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RESEARCH ARTICLE

P-tau/Aβ42 and Aβ42/40 ratios in CSF are equally predictive of amyloid PET status

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Abstract

Introduction: Measurement of amyloid beta (Aβ40 and Aβ42) and tau (phosphorylated tau [p-tau] and total tau [t-tau]) in cerebrospinal fluid (CSF) can be utilized to differentiate clinical and preclinical Alzheimer's disease dementia (AD) from other neurodegenerative processes.

Methods: CSF biomarkers were measured in 150 participants from the Mayo Clinic Study of Aging and the Alzheimer's Disease Research Center. P-tau/Aβ42 (Roche Elecsys, Fujirebio LUMIPULSE) and Aβ42/40 (Fujirebio LUMIPULSE) ratios were compared to one another and to amyloid positron emission tomography (PET) classification.

Results: Strong correlation was observed between LUMIPULSE p-tau/Aβ42 and Aβ42/40, as well as Elecsys and LUMIPULSE p-tau/Aβ42 and Aβ42/40 (Spearman's $\rho = -0.827$, -0.858, and 0.960, respectively). Concordance between LUMIPULSE p-tau/Aβ42 and Aβ42/40 was 96% and between Elecsys p-tau/Aβ42 and both LUMIPULSE ratios was 97%. All ratios had > 94% overall, positive, and negative percent agreement with amyloid PET classification.

Discussion: These data suggest that p-tau/Aβ42 and Aβ42/40 ratios provide similar clinical information in the assessment of amyloid pathology.

KEYWORDS
Alzheimer's disease dementia, amyloid positron emission tomography, amyloid beta 42/40, cerebrospinal fluid biomarkers, Elecsys, Immunoassay, LUMIPULSE, p-tau/Aβ42

1 | BACKGROUND

Alzheimer's disease (AD) is characterized by neuronal and synaptic degeneration resulting from the formation of extracellular amyloid plaques comprised of amyloid beta (Aβ) fibrils and intracellular aggregates of hyperphosphorylated tau.[1–3] Established biomarkers of these proteins are used in research and clinical trials. Positron emission tomography (PET) imaging can be used to visualize the presence of amyloid lesions in the cerebral cortex.[4–6] Amyloid PET tracers have been shown to have high agreement with histopathologic amyloid aggregates and are currently used as the standard to evaluate the clinical utility of AD cerebrospinal fluid (CSF) biomarkers.[7–9] In CSF, the combination of low concentrations of Aβ42 and high concentrations of total tau (t-tau) and phosphorylated tau (p-tau) reflect the

pathophysiological hallmarks of AD and have shown promising potential for the evaluation of AD in symptomatic and pre-symptomatic individuals.[2,10] At present, CSF AD biomarkers are included in trials of potential disease-modifying therapies for purposes of identifying eligible participants, monitoring therapeutic target engagement, and evaluating clinical trial outcome.[11–21]

Despite the routine use of these biomarkers in research and clinical trials, the use of CSF biomarkers in clinical practice has proven challenging due to assay limitations leading to between-laboratory and lot-to-lot variation. In recent years, assays to measure AD CSF biomarkers have been developed for measurement on high-throughput automated platforms, including the Roche Elecsys and Fujirebio LUMIPULSE assays, resulting in a more consistent analytical process. The Roche Elecsys CSF assays include β-Amyloid (1-42), pTau (181P) and total-Tau, while the Fujirebio LUMIPULSE G assays include β-Amyloid 1-42, β-Amyloid 1-40, pTau 181, and total Tau assays. Although these newer assays have been shown to be analytically superior to previous generation assays, there are no standardized cut-offs for identification of amyloid positivity associated with a diagnosis of AD dementia and/or mild cognitive impairment (MCI). Multiple studies have evaluated agreement of Aβ42, Aβ40, t-tau, p-tau, and their ratios with amyloid PET using various automated platforms.[22–26] In these studies, the use of p-tau/Aβ42 and Aβ42/40 ratios showed a higher percent agreement with normal/abnormal amyloid PET classification than individual biomarkers.[23–26] While different assay manufacturers may recommend the use of different ratios based on the assay availability on the respective automated platforms, studies have not compared the performance of the Elecsys and LUMIPULSE assays in the same participant cohort to assess if the use of the p-tau/Aβ42 ratio or the Aβ42/40 ratio provides superior agreement with amyloid PET classification. The goal of this study was to determine whether different biomarker ratios (p-tau/Aβ42 and Aβ42/Aβ40) and assays (Roche Elecsys and Fujirebio LUMIPULSE) differ in their ability to predict amyloid PET positivity in the same well-characterized participant cohort.

