ANNALS of Clinical and Translational Neurology
Open Access
ANA American Neurological Association since 1875

RESEARCH ARTICLE

Antemortem CSF Aβ42/Aβ40 ratio predicts Alzheimer's disease pathology better than Aβ42 in rapidly progressive dementias

Simone Baiardi[1,a], Samir Abu-Rumeileh[1,a], Marcello Rossi[2], Corrado Zenesini[2], Anna Bartoletti-Stella[2], Barbara Polischi[2], Sabina Capellari[1,2] & Piero Parchi[2,3]

[1]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna 40123, Italy
[2]IRCCS, Istituto delle Scienze Neurologiche di Bologna, Bologna 40139, Italy
[3]Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna 40138, Italy

Correspondence
Piero Parchi, IRCCS Istituto delle Scienze Neurologiche di Bologna, Ospedale Bellaria, Via Altura 1/8, 40139 Bologna, Italy.
Tel: +39-051-4966740;
Fax: +39-051-4966208;
E-mail: piero.parchi@unibo.it

Funding Information
This work was supported by the Italian Ministry of Health (grant RF2011-02351092) and by the University of Bologna (grant RFO).

Received: 16 October 2018; Accepted: 23 October 2018

Annals of Clinical and Translational Neurology **2019; 6(2): 263–273**

doi: 10.1002/acn3.697

[a]These authors contributed equally as first authors.

Abstract

Objective: Despite the critical importance of pathologically confirmed samples for biomarker validation, only a few studies have correlated CSF Aβ42 values in vivo with postmortem Alzheimer's disease (AD) pathology, while none evaluated the CSF Aβ42/Aβ40 ratio. We compared CSF Aβ42 and Aβ42/Aβ40 ratio as biomarkers predicting AD neuropathological changes in patients with a short interval between lumbar puncture and death. **Methods**: We measured CSF Aβ40 and Aβ42 and assessed AD pathology in 211 subjects with rapidly progressive dementia (RPD) and a definite postmortem diagnosis of Creutzfeldt-Jakob disease ($n = 159$), AD ($n = 12$), dementia with Lewy bodies (DLB, $n = 4$), AD/DLB mixed pathologies ($n = 5$), and various other pathologies ($n = 31$). **Results**: The score reflecting the severity of Aβ pathology showed a better correlation with ln(Aβ42/Aβ40) ($R^2 = 0.506$, $\beta = -0.713$, $P < 0.001$) than with ln(Aβ42) ($R^2 = 0.206$, $\beta = -0.458$, $P < 0.001$), which was confirmed after adjusting for covariates. Aβ42/Aβ40 ratio showed significantly higher accuracy than Aβ42 in the distinction between cases with or without AD pathology (AUC 0.818 ± 0.028 vs. 0.643 ± 0.039), especially in patients with Aβ42 levels ≤495 pg/mL (AUC 0.888 ± 0.032 vs. 0.518 ± 0.064). Using a cut-off value of 0.810, the analysis of Aβ42/Aβ40 ratio yielded 87.0% sensitivity, 88.2% specificity in the distinction between cases with an intermediate-high level of AD pathology and those with low level or no AD pathology. **Interpretation**: The present data support the use of CSF Aβ42/Aβ40 ratio as a biomarker of AD pathophysiology and noninvasive screener for Aβ pathology burden, and its introduction in the research diagnostic criteria for AD.

Introduction

The pathological hallmarks of Alzheimer's disease (AD) include the extracellular deposition of protein amyloid beta (Aβ) in brain parenchyma and blood vessel walls, and the intraneuronal accumulation of hyperphosphorylated tau (p-tau).[1] According to the prevailing amyloid hypothesis, aggregation and tissue deposition of Aβ precede p-tau driven neurofibrillary degeneration and anticipate the clinical onset of the disease by several years.[2–4] Positron emission tomography (PET) with fibrillary Aβ-specific radiotracers and the cerebrospinal fluid (CSF) quantitative assay of the 42 amino acid form of Aβ (Aβ42) provide in vivo evidence of brain Aβ deposition and are included in current research diagnostic criteria for AD.[5,6] However, despite the analytical and clinical validation and the established inverse correlation with amyloid-related neuropathological changes, the detection of CSF Aβ42 still suffers from limitations.[7,8] Aβ42 is highly labile and prone to aggregate, which makes its concentrations susceptible to variation in the preanalytical processing.[9–11] Furthermore, CSF Aβ levels may vary significantly among individuals, leading to a misinterpretation of test results in the presence of constitutively high or low

quantities of Aβ42 relative to chosen diagnostic "cut-off values". These and perhaps other factors might also be responsible for the reported lack of optimal correlation between CSF Aβ42 values and amyloid-PET[12] or neuropathological findings.[8,13] To overcome these limits, several authors proposed to evaluate the ratio between Aβ42 and the 40 amino acid form of Aβ(Aβ40) CSF concentrations instead of that of Aβ42 alone.[14–18] Given that Aβ40 is a more stable peptide which is not significantly decreased in AD,[19] it has been hypothesized that the CSF Aβ42/Aβ40 ratio may improve the diagnostic accuracy by allowing the normalization with respect to the sources of Aβ42 level variability unrelated to the AD pathology burden. Increasing evidence supports, indeed, the better diagnostic performance of Aβ42/Aβ40 ratio in comparison to Aβ42 alone.[15,16,20] Specifically, the Aβ42/Aβ40 ratio showed a better accordance with amyloid-PET findings[17,21–24] and an improved diagnostic accuracy also in a clinical setting when CSF biomarker assays gave discordant results.[18,25,26] However, to date, the relationship between antemortem CSF Aβ42/Aβ40 ratio with postmortem AD pathology has not been systematically studied. Aiming to contribute to the issue of the added diagnostic value of CSF Aβ42/Aβ40 ratio in the clinical setting, we tested the hypothesis that the CSF Aβ42/Aβ40 ratio better predicts the burden of AD-related neuropathological changes than CSF Aβ42 alone. To this aim, we took advantage of a large series of neuropathologically verified cases of Creutzfeldt-Jakob disease (CJD) and other rapidly progressive dementias (RPDs), in which the CSF biomarker assessment was performed, on average, only a few months before death.

