study. Current alcohol or substance misuse.</td></tr>
</table>

Supplementary Table 5: Agreement between CSF biomarkers and visual PET by patient cohort in the BioFINDER study. *NPA = negative percent agreement; NPV = negative predictive value; OPA = overall percent agreement; PPA = positive percent agreement; PPV = positive predictive value.*

CSF biomarker	Cohort	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	PET scans rated positive, %
Aβ(1–42)	NC	92·3 (64·0–99·8)	83·3 (74·9–89·8)	84·3 (76·6–90·3)	40·0 (22·7–59·4)	98·9 (94·0–100·0)	10·7
	SCD	89·7 (72·6–97·8)	72·5 (62·2–81·4)	76·7 (68·1–83·9)	51·0 (36·6–65·2)	95·7 (87·8–99·1)	24·2
	MCI	92·4 (84·2–97·2)	71·6 (59·9–81·5)	82·4 (75·4–88·0)	77·7 (67·9–85·6)	89·8 (79·2–96·2)	51·6
pTau/Aβ(1–42)	NC	76·9 (46·2–95·0)	88·9 (81·4–94·1)	87·6 (80·4–92·9)	45·5 (24·4–67·8)	97·0 (91·4–99·4)	10·7
	SCD	86·2 (68·3–96·1)	90·1 (82·1–95·4)	89·2 (82·2–94·1)	73·5 (55·6–87·1)	95·3 (88·5–98·7)	24·2
	MCI	92·4 (84·2–97·2)	87·8 (78·2–94·3)	90·2 (84·3–94·4)	89·0 (80·2–94·9)	91·5 (82·5–96·8)	51·6
tTau/Aβ(1–42)	NC	84·6 (54·6–98·1)	88·9 (81·4–94·1)	88·4 (81·3–93·5)	47·8 (26·8–69·4)	98·0 (92·8–99·8)	10·7
	SCD	86·2 (68·3–96·1)	90·1 (82·1–95·4)	89·2 (82·2–94·1)	73·5 (55·6–87·1)	95·3 (88·5–98·7)	24·2
	MCI	92·4 (84·2–97·2)	87·8 (78·2–94·3)	90·2 (84·3–94·4)	89·0 (80·2–94·9)	91·5 (82·5–96·8)	51·6

Supplementary Table 6: Summary statistics of Aβ(1–42), tTau and pTau values for two pre-analytical protocols used in the BioFINDER and the ADNI cohorts. *Aβ(1–42) measurements were above the technical limit of the assay for three patients' samples prepared according to the BioFINDER protocol; these samples were excluded from the analysis, resulting in an analysis population of n=17 for Aβ(1–42).*

CSF biomarker		"BioFINDER" pre-analytical handling	"ADNI" pre-analytical handling
Aβ(1–42), n		17	17
	Median pg/mL (MAD)	852 (354)	667 (332)
	Min-Max pg/mL	428–1455	315–1152
pTau, n		20	20
	Median pg/mL (MAD)	11·5 (4·8)	11·8 (4·9)
	Min-Max pg/mL	8·2–23·9	8·2–24·0
tTau, n		20	20
	Median pg/mL (MAD)	159 (66)	161 (71)
	Min-Max pg/mL	96–246	89–249

Supplementary Table 7: Agreement between CSF biomarkers and visual PET by patient cohort in the ADNI cohort. *NPA = negative percent agreement; NPV = negative predictive value; OPA = overall percent agreement; PPA = positive percent agreement; PPV = positive predictive value. Pre-analytically adjusted cutoffs optimized from BioFINDER primary analysis population.*

