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Featured Article

Cerebrospinal fluid biomarkers measured by Elecsys assays compared to amyloid imaging

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Abstract

Introduction: Levels of amyloid β peptide 42 (Aβ42), total tau, and phosphorylated tau-181 are well-established cerebrospinal fluid (CSF) biomarkers of Alzheimer's disease, but variability in manual plate-based assays has limited their use. We examined the relationship between CSF biomarkers, as measured by a novel automated immunoassay platform, and amyloid positron emission tomography.

Methods: CSF samples from 200 individuals underwent separate analysis for Aβ42, total tau, and phosphorylated tau-181 with automated Roche Elecsys assays. Aβ40 was measured with a commercial plate-based assay. Positron emission tomography with Pittsburgh Compound B was performed less than 1 year from CSF collection.

Results: Ratios of CSF biomarkers (total tau/Aβ42, phosphorylated tau-181/Aβ42, and Aβ42/Aβ40) best discriminated Pittsburgh Compound B–positive from Pittsburgh Compound B–negative individuals.

Discussion: CSF biomarkers and amyloid positron emission tomography reflect different aspects of Alzheimer's disease brain pathology, and therefore, less-than-perfect correspondence is expected. Automated assays are likely to increase the utility of CSF biomarkers.

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Keywords: Alzheimer's disease; Biomarker; Cerebrospinal fluid; Cutoff; Amyloid

1. Introduction

Alzheimer's disease (AD) refers to the progressive brain disease that is characterized by amyloid plaques that are comprised primarily of amyloid β peptide 42 (Aβ42) and neurofibrillary tangles that are comprised primarily of tau, including phosphorylated forms of tau. Individuals with AD are typically asymptomatic (have no apparent cognitive decline) for one to two decades during the preclinical phase of the disease [1,2]. As the disease progresses, individuals enter the symptomatic phase when they develop cognitive decline that culminates in dementia. Fluid biomarkers can identify individuals with AD brain pathology who are in either the preclinical phase or the symptomatic phase of the disease. Decreases in cerebrospinal fluid (CSF) Aβ42 levels and increases in CSF total tau (tTau) and phosphorylated tau-181 (pTau) may be the earliest markers

of AD brain pathology [3–6]. CSF Aβ42, tTau, or pTau individually, and especially the ratios of CSF tTau/Aβ42 and pTau/Aβ42, predict future cognitive decline of cognitively normal adults [7,8] and individuals diagnosed with mild cognitive impairment due to AD [9–12].

It is likely that the use of AD biomarkers will continue to increase in clinical practice and clinical trials. As demonstrated by clinicopathological series, the clinical diagnosis of AD can be incorrect [13], so biomarkers may be helpful in establishing an accurate diagnosis. CSF biomarkers are especially useful when the etiology of cognitive impairment is uncertain and AD is a possible cause [14]. Drug trials now routinely test CSF or imaging biomarkers in potential participants after it was found that many individuals enrolled in past AD drug trials did not have AD brain pathology [15–18]. CSF biomarkers are also being used in clinical trials to verify that drugs are having expected biological effects and may eventually be used as surrogate end points [15,16]. When an effective drug for AD is available, CSF biomarkers will become even more important in guiding the diagnosis and management of patients.

CSF Aβ42, tTau, and pTau were the first biomarkers described for AD [19], and now, molecular imaging biomarkers have also become well established [20,21]. Radiotracers that bind to β-amyloid (e.g., Pittsburgh Compound B [PIB], florbetapir, florbetaben, and flutemetamol) or aggregated tau (e.g., flortaucipir) can visualize plaques and tangles, respectively, with positron emission tomography (PET). Although these PET imaging techniques provide information regarding the degree and spatial distribution of brain pathology, there are limitations to their use, including high cost, limited access, use of radiation, and imaging of only a single type of pathology per scan [22,23]. A number of studies have previously evaluated the relationship between CSF biomarkers of AD and amyloid PET and found a strong inverse correlation between levels of CSF Aβ42 and binding of amyloid PET tracers [3,5,6,24–33]. The ratio of Aβ42 with another AD biomarker (e.g., tTau/Aβ42, pTau/Aβ42, or Aβ42/Aβ40) may provide the best correlation with amyloid PET measures [24,30,32].

The use of CSF biomarkers has been limited by a number of technical factors. There has been substantial variability in the intralaboratory and interlaboratory performance of the three most commonly used commercial enzyme-linked immunosorbent assays (ELISAs) for CSF Aβ42, tTau, and pTau: INNOTEST, AlzBio3, and Meso Scale Discovery. Issues with these assays include high lot-to-lot variability [34] and between-laboratory variability associated with differences in laboratory procedures and analytical techniques because the assays are run manually [12,35–37]. Practically, because most assays are based on the 96-well plate immunoassay format, laboratories must await a large number of samples to financially justify analysis, which in turn leads to delays in obtaining results. The lack of standardized reference materials for quantitation of these analy-

tes has made it difficult to compare absolute values across assays and studies [38]. Taken together, these issues have prevented the establishment of universal diagnostic cutoffs for CSF biomarkers and decreased the potential utility of CSF biomarkers in the clinic and in clinical trials.