Methods

Patient selection

We studied patients affected by RPD referred for diagnosis to the Laboratory of Neuropathology (NP-Lab) at the Institute of Neurological Sciences of Bologna between January 2005 and December 2017. Inclusion criteria were limited to the availability of sufficient and qualitatively adequate postmortem brain tissue for AD neuropathological diagnosis and staging, and a CSF sample of sufficient volume to complete all assays and collected within 36 months from death. The screening of NP-Lab database yielded a total of 211 suitable cases (Fig. S1). Primary neuropathological diagnosis included CJD ($n = 159$), AD ($n = 12$), dementia with Lewy bodies (DLB, $n = 4$), AD/DLB mixed pathologies ($n = 5$), encephalitis ($n = 7$), vascular dementia ($n = 6$), primary central nervous system malignancy ($n = 5$), Wernicke's encephalopathy ($n = 3$), hypoxic encephalopathy ($n = 2$), progressive supranuclear palsy ($n = 1$), variably protease-sensitive prionopathy ($n = 1$), and progressive multifocal leukoencephalopathy ($n = 1$). Finally, in five cases, neuropathological investigations did not reveal a significant specific pathology to reach a definitive diagnosis.

All subjects gave written informed consent for the use of their clinical data for research purposes and the study was approved by the local ethical review board.

Neuropathological examination

Neuropathological examination was performed using standardized procedures as described.[13] Briefly, tissues from the right hemisphere, brainstem, and cerebellum were rapidly frozen at −80°C, while the left parts of the brain were fixed in 10% formalin. Samples from the fixed hemisphere were taken from 23 brain regions, according to a standardized protocol.[27]

Histopathological examination was performed on seven μm thick sections of formalin-fixed and paraffin-embedded brain tissue blocks. All sections were stained hematoxylin-eosin for screening. Also, immunohistochemistry with antibodies specific for PrP (3F4, dilution 1:400, Signet Labs), hyperphosphorylated tau (AT8, dilution 1:100, Innogenetics), and Aβ (4G8, dilution 1:5000, Signet Labs) were applied to all cases. To this aim, several brain regions were stained, mainly following established consensus criteria.[1,28–30] Additionally, stainings for alpha-synuclein (LB509, dilution 1:100, Thermo Fisher Scientific), hyperphosphorylated TDP-43 (phospho Ser 409/410-1 polyclonal antibody, 1:1000, CosmoBio Co), anti-HLA-DR (CR3/43, 1:400, Agilent Dako), GFAP (6F2, 1:100, Agilent Dako), CD3 (SP7, 1:200, Thermo Fisher Scientific), and CD8 (CD8/144B, 1:100, Agilent Dako) were carried out in selected cases. Finally, Thioflavin S staining was performed to assess neuritic plaques in all cases showing AT8 positive immunoreactivity (even minimal) in the cerebral neocortex.

An experienced neuropathologist (P.P.) formulated the final diagnosis, assigned the amyloid phase according to Thal,[30,31] the Braak's stage of neurofibrillary pathology,[32] the CERAD neuritic plaques score,[33] and classified each case according to the level of AD neuropathologic change (ABC score).[1] To obtain a more continuous measure of Aβ brain load, we also calculated a score after evaluating semiquantitatively immunostained sections from the cerebral cortex (one from each lobe: frontal, temporal, parietal, and occipital), amygdala, hippocampus (CA1 region), striatum, midbrain, and cerebellum. Parenchymal Aβ pathology (Fig. S2) was graded (0–6) as follows: 0-entirely negative; 2-rare or sparse deposits (2–4 plaques in at least one 100× microscopic field); 4-moderate

number of deposits (5–10 plaques); 6-multiple deposits, disseminate (>10 plaques). An additional point was added when core plaques were also noted. Cerebral amyloid angiopathy (CAA) (Fig. S2) was evaluated in leptomeninges and parenchyma as follows: 0-entirely negative; 1-up to two vessels focally involved; 3-more than half of the vessels involved; and 2-intermediate between 1 and 3. For each case, a cumulative Aβ score (0–90) was calculated. Similarly, we formulated a score for AD-related tau neuropathological change by semiquantitative evaluation of p-tau immunoreactivity (0-no immunoreactivity; 1-mild; 2-moderate; 3-prominent) in six brain regions, namely transentorhinal cortex, entorhinal cortex, parahippocampal gyrus, middle temporal gyrus, middle frontal gyrus, and occipital cortex. Fine neuritic (threads) tau deposits, neurofibrillary tangles, and thick neuritis, which are part of neuritic plaques, were analyzed separately. Finally, a cumulative score (0–54) (AD tau score) was given.

CSF analysis

Antemortem CSF was obtained by lumbar puncture at the L3-L4 or L4-L5 interspinal space following a standard procedure, centrifuged at 1000*g* for 10 min when showing even mild signs of blood contamination, divided into aliquotes, and stored in polypropylene tubes at −80°C until analysis.

CSF Aβ42, and Aβ40 levels were analyzed using commercially available ELISA kits (INNOTEST Aβ1–42, and INNOTEST Aβ1–40; Innogenetics/Fujirebio) according to the manufacturer's instructions.

For the interpretation of Aβ42 results, we used an in-house cut-off value of Aβ42 ≤ 495 pg/mL. The calculation of this cut-off and further details about the pre- and analytical variables are reported in supplementary methods in Appendix S1. The ratio of Aβ42 to Aβ40 was calculated according to a previously published formula $[(A\beta42)/(A\beta40)\times10]$.[15]

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 21 (IBM, Armonk, NY, USA) and Stata SE version 14.2 (StataCorp LLC, Texas, USA). Depending on the data distribution, results were expressed as mean and standard deviation or median and interquartile range (IQR). Due to the non-normal distribution of biomarker values, Mann-Whitney *U* test and Kruskal-Wallis test were applied to test the differences between two or more groups. A Bonferroni correction was applied to multiple comparisons. For both univariate and multivariate linear regression models, Aβ42 and Aβ42/Aβ40 values were transformed into a natural logarithmic scale (ln) to obtain a normal data distribution.

Using univariate linear regression models, we tested the effect of preanalytical variables on CSF biomarker results.