HIGHLIGHTS

- Cerebrospinal fluid Alzheimer's disease biomarkers were measured on Roche and Fujirebio platforms.
- Phosphorylated tau (p-tau)/amyloid beta (Aβ)42 and Aβ42/40 analyte ratios were compared on a cohort of 150 individuals.
- Both p-tau/Aβ42 and Aβ42/40 ratios showed similar agreement with amyloid positron emission tomography.

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature on Alzheimer's disease (AD) cerebrospinal fluid (CSF) biomarkers using Elecsys and LUMIPULSE assays available in PubMed. Several studies have evaluated the use of amyloid beta (Aβ)40, Aβ42, total tau (t-tau), and phosphorylated tau (p-tau), and various ratios but none so far have done a side-by-side CSF marker comparison on the same sample cohort between these assays.
2. **Interpretation**: Our data indicate that the Elecsys and LUMIPULSE p-tau/Aβ42 and LUMIPULSE Aβ42/40 ratios in CSF show excellent concordance and overall, positive, and negative percent agreement with amyloid PET classification in a cohort of clinically characterized participants.
3. **Future directions**: Our research provides evidence supporting the use of either ratio, p-tau/Aβ42 and Aβ42/40, in CSF to assess amyloid pathology as an alternative to amyloid PET imaging. Replication of results in a larger participant cohort would strengthen our findings.

2 | METHODS

2.1 | Participants

Participants' CSF samples were collected at the Mayo Clinic in Rochester, Minnesota, between 2016 and 2020. A total of 150 CSF samples were included from the Mayo Clinic Study of Aging (MCSA) and the Alzheimer's Disease Research Center (ADRC). The MCSA is a longitudinal population-based study of residents of Olmsted County, Minnesota.[27,28] MCSA participants are evaluated every 15 months by a study coordinator, a physician, and a neuropsychologist. Final clinical diagnoses were established by consensus using previously published criteria.[27,29–31] The ADRC is a Mayo Clinic–based longitudinal study in which participants with a variety of neurodegenerative disease diagnoses made by behavioral neurologists are invited to participate. Both protocols have been approved by the institutional review boards of Mayo Clinic and Olmsted Medical Center. Written informed consent was obtained from all participants. Only participants clinically categorized as cognitively unimpaired (CU, $n = 107$), MCI ($n = 22$), or AD dementia (AD, $n = 21$) were included in this study. A subset of the participants ($n = 128$; 85%) also underwent ^{11}C Pittsburgh compound B PET (^{11}C PiB PET) imaging within 1 year of lumbar puncture.

2.2 | CSF collection/processing

CSF samples were collected from fasting participants during early morning hours. Lumbar punctures were performed using a 20- or 22-gauge Quincke needle in the lateral decubitus position from the L3 and L4 intravertebral space.[32] CSF was stored in polypropylene tubes at –80°C for durations ranging from approximately 9 months to 4 years. Because multiple aliquots from the same collection were available per patient, a separate tube was used for each platform. On the day of analysis, samples were thawed at room temperature, inverted 6 to 10

times, and vortexed for 20 seconds immediately prior to analysis on each instrument described below. Testing was performed on the same day on both platforms for individual patients.

2.3 | Elecsys assays

Aβ42, p-tau, and t-tau were quantified using Roche Elecsys β-Amyloid (1-42), pTau (181P)), and total-Tau assays, per manufacturer's instructions, on a Roche cobas 6000 e 601 module (Roche Diagnostics). Prior to use, the analytical performance of the assays was deemed acceptable through internal verification studies. Vendor-supplied quality control materials were measured at the beginning of each day of testing and were within target ranges before analysis of samples. In-house cutoffs derived using a group of 524 participants from the MCSA and ADRC, different to the ones included here, were applied as follows: Aβ42 (1026 pg/mL), p-tau (21.7 pg/mL), t-tau (238 pg/mL), p-tau/Aβ42 ratio (0.023) (manuscript in preparation).

