Cerebrospinal Fluid Aβ$_{42/40}$ Corresponds Better than Aβ$_{42}$ to Amyloid PET in Alzheimer's Disease

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Abstract.
Background: Decreased concentrations of amyloid-β 1-42 (Aβ$_{42}$) in cerebrospinal fluid (CSF) and increased retention of Aβ tracers in the brain on positron emission tomography (PET) are considered the earliest biomarkers of Alzheimer's disease (AD). However, a proportion of cases show discrepancies between the results of the two biomarker modalities which may reflect inter-individual differences in Aβ metabolism. The CSF Aβ$_{42/40}$ ratio seems to be a more accurate biomarker of clinical AD than CSF Aβ$_{42}$ alone.
Objective: We tested whether CSF Aβ$_{42}$ alone or the Aβ$_{42/40}$ ratio corresponds better with amyloid PET status and analyzed the distribution of cases with discordant CSF-PET results.
Methods: CSF obtained from a mixed cohort ($n = 200$) of cognitively normal and abnormal research participants who had undergone amyloid PET within 12 months ($n = 150$ PET-negative, $n = 50$ PET-positive according to a previously published cut-off) was assayed for Aβ$_{42}$ and Aβ$_{40}$ using two recently developed immunoassays. Optimal CSF cut-offs for amyloid positivity were calculated, and concordance was tested by comparison of the areas under receiver operating characteristic (ROC) curves (AUC) and McNemar's test for paired proportions.

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Results: CSF $A\beta_{42/40}$ corresponded better than $A\beta_{42}$ with PET results, with a larger proportion of concordant cases (89.4% versus 74.9%, respectively, $p < 0.0001$) and a larger AUC (0.936 versus 0.814, respectively, $p < 0.0001$) associated with the ratio. For both CSF biomarkers, the percentage of CSF-abnormal/PET-normal cases was larger than that of CSF-normal/PET-abnormal cases.

Conclusion: The CSF $A\beta_{42/40}$ ratio is superior to $A\beta_{42}$ alone as a marker of amyloid-positivity by PET. We hypothesize that this increase in performance reflects the ratio compensating for general between-individual variations in CSF total $A\beta$.

Keywords: Alzheimer's disease, amyloid-β, biomarker, cerebrospinal fluid, positron emission tomography

INTRODUCTION

Reduced concentrations of amyloid-β (Aβ) 1-42 in the cerebrospinal fluid (CSF) are associated with increased retention of Aβ tracers in the brain via positron emission tomography (PET) [1–5] and both are considered the earliest biomarkers of Alzheimer's disease (AD) [6–10]. However, several published studies show that results of the two measures are discordant in a proportion of individuals in the cohorts evaluated to date, most notably as CSF $A\beta_{42}$-abnormal/amyloid PET-normal (reviewed in [11]). Such discordance may reflect assay methodological variability and/or imprecision, or these biomarker modalities may reflect partly different pathophysiologic events [12].

Results from several groups demonstrate that normalization of the CSF $A\beta_{42}$ concentration to the level of total Aβ peptides (or the most abundant isoform, Aβ 1-40 [$A\beta_{40}$]) in the CSF by means of an $A\beta_{42/40}$ ratio improves the accuracy of clinical AD diagnosis [13–17] by "controlling for" inter-individual differences in overall levels of CSF Aβ. This led us to hypothesize that the CSF concentration of $A\beta_{42}$ depends not only on the pathophysiological status of a given individual, hence suggesting the presence or absence of AD pathology, but also on the total amount of Aβ peptides in the CSF, i.e., the total production of Aβ in the brain. At least two additional arguments, derived from experience regarding pre-analytical sample handling and data interpretation, further support the application of the $A\beta_{42/40}$ ratio for AD diagnostic purposes: (a) $A\beta_{42/40}$ is less prone to the error of misinterpretation when inappropriate test tubes to collect CSF are used [18] (likely due to the similar amount of absorption of both Aβ species); and (b) in two studies that applied the $A\beta_{42/40}$ ratio measured with the same ELISA assays to predict AD in mild cognitive impairment (MCI) subjects [15] or in early symptomatic AD [19], virtually identical $A\beta_{42/40}$ cut-offs were obtained despite differences in absolute $A\beta_{42}$ concentrations.

