Journal of Alzheimer's Disease 21 (2010) 1119–1128
DOI 10.3233/JAD-2010-100207
IOS Press

Evaluation of CSF Biomarkers as Predictors of Alzheimer's Disease: A Clinical Follow-Up Study of 4.7 Years

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Handling Associate Editor: Mony de Leon

Accepted 13 May 2010

Abstract. In this study, we determined the diagnostic accuracy of cerebrospinal fluid (CSF) biomarkers to predict development of Alzheimer's disease (AD) within five years in patients with mild cognitive impairment (MCI). To do so, the levels of tau, phosphorylated tau, Aβ_{42}, Aβ_{40}, Aβ_{38}, sAβPPα, and sAβPPβ were analyzed in 327 CSF samples obtained at baseline from patients with AD ($n = 94$), MCI ($n = 166$), depressive disorder ($n = 29$), and cognitively healthy controls ($n = 38$). In the cohort with MCI at baseline, 33% subsequently developed AD and 16% developed other types of dementia; however, 51% were still cognitively stable after a follow-up of 4.7 years (range 3.0–7.2). Optimal cut-offs for each biomarker or combinations of biomarkers were defined in the AD, control, and depressive disorder groups. Several combinations resulted in sensitivity and specificity levels $>$ 85% for differentiation of AD from controls and depressive disorder. Using the previously established cut-offs, a combination of Aβ_{42} and tau could predict future development of AD in MCI patients with a sensitivity of 88%, specificity 82%, positive predictive value 71%, and negative predictive value 94%. MCI patients with both low Aβ_{42} and high tau levels had a substantially increased risk of developing AD (OR 20; 95% CI 6–58), even after adjustment for confounding factors. Ultimately, CSF biomarkers can stratify MCI patients into those with very low or high risk for future development of AD. However, the specificities and positive predictive values are still too low to be able to diagnose AD before the patients fulfill the clinical criteria.

Keywords: Alzheimer's disease, amyloid-β, biomarkers, cerebrospinal fluid, early diagnosis, mild cognitive impairment

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia, and the global prevalence is estimated to increase considerably over the next few decades [1]. Pathological hallmarks of AD are senile plaques containing amyloid-β (Aβ) and neurofibrillary tangles containing tau protein. According to the amyloid cascade hypothesis, the 42 amino acid residues long isoform of Aβ_{1-42}(Aβ_{42}) initiates a cascade of pathological events in AD, ultimately resulting in synaptic dysfunction, neuronal loss, and brain atrophy [2–4]. Aβ_{42} is produced through orchestrated β-secretase and

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ISSN 1387-2877/10/$27.50

γ-secretase cleavages of the large trans-membranous amyloid-β protein precursor (AβPP). Through variability in the γ-secretase cleavage site, AβPP processing may also yield peptides with other C-terminal amino acids, such as Aβ_{1-38} and Aβ_{1-40}. Processing of AβPP also produces the N-terminal soluble fragments, including sAβPPα and sAβPPβ [2–4].

The underlying disease process probably starts decades before the clinical onset of the disease [4–6]. Disease modifying therapies such as γ- and β-secretase inhibitors or vaccination regimens are more likely to be effective if initiated during the early phases of AD when the neurodegeneration is not too severe. Therefore, there is an urgent need for methods to accurately detect AD pathology before the affected subjects have become demented [4–6].

Mild cognitive impairment (MCI) is a heterogeneous syndrome, where approximately 30-50% of the patients will develop AD within five years [7]. Several studies on cohorts with MCI have shown that abnormal levels of cerebrospinal fluid (CSF) tau and Aβ_{1-42} are associated with subsequent development of AD [8–17]. However, many of these studies have had relatively short periods of clinical follow-up. In MCI populations, it probably takes at least five years before most of the patients with preclinical AD have become demented and thereby may be diagnosed with clinical AD. Studies with short follow-up time will therefore underestimate the true prevalence of incipient AD. Consequently, if a biomarker can detect AD pathology many years before conversion to dementia will occur, the estimated specificity (and positive predictive value) of that biomarker will be falsely low in such studies. To our knowledge only one study has previously investigated the diagnostic value of CSF biomarkers in a MCI population, who have been followed up clinically after an average of more than four years [10]. A limitation of that particular study was however that the cut-off levels for CSF tau and Aβ_{42} were established in the same cohort of patients that was then used to evaluate the diagnostic performance of the biomarkers to detect incipient AD.

