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

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Word count: 4371
Abstract

Background: Amyloid βp40 (Aβp40) is the most abundant Aβ peptide in the brain. The cerebrospinal fluid (CSF) level of Aβp40 might therefore be considered to most closely reflect the total Aβ load in the brain. Both in Alzheimer’s disease (AD) and in normal aging the Aβ load in the brain has a large inter-individual variability. Relating Aβ42 to Aβ40 levels might consequently provide a more valid measure for reflecting the change in Aβ metabolism in dementia patients than the CSF Aβ42 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 Aβ42/Aβ40 ratio in differentiating AD from controls, VaD, DLB and FTD.

Methods: We analysed the CSF Aβ42/Aβ40 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 Aβ40 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). Aβ40 levels in AD patients were not significantly different from those in controls (4035 pg/ml; p=0.384). The Aβ42/Aβ40 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 Aβ42/Aβ40 ratio was used instead of Aβ42 concentrations alone (p<0.01) The Aβ42/Aβ40 ratio performed equally well as the combination of Aβ42, 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 Aβ42/Aβ40 ratio was used instead of Aβ42 alone.
Conclusion: The CSF $\text{A} \beta_{42}/\text{A} \beta_{40}$ ratio improves differentiation of AD patients from VaD, DLB and non-AD dementia patients, when compared to $\text{A} \beta_{42}$ alone, and is a more easily interpretable alternative to the combination of $\text{A} \beta_{42}$, p-tau and t-tau when differentiating AD from either FTD or non-AD dementia.

Key words: Abeta40 protein, $\text{A} \beta_{42}/\text{A} \beta_{40}$ 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 ß42 (Aß42), phosphorylated tau[18] (p-tau[18]) 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 Aß42 to the concentration of Aß40 may improve diagnostic accuracy of CSF analysis.[8] Aß40 is the most abundant Aß peptide in human CSF, whereas Aß42 accounts for only 10% of the total Aß population.[6, 9-11] As such, CSF Aß40 concentrations could be considered as the closest possible reflection of the total Aß load in the brain. This total CSF Aß load shows a large inter-individual variability.[12] Patients with a low total Aß load and therefore a low CSF Aß42 concentration[11], might receive an inappropriate diagnosis of AD if only CSF Aß42 is considered and an absolute cut-off value is used. Similarly, in patients with AD but with a high total Aß load, CSF Aß42 concentrations may not decrease below the cut-off value and in those cases rejection of the diagnosis AD might be incorrect. Since the total Aß concentration was found not to change in various dementia disorders,[8, 10, 13] and Aß40 concentrations were shown to be similar in groups of AD patients, controls and non-AD dementia patients,[13-17] hypothetically the ratio of Aß42 to Aß40 reflects the Aß changes in the brain more accurately than Aß42 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 Aβ42/Aβ40 ratio compared to Aβ42 alone,[15, 17, 18] although these results were not confirmed in other studies.[19, 20] The Aβ42/Aβ40 ratio has previously been studied in patients with FTD and AD, and a significantly decreased Aβ40 was found in FTD patients, resulting in an increased Aβ42/Aβ40 ratio compared to AD patients.[21] In a recent review it was concluded that levels of CSF Aβ40 or Aβ42 cannot usefully discriminate between AD and DLB,[22] although one study found that the Aβ42/Aβ40 ratio improved diagnostic accuracy of AD versus DLB relative to Aβ42 alone.[23] To our knowledge, the Aβ42/Aβ40 ratio has not been investigated in relation to VaD.

We hypothesized that differentiation between dementia syndromes would improve by using the CSF Aβ42/Aβ40 ratio since it may eliminate inter-individual differences in total Aβ load. Therefore, we explored the CSF Aβ40 concentration and Aβ42/Aβ40 ratio in patients with AD, VaD, DLB, FTD and in controls, and we investigated the diagnostic value of the Aβ42/Aβ40 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 $\text{A}_{40}$ and $\text{A}_{42}$ analysis. Data on t-tau and p-tau$_{181}$ 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 $\text{A}_{42}$, p-tau$_{181}$ and t-tau when establishing the clinical diagnosis, but they were never aware of $\text{A}_{20}$ 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 $-80 \, ^\circ\mathrm{C}$ until analysis. Levels of $\text{A}_{42}$, t-tau, and p-tau$_{181}$ 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%. CSF Aβ40 was analysed by using a commercial assay based on the Luminex technology (BioSource, Invitrogen Ltd, Paisley, UK). Inter-assay coefficient of variation for Aβ40 was 5.4 % (at 2875 pg/ml; n=32). The lower limit of detection was for Aβ42 50 pg/ml and for Aβ40 22 pg/ml.