Considering these data, we hypothesized that the CSF $A\beta_{42/40}$ ratio might reflect amyloid PET status (positive versus negative) better than the $A\beta_{42}$ concentration alone. To test this hypothesis, we used dichotomized PET results to calculate the optimal cut-offs for $A\beta_{42}$ concentrations and $A\beta_{42/40}$ ratios measured with two novel, recently validated ELISAs [14], and then compared the concordance of each of the two CSF biomarkers with the results of the amyloid PET. We also compared the distribution of the discordant results, i.e., the percentage of cases with pathologic CSF findings and normal PET results versus the percentage with normal CSF and pathologic PET results. We proposed that the results from these analyses might provide insight into the possible mechanism(s) for the observed known discordance between amyloid imaging and levels of CSF $A\beta_{42}$ and, thus, may be informative for pathologic disease staging.

MATERIALS AND METHODS

Participants

All procedures were approved by the Institutional Review Board and the Washington University Human Research Protection Office, and written informed consent was obtained from each participant. Participants were community-dwelling volunteers enrolled in studies of normal aging and dementia at the Knight Alzheimer's Disease Research Center (ADRC) at Washington University in St. Louis. Participants were 45–83 years of age at baseline and had no neurological, psychiatric or systemic medical illness that might compromise longitudinal study participation, nor medical contraindication to lumbar puncture (LP) or PET. All participants underwent cognitive assessments (including the Mini-Mental State Examination (MMSE) [20]), neurological evaluations and clinical assessments that included the Clinical Dementia Rating (CDR) [21].

To be included in the present analysis, baseline clinical and cognitive assessment, and LP and amyloid PET (via Pittsburgh Compound B, PiB) within 12 months of each other had to be available (mean ± SD, 67 ± 78 days). Two-hundred CSF samples were selected *a priori* based on cortical amyloid load via PET (25% amyloid PET-positive and 75% amyloid PET-negative, see below), independent of and blinded to participant demographics and clinical status. Given published reports of greater CSF-PET discordance in amyloid PET-negative individuals, greater numbers of amyloid-negative participant samples were evaluated so to enrich for possible and more common discordant (PiB-negative/Aβ$_{42}$-positive) cases. *APOE* genotype was obtained from the Knight ADRC Genetics Core [22].

CSF collection, processing, and analysis

CSF was collected and processed according to standardized protocols. Samples (20–30 mL) were collected using LP procedures (22 G × 3 1/2 inch atraumatic Sprotte spinal needle, Pajunk, #001151-30 C) via gravity drip into a 50 mL conical polypropylene tube (Corning #430829) at 8 : 00am after overnight fasting, gently inverted to avoid possible rostro-caudal gradient effects and immediately put on wet ice. Within 1 h of collection, samples were briefly centrifuged at low speed (2000× *g*, 5 min at 4°C) to pellet any cellular debris. All but the bottom 500 μL was transferred to a new 50 mL polypropylene tube, aliquoted (500 μL) into sterile 2 mL polypropylene screw-cap tubes with O-rings (Corning #430915), and immediately frozen at –80°C until analysis. The interval between sample collection and batch analysis varied from 1–9 years, with equal representation of storage intervals among the different amyloid PET groups (PiB+ versus PiB-). Samples underwent a single freeze-thaw cycle prior to assay, were thawed on wet ice (∼3 h) followed by vortexing prior to blinded analysis, and were run in duplicate. Aβ$_{42}$ and Aβ$_{40}$ concentrations were measured with immunoassays from IBL International (Hamburg, Germany) according to the manufacturer's protocols. To be included in the statistical analyses, the results had to pass quality control (QC) criteria consistent with the Alzheimer's Disease Neuroimaging Initiative (ADNI) study [23], including coefficients of variability (CV) of duplicates ≤25% and the results of the kit's run validation samples ("kit controls") within the acceptable range as defined by the manufacturer. Samples also had data available for Aβ$_{42}$, Tau, and

pTau181 using INNOTEST ELISA kits (Fujirebio, Ghent, Belgium).