Next-generation automated assay platforms are being developed to overcome the shortcomings of previous assay systems. Roche Diagnostics has developed Elecsys assays that utilize the automated cobas 601 analyzer. This assay platform exhibits high degrees of precision, accuracy, reliability, and reproducibility, with very low variability, in large part due to its automation [39]. We tested this novel assay platform using CSF samples obtained from individuals who had also undergone amyloid PET. CSF Aβ42, tTau, and pTau were measured separately with Elecsys assays. CSF Aβ40 was measured with a standard plate-based ELISA [24]. We then examined the relationship between cortical amyloid load as defined by PIB PET and CSF Aβ42, Aβ40, tTau, pTau, and three ratios of Aβ42 with another AD biomarker (tTau/Aβ42, pTau/Aβ42, and Aβ42/Aβ40).

2. Materials and methods

2.1. Participants, standard protocol approvals, and consents

Participants were community-dwelling volunteers enrolled in studies of normal aging and dementia at the Knight Alzheimer's Disease Research Center at Washington University in St. Louis. Participants had no neurological, psychiatric, or systemic medical illness that might compromise longitudinal study participation and no medical contraindication to lumbar puncture (LP) or PET. All participants underwent clinical assessments that included the Clinical Dementia Rating (CDR) [40]. *APOE* genotype was obtained from the Knight Alzheimer's Disease Research Center Genetics Core [41]. All procedures were approved by the Washington University Human Research Protection Office, and written informed consent was obtained from each participant.

Participants included in this study underwent a clinical assessment, LP, and PIB PET within a 365-day period. Of participants who met these criteria, 200 were selected based on cortical amyloid load by PIB PET (25% PIB-positive and 75% PIB-negative based on a previously established cutoff [42]). We chose to include more PIB-negative participant samples to enrich for discordant (PIB-negative and CSF biomarker positive) cases. Furthermore, we chose participants with a broad range of CSF Aβ42 values based on previous data from plate-based assays. Selection was independent of participant demographics and clinical status.

2.2. CSF collection, processing, and analysis

CSF was collected under standardized operating procedures. Participants underwent LP at 8 AM after overnight fasting. Twenty to 30 mLs of CSF was collected in a 50-mL

polypropylene tube via gravity drip using an atraumatic Sprotte 22 gauge spinal needle. The entire sample was gently inverted to disrupt potential gradient effects and centrifuged at low speed to pellet any cellular debris. Five hundred microliters of CSF was aliquoted into polypropylene tubes and stored at −80°C as previously described [5].

Aβ42, tTau, and pTau were measured with the corresponding Elecsys immunoassays utilizing the Roche cobas e 601 analyzer—a fully automated system. The Elecsys immunoassays are electrochemiluminescence immunoassays using a quantitative sandwich principle with a total assay duration of 18 minutes. Pristine aliquots from the selected cohort were measured according to the Roche study protocol (RD002967) written specifically to measure these samples. Aβ40 concentrations were measured with a plate-based ELISA from IBL International (Hamburg, Germany) according to the manufacturer's protocol. A single lot of assays for each analyte (either Elecsys for Aβ42, tTau, and pTau or IBL for Aβ40) was used to measure all samples to avoid lot-to-lot variability.

2.3. Amyloid PET imaging

Participants underwent a 60-minute dynamic scan with [11][C] PIB [43]. PET imaging was performed with a Siemens 962 HR + ECAT PET or Biograph 40 scanner (Siemens/CTI, Knoxville, KY). Structural magnetic resonance imaging using MPRAGE T1-weighted images was also acquired. Structural magnetic resonance images were processed using FreeSurfer [44] (http://freesurfer.net/) to derive cortical and subcortical regions of interest used in the PET processing [45,46]. Regional PIB values were converted to standardized uptake value ratios (SUVRs) using cerebellar gray as a reference and partial volume corrected using a regional spread function approach [45,46]. Values from the left and right lateral orbitofrontal, medial orbitofrontal, precuneus, rostral middle frontal, superior frontal, superior temporal, and middle temporal cortices were averaged together to represent a mean cortical SUVR. PIB positivity was defined as a mean cortical SUVR > 1.42 [42], which is commensurate with a mean cortical binding potential of 0.18 that has previously been used to define PIB positivity [45].

2.4. Statistical analyses

Characteristics of PIB-positive and PIB-negative groups were compared using t-tests for continuous variables and χ^2 tests for categorical variables. Performance of the Elecsys assay has not yet been formally established for measuring Aβ42 concentrations < 200 pg/mL or > 1700 pg/mL. None of the samples used for this study had Aβ42 concentrations < 200 pg/mL. Concentrations of Aβ42 > 1700 pg/mL were extrapolated based on the calibration curve. These values are restricted to research use and are not for clinical decision making. Values for CSF biomarkers, including single analytes and ratios, were compared to PIB PET SUVR using Spearman correlation.

Receiver operating characteristic (ROC) analyses were performed to determine the cutoffs for each CSF biomarker analyte and ratio that best distinguished PIB-positive from PIB-negative individuals. Positive percent agreement (PPA) was defined as the percent of PIB-positive individuals who were positive by a CSF biomarker measure. Negative percent agreement (NPA) was defined as the percent of PIB-negative individuals who were negative by a CSF biomarker measure. Overall percent agreement was defined as the sum of the PIB-positive individuals who were positive by a CSF biomarker measure and the PIB-negative individuals who were negative by a CSF biomarker measure divided by the entire cohort size. The CSF biomarker single analyte or ratio value with the highest Youden index (PPA + NPA − 1) was selected as the cutoff value. Analyses were performed with GraphPad Prism version 6.07 (GraphPad Software, La Jolla, CA).