CSF biomarker	Cohort	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)	PPV, % (95%CI)	NPV, % (95% CI)	PET scans rated positive, %
Aβ(1–42)	CN	82·1 (63·1–93·9)	86·2 (79–91·6)	85·4 (79–90·5)	56·1 (39·7–71·5)	95·7 (90·3–98·6)	17·7
	SMC	66·7 (44·7–84·4)	90 (80·5–95·9)	84 (75–90·8)	69·6 (47·1–86·8)	88·7 (79–95)	25·5
	EMCI	81·3 (72·6–88·2)	85·5 (79·1–90·5)	83·8 (78·9–88)	78·4 (69·6–85·6)	87·6 (81·5–92·2)	39·3
	LMCI	78·4 (69·2–86)	80 (66·3–90)	78·9 (71·6–85·1)	88·9 (80·5–94·5)	64·5 (51·3–76·3)	67·1
	AD	93·9 (87·8–97·5)	78·6 (49·2–95·3)	92·2 (86·1–96·2)	97·3 (92·2–99·4)	61·1 (35·7–82·7)	89·1
pTau/Aβ(1–42)	CN	82·1 (63·1–93·9)	93·1 (87·3–96·8)	91·1 (85·6–95·1)	71·9 (53·3–86·3)	96 (91–98·7)	17·7
	SMC	66·7 (44·7–84·4)	92·9 (84·1–97·6)	86·2 (77·5–92·4)	76·2 (52·8–91·8)	89 (79·5–95·1)	25·5
	EMCI	79·4 (70·5–86·6)	94·5 (89·9–97·5)	88·6 (84·2–92·1)	90·4 (82·6–95·5)	87·6 (81·9–92·1)	39·3
	LMCI	90·2 (82·7–95·2)	88 (75·7–95·5)	89·5 (83·5–93·9)	93·9 (87·1–97·7)	81·5 (68·6–90·7)	67·1
	AD	99·1 (95·2–100)	85·7 (57·2–98·2)	97·7 (93·3–99·5)	98·3 (93·9–99·8)	92·3 (64–99·8)	89·1
tTau/Aβ(1–42)	CN	75 (55·1–89·3)	94·6 (89·2–97·8)	91·1 (85·6–95·1)	75 (55·1–89·3)	94·6 (89·2–97·8)	17·7
	SMC	62·5 (40·6–81·2)	92·9 (84·1–97·6)	85·1 (76·3–91·6)	75 (50·9–91·3)	87·8 (78·2–94·3)	25·5
	EMCI	72·9 (63·4–81)	96·4 (92·3–98·7)	87·1 (82·6–90·9)	92·9 (85·1–97·3)	84·6 (78·6–89·4)	39·3
	LMCI	89·2 (81·5–94·5)	90 (78·2–96·7)	89·5 (83·5–93·9)	94·8 (88·3–98·3)	80·4 (67·6–89·8)	67·1
	AD	97·4 (92·5–99·5)	85·7 (57·2–98·2)	96·1 (91·1–98·7)	98·2 (93·8–99·8)	80 (51·9–95·7)	89·1

Supplementary Table 8: Performance of the CSF biomarkers vs. visual PET in ADNI for cutoffs optimized within the ADNI cohort. *NPA = negative percent agreement; OPA = overall percent agreement; PPA = positive percent agreement. Values in brackets are 95% confidence intervals.*

CSF biomarker	Cutoff	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)
Aβ(1–42)	976·6 pg/mL	92·2 (88·9–94·8)	80·9 (76·0–85·2)	87·0 (84·2–89·5)
pTau/Aβ(1–42)	0·0251	91·9 (88·5–94·6)	90·6 (86·8–93·7)	91·3 (88·9–93·4)
tTau/Aβ(1–42)	0·27	91·9 (88·5–94·6)	90·0 (86·0–93·1)	91·0 (88·5–93·1)

Supplementary Table 9: Agreement between CSF biomarkers and SUVR-based classification. *For BioFINDER ([^{18}F]flutemetamol tracer) the SUVR cutoff 1·24 was used, for ADNI ([^{18}F]florbetapir tracer) 1·16· NPA = negative percent agreement; OPA = overall percent agreement; PPA = positive percent agreement. Cutoffs optimized from BioFINDER primary analysis population.*

