

Cerebrospinal Fluid A β ₁₋₄₀ Improves Differential Dementia Diagnosis in Patients with Intermediate P-tau_{181P} Levels

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Abstract. It is assumed that the concentration of amyloid- β ₁₋₄₀ (A β ₁₋₄₀) in cerebrospinal fluid (CSF) reflects the total amount of A β protein in the brain and thus allows a better interpretation of inter-individual differences in A β quantity than the A β ₁₋₄₂ concentration. In this study, A β ₁₋₄₀ was added to the existing CSF biomarker panel of A β ₁₋₄₂, total tau (T-tau), and phosphorylated tau (P-tau_{181P}) in order to test whether the accuracy of the differential dementia diagnosis improved. The concentration of A β ₁₋₄₀ (INNOTEST® β -amyloid(1–40) prototype version, Innogenetics NV, Belgium) and the other biomarkers (INNOTEST®) was determined in CSF samples from 80 Alzheimer's disease (AD) patients, 75 non-AD dementia patients, and 30 controls. A large proportion of the study population had autopsy-confirmed neurodegeneration (AD: 73/80 = 91%; non-AD: 38/75 = 51%). The levels of A β ₁₋₄₀ were decreased in AD (10856 \pm 4745 pg/mL) and non-AD patients (10519 \pm 4491 pg/mL) when compared to controls (14760 \pm 7846 pg/mL) (p = 0.002 and p = 0.001). The A β ₁₋₄₂/A β ₁₋₄₀ ratio was significantly decreased in AD (0.043 \pm 0.021) as compared to non-AD patients (0.064 \pm 0.027; p < 0.001) and controls (0.053 \pm 0.023; p < 0.001). In order to differentiate AD from non-AD patients, a decision tree was constructed. The diagnostic accuracy of the decision tree that contained A β ₁₋₄₂, A β ₁₋₄₀, P-tau_{181P}, and the A β ₁₋₄₂/A β ₁₋₄₀ ratio was significantly better than the diagnostic accuracy (80% versus 74%) of the decision tree without A β ₁₋₄₀ and the A β ₁₋₄₂/A β ₁₋₄₀ ratio (p < 0.001). In conclusion, no difference in A β ₁₋₄₀ CSF levels was found between AD and non-AD patients, but adding CSF A β ₁₋₄₀ and the CSF A β ₁₋₄₂/A β ₁₋₄₀ ratio to a biomarker-based decision tree, might have an added value for discriminating AD from non-AD patients in case of intermediate CSF P-tau_{181P} values.

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INTRODUCTION

At the moment, many efforts are being made to improve the diagnostic accuracy for Alzheimer's disease (AD), and it will be of great importance to distinguish between AD and other types of dementia (non-AD).

In the new research criteria for AD diagnosis [1], biological markers play an important role. Total tau (T-tau) and phosphorylated tau (P-tau_{181P}) are two biological markers that are frequently used in AD diagnosis as they both are increased in AD compared to controls, reflecting neuronal degeneration and the formation of neurofibrillary tangles [2]. Because senile plaques, another neuropathological hallmark of AD, are mainly composed of amyloid- β_{1-42} ($A\beta_{1-42}$), a lot of attention has been directed to detect these protein accumulations as well.

A decrease in cerebrospinal fluid (CSF) levels of $A\beta_{1-42}$ has been observed in AD patients [3]. When using a combination of CSF $A\beta_{1-42}$ and T-tau levels, sensitivity and specificity values of >80% are reached to discriminate AD from healthy controls, both in clinically diagnosed [4] and autopsy-confirmed subjects [5]. However, its value to discriminate AD from non-AD dementias is limited due to overlapping CSF $A\beta_{1-42}$ levels [6].