3. Results

3.1. Participant characteristics

CSF samples from 198 individuals were analyzed (see Table 1 for participant characteristics). Samples from two participants in the selected cohort were omitted because of failure of PIB PET quality control (i.e., movement artifact or out of LP-PET window of 365 days). The average absolute interval from LP to PIB PET was 67 ± 78 days (mean ± standard deviation). Most of the participants (n = 176, 89%) were cognitively normal at the time of CSF collection with a CDR of 0, but some (n = 22, 11%)

Table 1
Participant characteristics

Characteristic	PIB negative	PIB positive	P
n	148	50	
CDR 0/0.5/1/2/3	141/7/0/0/0	35/11/3/1/0	
CDR > 0 (%)*	5%	30%	<.0001
MMSE[†]	29.1 ± 1.2	28.0 ± 3.0	<.001
Age at LP (years)[†]	64.2 ± 9.6	72.5 ± 7.2	<.0001
Gender (% male)*	34%	58%	<.01
Education (years)[†]	15.9 ± 2.5	15.5 ± 3.0	N.S.
APOE ε4 positive (%)*	31%	56%	<.01
PIB mean cortical SUVR[†]	1.04 ± 0.12	2.40 ± 0.70	<.0001
Elecsys Aβ42, pg/mL[†]	1428 ± 610	789 ± 256	<.0001
IBL Aβ40, pg/mL[†]	13,950 ± 4347	15,310 ± 4147	.06
Elecsys tTau, pg/mL[†]	191 ± 76	309 ± 127	<.0001
Elecsys pTau, pg/mL[†]	16.7 ± 7.8	30.3 ± 14.8	<.0001
Elecsys tTau/Aβ42[†]	0.150 ± 0.090	0.420 ± 0.173	<.0001
Elecsys pTau/Aβ42[†]	0.013 ± 0.010	0.041 ± 0.020	<.0001
Elecsys Aβ42/IBL Aβ40[†]	0.103 ± 0.028	0.052 ± 0.014	<.0001

Abbreviations: CDR, clinical dementia rating; MMSE, Mini–Mental State Examination; PIB, Pittsburgh Compound B; tTau, total tau; pTau, phosphorylated tau-181; Aβ42, amyloid β peptide 42; SUVR, standardized uptake value ratio; LP, lumbar puncture; IBL, IBL International (Hamburg, Germany).

*Percent, *P* values by χ^2 test.
[†]Mean ± standard deviation, *P* values by student's t-test.

had very mild (CDR 0.5) or mild (CDR 1) dementia. By design, 50 ($\sim$25%) of the individuals were PIB-positive (mean cortical SUVR $>$ 1.42). As expected, individuals who were PIB-positive were more likely to be cognitively impaired (30% vs. 5%, $P <$ .0001), older (72.5 $\pm$ 7.2 vs. 64.2 $\pm$ 9.6 years, $P <$ .0001), and carry an *APOE* ε4 allele (56% vs. 31%, $P <$ .01). In addition, PIB-positive individuals were more likely to be male ($P <$ .01) in this cohort.

3.2. Correlations between CSF biomarker measures and PIB binding

The Roche cobas e 601 analyzer was used to measure levels of Aβ42, tTau, and pTau with the corresponding Elecsys assays. At the time of analysis, this platform did not have an Aβ40 assay available. Therefore, Aβ40 levels were measured with the IBL Aβ40 ELISA kit. The values for Aβ42, Aβ40, tTau, or pTau versus PIB mean cortical SUVR were plotted (Fig. 1, upper panels). By Spearman correlation analysis, PIB binding was negatively correlated with CSF Aβ42 (r = −0.45, $P <$ .0001) and positively correlated with CSF Aβ40 (r = 0.20, $P <$ .01), tTau (r = 0.46, $P <$ .0001), and pTau (r = 0.51, $P <$ .0001). PIB binding was positively correlated with tTau/Aβ42 (r = 0.66) and pTau/Aβ42 (r = 0.66) and negatively correlated with Aβ42/Aβ40 (r = −0.63), all at $P <$ .0001 (Fig. 2, upper panels). Notably, tTau and pTau were almost perfectly correlated (r = 0.98, $P <$ .0001).

3.3. Determination of cutoffs for CSF biomarker measures

ROC analyses were performed to determine the cutoffs for each biomarker analyte and ratio that best distinguished

PIB status (positive or negative). Because PIB PET is not the gold standard for brain amyloid deposition (autopsy is the gold standard), we refer to PPA rather than sensitivity and NPA rather than specificity. The cutoffs selected are depicted in the lower panels of Fig. 1 for Aβ42 (A), Aβ40 (B), tTau (C), and pTau (D) and Fig. 2 for tTau/Aβ42 (A), pTau/Aβ42 (B), and Aβ42/Aβ40 (C). The lower panels also indicate the associated PPA, NPA, and overall percent agreement for each CSF measure with PIB status. The ROC curves and a summary of cutoff characteristics for all biomarker measures are shown in Fig. 3. Inspection of the ROC curves shows that Aβ42/Aβ40 and Aβ42 have a lower NPA for a given PPA at most potential cutoff values compared to the other ratios or single analytes, respectively (e.g., at a cut point with a PPA of 0.50 for all analytes, the NPA for Aβ42/Aβ40 is lower than for tTau/Aβ42 and pTau/Aβ42 and the NPA for Aβ42 is lower than for tTau and pTau). For the cutoff values selected, the PPAs for tTau/Aβ42, pTau/Aβ42, and Aβ42/Aβ40 were high (0.92–0.96) with somewhat lower NPAs (0.82–0.89). The PPA and NPA for Aβ42, tTau, and pTau as single analytes (0.68–0.90 for PPA and 0.73–0.83 for NPA) were not as high as the three ratios but were superior to Aβ40 (0.60 for PPA and 0.58 for NPA).

