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Diagnostic Accuracy of Cerebrospinal Fluid Amyloid-β Isoforms for Early and Differential Dementia Diagnosis

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Abstract.
Background: Overlapping cerebrospinal fluid biomarkers (CSF) levels between Alzheimer's disease (AD) and non-AD patients decrease differential diagnostic accuracy of the AD core CSF biomarkers. Amyloid-β (Aβ) isoforms might improve the AD versus non-AD differential diagnosis.
Objective: To determine the added diagnostic value of Aβ isoforms, $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, and $A\beta_{1\text{-}40}$, as compared to the AD CSF biomarkers $A\beta_{1\text{-}42}$, T-tau, and P-tau_{181P}.
Methods: CSF from patients with dementia due to AD ($n = 50$), non-AD dementias ($n = 50$), mild cognitive impairment due to AD ($n = 50$) and non-demented controls ($n = 50$) was analyzed with a prototype multiplex assay using MSD detection technology. The non-AD group consisted of frontotemporal dementia (FTD; $n = 17$), dementia with Lewy bodies (DLB; $n = 17$), and vascular dementia ($n = 16$).
Results: $A\beta_{1\text{-}37}$ and $A\beta_{1\text{-}38}$ increased accuracy to differentiate AD from FTD or DLB. $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, and $A\beta_{1\text{-}40}$ levels correlated with Mini-Mental State Examination scores and disease duration in dementia due to AD. The $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ ratio improved diagnostic performance of $A\beta_{1\text{-}42}$ in most differential diagnostic situations. $A\beta_{1\text{-}42}$ levels were lower in *APOE* ε4 carriers compared to non-carriers.

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Conclusions: Aβ isoforms help to differentiate AD from FTD and DLB. Aβ isoforms increase diagnostic performance of $A\beta_{1\text{-}42}$. In contrast to $A\beta_{1\text{-}42}$, Aβ isoforms seem to be correlated with disease severity in AD. Adding the Aβ isoforms to the current biomarker panel could enhance diagnostic accuracy.

Keywords: Alzheimer's disease, amyloid, biological markers, cerebrospinal fluid, diagnosis, differential, mild cognitive impairment

INTRODUCTION

Amyloid plaques, one of the major neuropathological hallmarks of Alzheimer's disease (AD), mainly consist of aggregates of carboxyterminally elongated forms of amyloid-β (Aβ) peptides [1], resulting from cleavage of the transmembrane amyloid-β protein precursor by β- and γ-secretases [2]. The most abundant Aβ peptides in cerebrospinal fluid (CSF) are $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ [3], of which $A\beta_{1\text{-}42}$ is the most pathological in AD as it is most prone to aggregation into Aβ plaques [4].

The combined assessment of CSF $A\beta_{1\text{-}42}$, total tau protein (T-tau), and tau phosphorylated at threonine 181 ($P\text{-}tau_{181P}$) increases diagnostic certainty for AD [5]. Compared to controls, the AD CSF biomarker profile consists of decreased $A\beta_{1\text{-}42}$ and increased T-tau and/or $P\text{-}tau_{181P}$ concentrations. However, when compared to non-AD dementias, these differences are less pronounced as the concentrations in patients with non-AD dementias are generally intermediate between those found in controls and AD patients, indicating an overlap between AD and non-AD patients [6].

Determining CSF Aβ isoforms might improve the AD versus non-AD differential diagnosis, as some evidence exists that $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ or $A\beta_{1\text{-}42}/A\beta_{1\text{-}38}$ ratios improve discriminating AD from non-AD dementias in comparison to $A\beta_{1\text{-}42}$ alone [7, 8]. Indeed, several studies have shown the CSF levels of $A\beta_{1\text{-}38}$ are decreased in frontotemporal dementia (FTD) as compared to AD and non-demented controls [9, 10]. Using the $A\beta_{1\text{-}42}/A\beta_{1\text{-}38}$ ratio, FTD could be differentiated from AD with a sensitivity and specificity of 82% [9]. As AD pathology is common in dementia with Lewy bodies (DLB) and the presence of senile plaques in DLB patients is associated with low CSF $A\beta_{1\text{-}42}$ concentrations, the determination of CSF $A\beta_{1\text{-}42}$ levels is of limited value for discriminating AD and DLB [11]. However, it has been shown that the ratios of $A\beta_{1\text{-}42}/A\beta_{1\text{-}37}$ and $A\beta_{1\text{-}42}/A\beta_{1\text{-}38}$ can differentiate between AD and DLB [12, 13].

In this study, the Aβ isoforms $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$ were analyzed and four research questions were explored: 1) Do Aβ isoforms correlate with disease severity in AD?; 2) Do the Aβ isoforms levels differ between apolipoprotein E (*APOE*) ε4 carriers and non-carriers?; 3) Does the ratio of $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ increase the diagnostic performance of $A\beta_{1\text{-}42}$ alone?; 4) What is the added diagnostic value of the Aβ isoforms?