As $A\beta_{1-40}$ is the most abundant $A\beta$ peptide in CSF, it is assumed that the concentration of $A\beta_{1-40}$ reflects the total amount of $A\beta$ protein in the brain [7]. The ratio of $A\beta_{1-42}/A\beta_{1-40}$ might be helpful to eliminate large inter-individual differences of absolute $A\beta_{1-42}$ CSF levels [7, 8]. Furthermore, some evidence already exists, claiming that the $A\beta_{1-42}/A\beta_{1-40}$ ratio improves the discrimination of AD from non-AD dementias as compared to $A\beta_{1-42}$ alone [9]. However, conflicting results have been published; Spies et al. [9] and Verwey et al. [10] showed that $A\beta_{1-40}$ improved diagnostic accuracy; however, these results are not supported by another study [11]. In all these studies, populations consisted of clinically diagnosed patients. The aim of the current study was to establish the added diagnostic value of $A\beta_{1-40}$ and the $A\beta_{1-42}/A\beta_{1-40}$ ratio to a combination of *APOE* and CSF levels of $A\beta_{1-42}$, T-tau, and P-tau_{181P} for the differential diagnosis of dementia in a patient population of whom a significant subset had autopsy-confirmed diagnoses.

METHODS

Study population

CSF samples of AD, non-AD, and control patients were selected from the Biobank of the Institute Born-Bunge (Antwerp, Belgium). The AD population was age- and gender-matched with the non-AD group. Data on age at sampling, gender, *APOE* genotype, and Mini-Mental State Exam score within 3 months of lumbar puncture were available.

Clinical diagnostic criteria

The diagnosis of probable AD was made according to the NINCDS-ADRDA criteria [12]. The combination of AD and cerebrovascular disease (AD + CVD) was diagnosed when patients fulfilled the criteria of probable AD according to NINCDS/ADRDA criteria and, in addition, displayed CVD on brain CT and/or MRI that, however, did not meet the criteria of relevant CVD according to NINDS-AIREN criteria of vascular dementia [13], thus excluding multiple large-vessel infarcts, strategically placed infarcts, multiple basal ganglia, and white matter lacunes or extensive white matter lesions. Vascular dementia (VaD) was diagnosed according to the NINDS-AIREN criteria [13]. For the diagnosis of probable frontotemporal dementia (FTD), the criteria described by Neary [14] were applied. For the diagnosis of probable dementia with Lewy bodies (DLB), the criteria of McKeith [15] were used. The control group was recruited among hospitalized patients at the neurology department, and did not present with central nervous system pathology after neurological work-up. All control subjects were screened for cognitive deficits and in case of doubt ($n = 10$), a neuropsychological examination was performed in order to exclude cognitive deficits.

Neuropathological criteria

As described previously [16], all pathological diagnoses were established by the same neuropathologist (JJM), who was blinded for the CSF results. For the diagnosis of AD, the neuropathological criteria of Braak and Braak [17] and Braak et al. [18] were applied. The pathological criteria of McKeith et al. [15]

were applied for the diagnosis of DLB. FTD was neuropathologically diagnosed according to the Jackson and Lowe [19] and Markesbery [20] criteria. VaD was neuropathologically diagnosed using the Markesbery [20] criteria.

CSF sampling and storage

CSF was obtained by lumbar puncture at the L3/L4 or L4/L5 interspace. CSF samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. The samples were collected at the Antwerp memory Clinic at Hospital Network Antwerp (ZNA) Middelheim and Hoge Beuken according to a standard protocol [5, 21].

CSF analysis

CSF levels of A β ₁₋₄₂, T-tau, and P-tau_{181P} were determined with commercially available single-analyte ELISA kits (INNOTEST® β -AMYLOID₍₁₋₄₂₎, INNOTEST® hTAU-Ag, and INNOTEST® PHOSPHO-TAU_(181P), Innogenetics, Ghent, Belgium). CSF A β ₁₋₄₀ levels were determined by means of ELISA, using 2G3 (A β 40)-3D6 (A β 1) antibodies (INNOTEST® β -AMYLOID₍₁₋₄₀₎ Prototype version, Innogenetics, Ghent, Belgium), this prototype kit was also used in a recent published study [22]. With each assay, the clinical samples, together with a blank (sample diluents), the calibrator solutions and the appropriate controls, were tested strictly following the test instructions provided in the kit inserts. All samples were run in duplicate. If the intra-assay range-to-average was $>20\%$ (calculated as $[(\text{value 1} - \text{value 2}) \times 100] / \text{average}$), the samples were retested. If concentrations were out-of-range, the value was set equal to the highest/lowest concentration of the calibration curve. 17 patients had out-of-range concentrations: 1 control A β ₁₋₄₀ <50000 pg/ml, 1 DLB T-tau <75 , 9 AD T-tau >1200 pg/ml, 3 VaD T-tau >1200 pg/ml, 1 FTD T-tau >1200 pg/ml, 1 DLB P-tau_{181P} <15.6 pg/ml, and 1 VaD P-tau_{181P} <15.6 pg/ml.