2.4 | LUMIPULSE assays

Aβ42, Aβ40, p-tau, and t-tau were quantified using Fujirebio LUMIPULSE G β-Amyloid 1-42, β-Amyloid 1-40, pTau 181, and total Tau assays, per manufacturer's instructions, on a LUMIPULSE G1200 analyzer (Fujirebio). Prior to use, the analytical performance of the assays was deemed acceptable through internal verification studies. Vendor-supplied quality control materials were measured at the beginning of each day of testing and were within target ranges before analysis of samples. The LUMIPULSE Aβ1-42 assay has been standardized according to certified reference materials (CRM) developed by the International Federation of Clinical Chemistry and Laboratory Medicine working group for CSF proteins (IFCC WG-CSF).[25] Cutoffs applied were as follows: Aβ42 (916 pg/mL), p-tau (63 pg/mL), t-tau (456 pg/mL), Aβ42/40 ratio (0.062), and p-tau/Aβ42 ratio (0.068).[26] A cutoff for Aβ40 was not applied as it does not differ significantly across the AD dementia clinical spectrum.

2.5 | ^{11C} Pittsburgh compound B PET imaging

PET imaging was performed with ^{11}C PiB using either a GE Healthcare or Siemens PET/CT imaging system. Imaging consisted of four 5-minute dynamic frames acquired 40 to 60 minutes after injection. Amyloid PET was analyzed using an in-house automated image processing pipeline as previously described.[28] Briefly, image voxel values were extracted from automatically labeled regions of interest. A "global" amyloid PET standardized uptake value ratio (SUVR) was calculated as the voxel-number weighted average of the median uptake across the following target regions: prefrontal, orbitofrontal, parietal, temporal, anterior cingulate, and posterior cingulate/precuneus divided by the median uptake in a cerebellar crus reference region. Target regions were white matter and gray matter sharpened to exclude CSF voxels; the reference region was gray matter sharpened. Partial volume correction was not used. For amyloid PET, results were considered abnormal if the SUVR was greater than or equal to 1.48.[28,33]

2.6 | Statistical analyses

Data analysis was performed using Analyse-it for Excel version 5.66 (Microsoft), SAS version 9.4 (SAS Institute), R version 3.6.2 (R Foundation for Statistical Computing), and JMP Pro 14.1.0 (SAS Institute). Individual biomarker concentrations and calculated marker ratios were tested for differences across clinical diagnoses with Kruskal-Wallis tests using an alpha level of 0.05. This was followed by Wilcoxon rank sum tests for pairwise comparisons of MCI and AD to CU using a Bonferroni corrected alpha level of 0.025. For the comparison of both LUMIPULSE and Elecsys p-tau/Aβ42 with the LUMIPULSE Aβ42/40 ratio, points were plotted on a correlation scatterplot and evaluated using a power regression model and Spearman's correlation, ρ. Elecsys and LUMIPULSE p-tau/Aβ42 ratios were compared using Passing–Bablok regression analysis and Spearman's correlation, ρ. For the aforementioned statistical analyses, Elecsys Aβ42 values of $>$ 1700 pg/mL and Elecsys p-tau values of $<$ 8.00 pg/mL were omitted as true concentrations could not be extrapolated from instrument results.

Concordance between the ratios was assessed using previously established as well as internally derived cutoffs.[26] Cutoffs were established based on receiver operating characteristic analysis of amyloid PET and the CSF ratios. The ratio value that showed the highest Youden index (positive percent agreement + negative percent agreement − 1) was selected. For the LUMIPULSE p-tau/Aβ42 ratio, the best overall agreement with normal/abnormal amyloid PET was obtained at a ratio of 0.068. Ratios of $>$ 0.068 were categorized as abnormal. For the LUMIPULSE Aβ42/40 ratio, the best overall agreement with normal/abnormal amyloid PET was obtained at a ratio of 0.062.[26] Ratios equal to or less than 0.062 were categorized as abnormal. For the Elecsys p-tau/Aβ42 ratio, best overall agreement with normal/abnormal amyloid PET was obtained at a value of 0.023 and values greater than 0.023 were categorized as abnormal. For Elecsys p-tau values of $<$ 8.00 pg/mL, a value of 7.99 pg/mL was assigned for p-tau/Aβ42 calculations. Similarly, a value of 1701 pg/mL was assigned to Elecsys Aβ42 values of $>$ 1700 pg/mL for p-tau/Aβ42 calculations.

The agreement between Elecsys and LUMIPULSE ratios with amyloid PET was expressed in the terms of overall percent agreement (OPA), positive percent agreement (PPA), and negative percent agreement (NPA). OPA was defined as the sum of the amyloid PET–positive individuals who were positive by the CSF ratio and the amyloid PET–negative individuals who were negative by the CSF ratio divided by the entire cohort size. PPA was defined as the percent of amyloid PET–positive individuals who were positive by the CSF ratio. NPA was defined as the percent amyloid PET–negative individuals who were negative by the CSF ratio.