For multivariate linear regression models, we used the Aβ score as a dependent variable and the ln(Aβ42) or the ln(Aβ42/Aβ40) as an independent variable. We tested by univariate models the possible contribution of each demographic variable in predicting the Aβ score and then added to the multivariate model only those with significant associations, using a stepwise approach with the application of the Likelihood ratio test at each step. We considered as possible covariates the age, sex, disease duration, interval between CSF collection and death, ApoE ε4 allele (presence or absence), prion disease (presence or absence), and AD tau score. Finally, we applied Bayesian information criteria (BIC)[34] to select the best performing model in the comparison between the one with ln(Aβ42) and the one with ln(Aβ42/Aβ40).

ROC analyses were obtained to compare the diagnostic value of Aβ42 and Aβ42/Aβ40 ratio. The optimal cut-off value for biomarkers was chosen using the maximized Youden index. The Delong test[35] was used to compare

Table 1. Demographics, CSF biomarker data, and AD-related neuropathology scores.

Demographics	
Mean age at onset – years ± SD	68.5 ± 9.3
Female – *n* (%)	106 (50.2%)
Median disease duration - months (IQR)	4 (2.5–9)
Median interval between LP and death - months (IQR)	1.5 (1–4)
ApoE genotype – *n* (%)	ε4/ε4: 5 (2.4); ε3/ε4: 30 (14.2); ε2/ε4: 1 (0.4); ε3/ε3: 158 (74.9); ε2/ε3: 17 (8.1)
CSF biomarker values	
Aβ42 pg/mL - median (IQR)	522 (373–763)
Aβ40 pg/mL - median (IQR)	5030 (3406–6951)
Aβ42/Aβ40 - median (IQR)	1.098 (0.806–1.435)
AD-related neuropathology scores	
Thal's Aβ phase[30] - *n* (%)	0: 80 (37.9); 1–2: 56 (26.6); 3: 33 (15.6); 4–5: 42 (19.9)
Braak's NF stage[31] - *n* (%)	0: 114 (54.0); I-II: 58 (27.5); III-IV: 34 (16.1); V-VI: 5 (2.4)
Level of AD neuropathological change (ABC score)[1] - *n* (%)	Not: 82 (38.9); Low: 95 (45.0); Intermediate: 29 (13.7); High: 5 (2.4)
CAA – *n* (%)	Negative: 148 (70.1); Type 1: 4 (1.9); Type 2: 59 (28.0)
Aβ score – median (IQR)	7.0 (0–30)
AD tau score - median (IQR)	3 (0.5–8)

Aβ, amyloid beta; AD, Alzheimer's disease; CAA; cerebral amyloid angiopathy; IQR, interquartile range; LP; lumbar puncture; n, number of cases; NF, neurofibrillary pathology; SD, standard deviation.

Table 2. Distribution of Aβ42 an Aβ40 levels across different diagnostic groups.

	AD+AD/DLB	Prion disease	Other RPDs	Inflammatory Diseases
N	17	160	26	8
Aβ42	270 (202–370)	568 (404–772)	506 (312–778)	402 (370–901)
Aβ40	4254 (3057–7009)	5054 (3392–6855)	5094 (3663–7939)	4737 (3095–8861)

Data are expressed as median and interquartile range.
Aβ, amyloid beta; AD, Alzheimer's disease; DLB, dementia with Lewy bodies; RPD, rapidly progressive dementia.

the areas under the curve (AUC) of Aβ42 and Aβ42/Aβ40 ratio. Differences were considered statistically significant at $P < 0.05$.

Results

Demographics, CSF biomarker values, and AD-related neuropathology scores (Table 1)

Patients with AD or AD/DLB mixed pathology showed significantly lower Aβ42 levels than those with prion disease ($P < 0.001$), inflammatory diseases ($P = 0.013$), and other RPDs ($P = 0.001$) (Table 2). Conversely, the levels of Aβ42 did not vary significantly among non-AD diagnostic groups (e.g., prion disease, inflammatory diseases and other RPDs). There were also no significant differences in the CSF levels of Aβ40 between AD or AD/DLB, prion disease, inflammatory diseases, and the other RPD groups. The results of CSF Aβ42 and Aβ40 analysis in patients with and without CAA and the effects of preanalytical variables are shown in Appendix S1.

Relationship between CSF Aβ42 levels and Aβ42/Aβ40 values with AD neuropathology

Patients with no Aβ deposits had higher levels of Aβ42 (666 pg/mL, IQR 405–835) than those with Aβ deposits (465 pg/mL, IQR 355–655) with 33.8% of subjects of the first group and 53.7% of the latter having Aβ42 levels below the threshold of 495 pg/mL. A comparison between subjects with high, intermediate, and low degree of AD pathology showed Aβ42 levels below threshold in, respectively, 80% ($n = 4$), 86.2% ($n = 25$) and 44.2% ($n = 42$) of cases. Similarly, 88.1% of subjects in Thal's phase 4 or 5 ($n = 37$), 48.3% of those in phase 3 ($n = 16$) and 33.6% of those in phase 1–2 ($n = 19$) had Aβ42 levels ≤495 pg/mL. As for Aβ42, the Aβ42/Aβ40 ratio showed higher values in subjects without Aβ deposits (1.383, IQR 1.111–1.855) than in those with Aβ deposits (0.893, IQR 0.682–1.179). Values of Aβ42/Aβ40 ratio and Aβ42 levels according to each Thal's phase of Aβ deposition are shown in Figure 1.