Cohort	CSF biomarker	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)
BioFINDER	Aβ(1–42)	88·0 (80·3–93·4)	84·8 (77·3–90·6)	86·3 (81·2–90·4)
	pTau/Aβ(1–42)	85·2 (77·1–91·3)	97·6 (93·1–99·5)	91·8 (87·6–95·0)
	tTau/Aβ(1–42)	85·2 (77·1–91·3)	97·6 (93·1–99·5)	91·8 (87·6–95·0)
ADNI	Aβ(1–42)	79·2 (74·6–83·2)	84·3 (79·5–88·3)	81·4 (78·2–84·3)
	pTau/Aβ(1–42)	84·7 (80·5–88·2)	93·6 (90·0–96·1)	88·5 (85·8–90·9)
	tTau/Aβ(1–42)	80·8 (76·4–84·7)	93·9 (90·5–96·4)	86·5 (83·6–89·1)

Supplementary Table 10: Agreement between CSF biomarkers and SUVR-based classification by patient cohort in the BioFINDER study. *NPA = negative percent agreement; NPV = negative predictive value; OPA = overall percent agreement; PPA = positive percent agreement; PPV = positive predictive value. Cutoffs optimized from primary analysis population.*

CSF biomarker	Cohort	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)	PPV, % (95%CI)	NPV, % (95% CI)	PET scans rated positive by SUVR, %
Aβ(1–42)	NC	69·6 (47·1–86·8)	86·5 (78·0–92·6)	83·2 (75·2–89·4)	55·2 (35·7–73·6)	92·2 (84·6–96·8)	19·3
	SCD	86·8 (71·9–95·6)	85·7 (75·3–92·9)	86·1 (78·1–92·0)	76·7 (61·4–88·2)	92·3 (83·0–97·5)	35·2
	MCI	89·9 (80·2–95·8)	83·3 (70·7–92·1)	87·0 (79·7–92·4)	87·3 (77·3–94·0)	86·5 (74·2–94·4)	56·1
pTau/Aβ(1–42)	NC	78·3 (56·3–92·5)	95·8 (89·7–98·9)	92·4 (86·1–96·5)	81·8 (59·7–94·8)	94·8 (88·4–98·3)	19·3
	SCD	78·9 (62·7–90·4)	97·1 (90·1–99·7)	90·7 (83·6–95·5)	93·8 (79·2–99·2)	89·5 (80·3–95·3)	35·2
	MCI	88·4 (78·4–94·9)	98·1 (90·1–100·0)	92·7 (86·6–96·6)	98·4 (91·3–100·0)	86·9 (75·8–94·2)	56·1
tTau/Aβ(1–42)	NC	78·3 (56·3–92·5)	95·8 (89·7–98·9)	92·4 (86·1–96·5)	81·8 (59·7–94·8)	94·8 (88·4–98·3)	19·3
	SCD	78·9 (62·7–90·4)	97·1 (90·1–99·7)	90·7 (83·6–95·5)	93·8 (79·2–99·2)	89·5 (80·3–95·3)	35·2
	MCI	88·4 (78·4–94·9)	98·1 (90·1–100·0)	92·7 (86·6–96·6)	98·4 (91·3–100·0)	86·9 (75·8–94·2)	56·1

