FEATURED ARTICLE

Robustness of CSF A β 42/40 and A β 42/P-tau181 measured using fully automated immunoassays to detect AD-related outcomes

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Abstract

Introduction: This study investigated the comparability of cerebrospinal fluid (CSF) cutoffs for Elecsys immunoassays for amyloid beta $(A\beta)42/A\beta40$ or $A\beta42/phosphorylated$ tau (p-tau)181 and the effects of measurement variability when predicting Alzheimer's disease (AD)-related outcomes (i.e., $A\beta$ -positron emission tomography [PET] visual read and AD neuropathology).

Methods: We studied 750 participants (BioFINDER study, Alzheimer's Disease Neuroimaging Initiative [ADNI], and University of California San Francisco [UCSF]). Youden's index was used to identify cutoffs and to calculate accuracy (A β -PET visual read as outcome). Using longitudinal variability in A β -negative controls, we identified

Antoine Leuzy and Niklas Mattsson-Carlgren contributed equally to this study.

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a gray zone around cut-points where the risk of an inconsistent predicted outcome was >5%.

Results: For A β 42/A β 40, cutoffs across cohorts were <0.059 (BioFINDER), <0.057 (ADNI), and <0.058 (UCSF). For A β 42/p-tau181, cutoffs were <41.90 (BioFINDER), <39.20 (ADNI), and <46.02 (UCSF). Accuracy was \approx 90% for both A β 42/A β 40 and A β 42/p-tau181 using these cutoffs. Using A β -PET as an outcome, 8.7% of participants fell within a gray zone interval for A β 42/A β 40, compared to 4.5% for A β 42/p-tau181. Similar findings were observed using a measure of overall AD neuropathologic change (7.7% vs. 3.3%). In a subset with CSF and plasma A β 42/40, the number of individuals within the gray zone was \approx 1.5 to 3 times greater when using plasma A β 42/40.

Discussion: CSF A β 42/p-tau181 was more robust to the effects of measurement variability, suggesting that it may be the preferred Elecsys-based measure in clinical practice and trials.

KEYWORDS

Alzheimer's disease, amyloid beta 42/amyloid beta 40, amyloid beta 42/phosphorylated tau 181, amyloid beta positron emission tomography, cerebrospinal fluid, Elecsys, plasma

1 | INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia and is characterized neuropathologically by the presence of extracellular amyloid beta (A β) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau.¹ These features can be detected in vivo using well-validated cerebrospinal fluid (CSF)-based biomarkers, including A β 42 in ratio to A β 40 (A β 42/A β 40) and tau phosphorylated at threonine 181 (A β 42/p-tau181).² Because the accuracy of clinical criteria for AD is suboptimal (when using autopsy data as a reference standard),^{3,4} CSF biomarkers are increasingly used in the workup of patients with cognitive impairment to rule out AD as an underlying cause, and have been incorporated in research diagnostic guidelines for AD⁵⁻⁷ and clinical trials for participant enrichment.

Despite the broad use of CSF A β and p-tau181 in clinical practice in several countries, significant variability in measured concentrations has been reported between laboratories and across sample batches,^{8,9} hampering the establishment of uniform global cutoff values that can be used across sites for defining abnormality. This variability can be traced to (1) pre-analytical (i.e., due to differences in the collection, handling, and storage of samples),¹⁰ (2) analytical (i.e., due to differences in how the assays are run as well as variability in kit components between assay lots),^{8,11} and (3) biological/patient-related (i.e., confounding factors inherent to a given patient, such as age) factors. For a given CSF biomarker, the variability stemming from these three sources results in a "total error" for that measure that can be established by collecting and testing samples on at least two different occasions. The total allowable error (TAE) of a biomarker, by contrast, is the maximum total error for which a biomarker still performs well in its intended clinical use.¹²

While biological/patient-related factors do not appear to pose a significant problem for CSF AD biomarkers,^{13,14} pre-analytical (e.g., sample handling and storage) and analytical (e.g., between/within differences in laboratory procedures) variability is known to affect biomarker values from enzyme-linked immunosorbent assays (ELISA),¹⁵ which are widely used for routine CSF biomarker analysis. This has been shown to result in interlaboratory variation up to 15%.¹⁵ This can be addressed through the use of standardized protocols for CSF collection and storage¹⁶ and fully automated platforms such as the Roche Elecsys immunoassays that have high test-retest reliability (<5%) and low laboratory- and kit-associated variability.¹³ These show excellent concordance with manual ELISAs¹⁷ and have been well validated against Aβ-positron emission tomography (PET)¹⁸⁻²⁰ and post mortem data.²¹ Despite the low variability levels described for fully automated assays, total error levels may result in variable classification (i.e., an individual is classified as having normal AD biomarker findings at one time point and abnormal at another), increasing the diagnostic uncertainty at the individual patient level.