Statistical analyses

To normalize the biomarker data, concentrations were log-transformed. For the comparison of the demographic variables between the AD, non-AD, and control groups, an Anova test with Bonferroni correction was used. A Chi-square test was used to compare gender distribution and APOE ϵ 4 carriers between the

different groups. Kruskal-Wallis and *post-hoc* Mann-Whitney U tests were used to compare biomarker concentrations between AD and the different non-AD groups. Receiver operating characteristic curves (ROC) were applied to define optimal CSF biomarker cut-off values to discriminate between the different groups. The ideal cut-off value was defined as the maximum sum of sensitivity and specificity. To combine the different biomarkers, decision trees were made using the Chi-squared automatic interaction detection method (CHAID). The maximum tree depth was set to three levels, the significance (Pearson) for splitting nodes and merging categories was set to 0.05, the maximum number of iterations was 100, the minimum number of cases in parent nodes was 10 and for a child node 5 and the minimum change in expected cell frequencies was 0.001. The sensitivity, specificity, and diagnostic accuracy between different models was compared using a McNemar test. A probability level <0.05 was considered significant. Statistical analyses were performed using SPSS 18 (SPSS Inc., Chicago, USA).

RESULTS

Population and biomarker results

The study population consisted of 80 AD, 75 non-AD, and 30 control subjects. A large proportion of the study population had autopsy-confirmed neurodegeneration (AD: $73/80 = 91\%$; non-AD: $38/75 = 51\%$). The delay between lumbar puncture and autopsy was 1.7 ± 2.6 years in AD and 1.3 ± 1.6 years in non-AD patients. An overview of the population is given in Fig. 1. The demographic and clinical data on the AD, non-AD, and control population are summarized in Table 1.

There was no significant difference in A β ₁₋₄₀ CSF levels between AD and non-AD patients ($p = 1.000$). However, when compared to controls, both the AD and non-AD groups presented with decreased CSF A β ₁₋₄₀ levels ($p = 0.002$ and $p = 0.001$). A β ₁₋₄₂ ($p < 0.001$) and the A β ₁₋₄₂/A β ₁₋₄₀ ratio ($p < 0.001$) were significantly decreased in AD as compared to non-AD patients and controls.

The levels of A β ₁₋₄₂ and A β ₁₋₄₀ and the A β ₁₋₄₂/A β ₁₋₄₀ ratio were compared between AD and the other dementia types separately (Fig. 2). A significant decrease in the A β ₁₋₄₂ levels and the A β ₁₋₄₂/A β ₁₋₄₀ ratio was found in AD compared to FTD ($p < 0.001$) and VaD patients ($p < 0.001$). No significant difference was found between AD and DLB patients.

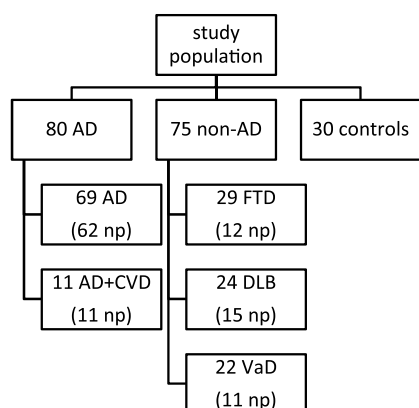


Fig. 1. Overview of the study population. AD, Alzheimer's disease; np, neuropathologically confirmed; AD + CVD, Alzheimer's disease + cerebrovascular disease; FTD, frontotemporal dementia; DLB, dementia with Lewy bodies, VaD, vascular dementia.