In vivo amyloid imaging

Participants received a single intravenous bolus injection of ^{11}[C] PiB (between 4.7 and 19.0 mCi). PET imaging [24] was conducted with a Siemens 962 HR+ ECAT PET or Biograph 40 scanner (Siemens/CTI, Knoxville KY), and magnetic resonance imaging (MRI) using MPRAGE T1-weighted images (1 mm × 1 mm × 1.25 mm) was obtained for anatomic reference as described previously [25, 26]. Participants underwent a 60-min dynamic scan with ^{11}[C] PiB, and PET data was processed using regions of interest (ROIs) derived from structural MRIs using Freesurfer [25, 26]. Regional time-activity curves were extracted for each ROI, and binding potentials were calculated using Logan graphical analysis [27] with cerebellar gray matter as the reference region. The mean cortical binding potential (MCBP) was calculated based on a set of selected cortical regions known to have high levels of deposition in AD [28], including precuneus, rostral middle frontal cortex, superior frontal cortex, superior temporal cortex, middle temporal cortex, lateral orbitofrontal cortex, and medial orbitofrontal cortex [25]. A MCBP cutoff of >0.18 for positivity was chosen to be consistent with prior studies [9, 28–30].

Statistical analyses

Sample size was calculated with the negative/positive ratio, power, and significance set to 3 : 1, 0.9, and 0.05, respectively, and the areas under the receiver operating characteristic (ROC) curves (AUCs) of 0.974 (Aβ$_{42/40}$) and 0.827 (Aβ$_{42}$) [14]. Unless otherwise stated, results are presented as means ± standard deviations (SD) or 95% confidence intervals (CI). CSF Aβ$_{42}$ and Aβ$_{42/40}$ cut-offs, and the corresponding sensitivities and specificities for PiB PET-positivity, were calculated based on the dichotomous PET grouping at the maximal Youden Indices, using a cut-off for PiB positivity of 0.18 [28]. Comparisons of continuous variables were performed with Student's *t*-test or Welch test in cases of unequal variances; dichotomous variables were compared with chi-squared test. AUCs of the ROC curves were compared with DeLong test [32]. McNemar's test for paired proportions was used for testing differences between the proportions of CSF-PiB concordant cases, and sensitivities and specificities. All

tests were two-tailed. Logistic regression models were applied with logit of probability of PET-positivity (logit (π_{PET+})) as the outcome, and the CSF biomarkers and age as continuous, and *APOE ε4* status (presence of $\varepsilon4$ allele = 1) and sex (males = 1) as dichotomous predictors. Covariates were selected either for epidemiological reasons (age, sex, APOE) or due to observed significances between PET+ and PET- groups (Aβ$_{40}$ concentrations). Mann-Whitney U test was used to compare LP/PET intervals between "CSF/PET concordant" and "CSF/PET discordant" groups separately for Aβ$_{42}$ and Aβ$_{42/40}$. MedCalc 13.1 (MedCalc Software bvba, Ostend, Belgium) and Stata 14.1 (StatCorp, College Station, TX) were used for statistical analyses. Significance was defined as $p < 0.05$.

RESULTS

Demographic data and CSF biomarkers

As expected given the 3 : 1 (PiB-:PiB+) selection criterion based on amyloid PET positivity, the cohort was comprised of 88% cognitively normal (CDR 0) and 12% cognitively abnormal (CDR >0; $n = 19$ CDR 0.5, $n = 3$ CDR 1, $n = 1$ CDR 2) individuals. The cohort demographics and biomarker data are shown in Table 1. One sample did not pass QC so was excluded from statistical analysis. Not unexpectedly, the amyloid-positive (PiB+) group was older than the amyloid-negative (PiB–) group ($p < 0.001$), contained a higher percentage of *APOE ε4* carriers ($p < 0.01$), and had a lower mean score on the MMSE ($p = 0.02$). The PiB- group contained a greater percentage of women than the PiB+ group (65% versus 43%, respectively), but there were no differences in the years of education ($p = 0.28$). Median absolute intervals between the time of CSF collection and PiB

PET scan did not differ significantly between the two groups (24 versus 33 days, U = 3408, $p = 0.45$), and in both groups PET was performed before LP in similar proportions of the cases (36.7% and 38.7% of PET+ and PET- participants, respectively, $\chi^2(1) = 0.058$, $p = 0.81$).