The potential diagnostic accuracy of the Aβ peptides, $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}$, was assessed for differential dementia diagnoses as well as for early AD diagnosis. In addition, in order to evaluate the added value of the Aβ isoforms, their diagnostic values were compared to the diagnostic values of $A\beta_{1\text{-}42}$, T-tau, and $P\text{-}tau_{181P}$.

METHODS

Study population

Samples from patients and controls were selected from the Biobank of the Institute Born-Bunge. Only samples from patients recruited in the Memory Clinic and Department of Neurology of Hospital Network Antwerp (ZNA) were selected to avoid inter-center variability due to possible differences in pre-analytical steps. Patients with dementia due to AD ($n = 50$), mild cognitive impairment (MCI) due to AD ($n = 50$), and patients with non-AD dementias ($n = 50$) were included. The non-AD group consisted of 17 patients with FTD, 17 DLB patients, and 16 patients with vascular dementia (VaD).

Patients with MCI and dementia due to AD were diagnosed according to the NIA-AA criteria [14, 15], with at least intermediate probability of AD etiology (based on the CSF biomarkers or hippocampal volume on MRI). MCI due to AD and dementia due to AD will hereafter be referred to as 'MCI' and 'AD', respectively. FTD, DLB, and VaD were diagnosed according to the criteria described by Neary et al. [16], the clinical diagnostic criteria of McKeith et al. [17], and the NINDS-AIREN criteria [18], respectively.

The control group consisted of cognitively healthy elderly ($n = 35$) in whom cognitive deterioration was ruled out by means of neuropsychological screening. Cognitively healthy elderly also fulfilled the following inclusion criteria: 1) no neurological or psychiatric antecedents and 2) no central nervous system disease

following extensive clinical examination. The control group also consisted of patients with neurological diseases in whom neurodegenerative disorders were ruled out by means of an extensive neurological work-up ($n = 15$). The study was approved by the local ethics committee (University Hospital Antwerp) and all subjects gave their written informed consent.

CSF sampling

Lumbar puncture (LP), CSF sampling and handling have been performed according to a standard protocol [19]. CSF samples were stored at −80°C until analysis.

CSF biomarker analyses

CSF biomarker analyses of $A\beta_{1\text{-}42}$, T-tau, and P-tau_{181P} were performed using commercially available single parameter ELISA kits (INNOTEST®, Fujirebio Europe, Ghent, Belgium) at the BIODEM lab as previously described [20]. CSF biomarker analyses of $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, and $A\beta_{1\text{-}40}$ were performed at QPS Netherlands BV (Groningen, The Netherlands) with a prototype multiplex assay developed by Janssen Research and Development that uses Meso Scale Discovery (MSD) detection technology as previously described [21].

Briefly, the multiplex assay involved a sandwich immunoassay with electrochemoluminescence detection. Standards of human $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, and $A\beta_{1\text{-}40}$ (AnaSpec, San Jose, USA) were dissolved in dimethylsulphoxide at 0.1 mg/mL and stored at −80°C. For use in the assay, peptides were further diluted in casein buffer (0.1% casein in PBS). Purified monoclonal antibodies specific for $A\beta_{1\text{-}37}$ (JRD/Aβ37/3), $A\beta_{1\text{-}38}$ (J&JPRD/Aβ38/5), and $A\beta_{1\text{-}40}$ (JRF/cAβ40/28) were coated on MSD 4-plex 96-well plates on spatially distinct spots. Plates were blocked with casein buffer for 1–4 h at room temperature. After washing, standards, quality control samples, and 1/2 prediluted CSF samples were incubated overnight at 4°C together with MSD SULFO-TAG™-labeled human-specific detection antibody JRF/AβN/25. JRF/AβN/25 detects an end-specific epitope of Aβ leading to the detection of full-length Aβ peptides ($A\beta_{1\text{-}x}$). After overnight incubation, plates were washed, after which 2x Read Buffer (MSD) was added according to the manufacturer's recommendations and plates were read on MSD Sector Imager 6000. $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, and $A\beta_{1\text{-}40}$ concentrations were determined by interpolation from the standard curve using MSD Workbench software and 4 parameter logistic model with $1/Y^2$ weighting function. All calibration standards and CSF samples were analyzed in duplicate. Only mean values with a replicate well coefficient of variation (CV) of less than or equal to 20.0% were accepted. The samples of the different diagnostic groups were tested randomized over multiple plates. The means for the interplate CV for the quality control samples were less than 12% for all analytes. The upper and lower limit of quantification, determined as the highest and lowest calibrator concentration for which overall CV and bias were ≤25.0%, was 4.57 pg/mL and 10 000 pg/mL, respectively, for all measured Aβ peptides.

