



Featured Article

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

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Abstract

Introduction: We studied whether fully automated Elecsys cerebrospinal fluid (CSF) immunoassay results were concordant with positron emission tomography (PET) and predicted clinical progression, even with cutoffs established in an independent cohort.

Methods: Cutoffs for Elecsys amyloid- β_{1-42} (A β), total tau/A β_{1-42} , and phosphorylated tau/A β_{1-42} were defined against [¹⁸F]flutemetamol PET in Swedish BioFINDER (n = 277) and validated against [¹⁸F]florbetapir PET in Alzheimer's Disease Neuroimaging Initiative (n = 646). Clinical progression in patients with mild cognitive impairment (n = 619) was studied.

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⁴Data used in 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 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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Results: CSF total tau/A β (1–42) and phosphorylated tau/A β (1–42) ratios were highly concordant with PET classification in BioFINDER (overall percent agreement: 90%; area under the curve: 94%). The CSF biomarker statuses established by predefined cutoffs were highly concordant with PET classification in Alzheimer's Disease Neuroimaging Initiative (overall percent agreement: 89%–90%; area under the curves: 96%) and predicted greater 2-year clinical decline in patients with mild cognitive impairment. Strikingly, tau/A β ratios were as accurate as semiquantitative PET image assessment in predicting visual read-based outcomes.

Discussion: Elecsys CSF biomarker assays may provide reliable alternatives to PET in Alzheimer's disease diagnosis.

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Keywords: CSF biomarkers; Amyloid PET concordance; Clinical progression; Biomarker validation; Amyloid- β (1–42); Total tau (tTau); Phosphorylated tau (pTau); Cutoffs

1. Introduction

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease. The pathologic hallmarks of AD include neuritic plaques composed of aggregated amyloid- β peptides (A β) surrounded by dystrophic neurites, and neurofibrillary tangles composed of hyperphosphorylated tau proteins, accompanied by neuronal and synaptic degeneration [1]. Currently, AD treatments only provide symptomatic benefit, but ongoing drug discovery efforts focus on developing disease-modifying drugs [2]. Disease-modifying drugs will likely be most efficacious in early stages of AD; therefore, early and accurate AD diagnosis is essential for successful disease-modifying therapy development. However, in current clinical practice, a diagnosis of probable AD is made based on clinical symptoms, largely by the exclusion of other causes of dementia [3,4], with postmortem evidence of AD pathology required to confirm the diagnosis. It is well established, from combined clinical and neuropathologic studies [5,6], and clinical trials using amyloid- β PET scans [7], that the accuracy of clinical criteria is suboptimal. Therefore, including biomarkers in the diagnostic workup of subjects could increase the accuracy of AD diagnosis, recognize earlier prodementia disease stages, inform the dementia diagnosis when symptoms are atypical, and enrich clinical trial populations.

The use of A β and tau protein biomarkers for AD diagnosis is recommended in recent research diagnostic guidelines for AD, the National Institute on Aging–Alzheimer's Association [8–10], and International Work Group 2 [11] criteria. To date, visual reads of amyloid- β PET scans is the only Food and Drug Administration–approved biomarker method to aid in the diagnosis of AD; specifically, a negative amyloid- β PET scan can be used to rule out AD [12]. Tau PET tracers are also currently in development for AD evaluation [13]. However, PET imaging is expensive and requires specialist units and equipment and confers a radioactive burden on the patient. Cerebrospinal fluid (CSF) biomarkers have shown good, but not complete, concordance with amyloid- β PET classification [14] and

may allow for robust, automated quantification of multiple pathologic markers of AD.

The A β (1–42), phosphorylated tau (pTau), and total tau (tTau) CSF biomarkers are able to distinguish patients with AD versus controls as outlined in a recent meta-analysis [15]. These CSF biomarkers may also indicate an increased risk of future clinical progression to AD in patients with mild cognitive impairment (MCI) [16–19]. Unfortunately, the currently available CSF assays for A β (1–42), pTau, and tTau are limited by considerable variability between laboratories and assay batches [20,21]. This has precluded the introduction of uniform, worldwide cutoff values and hindered the widespread introduction of CSF biomarkers into clinical practice. To improve the reliability of CSF biomarker measurement, Roche Diagnostics is developing fully automated Elecsys CSF immunoassays for A β (1–42) [22], as well as pTau and tTau (article in preparation) with high analytical performance and reduced variability across laboratories and batches. The Elecsys β -Amyloid (1–42) CSF immunoassay has been assessed in the Alzheimer's Association Quality Control program since 2014 [23], yielding mean between-laboratory coefficient of variation of approximately 4% (compared with >15% for manual assays).

Preanalytical procedures can influence the measured concentration of CSF biomarkers, preventing direct comparison of data between studies. In particular, A β (1–42) peptides are known to be prone to preanalytical influences such as tube type, freeze-thaw steps, transfer steps, and aliquot volume [24–27]. Therefore, differences in preanalytical protocols need to be considered when directly comparing CSF measurements from different cohorts.

In the present study, we evaluated whether the newly developed Elecsys CSF immunoassays for the biomarkers A β (1–42), pTau/A β (1–42), and tTau/A β (1–42) can be used to develop global cutoffs that can be transferred from one population to another, even when the CSF samples were analyzed in different laboratories. We first established the concordance of CSF biomarkers with amyloid PET classification by visual read in the Swedish BioFINDER study

($n = 277$; patients with mild cognitive symptoms (MCSs); [^{18}F]flutemetamol PET tracer) and then, adjusting cutoffs for preanalytical differences, we validated biomarker concordance with amyloid PET classification in patients from the Alzheimer's Disease Neuroimaging Initiative (ADNI) study ($n = 646$; patients with significant memory concern, MCI or AD; [^{18}F]florbetapir PET tracer). These biomarkers were also evaluated for prediction of clinical progression over 2 years in patients with MCI in ADNI.

2. Methods

To achieve our objectives, a three-part methodology was used in two independent cohorts (Fig. 1). In part 1, the concordance between CSF biomarkers and visual read amyloid- β PET in the BioFINDER population was determined and CSF biomarker cutoffs were established. In part 2, CSF samples derived from the same patients were handled according to two different preanalytical protocols before analysis (BioFINDER and ADNI) to determine a "CSF cutoff adjustment factor" to transfer cutoffs determined in the BioFINDER cohort (part 1) to the ADNI cohort (part 3). In part 3, the adjusted CSF cutoffs were applied to validate the concordance of the predefined CSF biomarker cutoffs with PET classification in an independent cohort from the ADNI study. Finally, the ability of the CSF biomarker status, established by predefined cutoffs, to predict future clinical progression in ADNI was also evaluated.

3. Study populations

3.1. Part 1: Training study (BioFINDER)

The BioFINDER (www.biofinder.se) study population included 728 patients (normal controls, with MCSs or AD; Supplementary Table 4) consecutively recruited between September 2010 and December 2014 at three different memory clinics as previously described [28,29]. The primary analysis population to assess PET concordance included 277 patients with MCSs who had amyloid- β PET images and CSF samples. Based on a neuropsychologic battery [28], this population was classified as subjective cognitive decline ($n = 120$, 43%) or MCI ($n = 153$, 55%), with unknown subclassification for $n = 4$ (1.4%) who had not undergone extensive neuropsychological testing. The characteristics of the study participants are given in Table 1 (primary analysis population) and Supplementary Table 1 (overall BioFINDER study population).