Inter-assay CVs, tested with two kit control samples, were 2.2% and 7.1% for Aβ$_{40}$, and 2.3% and 6.5% for Aβ$_{42}$. Median range-to-averages of the duplicate CSF samples measurements (expressing intra-assay variation) were 2.3% for Aβ$_{40}$ and 2.5% for Aβ$_{42}$.

By definition, the amount of cortical amyloid deposition (MCBP, mean ± SD) was higher in the PiB+ compared to the PiB– group ($p < 0.001$). As expected, PiB+ participants had significantly lower concentrations of CSF Aβ$_{42}$ ($p < 0.001$) and a lower mean Aβ$_{42/40}$ ratio ($p < 0.001$), as well as significantly higher concentrations of CSF Tau ($p < 0.001$) and pTau181 ($p < 0.001$). Interestingly, the PiB+ group exhibited a modest but significantly higher mean concentration of CSF Aβ$_{40}$ compared to the PiB- group ($p = 0.047$). Differences in LP/PET intervals between "CSF/PET concordant" and "CSF/PET discordant" groups were not significant ($p > 0.1$ for both CSF biomarkers).

Concordance between amyloid PET and CSF biomarkers

The relationships between CSF analyte values and cortical amyloid load (PiB MCBP) are shown in Fig. 1. The optimal CSF cut-offs that best differentiated the dichotomized PiB+ and PiB- groups were 735 pg/mL for Aβ$_{42}$, 11,600 pg/mL for Aβ$_{40}$, and 0.05 for the Aβ$_{42/40}$ ratio. At these cut-offs, the Aβ$_{40}$, Aβ$_{42}$ concentrations and the Aβ$_{42/40}$ ratio yielded sensitivities (with dichotomized PiB PET status defined as the reference) of 85.7% (CI: 72.8–94.1),
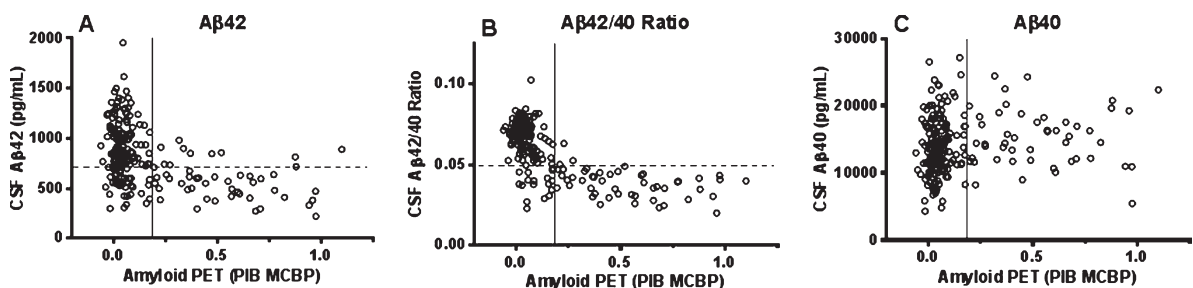

Fig. 1. Scatterplots of cortical amyloid PET load using [^{11}C] PiB and concentrations of CSF Aβ$_{42}$ (A), the Aβ$_{42/40}$ ratio (B), and Aβ$_{40}$ (C). Solid vertical line represents the *a priori* dichotomous cut-off for PiB positivity as published previously [28]. Dashed horizontal lines on panels A and B represent the best-performing cut-offs of the respective CSF biomarkers calculated in the present study.