Diagnostic performance of $A\beta_{1-42}$ and $A\beta_{1-40}$

For the differentiation between AD and non-AD patients, the optimal cut-off value for the $A\beta_{1-42}/A\beta_{1-40}$ ratio was 0.057 (sensitivity = 81%, specificity = 60%) and the optimal cut-off value for $A\beta_{1-42}$ was 517 pg/ml (sensitivity = 81%, specificity = 59%) (Fig. 3). The AUC \pm se of the $A\beta_{1-42}/A\beta_{1-40}$ ratio (0.749 \pm 0.039; 95% CI: 0.673–0.826) was not different from the AUC of $A\beta_{1-42}$ (0.747 \pm 0.039; 95% CI: 0.670–0.827).

The CSF biomarkers $A\beta_{1-42}$, $A\beta_{1-40}$, T-tau, P-tau_{181P}, the $A\beta_{1-42}/A\beta_{1-40}$ ratio and APOE $\epsilon 4$ carrier

data were entered in a decision tree model in order to optimally discriminate between AD and non-AD dementia patients. The final decision tree retained the following biomarkers: CSF $A\beta_{1-42}$, $A\beta_{1-40}$, P-tau_{181P}, and the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio (Fig. 4). In a first step, dementia patients were divided in three groups according to CSF P-tau_{181P} levels. In the group with low P-tau_{181P} levels, CSF $A\beta_{1-42}$ levels were used to subdivide this group. The group with intermediate CSF P-tau_{181P} levels was subdivided using the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio and the group with a low ratio was further divided according to the CSF $A\beta_{1-40}$ levels. A sensitivity and specificity of 80% was reached using this model and the accuracy of this tree was 80% for differentiating AD from non-AD dementias. In the neuropathologically confirmed population, the sensitivity and specificity was 79% and 76%, respectively.

When a decision tree was constructed without CSF $A\beta_{1-40}$ levels and without the CSF $A\beta_{1-42}/A\beta_{1-40}$ ratio, a sensitivity and specificity of 91% and 56% was achieved, which resulted in a diagnostic accuracy of 74%. When these results were compared with the sensitivity and specificity of the model including $A\beta_{1-40}$ and the $A\beta_{1-42}/A\beta_{1-40}$ ratio, the specificity decreased ($p < 0.001$), but the sensitivity increased ($p = 0.004$) and the diagnostic accuracy for discriminating AD from non-AD dementia patients improved significantly ($p < 0.001$). To assess the clinical value of $A\beta_{1-40}$, patients who had clinically ambiguous diagnoses (when the clinical diagnostic work-up was not able to discriminate between AD and non-AD

Table 1
Demographic, clinical, genetic and biomarker data

	AD (\pm CVD)	non-AD	Control	<i>p</i> -value
Gender (male/female)	43/37	43/32	12/18	0.270
Age (year)	75 \pm 10 <i>n</i> = 80	73 \pm 10 <i>n</i> = 75	74 \pm 9 <i>n</i> = 30	0.456
MMSE score (/30)	13 \pm 7*,\$ <i>n</i> = 72	17 \pm 8*,\$ <i>n</i> = 64	28 \pm 2\$,# <i>n</i> = 13	<0.001
APOE $\epsilon 4$ carriers (%)	36 (56%) <i>n</i> = 64	22 (33%) <i>n</i> = 66	6 (46%) <i>n</i> = 13	0.032
T-tau (pg/ml)	613 \pm 323*,\$ <i>n</i> = 80	392 \pm 273* <i>n</i> = 75	309 \pm 168\$ <i>n</i> = 30	<0.001
P-tau _{181P} (pg/ml)	80 \pm 46*,\$ <i>n</i> = 80	50 \pm 26* <i>n</i> = 75	55 \pm 28\$ <i>n</i> = 30	<0.001
$A\beta_{1-42}$ (pg/ml)	405 \pm 158*,\$ <i>n</i> = 80	611 \pm 249* <i>n</i> = 75	710 \pm 282\$ <i>n</i> = 30	<0.001
$A\beta_{1-40}$ (pg/ml)	10856 \pm 4745\$ <i>n</i> = 80	10519 \pm 4491# <i>n</i> = 75	14760 \pm 7846#,\$ <i>n</i> = 30	0.001
$A\beta_{1-42}/A\beta_{1-40}$	0.043 \pm 0.021*,\$ <i>n</i> = 80	0.064 \pm 0.027* <i>n</i> = 75	0.053 \pm 0.023\$ <i>n</i> = 30	<0.001