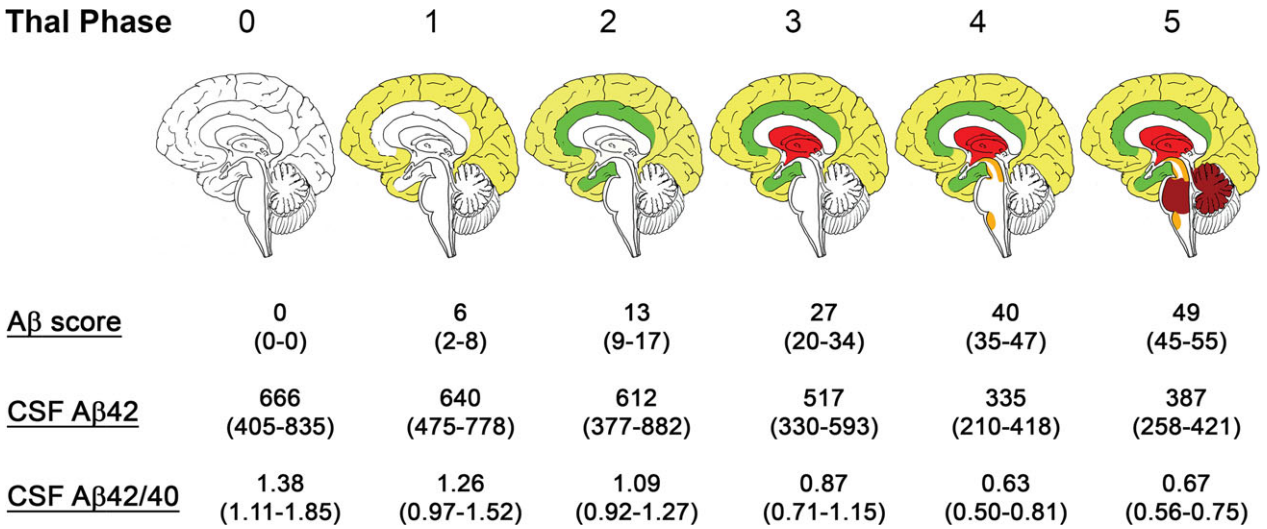

Figure 1. Comparison of Aβ neuropathological score, CSF Aβ42 levels (pg/mL), and CSF Aβ42/Aβ40 ratio across the Thal's phases of Aβ pathology.[31] Data are expressed as median and interquartile range (IQR).

Correlations between CSF biomarkers and amyloid-beta pathology

In the total cohort, the Aβ score showed a better correlation with ln(Aβ42/Aβ40) ($R^2 = 0.506$, $\beta = -0.713$, $P < 0.001$) than with ln(Aβ42) ($R^2 = 0.206$, $\beta = -0.458$, $P < 0.001$) (Table 3 and Figure 2). Accordingly, the model including ln(Aβ42/Aβ40) yielded a lower BIC value (1096.1) than that including ln(Aβ42) (1196.3), suggesting that ln(Aβ42/Aβ40) is a better explanatory variable. We found similar associations [ln(Aβ42): $R^2 = 0.547$, $\beta = -0.239$, $P < 0.001$; ln(Aβ42/Aβ40): $R^2 = 0.634$, $\beta = -0.460$, $P < 0.001$] and a lower BIC value for the model with ln(Aβ42/Aβ40) (1031.2) than that with ln (Aβ42) (1075.4), after accounting for covariates by multivariate regression analysis (Table 3).

When we considered only the cases with Aβ42 levels lower than ≤495 pg/mL ($n = 98$), the difference of the correlation coefficients between Aβ score and ln(Aβ42/Aβ40) ($R^2 = 0.591$, $\beta = -0.772$, $P < 0.001$) and Aβ score and ln (Aβ42) ($R^2 = 0.040$, $\beta = -0.224$, $P = 0.026$) increased further. The latter result was confirmed after adjusting for covariates [ln(Aβ42): $R^2 = 0.536$, $\beta = -0.036$, $P < 0.001$; ln(Aβ42/Aβ40): $R^2 = 0.686$, $\beta = -0.507$, $P < 0.001$]. In both analyses, either not-adjusted or adjusted for covariates, the BIC values of the models with ln(Aβ42/Aβ40) were lower (513.6 and 486.3, respectively) than those of the models with ln(Aβ42) (597.2 and 523.6).

Table 3. Univariate and multivariate regression models to predict postmortem cerebral amyloid-beta pathology.

Variable	Model R^2	Model *P* value	B (95% CI)	*SE*	Beta[1]	*P* value
A) Total cohort						
Univariate models						
Ln (Aβ42)	0.206	<0.001	−17.368 (−21.970 to −12.766)	2.335	−0.458	
Ln (Aβ42/Aβ40)	0.506	<0.001	−30.538 (−34.633 to −26.442)	2.077	−0.713	
Multivariate models						
Ln (Aβ42)	0.547	<0.001	−9.000 (−12.822 to −5.177)	1.939	−0.239	<0.001
Age at LP (y)			0.529 (0.327 to 0.732)	0.103	0.265	<0.001
AD tau score			0.925 (0.700 to 1.149)	0.114	0.468	<0.001
ApoEε4			6.667 (1.968 to 11.365)	2.383	0.135	0.006
Prion disease			4.435 (0.100 to 8.771)	2.199	0.102	0.045
Intercept			24.813 (−2.223 to 51.850)	13.712	–	0.072
Ln (Aβ42/Aβ40)	0.634	<0.001	−19.618 (−24.098 to −15.138)	2.272	−0.460	<0.001
Age at LP (y)			0.215 (0.023 to 0.408)	0.098	0.108	0.029
AD tau score			0.759 (0.553 to 0.966)	0.105	0.385	<0.001
ApoEε4			6.182 (1.971 to 10.394)	2.136	0.125	0.004
Prion disease			5.142 (1.247 to 9.038)	1.976	0.118	0.010
Intercept			−7.764 (−21.334 to 5.806)	6.882	–	0.261
B) Cohort with Aβ42 ≤ 495 pg/mL						
Univariate models						
Ln (Aβ42)	0.040	0.026	−14.875 (−27.961 to −1.790)	6.592	−0.224	
Ln (Aβ42/Aβ40)	0.591	<0.001	−31.042 (−36.227 to −25.857)	2.612	−0.772	
Multivariate models						
Ln (Aβ42)	0.536	<0.001	−2.376 (−12.252 to 7.501)	4.971	−0.036	0.634
Age at LP (y)			0.676 (0.343 to 1.010)	0.168	0.331	<0.001
AD tau score			0.922 (0.597 to 1.248)	0.164	0.501	<0.001
ApoEε4			5.452 (−1.335 to 12.240)	3.416	0.114	0.114
Prion disease			1.813 (−4.936 to 8.562)	3.397	0.041	0.595
Intercept			−21.190 (−81.537 to 39.156)	30.371	–	0.487
Ln (Aβ42/Aβ40)	0.686	<0.001	−20.293 (−26.440 to −14.146)	3.094	−0.507	<0.001
Age at LP (y)			0.335 (0.042 to 0.627)	0.147	0.164	0.025
AD tau score			0.588 (0.306 to 0.871)	0.142	0.320	<0.001
ApoEε4			4.264 (−1.321 to 9.850)	2.811	0.089	0.133
Prion disease			3.981 (−1.518 to 9.480)	2.767	0.090	0.154
Intercept			−9.834 (−29.956 to 10.289)	10.127	–	0.334

Optimal multivariate linear regression models using the Aβ postmortem pathology score as the dependent variable and either CSF ln(Aβ42) levels or ln(Aβ42/Aβ40) as independent variable after adjusting for covariates (age at LP, AD tau score, presence of prion disease, presence of ApoE ε4). Aβ, amyloid beta; AD, Alzheimer's disease; CI, interval of confidence; ln, natural logarithm; LP, lumbar puncture; SE, standard error; y, years.
[1]standardized Beta.