Disease severity in AD

Disease severity of AD was estimated by Mini-Mental State Examination (MMSE) scores and disease duration. MMSE tests were always performed 3 months before or after LP. If available, the yearly change in MMSE, i.e., the difference between the earliest MMSE score and the most recent one divided by their time interval, was also reported. Disease duration was considered as the difference between age at onset and age at LP.

APOE genotyping

The isolation of genomic DNA from peripheral blood lymphocytes was performed at the Genetic Service Facility (http://www.vibgeneticservicefacility.be) of the VIB Department of Molecular Genetics on a Magtration® System 8Lx. robotic platform. SNPs in *APOE* (rs429358 and rs7412, determining the ε2/ε3/ε4 polymorphism) were genotyped by Sanger sequencing.

Statistical analyses

Statistical analyses were performed using SPSS 20. First, a Kolmogorov-Smirnov test was performed to check for normal distribution. Since most variables did not follow a normal distribution, non-parametric tests were used. To compare gender distribution and *APOE* carrier status across the groups a Chi-square test was performed. A Kruskal-Wallis test was used to compare biomarker data over all groups. Subsequently, Mann-Whitney U tests were performed to compare groups separately. To assess correlations, Spearman's Rho correlation tests were performed. Receiver operating characteristic (ROC) curve analyses were used to obtain area under the curve (AUC) values and to define optimal cut-off values to discriminate MCI and AD

Table 1
Demographic, clinical, and biomarker data for all groups

	AD	Non-AD	MCI	Controls	FTD	VaD	DLB	*p*-value
Gender (F/M)	31 / 18	20 / 30	32 / 17	17 / 33	06-Nov	08-Aug	06-Nov	0.002
Age (y)	77 (70–82)	74 (70–78)	79 (72–82)	68 (61–73)	70 (66–73)	77 (72–81)	75 (73–81)	<0.001
% *APOE* ε4 carriers	56.8 $n=44$	26.8 $n=49$	40.0 $n=40$	27.3 $n=11$	29.4 $n=17$	31.3 $n=16$	25.0 $n=16$	0.04
MMSE at LP (/30)	19 (15–24) $n=47$	18 (14–24) $n=44$	25 (22–26) $n=43$	Not available	19 (14–25) $n=16$	16 (12–23) $n=15$	19 (16–23) $n=13$	<0.001
Age at onset (y)	76 (68–80)	71 (65–77)	76 (67–80)	/	65 (59–68)	75 (69–79)	73 (69–78)	0.04
Disease duration (y)	2 (1–4)	3 (1–4)	2 (1–4)	/	3 (2–6)	1 (1–3)	3 (2–3)	0.97
$A\beta_{1\text{-}42}$ (pg/mL)	476 (389–578)	583 (417–853)	514 (406–616)	834 (630–1059)	582 (407–853)	603 (512–870)	595 (430–806)	<0.001
T-tau (pg/mL)	561 (405–807)	281 (230–428)	491 (422–603)	246 (172–373)	296 (230–428)	296 (230–431)	250 (234–377)	<0.001
P-tau_{181P} (pg/mL)	78.0 (58.0–98.0)	41.6 (32.0–59.8)	79.0 (63.0–105.0)	42.9 (33.0–61.7)	40.0 (32.0–61.0)	46.7 (37.8–58.4)	41.0 (30.0–49.0)	<0.001
$A\beta_{1\text{-}37}$ (pg/mL)	701 (556–894)	544 (369–784)	943 (684–1135)	717 (530–885)	523 (384–706)	691 (471–905)	483 (369–758)	<0.001
$A\beta_{1\text{-}38}$ (pg/mL)	2174 (1598–2880)	1679 (1108–2543)	2724 (2108–3872)	2238 (1745–2832)	1607 (1235–2212)	2070 (1340–2777)	1353 (1024–1968)	<0.001
$A\beta_{1\text{-}40}$ (pg/mL)	6023 (4616–8447)	5120 (3757–7667)	8658 (7153–11310)	6380 (4599–8308)	4564 (3348–5799)	6680 (4045–7624)	4487 (3766–8005)	<0.001
$A\beta_{1\text{-}42}$/T-tau	0.79 (0.58–1.09)	2.18 (1.48–3.34)	0.99 (0.73–1.64)	3.30 (2.25–4.61)	2.06 (1.01–3.43)	2.27 (1.74–2.85)	2.41 (1.25–3.34)	<0.001
$A\beta_{1\text{-}42}$/P-tau_{181P}	6.10 (4.67–7.16)	14.71 (8.78–20.75)	6.54 (4.61–9.14)	18.33 (13.40–23.24)	14.6 (7.05–20.90)	14.45 (9.72–21.07)	14.87 (8.72–19.66)	<0.001
$A\beta_{1\text{-}38}/A\beta_{1\text{-}40}$	0.35 (0.31–0.38)	0.33 (0.28–0.35)	0.32 (0.29–0.35)	0.35 (0.32–0.38)	0.35 (0.30–0.37)	0.34 (0.31–0.37)	0.27 (0.24–0.33)	<0.001
$A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$	0.07 (0.06–0.09)	0.12 (0.08–0.17)	0.06 (0.04–0.08)	0.12 (0.11–0.16)	0.14 (0.09–0.17)	0.11 (0.08–0.15)	0.13 (0.08–0.17)	<0.001
$A\beta_{1\text{-}42}/A\beta_{1\text{-}38}$	0.21 (0.17–0.26)	0.40 (0.24–0.54)	0.20 (0.14-–0.26)	0.37 (0.29–0.47)	0.39 (0.24–0.48)	0.31 (0.25–0.50)	0.51 (0.28–0.60)	<0.001
$A\beta_{1\text{-}42}/A\beta_{1\text{-}37}$	0.66 (0.51–0.76)	01.13 (0.79–01.57)	0.62 (0.41–0.85)	01.19 (0.97–1.48)	01.17 (0.74–1.50)	0.92 (0.80–1.31)	1.22 (0.83–1.72)	<0.001
Relative $A\beta_{1\text{-}42}$	0.05 (0.04–0.06)	0.08 (0.05–0.11)	0.04 (0.03–0.05)	0.08 (0.07–0.10)	0.09 (0.06–0.11)	0.07 (0.06–0.10)	0.09 (0.05–0.11)	<0.001