In the present study, we aimed to (1) assess the comparability of CSF cutoffs for Elecsys-based A β 42/A β 40 and A β 42/p-tau181 across three separate cohorts to assess the impact of different preanalytical protocols and (2) assess the effects of variability over repeated CSF measurements (test-retest variability) on AD-related outcomes (i.e., A β -PET visual read and a measure of overall AD neuropathologic change). Further, given the interest in blood tests for A β pathology²² the performance of CSF A β 42/A β 40 was compared to that for plasma A β 42/A β 40 measured using Elecsys immunoassays in a subset of participants. We hypothesized that: (1) we would obtain similar cutoffs for A β 42/A β 40 and A β 42/p-tau181; (2) CSF A β 42/p-tau181 would prove more robust to longitudinal variation, compared to CSF A β 42/A β 40;

RESEARCH IN CONTEXT

- Systematic Review: The authors reviewed the available literature on PubMed for articles on fully automated assays for cerebrospinal fluid (CSF) amyloid beta (Aβ)42/Aβ40 and Aβ42/phosphorylated tau (p-tau)181. Despite the low variability levels described for fully automated assays, total error levels may result in variable classification. Further, uniform global cutoffs for defining abnormality have yet to be established.
- 2. Interpretation: Similar cutoffs could be applied across three cohorts for Elecsys CSF $A\beta 42/A\beta 40$ and $A\beta 42/p$ tau181. Compared to $A\beta 42/A\beta 40$, $A\beta 42/p$ -tau181 was more robust to the effects of measurement variability in that the percentage of participants showing a >5% chance (gray zone) of having a different predicted outcome ($A\beta$ -positron emission tomography visual read and a measure of overall Alzheimer's disease neuropathologic change) if they were to have two CSF measurements close in time was \approx 1.5 to 2 times greater for $A\beta 42/A\beta 40$. This suggests that $A\beta 42/p$ -tau181 may be the preferred Elecsys-based measure in clinical settings and clinical trials.
- 3. Future Directions: Further validation studies are required for gray zone cases.

and (3) CSF A β 42/A β 40 would prove superior to plasma A β 42/A β 40 in terms of the percentage of individuals showing uncertain outcomes.

2 | METHODS

2.1 | Participants

We included 750 participants with Elecsys CSF A_β42/A_β40 and A β 42/p-tau181 and A β -PET from three separate cohorts: 172 from the Swedish BioFINDER study (clinical trial no. NCT01208675), scanned with [¹⁸F]flutemetamol (85 cognitively unimpaired [CU] and 87 with mild cognitive impairment [MCI]), 318 from the Alzheimer's Disease Neuroimaging Initiative (ADNI; clinical trial NCT00106899; 54 CU controls and 264 MCI), scanned with [¹⁸F]florbetapir, and 260 from the University of California San Francisco (UCSF) Alzheimer's Disease Research Center (55 CU, 22 MCI, 37 AD dementia, and 146 with various non-AD disorders) who were scanned with [¹¹C]Pittsburgh compound B ([¹¹C]PiB). Inclusion criteria for individuals with and without cognitive impairment have been described elsewhere and are described in the supporting information. Written informed consent was obtained from all participants, either directly or from a legally authorized representative, with patient assent. Studies were approved by local institutional review boards.