In the presents study, the levels of total tau (T-tau), phosphorylated tau (P-tau), Aβ_{42}, Aβ_{40}, Aβ38, sAβPPα, and sAβPPβ were analyzed in 327 CSF samples obtained at baseline from patients with AD (n = 94), MCI (n = 166), depressive disorder (n = 29), and cognitively healthy controls (n = 38). The included patients with MCI at baseline have either subsequently developed a certain type of dementia or they have been cognitively stable for an average of 4.7 years (range 3.0–7.2 years). Following recommendations in the STARD criteria [18], we established the cut-off levels of the individual biomarkers or combinations of biomarkers when differentiating AD patients from controls and patients with depressive disorder. The ability of the biomarkers to predict future development of AD was then evaluated in the MCI cohort.

MATERIALS AND METHODS

Patients with AD, MCI, depression, and cognitively healthy controls

In the present study, performed at the Memory clinic of Malmö University Hospital, CSF samples were included from patients with AD (n = 94), MCI (n = 166), depressive disorder (n = 29), and from cognitively healthy controls (n = 38). At the clinical baseline visit, physicians with special interest in cognitive disorders performed a thorough physical, neurological, and psychiatric examination, as well as a clinical interview focusing on cognitive symptoms and ADL function. Furthermore, cognitive tests, analysis of apolipoprotein E (*APOE*) genotype, and imaging of the brain were done. Patients who received an AD diagnosis at baseline had to meet the DSM-IIIR criteria of dementia [19] and the criteria of probable AD defined by NINCDS-ADRDA [20]. The AD patients were followed over time with repeated clinical evaluations, which increases the clinical diagnostic accuracy.

Patients with MCI at baseline had to fulfill the criteria advocated by Petersen and colleagues [7], including: 1) memory complaint, preferably corroborated by an informant; 2) objective memory impairment adjusted for age and education, as judged by the physician; 3) preservation of general cognitive functioning, as determined by the clinician's judgment based on a structured interview with the patient and a Mini-Mental Status Examination (MMSE) score greater than or equal to 24; 4) zero or minimal impairment of daily life activities; and 5) not fulfilling the DSM-IIIR criteria of dementia. Patients with other causes of cognitive impairment, including subdural hematoma, brain tumor, CNS infection, schizophrenia, major depressive episode, and current alcohol abuse were not included. However, MCI subjects were allowed to show signs of white matter changes or silent brain infarcts, because these changes are frequent in elderly subjects with or without cognitive deficits. Similarly, MCI patients with mild to moderate depressive symptoms and low plasma concentrations of vitamin B12 or folate were not excluded.

The included patients with MCI at baseline have either subsequently developed a certain type of dementia or they have been cognitively stable for an average of 4.7 years (range 3.0–7.2 years). The patients with MCI who received a diagnosis of AD during clinical follow-up were required to meet the same criteria as those diagnosed with AD already at baseline (see above). Subjects who during follow-up were diagnosed as having vascular dementia (VaD) fulfilled the DSM-IIIR criteria of dementia and the requirements for probable VaD by NINDS-AIREN [21]. VaD of the subcortical type were diagnosed according to the recommendations by Erkinjuntti and co-workers [22]. The consensus criteria by McKeith and coworkers [23] were applied when diagnosing dementia with Lewy bodies.

Patients with depressive disorder fulfilled the DSM-IV criteria of depression [24]. They did not fulfill the criteria of MCI or dementia at the baseline visit or during clinical follow-up.

The control population consisted of healthy elderly volunteers, who were recruited in the city of Malmö, Sweden. Inclusion criteria were (i) absence of memory complaints or any other cognitive symptoms; (ii) preservation of general cognitive functioning; and (iii) no active neurological or psychiatric disease.

The clinical diagnoses of all patients were reviewed by a consensus group consisting of three medical doctors (JH, LM, and OH) with special interest in cognitive disorders. Out of the 327 subjects included in the present study, data from 30 cases have previously been published [16].

The study was approved by the Ethics Committee at the University of Lund and the patients and/or their relatives gave their informed consent (for research).