All data are median values with 25th and 75th quartiles between brackets, except for gender and % of *APOE* ε4 carriers. To compare gender distribution and *APOE* carrier status across the groups a Chi-square test was performed. A Kruskal-Wallis test was used to compare biomarker data over all groups. AD, Alzheimer's disease; Non-AD, dementia not due to Alzheimer's disease; MCI, mild cognitive impairment; FTD, frontotemporal dementia; VaD, vascular dementia; DLB, dementia with Lewy bodies; *APOE*, Apolipoprotein E; MMSE, Mini-Mental State Examination; LP, lumbar puncture; Relative $A\beta_{1\text{-}42}$, ratio of $A\beta_{1\text{-}42}$ to the sum of all Aβ isoforms.

from all other groups. The cut-off values were determined by calculating the maximal sum of sensitivity and specificity (i.e., maximizing the Youden index). In order to compare AUC values, DeLong tests were performed by using the pROC package [22] in the statistical software package R (R Core Team). Correction for multiple testing was not performed due to the small study population and the explorative nature of this study.

RESULTS

Study population: Demographic, clinical, and biomarker data

One AD patient and one MCI patient were excluded from statistical analyses because of all their Aβ isoforms concentrations being below the lowest range. The groups were not age- and gender-matched. Table 1 summarizes demographic, clinical, and biomarker data for all groups.

Correlation with MMSE

In the MCI group, none of the correlations were significant (Table 2). However, in the AD group, $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, and $A\beta_{1\text{-}40}$ correlated moderately with MMSE scores. Yearly change in MMSE correlated significantly but weakly ($p < 0.05$) with $A\beta_{1\text{-}42}$ in the MCI group.

Correlation with disease duration

In the AD population, the correlations of $A\beta_{1\text{-}38}$, $A\beta_{1\text{-}40}$, and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ with disease duration were weak but significant (Table 2).

Effect of APOE ε4

The Aβ isoforms levels were compared between subjects carrying one or two ε4 alleles ($n = 58$) and non-carriers ($n = 86$) (Table 3). $A\beta_{1\text{-}42}$ was significantly lower in ε4 carriers ($p < 0.001$), while $A\beta_{1\text{-}37}$, $A\beta_{1\text{-}38}$, and $A\beta_{1\text{-}40}$ were not significantly different.

In the MCI and AD populations separately none of the biomarkers differed significantly between ε4 carriers and non-carriers. However, when combining both diagnostic groups, $A\beta_{1\text{-}42}$ was significantly lower in carriers than non-carriers ($p < 0.05$). This was also found in the non-AD group ($p < 0.05$).

Diagnostic accuracy

The ROC curve analysis results of the best performing biomarkers are summarized in Table 4, while the remaining data are given in the Supplementary Material.

AD versus MCI

$A\beta_{1\text{-}42}$, T-tau, and P-tau_{181P} did not differentiate between MCI and AD, keeping in mind these analytes were used to define these groups. The AUC values of the Aβ isoforms were below 0.800 (Supplementary Table 1).

AD and MCI versus controls

The biomarkers performing best when comparing AD patients and controls were $A\beta_{1\text{-}42}$/T-tau and $A\beta_{1\text{-}42}$/P-tau_{181P}. $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ performed comparably to $A\beta_{1\text{-}42}$ for discriminating AD from controls (Table 5; Supplementary Table 2).