Levels of Aβ42 $>$ 1700 pg/mL were extrapolated and therefore estimated, so we performed alternative analyses to determine whether inaccuracies in high Aβ42 values could bias our results. We reanalyzed our data treating individuals with Aβ42 $>$ 1700 pg/mL as biomarker negative, regardless of the level of other analytes (Supplementary Fig. 1). Notably, all 40 individuals in our cohort with Aβ42 values $>$ 1700 pg/mL were PIB-negative. We found
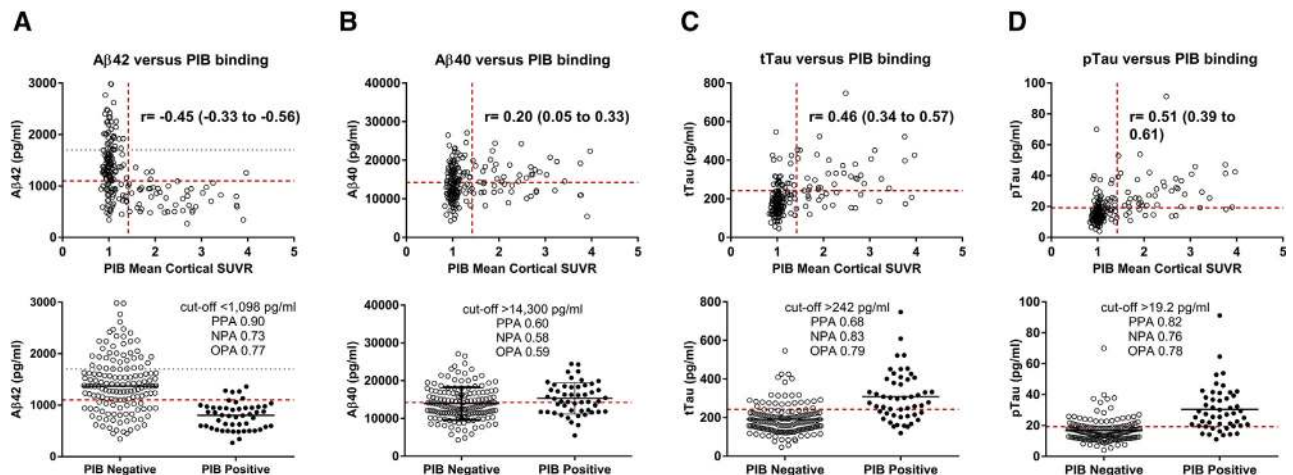

