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The cerebrospinal fluid amyloid $\beta_{42/40}$ ratio in the differentiation of Alzheimer's disease from non-Alzheimer's dementia

P.E. Spies*^{1,4}, D. Slats^{1,4}, J.M.C. Sjögren^{1,4,5}, B.P.H. Kremer³, F.R.J. Verhey⁶, M.G.M. Olde Rikkert^{1,4}, M.M. Verbeek^{2,3,4}

Radboud University Nijmegen Medical Centre, Donders Institute for Brain, Cognition and Behaviour,

¹Department of Geriatric Medicine, ²Department of Laboratory Medicine, ³Department of Neurology,

⁴Alzheimer Centre, Nijmegen, The Netherlands

⁵Schering-Plough Research Institute, Oss, The Netherlands

⁶Alzheimer Centre, Maastricht, The Netherlands

Corresponding author:

P. Spies, Department of Geriatric Medicine, 925, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: +31 24 361 6772, fax: +31 24 361 7408, e-mail: p.spies@ger.umcn.nl

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Abstract

Background: Amyloid β_{40} ($A\beta_{40}$) is the most abundant $A\beta$ peptide in the brain. The cerebrospinal fluid (CSF) level of $A\beta_{40}$ might therefore be considered to most closely reflect the total $A\beta$ load in the brain. Both in Alzheimer's disease (AD) and in normal aging the $A\beta$ load in the brain has a large inter-individual variability. Relating $A\beta_{42}$ to $A\beta_{40}$ levels might consequently provide a more valid measure for reflecting the change in $A\beta$ metabolism in dementia patients than the CSF $A\beta_{42}$ concentrations alone. This measure may also improve differential diagnosis between AD and other dementia syndromes, such as vascular dementia (VaD), dementia with Lewy bodies (DLB), and frontotemporal dementia (FTD).

Objective: To investigate the diagnostic value of the CSF $A\beta_{42}/A\beta_{40}$ ratio in differentiating AD from controls, VaD, DLB and FTD.

Methods: We analysed the CSF $A\beta_{42}/A\beta_{40}$ ratio, phosphorylated tau₁₈₁ and total tau in 69 patients with AD, 26 patients with VaD, 16 patients with DLB, 27 patients with FTD, and 47 controls.

Results: Mean $A\beta_{40}$ levels were 2850 pg/ml in VaD and 2830 pg/ml in DLB patients, both significantly lower than in AD patients (3698 pg/ml; $p < 0.01$). $A\beta_{40}$ levels in AD patients were not significantly different from those in controls (4035 pg/ml; $p = 0.384$). The $A\beta_{42}/A\beta_{40}$ ratio was significantly lower in AD patients than in all other groups ($p < 0.001$, ANCOVA). Differentiating AD from VaD, DLB and non-AD dementia improved when the $A\beta_{42}/A\beta_{40}$ ratio was used instead of $A\beta_{42}$ concentrations alone ($p < 0.01$). The $A\beta_{42}/A\beta_{40}$ ratio performed equally well as the combination of $A\beta_{42}$, phosphorylated tau₁₈₁ and total tau in differentiating AD from FTD and non-AD dementia. The diagnostic performance of the latter combination was not improved when the $A\beta_{42}/A\beta_{40}$ ratio was used instead of $A\beta_{42}$ alone.

Conclusion: The CSF $A\beta_{42}/A\beta_{40}$ ratio improves differentiation of AD patients from VaD, DLB and non-AD dementia patients, when compared to $A\beta_{42}$ alone, and is a more easily interpretable alternative to the combination of $A\beta_{42}$, p-tau and t-tau when differentiating AD from either FTD or non- AD dementia.

Key words: Abeta40 protein, $A\beta_{42}/A\beta_{40}$ ratio, Alzheimer's disease, vascular dementia, dementia with Lewy bodies.