The biomarkers performing best when comparing MCI patients and controls were $A\beta_{1\text{-}42}$/T-tau and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$. $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ as well as $A\beta_{1\text{-}42}/A\beta_{1\text{-}37}$ significantly increased the performance of $A\beta_{1\text{-}42}$ alone to discriminate MCI and controls (Table 5; Supplementary Table 2).

AD and MCI versus non-AD

The best performing biomarkers when comparing AD patients and non-AD dementias were the $A\beta_{1\text{-}42}$/T-tau and $A\beta_{1\text{-}42}$/P-tau_{181P} ratios. The AUC values of the $A\beta_{1\text{-}42}/A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}42}/A\beta_{1\text{-}37}$ ratios reached the 0.800 threshold and were significantly higher than the AUC of $A\beta_{1\text{-}42}$ alone (Table 5; Supplementary Table 3).

When comparing MCI with non-AD dementia patients, the best performing biomarkers were P-tau_{181P} and $A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$. $A\beta_{1\text{-}42}/A\beta_{1\text{-}38}$ and $A\beta_{1\text{-}42}/A\beta_{1\text{-}37}$ also significantly increased the power of $A\beta_{1\text{-}42}$ to discriminate between MCI and non-AD (Table 5; Supplementary Table 3).

AD and MCI versus FTD

The best biomarkers to distinguish AD and FTD were $A\beta_{1\text{-}42}/A\beta_{1\text{-}37}$ and the relative value of $A\beta_{1\text{-}42}$, i.e., the ratio of $A\beta_{1\text{-}42}$ to the sum of all Aβ isoforms (Supplementary Table 4). All ratios increased the performance of $A\beta_{1\text{-}42}$ significantly (Table 5).

Table 2
Correlation of the levels of the Aβ isoforms with MMSE scores, yearly MMSE score change and disease duration in the MCI and AD populations

	$A\beta_{1\text{-}37}$	$A\beta_{1\text{-}38}$	$A\beta_{1\text{-}40}$	$A\beta_{1\text{-}42}$	$A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$
Correlation with MMSE scores					
MCI population					
Correlation Coefficient	0.207	0.150	0.248	0.066	–0.047
p-value	0.182	0.338	0.109	0.672	0.765
n	43	43	43	43	43
AD population					
Correlation Coefficient	0.520	0.431	0.450	0.264	–0.214
p-value	0.000	0.003	0.002	0.073	0.148
n	47	47	47	47	47
Correlation with yearly change in MMSE scores					
MCI population					
Correlation Coefficient	0.239	0.231	0.099	0.362	0.187
p-value	0.148	0.162	0.554	0.026	0.261
n	38	38	38	38	38
AD population					
Correlation Coefficient	−0.135	−0.158	−0.047	−0.197	−0.106
p-value	0.405	0.330	0.772	0.223	0.514
n	40	40	40	40	40
Correlation with disease duration					
MCI population					
Correlation Coefficient	−0.063	0.031	0.013	0.032	−0.023
p-value	0.670	0.832	0.930	0.827	0.878
n	49	49	49	49	49
AD population					
Correlation Coefficient	0.255	0.370	0.388	0.034	−0.397
p-value	0.077	0.009	0.006	0.816	0.005
n	49	49	49	49	49

Median change in MMSE over time in the AD group was −1.2 (−3.8–(−0.3)) over a median time interval of 2.7 years (1.3–4.5). In the MCI population, the median MMSE change was −3.6 (−1.8–(−0.5)) over a median time interval of 3.6 years (2.4–5.8). AD, Alzheimer's disease; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination.

Table 3
Comparison of the Aβ isoforms between *APOE* ε4 carriers and non-carriers

	n	$A\beta_{1\text{-}37}$ (pg/mL)	$A\beta_{1\text{-}38}$ (pg/mL)	$A\beta_{1\text{-}40}$ (pg/mL)	$A\beta_{1\text{-}42}$ (pg/mL)
All groups					
Non-carrier	86	691 (497–894)	2,111 (1,456–2,804)	6,669 (4,426–8,585)	578 (433–824)
Carrier	58	707 (556–943)	2,186 (1,607–2,831)	6,251 (5,018–8,664)	469 (378–548)
p-value		0.273	0.489	0.824	0.000
AD population					
Non-carrier	19	593 (518–882)	1,934 (1,488–2,575)	5,497 (4,329–7,230)	500 (417–600)
Carrier	25	701 (591–888)	2,018 (1,797–2,631)	5,928 (5,018–7,580)	443 (321–508)
p-value		0.118	0.678	0.337	0.110
MCI population					
Non-carrier	24	865 (648–1,081)	2,661 (2,069–3,410)	8,650 (7,004–11,292)	520 (421–621)
Carrier	16	951 (698–1,119)	2,756 (2,268–3,605)	8,553 (7,671–10,104)	495 (330–577)
p-value		0.629	0.679	0.679	0.263
Combination MCI and AD					
Non-carrier	43	742 (536–980)	2,427 (1,644–3,267)	7,230 (5,326–9,535)	513 (417–606)
Carrier	41	727 (637–971)	2,300 (1,856–2,880)	6,843 (5,403–8,956)	462 (321–541)
p-value		0.579	0.961	0.690	0.040
Non-AD population					
Non-carrier	35	562 (414–768)	1,679 (1,108–2,576)	5,388 (3,821–7,667)	714 (509–915)
Carrier	14	558 (369–866)	1,715 (1,225–2,543)	5,120 (3,348–8,005)	502 (397–584)
p-value		0.982	0.965	0.912	0.026