Fig. 1. Single CSF analyte values compared to PIB binding. PIB binding was negatively correlated with CSF Aβ42 (A) and positively correlated with CSF Aβ40 (B), tTau (C), and pTau (D). Each point represents the analyte value and PIB mean cortical SUVR for one individual. The horizontal red dashed lines represent the cutoff values that best distinguish between PIB-positive and PIB-negative individuals. The horizontal gray dotted line represents the upper limit of quantitation for Aβ42 (A). For the upper panels, the vertical red dashed lines represent the established cutoff value for PIB positivity (SUVR $>$ 1.42). The Spearman correlation coefficient (r) with 95% confidence intervals is indicated. For the lower panels, individuals were dichotomized into PIB-negative (SUVR $\leq$ 1.42) and PIB-positive (SUVR $>$ 1.42) groups. The lower panels indicate the cutoff values and associated positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) for each CSF analyte with PIB binding. Abbreviations: CSF, cerebrospinal fluid; PIB, Pittsburgh Compound B; tTau, total tau; pTau, phosphorylated tau-181; Aβ42, amyloid β peptide 42; SUVR, standardized uptake value ratio.
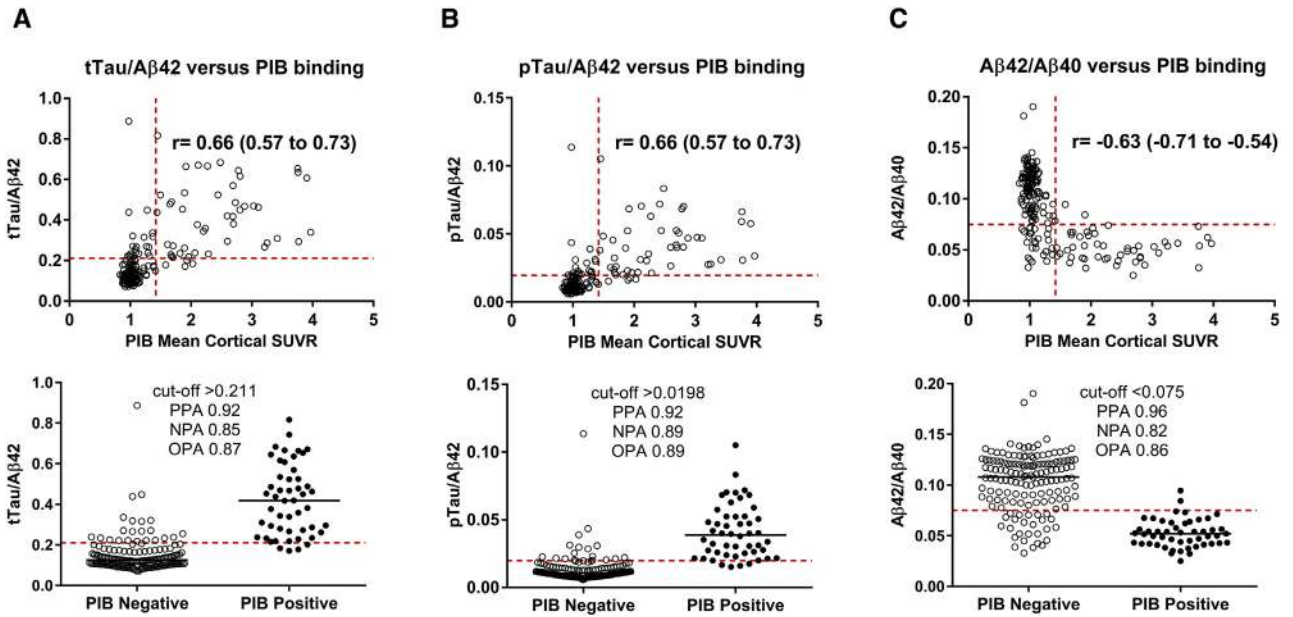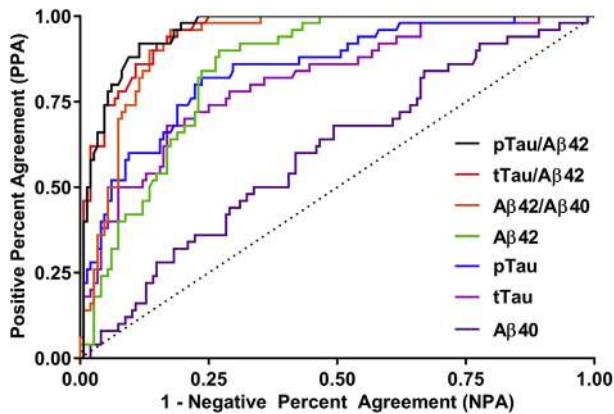
Fig. 2. CSF ratios compared to PIB binding. PIB binding is positively correlated with CSF tTau/Aβ42 (A), pTau/Aβ42 (B), and Aβ42/Aβ40 (C). Each point represents the ratio of analytes and PIB mean cortical SUVR for one individual. The horizontal red dashed lines represent the cutoff values that best distinguish between PIB-positive and PIB-negative individuals. For the upper panels, the vertical red dashed lines represent the established cutoff value for PIB positivity (SUVR > 1.42). The Spearman correlation coefficient (r) with 95% confidence intervals is indicated. For the lower panels, individuals were dichotomized into PIB-negative (SUVR ≤ 1.42) and PIB-positive (SUVR > 1.42) groups. The lower panels indicate the cutoff values and associated positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) for each CSF analyte with PIB binding. Abbreviations: CSF, cerebrospinal fluid; PIB, Pittsburgh Compound B; tTau, total tau; pTau, phosphorylated tau-181; Aβ42, amyloid β peptide 42; SUVR, standardized uptake value ratio.

	cut-off	PPA	NPA	AUC
pTau/Aβ42	>0.0198	0.92 (0.81-0.98)	0.89 (0.82-0.93)	0.96 (0.93-0.98)
tTau/Aβ42	>0.211	0.92 (0.81-0.98)	0.85 (0.78-0.90)	0.95 (0.92-0.98)
Aβ42/Aβ40	<0.075	0.96 (0.86-1.00)	0.82 (0.75-0.88)	0.93 (0.89-0.96)
Aβ42	<1,098 pg/ml	0.90 (0.78-0.97)	0.73 (0.65-0.80)	0.85 (0.80-0.90)
pTau	>19.2 pg/ml	0.82 (0.69-0.91)	0.76 (0.69-0.83)	0.84 (0.78-0.91)
tTau	>242 pg/ml	0.68 (0.53-0.80)	0.83 (0.76-0.89)	0.81 (0.74-0.88)
Aβ40	>14,300 pg/ml	0.60 (0.45-0.74)	0.58 (0.50-0.66)	0.60 (0.51-0.69)

Fig. 3. Receiver operator characteristic (ROC) curves for CSF biomarkers compared to PIB binding. For ROC analysis, individuals were dichotomized into PIB-negative (SUVR ≤ 1.42) and PIB-positive (SUVR > 1.42) groups. For each CSF biomarker measure, the table indicates the cutoff values and associated positive percent agreement (PPA), negative percent agreement (NPA), and area under the ROC curve (AUC) for the measure compared to PIB status. 95% confidence intervals are included in parentheses. Abbreviations: CSF, cerebrospinal fluid; PIB, Pittsburgh Compound B; SUVR, standardized uptake value ratio.

minimal changes in the results, likely because individuals with Aβ42 > 1700 pg/mL typically do not have significant AD brain pathology and therefore rarely have elevated tTau or pTau. The only small differences we found were that the NPA for tTau/Aβ42 increased from 0.85 to 0.86 and the NPA for Aβ42/Aβ40 increased from 0.82 to 0.83 when individuals with Aβ42 > 1700 pg/mL were considered biomarker negative. We therefore concluded that estimation of Aβ42 values > 1700 pg/mL did not affect concordance with PIB PET.