Analysis of baseline CSF

CSF was collected in polypropylene tubes, stored at $-80°$C and analyzed after the clinical follow-up of the study was completed. The procedure followed The Alzheimer's Association Flow Chart for LP and CSF sample processing [25]. The levels of total tau, tau phosphorylated at Thr_{181} (P-tau) and Aβ_{42} were determined using xMAP technology as previously described [26]. In the present study the results were not adjusted to match those obtained from conventional ELISA measurements. In eight cases (7 MCI and 1 depression), xMAP analysis resulted in technical errors and these samples were excluded from the study. CSF Aβ_{38}, Aβ_{40}, and Aβ_{42} levels were analyzed by electrochemiluminescence technology (Meso Scale Discovery [MSD], Gaithersburg, Maryland, USA), using the MS6000 Human Abeta 3-Plex Ultra-Sensitive Kit, following the recommendations by the manufacturer. Measurement of β-secretase cleaved soluble AβPP (sAβPPβ) and α-secretase cleaved soluble AβPP (sAβPPα) in CSF was performed by electrochemiluminescence technology (Meso Scale Discovery, Gaithersburg, Maryland, USA), using the MS6000 Human sAβPPα/sAβPPβKit, following the recommendations by the manufacturer. In 7 cases (4 MCI, 2 AD, and 1 depression) MSD analysis resulted in technical errors for the analysis of Aβ_{42}. Data from these samples were included in the manuscript, except when MSD Aβ_{42} levels were evaluated.

Statistical analyses

The statistical analyses were accomplished with SPSS for Windows, version 17.0.1 (SPSS Inc, Chicago, Illinois), unless otherwise specified. To compare demographic and CSF baseline data between groups, a non-parametric Kruskal-Wallis test was performed followed by Mann-Whitney U-test for continuous variables with correction for multiple comparisons (see Table 1). Pearson's x^2 test was used for dichotomous variables. The Spearman correlation coefficient was used for bivariate correlation analyses. Receiver operating characteristic (ROC) curves were drawn by plotting the true-positive fraction (sensitivity) against false-positive fraction (100% – specificity). When evaluating the ability of CSF biomarkers to distinguish patients with AD from controls and cases with depression, the latter two were treated as one group as CSF biomarker levels did not differ significantly between these groups. The area under the curve (AUC) was calculated using Medcalc for Windows, version 11.1 (Medcalc Software, Mariakerke, Belgium). The MedCalc software was also used to assess the statistical difference of diagnostic performance between the different biomarkers or biomarker ratios (continuous variables only), according to the method developed by DeLong et al. [27]. We used the cut-off levels of the individual biomarkers or combinations of biomarkers that resulted in the highest Youden's index (sensitivity+specificity-1) [28] when differentiating AD patients from controls and patients with depressive disorder. It is important to note that the optimal cut-offs for a certain biomarker (or a certain ratio of biomarkers) is usually not the exactly same when the biomarker (or biomarker ratio) is used alone as a continuous variable compared to when it is used in combination with another biomarker, because

Table 1
Demographic data and levels of CSF biomarkers of included subjects with successful xMAP analysis

	Controls ($n = 38$)	Depression ($n = 28$)	AD ($n = 94$)	Stable MCI ($n = 82$)	MCI-AD ($n = 52$)	MCI-other ($n = 25$)
Females	27 (71%)	14 (50%)	61 (65%)	46 (56%)	34 (65%)	11 (44%)
Age, y	77 (8.2)	58 (8.4)[a]	77 (7.1)[b]	69 (7.5)[a,b,c]	76 (7.8)[b,d]	72 (6.7)[b,c]
Carrier of *APOE ε4*	10 (26%)	8 (29%)	64 (68%)[a,b]	37 (45%)[c]	40 (77%)[a,b,d]	12 (48%)
MMSE score at baseline	28.3 (1.8)	27.9 (2.2)	19.0 (3.9)[a,b]	28.3 (1.3)[c]	26.1 (1.6)[a,b,c,d]	26.9 (2.0)[c]
Aβ_{42xMAP}	265 (74)	271 (53)	158 (41)[a,b]	249 (64)[c]	156 (57)[a,b,d]	221 (70)[c,e]
T-tau	91 (49)	54 (26)[a]	177 (113)[a,b]	81 (48)[c]	143 (68)[a,b,d]	82 (44)[c,e]
P-tau	31 (17)	29 (11)	54 (32)[a,b]	31 (16)[c]	51 (22)[a,b,d]	30 (13)[c,e]
Aβ42$_{MSD}$	1019 (435)	862 (386)	480 (247)[a,b]	799 (391)[c]	488 (255)[a,b,d]	688 (492)[a]
Aβ_{40}	11036 (2613)	8235 (2535)[a]	9384 (2653)[a]	9029 (2726)[a]	9133 (3016)[a]	7746 (2734)[a]
Aβ_{38}	2284 (833)	1570 (805)[a]	1864 (748)	1807 (758)[a]	1950 (747)	1480 (734)[a]
sAβPPα	787 (350)	689 (274)	767 (371)	720 (353)	802 (360)	589 (264)
sAβPPβ	244 (112)	193 (80)	238 (125)	237 (113)	249 (109)	192 (90)