3.2. Part 2: Preanalytical protocol comparison and cutoff adjustment

CSF samples were collected at Skåne University Hospital from January 2016 to April 2016 from $n = 20$ subjects (≥ 18 years) undergoing diagnostic lumbar puncture due to suspicion of normal pressure hydrocephalus. These subjects were chosen as they provided sufficient residual CSF volume (≥ 40 mL) to conduct parallel assessment of the two preanalytical protocols. CSF samples were handled according to the two different preanalytical protocols (BioFINDER and ADNI), as detailed in Supplementary Table 2.

3.3. Part 3: Validation study (ADNI)

The ADNI study population comprised 918 subjects (cognitively normal, with significant memory concern, early mild cognitive impairment or late mild cognitive impairment, or AD) from ADNI-GO and ADNI-2. The primary analysis population for amyloid- β PET concordance analysis with Elecsys CSF measurement included 646 participants from ADNI-GO and ADNI-2 with significant memory concern, early mild cognitive impairment, late mild cognitive impairment, or AD (Supplementary Table 4); all participants had amyloid- β PET images and CSF samples. The characteristics of the study participants are given in Table 1 (primary analysis population) and Supplementary Table 1 (ADNI study population).

3.4. Clinical progression prediction

The clinical dementia rating-sum of boxes (CDR-SB) scores of 619 participants from ADNI-1, ADNI-GO, and ADNI-2 cohorts with early ($n = 277$) or late ($n = 342$) MCI at baseline were tracked in the ADNI database over 2 years. Four hundred ninety-four patients had CDR-SB scores at baseline and 24 months.

3.5. PET image analysis

For BioFINDER, cerebral amyloid- β deposition was visualized with the PET tracer [^{18}F]flutemetamol. The tracer was manufactured, and PET scanning was conducted as previously described [28,29]. For ADNI images, cerebral A β deposition was visualized with the PET tracer [^{18}F]florbetapir. PET imaging was performed within 2 weeks before or after the baseline clinical assessments, as described previously [30].

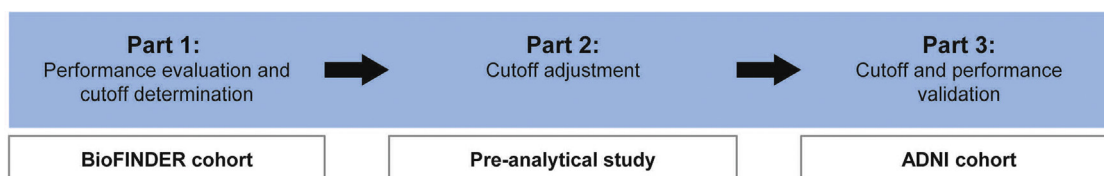


Fig. 1. Schematic of three-part strategy for evaluating CSF biomarker concordance with amyloid PET concordance. Abbreviations: CSF, cerebrospinal fluid; ADNI, Alzheimer's Disease Neuroimaging Initiative.

Table 1
Demographics and characteristics of Elecsys CSF measurements for BioFINDER and ADNI primary analysis populations, overall and by cohort

Parameter	BioFINDER			ADNI				
	SCD (N = 120)	MCI (N = 153)	Primary analysis population* (N = 277) [†]	SMC (N = 94)	EMCI (N = 272)	LMCI (N = 152)	AD (N = 128)	Primary analysis population [‡] (N = 646)
Cohort, n (%)	120	153	277	94	272	152	128	646
ADNI-GO				0 (0.0)	115 (42.3)	0 (0.0)	0 (0.0)	115 (17.8)
ADNI-2				94 (100.0)	157 (57.7)	152 (100.0)	128 (100.0)	531 (82.2)
Age, mean years (SD)	69.7 (5.41)	70.8 (5.45)	70.3 (5.45)	72.1 (5.43)	71.1 (7.37)	72.2 (7.43)	74.3 (8.35)	72.1 (7.42)
Gender, n (%)								
Male	61 (50.8)	100 (65.4)	162 (58.5)	38 (40.4)	152 (55.9)	82 (53.9)	76 (59.4)	348 (53.9)
Female	59 (49.2)	53 (34.6)	115 (41.5)	56 (59.6)	120 (44.1)	70 (46.1)	52 (40.6)	298 (46.1)
Education (years), n	120	151	273	94	272	152	128	646
Mean (SD)	12.8 (3.46)	11.2 (3.33)	11.9 (3.47)	16.7 (2.47)	15.9 (2.64)	16.7 (2.53)	15.7 (2.65)	16.2 (2.62)
APOE ϵ 4 risk alleles, n (%)	119	153	276	94	272	152	128	646
0 ϵ 4	67 (56.3)	81 (52.9)	150 (54.3)	62 (66.0)	157 (57.7)	64 (42.1)	42 (32.8)	325 (50.3)
1 ϵ 4	45 (37.8)	53 (34.6)	100 (36.2)	31 (33.0)	95 (34.9)	62 (40.8)	60 (46.9)	248 (38.4)
2 ϵ 4	7 (5.9)	19 (12.4)	26 (9.4)	1 (1.1)	20 (7.4)	26 (17.1)	26 (20.3)	73 (11.3)
MMSE, mean score (SD)	28.6 (1.36)	27.2 (1.78)	27.8 (1.76)	29.0 (1.24)	28.3 (1.58)	27.6 (1.83)	23.2 (2.05)	27.2 (2.68)
Visual PET, n (%)	120	153	277	94	272	152	128	646
Negative	91 (75.8)	74 (48.4)	167 (60.3)	70 (74.5)	165 (60.7)	50 (32.9)	14 (10.9)	299 (46.3)
Positive	29 (24.2)	79 (51.6)	110 (39.7)	24 (25.5)	107 (39.3)	102 (67.1)	114 (89.1)	347 (53.7)
SUVr, n	108	123	233	94	272	152	127	645
Mean (SD)	1.26 (0.294)	1.44 (0.365)	1.36 (0.344)	1.16 (0.207)	1.21 (0.236)	1.35 (0.270)	1.51 (0.247)	1.29 (0.272)
Elecsys CSF biomarker, n	120	153	277	94	272	152	128	646
A β (1–42), median pg/mL (MAD)	1340 (534)	951 (508)	1048 (593)	1325 (557)	1066 (572)	784 (288)	595 (214)	862 (453)
pTau, median pg/mL (MAD)	18.5 (7.53)	21.5 (11.36)	20.0 (9.38)	19.0 (7.86)	20.7 (8.93)	28.0 (12.86)	33.8 (13.00)	24.2 (12.2)
tTau, median pg/mL (MAD)	217 (87.8)	255 (112.7)	240 (100)	217 (80.5)	234 (91.8)	291 (128.7)	340 (135.4)	258 (107)
pTau/A β (1–42), median (MAD)	0.013 (0.006)	0.029 (0.026)	0.016 (0.011)	0.015 (0.008)	0.017 (0.011)	0.037 (0.029)	0.058 (0.024)	0.029 (0.026)
tTau/A β (1–42), median (MAD)	0.157 (0.070)	0.321 (0.259)	0.197 (0.138)	0.165 (0.075)	0.202 (0.125)	0.391 (0.270)	0.575 (0.255)	0.317 (0.258)

Abbreviations: CSF, cerebrospinal fluid; ADNI, Alzheimer's Disease Neuroimaging Initiative; AD, Alzheimer's disease; EMCI, early mild cognitive impairment; LMCI, late mild cognitive impairment; MAD, median absolute deviation; MCI, mild cognitive impairment; MCS, mild cognitive symptom; SCD, subjective cognitive decline; SMC, significant memory concern; APOE, apolipoprotein E; MMSE, Mini-Mental State Examination; SUVr, standardized uptake value ratio; tTau, total tau; pTau, phosphorylated tau.