Data are shown as mean \pm SD. An Anova with Bonferroni correction was used to compare the different groups with exception of the gender distribution and APOE $\epsilon 4$ carriers (Chi-square with Bonferroni correction). Significant differences are indicated with the following symbols: *AD versus non-AD; \$AD versus control; # non-AD versus control.

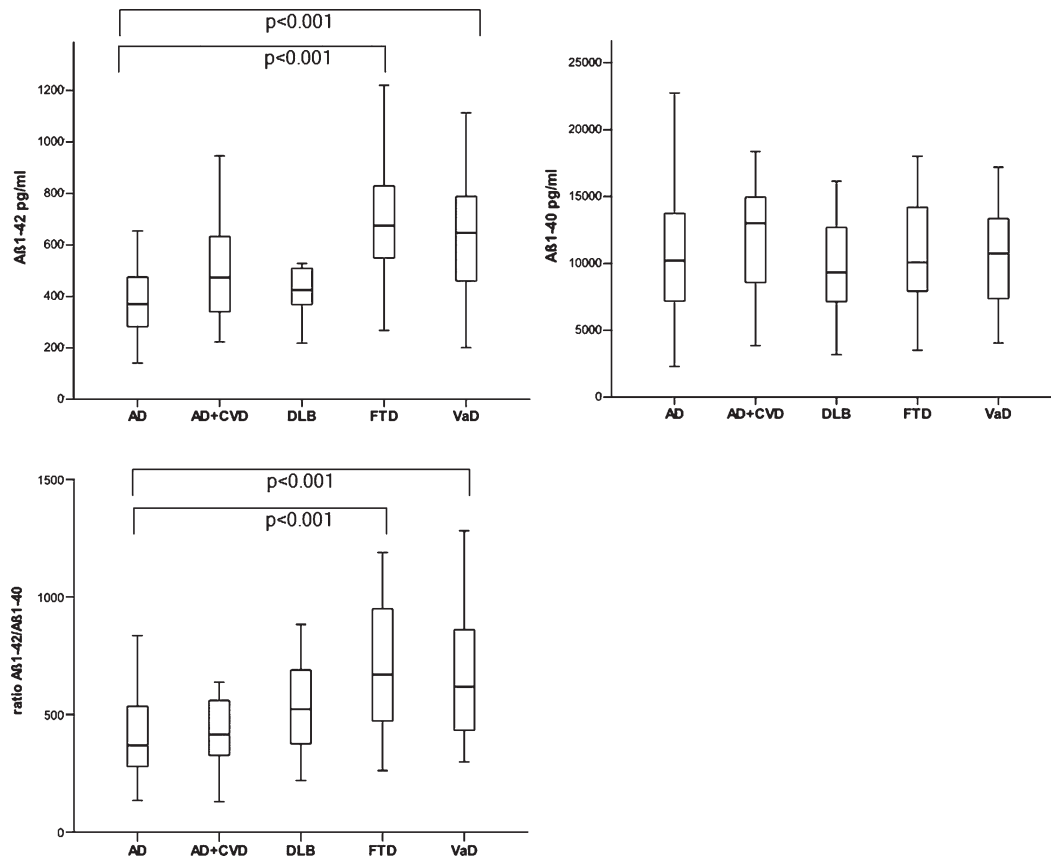


Fig. 2. Boxplots of the CSF $A\beta_{1-42}$, $A\beta_{1-40}$ concentration and ratio from the different types of dementia.