Introduction

While making an accurate diagnosis is important for management of dementia, differentiating between Alzheimer's disease (AD) and other types of dementia using clinical criteria is the true clinical challenge: difficult and prone to inaccuracy. The NINCDS-ADRDA criteria for AD[1] have been validated against neuropathological standards and turned out to have a specificity of 23-69% for differentiating AD from other dementias.[2, 3]

Analysis of cerebrospinal fluid (CSF) has been used increasingly to differentiate the various dementia disorders.[4] Although CSF analysis of amyloid β_{42} ($A\beta_{42}$), phosphorylated tau₁₈₁ (p-tau₁₈₁) and total tau (t-tau) is efficacious in distinguishing between AD dementia and non-demented controls, it is inaccurate in separating AD from other types of dementia.[5-7] CSF biomarker profiles of AD, vascular dementia (VaD), frontotemporal dementia (FTD), and dementia with Lewy bodies (DLB) overlap, which makes discrimination between these various dementias based on CSF analysis alone difficult.

It has been suggested that relating $A\beta_{42}$ to the concentration of $A\beta_{40}$ may improve diagnostic accuracy of CSF analysis.[8] $A\beta_{40}$ is the most abundant $A\beta$ peptide in human CSF, whereas $A\beta_{42}$ accounts for only 10% of the total $A\beta$ population.[6, 9-11] As such, CSF $A\beta_{40}$ concentrations could be considered as the closest possible reflection of the total $A\beta$ load in the brain. This total CSF $A\beta$ load shows a large inter-individual variability.[12] Patients with a low total $A\beta$ load and therefore a low CSF $A\beta_{42}$ concentration [11], might receive an inappropriate diagnosis of AD if only CSF $A\beta_{42}$ is considered and an absolute cut-off value is used. Similarly, in patients with AD but with a high total $A\beta$ load, CSF $A\beta_{42}$ concentrations may not decrease below the cut-off value and in those cases rejection of the diagnosis AD might be incorrect. Since the total $A\beta$ concentration was found not to change in various dementia disorders,[8, 10, 13] and $A\beta_{40}$ concentrations were shown to be similar in groups of AD patients, controls and non-AD dementia patients,[13-17] hypothetically the ratio of $A\beta_{42}$ to $A\beta_{40}$ reflects the $A\beta$ changes in the brain more accurately than $A\beta_{42}$ alone.

It was found in several studies that differentiation between AD and healthy controls, subjects with non-AD dementia, or subjects with other neurological disorders, improved when using the CSF $A\beta_{42}/A\beta_{40}$ ratio compared to $A\beta_{42}$ alone,[15, 17, 18] although these results were not confirmed in other studies.[19, 20] The $A\beta_{42}/A\beta_{40}$ ratio has previously been studied in patients with FTD and AD, and a significantly decreased $A\beta_{40}$ was found in FTD patients, resulting in an increased $A\beta_{42}/A\beta_{40}$ ratio compared to AD patients.[21] In a recent review it was concluded that levels of CSF $A\beta_{40}$ or $A\beta_{42}$ cannot usefully discriminate between AD and DLB,[22] although one study found that the $A\beta_{42}/A\beta_{40}$ ratio improved diagnostic accuracy of AD versus DLB relative to $A\beta_{42}$ alone.[23] To our knowledge, the $A\beta_{42}/A\beta_{40}$ ratio has not been investigated in relation to VaD.

We hypothesized that differentiation between dementia syndromes would improve by using the CSF $A\beta_{42}/A\beta_{40}$ ratio since it may eliminate inter-individual differences in total $A\beta$ load. Therefore, we explored the CSF $A\beta_{40}$ concentration and $A\beta_{42}/A\beta_{40}$ ratio in patients with AD, VaD, DLB, FTD and in controls, and we investigated the diagnostic value of the $A\beta_{42}/A\beta_{40}$ ratio in differentiating AD from each of these groups individually as well as from the total group of non-AD dementia patients.

Materials and methods

Patients

Sixty-nine AD, 26 VaD, 27 FTD, and 16 DLB patients were included in this study that was based on the CSF database of the Alzheimer Centre of the Radboud University Nijmegen Medical Centre. This database contains clinical data as well as biobanked CSF and serum of consecutive patients. All patients or their legal representative had given informed consent for lumbar puncture. We included all patients with a clinically clear-cut dementia diagnosis, whose CSF was available for A β ₄₀ and A β ₄₂ analysis. Data on t-tau and p-tau₁₈₁ were also obtained. A diagnosis was made by a multidisciplinary team, which consisted of a geriatrician, neurologist, neuropsychologist, and – if needed – an old-age psychiatrist. The panel used the accepted clinical diagnostic criteria, i.e. the NINCDS-ADRDA criteria for probable AD, the NINDS-AIREN criteria for probable VaD, the 1998 consensus on clinical diagnostic criteria for FTD and the 1996 consensus guidelines for the clinical and pathologic diagnosis of DLB.[1, 24-26] In less than 10% of the cases, the clinicians were aware of the CSF results for A β ₄₂, p-tau₁₈₁ and t-tau when establishing the clinical diagnosis, but they were never aware of A β ₄₀ levels.