To obtain estimates of the total variability of AB42/AB40 and also included 315 CU individuals from BioFINDER with biannual longitudinal CSF data extending up to 8 years (partially overlapping with the main cohort, but without requiring A β -PET). To focus on non–ADrelated variability, we defined $A\beta$ positivity in this group at baseline using a stringent threshold derived with Gaussian mixture modeling (GMM) of CSF A\u03c342/A\u03c340 at baseline. The GMM analysis identified two modes, representing the Aβ-negative and Aβ-positive CUs. We used the upper 95% confidence limit of the leftward (i.e., $A\beta$ -positive) distribution as the cutoff for subject inclusion. Sensitivity analyses for estimates of variability were also performed using an alternative cutoff to define $A\beta$ negativity, generated by the maximum separation of modes in the GMM analysis. A final sensitivity analysis for estimates of variability was done using an additional dataset from 38 participants randomly selected from the clinical practice of the Memory Clinic at Skåne University Hospital (test-retest cohort) who completed two lumbar punctures (morning and evening) and blood sampling within 6 to 10 weeks (average 7.4 \pm 1.05 weeks). The collection procedure, amount of fluid collected, and pre-analytical handling protocol was identical across visits. Approximately half (47.4%) were A β -positive.

2.2 | CSF biomarkers

Lumbar puncture and CSF handling in BioFINDER has previously been described in detail.²³ After collection and centrifugation at three centers, CSF supernatant was stored in 1 ml aliguots in polypropylene tubes at <-60°C. In ADNI. CSF collection was performed as described in the ADNI procedures manual (http://www.adni-info.org/). Within 1 hour of collection CSF samples were frozen on dry ice and shipped 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 \leq -80°C. For UCSF samples, CSF was obtained following ADNI protocols,²⁴ and transferred from collection tubes into polypropylene tubes and frozen within 1 hour of sampling. A β 42, A β 40, and p-tau181 were measured at three different centers using the Elecsys immunoassays on a Cobas e 601 analyzer: the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden (BioFINDER); the Biomarker Research Laboratory, University of Pennsylvania, USA (ADNI); and the Clinical Chemistry Department at Skåne University Hospital, Malmö, Sweden (UCSF), according to the preliminary kit manufacturer's instructions and as described previously.25

2.3 \mid A β -PET acquisition and processing

For BioFINDER, [¹⁸F]flutemetamol studies were performed using a Philips Gemini TF PET/computed tomography (CT) scanner (Philips Medical Systems), 90 to 110 minutes post-injection; data were acquired in list mode and binned into frames using an iterative Vue Point HD algorithm (six subsets, 18 iterations with 3 mm filter and no time-of-flight correction).

For ADNI, [¹⁸F]florbetapir data were acquired 50 to 70 minutes post-injection.²⁶ For UCSF, [¹¹C]PiB data were acquired 50 to 70 minutes post-injection on a Siemens Biograph PET/CT scanner. All participants completed T1-weighted magnetization-prepared rapid gradient echo magnetic resonance imaging scans²⁷ for PET image co-registration and template normalization.

2.4 Visual read of Aβ-PET images

Banked [¹⁸F]flutemetamol (BioFINDER) or [¹⁸F]florbetapir (ADNI) PET images were re-evaluated by three independent readers at Molecular NeuroImaging, New Haven, Connecticut, USA.²³ A similar approach was used for [¹¹C]PiB.²⁸

2.5 | Neuropathology

Neuropathological assessments were available for 90 participants from the UCSF cohort. Assessments were performed according to previously described procedures²⁹—blinded to CSF results³⁰—and included the AD Thal amyloid phase,³¹ indicating topographical extent of A β plaque pathology; Braak neurofibrillary tangle stage,³² indicating the topographical extent of tau neurofibrillary pathology; and Consortium to Establish a Registry for Alzheimer's Disease (CERAD) score,³³ indicating the density of neocortical neuritic plaques. Thal phase, Braak stage, and CERAD score were aggregated in the Alzheimer's Disease Neuropathological Change (ADNC) score.³⁰ ADNC has four levels: none, low, intermediate, or high. These were combined into none/low (negative ADNC) and intermediate/high (positive ADNC).

2.6 Statistical analyses

All analyses were performed in R, version 4.1.2. Youden's index was used to identify optimal cutoffs for A β 42/A β 40 and A β 42/p-tau181 that separated A β -positive and A β -negative individuals based on A β -PET visual read. Using these cutoffs, sensitivity, specificity, and accuracy (i.e., percentage of correctly classified individuals) were calculated within and between cohorts.