Statistical analysis
Since p-tau181, t-tau, Aβ42, Aβ40, and the Aβ42/Aβ40 ratio were not all normally distributed, they were log-transformed. Differences in p-tau181, t-tau, Aβ42, Aβ40 and the Aβ42/Aβ40 ratio were analysed using 2-way ANCOVA. Since sex and age did not contribute to the differences in Aβ42, Aβ40 and the Aβ42/Aβ40 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 Aβ42/Aβ40 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 Aβ_{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 Aβ_{40} levels between AD patients and FTD patients (p=0.981) or controls (p=0.384). In AD patients, the concentration of CSF Aβ_{42} was significantly lower than in VaD and FTD patients and controls (p<0.001), whereas the concentration of p-tau_{181} and t-tau was significantly higher than in all other groups (p<0.001). The Aβ_{42}/Aβ_{40} ratio was significantly lower in AD patients than in all other groups (p<0.001) (see Table 1 and Fig. (1)).

We investigated the diagnostic value of the Aβ_{42}/Aβ_{40} ratio in several comparisons. First, we investigated if the use of the Aβ_{42}/Aβ_{40} ratio would be superior to the use of Aβ_{42} alone. Differentiation of AD from either VaD, DLB or non-AD dementia significantly improved when using the Aβ_{42}/Aβ_{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 Aβ_{42}/Aβ_{40} ratio compared to the combination of Aβ_{42}/Aβ_{40} ratio p-tau_{181} 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 Aβ_{42}/Aβ_{40} ratio or the combination of Aβ_{42}, p-tau_{181} and t-tau (p>0.05). Differentiation of AD from either controls, VaD or DLB was better using the combination of Aβ_{42}, p-tau_{181} and t-tau than using the Aβ_{42}/Aβ_{40} ratio (p<0.05). Third, we analysed if replacement of Aβ_{42} by the Aβ_{42}/Aβ_{40} ratio in the combination with p-tau_{181} 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.
Discussion

We explored the CSF Aβ40 concentrations in patients with different types of dementia and in controls, and examined the value the Aβ42/Aβ40 ratio in differentiating AD from controls, from FTD, DLB, VaD, and from these latter three groups combined. The observed patterns of Aβ40 and Aβ42 in the various dementia groups are remarkable. As expected, the AD group was characterized by normal Aβ40 and low Aβ42 levels, but contrary to our expectations, the VaD group had low Aβ40 and intermediate Aβ42 levels, whereas the DLB group was characterized by low concentrations of both Aβ40 and Aβ42 levels. The FTD group had normal Aβ40 and Aβ42 levels.

Only one study has reported the CSF Aβ40 concentration in patients with VaD. In accordance with our results, a lower Aβ40 concentration in VaD patients compared to AD patients was found.[28] Multiple explanations for this low Aβ40 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 Aβ40 and Aβ42 [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 Aβ40 across the blood-brain barrier, leading to vascular deposits of Aβ40.[32]

Our finding of a lower Aβ40 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 Aβ metabolism, leading to an overall decrease in Aβ synthesis. Results from in vitro research indicate an interaction between α-synuclein and Aβ.[33, 34] Neuropathological studies found a positive correlation between Aβ plaque burden and α-synuclein load.[35, 36] Aβ40 plaque level was greater in cases with α-synuclein aggregates compared to cases lacking α-synuclein aggregates, while no difference was found in Aβ42 plaque burden.[35] A greater Aβ40 plaque burden in DLB might explain the decreased Aβ40 concentration in CSF that we found.

The few studies that measured Aβ40 concentration in FTD patients report varying results. Our results are supported by previous findings of similar concentrations of Aβ40 in FTD patients and AD patients.[37] In contrast with this, another group reported lower Aβ40 concentrations in FTD patients.
compared to AD patients. 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 Ap42/Ap40 ratio under the assumption that Ap40 closely represents the total cerebral Aβ load. Since we found different Ap40 concentrations in the various dementia groups, it can be debated if the Ap42/Ap40 ratio is a good representation of the Ap42 fraction of the total Aβ load and thus eliminates inter-individual differences in total Aβ concentrations. Nevertheless, differentiating AD from VaD, DLB and non-AD dementia improved when we used this ratio compared to Ap42 alone, probably due to the differences in Ap40 concentrations. Results obtained by applying the Ap42/Ap40 ratio fulfilled the criteria for biomarkers as established by the Working Group on molecular and biochemical markers of Alzheimer’s disease in differentiating AD from VaD, FTD, DLB, and non-AD, since both sensitivity and specificity were >80%.