The control group consisted of 47 non-demented patients who underwent lumbar puncture for various complaints. Most frequently diagnosed were headache (n=10), polyneuropathy (n=5), radiculopathy (n=4), vertigo (n=3), and delirium (n=2). The remaining controls had a variety of complaints but turned out not to have central neurological problems. CSF cell count, glucose, lactate, haemoglobin, bilirubin, total protein and oligoclonal IgG bands were normal in all controls. Patient characteristics are listed in table 1.

CSF

CSF was collected by lumbar puncture in polypropylene tubes, transported within 30 minutes to the adjacent laboratory at room temperature, centrifuged after routine investigations, and immediately aliquoted and stored at –80 °C until analysis. Levels of A β ₄₂, t-tau, and p-tau₁₈₁ in CSF were measured using enzyme linked immunosorbent assays (Innogenetics NV, Gent, Belgium). Inter-assay

coefficients of variation for these assays were 3.8 – 8.4%.[27] CSF A β ₄₀ was analysed by using a commercial assay based on the Luminex technology (BioSource, Invitrogen Ltd, Paisley, UK). Inter-assay coefficient of variation for A β ₄₀ was 5.4 % (at 2875 pg/ml; n=32). The lower limit of detection was for A β ₄₂ 50 pg/ml and for A β ₄₀ 22 pg/ml.

Statistical analysis

Since p-tau₁₈₁, t-tau, A β ₄₂, A β ₄₀, and the A β ₄₂/A β ₄₀ ratio were not all normally distributed, they were log-transformed. Differences in p-tau₁₈₁, t-tau, A β ₄₂, A β ₄₀ and the A β ₄₂/A β ₄₀ ratio were analysed using 2-way ANCOVA. Since sex and age did not contribute to the differences in A β ₄₂, A β ₄₀ and the A β ₄₂/A β ₄₀ ratio between dementia groups and controls, both (sex and age) were removed from the model and the ANCOVA was thereafter rerun. Differences between AD and the other groups were further explored using Dunnett's post-hoc test.

We used logistic regression analysis to assess the value of the A β ₄₂/A β ₄₀ ratio compared to other combinations of CSF biomarkers in differentiating AD from controls, from VaD, FTD, DLB, respectively, and from these three groups combined ('non-AD dementia'). Sex and age contributed significantly to the logistic regression models and therefore the models were corrected for these factors. Receiver operating characteristic (ROC) curves were calculated and the areas under the ROC curves were compared using MedCalc (Mariakerke, Belgium). Sensitivity and specificity were determined based on the highest Youden index, i.e. the point on the ROC curve at which sensitivity + specificity – 1 is maximized. All other statistical analyses were carried out using SPSS, version 16.0.

Results

The mean level of CSF $A\beta_{40}$ was significantly lower in VaD patients than in AD patients ($p < 0.001$), as well as in DLB patients compared to AD patients ($p = 0.008$) (see Table 1 and Fig. (1)). There was no significant difference in $A\beta_{40}$ levels between AD patients and FTD patients ($p = 0.981$) or controls ($p = 0.384$). In AD patients, the concentration of CSF $A\beta_{42}$ was significantly lower than in VaD and FTD patients and controls ($p < 0.001$), whereas the concentration of p-tau₁₈₁ and t-tau was significantly higher than in all other groups ($p < 0.001$). The $A\beta_{42}/A\beta_{40}$ ratio was significantly lower in AD patients than in all other groups ($p < 0.001$) (see Table 1 and Fig. (1)).

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We investigated the diagnostic value of the $A\beta_{42}/A\beta_{40}$ ratio in several comparisons. First, we investigated if the use of the $A\beta_{42}/A\beta_{40}$ ratio would be superior to the use of $A\beta_{42}$ alone.