All data are median values with 25th and 75th quartiles between brackets, except for N. AD, Alzheimer's disease; Non-AD, dementia not due to Alzheimer's disease; MCI, mild cognitive impairment.

Table 4
Best performing biomarkers for all differential diagnostic situations based on ROC curve analyses

	AD versus controls					MCI versus controls			
	AUC	cut-off	sens [%]	spec [%]		AUC	cut-off	sens [%]	spec [%]
$A\beta_{1-42}$/T-tau	0.968	<1.708	93.9	92.0	$A\beta_{1-42}$/T-tau	0.922	<1.861	83.7	90.0
$A\beta_{1-42}$/P-tau$_{181P}$	0.930	<10.122	91.8	86.0	$A\beta_{1-42}/A\beta_{1-40}$	0.924	<0.1024	91.8	84.0
	AD versus non-AD					MCI versus non-AD			
$A\beta_{1-42}$/T-tau	0.842	<1.420	87.8	76.0	P-tau$_{181P}$	0.857	>57.50 pg/mL	89.8	74.0
$A\beta_{1-42}$/P-tau$_{181P}$	0.840	<9.440	87.8	72.0	$A\beta_{1-42}/A\beta_{1-40}$	0.845	<0.1022	91.8	62.0
	AD versus FTD					MCI versus FTD			
Relative $A\beta_{1-42}$	0.831	<0.0768	91.8	58.8	Relative $A\beta_{1-42}$	0.875	<0.0491	67.3	94.1
$A\beta_{1-42}/A\beta_{1-37}$	0.851	<0.7351	69.4	94.1	$A\beta_{1-42}/A\beta_{1-40}$	0.882	<0.0944	85.7	75.0
	AD versus VaD					MCI versus VaD			
$A\beta_{1-42}$/T-tau	0.902	<1.589	89.8	87.5	P-tau$_{181P}$	0.881	>59.90 pg/mL	85.7	81.3
$A\beta_{1-42}$/P-tau$_{181P}$	0.912	<8.096	79.6	93.8	$A\beta_{1-42}$/P-tau$_{181P}$	0.860	<8.092	67.3	93.8
	AD versus DLB					MCI versus DLB			
$A\beta_{1-42}/A\beta_{1-38}$	0.843	<0.3957	95.9	70.6	P-tau$_{181P}$	0.855	>49.50 pg/mL	95.9	76.5
$A\beta_{1-42}$/T-tau	0.838	<1.222	83.7	76.5	$A\beta_{1-38}$	0.855	>1850.00 pg/mL	87.8	70.6

AD, Alzheimer's disease; MCI, mild cognitive impairment; AUC, area under the curve; sens, sensitivity; spec, specificity.

Table 5
Significance levels (p-values) of the AUC value comparisons of the Aβ isoforms ratios with $A\beta_{1-42}$ alone

Differential diagnosis	$A\beta_{1-42}/A\beta_{1-40}$	$A\beta_{1-42}/A\beta_{1-38}$	$A\beta_{1-42}/A\beta_{1-37}$
AD versus controls	0.857	0.688	0.918
MCI versus controls	0.002	0.102	0.049
AD versus non-AD	0.113	0.049	0.016
MCI versus non-AD	0.000	0.000	0.000
AD versus FTD	0.025	0.039	0.008
MCI versus FTD	0.000	0.002	0.001
AD versus VaD	0.979	0.899	0.899
MCI versus VaD	0.034	0.133	0.103
AD versus DLB	0.392	0.058	0.061
MCI versus DLB	0.009	0.002	0.002

DeLong tests were performed by using the pROC package in the statistical software package R to compare the AUC values. AUC, area under the curve; AD, Alzheimer's disease; Non-AD, dementia not due to Alzheimer's disease; MCI, mild cognitive impairment; FTD, frontotemporal dementia; VaD, vascular dementia; DLB, dementia with Lewy bodies.