TABLE 1 Clinical characteristics and biomarker distribution of participants

	CU ($n = 107$)	MCI ($n = 22$)	AD ($n = 21$)	Kruskal-Wallis *P*-value
Sex, *N* (%), (M, F)	62 (58%), 45 (42%)	15 (68%), 7 (32%)	12 (57%), 9 (43%)	
Age, mean (SD)	66.2 (12.2)	72.8 (11.4)	66.2 (9.7)	
Kokmen STMS score, mean (SD)	36.4 (1.6)	32.0 (2.5)	20.7 (9.3)	
SUVR, mean (SD)[a]	1.48 (0.38)	1.66 (0.55)	2.53 (0.38)	
Elecsys Aβ42 (pg/mL), median [IQR][b]	1376 [1021, > 1700]	1078 [678, > 1700]	559 [489, 696]**	<.0001
Elecsys p-tau (pg/mL), median [IQR][c]	15.6 [12.4, 21.1]	21.3 [14.7, 25.4]	35.2 [25.2, 44.5]#	<.0001
Elecsys t-tau (pg/mL), median [IQR]	189 [149, 253]	256 [170, 298]	325 [254, 451]¶	<.0001
Elecsys p-tau/Aβ42, median [IQR][b,c]	0.012 [0.009, 0.016]	0.014 [0.012, 0.037]	0.054 [0.046, 0.088]**	<.0001
LUMIPULSE Aβ40 (pg/mL), median [IQR]	11068 [8923, 13754]	12439 [8620, 14217]	10006 [8618, 11994]	.3702
LUMIPULSE Aβ42 (pg/mL), median [IQR]	926 [718, 1169]	716 [530, 1191]	433 [358, 500]**	<.0001
LUMIPULSE p-tau (pg/mL), median [IQR]	34.1 [25.7, 45.4]	46.2 [30.2, 62.6]	106.8 [75.6, 140.1]**	<.0001
LUMIPULSE t-tau (pg/mL), median [IQR][d]	230 [180, 344]	312 [211, 463]	719 [487, 920]**	<.0001
LUMIPULSE p-tau/Aβ42, median [IQR]	0.033 [0.028, 0.045]	0.039 [0.037, 0.154]	0.260 [0.192, 0.360]**	<.0001
LUMIPULSE Aβ42/40, median [IQR]	0.094 [0.078, 0.097]	0.087 [0.051, 0.098]	0.042 [0.037, 0.047]**	<.0001

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease dementia; CU, cognitively unimpaired; F, female; IQR, interquartile range; M, male; MCI, mild cognitive impairment; p-tau, phosphorylated tau; SD, standard deviation; STMS, Short Test of Mental Status; SUVR, standardized uptake value ratio;t-tau, total tau.

[a]SUVR data available on CU ($n = 92$), MCI ($n = 20$), AD ($n = 16$).

[b]Elecsys Aβ42 measurements of > 1700 pg/mL were considered 1701 pg/mL for calculation purposes ($n = 33$ [22%]).

[c]Elecsys p-tau measurements of < 8.00 pg/mL were considered 7.99 pg/mL for calculation purposes ($n = 3$ [2%]).

[d]3 LUMIPULSE t-tau results omitted from data due to heterophile interference.

Statistical significance of MCI and AD versus CU tested with pairwise Wilcoxon ranks sum tests is denoted with symbols: ¶$P < .05$, #$P < .01$, **$P < .001$.

3 | RESULTS

3.1 | Study participants

Table 1 summarizes the clinical characteristics and observed biomarker distributions of participants. The clinical diagnosis of participants was predominately CU ($n = 107$) with an approximately equal number of participants with diagnoses of MCI ($n = 22$) and AD dementia ($n = 21$). The majority of participants in each diagnostic group were male (58% CU, 68% MCI, 57% AD), with overall mean ages ranging from 66.2 to 72.8 years. The average Kokmen Short Test of Mental Status score was 36.4 in CU participants, 32.0 in MCI, and 20.7 in AD dementia. In participants with amyloid PET results ($n = 128$), CU participants had the lowest proportion of abnormal amyloid PET results (23%), followed by MCI (30%), and AD dementia (100%).