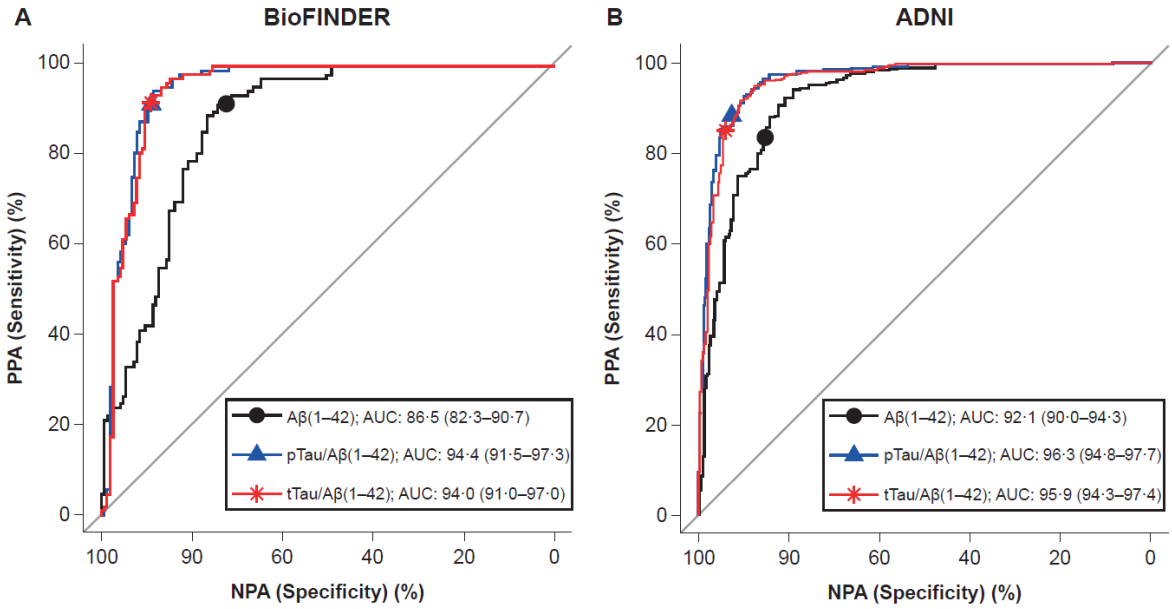
Supplementary Table 11: Agreement between CSF biomarkers vs. SUVR-based classification by patient cohort in the ADNI study*. NPA = negative percent agreement; NPV = negative predictive value; OPA = overall percent agreement; PPA = positive percent agreement PPV = positive predictive value. Cutoffs optimized from BioFINDER primary analysis population.*

CSF biomarker	Cohort	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)	PPV, % (95%CI)	NPV, % (95% CI)	PET scans rated positive by SUVR, %
Aβ(1–42)	CN	60·4 (45·3–74·2)	89·1 (81·7–94·2)	80·4 (73·3–86·3)	70·7 (54·5–83·9)	83·8 (75·8–89·9)	30·4
	SMC	56·7 (37·4–74·5)	90·6 (80·7–96·5)	79·8 (70·2–87·4)	73·9 (51·6–89·8)	81·7 (70·7–89·9)	31·9
	EMCI	74·6 (65·7–82·1)	85·1 (78·4–90·3)	80·5 (75·3–85·1)	79·3 (70·5–86·4)	81·4 (74·5–87·1)	43·4
	LMCI	76·9 (67·6–84·6)	79·2 (65–89·5)	77·6 (70·2–84)	88·9 (80·5–94·5)	61·3 (48·1–73·4)	68·4
	AD	92 (85·4–96·3)	64·3 (35·1–87·2)	89 (82·2–93·8)	95·4 (89·6–98·5)	50 (26–74)	89
pTau/Aβ(1–42)	CN	60·4 (45·3–74·2)	97·3 (92·2–99·4)	86·1 (79·7–91·1)	90·6 (75–98)	84·9 (77·5–90·7)	30·4
	SMC	56·7 (37·4–74·5)	93·8 (84·8–98·3)	81·9 (72·6–89·1)	81 (58·1–94·6)	82·2 (71·5–90·2)	31·9
	EMCI	74·6 (65·7–82·1)	96·1 (91·7–98·6)	86·8 (82·2–90·6)	93·6 (86·6–97·6)	83·1 (76·8–88·3)	43·4
	LMCI	90·4 (83–95·3)	91·7 (80–97·7)	90·8 (85–94·9)	95·9 (89·9–98·9)	81·5 (68·6–90·7)	68·4
	AD	97·3 (92·4–99·4)	71·4 (41·9–91·6)	94·5 (89–97·8)	96·5 (91·3–99)	76·9 (46·2–95)	89
tTau/Aβ(1–42)	CN	56·3 (41·2–70·5)	99·1 (95–100)	86·1 (79·7–91·1)	96·4 (81·7–99·9)	83·8 (76·4–89·7)	30·4
	SMC	53·3 (34·3–71·7)	93·8 (84·8–98·3)	80·9 (71·4–88·2)	80 (56·3–94·3)	81·1 (70·3–89·3)	31·9
	EMCI	66·1 (56·8–74·6)	96·1 (91·7–98·6)	83·1 (78·1–87·3)	92·9 (85·1–97·3)	78·7 (72·2–84·3)	43·4
	LMCI	89·4 (81·9–94·6)	93·8 (82·8–98·7)	90·8 (85–94·9)	96·9 (91·1–99·4)	80·4 (67·6–89·8)	68·4
	AD	95·6 (90–98·5)	71·4 (41·9–91·6)	92·9 (87–96·7)	96·4 (91·1–99)	66·7 (38·4–88·2)	89