Table 1
Demographic and biomarker data

Characteristic	PiB+	PiB–	p
N	49	150	NA
Female, %	43%	65%	<0.01*
Age at LP, y	72.4 ± 7.3	64.2 ± 9.6	<0.001
% APOE ε4 carriers	58%	31%	<0.01*
Education, y	15.4 ± 2.9	15.9 ± 2.5	0.283
MMSE	28.00 ± 3.03	29.07 ± 1.21	0.02
Median LP-PiB PET interval (IQR), days	24 (16 – 64)	33 (12–94)	0.45†
PiB MCBP	0.5275 ± 0.2454	0.0473 ± 0.0502	<0.001
CSF Aβ$_{40}$, pg/mL	15 280 ± 4 160	13 890 ± 4 260	0.047
CSF Aβ$_{42}$, pg/mL	577 ± 184	887 ± 292	<0.001
CSF Aβ$_{42/40}$	0.038 ± 0.009	0.065 ± 0.013	<0.001
CSF Tau, pg/mL	492 ± 392	234 ± 125	<0.001
CSF pTau181, pg/mL	75.8 ± 35.3	47.2 ± 19.1	<0.001

*χ^2 test. †Mann-Whitney test. APOE, apolipoprotein E; CSF, cerebrospinal fluid; IQR, interquartile range; LP, lumbar puncture; MCBP, mean cortical binding potential; MMSE, Mini-Mental State Examination (0–30, with 30 a perfect score) at clinical assessment closest in time to LP; NA, not applicable; PET, positron emission tomography; PiB, Pittsburgh Compound B. Unless otherwise indicated, values represent the mean ± SD.

81.6% (CI: 68.0–91.2) and 95.9% (CI: 86.0–99.5), respectively, with the corresponding specificities of 34.0% (CI: 26.5–42.2), 72.0% (CI: 64.8–79.6), and 88.0% (CI: 81.7–92.7), corresponding to accuracies of 46.7% for Aβ$_{40}$, 74.9% for Aβ$_{42}$, and 89.4% for the Aβ$_{42/40}$ ratio.

For both Aβ$_{42}$ and Aβ$_{42/40}$, the percentage of cases with abnormal CSF findings but normal PET results (false positives: 20.6% and 9.0% for Aβ$_{42}$ and Aβ$_{42/40}$, respectively), clustering in the lower left quadrant of Fig. 1, was larger than the percentage of cases with normal CSF findings and abnormal PET (false negatives: 4.5% and 1.5% for Aβ$_{42}$ and Aβ$_{42/40}$, respectively), clustering in the upper right quadrant of Fig. 1. Detailed results are presented in Table 2.

McNemar test for paired proportions revealed a highly significantly larger proportion of Aβ$_{42/40}$-PET concordant cases compared to Aβ$_{42}$-PET concordant cases ($\chi^2(1) = 19.56$, $p < 0.001$), with an odds ratio of discordant cases of 5.14 (CI: 2.26 to 13.69).

ROC curves yielded AUC's of 0.599 for Aβ$_{40}$, 0.814 for Aβ$_{42}$ and 0.936 for the Aβ$_{42/40}$ ratio (Fig. 2), with the Aβ$_{42/40}$ ratio demonstrating significantly higher correspondence with PiB status compared to the concentrations of Aβ$_{42}$ ($p < 0.001$) and Aβ$_{40}$ ($p < 0.001$) (Table 2).

Logistic regression models

Logistic regression models are presented in Supplementary Table 1. In both models (with Aβ$_{42}$ and Aβ$_{42/40}$, respectively), when adjusted for Aβ$_{40}$,
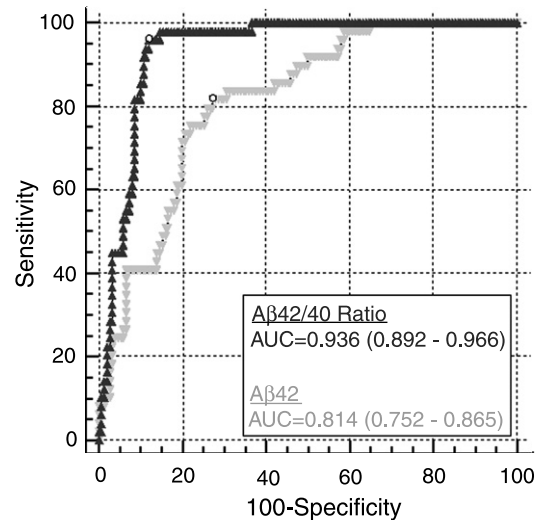