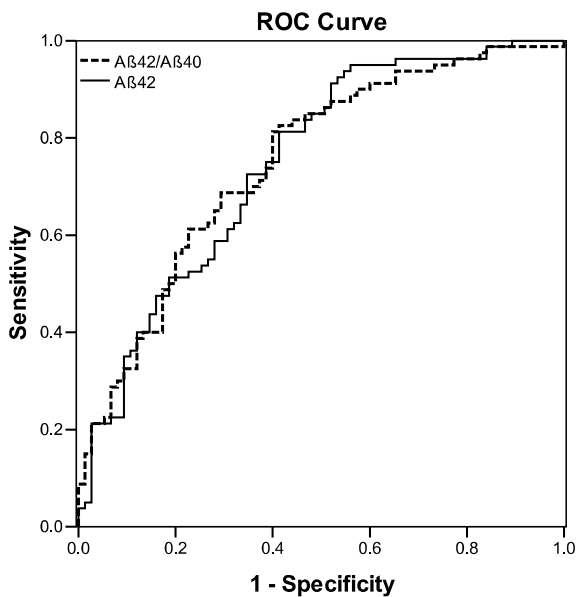


Fig. 3. ROC curves were made to determine the optimal cut-off value to discriminate between AD and non-AD patients.

dementia) were selected from the neuropathologically confirmed patients ($n = 16$). By using the decision tree without $A\beta_{1-40}$, only 7/16 patients were correctly diagnosed. When $A\beta_{1-40}$ was added to the decision tree, the number of correctly classified patients rose to 10/16.

DISCUSSION

The present study was designed to establish the added diagnostic value of $A\beta_{1-40}$ and the $A\beta_{1-42}/A\beta_{1-40}$ ratio to a combination of *APOE* genotype and CSF levels of $A\beta_{1-42}$, T-tau, and P-tau_{181P} for the differential diagnosis of dementia in a patient population of whom a significant subset had autopsy-confirmed neurodegeneration.

Biomarker results

When comparing the CSF levels of $A\beta_{1-42}$, $A\beta_{1-40}$, T-Tau, P-tau_{181P}, and the $A\beta_{1-42}/A\beta_{1-40}$ ratio between AD and non-AD patients, all biomarkers were