To assess classification stability and identify a gray zone around cut points where there is a risk of misclassification (i.e., those with a >5% chance of having a different predicted outcome due to measurement variability if they were to have two CSF measurements close in time), we first determined the longitudinal variability of A β 42/A β 40 and A β 42/p-tau181 in A β -negative CU individuals. This was calculated as the standard deviation (SD) of the average relative percent change between biomarker values across measurements. Next, we randomly varied CSF biomarker values for each participant based on a random sample from a normal distribution with mean equal to zero and SD equal to the longitudinal variability estimate for each biomarker. This simulation was run over 1000 bootstrap trials to obtain 95% confidence interval (CI)-based gray zones for cutoffs. We report the percentage of study participants whose predicted outcomes (A β -PET visual read and AD neuropathologic change) changed when biomarkers were randomly varied. The proportions of individuals within the gray zones for A β 42/A β 40 and A β 42/p-tau181 were compared using Fisher's exact test. In addition, constrained generalized additive models were used to examine the estimated risk of having an abnormal A β -PET scan across different levels of CSF A β 42/A β 40 and A β 42/p-tau181. These models were fit using the R cgam package version 1.17.³⁴ Last, in the subset of individuals from BioFINDER that had both plasma and CSF A β 42/A β 40 (n = 139), a receiver operating characteristic (ROC) analysis was performed using CSF A β 42/A β 40 status as outcome and plasma A β 42/A β 40 as predictor.

2.7 | Plasma Aβ42/Aβ40 in BioFINDER

Blood samples collected at the same time as CSF samples were available in a subset of participants from BioFINDER (n = 139) and in the test-retest cohort (n = 38). Non-AD-related variability in plasma A β 42/A β 40 was determined in the same fashion as for CSF (i.e., SD of the average relative percent change between biomarker values across measurements) in the subset of A β -negative CU participants with available longitudinal plasma samples (n = 248). Details surrounding plasma collection have been described previously³⁵ and are included in the supporting information. Plasma A β 42 and A β 40 were measured using the Elecsys immunoassays on a Cobas e 601 analyzer at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden. Performance of plasma A β 42/A β 40 was compared to that for CSF A β 42/A β 40 in the same subjects, using A β -PET visual read as outcome (n = 46).

3 | RESULTS

3.1 | Accuracies of CSF biomarkers to predict $A\beta$ -PET

Participant selection and characteristics are summarized in Figure S1 in supporting information and Table 1. Youden index-based cutoffs (A β -PET visual read as outcome) by cohort were as follows: BioFINDER (<0.059[95% CI, 0.052-0.063] for A β 42/A β 40 and <41.90 [95% CI, 34.26-54.98] for A β 42/p-tau181), ADNI (<0.056 [95% CI, 0.052-0.060] for A β 42/A β 40 and <39.20 [95% CI, 29.02-56.29] for A β 42/A β 40 and <39.20 [95% CI, 0.050-0.067] for A β 42/A β 40 and <46.02 [95% CI, 34.20-51.54] for A β 42/p-tau181). Using these cutoffs, average accuracies of \approx 90% were achieved for both A β 42/A β 40 (range 90.70%-94.03%) and A β 42/p-tau181 (range 89.77%-94.13%). Accuracies—along with sensitivities/specificities are reported in Table 2. Cross-validation of cutoffs between cohorts showed that accuracies were stable across cohorts and cutoffs (Figure S2 in supporting information). When using common cutoffs—defined as

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TABLE 1 Participant characteristics

	BioFINDER	ADNI	UCSF
Total n	172	318	260
CU/CI, n	85/87	54/264	54/206
Age, years	70.39 (5.40)	71.06 (7.16)	65.02 (8.92)
Female, n (%)	72 (42%)	163 (51%)	122 (47%)
Education, years	11.63 (3.27)	16.16 (2.77)	16.84 (3.09)
APOE ε4+, n (%)	79 (46%)	170 (53%)	96 (34%)*
MMSE	27.94 (1.70)	27.95 (1.79)	24.35 (5.52)**
Elecsys Αβ42/Αβ40	0.068 (0.030)	0.053 (0.023)	0.070 (0.025)
Elecsys Aβ42/P-tau181	63.61 (43.49)	42.74 (33.04)	67.87 (39.10)
A β -PET, visual read, n (%)	77 (45%)	191 (60%)	82 (32%)

Abbreviations: $A\beta$, amyloid beta; ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE, apolipoprotein E; CI, cognitively impaired; CU, cognitively unimpaired; MMSE, Mini-Mental State Examination; PET, positron emission tomography; p-tau, phosphorylated tau; UCSF, University of California San Francisco.

*APOE data missing for 26 participants.