Fig. 2. Receiver operating characteristic (ROC) curves of the CSF Aβ$_{42}$ concentration and Aβ$_{42/40}$ ratio. Values for areas under the ROC curves (AUC) correspond to mean ± 95% CI. Open circles represent the combinations of the sensitivities and the specificities at the maximal Youden Indices.

age, APOE ε4 status, and sex, the effects of Aβ$_{42}$ and Aβ$_{42/40}$ remained highly significant ($p < 0.001$). Consistent with increased Aβ$_{40}$ in the PiB+ group, the effect of Aβ$_{40}$ was positive and significant ($p < 0.001$) in the Aβ$_{42}$ model, but became non-significant in the Aβ$_{42/40}$ model ($p = 0.36$). In both models, the effects of age were positive and significant. Male sex was a positive predictor but only with borderline significance, and presence of at least one APOE ε4 allele was a non-significant covariate in both models ($p > 0.05$).

Table 2
Analyses of concordance of CSF Aβ$_{42}$ and the Aβ$_{42/40}$ ratio with amyloid PET status

Parameter	Aβ$_{42}$	Aβ$_{42/40}$
Cut-off at the max. YI	735 pg/mL	0.05
Sensitivity at the max. YI (95% CI)	81.6% (68.0–91.2)	95.9% (86.0–99.5)
Specificity at the max. YI (95% CI)	72.7% (64.8–79.6)	88.0% (81.7–92.7)
Positive PV (95% CI)	49.4% (38.1–60.7)	72.3% (59.8–82.7)
Negative PV (95% CI)	92.4% (86.0–96.5)	98.5% (94.7–99.8)
Accuracy (concordance proportion)	74.9%	89.4%*
%False Pos. (N)/%False Neg. (N)	20.6% (41)/4.5% (9)	9.0% (18)/1.5% (3)
Area Under the ROC curve (95% CI)	0.814 (0.752–0.865)	0.936† (0.892–0.966)

*$p < 0.001$ compared to the Aβ$_{42}$ concentrations (McNemar test for paired proportions); †$p < 0.001$ compared to the Aβ$_{42}$ concentrations (DeLong test for differences in AUC's); False positive, abnormal CSF/normal PET; false negative, normal CSF/abnormal PET; CSF, cerebrospinal fluid; PET, positron emission tomography; PV, predictive value; ROC, receiver operating characteristic; YI, Youden index.

DISCUSSION

The main finding in this study is significantly better concordance of the CSF Aβ$_{42/40}$ ratio, compared to CSF Aβ$_{42}$ alone, with cortical Aβ deposition as measured by [^{11}C] PiB PET. This finding is consistent with our [14] and others' [16, 17] reports of better diagnostic accuracy of the Aβ$_{42/40}$ ratio compared to Aβ$_{42}$ in early AD, as well as with CSF biomarkers of neurodegeneration, Tau and pTau181 [13]. We hypothesize that the concentration of Aβ$_{42}$ in the CSF depends not only on the physiological amyloid status of a given individual (presence or absence of amyloid pathology) but also on the total amount of Aβ peptides that are present. By normalizing to the concentration of Aβ$_{40}$, the most abundant Aβ species in the CSF, the ratio removes the potential confound of differences in overall Aβ concentration and provides a better index of underlying amyloid-related pathology. Two experimental observations further support the application of the Aβ$_{42/40}$ ratio for AD diagnostics: (a) Aβ$_{42/40}$ is less prone to the error of misinterpretation when non-polypropylene test tubes (as are often found in standard clinical LP trays) are used to collect CSF [18] likely due to the fact that both Aβ isoforms seem to absorb to the tube surface to a similar extent; and (b) two studies using a different set of ELISAs (produced by a currently non-existing vendor and based on N-terminally non-specific antibodies) that have applied the Aβ$_{42/40}$ ratio to predict AD in MCI subjects [15] and discriminate early symptomatic AD from controls [19] reported almost identical Aβ$_{42/40}$ cut-offs (0.095 and 0.098, respectively), whereas the corresponding cut-offs for Aβ$_{42}$ alone differed by more than 15% (640 pg/mL and 550 pg/mL, respectively).