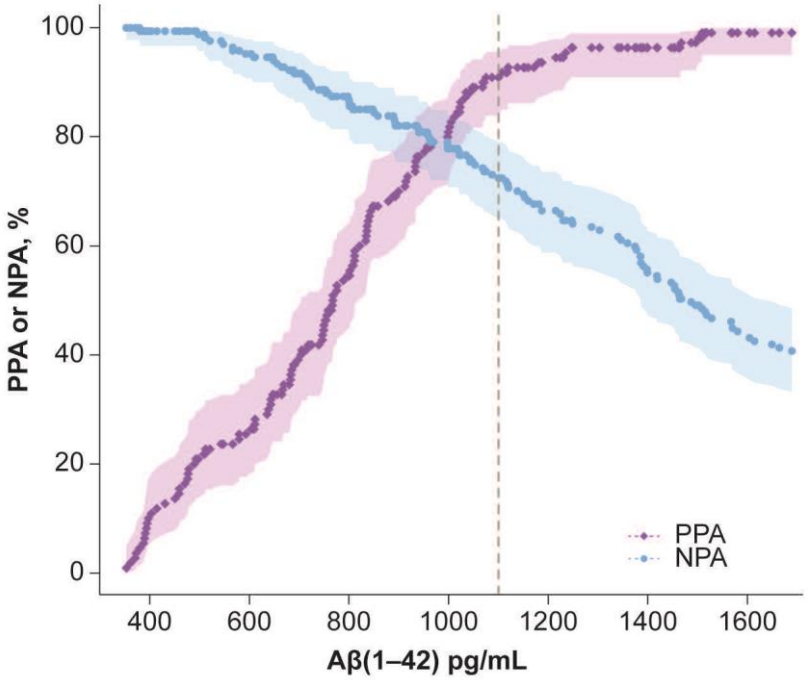
Supplementary Table 12: Prediction of clinical decline by the Elecsys biomarkers. *The pre-specified (and pre-analytically adjusted) cutoffs were used. Clinical decline was defined based on change in CDR-SB over 2 years (see methods for detail).*

	CDR-SB score change from baseline at 2 years, estimate (95% CI)		
Biomarker	**Biomarker negative**	**Biomarker positive**	**Difference between biomarker negative and biomarker positive**
Aβ(1–42)	0·31 (0·16–0·46)	1·4 (1·3–1·6)	1·1 (0·9–1·3)
pTau/ Aβ(1–42)	0·17 (-0·02–0·32)	1·6 (1·4–1·7)	1·4 (1·2–1·6)
tTau/ Aβ(1–42)	0·21 (0·07–0·35)	1·6 (1·5–1·8)	1·4 (1·3–1·6)