Data are mean (SD) or number (%). CSF biomarker levels are given in pg/ml.
[a] $p < 0.003$ vs. Controls; [b] $p < 0.003$ vs. Depression; [c] $p < 0.003$ vs AD; [d] $p < 0.003$ vs. Stable MCI; [e] $p < 0.003$ vs MCI-AD.
Abbreviations: Stable MCI, MCI patients with stable cognitive functions during a follow-up period of 3.0–7.2 years; MCI-AD, MCI patients who developed Alzheimer's disease during follow-up; APOE, apolipoprotein E; MMSE, Mini-Mental State Examination. Aβ_{42xMAP}, Aβ_{42} quantified with xMAP technology; Aβ_{42MSD}, Aβ_{42} quantified with MSD technology; T-tau, total tau; P-tau, phosphorylated tau.

the latter approach includes two different continuous variables with two different cut-offs. Using the previously established cut-offs, the diagnostic accuracy of the biomarkers or combination of biomarkers were then evaluated in the MCI-cohort. Binary logistic regression models were used to study whether abnormal levels of CSF biomarkers in MCI patients were associated with subsequent development of AD. The analyses were carried out with adjustment for potential confounding of the baseline demographic variables, i.e., age, gender, MMSE, and *APOE ε4* carrier status (carriers of zero, one, or two *APOE ε4* alleles). Furthermore, a backward stepwise binary regression model was used to simultaneously estimate the impact of the baseline variables (pathological CSF, age, gender, MMSE total score, and *APOE ε4* carrier status) on the conversion to AD among MCI subjects.

RESULTS

Subjects and biomarker levels

In the present study, CSF samples were included from patients with AD ($n = 94$), MCI ($n = 166$), depressive disorder ($n = 29$), and from cognitively healthy controls ($n = 38$). Out of 166 patients with MCI at baseline, 85 (51%) were cognitively stable when clinically followed up after an average of 4.7 years (3.0–7.2 years). However, 55 (33%) of the MCI patients subsequently developed AD and 26 (16%) developed other types of dementias, including vascular dementia ($n = 17$), dementia with Lewy bodies ($n = 4$), progressive supranuclear palsy ($n = 3$), semantic dementia ($n = 1$), and normal pressure hydrocephalus ($n = 1$). In eight cases (7 MCI and 1 depression) xMAP analysis of Aβ_{42}, T-tau, and P-tau were missing, and these samples were excluded from the study. The demographic data and biomarker levels of all included subjects are shown in Table 1. The frequencies of *APOE ε4* carriers were significantly higher, and the baseline MMSE scores were significantly lower, in the groups with AD patients and MCI patients who subsequently developed AD (MCI-AD) (Table 1). Moreover, both AD patients and the MCI patients who later on developed AD (MCI-AD) had higher baseline levels of T-tau and P-tau and lower levels of Aβ_{42} compared with cognitively healthy controls, patients with depressive disorder, cognitively stable MCI patients, and MCI patients who developed other forms of dementias than AD ($p < 0.0001$) (Table 1). In the control group, there were no significant correlations between CSF biomarkers and age or baseline MMSE score.

Tau and P-tau did not significantly differ between carriers of zero, one, or two *APOE ε4* alleles in the studied diagnostic groups. Figure 1 depicts the levels of Aβ_{42} in the different diagnostic groups, stratified by the number of *APOE ε4* alleles (see also Fig. 2 for specific *APOE* genotypes). In the control and depression groups, there were no significant differences in Aβ_{42} levels between subjects who were carriers of zero, one, or two *APOE ε4* alleles; however, in the AD and MCI-