Differentiation of AD from either VaD, DLB or non-AD dementia significantly improved when using the $A\beta_{42}/A\beta_{40}$ ratio ($p < 0.01$). In contrast, differentiation of AD from either FTD or controls did not improve ($p > 0.05$). Second, we analysed the diagnostic value of the $A\beta_{42}/A\beta_{40}$ ratio compared to the combination of $A\beta_{42}/A\beta_{40}$ ratio p-tau₁₈₁ and t-tau, which is a frequently used combination of CSF biomarkers in AD research. Differentiation of AD from either FTD or non-AD dementia was equally good for the $A\beta_{42}/A\beta_{40}$ ratio or the combination of $A\beta_{42}$, p-tau₁₈₁ and t-tau ($p > 0.05$). Differentiation of AD from either controls, VaD or DLB was better using the combination of $A\beta_{42}$, p-tau₁₈₁ and t-tau than using the $A\beta_{42}/A\beta_{40}$ ratio ($p < 0.05$). Third, we analysed if replacement of $A\beta_{42}$ by the $A\beta_{42}/A\beta_{40}$ ratio in the combination with p-tau₁₈₁ and t-tau would improve results. Differentiation of AD from both controls and from all dementia groups was equally good for both combinations ($p > 0.05$). ROC curves are shown in Fig. (2) and Fig. (3). Sensitivity and specificity, AUC and likelihood ratios of each parameter are shown in Table 2.

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Discussion

We explored the CSF $A\beta_{40}$ concentrations in patients with different types of dementia and in controls, and examined the value the $A\beta_{42}/A\beta_{40}$ ratio in differentiating AD from controls, from FTD, DLB, VaD, and from these latter three groups combined. The observed patterns of $A\beta_{40}$ and $A\beta_{42}$ in the various dementia groups are remarkable. As expected, the AD group was characterized by normal $A\beta_{40}$ and low $A\beta_{42}$ levels, but contrary to our expectations, the VaD group had low $A\beta_{40}$ and intermediate $A\beta_{42}$ levels, whereas the DLB group was characterized by low concentrations of both $A\beta_{40}$ and $A\beta_{42}$ levels. The FTD group had normal $A\beta_{40}$ and $A\beta_{42}$ levels.

Only one study has reported the CSF $A\beta_{40}$ concentration in patients with VaD. In accordance with our results, a lower $A\beta_{40}$ concentration in VaD patients compared to AD patients was found.[28] Multiple explanations for this low $A\beta_{40}$ concentration are possible. For example, it may be caused by the ischemic damage that has occurred in VaD patients, since cerebral ischemia has been shown to result in plaque formation and accumulation of $A\beta_{40}$ and $A\beta_{42}$ [29-31] and, consequently, leads to a reduced CSF concentration. In addition, atherosclerosis, one of the risk factors for VaD, may inhibit the clearance of $A\beta_{40}$ across the blood-brain barrier, leading to vascular deposits of $A\beta_{40}$. [32]

Our finding of a lower $A\beta_{40}$ concentration in DLB patients compared to AD is in accordance with the literature.[12] Possibly, the intraneuronal α -synuclein aggregates that are found in DLB affect $A\beta$ metabolism, leading to an overall decrease in $A\beta$ synthesis. Results from in vitro research indicate an interaction between α -synuclein and $A\beta$. [33, 34] Neuropathological studies found a positive correlation between $A\beta$ plaque burden and α -synuclein load. [35, 36] $A\beta_{40}$ plaque level was greater in cases with α -synuclein aggregates compared to cases lacking α -synuclein aggregates, while no difference was found in $A\beta_{42}$ plaque burden. [35] A greater $A\beta_{40}$ plaque burden in DLB might explain the decreased $A\beta_{40}$ concentration in CSF that we found.

The few studies that measured $A\beta_{40}$ concentration in FTD patients report varying results. Our results are supported by previous findings of similar concentrations of $A\beta_{40}$ in FTD patients and AD patients. [37] In contrast with this, another group reported lower $A\beta_{40}$ concentrations in FTD patients

compared to AD patients.[21] The varying results might be explained by the inclusion of heterogeneous FTD patient groups in research, comprising patients with clinically diagnosed FTD, semantic dementia and primary progressive aphasia, presumably with different neuropathological substrates.