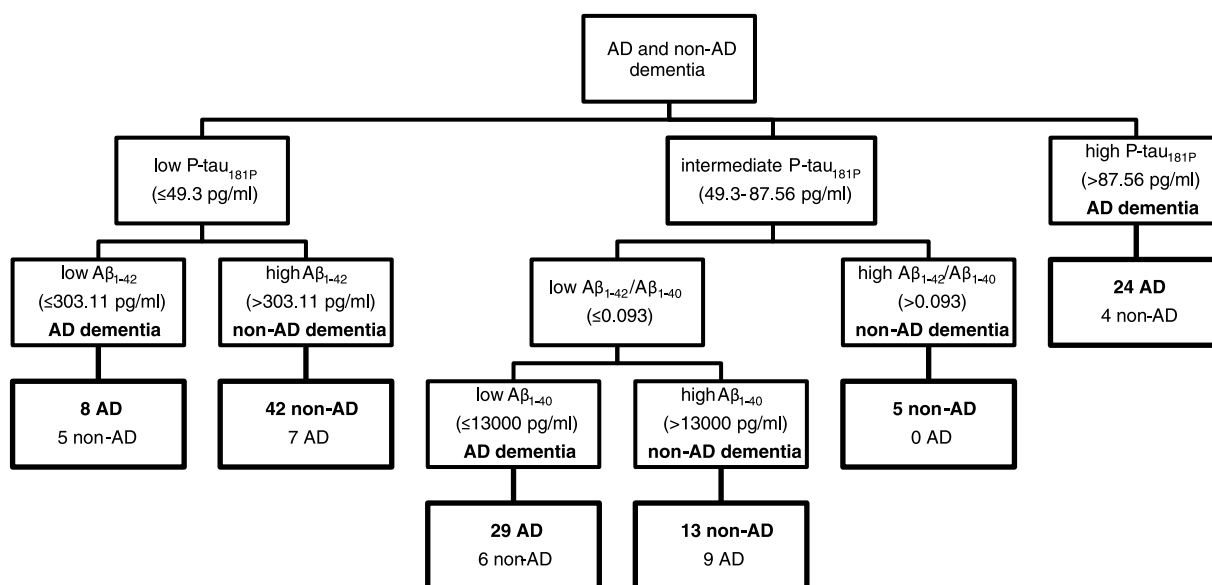


Fig. 4. Decision tree.

significantly different with exception of $A\beta_{1-40}$. As expected, the AD group was characterized by decreased CSF $A\beta_{1-42}$ and elevated CSF T-tau and P-tau_{181P} levels. The $A\beta_{1-42}/A\beta_{1-40}$ ratio was decreased in AD when compared to non-AD patients and controls. This result is consistent with the research of Spies et al. [9] in CSF, and a decrease has also been reported in plasma [23]. The CSF levels of $A\beta_{1-40}$ in both AD and non-AD patients were decreased when compared to controls. In other studies that examined CSF levels of $A\beta_{1-40}$ in AD patients and controls, no significant differences were found [8–10, 24, 25]. However, decreased CSF levels of $A\beta_{1-40}$ were previously found in non-AD patients when compared to controls. Pijnenburg et al. [25] and Verwey et al. [10] found decreased levels of CSF levels of $A\beta_{1-40}$ in FTD and Spies et al. [9] found decreased levels in VaD and DLB. Decreased levels of $A\beta_{1-40}$ have also been found in patients with cerebral amyloid pathology [24]. There are various mechanisms that could lead to changes in $A\beta_{1-40}$ levels, like an alteration in the production of amyloid peptide, changes in the breakdown and clearance or retention in senile plaques. It is unknown which mechanism is responsible for the decrease in $A\beta_{1-40}$ in AD and non-AD and it could be possible that the reason for the decrease is different in the different pathologies. Another explanation for the decreased levels can be related to the formation of $A\beta$ dimers or oligomers that escape detection [26]. This could also explain the different results between studies because other

antibodies were used and it is possible that some epitopes remain available while others are blocked.

Further analyses showed that in FTD and VaD patients, the CSF levels of $A\beta_{1-42}$ and the $A\beta_{1-42}/A\beta_{1-40}$ ratio were significantly different when compared to AD patients. No significant differences in $A\beta_{1-40}$ levels were found between AD and the other non-AD dementias. Other studies comparing CSF $A\beta_{1-40}$ levels between FTD and AD also found no significant difference between both groups [9, 10]. However, decreased CSF levels of $A\beta_{1-40}$ have been reported in clinically diagnosed VaD and DLB patients when compared to AD patients [9]. The comparable CSF $A\beta_{1-42}$ levels in DLB and AD patients can be explained by AD co-pathology in the brain of DLB patients as we recently demonstrated in autopsy-confirmed patients [27].

To assess the additional diagnostic value of CSF $A\beta_{1-40}$ for the AD versus non-AD differential diagnosis, ROC curves were made to find the optimal cut-off value for $A\beta_{1-40}$ and the $A\beta_{1-42}/A\beta_{1-40}$ ratio to discriminate between AD and non-AD patients. When applying these cut-offs, CSF $A\beta_{1-40}$ levels and the $A\beta_{1-42}/A\beta_{1-40}$ ratio did not yield better results when compared to CSF $A\beta_{1-42}$ levels.

Taken together, these results indicate that there are no differences in CSF $A\beta_{1-40}$ levels between (mostly) autopsy-confirmed AD and non-AD dementia patients. On its own or in combination with $A\beta_{1-42}$, $A\beta_{1-40}$ could not improve the differential diagnosis of AD.