**MMSE data missing for 21 participants.

TABLE 2 Performance of Youden index-based cutoffs

			Performance [95% CI]		
CSF measure	Cohort	Cutoff [95% CI] (Youden-based)	Sensitivity	Specificity	Accuracy
Αβ42/Αβ40	BioFINDER	<0.059 [0.052, 0.063]	93.97 [90.45, 96.98]	83.92 [79.22, 88.24]	88.33 [85.24, 91.41]
	ADNI	<0.056 [0.051, 0.060]	98.24 [96.48, 99.56]	87.40 [81.11, 92.91]	94.34 [91.82, 96.54]
	UCSF	<0.058 [0.050, 0.067]	95.12 [90.24, 98.78]	91.01 [86.52, 94.94]	93.08 [90.0, 96.15]
Aβ42/p-tau181	BioFINDER	<41.90 [34.26, 54.98]	91.46 [87.44, 94.97]	86.27 [81.57, 90.21]	88.55 [85.02, 90.97]
	ADNI	<39.20 [29.40, 56.20]	95.81 [92.67, 96.54]	91.34 [86.61, 96.10]	94.03 [91.19, 96.54]
	UCSF	<46.02 [34.20, 51.54]	95.12 [90.21, 98.78]	92.70 [88.76, 96.63]	93.46 [90.38, 96.15]

Note: Youden-based cutoffs were based on A β -PET visual read as outcome.

Abbreviations: Aβ, amyloid beta; ADNI, Alzheimer's Disease Neuroimaging Initiative; CI, confidence interval; CSF, cerebrospinal fluid; PET, positron emission tomography; p-tau, phosphorylated tau; UCSF, University of California San Francisco.

the average cutoff across cohorts (i.e., 0.058 for $A\beta 42/A\beta 40$ and 42.37 for $A\beta 42/p$ -tau181)—no significant differences were seen in accuracy across cohorts (Table S1 in supporting information).

3.2 | Longitudinal variability and robustness of predictions using CSF biomarkers

Using longitudinal CSF data from a separate cohort of A β -negative CU individuals (n = 269), the observed variability (SD of the average relative percent change between biomarker values) was 11.18% for A β 42/A β 40 and 19.35% for A β 42/p-tau181.

Using these variability estimates we then estimated the robustness of A β 42/A β 40 and A β 42/p-tau181 by calculating the percentage of participants with uncertain predicted outcomes. Based on 95% Cls for A β 42/A β 40 and A β 42/p-tau181 cut points, we found that, on average, a significantly higher proportion of participants (8.71%) fell within this uncertain interval for A β 42/A β 40 (11.04% for BioFINDER, 8.17% for ADNI, 6.92% for UCSF) compared to A β 42/p-tau181 (average 4.45%: 5.81% for BioFINDER, 4.08% for ADNI, 3.46% for UCSF) using A β -PET visual read as outcome (P < 0.01; Figure 1). Similar results were obtained when performing sensitivity analyses using estimates of variability from A β -negative participants defined using a GMM-based cutoff (Table S2 in supporting information), when using variability estimates from the test-retest cohort (Table S3 in supporting information), and when varying the risk level of an inconsistent predicted outcome (1%, 10%, and 15%; Table S4 in supporting information).

Data points for A β 40, A β 42, and p-tau181 in relation to cutoffs for A β 42/A β 40 and A β 42/p-tau181 are shown in Figure S3 in supporting information. Average concordance between A β 42/A β 40 and A β 42/p-tau181 (i.e., percentage of individuals positive or negative for both measures using cutoffs from Table 2) across cohorts was



FIGURE 1 Scatterplots ($A\beta 42/A\beta 40$ vs. $A\beta 42/p$ -tau181) showing Youden index-based cutoffs for $A\beta$ -PET positivity and associated gray zones. Findings are shown for BioFINDER (A), ADNI (B) and UCSF (C) cohorts. In each panel, the solid lines indicate the Youden-based cutoffs (set using positive $A\beta$ -PET visual read as outcome) while the dot-dashed lines indicate gray zones where there was a >5% chance of misclassification due to the variability of each CSF biomarker. $A\beta$, amyloid beta; ADNI, Alzheimer's Disease Neuroimaging Initiative; CI, cognitively impaired; CSF, cerebrospinal fluid; CU, cognitively unimpaired; PET, positron emission tomography; p-tau, phosphorylated tau; UCSF, University of California San Francisco

94.43% (BioFINDER, 93.60%; ADNI, 96.22%; UCSF, 93.46%). Using constrained generalized additive models, more abnormal ratio values of A β 42/p-tau181 were shown to carry a higher risk of an abnormal A β -PET scan, compared to CSF A β 42/A β 40 (Figure 2, Figures S4 and S5 in supporting information).