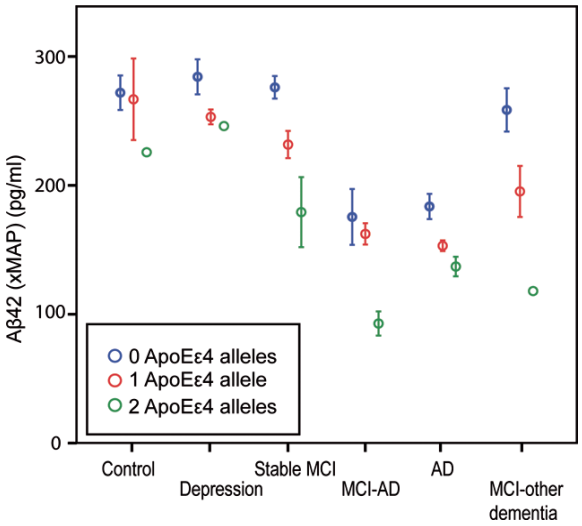
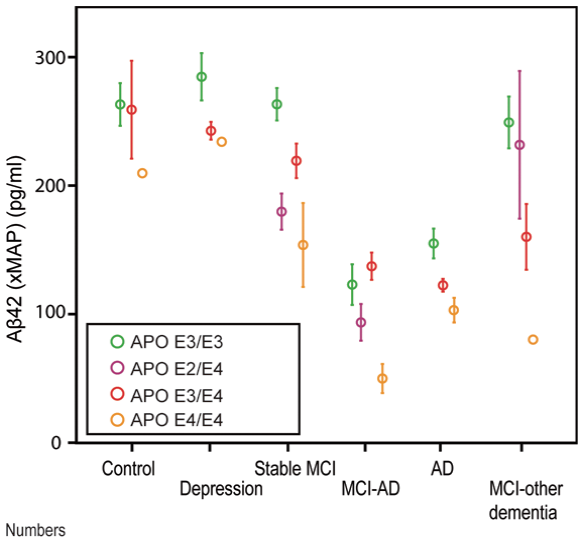


Fig. 1. Figure 1 depicts the levels of Aβ_{42} in the different diagnostic groups, stratified by the number of *APOE ε4* alleles. The levels of Aβ_{42} differed significantly between subjects with *AD* when compared to controls, cases with depression, or cases with stable MCI, respectively, even when analyzing the subgroups with zero or one *APOE ε4* alleles separately (p<0.01). Similarly, the levels of Aβ_{42} differed significantly between subjects with *MCI-AD* when compared to controls, cases with depression, or cases with stable MCI, respectively, even when analyzing the subgroups with zero or one *APOE ε4* alleles separately (p<0.01). Error bars represent SEM.

AD groups, Aβ_{42} levels were significantly lower in subjects with two alleles of *APOE ε4* compared to those with zero alleles ($p < 0.01$). Importantly, the levels of Aβ_{42} differed significantly between subjects with AD or MCI-AD when compared to controls, cases with depression or cases with stable MCI, respectively, even when analyzing the subgroups with zero or one *APOE ε4* alleles separately ($p < 0.01$) (Table 2). The subgroup with two *APOE ε4* alleles could not be analyzed separately as it contained only 1 control and 1 case with depression and five cases with either stable MCI or MCI-AD. However, when analyzing individuals with either one or two *APOE ε4* alleles as a group, we found very significant differences in the levels of Aβ_{42} between subjects with AD or MCI-AD when compared to controls, cases with depression or stable MCI cases, respectively ($p < 0.001$).

Biomarkers for differentiation of AD from controls and depression

ROC analysis resulted in areas under the ROC curves (AUC) above 0.87 for several biomarkers or ratios of biomarkers when differentiating AD from controls and depression (Table 3). None of the biomarkers resulted



Numbers						
E3/E3	27	17	36	9	27	13
E2/E4	0	0	2	3	0	2
E3/E4	9	7	30	32	52	9
E4/E4	1	1	5	5	12	1

Fig. 2. The figure depicts the levels of Aβ_{42} in the different diagnostic groups, stratified by the main *APOE* genotypes. The levels of Aβ_{42} differed significantly between subjects with *AD* when compared to controls, cases with depression or cases with stable MCI, respectively, even when analyzing the subgroups with E3/E3 or E3/E4 separately (p<0.01). Similarly, the levels of Aβ_{42} differed significantly between subjects with *MCI-AD* when compared to controls, cases with depression or cases with stable MCI, respectively, even when analyzing the subgroups with E3/E3 or E3/E4 separately (p<0.01). The groups with E2/E4 and E4/E4 genotypes are too small to be able to reliably study effect of diagnosis on Aβ_{42} levels. Error bars represent SEM.

in significantly greater AUCs than that of Aβ_{42} alone, when it was quantified with xMAP technology (Table 3) ($p > 0.05$). However, both Aβ_{42} and T-tau performed better than P-tau ($p < 0.001$). Moreover, the T-tau/Aβ_{40} ratio was superior to T-tau alone ($p < 0.001$) when the biomarkers were used as continuous variables.