3.2 | Correlation between ratios

A negative correlation was observed between the LUMIPULSE p-tau/Aβ42 and LUMIPULSE Aβ42/40 ratios, with Spearman's ρ (95% confidence interval [CI]) of −0.827 (−0.873; −0.767). The best fit for the relationship was obtained using the power fit equation LUMIPULSE Aβ42/40 = 0.022 LUMIPULSE (p-tau/Aβ42)$^{-0.413}$ (Figure 1A). A positive correlation was observed between p-tau/Aβ42 ratio measurements between the platforms, with Spearman's ρ (95% CI) of 0.960 (0.942; 0.972) (Figure 1B). A strong negative correlation was observed between the Elecsys p-tau/Aβ42 and LUMIPULSE Aβ42/40 ratios, with Spearman's ρ (95% CI) of −0.858 (−0.901; −0.800). The best fit for the relationship was obtained using the power fit equation LUMIPULSE Aβ42/Aβ40 = 0.011 Elecsys (p-tau/Aβ42) $^{-0.467}$ (Figure 1C).

TABLE 2 Concordance between LUMIPULSE CSF ratio classifications

	LUMIPULSE Aβ42/40	
LUMIPULSE p-tau/Aβ42	Normal > 0.062	Abnormal ≤ 0.062
Normal ≤ 0.068	103	3
Abnormal > 0.068	3	41

Abbreviations: Aβ, amyloid beta; CSF, cerebrospinal fluid; p-tau, phosphorylated tau.

3.3 | Concordance between ratios

Concordance between the LUMIPULSE p-tau/Aβ42 and Aβ42/40 ratios was 96% based on preselected cutoffs (Table 2). In CU participants, two individuals were classified as normal by the LUMIPULSE p-tau/Aβ42 ratio (ratios of 0.067, 0.053) and abnormal by the LUMIPULSE Aβ42/40 ratio (ratios of 0.059, 0.062), whereas one CU individual was classified as abnormal by the LUMIPULSE p-tau/Aβ42 ratio (0.075) and normal by the LUMIPULSE Aβ42/40 ratio