In the UCSF neuropathology subset—in which the mean interval between lumbar puncture and death was 2.9 years (SD 1.8 years, range 0.2–7.5)—a trend (P = 0.08) toward a significantly higher proportion of participants with uncertain outcomes was found for A β 42/A β 40 (8.89%) compared to A β 42/p-tau181 (3.30%) using the previously defined variability estimates for A β 42/A β 40 and A β 42/p-tau181 and ADNC score as outcome (negative:none/low [70%]; positive:intermediate/high [30%]).

3.3 | CSF biomarker status in relation to A β -PET and ADNC scores

To examine whether participants in the gray zone for CSF A β 42/A β 40 and A β 42/p-tau181 were close to the cutoffs for A β -PET, we plotted A β 42/A β 40 and A β 42/p-tau181 status (negative, positive, or in the gray zone using A β -PET visual read as outcome) in relation to A β -PET standardized uptake value ratio and ADNC scores (Figure S6 in supporting information). Using a priori cutoffs for A β -PET,^{36,37} an average of 73.38% (BioFINDER-1, 73.68%; ADNI, 73.08%) and 91.16% (BioFINDER-1, 90%; ADNI, 92.30%) of gray zone cases were A β -PET positive using A β 42/A β 40 and A β 42/p-tau181, respectively. In the UCSF cohort, 14.81% and 7.41% of gray zone cases had intermediate/high ADNC scores for A β 42/A β 40 and A β 42/p-tau181, respectively.

3.4 Longitudinal variability and robustness of predictions using plasma biomarkers

and plasma (Table S5 in supporting information), 9.35% fell within the interval associated with >5% chance of having a different predicted A_β-PET visual read over time. For plasma-in which the longitudinal variation for A\u03c642/A\u03c640 was 7.49\u03c8 in A\u03c6-negative CU participants (average follow-up, 3.65 ± 1.57 years)-30.93% of participants fell within the interval associated with a >5% chance of having a different predicted outcome over time when using $A\beta$ -PET visual read as outcome. Improved findings (28.78% with uncertain outcome) were obtained when plasma A_β42/A_β40 variation was derived from the test-retest cohort (4.10%). Data points for $A\beta 40$ and $A\beta 42$ (CSF and plasma) are shown in relation to the cutoffs for Aβ42/Aβ40 in Figure S7 in supporting information. ROC analysis using using the Youden-based cutoff of <0.0610) showed that plasma Aβ42/Aβ40 had an area under the curve (AUC) of 0.834 (95% CI, 0.762-0.907).

3.5 Online individualized risk prediction tool

We provide an illustrative online tool at https://brainapps.shinyapps.io/ CSFpredict_GZ/, where individualized predictions can be made for A β -PET using CSF A β 42/A β 40 and A β 42/p-tau181. Illustrative cases showing positive, negative, and gray zone CSF results for A β 42/p-tau181 are shown in Figure 3.

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FIGURE 2 Estimated risk of A β -PET positivity for CSF A β 42/A β 40 and A β 42/p-tau181 in BioFINDER. The estimated risk of being A β -PET positive is shown for CSF A β 42/A β 40 (A and B) and A β 42/P-tau181 (C and D), assuming a prevalence of \approx 30% A β -PET positivity (dashed gray line). Results are shown using ratios (A and C) and percentiles (B and D). While the risk of being A β -PET positive was low for both A β 42/A β 40 and A β 42/p-tau181 at higher (normal) levels, the risk of A β -PET positivity was greater for A β 42/p-tau181 at lower (abnormal) levels. A β , amyloid beta; ADNI, Alzheimer's Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; PET, positron emission tomography; p-tau, phosphorylated tau; UCSF, University of California San Francisco