*MCS patients with visual PET and CSF measurement available.

[†]Four patients of the BioFINDER primary analysis population did not have the subclassification into SCD or MCI.

[‡]SMC, EMCI, LMCI, and AD patients with visual PET and CSF measurement available.

3.5.1. Visual read analysis

Banked [^{18}F]flutemetamol (BioFINDER) or [^{18}F]florbetapir (ADNI) PET images were re-evaluated by three independent readers at MNI, New Haven, USA. Further details are provided in the [Supplementary Methods](#).

3.5.2. Standardized uptake value ratio analysis

The same banked amyloid- β PET images from BioFINDER and ADNI were quantitatively assessed at MNI, New Haven, USA. Standardized uptake value ratios (SUVRs) were calculated with a standardized cortical anatomical automatic labeling volume-of-interest template placed on spatially normalized image volumes using a whole-cerebellum reference region, as previously described [31]. Composite SUVRs were calculated as the unweighted mean of the left and right lateral temporal, frontal, posterior cingulate/precuneus, and parietal cortices.

3.6. CSF collection and biomarker measurement

In BioFINDER, CSF samples were collected per the Alzheimer's Association Flow Chart for CSF biomarkers [32]. Lumbar CSF samples were collected at three centers and centrifuged, and the supernatant was stored in 1-mL aliquots in polypropylene tubes at $\leq -60^\circ\text{C}$. Only never-before-thawed samples that had been stored in Sarstedt tubes ($n = 277$ with MCSs; the primary population) were included in the present study; samples that had been stored in NUNC tubes ($n = 10$) were excluded because of differences in $\text{A}\beta(1-42)$ levels putatively arising from differences in binding of $\text{A}\beta(1-42)$ to the tube walls.

In ADNI, lumbar puncture was performed as described in the ADNI procedures manual (<http://www.adni-info.org/>). CSF samples were frozen on dry ice within 1 hour after collection and shipped overnight on dry ice to the ADNI Biomarker Core laboratory at the University of Pennsylvania Medical Center. Aliquots (0.5 mL) were prepared from these and stored in barcode-labeled polypropylene vials at -80°C . Never-before-thawed aliquots of CSF samples collected between July 7, 2007 and December 18, 2013 were used in this study.

CSF samples were measured using the Elecsys β -amyloid(1-42) CSF [22], and the Elecsys phosphotau (181P) CSF and Elecsys total-tau CSF immunoassays on a cobas e 601 analyzer (software version 05.02) at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden (BioFINDER) or at the Biomarker Research Laboratory, University of Pennsylvania, USA (ADNI), according to the preliminary kit manufacturer's instructions and as described in previous studies [22].

3.7. Statistical analysis

In part 1, cutoffs for the CSF biomarkers $\text{A}\beta(1-42)$, pTau/ $\text{A}\beta(1-42)$, and tTau/ $\text{A}\beta(1-42)$ were determined to optimize concordance with visual read PET classification in BioFINDER based on performance and robustness (see

[Supplementary Materials](#) for further details). Throughout the article, concordance was measured using the agreement measures—overall percent agreement (OPA), positive percent agreement (PPA, “sensitivity”), and negative percent agreement (NPA, “specificity”).

In part 2 of the preanalytical study, the measured concentrations were averaged within each patient (across four aliquots each) and preanalytical handling procedure (BioFINDER, ADNI). The two preanalytical protocols were compared by means of average proportional difference and 95% CI according to paired t-tests, Pearson's correlation coefficients, and Passing-Bablok regression.

In part 3, the performance of the cutoffs predefined in the BioFINDER cohort and adjusted for the ADNI preanalytical protocol was evaluated by assessing concordance of the CSF biomarkers with PET visual read-based and SUVR-based classification.

A linear mixed-effects model (with random intercept) of CDR-SB score over 2 years (with visit time points at baseline, six, 12, and 24 months as a categorical variable) was used to analyze the predictive properties of CSF biomarkers. The model was adjusted for age, gender, education, baseline CDR-SB score, and interaction term baseline CDR-SB score: visit time point. As a sensitivity analysis, the model was additionally adjusted for apolipoprotein E (*APOE*) $\epsilon 4$ genotype (the number of $\epsilon 4$ alleles).

Some $\text{A}\beta(1-42)$ measurement values were beyond the upper technical limit of the immunoassay and were handled as described in the [Supplementary Methods](#).

3.8. Role of the funding source

The study was funded by Roche Diagnostics GmbH. The study was only possible due to the generous support of ADNI and the Swedish BioFINDER study in providing samples. T.B., V.L., V.C., U.E., R.B., K. Buck, K.Z., and C.R. are current or former employees of Roche Diagnostics. Roche Diagnostics also supported reporting of study results by procuring medical writing support. All authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

4. Results

4.1. Part 1: CSF biomarker concordance with amyloid- β PET in BioFINDER and determination of CSF biomarker cutoffs

The aim of part 1 was to determine cutoffs for CSF biomarker concordance with amyloid- β PET visual read classification. The cohort characteristics and demographics from the BioFINDER cohort are shown in [Table 1](#) (see [Supplementary Table 1](#) for further details).

For the visual read analysis, majority voting of three independent reads resulted in $N = 110$ (40%) positive, and $N = 167$ (60%) negative PET reads. Interreader agreement