We investigated the $A\beta_{42}/A\beta_{40}$ ratio under the assumption that $A\beta_{40}$ closely represents the total cerebral $A\beta$ load. Since we found different $A\beta_{40}$ concentrations in the various dementia groups, it can be debated if the $A\beta_{42}/A\beta_{40}$ ratio is a good representation of the $A\beta_{42}$ fraction of the total $A\beta$ load and thus eliminates inter-individual differences in total $A\beta$ concentrations. Nevertheless, differentiating AD from VaD, DLB and non-AD dementia improved when we used this ratio compared to $A\beta_{42}$ alone, probably due to the differences in $A\beta_{40}$ concentrations. Results obtained by applying the $A\beta_{42}/A\beta_{40}$ ratio fulfilled the criteria for biomarkers as established by the Working Group on molecular and biochemical markers of Alzheimer's disease[38] in differentiating AD from VaD, FTD, DLB, and non-AD, since both sensitivity and specificity were >80%.

The $A\beta_{42}/A\beta_{40}$ ratio seems to be a good alternative for the combination of $A\beta_{42}$, p-tau₁₈₁ and t-tau, to distinguish AD from either FTD or non-AD dementia. The $A\beta_{42}/A\beta_{40}$ ratio has the advantage that only two analyses are needed instead of three, and, consequently, the results are easier to interpret. The differentiation of AD from non-AD dementia is a frequently encountered problem in clinical practice, when dementia can be established, but the differential diagnosis encompasses AD and another type of dementia. Thus, the use of the CSF $A\beta_{42}/A\beta_{40}$ ratio may help in this clinical decision making. However, the use of four biomarkers, i.e. the $A\beta_{42}/A\beta_{40}$ ratio combined with p-tau₁₈₁ and t-tau, did not improve differentiation when compared to the combination of the three currently used biomarkers $A\beta_{42}$, p-tau₁₈₁ and t-tau.

Some bias may have occurred in our analysis since in less than 10% of the patients the results of the CSF analysis for $A\beta_{42}$, t-tau and p-tau₁₈₁ were known to the clinicians. However, given the small fraction of patients this applies to, we believe that this did not affect our results, also since our sensitivity and specificity results for discriminating AD from other dementias using $A\beta_{42}$ are comparable with the literature.[18, 39, 40] The number of patients in our non-AD groups were moderate, yet not very different from the numbers mentioned in other studies.[12, 21, 28, 37] Besides,

the significant results that we found had a p-value <0.01 , suggesting that these results are not expected to change with an increasing number of patients.

In summary, the $A\beta_{42}/A\beta_{40}$ ratio is more accurate for differentiating AD from other types of dementia than $A\beta_{42}$ alone, and can be a good and easier interpretable alternative for the combination of the established biomarkers $A\beta_{42}$, p-tau₁₈₁ and t-tau for the differentiation of AD from non-AD dementia, which may help to accept or refute the clinical diagnosis of AD.

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Table 1 Clinical characteristics and results of CSF analysis

	controls	AD	VaD	FTD	DLB
males/females (n)	23/24	34/35	17/9	19/8	12/4
age (y)	61 ± 8	69 ± 8	72 ± 9	65 ± 7	76 ± 8
disease duration (mo)	n.a.	29 ± 23	35 ± 29	34 ± 21	34 ± 27
		n=60	n=20	n=26	n=8
t-tau (pg/ml)*	221 ± 80	710 ± 390	391 ± 526	448 ± 315	257 ± 98
p-tau ₁₈₁ (pg/ml)*	49 ± 16	111 ± 52	46 ± 16	70 ± 36	53 ± 17
Aβ ₄₂ (pg/ml)*	840 ± 260	428 ± 127	631 ± 270	786 ± 229	499 ± 179
Aβ ₄₀ (pg/ml)*	4035 ± 1177	3698 ± 1096	2850 ± 1031	3590 ± 1038	2830 ± 697
Aβ ₄₂ /Aβ ₄₀ ratio*	0.21 ± 0.04	0.12 ± 0.04	0.23 ± 0.08	0.23 ± 0.06	0.18 ± 0.06

Values are expressed as means ± SD.

*, p-value <0.001 by ANOVA.