4 DISCUSSION

As universal cutoffs are a key prerequisite for the widespread use of Aβ42/Aβ40 and Aβ42/p-tau181 in clinical practice, we examined how comparable cutoffs were across cohorts and the impact of different cutoffs on accuracy (i.e., percentage of correctly classified individuals). Despite the CSF samples having been analyzed in different laboratories using several preanalytical protocols-and there being differences in cohort composition and A_β-PET tracers—we did not observe significant differences in either cutoffs or accuracies across measures and cohorts, nor in accuracies using a common (average) cutoff across cohorts for A
^β42/A
^β40 and A
^β42/p-tau181. These findings are in line with previous work using CSF Elecsys AD biomarkers, in which it was shown that a cutoff combining $A\beta 42$ and p-tau181 established in BioFINDER and validated in ADNI showed high concordance with visual read A β -PET classification.²³ Though these results suggest, overall, that Elecsys $A\beta 42/A\beta 40$ and $A\beta 42/p$ -tau181 may prove relatively robust to methodological differences, numeric differences of $\approx 3\%$ between cohorts indicate that a unified preanalytical protocol for CSF handling may nevertheless be optimal to facilitate the establishment of global cutoff values required for routine use of CSF biomarkers in clinical practice.^{38,39}

Across the investigated outcomes (Aβ-PET visual read, and ADNC score), the percentage of individuals at risk for misclassification was lower for A\u03c342/p-tau181, compared to A\u03c342/A\u03c340. The superior performance of A_β42/p-tau181 may reflect the greater TAE of this measure. Despite having a greater estimate of measurement variability (19.35% vs. 11.18% for A\u03c642/A\u03c640 using the SD of the average relative percent change between biomarker measurements from longitudinal data in A\beta-negative CU individuals), fewer subjects fell within the range associated with a > 5% chance of varying back and forth across the cutoff threshold. This can likely be explained by the fact that data points for A β 42/A β 40 are closer to the cutoff than for A β 42/ptau181. The superiority of A β 42/p-tau181 when using A β -PET visual read and ADNC score as outcomes may also be due to this marker combining measures of two different pathologic processes into a single measure. As CSF A β 42 and p-tau181 concentrations are thought to change at different points in the disease course (earlier for A
^β42, later for p-tau181),⁴⁰ their combination may improve performance when predicting measures of disease stage,⁴¹ such as A β -PET status, measures of clinical progression, or post mortem estimates of AD pathology burden. Moreover, in clinical settings in which $A\beta$ -PET is increasingly used, risk curves based on constrained generalized additive models clearly showed that abnormal A β 42/p-tau181 ratio values carried a



CSF biomarker prediction of amyloid PET positivity



(B)

CSF biomarker prediction of amyloid PET positivity



FIGURE 3 Online individualized risk prediction tool for $A\beta$ -PET. BioFINDER-based individualized risk predictions are shown for $A\beta$ -PET using CSF $A\beta$ 42/ $A\beta$ 40 and $A\beta$ 42/p-tau181 levels that are (A) positive, (B) negative, and (C) in the gray zone for either marker. Cutoffs and associated intervals used to define gray zones were 0.059 [0.052, 0.063] for CSF $A\beta$ 42/ $A\beta$ 40 and 41.90 [34.40, 54.98] for CSF $A\beta$ 42/p-tau181. $A\beta$, amyloid beta; CI, confidence interval; CSF, cerebrospinal fluid; PET, positron emission tomography; p-tau, phosphorylated tau

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higher risk of A β -PET positivity compared to A β 42/A β 40. As a result, this measure should provide greater confidence to physicians when A β status is of diagnostic relevance. Where possible, however, combining measures of A β , tau, and neurodegeneration⁶ may improve prognostic value compared to the use of A β 42/p-tau181 alone, though further work addressing this is required. Further, in clinical trials in preclinical AD, A β 42/A β 40 may prove more sensitive to very early A β pathology compared to A β 42/p-tau181.⁴²

In the present study we estimated the percentage of cases whose predicted status (normal vs. abnormal) on different AD-related outcomes would have a >5% chance to differ after repeated lumbar punctures. Our results call attention to the importance of such gray