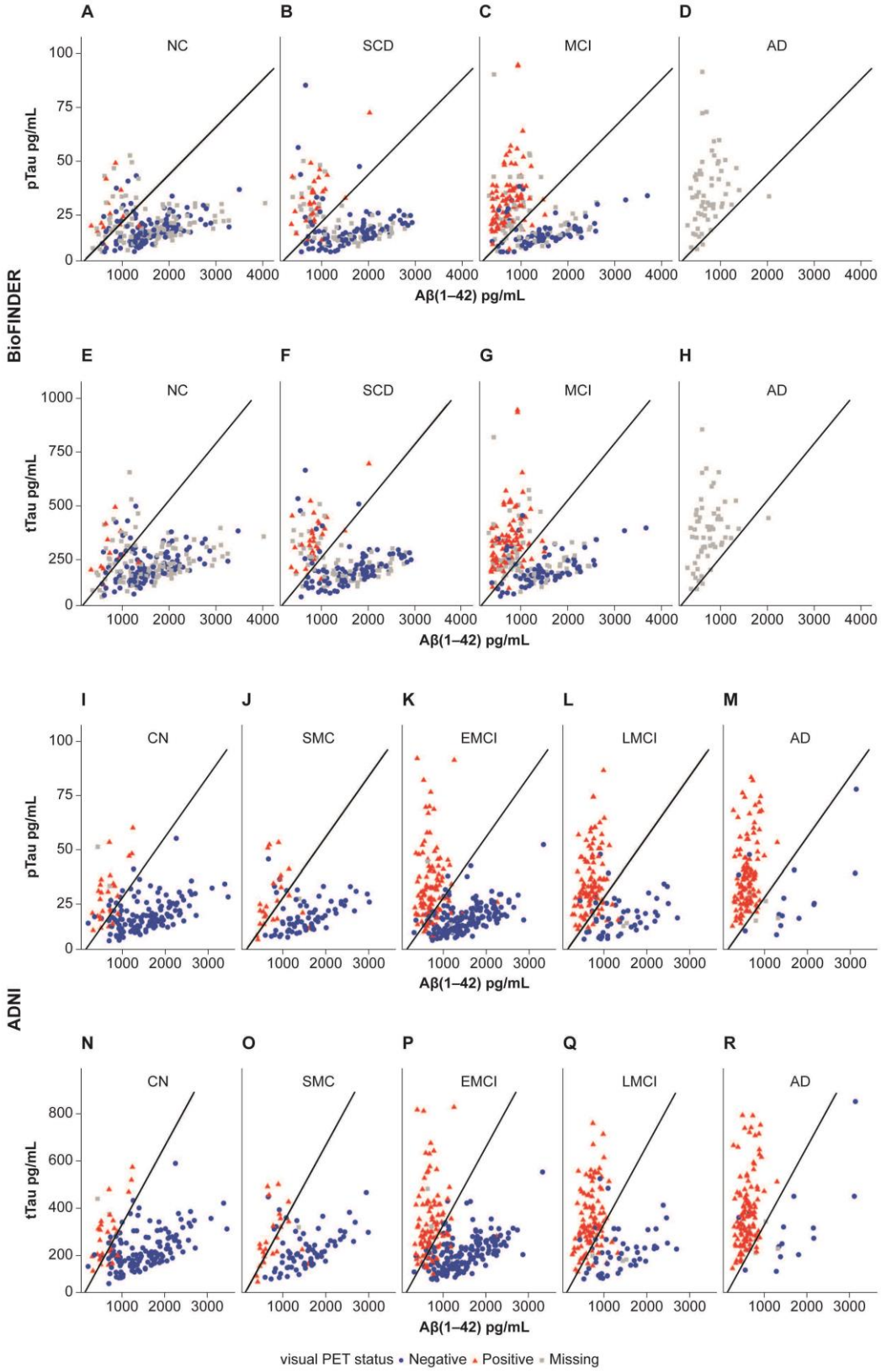
Supplementary figures



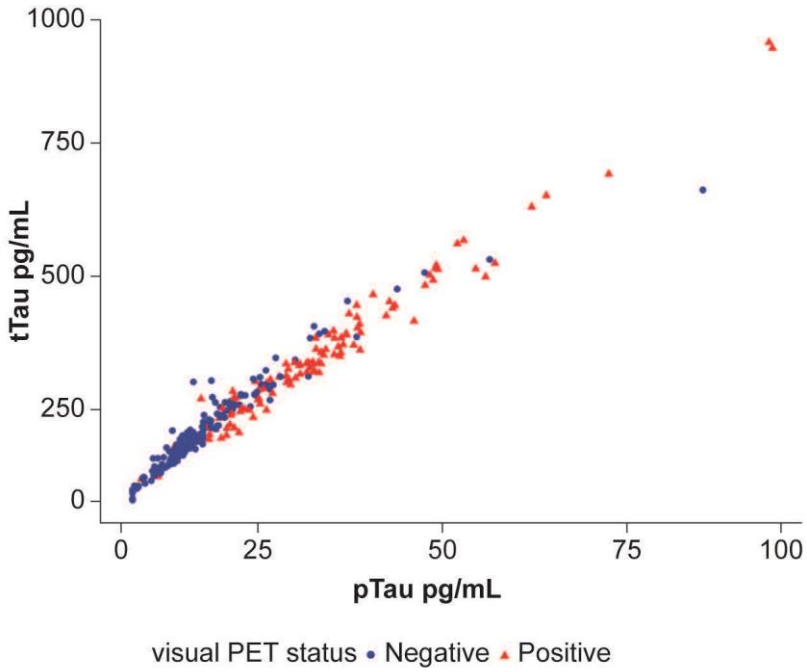
Supplementary Figure 1: Receiver Operation Characteristic (ROC) curve analysis for CSF Aβ(1–42), pTau/Aβ(1–42) and tTau/Aβ(1–42). *Graphs show curves from BioFINDER (A) and ADNI (B) cohorts. Cutoff values represented by symbols. Black lines and dots: Aβ(1–42), blue lines and