3.4. Concordance of CSF ratios and PIB binding

Because the three CSF ratios (tTau/Aβ42, pTau/Aβ42, and Aβ42/Aβ40) performed well in discriminating PIB-positive and PIB-negative individuals, we next examined the degree to which the CSF ratios were concordant with other CSF ratios and with PIB status (Table 2). Biomarker status (positive or negative according to the cutoffs previously discussed) was visualized in scatterplots of CSF tTau versus Aβ42 (Fig. 4A), pTau versus Aβ42 (B), and Aβ40 versus Aβ42 (C). There was a concordance of all three CSF ratios and PIB PET in 166 of 198 individuals in our cohort (84%): all three CSF ratios were positive in 46 of the 50 PIB-positive individuals (92%) and all three CSF ratios were negative in 120 of the 148 PIB-negative individuals (81%). Four individuals were PIB-positive but either all three CSF ratios were negative (two individuals) or Aβ42/

Table 2
Concordance between CSF tTau/Aβ42, pTau/Aβ42, Aβ42/Aβ40, and PIB PET

Characteristic	n (% of PIB group)	CDR 0/0.5/1/2/3	PIB SUVR >1.42	tTau/Aβ42 >0.211	pTau/Aβ42 >0.0198	Aβ42/Aβ40 <0.075
PIB positive, n = 50						
PIB + and all CSF ratios +	46 (92)	31/11/3/1/0	2.45 ± 0.71	0.440 ± 0.165	0.0434 ± 0.0194	0.050 ± 0.011
PIB +, all CSF ratios −	2 (4)	2/0/0/0/0	1.74 ± 0.23	0.175 ± 0.005	0.0156 ± 0.0006	0.089 ± 0.007
tTau/Aβ42 and pTau/Aβ42 −, Aβ42/Aβ40 +	2 (4)	2/0/0/0/0	1.98 ± 0.07	0.193 ± 0.012	0.0172 ± 0.0006	0.069 ± 0.003
PIB negative, n = 148						
All CSF ratios −	120 (81)	114/6/0/0/0	1.03 ± 0.10	0.120 ± 0.030	0.0104 ± 0.0026	0.114 ± 0.019
All CSF ratios +	16 (11)	16/0/0/0/0	1.16 ± 0.14	0.334 ± 0.162	0.0322 ± 0.0228	0.050 ± 0.011
Aβ42/Aβ40 +, tTau/Aβ42, and pTau/Aβ42 −	6 (4)	6/0/0/0/0	0.98 ± 0.08	0.189 ± 0.013	0.0165 ± 0.0019	0.067 ± 0.005
Aβ42/Aβ40 and tTau/Aβ42 +, pTau/Aβ42 −	4 (3)	3/1/0/0/0	1.06 ± 0.16	0.222 ± 0.003	0.0192 ± 0.0003	0.066 ± 0.007
tTau/Aβ42 and pTau/Aβ42 +, Aβ42/Aβ40 −	1 (1)	1/0/0/0/0	1.05	0.222	0.0203	0.083
tTau/Aβ42 +, Aβ42/Aβ40, and pTau/Aβ42 −	1 (1)	1/0/0/0/0	1.32	0.223	0.0183	0.084

Abbreviations: CSF, cerebrospinal fluid; CDR, clinical dementia rating; PIB, Pittsburgh Compound B; tTau, total tau; pTau, phosphorylated tau-181; Aβ42, amyloid β peptide 42; SUVR, standardized uptake value ratio; PET, positron emission tomography.

Aβ40 was positive but tTau/Aβ42 and pTau/Aβ42 were negative (two individuals). Sixteen individuals were PIB-negative, but all three CSF ratios were positive. Twelve individuals were PIB-negative and had partial discordance of the CSF ratios; most (10 of 12) had high Aβ42/Aβ40.

There was a concordance of PIB PET and all three CSF ratios in 21 of the 22 individuals in our cohort with cognitive impairment (CDR > 0): Fifteen individuals were positive by all measures and six were negative by all measures. One individual rated CDR 0.5 was PIB-negative, Aβ42/Aβ40 and tTau/Aβ42 positive, but pTau/Aβ42 negative. The six individuals rated CDR > 0 who were negative by both PET PIB and all three CSF ratios likely have a non-AD cause of their cognitive symptoms. In all 198 cases, tTau/Aβ42 was positive if pTau/Aβ42 was positive, but tTau/Aβ42 was positive in some individuals (n = 5) when pTau/Aβ42 was negative. Notably, many of the individuals with partial discordance of the CSF ratios had values close to the cutoffs and therefore may be in a transitional or borderline stage (see Table 2).

4. Discussion

Overall, we found a high concordance between PIB PET and CSF biomarkers of AD as measured by the Elecsys assays. Ratios of CSF biomarkers that included Aβ42 (tTau/Aβ42, pTau/Aβ42, and Aβ42/Aβ40) best distinguished PIB-positive from PIB-negative individuals. All three CSF ratios were positive in 46 of the 50 PIB-positive individuals (92%), and all three CSF ratios were negative in 120 of the 148 PIB-negative individuals (81%). Out of the 32 individuals (16% of the cohort) with discordance between the three CSF ratios and PIB PET, 28 individuals were negative by PIB but positive by at least one CSF ratio.