The analyses of CSF Aβ isoforms in the current study were performed with two novel assays that

were validated recently by our group and approved for human *in vitro* diagnostic (IVD) use in Europe [14]. The assays exhibit very good analytical performance, both apply the same protocol for sample dilution, incubation, and washing steps, and require only a small volume (25 μL) of CSF for both biomarkers measured in duplicate. Interestingly, we observed small but significantly ($p = 0.047$) elevated levels of CSF Aβ$_{40}$ in the PiB+ group compared to the PiB- group. This is consistent with similarly slight, but significant, increases observed in the MCI/AD group compared to controls when measured with the same assay [14]. Also, in a recent study by Dorey et al. [33] investigating the performance of these markers for correspondence with clinical diagnosis, CSF Aβ$_{40}$ concentrations were higher in AD than non-AD patients, and inclusion of CSF Aβ$_{40}$ as an Aβ$_{42/40}$ ratio corrected 76.2% of misinterpreted cases in AD patients with normal CSF Aβ$_{42}$ concentrations and 94.7% of cases when Aβ$_{40}$ was used alone. When we adjusted for the Aβ$_{42/40}$ ratio, but not for Aβ$_{42}$ alone, the Aβ$_{40}$ concentration became insignificant as a predictor of amyloid PET status in the logistic regression models, whereas both Aβ$_{42}$ and the Aβ$_{42/40}$ ratio remained highly significant as predictors of amyloid PET after adjustment for Aβ$_{40}$, age, *APOE ε*4 carriage, and sex. After adjustment for other covariates, older age remained a significant predictor of cortical amyloid-PET positivity, consistent with age being the strongest risk factor for AD. Odds ratio of Aβ$_{42}$-PET versus Aβ$_{42/40}$-PET discordant cases, resulting from McNemar test, indicates that the odds to falsely predict the PET outcome is five-fold lower with the Aβ$_{42/40}$ ratio compared to Aβ$_{42}$ alone.

Both biomarker modalities (CSF analysis and amyloid PET) have their practical advantages and disadvantages in terms of cost (actual and reimbursable), perceived invasiveness, and availability in clinical as

well as research settings [34], and our results do not endorse one or the other as a "gold standard" for amyloid biomarker positivity. From a scientific point of view, the discordance between CSF Aβ$_{42}$ and amyloid PET (typically observed as false positives, i.e., abnormal/low CSF Aβ$_{42}$ in the absence of amyloid-PET positivity) has been hypothesized to be due to possible methodological variability/limitations in the CSF assays, differences in the temporal trajectories of processes identified by the two biomarker modalities (e.g., CSF levels of soluble Aβ$_{42}$ becoming abnormal prior to aggregated Aβ$_{42}$ [amyloid] becoming detectable by PET), and/or inter-individual differences in overall levels of Aβ peptides [9, 12]. These possible scenarios are not mutually exclusive. Our use of well-performing assays that utilize the same dilution factor for both Aβ$_{42}$ and Aβ$_{40}$ minimizes methodological variability as a source of discordance between CSF Aβ$_{42}$ and amyloid PET results and increases the confidence of the ratio as being an accurate measure of the proportion of each of these species in CSF. In so doing, the increase in concordance that we observe for the Aβ$_{42/40}$ ratio suggests much of the "false positives" as defined by CSF Aβ$_{42}$ is due to lower overall levels of Aβ in these individuals, whereas the "false negatives" can be attributed in large part to higher overall CSF Aβ levels. Indeed, the negative predictive value of the Aβ$_{42/40}$ ratio in this study (98%) approached 100%, thus supporting its use for ruling out the presence of underlying amyloid pathology. While use of the ratio also significantly increased the positive predictive value for cortical amyloid PET positivity, 9% of individuals in this cohort were still classified as amyloid-positive by CSF Aβ$_{42/40}$ but amyloid-negative by PET (false negative), consistent with CSF Aβ$_{42}$ levels dropping prior to cortical amyloid being detectable by PiB PET. That all but one participant in this discordant group (whether CSF abnormality was defined by Aβ$_{42}$ alone or the Aβ$_{42/40}$ ratio) were cognitively normal is consistent with findings from a recent study in which low CSF Aβ$_{42}$ but normal amyloid PET was much more frequent in cognitively normal individuals and early MCI cases than in the late MCI stage or AD dementia [12]. Longitudinal CSF and amyloid PET biomarker evaluation will be required to test whether levels of CSF Aβ$_{42}$ indeed become abnormal (drop) before amyloid-positivity is detected with PET as has been suggested by a recent study by Vlassenko and colleagues [30]. That study, however, did not assess CSF Aβ$_{40}$ so comparison of the two CSF measures was not possible. Results of a recent