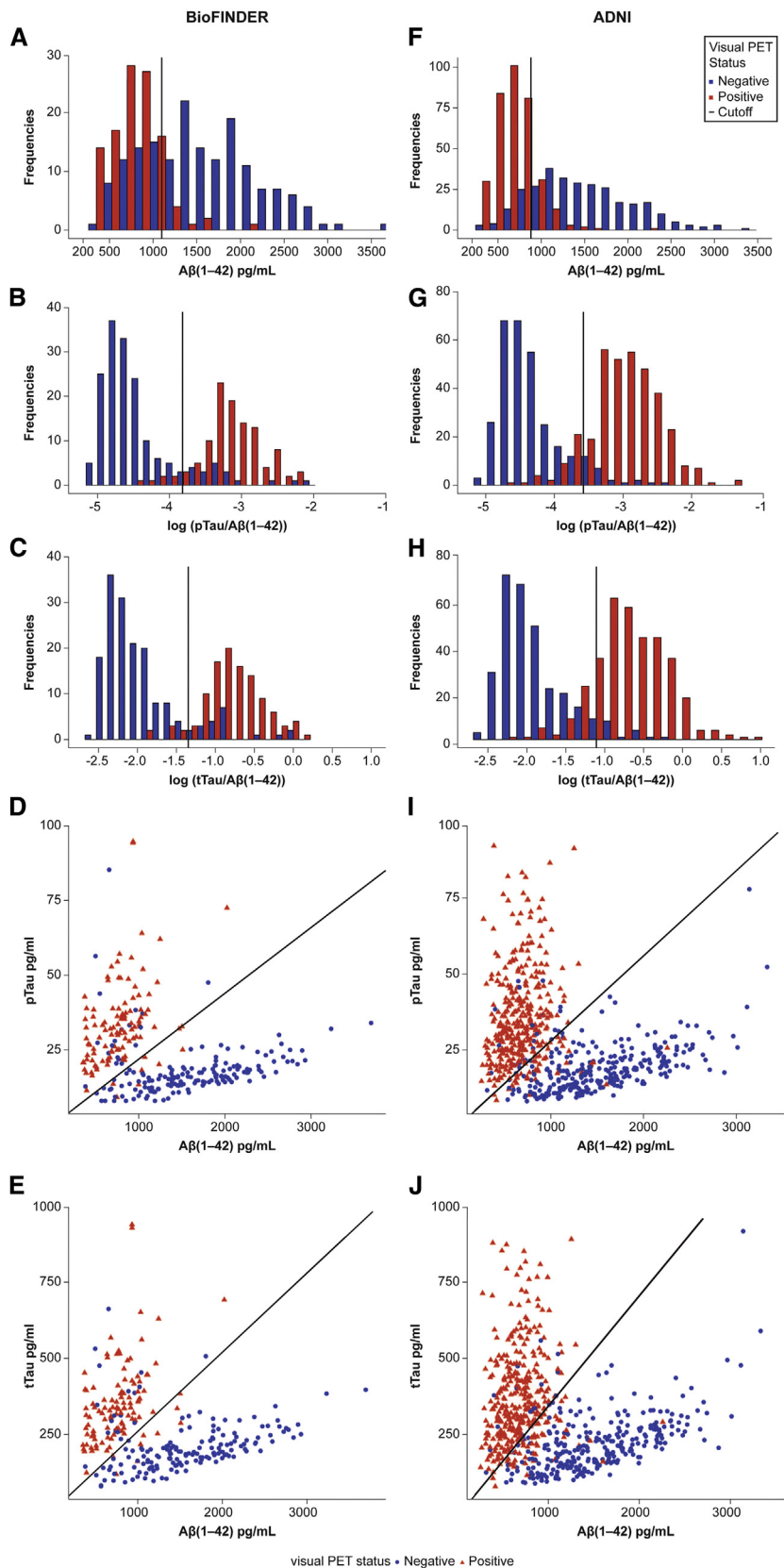


Fig. 2. Distribution of the CSF biomarkers colored by PET visual read classification. (A–C) (BioFINDER cohort) and (F–H) (ADNI cohort): Frequency distribution of $A\beta(1-42)$, $\log(p\text{Tau}/A\beta(1-42))$ and $\log(t\text{Tau}/A\beta(1-42))$, respectively, by PET classification. (D and E) (BioFINDER cohort) and (I and J) (ADNI cohort): Scatterplots of $A\beta(1-42)$ versus $p\text{Tau}$ (D and I) and $t\text{Tau}$ (E and J) with the cutoffs for the respective ratio $p\text{Tau}/A\beta(1-42)$ (BioFINDER: 0.022, ADNI: 0.028) and $t\text{Tau}/A\beta(1-42)$ (BioFINDER: 0.26, ADNI: 0.33) shown as diagonal lines. $n = 277$ (BioFINDER A–E) and $n = 646$ (ADNI, F–J). Red bars or triangles, PET-positive; blue bars or dots, PET-negative. Abbreviations: CSF, cerebrospinal fluid; ADNI, Alzheimer's Disease Neuroimaging Initiative; tTau, total tau; pTau, phosphorylated tau; $A\beta$, amyloid β .

Table 2

Performance of CSF biomarker cutoffs versus visual amyloid- β PET in BioFINDER and ADNI

Cohort	CSF biomarker	Cutoff	PPA, %	NPA, %	OPA, %	AUC, %
BioFINDER	A β (1–42)	1100 pg/mL	90.9 (83.9–95.6)	72.5 (65.0–79.1)	79.8 (74.6–84.4)	86.5 (82.3–90.7)
	pTau/A β (1–42)	0.022	90.9 (83.9–95.6)	89.2 (83.5–93.5)	89.9 (85.7–93.2)	94.4 (91.5–97.3)
	tTau/A β (1–42)	0.26	90.9 (83.9–95.6)	89.2 (83.5–93.5)	89.9 (85.7–93.2)	94.0 (91.0–97.0)
ADNI	A β (1–42)	880 pg/mL	83.6 (79.3–87.3)	85.3 (80.8–89.1)	84.4 (81.3–87.1)	92.1 (90.0–94.3)
	pTau/A β (1–42)	0.028	88.2 (84.3–91.4)	92.6 (89.1–95.3)	90.3 (87.7–92.4)	96.3 (95.2–98.0)
	tTau/A β (1–42)	0.33	85.0 (80.8–88.6)	94.0 (90.7–96.4)	89.2 (86.5–91.5)	96.3 (94.8–97.7)

Abbreviations: CSF, cerebrospinal fluid; ADNI, Alzheimer's Disease Neuroimaging Initiative; AUC, area under the curve; NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement; tTau, total tau; pTau, phosphorylated tau.

NOTE. Values in brackets are 95% confidence intervals.

was high (interreader mean OPA = 90.1% [min 87.7, max 94.8]; see [Supplementary Results](#); [Supplementary Table 3](#)).

The distribution of CSF biomarker concentration appeared to correspond with the two PET classification groups ([Fig. 2A–C](#); area under the curve: 87%–94%; [Supplementary Fig. 1A](#)). Cutoffs for A β (1–42), pTau/A β (1–42), and tTau/A β (1–42) were specified at values that best separated the PET-positive and PET-negative groups and were robust to changes in measurement levels (see [Section 2](#)). For example, with respect to CSF A β (1–42) levels, a lower cutoff would lead to a steep decline in PPA, without substantial increase in NPA ([Supplementary Fig. 2](#)). Therefore, a compromise for the cutoff 1100 pg/mL was chosen [A β (1–42) \leq 1100 pg/mL: test positive; >1100 pg/mL: test negative] with high (91%) PPA and 72% NPA ([Table 2](#); [Supplementary Fig. 2](#)). Based on similar considerations, the pTau/A β (1–42) and tTau/A β (1–42) ratio cutoffs were defined as follows: pTau/A β (1–42) = 0.022, tTau/A β (1–42) = 0.26 ([Table 2](#)).

The distributions of CSF levels of pTau or tTau versus A β (1–42) revealed two clusters that corresponded to the PET classification ([Fig. 2D and E](#)). A diagonal line reflecting the pTau/A β (1–42) or tTau/A β (1–42) cutoffs ([Fig. 2D and E](#)) discriminated between a PET-positive and PET-negative classification better than a vertical line reflecting the A β (1–42) single biomarker cutoff. This was consistent across clinical cohorts ([Supplementary Fig. 3A–H](#); [Supplementary Table 5](#)). Specifically, in the primary analysis population, CSF pTau/A β (1–42) and tTau/A β (1–42) cutoffs showed higher NPA (89%) than CSF A β (1–42) alone (73%), at the same PPA (91%), resulting in OPA values of 90% ([Table 2](#)). A strong correlation between pTau and tTau CSF measurements was seen ([Supplementary Fig. 4](#)). There was no clear preference for either CSF tau biomarker when comparing the pTau/A β (1–42) and tTau/A β (1–42) with PET ([Fig. 2D and E](#); [Table 2](#)).