The $A\beta_{1-42}/A\beta_{1-40}$ ratio was the best biomarker to distinguish MCI and FTD. $A\beta_{1-40}$, $A\beta_{1-42}/A\beta_{1-38}$, and $A\beta_{1-42}/A\beta_{1-37}$ also performed well, with all ratios significantly increasing the performance of $A\beta_{1-42}$ (Table 5; Supplementary Table 4).

AD and MCI versus VaD

The best biomarkers to distinguish AD and VaD were $A\beta_{1-42}$/T-tau and $A\beta_{1-42}$/P-tau$_{181P}$, comparable to the AD versus controls situation. The diagnostic accuracy of $A\beta_{1-42}$ was not increased by ratios with the other Aβ isoforms (Table 5; Supplementary Table 5).

The best performing biomarker when differentiating MCI and VaD was P-tau$_{181P}$. The $A\beta_{1-42}/A\beta_{1-40}$ ratio increased the diagnostic accuracy of $A\beta_{1-42}$ significantly (Table 5; Supplementary Table 5).

AD and MCI versus DLB

The best biomarkers to differentiate between AD and DLB were $A\beta_{1-42}/A\beta_{1-38}$ and $A\beta_{1-42}$/T-tau (Supplementary Table 6). Similar performances were found for T-tau, $A\beta_{1-42}/A\beta_{1-37}$, P-tau$_{181P}$, and $A\beta_{1-38}/A\beta_{1-40}$. The diagnostic accuracy of $A\beta_{1-42}$ was not increased by any isoform ratio (Table 5).

The best performing biomarkers to differentiate MCI and DLB were P-tau$_{181P}$ and $A\beta_{1-38}$. The $A\beta_{1-42}/A\beta_{1-38}$ ratio and $A\beta_{1-37}$ performed similarly. The performance of $A\beta_{1-42}$ was substantially increased by the ratios with the other Aβ isoforms (Table 5; Supplementary Table 6).

DISCUSSION

This study was set up to investigate the potential diagnostic value of the Aβ peptides, $A\beta_{1-37}$, $A\beta_{1-38}$, and $A\beta_{1-40}$, for differential dementia diagnosis as well as for early AD diagnosis. In addition, in order to evaluate the added value of the Aβ isoforms, their diagnostic values were compared to the diagnostic values of $A\beta_{1-42}$, T-tau, and P-tau_{181P}.

The four research questions posed in this study will be further discussed in this section. The research questions regarding diagnostic performance of $A\beta_{1-42}/A\beta_{1-40}$ and with regard to the added value of the Aβ isoforms are combined in the subsection 'Diagnostic performance' as they largely coincide.

Correlation with disease severity in AD

When assessing the correlation of the Aβ isoforms as well as the $A\beta_{1-42}/A\beta_{1-40}$ ratio with MMSE scores and disease duration, significant weak to moderate correlations were found in the AD population, except for the $A\beta_{1-42}/A\beta_{1-40}$ ratio. On the other hand, no significant correlations in the MCI population were found. Similar results were found by Mulugeta et al. [13], though they had to combine all investigated patients in order to find significant correlations. Our results imply there might be a correlation of the Aβ isoforms with disease severity in AD and the Aβ isoforms could have a prognostic value in AD. However, this needs further investigation in larger, independent cohorts before any conclusions can be drawn.

Difference between APOE ε4 carriers and non-carriers

The Aβ isoforms, $A\beta_{1-37}$, $A\beta_{1-38}$, and $A\beta_{1-40}$, were not different between ε4 carriers and non-carriers. The levels of $A\beta_{1-42}$ were always lower in ε4 carriers as compared to non-carriers, although this difference was not always significant. In the AD and MCI groups separately, none of the biomarkers were significantly different between carriers and non-carriers. However, when combining both AD and MCI groups, there was a significant difference in the level of $A\beta_{1-42}$ between carriers and non-carriers, which could be a confirmation of results found in a study on autopsy-confirmed AD patients [23]. This change in significance could be caused by the higher power when combining both groups. In the pooled non-AD population a significant difference was found in levels of $A\beta_{1-42}$. This difference might be explained by the fact that ε4 is a risk factor for AD co-pathology in the brain of non-AD dementias as well [11].

Diagnostic performance

$A\beta_{1-42}$, T-tau, and P-tau_{181P} did not differentiate between MCI and AD. This was to be expected, since both groups have AD and these biomarkers have almost reached their maximal increase or decrease in MCI, only changing minimally with disease evolution as from the MCI stage. Interestingly, comparable differences were found regarding the Aβ isoforms when comparing MCI and AD with controls and the non-AD groups. This once more points to the common AD pathophysiology in MCI and AD groups. Based on the ROC analyses, the Aβ isoforms were able to differentiate between MCI and AD groups, although the AUC values were below 0.800. This might be explained by the moderate correlation of the Aβ isoforms with disease severity. Both results might point to changes of these isoforms with AD progression, in contrast to $A\beta_{1-42}$ that remains stable.