Previous reports have identified amyloid PET–negative individuals with positive CSF biomarkers [3,5,6,42]. Recent work has demonstrated that amyloid PET–negative but CSF biomarker–positive individuals have increased rates of amyloid accumulation, suggesting these individuals have early AD brain pathology and are likely to develop amyloid PET positivity [3,42]. While CSF biomarkers and amyloid PET are both markers of amyloid pathology, CSF biomarkers indicate the state of Aβ42 production and clearance at the time of LP while amyloid PET images the accumulation of neuritic amyloid plaques over many years. In addition, amyloid PET tracers are designed to bind to neuritic amyloid plaques [47], whereas CSF biomarkers could be more sensitive to deposition of both neuritic amyloid plaques and diffuse amyloid plaques [48]. It appears likely that CSF biomarkers become positive very early in the course of the disease, before sufficient amyloid has accumulated to create an amyloid PET signal. Therefore, less-than-perfect correspondence of PIB PET and CSF biomarkers is expected and may reflect differences in AD brain pathology.

Notably, we found that in cases of partial discordance between CSF biomarker ratios and PIB PET (when some, but not all, CSF biomarker ratios agreed with PIB PET), Aβ42/Aβ40 was typically the positive ratio (nine of 11 cases) in PIB-negative cases and was the sole positive ratio in two PIB-positive cases. These findings suggest that abnormal Aβ42/Aβ40 may be the earliest indicator of amyloid brain pathology, potentially reflecting stage 1 of preclinical AD (amyloid deposition but no abnormalities in tTau or pTau) [49]. Compared to Aβ42, the ratio of Aβ42/Aβ40 may better reflect deposition of amyloid because it may normalize for individual variation in overall amyloid production [24]. However, many of the cases with partial discordance of CSF ratios have borderline values and selecting different cutoffs would change the concordance of the ratios somewhat. Larger studies are required to determine whether Aβ42/Aβ40 becomes altered at an earlier stage than tTau/Aβ42 and pTau/Aβ42. Interestingly, we also found that tTau and pTau were almost perfectly correlated (r = 0.98, P < .0001) in our cohort. It is possible that tTau and pTau may be less highly correlated in a cohort enriched for non-AD dementia—this is a topic for future studies.

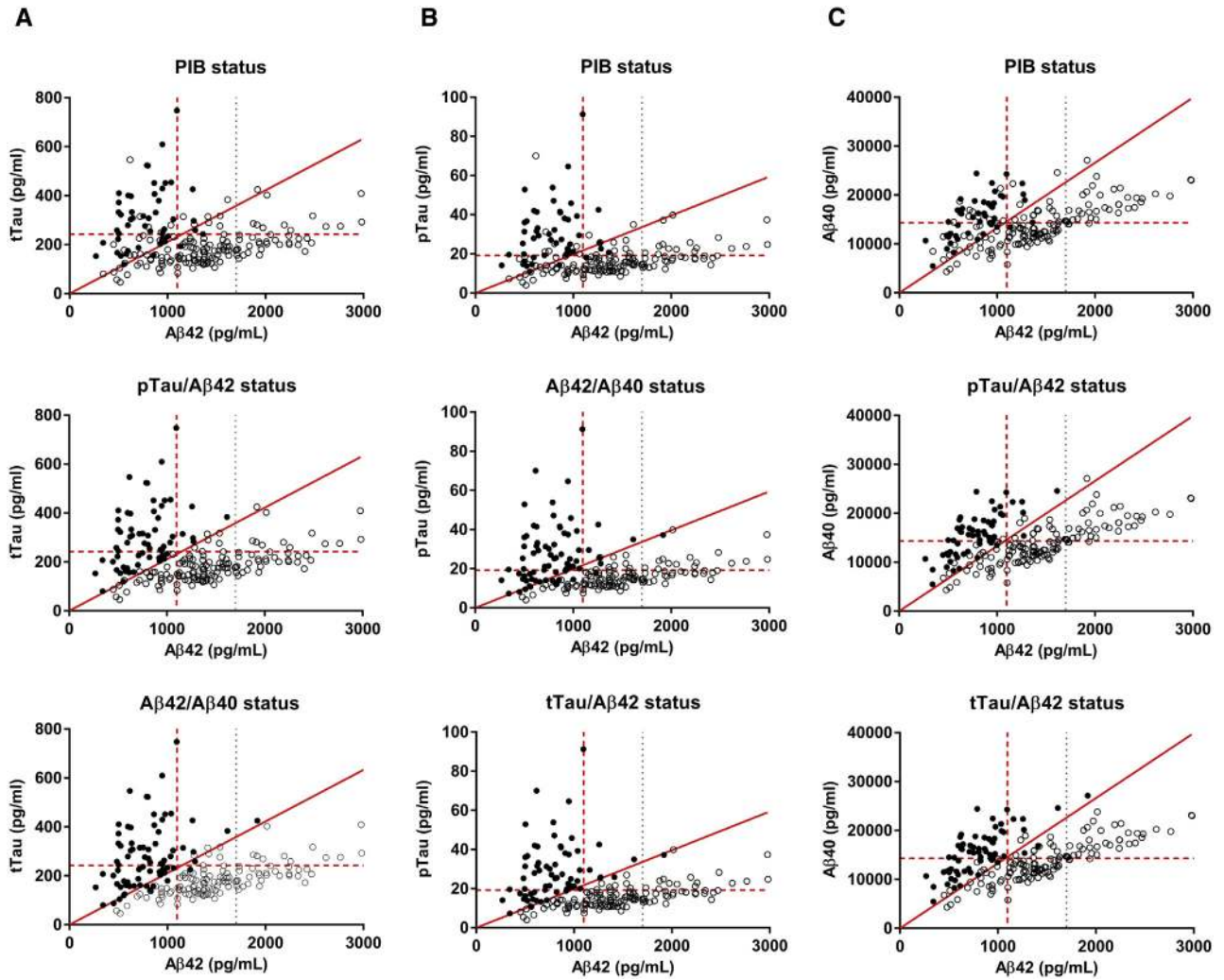S.E. Schindler et al. / Alzheimer's & Dementia 14 (2018) 1460-1469