4.2. Part 2: Preanalytical comparison and cutoff adjustment for the two preanalytical CSF handling protocols

In part 2, we assessed systematic differences in A β (1–42), pTau, or tTau levels in CSF samples derived from the

same patients and handled by two different preanalytical protocols (BioFINDER and ADNI). Measurement of CSF A β (1–42) levels revealed systematic differences (on average, \sim 24%) between the values measured after handling by BioFINDER or ADNI protocols, whereas no meaningful difference was observed in CSF pTau or tTau concentrations (1%–3%; [Supplementary Table 6](#)). To account for the preanalytical differences, a cutoff adjustment factor of 0.8 (using the upper confidence limit of the systematic bias) was calculated for A β (1–42) from the BioFINDER (part 1) to the ADNI cohort ([Supplementary Fig. 5D](#)); the pTau/A β (1–42) and tTau/A β (1–42) cutoffs were also transferred using the inverse adjustment factor 0.8^{-1} (see [Supplementary Methods and Results](#) for further details). This resulted in adjusted CSF biomarker cutoffs to be validated in the ADNI cohort in part 3: A β (1–42) = 880 pg/mL, pTau/A β (1–42) = 0.028, tTau/A β (1–42) = 0.33; these cutoffs were determined before the ADNI cohort was analyzed.

4.3. Part 3: Validation of amyloid- β PET concordance in ADNI

The aim of part 3 was to validate the PET concordance of CSF A β (1–42), pTau/A β (1–42), and tTau/A β (1–42) in the ADNI cohort (n = 646) using the predefined adjusted cutoffs determined in part 2. Characteristics and demographics of the ADNI cohort are shown in [Table 1](#) (see [Supplementary Table 1](#) for further details). It is worth noting that the median biomarker values were quite similar in the BioFINDER and ADNI cohorts ([Table 1](#)) and showed similar data distributions ([Fig. 2](#)). Using the predefined transferred cutoffs, the CSF biomarkers A β (1–42), pTau/A β (1–42), and tTau/A β (1–42) distinguished between the PET classifications ([Fig. 2F–H](#), respectively) with high PPA and NPA, OPA values of 84%–90%, and area under the curve values of 92%–96% ([Table 2](#); [Supplementary Fig. 1B](#)). The CSF pTau/A β (1–42) ratio performed slightly better than the tTau/A β (1–42) ratio; both ratios showed superior performance than A β (1–42) alone, consistent with BioFINDER (part 1).

The distributions of pTau and tTau versus A β (1–42) indicated that these CSF biomarkers were concordant with PET

classification across clinical cohorts (including cognitively normal subjects) in ADNI (Supplementary Fig. 3I–R). With increasing prevalence of PET positivity with more severe disease stage, there was a corresponding trend toward an increase in PPV and a decrease in NPV (Supplementary Table 7).

A cutoff determination analogous to part 1 was performed for the ADNI study population as a sensitivity analysis. The resulting CSF biomarker cutoffs were 977 pg/mL, 0.025, and 0.27 for A β 42, pTau/A β 42, and tTau/A β 42, respectively, and had a high overall agreement with visual read amyloid PET classification (Supplementary Table 8).

4.4. SUVR amyloid- β PET concordance

In addition to qualitative visual read, quantitative SUVR amyloid- β PET values were also investigated. SUVR-based and visual read-based classification showed high agreement at the SUVR cutoffs defined by mixture modeling (BioFINDER: PPA = 98.8%, NPA = 84.4%, OPA = 89.7%; ADNI: PPA = 95.1%, NPA = 88.0%, OPA = 91.8%). Using an SUVR classification cutoff and the predefined CSF biomarker cutoffs, high concordance for all three biomarkers was observed for both BioFINDER and ADNI cohorts (Fig. 3). The overall agreement of the CSF biomarkers with SUVR-based classification was similar in ADNI but

slightly higher in BioFINDER than with visual read-based PET classification (Supplementary Table 9). For example, for A β (1–42), in the BioFINDER study, CSF biomarker agreement with SUVR was 86% (vs. 80% with visual read); for pTau/A β (1–42) and tTau/A β (1–42) ratios, it was 92% (vs. 90% with visual read). High agreement between the CSF biomarkers and SUVR-based classification was also observed across clinical cohorts in the BioFINDER (Supplementary Table 10) and ADNI (Supplementary Table 11) studies.

4.5. Clinical progression predicted by predefined CSF biomarker cutoffs in MCI patients in ADNI cohort

To study whether CSF biomarker status, established by predefined cutoffs, could predict clinical progression, the ADNI MCI population ($n = 619$) was examined. There was a significant difference in progression (as defined by change in CDR-SB, a measure of cognition and function, from baseline to two years) between biomarker-positive and biomarker-negative patients (Fig. 4; Supplementary Table 12); this was true for all three CSF biomarkers. Biomarker-positive patients progressed by 1.4–1.6 points over 2 years, whereas biomarker-negative patients' progression was significantly less than 0.5 (Supplementary Table 12). This was also the case when the model was