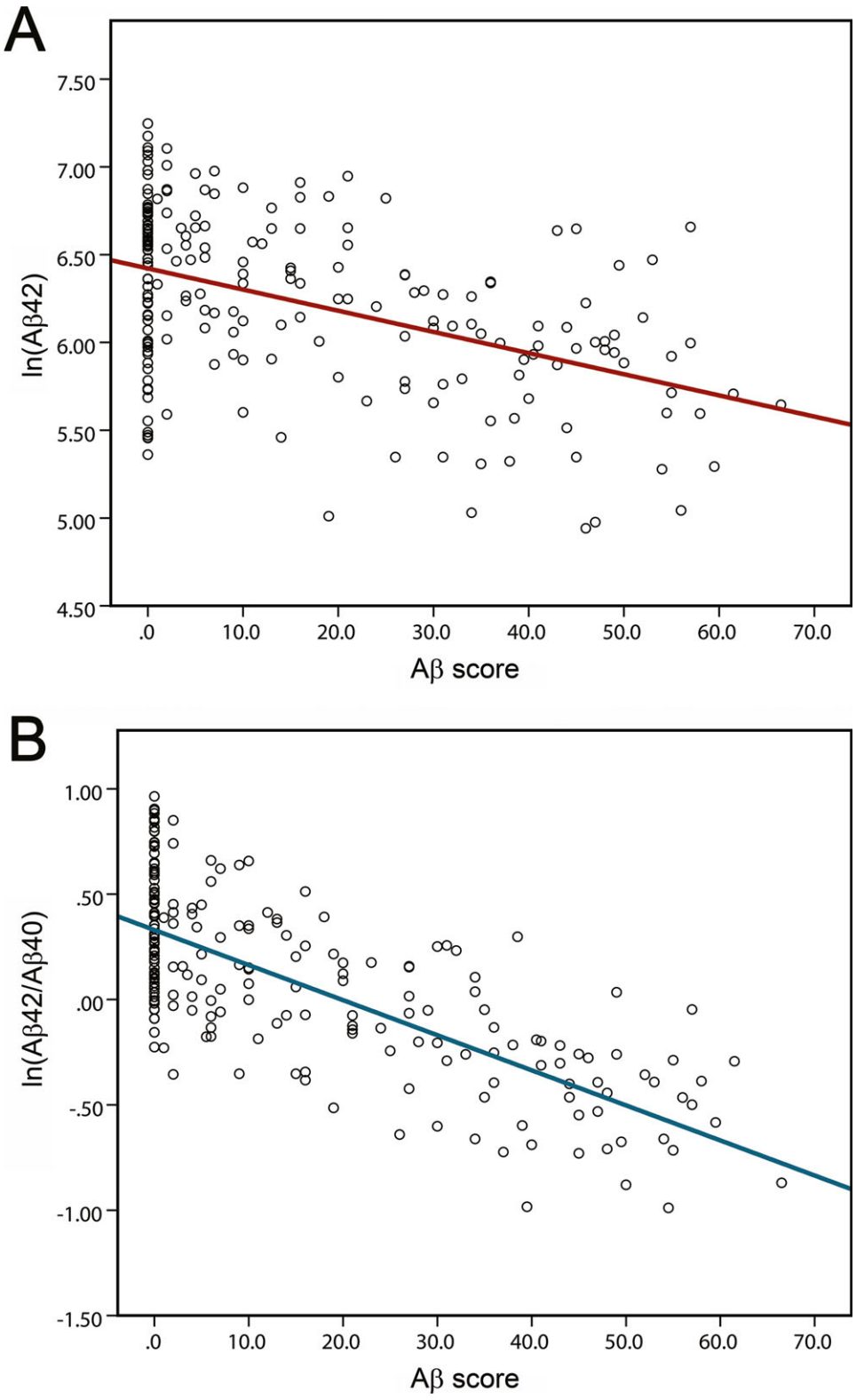

Figure 2. Correlations between CSF ln(Aβ42) levels, ln(Aβ42/Aβ40) values, and Aβ-related pathology. (A) A significant negative correlation is seen between CSF ln(Aβ42) levels and Aβ pathology score ($R^2 = 0.206$, $\beta = -0.458$, $P < 0.001$). (B) In comparison to (A) the negative correlation between CSF ln(Aβ42/Aβ40) and Aβ pathology score is significantly higher ($R^2 = 0.506$, $\beta = -0.713$, $P < 0.001$).

Table 4. ROC analyses in the total cohort.

	Analysis				*P* value
	AUC	Cut-off	Sens (%)	Spec (%)	(Delong test)
(1) Thal's phase: 3–5 vs. 0–2					
Aβ42	0.755 ± 0.034	<466	72.5	69.3	0.0001
Aβ42/Aβ40	0.905 ± 0.021	<0.955	84.6	80.0	
(2) ABC score: AD+ vs. AD-					
Aβ42	0.643 ± 0.039	<622	56.1	71.3	0.0004
Aβ42/Aβ40	0.818 ± 0.028	<1.131	72.0	69.8	
(3) ABC score: Intermediate-high vs. not-low					
Aβ42	0.808 ± 0.035	<405	75.7	76.5	0.0174
Aβ42/Aβ40	0.900 ± 0.027	<0.810	87.0	88.2	

Aβ, amyloid beta; AD, Alzheimer's disease; AUC, area under the curve; Sens, sensitivity; Spec, specificity.

Diagnostic accuracy of CSF amyloid-beta-related biomarkers

Based on our intralaboratory Aβ42 cut-off value, we extended the assessment of the relative diagnostic accuracy of each amyloid-related biomarker to the group of patients with Aβ42 levels lower than 495 pg/mL. In this group and in the total cohort, we performed ROC analyses according to: (1) the presence of Thal's phase 3–5 versus 0–2, (2) the presence or absence of AD pathology as assessed by the ABC score (AD+ vs. AD-), and (3) the presence of a intermediate-high versus low-not AD pathology.