study by Palmqvist et al. also suggest that decreases in CSF Aβ$_{42}$ precede alterations in amyloid PET [35]. Interestingly, another recent study using different ELISAs also reported better correspondence of the Aβ$_{42/40}$ ratio with Aβ PET, however, with a higher proportion of Aβ–/PET+ individuals compared to the present study [36]. This discrepancy is likely due to the differences in the cohorts (higher numbers of cognitively normal individuals expected to be in the preclinical period in the current cohort) and/or different methodological issues. Another study reported no significant differences in concordances between Aβ$_{42/40}$ and Aβ$_{42}$ with amyloid PET, although the AUC for Aβ$_{42/40}$ was slightly larger than that for Aβ$_{42}$ alone [37]. This lack of significance might be attributed to the lower statistical power (a much smaller cohort, $n = 38$) and/or the use of different ELISAs and PET tracer (^{18}F-flutemetamol) in that study. Similarly, another smaller study concluded that the Aβ$_{42/40}$ ratio had a "large" effect size (defined as Cohen's $f^2 = 0.35$), compared to a "medium" effect size (defined as Cohen's $f^2 = 0.15$), for Aβ$_{42}$, in predicting concurrent PiB burden [38]. Aggregation of soluble Aβ into diffuse, non-fibrillar plaques that are not identifiable by amyloid PET imaging may also contribute to the "false negative" discordant cases [39]. If confirmed, a finding of CSF alterations preceding those in PET would support the CSF Aβ$_{42/40}$ ratio (and perhaps even Aβ$_{42}$ alone, albeit with less accuracy) as the earliest detectable marker of β-amyloidosis.

Our study is not without limitations. Although amyloid PET in this study is considered to be the reference to define the presence of amyloid, positivity was defined according to dichotomous, research-based thresholds in a defined set of cortical regions. Also, since samples were specifically selected to over-represent possible CSF/PET discordance (by evaluating 25% PET-positive and 75% PET-negative cases), the cohort does not reflect the general distribution of amyloid PET positivity in the general population. Furthermore, because of this strategy, the cohort was significantly skewed toward cognitively normal individuals (88% cognitively normal, 12% cognitively abnormal). Additional study is needed in larger cohorts comprised of greater numbers of cognitively abnormal individuals, as well as those clinically diagnosed with non-AD dementias in order to better evaluate the CSF/PET concordance in non-AD populations. Finally, we performed statistical analyses of concordance after calculating optimal cut-off values for both CSF biomarkers. As this was designed to be a

biomarker concordance study, independent of clinical status, the diagnostic value of any of the biomarkers and modalities studied here remains to be evaluated.

In summary, the CSF Aβ$_{42/40}$ ratio performs better, compared to CSF Aβ$_{42}$ alone, in identifying individuals with versus without underlying cortical amyloid as defined by PiB PET. The ratio outperforms the single Aβ$_{42}$ measure in all metrics, including sensitivity, specificity, positive and negative predictive values, and overall accuracy. Of special note is its better concordance in individuals classified as amyloid-negative by PET, suggesting that the higher discordance between CSF Aβ$_{42}$ alone and amyloid PET-positivity may reflect overall lower levels of Aβ$_{42}$ in some individuals as opposed to its aggregation into plaques (or oligomeric forms that are likely not detected with the current assays [40]. The CSF Aβ$_{42/40}$ ratio may, therefore, be useful as a proxy for amyloid status in AD clinical trials and eventually in clinical care settings.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-160722.

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