AD, Alzheimer disease; VaD, vascular dementia; FTD, frontotemporal dementia; DLB, dementia with Lewy bodies; t-tau, total tau; p-tau₁₈₁, phosphorylated tau₁₈₁; n.a., not applicable

Table 2 Sensitivities and specificities, AUC and likelihood ratios for discriminating Alzheimer’s disease from controls and from other types of dementia, using different combinations of CSF biomarkers

	AD vs controls			AD vs VaD			AD vs FTD			AD vs DLB			AD vs non-AD		
	sens. spec.	AUC	LR	sens. spec.	AUC	LR	sens. spec.	AUC	LR	sens. spec.	AUC	LR	sens. spec.	AUC	LR
Aβ₄₂	93% 87%	0.949	7.3	83% 69%	0.785	2.7	94% 85%	0.941	6.4	65% 75%	0.755	2.6	83% 74%	0.811	3.2
Aβ₄₂/Aβ₄₀ ratio	93% 87%	0.947	7.3	93% 84%	0.900*	5.8	99% 83%	0.936	5.9	85% 85%	0.893*	5.5	90% 82%	0.903*	5.1
Aβ₄₂, p-tau, t-tau	97% 98%	0.994 [†]	45.6	93% 96%	0.963 [†]	23.1	93% 88%	0.953	7.4	90% 100%	0.966 [†]	n.c.	93% 84%	0.924	5.7
Aβ₄₂/Aβ₄₀ ratio, p-tau, t-tau	93% 98%	0.980	43.5	91% 96%	0.957	22.8	99% 79%	0.936	4.7	90% 100%	0.970	n.c.	87% 90%	0.923	9.0

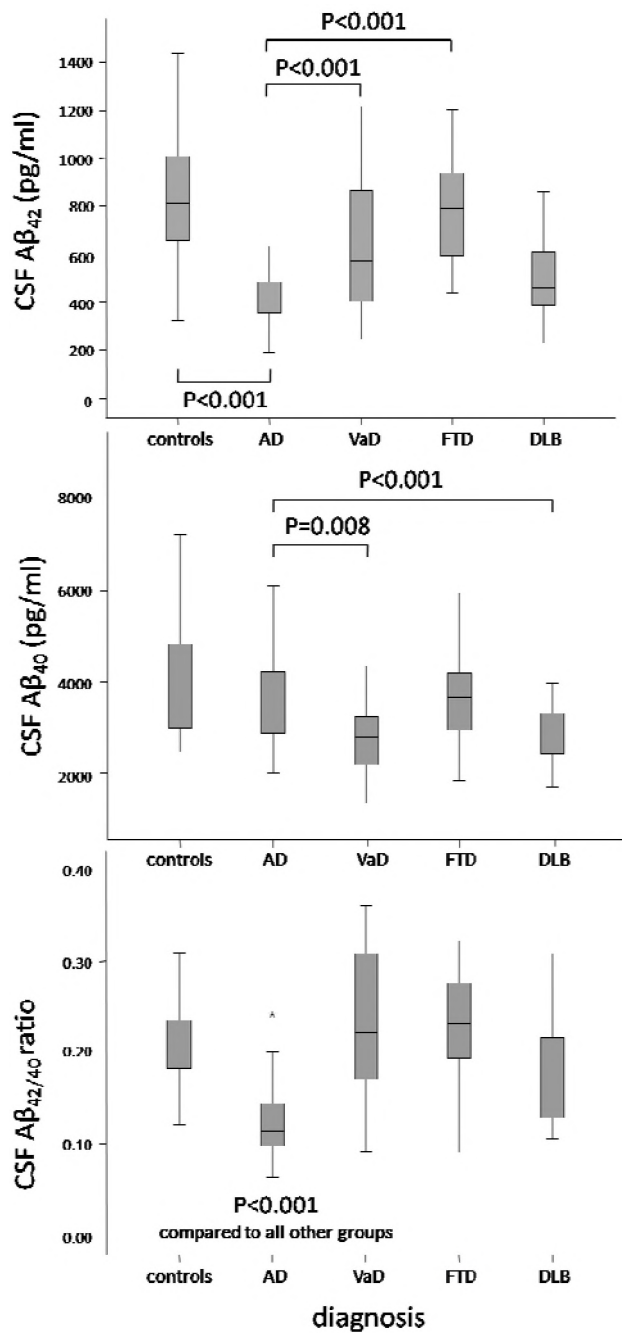
AD, Alzheimer disease; VaD, vascular dementia; FTD, frontotemporal dementia; DLB, dementia with Lewy bodies; non-AD, combined group of patients with VaD, FTD or DLB.