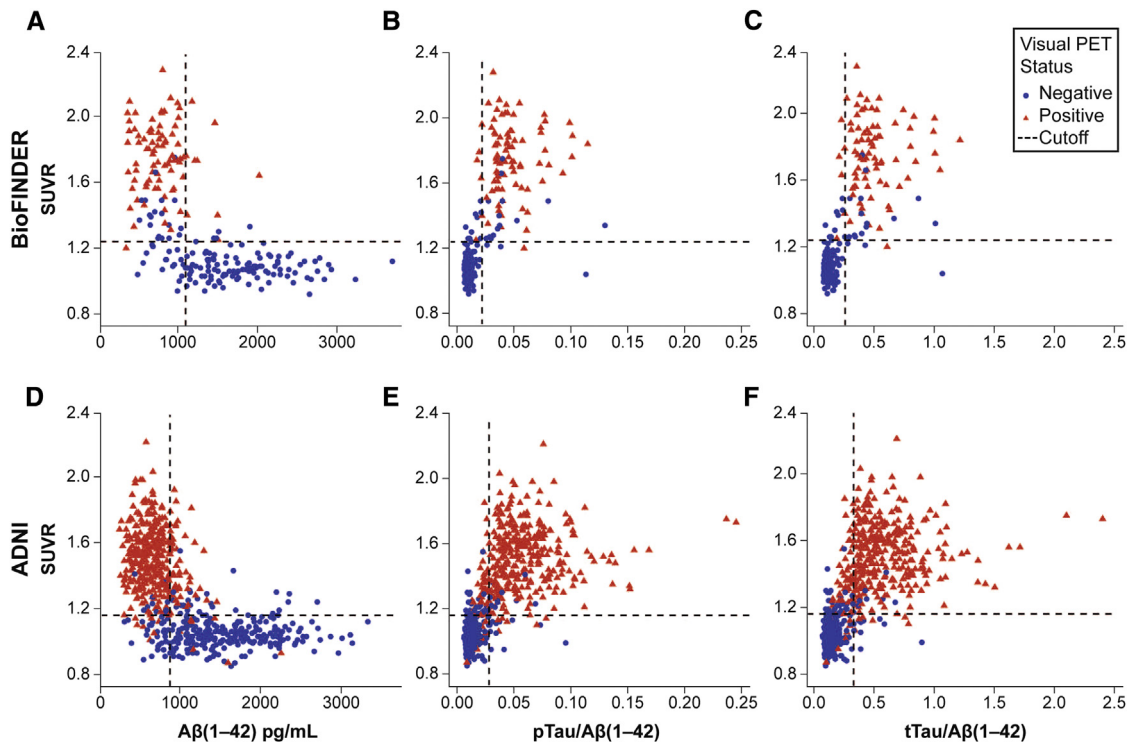


Fig. 3. Scatterplots of CSF biomarkers versus SUVRs in BioFINDER (A–C) and ADNI (D–F). Color and symbols indicate visual read PET-positive (red triangles) and PET-negative (blue dots) patients; vertical and horizontal dashed lines correspond to SUVR and CSF biomarker cutoff values, respectively. (A and D) A β (1–42), (B and E) pTau/A β (1–42) ratio, and (C and F) tTau/A β (1–42) ratio. Number of samples is reduced to $N = 233$ in BioFINDER and $N = 645$ in ADNI as the SUVRs were not available for all patients with PET scans. Abbreviations: CSF, cerebrospinal fluid; SUVR, standardized uptake value ratio; tTau, total tau; pTau, phosphorylated tau; A β , amyloid β .

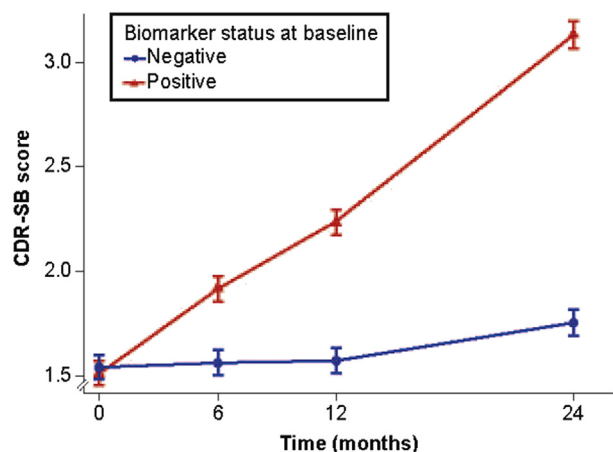


Fig. 4. Time course of pTau/A β (1–42) ratio in patients with MCI in the ADNI cohort over 2 years. LS-means with standard errors by biomarker group (red: pTau/A β (1–42) biomarker-positive at baseline; blue: pTau/A β (1–42) biomarker-negative at baseline). Increasing CDR-SB score indicates a clinical decline. N = 619. No adjustment for ApoE4 status. Abbreviations: pTau, phosphorylated tau; A β , amyloid β ; ADNI, Alzheimer's Disease Neuroimaging Initiative; MCI, mild cognitive impairment; CDR-SB, clinical dementia rating–sum of boxes; APOE, apolipoprotein E.

additionally adjusted for *APOE* ϵ 4 status (data not shown). The data revealed a trend for pTau/A β (1–42) and tTau/A β (1–42) ratios showing a greater difference in progression between “biomarker-negative” and “biomarker-positive” groups than A β (1–42) alone.

5. Discussion

In this study, we used a three-part strategy to demonstrate CSF biomarker concordance with amyloid- β PET in both the BioFINDER and ADNI studies. In part 1, we determined cutoffs for CSF A β (1–42), pTau/A β (1–42), and tTau/A β (1–42) for concordance with PET visual read in the BioFINDER cohort. Because of preanalytical protocol variations, in part 2, we calculated an adjustment factor to transfer the BioFINDER-determined cutoffs to the ADNI cohort. In part 3, we validated the predefined adjusted cutoffs in the ADNI cohort. Finally, we also showed that CSF biomarker status, established by prespecified cutoffs, had high agreement with SUVR PET classification and that the CSF biomarkers predicted future clinical progression in MCI patients.

These data showed that we could transfer CSF biomarker cutoffs from one independent cohort to another, although (1) the CSF samples were analyzed in different laboratories, (2) different preanalytical protocols were used, (3) the populations were different, and (4) different PET tracers were used. Furthermore, with the same predefined adjusted cutoffs, the biomarkers A β (1–42), pTau/A β (1–42), and tTau/A β (1–42) could clearly separate the MCI patients in the ADNI cohort who deteriorated clinically over 24 months from those who remained stable. The ability to accurately predict future disease progression using a fluid biomarker

test is relevant for both routine clinical diagnosis and the selection of patients for clinical trials.

Taking into account that postmortem pathology is the true gold standard for the detection of amyloid pathology, the interreader reliability of PET visual read was good (mean OPA 90.1% in BioFINDER and 94.0% in ADNI), but not “perfect.” This demonstrates the limitation of the visual PET method as it is partly subjective and reader dependent. However, because the amyloid- β PET visual read was used as a surrogate for amyloid pathology, the real gold standard, the OPA of the CSF assays to amyloid- β PET visual read, cannot be better than the average interreader OPA of amyloid- β PET (90.1%–93.4%), similar to the agreements between visual read-based and SUVR-based classifications of the same amyloid PET images (OPA = 89.7%–91.8%). In this context, it is interesting to note the Elecsys CSF tau/A β (1–42) ratios demonstrated high concordance with amyloid- β PET visual read-based (OPA 89.9% in BioFINDER and 89.2%–90.3% in ADNI) and SUVR-based PET (OPA = 91.8% in BioFINDER and 86.5%–88.5% in ADNI). That is, the concordance between CSF tau/A β (1–42) and amyloid PET was almost as strong as the concordance between SUVR-based and visual read-based classifications of the same PET images.

The Elecsys immunoassays showed high precision in that CSF cutoffs could be transferred from one independent study to another using a cutoff adjustment factor, even when the CSF samples were handled using different protocols and analyzed in different laboratories, although, in principle, the need to adjust cutoffs between different studies would be eliminated if a universal preanalytical protocol for CSF handling were introduced. However, this study is a step toward identifying uniform, global cutoff values to enable the introduction of CSF biomarkers into clinical practice.

This study showed a high concordance of CSF A β (1–42) with amyloid- β PET, which is supported by previous studies using other CSF assays. A previous analysis of the BioFINDER study demonstrated a 92.5% concordance between CSF A β (1–42) and PET SUVR categorization [33]. Moreover, a recent study on patients with AD and healthy controls demonstrated 86.9% total agreement for PET visual read based on precalculated CSF biomarker cutoffs [34].

The higher NPA of tau/A β (1–42) ratios than A β (1–42) alone seen in this study indicates that the CSF biomarker ratios may have greater diagnostic utility. This is supported by previous literature, as outlined in a recent review [35]. For example, in a recent study, the tTau/A β (1–42) ratio increased concordance with PIB PET SUVR from 85.2% (κ statistic = 0.703, CI 0.51–0.89) with CSF A β (1–42) to 92.5% (κ statistic = 0.849, CI 0.71–0.99) [36]. This has also been shown for the pTau/A β (1–42) ratio, where in 103 mostly cognitively normal participants, CSF pTau/A β (1–42) showed greater sensitivity for detection of PIB+ compared with A β (1–42) alone [37].