When comparing MCI and AD and controls, analyzing Aβ isoforms has an added value, as $A\beta_{1-42}/A\beta_{1-40}$ performed slightly better as compared to $A\beta_{1-42}$ for discriminating AD from controls and substantially better for discriminating MCI and controls. However, since the AUC value of $A\beta_{1-42}/A\beta_{1-40}$ is comparable or lower than those of $A\beta_{1-42}$/T-tau and $A\beta_{1-42}$/P-tau_{181P}, the added diagnostic value of the Aβ isoforms is considered to be limited.

According to our in-house validated $A\beta_{1-42}$ cut-off to discriminate AD from cognitively healthy elderly (638.5 pg/mL), five patients had normal $A\beta_{1-42}$ levels. However, their $A\beta_{1-42}/A\beta_{1-40}$ ratio was decreased as compared to controls, although this difference was not significant ($p > 0.05$), probably due to the small number of patients. We hypothesize that the $A\beta_{1-42}/A\beta_{1-40}$ ratio has a diagnostic value in AD patients having normal values of $A\beta_{1-42}$, since the $A\beta_{1-42}/A\beta_{1-40}$ ratio is decreased in these patients as compared to controls [24, 25], which should be further investigated in larger cohorts.

Given our results for AD versus FTD, analyzing $A\beta_{1-37}$ has an added diagnostic value. It should also be noted our results for $A\beta_{1-38}$ are comparable to those of Gabelle et al. [10]. However, in contrast to Gabelle et al. [10], we found no added value of $A\beta_{1-38}$ for the differential diagnosis of AD and FTD given the relatively low AUC (not exceeding 0.800). In addition, we found similar sensitivity but lower specificity values for $A\beta_{1-42}/A\beta_{1-38}$ as Bibl et al. [9] for discriminating AD and FTD.

To differentiate AD and VaD, the core AD biomarkers performed best. The $A\beta_{1-42}/A\beta_{1-40}$ ratio increased the diagnostic accuracy of $A\beta_{1-42}$ alone, pointing to a diagnostic value of the Aβ isoforms. However, since the AUC values were not higher than those of $A\beta_{1-42}$/T-tau and $A\beta_{1-42}$/P-tau_{181P} in the AD versus VaD situation and P-tau_{181P} and $A\beta_{1-42}$/P-tau_{181P} in the MCI versus VaD situation, the added diagnostic value is limited.

As the AUC value of $A\beta_{1-42}/A\beta_{1-38}$ when comparing AD and DLB was only a little higher than the AUC value of $A\beta_{1-42}$/T-tau, the added diagnostic value of $A\beta_{1-38}$ is only limited. This also held true for MCI and DLB, as the best performing biomarkers P-tau_{181P} and $A\beta_{1-38}$ had equal AUC values. The performance of $A\beta_{1-42}/A\beta_{1-38}$ confirmed earlier findings [12, 13]. Although these previous studies pointed to a disease specific peptide pattern, our study shows that the added diagnostic value of such a pattern is questionable.

Regarding the pooled non-AD group, the Aβ isoforms had no added diagnostic value, which is probably due to the fact this group is a combination of three pathophysiologically different disorders and the Aβ isoforms might behave differently in these different neurodegenerative disorders. Although the ratios of $A\beta_{1-42}$ increased the discriminative power of $A\beta_{1-42}$, analyzing Aβ isoforms did not have an added value for differentiating MCI or AD from pooled non-AD dementias as the routine biomarkers still performed better.

In summary, the diagnostic performance of $A\beta_{1-42}$ increased when calculating the $A\beta_{1-42}/A\beta_{1-40}$ ratio. This was the case when comparing the AD groups with FTD and when comparing MCI with non-AD in general, but also FTD, DLB, and VaD separately and controls. Furthermore it was shown there is an added diagnostic value of the Aβ isoforms for differentiating AD and FTD. The added diagnostic value was only limited when comparing the AD groups with VaD, DLB, and controls. Rather, altered $A\beta_{1-42}/A\beta_{1-40}$ ratios in CSF might be specific for AD since both peptides are representative for the two possible cleavage routes of the protease γ-secretase [26].

The present findings should be replicated and confirmed in a larger and independent cohort of patients, including autopsy-confirmed cases.

CONCLUSION

In conclusion, the Aβ isoforms could help in some differential diagnostic situations. Adding the Aβ isoforms to the current biomarker panel could enhance diagnostic accuracy. This is the case for discriminating AD from FTD and MCI from all other diagnoses and to diagnose AD in patients with normal $A\beta_{1-42}$ levels. In contrast to $A\beta_{1-42}$, Aβ isoforms seem to be correlated with disease severity in AD.

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SUPPLEMENTARY MATERIAL

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