The Ap42/Ap40 ratio seems to be a good alternative for the combination of Ap42, p-tau181 and t-tau, to distinguish AD from either FTD or non-AD dementia. The Ap42/Ap40 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 Ap42/Ap40 ratio may help in this clinical decision making. However, the use of four biomarkers, i.e. the Ap42/Ap40 ratio combined with p-tau181 and t-tau, did not improve differentiation when compared to the combination of the three currently used biomarkers Ap42, 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 Ap42, 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 Ap42 are comparable with the literature. The number of patients in our non-AD groups were moderate, yet not very different from the numbers mentioned in other studies.
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 $\text{A}β_{42}/\text{A}β_{40}$ ratio is more accurate for differentiating AD from other types of dementia than $\text{A}β_{42}$ alone, and can be a good and easier interpretable alternative for the combination of the established biomarkers $\text{A}β_{42}$, $p$-tau$_{181}$ and t-tau for the differentiation of AD from non-AD dementia, which may help to accept or refute the clinical diagnosis of AD.
Acknowledgements

The authors wish to thank the technicians of the Department of Laboratory Medicine for CSF analysis. The authors have all contributed to the work and agree with the presented findings. This work was supported by a grant from the Centre for Translational Molecular Medicine, LeARN project - a Dutch public-private partnership on the development and valorization of innovative diagnostic techniques for early detection of Alzheimer Disease; MV is supported by a Zon-MW Innovative Research grant (Vidi program no. 917.46.331). MSj is employed by Schering-Plough and has an affiliation to the Radboud University Nijmegen Medical Centre. There are no conflicts or interests with regard to the research performed in this study.
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### Table 1  Clinical characteristics and results of CSF analysis

<table>
<thead>
<tr>
<th></th>
<th>controls</th>
<th>AD</th>
<th>VaD</th>
<th>FTD</th>
<th>DLB</th>
</tr>
</thead>
<tbody>
<tr>
<td>males/females (n)</td>
<td>23/24</td>
<td>34/35</td>
<td>17/9</td>
<td>19/8</td>
<td>12/4</td>
</tr>
<tr>
<td>age (y)</td>
<td>61 ± 8</td>
<td>69 ± 8</td>
<td>72 ± 9</td>
<td>65 ± 7</td>
<td>76 ± 8</td>
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<tr>
<td>disease duration (mo)</td>
<td>n.a.</td>
<td>29 ± 23</td>
<td>35 ± 29</td>
<td>34 ± 21</td>
<td>34 ± 27</td>
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<tr>
<td></td>
<td>n=60</td>
<td>n=20</td>
<td>n=26</td>
<td>n=8</td>
<td></td>
</tr>
<tr>
<td>t-tau (pg/ml)*</td>
<td>221 ± 80</td>
<td>710 ± 390</td>
<td>391 ± 526</td>
<td>448 ± 315</td>
<td>257 ± 98</td>
</tr>
<tr>
<td>p-tau181 (pg/ml)*</td>
<td>49 ± 16</td>
<td>111 ± 52</td>
<td>46 ± 16</td>
<td>70 ± 36</td>
<td>53 ± 17</td>
</tr>
<tr>
<td>Aβ42 (pg/ml)*</td>
<td>840 ± 260</td>
<td>428 ± 127</td>
<td>631 ± 270</td>
<td>786 ± 229</td>
<td>499 ± 179</td>
</tr>
<tr>
<td>Aβ40 (pg/ml)*</td>
<td>4035 ± 1177</td>
<td>3698 ± 1096</td>
<td>2850 ± 1031</td>
<td>3590 ± 1038</td>
<td>2830 ± 697</td>
</tr>
<tr>
<td>Aβ42/Aβ40 ratio*</td>
<td>0.21 ± 0.04</td>
<td>0.12 ± 0.04</td>
<td>0.23 ± 0.08</td>
<td>0.23 ± 0.06</td>
<td>0.18 ± 0.06</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>AD vs controls</th>
<th>AD vs VaD</th>
<th>AD vs FTD</th>
<th>AD vs DLB</th>
<th>AD vs non-AD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sens. spec.</td>
<td>AUC</td>
<td>LR</td>
<td>sens. spec.</td>
<td>AUC</td>
</tr>
<tr>
<td>Aβ42</td>
<td>93%</td>
<td>0.949</td>
<td>7.3</td>
<td>83%</td>
<td>0.785</td>
</tr>
<tr>
<td>Aβ42/Aβ40 ratio</td>
<td>93%</td>
<td>0.947</td>
<td>7.3</td>
<td>93%</td>
<td>0.900*</td>
</tr>
<tr>
<td>Aβ42, p-tau, t-tau</td>
<td>97%</td>
<td>0.994*</td>
<td>45.6</td>
<td>93%</td>
<td>0.963*</td>
</tr>
<tr>
<td>Aβ42/Aβ40 ratio, p-tau, t-tau</td>
<td>93%</td>
<td>0.980</td>
<td>43.5</td>
<td>91%</td>
<td>0.957</td>
</tr>
</tbody>
</table>

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 Aβ42 level

‡ p<0.05 compared to AUC for Aβ42/Aβ40 ratio
Figure 1 Cerebrospinal fluid Aβ42 and Aβ40 concentrations and Aβ42/Aβ40 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 Aβ42, and the Aβ42/Aβ40 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 Aβ_{42}/Aβ_{40} ratio, the Aβ_{42}/Aβ_{40} ratio combined with p-tau_{181} and t-tau, and the combination of Aβ_{42}, p-tau_{181} 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.