triangles: pTau/Aβ(1–42) and red lines and asterisks: tTau/Aβ(1–42).*



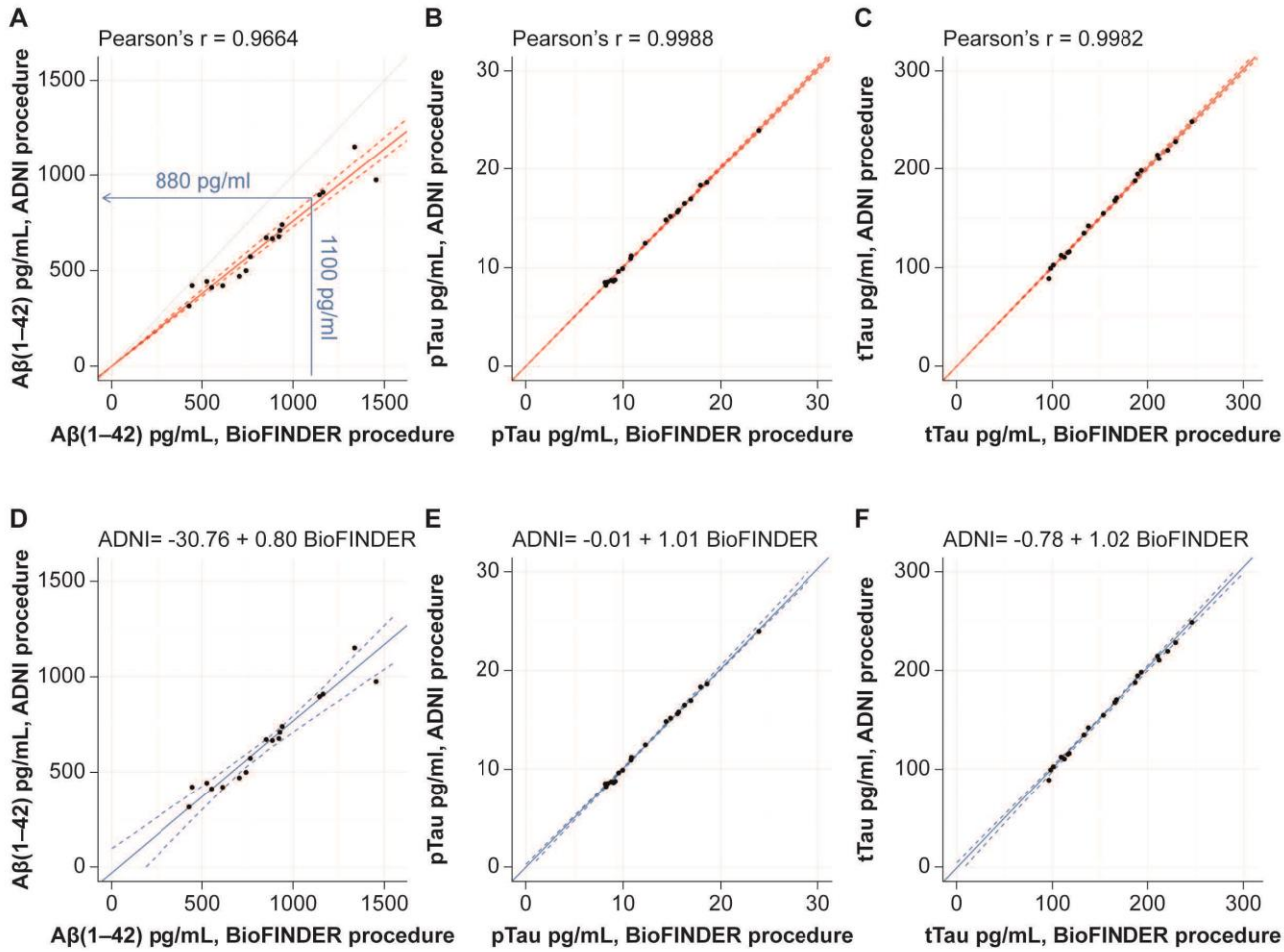
Supplementary Figure 2:PPA and NPA for CSF Aβ(1–42) over a range of possible CSF cutoffs in the BioFINDER cohort. *The selected cutoff 1100 pg/mL Aβ(1–42) is marked by the vertical dashed line. NPA = negative percent agreement; PPA = positive percent agreement.*