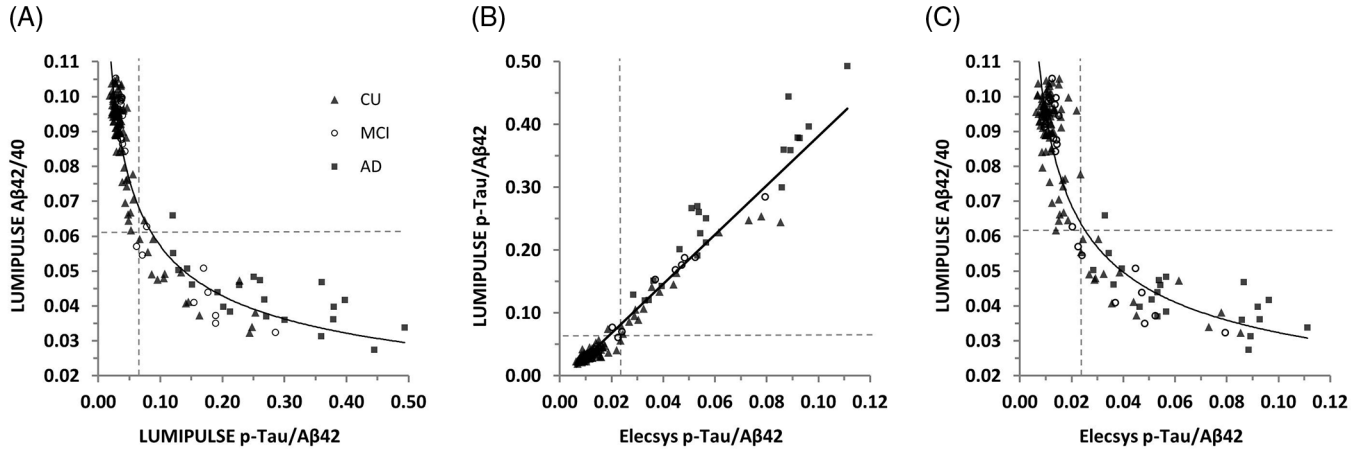

FIGURE 1 Comparison between LUMIPULSE and Elecsys cerebrospinal fluid (CSF) Alzheimer's disease biomarker ratios. A, Comparison of LUMIPULSE p-tau/Aβ42 ratio with LUMIPULSE Aβ42/40 ratio. Cutoffs of 0.068 and 0.062, respectively. Power best-fit equation LUMIPULSE Aβ42/40 = 0.022 LUMIPULSE (p-tau/Aβ42) $^{-0.413}$, $\rho = -0.827$, $R^2 = 0.914$. B, Comparison of Elecsys p-tau/Aβ42 ratio with LUMIPULSE p-tau/Aβ42 ratio. Cutoffs of 0.023 and 0.068, respectively. Passing-Bablok best-fit regression equation: LUMIPULSE p-tau/Aβ42 = 3.92(Elecsys p-tau/Aβ42)−0.01, $\rho = 0.960$. C, Comparison of Elecsys p-tau/Aβ42 ratio with LUMIPULSE Aβ42/40 ratio. Cutoffs of 0.023 and 0.062, respectively. Power best-fit equation LUMIPULSE Aβ42/40 = 0.011 Elecsys (p-tau/Aβ42) $^{-0.467}$, $\rho = -0.858$, $R^2 = 0.870$. Dashed lines represent cutoffs for the respective assay's ratio.

TABLE 3 Concordance between Elecsys and LUMIPULSE CSF ratio classifications

	LUMIPULSE p-tau/Aβ42		LUMIPULSE Aβ42/40	
Elecsys p-tau/Aβ42	Normal ≤ 0.068	Abnormal > 0.068	Normal > 0.062	Abnormal ≤ 0.062
Normal ≤ 0.023	104	2	104	2
Abnormal > 0.023	2	42	2	42

Abbreviations: Aβ, amyloid beta; CSF, cerebrospinal fluid; p-tau, phosphorylated tau.

(0.065). In the MCI category, one individual was classified as normal by the LUMIPULSE p-tau/Aβ42 ratio (0.061) and abnormal by the LUMIPULSE Aβ42/40 ratio (0.057). In the same category, one individual was classified as abnormal by the LUMIPULSE p-tau/Aβ42 ratio (0.078) and normal by the LUMIPULSE Aβ42/40 ratio (0.063). One individual with AD dementia was classified as abnormal by the LUMIPULSE p-tau/Aβ42 ratio (0.120) and normal by the LUMIPULSE Aβ42/40 ratio (0.066).

Concordance between Elecsys and LUMIPULSE p-tau/Aβ42 ratios was 97% based on the preselected cutoffs (Table 3). In CU participants, one individual was classified as normal by the Elecsys p-tau/Aβ42 ratio (0.019) and abnormal by the LUMIPULSE p-tau/Aβ42 ratio (0.075), whereas two CU individuals were classified as abnormal by the Elecsys p-tau/Aβ42 ratio (both ratios of 0.024) and normal by the LUMIPULSE p-tau/Aβ42 ratio (ratios of 0.067, 0.056). In the MCI category, one individual was classified as normal by the Elecsys p-tau/Aβ42 ratio (0.020) and abnormal by the LUMIPULSE p-tau/Aβ42 ratio (0.078). There was no disagreement in classification in the AD dementia group between the platforms when using the p-tau/Aβ42 ratios. In cases of disagreement between the ratios, the results were near the cutoff (0.023 for Elecsys and 0.068 for LUMIPULSE) for one or both ratios.

Concordance between Elecsys p-tau/Aβ42 and LUMIPULSE Aβ42/40 ratios was 97% (Table 3). Discrepant cases were observed in all three clinical categories (CU, MCI, and AD dementia). In the CU group, one individual was classified as normal by the Elecsys p-tau/Aβ42 ratio (0.014) and abnormal by the LUMIPULSE Aβ42/40 ratio (0.062). In the same group, one individual was classified as abnormal by the Elecsys p-tau/Aβ42 ratio (0.024) and normal by the LUMIPULSE Aβ42/40 ratio (0.078). One individual in the MCI group was classified was normal by the Elecsys p-tau/Aβ42 ratio (0.022) and abnormal by the LUMIPULSE Aβ42/40 ratio (0.057). In the AD dementia group, one individual was classified as abnormal by the Elecsys p-tau/Aβ42 ratio (0.033) and normal by the LUMIPULSE Aβ42/40 ratio (0.066). In most instances, the discrepancies between the ratios were due to results being near the cutoffs (0.023 for Elecsys and 0.062 for LUMIPULSE) for one of the ratios.