Sens., sensitivity; spec., specificity; AUC, area under the ROC curve; LR, likelihood ratio; n.c., not computable.

* p<0.01 compared to AUC for A β ₄₂ level

† p<0.05 compared to AUC for A β ₄₂/A β ₄₀ ratio

Figure 1 Cerebrospinal fluid A β_{42} and A β_{40} concentrations and A β_{42} /A β_{40} ratio in controls and patients with various types of dementia



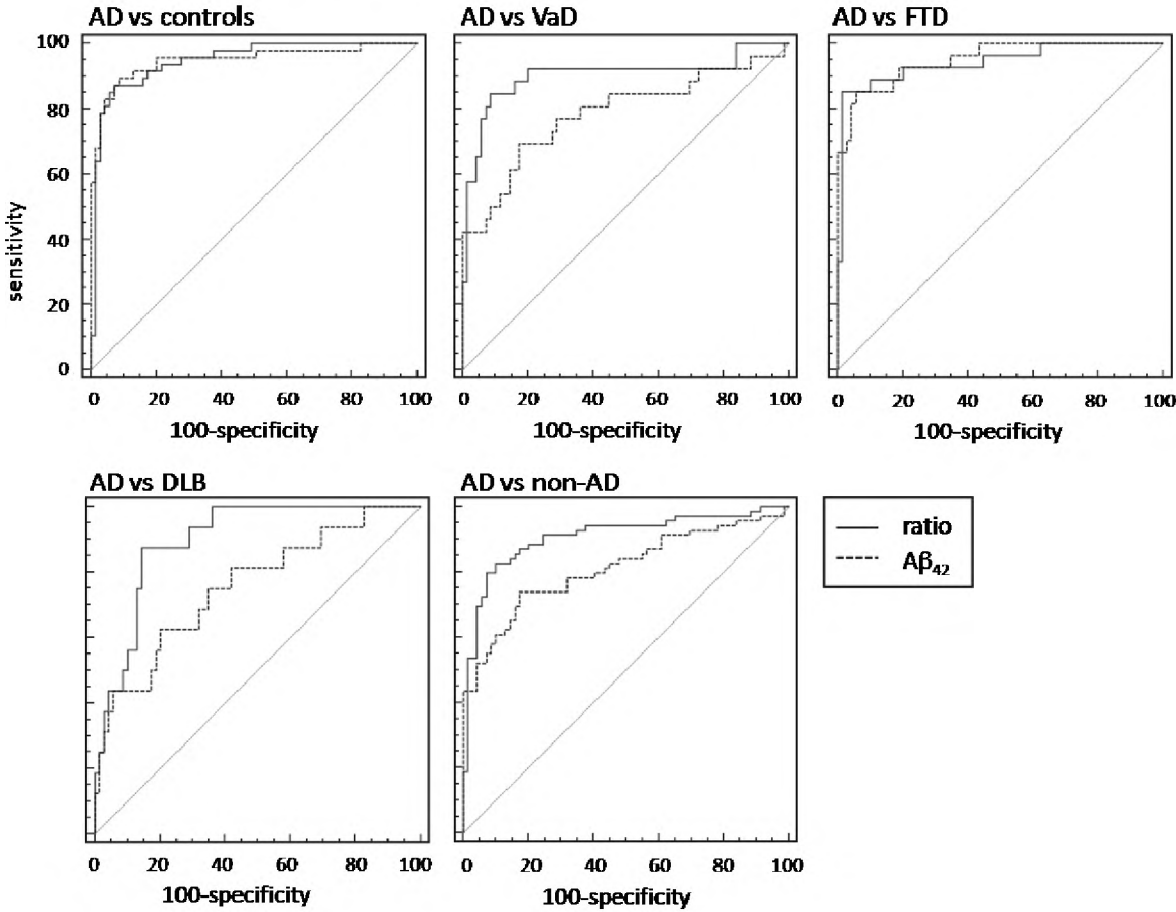
See text for statistical analyses.

Bold horizontal bar represents the median. Central box: 25th to 75th percentile (interquartile range).

Vertical bar bordered by highest and lowest value within 1.5 * interquartile range.