Supplementary Figure 3: Scatterplots of pTau and tTau vs. Aβ(1–42) per cohort in BioFINDER (A-H; n=721) and ADNI (I-R; n=819) *including all patients with CSF measurement (with and without visual PET read-out). Cutoffs optimized from primary analysis population.*



Supplementary Figure 4: Scatterplot pTau vs. tTau in BioFINDER cohort*. Red triangles, visual read PET-positive; blue dots, visual read PET-negative. N=277.*



Supplementary Figure 5: Results of pre-analytical experiment for Aβ(1–42) (A and D), pTau (B and E) and tTau (C and F). *Results of method comparison of biomarker levels in samples prepared according to the 'BioFINDER' (x-axis) and 'ADNI' (y-axis) pre-analytical protocols.* ***A-C*** *Solid red line shows linear dependencies ADNI = 0·76*BioFINDER (Aβ (1–42), A), ADNI = 1·007*BioFINDER (pTau, B), and ADNI = 1·006*BioFINDER (tTau, C), with factors corresponding to the mean percentage differences between the two protocols. The dashed red lines represent the confidence limits of the ADNI-BioFINDER comparisons. In A, the solid blue line shows the proposed adjustment of the BioFINDER cutoff (1100 pg/mL) to the ADNI cutoff (880 pg/mL) using the upper confidence limit of the mean percentage difference, between the two protocols, 0·8, as an adjustment factor.* ***D-F****: solid blue line shows regression line fitted using Passing-Bablok approach. Dashed lines show the corresponding 95% confidence bounds of the fitted line. D: Aβ (1–42). Regression coefficients (95% CI): intercept -30·76 (-179·41, 95·87) and slope 0·8 (0·64, 0·96). E: tTau. Regression coefficients (95% CI): intercept -0·78 (-8·39, 4·45) and slope 1·02 (0·98, 1·06). F: pTau. Regression coefficients (95% CI): intercept -0·03 (-0·46, 0·19) and slope 1·01 (1·00, 1·05). As there was no significant intercept, the adjustment factor was derived from the average proportional difference rather than the Passing-Bablok regression. Number of observations used for the analysis: for tTau and pTau N = 20, for Aβ(1–42) N=17 since three of 20 patients had measurements above the technical limit and were excluded from the analysis.*

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