3.4 | Agreement of ratios with amyloid PET

Agreement between the CSF ratios and amyloid PET was assessed (Table 4). The LUMIPULSE p-tau/Aβ42 ratio had an OPA of 92%, a PPA

TABLE 4 Agreement of CSF ratios with amyloid PET classifications

	LUMIPULSE p-tau/Aβ42		LUMIPULSE Aβ42/40		Elecsys p-tau/Aβ42	
Amyloid PET SUVR	Normal ≤ 0.068	Abnormal > 0.068	Normal > 0.062	Abnormal ≤ 0.062	Normal ≤ 0.023	Abnormal > 0.023
Normal < 1.48	84	1	83	2	83	2
Abnormal ≥ 1.48	9	34	10	33	10	33

Abbreviations: Aβ, amyloid beta; CSF, cerebrospinal fluid; PET, positron emission tomography; p-tau, phosphorylated tau; SUVR, standardized uptake value ratio.

TABLE 5 Participants with discrepant classifications between CSF ratio(s) and amyloid PET

Participant	Categorization	SUVR	LUMIPULSE tau/Aβ42	LUMIPULSE Aβ42/40	Elecsys p-tau/Aβ42
1	CU	1.33	0.056	0.078	0.024
2	CU	1.50	0.032	0.100	0.012
3	CU	1.49	0.034	0.095	0.011
4	CU	1.52	0.034	0.095	0.014
5	CU	1.52	0.037	0.093	0.010
6	CU	1.55	0.039	0.075	0.012
7	CU	1.57	0.052	0.067	0.017
8	CU	1.50	0.053	0.062	0.014
9	CU	1.51	0.067	0.059	0.024
10	CU	1.57	0.031	0.094	0.016
11	CU	1.64	0.075	0.065	0.019
12	MCI	1.39	0.071	0.055	0.024
13	MCI	1.37	0.061	0.057	0.022
14	MCI	1.52	0.078	0.063	0.020
15	AD	2.27	0.120	0.066	0.033

Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease dementia; CSF, cerebrospinal fluid; CU, cognitively unimpaired; MCI, mild cognitive impairment; PET, positron emission tomography; p-tau, phosphorylated tau; SUVR, standardized uptake value ratio.

of 79%, and an NPA 99%. The LUMIPULSE Aβ42/40 ratio had an OPA of 91%, a PPA of 77%, and an NPA of 98%. The Elecsys p-tau/Aβ42 ratio had an OPA of 91%, a PPA of 77%, and an NPA of 98%. Table 5 summarizes results of participants with one or more ratios discordant with amyloid PET classification.

4 | DISCUSSION

In this study, excellent concordance was demonstrated between LUMIPULSE ratios as well as between Elecsys and LUMIPULSE ratios. Additionally, excellent agreement of the ratios with amyloid PET classification was observed. Given the strong correlations between the ratios, the equation of the regression analysis may be used for conversion between the ratios. These equations, however, are specific to the data used in this study and may not be suitable for other cohorts or samples collected and processed using different preanalytical protocols. Additional studies may be necessary to further support the use of these conversion formulas to extrapolate the relationship between the biomarker ratios.

When evaluating the agreement of ratios with clinical diagnosis, both ratios and platforms investigated in this study performed similarly, supporting subsequent comparison between ratios. Both the Elecsys and LUMIPULSE p-tau/Aβ42 ratios were abnormal in 100% of participants classified into the AD dementia group while the LUMIPULSE Aβ42/40 ratio was abnormal in 95% of participants in the AD dementia group. The different ratios on each platform showed strong concordance with one another, with disagreement between normal/abnormal classifications often occurring with results near the ratio cutoffs. Generally, biomarker values near cutoffs require cautious interpretation as imprecision of the assays can play a role in the exact absolute value. For this reason, values near cutoffs need to the interpreted in the context of clinical history. Alternatively, the use of a gray zone based on the 95% CI may assist clinicians when evaluating biomarker ratios.

Both ratios and platforms showed strong agreement with amyloid PET classifications with OPA, PPA, and NPA of 94% or greater. Similar to published findings, the LUMIPULSE p-tau/Aβ42 ratio showed slightly better agreement with amyloid PET classification than the LUMIPULSE Aβ42/40 ratio.[26] Participants with disagreement between

normal/abnormal classifications were often observed when the SUVR and/or ratio results were near cutoffs. Disagreement was observed between ratios and amyloid PET results across all clinical diagnoses.