In the total cohort (N = 211), the Aβ42/Aβ40 ratio was superior to Aβ42 in the distinction between cases with or without AD pathology (AUC 0.818 ± 0.028 vs. 0.643 ± 0.039). As expected, the analyses (1) and (3) yielded similar results (Table 4). Most significantly, using cut-off values of 405 pg/mL for Aβ42 and 0.810 for Aβ42/Aβ40, ratio the analyses yielded 75.7% or 87.0% sensitivity and 76.5% or 88.2% specificity, respectively, in the distinction between cases with an intermediate-high level of AD pathology and those with low level and no AD pathology. AUC, sensitivity, and specificity values for all analyses are reported in Table 4. For every ROC analysis, the AUC of Aβ42/Aβ40 ratio was significantly higher than that of Aβ42 at Delong test.

When we restricted the analysis to patients with Aβ42 levels lower than 495 pg/mL, we observed a significant increase in the accuracy of Aβ42/Aβ40 ratio (AUC 0.939 ± 0.022) in the prediction of Thal's phases (3–5 vs. 0–2), whereas the accuracy of Aβ42 decreased (AUC

CSF Aβ42/Aβ40	n	Thal amyloid phase				Braak NF stage		
		0 (%)	1-2 (%)	3 (%)	4-5 (%)	0-II (%)	III-IV (%)	V-VI (%)
<0.4	4	0 (0.0)	0 (0.0)	1 (25.0)	3 (75.0)	1 (25.0)	2 (50.0)	1 (25.0)
0.4 – 0.6	17	0 (0.0)	0 (0.0)	3 (17.6)	14 (82.4)	7 (41.2)	8 (47.0)	2 (11.8)
0.6 – 0.8	30	1 (3.3)	3 (10.0)	9 (30.0)	17 (56.7)	15 (50.0)	13 (43.3)	2 (6.7)
0.8 – 1.0	37	8 (21.6)	16 (43.2)	7 (18.9)	6 (16.2)	32 (86.5)	5 (13.5)	0 (0.0)
1.0 – 1.2	40	18 (45.0)	13 (32.5)	8 (20.0)	1 (2.5)	37 (92.5)	3 (7.5)	0 (0.0)
1.2 – 1.4	24	13 (54.2)	5 (20.8)	5 (20.8)	1 (4.2)	22 (91.7)	2 (8.3)	0 (0.0)
1.4 – 1.6	23	10 (43.5)	13 (56.5)	0 (0.0)	0 (0.0)	23 (100)	0 (0.0)	0 (0.0)
1.6 – 1.8	9	7 (77.8)	2 (22.2)	0 (0.0)	0 (0.0)	9 (100)	0 (0.0)	0 (0.0)
1.8 – 2.0	10	6 (60.0)	4 (40.0)	0 (0.0)	0 (0.0)	10 (100)	0 (0.0)	0 (0.0)
>2.0	17	17 (100)	0 (0.0)	0 (0.0)	0 (0.0)	16 (94.1)	1 (5.9)	0 (0.0)

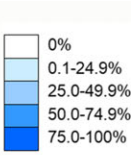

Figure 3. Distribution of AD pathological changes at each given range of CSF Aβ42/Aβ40 ratio values. Aβ, amyloid beta; NF, neurofibrillary pathology; n, number of cases.

0.613 ± 0.057) ($P < 0.001$). The same trend, with the ratio showing significantly higher values (AUC 0.888 ± 0.032; 0.869 ± 0.036) in comparison to Aβ42 (AUC 0.518 ± 0.064; 0.650 ± 0.058) ($P < 0.001$ and $P = 0.002$), was observed by considering the AD pathology score (analyses 2 and 3, respectively).

Details about the distribution of AD pathological changes in the patient cohort according to given ranges of values of CSF Aβ42/Aβ40 ratio are provided in Figure 3.

Discussion

The results of the present study demonstrate that, in patients with RPD of various etiology, the measurement of Aβ42/Aβ40 ratio in CSF samples taken shortly before death predicts better than CSF Aβ42 levels the burden of Aβ brain deposits. The assessment of diagnostic accuracy in pathologically verified case series represents the gold standard for biomarker validation. Nevertheless, only a few studies have consistently correlated Aβ42 values in vivo with postmortem Aβ pathology,[7,8] while none has, to date, specifically considered the predictive value of the Aβ42/Aβ40 ratio on Aβ pathology. Moreover, such studies are hampered by the long latency between the in vivo and postmortem assessments, which are especially relevant considering the current need to validate the diagnostic biomarkers for AD and other neurodegenerative diseases in the prodromal or even preclinical stage.[10,36,37] Brains affected by CJD and other RPDs provide the unique opportunity to correlate the CSF findings with the postmortem neuropathology within a short time interval.[13] Moreover, by including patients who were virtually asymptomatic before the onset of the RPD, such a series represents well the population in which the accuracy of in vivo markers for AD needs to be validated the most (i.e., elderly asymptomatic patients with various degree of AD pathology).[10,36,37]