The superiority of tau/A β (1–42) ratios over A β (1–42) alone may be due to a number of reasons. First, the tau/A β (1–42) ratios combine measures of two different pathologic processes into a single diagnostic biomarker. Second, the pTau or tTau ratios may reduce random error or variance in A β (1–42) measurements. There are natural fluctuations or variations in the production, secretion, and degradation of CSF proteins [38], and by normalizing the values of any protein to any other brain-derived protein, many of these natural variations in protein concentration may be compensated for. Third, tau and A β (1–42) markers change at different points in the disease [39], suggesting that A β (1–42) is an earlier marker than tau. It has been speculated that CSF A β (1–42) levels can be abnormal slightly earlier in the disease than amyloid- β PET visual read [40]. Therefore, combining A β (1–42) in a ratio with tau, a marker that is abnormal slightly later in the disease, may correspond better to amyloid- β PET visual read. A different line of research suggests an improved concordance with amyloid- β PET imaging when combining A β (1–42) in a ratio with shorter A β peptides [41,42]. Future studies are needed to compare the performance of tau/A β (1–42) ratios with, for example, a ratio A β (1–42)/A β (1–40) using the Elecsys immunoassays.

The present study indicates that CSF biomarkers, established by predefined cutoffs, were able to separate clinically progressing from clinically stable patients; this is consistent with previous studies. For example, the tTau/A β (1–42) ratio was shown to predict MCI conversion to probable AD over 1 year [43] and the baseline tTau/A β (1–42) ratio indicated progression from MCI to dementia over 4–6 years, with a PPA of 95% and a NPA of 83% [16]. Furthermore, a “CSF AD profile” at baseline significantly increased the risk of patient progression from MCI to dementia [19]. These data suggest that the CSF biomarker profile could be used to support the diagnosis of early-stage AD. Further studies are warranted to examine whether greater rates of cognitive and functional decline are observed when both a tau protein and A β (1–42) are pathologic versus when either A β (1–42) or a tau protein alone are pathologic [44,45].

We acknowledge the limitations of this study, which potentially impact the interpretation of these results. First, two prospective cohorts were used with two different predefined preanalytical protocols. Variations in preanalytical handling of CSF samples might influence the CSF AD biomarker levels [24], especially A β (1–42) [25]. However, these differences could be compensated for with the adjustment factor calculation in part 2, albeit using small sample sizes ($n = 17$, $n = 20$; under suspicion of hydrocephalus). The ADNI preanalytical protocol includes a large number of handling steps, which may not have been exactly replicated in our study. This may have introduced additional variability to the CSF biomarker quantification and may explain why the predefined, transferred cutoffs were not the same as the newly optimized cutoffs in ADNI

(see [Supplementary Results](#)). There were also slight differences in the subjective impairment and MCI populations between cohorts and two different PET ligands ([18 F]florbetapir and [18 F]flutemetamol) were used; despite these differences, the concordance was shown between CSF markers and PET classification in both cohorts. Such methodological differences are likely representative of the variability in current clinical practice. Second, the PET visual read analysis, used as the “gold standard” in this study, is a proxy for histopathology and partly subjective and reader dependent. Finally, the two methods compared here (PET and CSF) measure different species of A β —amyloid- β PET ligands bind to aggregated forms of A β , whereas soluble A β is measured by CSF immunoassays. However, these two pools of A β are thought to be closely related [46], and this is supported by the high concordance seen in this study.

In conclusion, the study demonstrates concordance of CSF A β (1–42), pTau/A β (1–42), and tTau/A β (1–42) biomarkers with amyloid- β PET across two different cohorts with different populations, different amyloid- β PET tracers, and preanalytical protocols, which we believe may herald the potential for harmonized global cutoffs for CSF A β (1–42), pTau/A β (1–42), and tTau/A β (1–42) biomarkers of AD. The cutoffs were validated with two different amyloid- β PET tracers against two methods of amyloid- β PET analysis—visual read and SUVR. In addition, CSF biomarkers identified patients who clinically progressed over the subsequent 2 years. However, before global Elecsys CSF AD biomarkers cutoffs can be implemented, a unified preanalytical protocol for CSF handling must be established. New, automated CSF biomarker assays have the potential to aid the clinical diagnosis of AD and provide a practical, reliable alternative to amyloid- β PET on a global level.

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Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jalz.2018.01.010>.

RESEARCH IN CONTEXT

1. Systematic review: Biomarkers of Alzheimer's disease are needed to improve the accuracy of disease diagnosis and to enrich clinical trial populations. Current cerebrospinal fluid (CSF) biomarker assays are limited by between-batch and between-laboratory variability, hindering widespread introduction.
2. Interpretation: Previous studies have demonstrated high concordance between CSF biomarkers and amyloid β PET; the present study illustrates this robustly using three novel, fully automated immunoassays in two independent cohorts—Swedish BioFINDER and Alzheimer's Disease Neuroimaging Initiative, with two different PET ligands. CSF biomarkers were also associated with clinical progression among mild cognitive impairment patients.
3. Future directions: High-precision, fully automated immunoassays offer an unprecedented opportunity to establish harmonized, global decision points for CSF biomarkers to aid Alzheimer's disease diagnosis and predict clinical decline as soon as a unified pre-analytical protocol has been established. This study also supports the use of amyloid- β PET and CSF tau/amyloid- β (1–42) biomarker ratios interchangeably.