Fig. 4. Concordance of CSF ratios and PIB binding. The status (positive or negative according to CSF ratios or PIB binding) was evaluated in scatterplots of CSF tTau versus Aβ42 (A), pTau versus Aβ42 (B), and Aβ40 versus Aβ42 (C). Each point represents CSF biomarkers in one individual. Solid points have a positive biomarker status (as defined in the plot titles) and open points have a negative status. The horizontal red dashed lines represent the cutoff values for CSF tTau (A), pTau (B), and Aβ40 (C). The vertical red dashed lines represent the cutoff value for Aβ42. The vertical gray dotted lines represent the upper limit of quantitation for Aβ42. The sloped solid red lines represent the cutoff values for tTau/Aβ42 (A), pTau/Aβ42 (B), and Aβ40/Aβ42 (C). Abbreviations: CSF, cerebrospinal fluid; PIB, Pittsburgh Compound B; tTau, total tau; pTau, phosphorylated tau-181; Aβ42, amyloid β peptide 42.

The Roche Elecsys assays and other automated assays for CSF biomarkers are likely to increase the utility of CSF biomarkers in research, clinical trials, and clinical diagnosis. Further studies are needed to examine the concordance between CSF biomarkers of AD as measured by the Elecsys assays and other amyloid PET tracers. Studies are also needed to evaluate whether CSF biomarkers of AD as measured by the Elecsys assays predict future cognitive decline. It is unclear whether the same cutoff values that correspond with amyloid PET status will also best predict cognitive decline. Finally, comparison of CSF biomarkers with brain autopsy data in cases with a short CSF collection to autopsy interval would be helpful in demonstrating that CSF biomarkers as measured by the Elecsys assays are strongly correlated with AD brain pathology.

Given the high degree of precision, accuracy, reliability, and reproducibility of the Elecsys assays [39], it is possible that an Elecsys CSF biomarker measure could be found that is reproducible across all sites worldwide and is highly predictive of AD brain pathology. It is important to note that preanalytical factors may affect CSF biomarker values, especially of Aβ42. Therefore, further refinement of CSF testing for AD will require rigorous standardization of preanalytical factors, including sample collection and processing. It will also be important to further define when it is appropriate for clinicians to perform CSF testing for AD. When a disease-modifying agent for AD becomes available, many patients will be interested in learning their amyloid status, and it will be important to have clear guidelines in place for all aspects of CSF testing.

Acknowledgments

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jalz.2018.01.013.

RESEARCH IN CONTEXT

1. Systematic review: Cerebrospinal fluid (CSF) biomarkers of Alzheimer's disease are used in research, in clinical trials, and to inform clinical diagnosis. Technical factors, including lot-to-lot variability in assays, have limited the utility of CSF assays.

2. Interpretation: CSF biomarker values measured with Roche Elecsys assays were compared to Pittsburgh Compound B (PIB) positron emission tomography. We found that ratios of CSF biomarkers that included amyloid β peptide 42 (Aβ42) (total tau/Aβ42, pTau/Aβ42, and Aβ42/Aβ40) best distinguished between individuals who were PIB-positive and PIB-negative. Discordance between CSF biomarkers and PIB positron emission tomography may occur because CSF biomarkers measure different aspects of Alzheimer's disease brain pathology.

3. Future directions: Automated assays for CSF biomarkers are likely to improve the reliability of CSF testing for Alzheimer's disease. Further work is needed to standardize preanalytical factors.
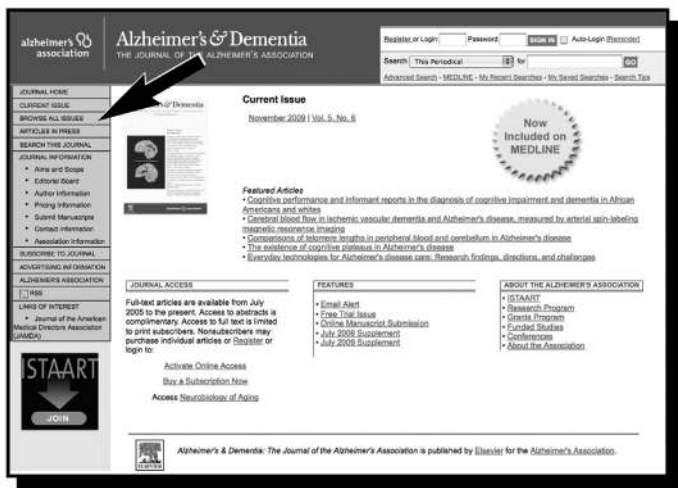
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