The use of CSF biomarker ratios (p-tau/Aβ42 and Aβ42/40) has been shown to be superior to the individual biomarkers compared to amyloid PET agreement.[23–26] The use of a ratio provides both analytical and clinical advantages when evaluating AD pathology. Analytically, the use of a ratio can help mitigate the effect that incorrect sample handling may have on amyloid levels.[34–36] In addition, the use of ratios could compensate for individual differences in amyloid precursor protein processing that otherwise might lead to false positive or false negative Aβ42 levels.[37] Moreover, the use of Aβ42/Aβ40 ratio has proven to partially mitigate the effect of some preanalytical confounders that have been described to alter the results of amyloid levels.[35] Clinically, the use of ratios can also help compensate for disruption of CSF dynamics that could lead to an abnormally low Aβ42 concentration. In our clinical experience with these assays patients presenting with a low Aβ42 concentration but a normal p-tau/Aβ42 ratio often have problems with CSF dynamics, such as normal pressure hydrocephalus, which is known to decrease protein levels in CSF, resulting in low Aβ42 levels.[38,39]

Strengths of our study include the overall sample size ($n = 150$) and concurrent measurement of samples on both Elecsys and LUMIPULSE assays. The ability to measure samples on both platforms without additional freeze/thaw cycles allowed for the comparison of measurements without concern of confounding effects from sample stability. All participants were categorized as CU, MCI, or AD dementia, which allowed for comparison of analytical results with clinical diagnoses. Additionally, the majority of the participants had amyloid PET results ($n = 128$, 85%).

Limitations of the study include limited participants with MCI ($n = 22$, 15%) or AD dementia ($n = 21$, 14%) classification. CSF samples were collected under optimal conditions for research, which may not be generalizable to collections in clinical settings in which patients may not be fasting and collections may be performed in non-morning hours. However, current literature suggests that there is not significant diurnal variation in CSF biomarkers related to AD.[40] Our cohort primarily included individuals of North European descent; studies suggest that ratios may perform differently in African American individuals where differences in tau protein concentration have been suggested.[41–43] Ratio classifications were compared to classification by amyloid PET imaging performed using ^{11}C PiB and not ^{18}F-labeled amyloid PET agents, which are Food and Drug Administration approved and used in most amyloid PET imaging studies and clinical settings.

A significant limitation in the interpretation of biomarkers and their ratios is that cutoffs are not universal due to the lack of standardization across assays. The Elecsys assays and three of the four LUMIPULSE assays are not currently standardized against CRMs or the other platform.[25,43] The LUMIPULSE Aβ42 assay has been standardized to CRMs developed by the IFCC WG-CSF.[25,43] Although the Elecsys Aβ42 assay used in this study has not been standardized to the IFCC WG-CSF CRM material, it has been standardized against the Joint Committee for Traceability in Laboratory Medicine–approved reference measurement procedure based on liquid chromatography-tandem mass spectrometry (LC-MS/MS).[44–46] Cutoffs for Elecsys assays were derived from internal studies performed with samples from a separate, but similar, internal cohort. LUMIPULSE cutoffs applied in our studies were derived from studies by Alcolea et al.[26] Both applied similar preanalytical processing procedures as those used in our study. Cutoffs for both assays were derived through comparison with amyloid status and Youden indices.[26] Assigning values of 1701 pg/mL for > 1700 Aβ42 results on Elecsys assays for the calculation of the p-tau/Aβ42 ratio may have resulted in a falsely elevated ratio, primarily in participants with high p-tau levels, underscoring the importance of interpreting ratios in the context of individual biomarker concentrations including t-tau, which is not included in the ratios.

In conclusion, while previous studies have evaluated the performance of the ratios in CSF, no studies exist comparing both ratios measured by the Elecsys and LUMIPULSE assays in the same participant cohort.[23–26] Our findings suggest that both ratios will provide the same clinical information when used to assess the presence of amyloid pathology. Additionally, the observed best-fit equation and good correlation between the two manufacturers' ratios may enable extrapolation between the ratio values in future studies.

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CONFLICTS OF INTEREST

R.C. Petersen is a consultant for Roche, Inc.; Biogen, Inc.; and Eisai, Inc. and is on a DSMB for Genentech. He receives funding for research from NIH. M.M. Mielke has consulted for Biogen and Brain Protection Company and receives research support from NIH and DOD. V.J. Lowe serves as a consultant for Bayer Schering Pharma, Life Molecular Imaging, Eisai, Inc., AVID Radiopharmaceuticals, and GE Healthcare and receives research support from GE Healthcare, Siemens Molecular Imaging, AVID Radiopharmaceuticals, and the NIH (NIA, NCI). C.R. Jack serves on an independent data monitoring board for Roche, has served as a speaker for Eisai, and consulted for Biogen, but he receives no personal compensation from any commercial entity. He receives research support from NIH and the Alexander Family Alzheimer's Disease Research Professorship of the Mayo Clinic.

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