According to our data, the Aβ42/Aβ40 ratio performs significantly better than Aβ42 alone in predicting the whole spectrum of AD pathological changes, not only the burden of Aβ deposits. However, the added value of the ratio appears to decrease with increasing severity of AD pathology, being highest in cases with low or even absent Aβ deposition, which especially underlines the importance of the Aβ42/Aβ40 ratio in the identification of the "false positive" samples with low Aβ42 reflecting causes other than the Aβ-related pathology. These confounders are well known and include not only preanalytical factors, such as the interval between collection and freezing, sample exposure to storage surfaces, and assay measurement variation,[10,11,38] and comorbidities affecting Aβ metabolism, but also the individual variability in CSF Aβ42 production. Indeed, evidence indicates that CSF Aβ peptide concentration varies between individuals and some subjects may be constitutively low Aβ42 producers.[16] Moreover, pathological changes other than Aβ deposition such as synaptic loss might contribute to the reduction of Aβ levels, including Aβ42.[39] Accordingly, in the clinical setting, the occurrence of indeterminate results, revealing abnormal Aβ42 levels and normal t-tau and p-tau, is a significant problem limiting the diagnostic accuracy of CSF biomarkers in suspected AD cases.[6,23,24] In this respect, the use of t-tau/Aβ42 or p-tau/Aβ42 ratios has shown a better diagnostic performance in comparison to the single biomarker determination,[10,40] and with an overall accuracy comparable to that of Aβ42/Aβ40 ratio.[41,42] However, given that CSF tau and Aβ reflect distinct pathological processes which have different importance regarding both specificity and time of appearance, the use of a ratio targeted to a single proteinopathy appears more rationale and might even be more accurate in preclinical AD. Indeed, according to the prevailing amyloid hypothesis, Aβ aggregation represents the primary and most specific pathological event in AD since tau deposition may occur secondarily to many other pathologies. Accordingly, AD biomarkers are increasingly being distinct in Aβ deposition, tau pathology, and neurodegeneration, following the A/T/N classification.[36,43] Consequently, the identification and implementation of the biomarkers with the highest specificity and accuracy in measuring the degree of Aβ pathology should be the primary goal,[36] especially in the earlier disease stages when tau pathology is not widespread yet. The results of a recent study showing that the accuracy of [18F]flortaucipir PET in the discrimination between AD and other neurodegenerative disorders is lower at the prodromal stage of AD support this conclusion.[44] In this regard, the Aβ42/Aβ40 ratio is emerging as the best current candidate CSF biomarker for Aβ pathology since it minimizes biases linked to preanalytical and analytical factors, and the inter-individual variability of Aβ production.[18,25,26] In this context, our results do not propose a novel marker, but rather provide extensive neuropathological data, from a large patient cohort, in support of the added value of the Aβ42/Aβ40 ratio in the diagnostic AD assessment. The results obtained recommend the use of the Aβ42/Aβ40 ratio in the clinical evaluation of AD pathology using CSF biomarkers, especially in cases with an "indeterminate" CSF profile, characterized by normal t-tau and p-tau and low Aβ42 levels.

Limitations of the present study mainly concern the inclusion of cases with CJD and other causes of RPD such as encephalitis, in which previous studies documented a reduction of mean CSF Aβ42 levels in comparison to normal subjects.[45,46] However, in our cohort, we found

comparable levels of CSF Aβ42 among the non-AD diagnostic groups. Additionally, we obtained the same correlation between amyloid CSF biomarkers and Aβ score after accounting for the presence of prion disease, which indicates that the findings are not only related to a CJD-specific effect. Finally, the influence of copathologies on Aβ levels in the CSF has also a significant impact in clinical practice given that mixed brain pathologies frequently affect the elderly population.[47] While the unbalanced cohort, mainly represented by CJD cases, is a recognized limit of our study, the focus on CJD and other RPDs provided the unique opportunity to analyze CSF data on samples collected, on average, only a few months before death and in several brains with a degree of Aβ pathology corresponding to presymptomatic AD.

We choose not to include CSF t-tau and p-tau data in our analyses because of the high proportion of CJD cases in the cohort. Indeed, CSF t-tau and, at least in some subtypes, p-tau levels may increase in CJD independently from the coexisting AD pathology. Moreover, previous data from our group demonstrated that in typical sCJD (e.g., MM1 subtype), in which p-tau CSF levels are not significantly elevated, p-tau fails to consistently discriminate between patients with or without age- or AD-related neurofibrillary pathology when tau deposition is limited to the medial temporal lobe (Braak stages I–III), the stages most commonly associated with early asymptomatic AD.[13]

In conclusion, the present data provide strong support for the use of CSF Aβ42/Aβ40 ratio as a biomarker for Aβ-related pathology (A category in the A/T/N scheme) in clinical practice and its introduction in the research diagnostic criteria for AD.

Acknowledgments

The authors thank Sabrina Boninsegna B.Sc., Silvia Piras, B.Sc. and Benedetta Carlà M.Sc. for their valuable technical assistance. This work was supported by the Italian Ministry of Health (grant RF2011-02351092) and by the University of Bologna (grant RFO).

Author contributions

Conception and design of the study (SB, SAR, and PP), acquisition and analysis of the data (SB, SAR, MR, CZ, ABS, BP, SC, and PP), and drafting the manuscript and figures (SB, SAR, and PP). Study supervision (PP).

Conflict of Interests

Nothing to report.

References

1. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 2012;123:1–11.
2. Jack CR Jr, Wiste HJ, Vemuri P, et al. Brain beta-amyloid measures and magnetic resonance imaging atrophy both predict time-to-progression from mild cognitive impairment to Alzheimer's disease. Brain 2010;133:3336–3348.
3. Scheltens P, Blennow K, Breteler MM, et al. Alzheimer's disease. Lancet 2016;388:505–517.
4. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:280–292.
5. Dubois B, Feldman HH, Jacova C, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. Lancet Neurol 2014;13:614–629.
6. McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011;7:263–269.
7. Strozyk D, Blennow K, White LR, Launer LJ. CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. Neurology 2003;60:652–656.
8. Tapiola T, Alafuzoff I, Herukka SK, et al. Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. Arch Neurol 2009;66:382–389.
9. Lewczuk P, Beck G, Esselmann H, et al. Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides. Clin Chem 2006;52:332–334.
10. Bjerke M, Engelborghs S. Cerebrospinal fluid biomarkers for early and differential Alzheimer's disease diagnosis. J Alzheimers Dis 2018;62:1199–1209.
11. Hansson O, Mikulskis A, Fagan AM, et al. The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: a review. Alzheimers Dement 2018;14:1313–1333.
12. Blennow K, Mattsson N, Schöll M, et al. Amyloid biomarkers in Alzheimer's disease. Trends Pharmacol Sci 2015;36:297–309.
13. Lattanzio F, Abu-Rumeileh S, Franceschini A, et al. Prion-specific and surrogate CSF biomarkers in Creutzfeldt-Jakob disease: diagnostic accuracy in relation to molecular