References

- [1] Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, Cummings JL. Alzheimer's disease. *Nat Rev Dis Primers* 2015; 1:15056.
- [2] Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, et al. Alzheimer's disease. *Lancet* 2016;388:505–17.
- [3] American Academy of Neurology. AAN Guideline Summary for clinicians: detection, diagnosis and management of dementia. 2004; July 2014. Available at: http://tools.aan.com/professionals/practice/pdfs/dementia_guideline.pdf. Accessed March 5, 2018.
- [4] Sachdev PS, Mohan A, Taylor L, Jeste DV. DSM-5 and mental disorders in older individuals: an overview. *Harv Rev Psychiatry* 2015; 23:320–8.
- [5] Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005–2010. *J Neuropathol Exp Neurol* 2012; 71:266–73.
- [6] Serrano-Pozo A, Qian J, Monsell SE, Blacker D, Gomez-Isla T, Betensky RA, et al. Mild to moderate Alzheimer dementia with insufficient neuropathological changes. *Ann Neurol* 2014;75:597–601.
- [7] Salloway S, Sperling R, Fox NC, Blennow K, Klunk W, Raskind M, et al. Two Phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med* 2014;370:322–33.
- [8] McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:263–9.
- [9] Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:280–92.
- [10] Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:270–9.
- [11] Dubois B, Feldman HH, Jacova C, Hampel H, Molinuevo JL, Blennow K, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet Neurol* 2014;13:614–29.
- [12] Mosconi L, McHugh PF. FDG- and amyloid-PET in Alzheimer's disease: is the whole greater than the sum of the parts? *Q J Nucl Med Mol Imaging* 2011;55:250–64.
- [13] Saint-Aubert L, Lemoine L, Chiotis K, Leuzy A, Rodriguez-Vieitez E, Nordberg A. Tau PET imaging: present and future directions. *Mol Neurodegener* 2017;12:19.
- [14] Toledo JB, Bjerke M, Da X, Landau SM, Foster NL, Jagust W, et al. Alzheimer's Disease Neuroimaging Initiative Investigators. Nonlinear association between cerebrospinal fluid and florbetapir F-18 beta-amyloid measures across the spectrum of Alzheimer disease. *JAMA Neurol* 2015;72:571–81.
- [15] Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol* 2016; 15:673–84.
- [16] Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. *Lancet Neurol* 2006;5:228–34.
- [17] van Rossum IA, Vos SJ, Burns L, Knol DL, Scheltens P, Soininen H, et al. Injury markers predict time to dementia in subjects with MCI and amyloid pathology. *Neurology* 2012;79:1809–16.
- [18] Buchhave P, Minthon L, Zetterberg H, Wallin AK, Blennow K, Hansson O. Cerebrospinal fluid levels of beta-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch Gen Psychiatry* 2012;69:98–106.
- [19] Visser PJ, Verhey F, Knol DL, Scheltens P, Wahlund LO, Freund-Levi Y, et al. Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study. *Lancet Neurol* 2009;8:619–27.
- [20] Vos SJ, Visser PJ, Verhey F, Aalten P, Knol D, Ramakers I, et al. Variability of CSF Alzheimer's disease biomarkers: implications for clinical practice. *PLoS One* 2014;9:e100784.
- [21] Mattsson N, Andreasson U, Persson S, Arai H, Batish SD, Bernardini S, et al. The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimers Dement* 2011;7:386–395.e6.
- [22] Bittner T, Zetterberg H, Teunissen CE, Ostlund RE Jr, Militello M, Andreasson U, et al. Technical performance of a novel, fully automated electrochemiluminescence immunoassay for the quantitation of beta-amyloid (1-42) in human cerebrospinal fluid. *Alzheimers Dement* 2016;12:517–26.
- [23] The Alzheimer's Association & The University of Gothenburg. The Alzheimer's Association QC program for CSF biomarkers. 2017; 2017.
- [24] Fourier A, Portelius E, Zetterberg H, Blennow K, Quadrio I, Perret-Liaudet A. Pre-analytical and analytical factors influencing Alzheimer's disease cerebrospinal fluid biomarker variability. *Clin Chim Acta* 2015;449:9–15.
- [25] Vanderstichele HM, Janelidze S, Demeyer L, Coart E, Stoops E, Herbst V, et al. Optimized standard operating procedures for the

- analysis of cerebrospinal fluid Abeta42 and the ratios of Abeta isoforms using low protein binding tubes. *J Alzheimers Dis* 2016; 53:1121–32.
- [26] Bjerke M, Portelius E, Minthon L, Wallin A, Anckarsäter H, Anckarsäter R, et al. Confounding factors influencing amyloid Beta concentration in cerebrospinal fluid. *Int J Alzheimers Dis* 2010; 2010:1–11.
- [27] Toombs J, Paterson RW, Lunn MP, Nicholas JM, Fox NC, Chapman MD, et al. Identification of an important potential confound in CSF AD studies: aliquot volume. *Clin Chem Lab Med* 2013; 51:2311–7.
- [28] Mattsson N, Insel PS, Palmqvist S, Stomrud E, van Western D, Minthon L, et al. Increased amyloidogenic APP processing in APOE ε4-negative individuals with cerebral β-amyloidosis. *Nat Commun* 2016;7:10918.
- [29] Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, van Westen D, Jeromin A, et al. Plasma beta-amyloid in Alzheimer's disease and vascular disease. *Sci Rep* 2016;6:26801.
- [30] Jagust WJ, Landau SM, Koeppe RA, Reiman EM, Chen K, Mathis CA, et al. The Alzheimer's Disease Neuroimaging Initiative 2 PET Core: 2015. *Alzheimers Dement* 2015;11:757–71.
- [31] Barthel H, Gertz HJ, Dresel S, Peters O, Bartenstein P, Buerger K, et al. Florbetaben Study Group. Cerebral amyloid-beta PET with florbetaben (18F) in patients with Alzheimer's disease and healthy controls: a multicentre phase 2 diagnostic study. *Lancet Neurol* 2011; 10:424–35.
- [32] Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nature reviews. Neurology* 2010;6:131–44.
- [33] Palmqvist S, Zetterberg H, Blennow K, Vestberg S, Andreasson U, Brooks DJ, 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; 71:1282–9.
- [34] Mo Y, Stromswold J, Wilson K, Holder D, Sur C, Laterza O, et al. A multinational study distinguishing Alzheimer's and healthy patients using cerebrospinal fluid tau/Abeta42 cutoff with concordance to amyloid positron emission tomography imaging. *Alzheimers Dement (Amst)* 2017;6:201–9.
- [35] Mattsson N, Lonneborg A, Boccardi M, Blennow K, Hansson O Geneva Task Force for the Roadmap of Alzheimer's Biomarkers. Clinical validity of cerebrospinal fluid Abeta42, tau, and phospho-tau as biomarkers for Alzheimer's disease in the context of a structured 5-phase development framework. *Neurobiol Aging* 2017;52:196–213.
- [36] Wang MJ, Yi S, Han JY, Park SY, Jang JW, Chun IK, et al. Analysis of cerebrospinal fluid and [11C]PIB PET biomarkers for Alzheimer's disease with updated protocols. *J Alzheimers Dis* 2016;52:1403–13.
- [37] Fagan AM, Shaw LM, Xiong C, Vanderstichele H, Mintun MA, Trojanowski JQ, et al. Comparison of analytical platforms for cerebrospinal fluid measures of Beta-amyloid 1-42, total tau, and p-tau181 for identifying Alzheimer disease amyloid plaque pathology. *Arch Neurol* 2011;68:1137–44.
- [38] Lucey BP, Fagan AM, Holzman DM, Morris JC, Bateman RJ. Diurnal oscillation of CSF Aβ and other AD biomarkers. *Mol Neurodegener* 2017;12:36.
- [39] Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* 2010;9:119–28.
- [40] Palmqvist S, Mattsson N, Hansson O Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid analysis detects cerebral amyloid-beta accumulation earlier than positron emission tomography. *Brain* 2016;139:1226–36.
- [41] Janelidze S, Zetterberg H, Mattsson N, Palmqvist S, Vanderstichele H, Lindberg O, et al. Swedish BioFINDER study group. CSF Abeta42/Abeta40 and Abeta42/Abeta38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol* 2016;3:154–65.
- [42] Lewczuk P, Leleental N, Spitzer P, Maler JM, Kornhuber J. Amyloid-beta 42/40 cerebrospinal fluid concentration ratio in the diagnostics of Alzheimer's disease: validation of two novel assays. *J Alzheimers Dis* 2015;43:183–91.
- [43] Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* 2009; 65:403–13.
- [44] Lewczuk P, Zimmermann R, Wiltfang J, Kornhuber J. Neurochemical dementia diagnostics: a simple algorithm for interpretation of the CSF biomarkers. *J Neural Transm (Vienna)* 2009;116:1163–7.
- [45] Lewczuk P, Kornhuber J, German Dementia Competence Network, Toledo JB, Trojanowski JQ, Knapik-Czajka M, Peters O, et al. Validation of the Erlangen Score Algorithm for the prediction of the development of dementia due to Alzheimer's disease in pre-dementia subjects. *J Alzheimers Dis* 2015;48:433–41.
- [46] Blennow K, Mattsson N, Scholl M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. *Trends Pharmacol Sci* 2015; 36:297–309.