Diagnostic biomarker-based paradigm

In search of the best possible sequence and combination of biomarkers to discriminate between AD and non-AD patients, a decision tree was constructed. In this model, $A\beta_{1-42}$, $A\beta_{1-40}$, P-tau_{181P}, and the $A\beta_{1-42}/A\beta_{1-40}$ ratio were used to discriminate AD from non-AD patients. T-tau was entered but not retained in the model, which is in accordance with a biomarker-based logistic regression model for AD versus non-AD differential dementia diagnosis [5]. This can be explained by the fact that T-tau is a general marker for neurodegeneration, and is not a specific marker for AD. P-tau_{181P}, on the other hand, is a more specific biomarker for AD and it is the biomarker that was used in the first step of the decision tree. In case of intermediate CSF P-tau_{181P} levels, CSF $A\beta_{1-40}$ levels and the $A\beta_{1-42}/A\beta_{1-40}$ ratio were applied to discriminate between AD and non-AD patients. In case of a low $A\beta_{1-42}/A\beta_{1-40}$ ratio, $A\beta_{1-40}$ CSF levels were used to discriminate between AD and non-AD. This is consistent with the research of Wiltfang et al. [7] who showed that a low or high $A\beta$ load could lead to misinterpretation of the neurochemical diagnosis. Although no significant difference in CSF $A\beta_{1-40}$ levels was found in the total AD and non-AD population, in the group of dementia patients with intermediate P-tau_{181P} levels, there was a significant difference in $A\beta_{1-40}$ levels. Hence $A\beta_{1-40}$ could be used to differentiate between AD and non-AD in the decision tree. So when CSF P-tau_{181P} levels could not distinguish between AD and non-AD patients, adding CSF $A\beta_{1-40}$ levels had an added value for the AD versus non-AD differential diagnosis.

A decision tree without $A\beta_{1-40}$ levels and the $A\beta_{1-42}/A\beta_{1-40}$ ratio was also created and achieved a sensitivity of 81%, a specificity of 56%, and diagnostic accuracy of 74%. When comparing these results to the decision that contained $A\beta_{1-40}$ levels and the $A\beta_{1-42}/A\beta_{1-40}$ ratio, the sensitivity was slightly reduced (from 91% to 80%), while the specificity and diagnostic accuracy was improved (from 56% to 80%; 74 to 80%). In the neuropathologically confirmed population, the sensitivity of the decision tree was 79% (58/73) and the specificity 76% (29/38), resulting in a diagnostic accuracy of 78%. The decision tree with $A\beta_{1-40}$ levels and the $A\beta_{1-42}/A\beta_{1-40}$ ratio was also better in discriminating in patients with an ambiguous clinical diagnosis.

Although no differences in $A\beta_{1-40}$ levels could be found between the AD and non-AD groups, adding CSF $A\beta_{1-40}$ levels and the $A\beta_{1-42}/A\beta_{1-40}$ ratio to a

biomarker-based paradigm improved the overall diagnostic accuracy that reached the recommendations of The Ronald and Nancy Reagan Research Institute of Alzheimer's Association and the National Institute on Aging Working Group [28].

Limitations and future

Differences between the present and other study results might be explained by different analytical methods to measure CSF $A\beta_{1-40}$ levels. Moreover, as other studies have included clinically diagnosed patients, one cannot rule out that AD patient groups contained non-AD patients and vice versa. In our study, a large part of the study population had a definite etiological dementia diagnosis (91% of the AD and 51% of the non-AD population) and the clinically diagnosed patients were followed up which contributed to the diagnostic accuracy, which is an advantage of the present study. However, the control patients that were used in this study were not neuropathologically confirmed so it is possible that they presented with a preclinical stage of dementia. This biomarker-based paradigm should be validated in an independent study, and to assess the value of this model in clinical practice, a prospective study should be designed.

CONCLUSION

No difference in $A\beta_{1-40}$ CSF levels was found between AD and non-AD patients, but adding CSF $A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratios to the CSF AD biomarkers, $A\beta_{1-42}$, T-tau, and P-tau_{181P}, might have an added value for discriminating AD from non-AD dementia patients in case of intermediate CSF P-tau_{181P} levels in a (largely) autopsy-confirmed population.

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