subtypes and analysis of neuropathological correlates of p-tau and Aβ42 levels. Acta Neuropathol 2017;133:559–578.
14. Wiltfang J, Esselmann H, Smirnov A, et al. Beta-amyloid peptides in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Ann Neurol 2003;54:263–267.
15. Hansson O, Zetterberg H, Buchhave P, et al. Prediction of Alzheimer's disease using the CSF Abeta42/Abeta40 ratio in patients with mild cognitive impairment. Dement Geriatr Cogn Disord 2007;23:316–320.
16. Wiltfang J, Esselmann H, Bibl M, et al. Amyloid beta peptide ratio 42/40 but not A beta 42 correlates with phospho-Tau in patients with low- and high-CSF A beta 40 load. J Neurochem 2007;101:1053–1059.
17. Janelidze S, Zetterberg H, Mattsson N, et al. CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: better diagnostic markers of Alzheimer disease. Ann Clin Transl Neurol 2016;3:154–165.
18. Dumurgier J, Schraen S, Gabelle A, et al. Cerebrospinal fluid amyloid-β 42/40 ratio in clinical setting of memory centers: a multicentric study. Alzheimers Res Ther. 2015;7:30.
19. Olsson B, Lautner R, Andreasson U, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. Lancet Neurol 2016;15:673–684.
20. Lewczuk P, Esselmann H, Otto M, et al. Neurochemical diagnosis of Alzheimer's dementia by CSF Abeta42, Abeta42/Abeta40 ratio and total tau. Neurobiol Aging 2004;25:273–281.
21. Leuzy A, Chiotis K, Hasselbalch SG, et al. Pittsburgh compound B imaging and cerebrospinal fluid amyloid-beta in a multicentre European memory clinic study. Brain 2016;139:2540–2553.
22. Pannee J, Portelius E, Minthon L, et al. Reference measurement procedure for CSF amyloid beta (Aβ)(1-42) and the CSF Aβ(1-42)/Aβ(1-40) ratio - a cross-validation study against amyloid PET. J Neurochem 2016;139:651–658.
23. Lewczuk P, Matzen A, Blennow K, et al. Cerebrospinal fluid Aβ42/40 corresponds better than Aβ42 to amyloid PET in Alzheimer's disease. J Alzheimers Dis 2017;55:813–822.
24. Niemantsverdriet E, Ottoy J, Somers C, et al. The cerebrospinal fluid Aβ1-42/Aβ1-40 ratio improves concordance with amyloid-PET for diagnosing alzheimer's disease in a clinical setting. J Alzheimers Dis 2017;60:561–576.
25. Sauvée M, DidierLaurent G, Latarche C, et al. Additional use of $A\beta_{42}/A\beta_{40}$ ratio with cerebrospinal fluid biomarkers P-tau and $A\beta_{42}$ increases the level of evidence of Alzheimer's disease pathophysiological process in routine practice. J Alzheimers Dis 2014;41:377–386.
26. Dorey A, Perret-Liaudet A, Tholance Y, et al. Cerebrospinal fluid Aβ40 improves the interpretation of Aβ42 concentration for diagnosing Alzheimer's disease. Front Neurol 2015;6:247.
27. Parchi P, Strammiello R, Notari S, et al. Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrPSc types: an updated classification. Acta Neuropathol 2009;118:659–671.
28. Parchi P, de Boni L, Saverioni D, et al. Consensus classification of human prion disease histotypes allows reliable identification of molecular subtypes: an inter-rater study among surveillance centres in Europe and USA. Acta Neuropathol 2012;124:517–529.
29. Alafuzoff I, Arzberger T, Al-Sarraj S, et al. Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. Brain Pathol 2008;18:484–496.
30. Alafuzoff I, Thal DR, Arzberger T, et al. Assessment of beta-amyloid deposits in human brain: a study of the BrainNet Europe Consortium. Acta Neuropathol 2009;117:309–320.
31. Thal DR, Rüb U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology 2002;58:1791–1800.
32. Braak H, Alafuzoff I, Arzberger T, et al. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 2006;112:389–404.
33. Mirra SS, Heyman A, McKeel D, et al. Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479–486.
34. Raftery AE. Bayesian model selection in social research. Sociol Methodol 1995;25:111–163.
35. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–845.
36. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018;14:535–562.
37. Hsu D, Marshall GA. Primary and secondary prevention trials in Alzheimer disease: looking back moving forward. Curr Alzheimer Res 2017;14:426–440.
38. Toombs J, Foiani MS, Wellington H, et al. Amyloid β peptides are differentially vulnerable to preanalytical surface exposure, an effect incompletely mitigated by the use of ratios. Alzheimers Dement 2018;10:311–321.
39. Blennow K, Biscetti L, Eusebi P, Parnetti L. Cerebrospinal fluid biomarkers in Alzheimer's and Parkinson's diseases—From pathophysiology to clinical practice. Mov Disord 2016;31:836–847.
40. Mattsson N, Lönneborg A, Boccardi M, et al. Clinical validity of cerebrospinal fluid Aβ42, tau, and phospho-tau as biomarkers for Alzheimer's disease in the context of a

structured 5-phase development framework. Neurobiol Aging 2017;52:196–213.
41. Struyfs H, Van Broeck B, Timmers M, et al. Diagnostic accuracy of cerebrospinal fluid amyloid-β isoforms for early and differential dementia diagnosis. J Alzheimers Dis 2015;45:813–822.
42. Janelidze S, Pannee J, Mikulskis A, et al. Concordance between different amyloid immunoassays and visual amyloid positron emission tomographic assessment. JAMA Neurol 2017;74:1492–1501.
43. Jack CR Jr, Bennett DA, Blennow K, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. Neurology 2016;87:539–547.
44. Ossenkoppele R, Rabinovici GD, Smith R, et al. Discriminative accuracy of [18F]flortaucipir positron emission tomography for Alzheimer disease vs other neurodegenerative disorders. JAMA 2018;320:1151–1162.
45. Otto M, Esselmann H, Schulz-Shaeffer W, et al. Decreased beta-amyloid1-42 in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Neurology 2000;54:1099–1102.
46. Spitzer P, Lang R, Oberstein TJ, et al. A specific reduction in Aβ(1-42) vs. a universal loss of Aβ peptides in CSF differentiates Alzheimer's disease from meningitis and multiple sclerosis. Front Aging Neurosci 2018;10:152.
47. Kovacs GG, Alafuzoff I, Al-Sarraj S, et al. Mixed brain pathologies in dementia: the BrainNet Europe consortium experience. Dement Geriatr Cogn Disord 2008;26:343–350.

Supporting Information

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

Appendix S1. Methods: CSF analysis (analytical and pre-analytical details). Results: analysis of the effect of cerebral amyloid angiopathy and of preanalytical variables on CSF biomarker results. Figure 1: study flow chart. Figure 2: assessment of Aβ and p-tau pathology.

