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

Simone Baiardini1, Samir Abu-Rumeileh1, Marcello Rossi2, Corrado Zenesini2, Anna Bartoletti-Stella2, Barbara Polischi2, Sabina Capellari1,2 & Piero Parchi2,3

1Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna 40123, Italy
2IRCCS, Istituto delle Scienze Neurologiche di Bologna, Bologna 40139, Italy
3Department 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

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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) (R² = 0.506, β = −0.713, P < 0.001) than with ln(Aβ42) (R² = 0.206, β = −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 fibrillar 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 or neuropathological findings. 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 Aβ 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 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. 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.

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. 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 semi-quantitative 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 rpm for 10 min when showing even mild signs of blood contamination, divided into aliquots, 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 [(Aβ42)/(Aβ40)×10].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)14 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 test15 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/ε2: 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 phase30 – n (%)               | 0: 80 (37.9); 1–2: 56 (26.6); 3: 33 (15.6); 4–5: 42 (19.9) |
| Braak’s NF stage31 – 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 (%)                   | 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.
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.

|          | 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.

**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 \( \leq 495 \) 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.

![Thal Phase](image)

**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 |
| ApoE4                         | 6.667 (1.968 to 11.365) | 2.383 | 0.135 | 0.006 |
| Prion disease                 | 4.435 (1.000 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 |
| ApoE4                         | 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 (−17.961 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 |
| ApoE4                         | 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 |
| ApoE4                         | 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 ApoE4). 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.

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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 ($R^2 = 0.506$, $\beta = -0.713$, $P < 0.001$).
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 an 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 0.818 ± 0.028 vs. 0.643 ± 0.039).

Table 4. ROC analyses in the total cohort.

| Analysis                | AUC     | Cut-off | Sens (%) | Spec (%) | P value (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.

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, 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.

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. 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).

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, 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 Aβ producers. Moreover, pathological changes other than Aβ deposition such as synaptic loss might contribute to the reduction of Aβ levels, including Aβ42. 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. 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 and with an overall accuracy comparable to that of Aβ42/Aβ40 ratio. 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. 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, 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. 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. 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. 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. 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.

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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.

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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.