zones when using CSF AD biomarkers. While the number of participants falling within this zone was relatively low (e.g., 11.04%, on average, for A β 42/A β 40, and 5.81% for A β 42/p-tau181 when using A β -PET status as outcome), these figures nevertheless translate into significant case numbers when extrapolated to scenarios in which these biomarkers are used in clinical practice. Gray zones have been previously described for A β 42/p-tau181 measured using INNOTEST ELISAs in patients with dementia,⁴³ and have also been described in recommendations for the use of CSF in the diagnostic workup of MCI patients.⁴⁴ Similar to other areas of medicine, including oncology and cardiology,⁴⁵⁻⁴⁷ recommendations have included that the analysis be repeated or that an imaging-based investigation be performed. The upper and lower boundaries defining the gray zone for a given marker could even be used in the clinical chemistry laboratory providing the CSF results to clinicians, such that a gray zone result would result in immediate re-testing of the sample. Should the result still fall within the gray zone for that measure, a new lumbar puncture could be ordered, or an imaging biomarker ordered. Our findings on the percentage of gray zone cases that were A β -PET positive support this approach, particularly for A β 42/p-tau181 (where \approx 90% were A β -PET positive, compared to \approx 73% for A β 42/A β 40).

Though measuring $A\beta$ reliably in blood has proven challenging using traditional ELISA technology,⁴⁸ plasma A β 42/40 determined using high-precision immunoprecipitation-coupled mass spectrometry (IP-MS) has been shown to correlate with A β -PET and to accurately identify individuals who are A β -positive using PET.⁴⁹ More recent work using fully automated immunoassay platforms-including the Elecsys platform from Roche-have shown accuracies for predicting $A\beta$ -PET that approach those for MS-based A β 42/40 measures.³⁵ We therefore compared the performance of CSF $A\beta 42/40$ to that for plasma A β 42/40, both measured using the Elecsys platform. Compared to CSF A β 42/40, the number of participants that fell within the range associated with >5% chance of having a different predicted outcome over time was ≈ 1.5 to 3 times greater when using plasma A β 42/40. This is likely because the data points for $A\beta 42/A\beta 40$ in plasma are closer to the cutoff than those for CSF. Though we were unable to compare CSF A_β42/40 with plasma A_β42/40 measured using a different immunoassay, the difference in performance between CSF and plasma would likely have been even greater as a recent head-tohead comparison of several IP-MS and immunoassays showed that $A\beta 42/40$ measured using Elecsys immunoassays was the best performing in terms of AUC values using CSF AB42/40 and AB-PET status as outcome.⁵⁰ The superior performance of A β 42/40 in CSF, compared to plasma, can be attributed to the fact that $A\beta$ brain pathology results in a decrease in plasma $A\beta 42/40$ of 8% to 15%, compared to a decrease of \approx 50% in CSF.^{2,22} This difference is thought to relate to peripheral Aß production, binding to peripheral blood proteins that are present at approximately 200-fold higher concentrations in plasma than in CSF, and liver metabolic rates.²²

Strengths of this study include its large sample size, the use of three separate cohorts, and the use of multiple AD-related outcomes. Moreover, CSF biomarkers were measured using the same type of (Elecsys) platform at three different sites and the variability estimates for A³42/A³40 and A³42/p-tau181 were determined using several approaches. Also, we compared the performance of $A\beta 42/A\beta 40$ in CSF and plasma measured in the same individuals using the same immunoassays. We also provide an online prediction tool to illustrate the possible future use of such a tool in clinical settings, though future studies will be required to address its performance in other cohorts. A limitation of this study was that there were differences in the clinical composition of cohorts and the outcome used to set Youdenbased cutoffs (i.e., $A\beta$ -PET visual read) involved three different $A\beta$ -PET tracers ([¹⁸F]flutemetamol, [¹⁸F]florbetapir, and [¹¹C]PIB); despite these differences, however, the percentage of correctly classified individuals (i.e., accuracy) was similar across cohorts. Moreover, such

methodological differences are likely representative of the variability in current clinical practice. Further, though A β -PET visual read analysis has been shown to be well correlated with A β pathology *post mortem*,⁵¹ visual read remains a proxy for histopathology and is partially subjective and reader dependent. Last, the included pathology cohort was small, which likely explains the trend level finding when using ADNC scores as outcome. This warrants further work in a larger *post mortem* cohort.

In conclusion, we here showed that similar cutoffs could be applied across centers to Elecsys CSF A β 42/A β 40 and A β 42/p-tau181 and that A β 42/p-tau181 was more robust to the effects of measurement variability in terms of AD-related outcomes than A β 42/A β 40, using three separate cohorts with different populations, preanalytical protocols, and A β -PET tracers. These findings suggest that A β 42/p-tau181 may be the preferred Elecsys-based measure in clinical settings and clinical trials. Future studies are required, however, addressing gray zone cases.

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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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