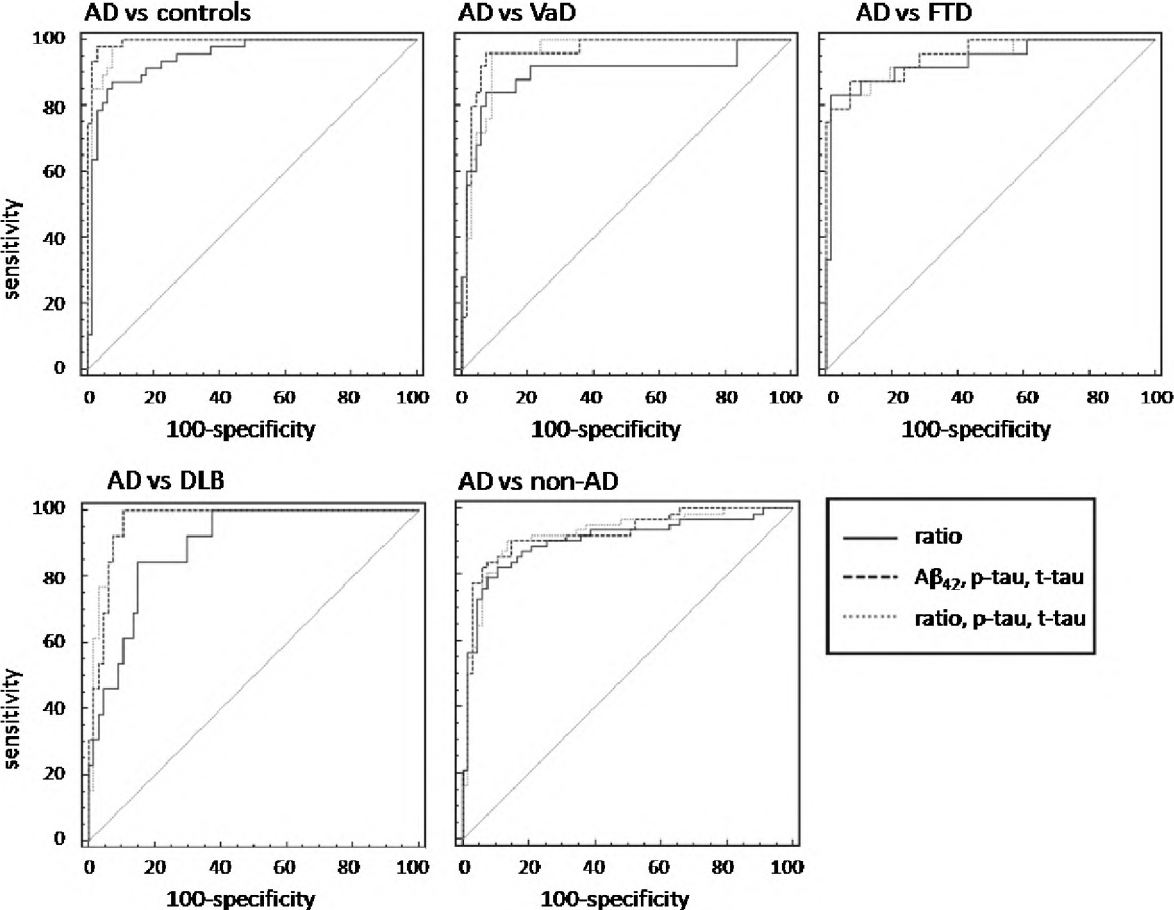
AD, Alzheimer disease; VaD, vascular dementia; FTD, frontotemporal dementia; DLB, dementia with Lewy bodies

Figure 2 Receiver Operating Characteristic curves comparing $A\beta_{42}$, and the $A\beta_{42}/A\beta_{40}$ ratio in AD versus controls and versus various types of dementia



AD, Alzheimer disease; VaD, vascular dementia; FTD, frontotemporal dementia; DLB, dementia with Lewy bodies.

Figure 3 Receiver Operating Characteristic curves comparing the $A\beta_{42}/A\beta_{40}$ ratio, the $A\beta_{42}/A\beta_{40}$ ratio combined with p-tau₁₈₁ and t-tau, and the combination of $A\beta_{42}$, p-tau₁₈₁ and t-tau in AD versus controls and versus various types of dementia



AD, Alzheimer disease; VaD, vascular dementia; FTD, frontotemporal dementia; DLB, dementia with Lewy bodies.