Youden's index was then used to establish optimal cut-offs when separating AD patients from controls and patients with depression, resulting in sensitivity and specificity levels $> 85\%$ for many of the combinations of biomarkers (Table 3). The highest Youden's index (0.80) was achieved when either using the Aβ_{42MSD}/Tau ratio (< 7.3) or when using the combination of Aβ_{42xMAP} (< 209 ng/ml) and T-tau/Aβ_{40} ratio (> 0.088) (Table 3, Fig. 3).

Prediction of AD among MCI patients

ROC analysis resulted in AUCs $\geqslant 0.84$ for several biomarkers or ratios of biomarkers when predicting

Table 2
Baseline levels of Aβ_{42}, age, and MMSE scores in the different diagnostic groups, stratified by the number of *APOE ε4* alleles

	Controls	Depression	Stable MCI	MCI-AD	AD	MCI-other
Aβ_{42}						
0 *APOEε4* alleles	267 ± 69	280 ± 60	272 ± 57	$173 \pm 74^{a,b,c}$	$180 \pm 53^{a,b,c}$	$254 \pm 59^{d,e}$
	($n = 28$)	($n = 20$)	($n = 45$)	($n = 12$)	($n = 30$)	($n = 13$)
1 *APOEε4* allele	262 ± 93	249 ± 15	228 ± 59	$160 \pm 48^{a,b,c}$	$150 \pm 29^{a,b,c}$	192 ± 64
	($n = 9$)	($n = 7$)	($n = 32$)	($n = 35$)	($n = 52$)	($n = 11$)
2 *APOEε4* alleles	222	242	176 ± 60	91 ± 21^{f}	135 ± 26	116
	($n = 1$)	($n = 1$)	($n = 5$)	($n = 5$)	($n = 12$)	($n = 1$)
Age						
0 *APOEε4* alleles	76 ± 7	58 ± 9^{a}	70 ± 8^{b}	$79 \pm 8^{b,c}$	75 ± 8^{b}	$72 \pm 6^{b,e}$
1 *APOEε4* allele	80 ± 11	56 ± 3^{a}	$69 \pm 7^{a,b}$	$75 \pm 8^{b,c}$	$78 \pm 6^{b,c}$	71 ± 8^{b}
2 *APOEε4* alleles	82	75	70 ± 7	73 ± 7	74 ± 7	84

Data are mean (SD) or number (%). Aβ_{42} levels are given in pg/ml.
[a] $p < 0.003$ vs. Controls; [b] $p < 0.003$ vs. Depression; [c] $p < 0.003$ vs. Stable MCI; [d] $p < 0.003$ vs. AD; [e] $p < 0.003$ vs. MCI-AD; [f] $p < 0.003$ vs. Stable MCI.
Abbreviations: Stable MCI, MCI patients with stable cognitive functions during a follow-up period of 3.0–7.2 years; MCI-AD, MCI patients who developed Alzheimer's disease during follow-up; *APOE*, apolipoprotein E; Aβ_{42}, Aβ_{42} quantified with xMAP technology.

Table 3
Diagnostic accuracy of CSF biomarkers for differentiation of patients with Alzheimer's disease from cognitively healthy controls and patients with depression

	AUC (95% CI)	Optimal cut off(s)	Youden's index	Sensitivity, %	Specificity, %
Biomarkers as continuous variables					
Aβ_{42MSD}/Tau	0.94 (0.89–0.97)	< 7.3	0.80	97	83
Aβ_{42MSD}/Aβ_{40}	0.91 (0.86–0.95)	< 0.069	0.79	93	86
Aβ_{42xMAP}/T-tau	0.94 (0.89–0.97)	< 2.5	0.78	96	82
T-tau/Aβ_{40}	0.94 (0.90–0.97)	> 0.010	0.75	89	86
Aβ_{42xMAP}	0.92 (0.87–0.96)	< 209	0.74	91	83
Aβ_{42MSD}/Aβ_{38}	0.89 (0.83–0.93)	< 0.37	0.69	87	82
Aβ_{42MSD}/P-tau	0.89 (0.83–0.93)	< 21	0.69	87	82
Aβ_{42xMAP}/P-tau	0.88 (0.82–0.93)	< 6.6	0.66	83	83
Aβ_{42MSD}	0.88 (0.82–0.93)	< 523	0.62	73	89
T-tau	0.87 (0.81–0.92)	> 100	0.61	78	83
Aβ_{42xMAP}/Aβ_{40}	0.83 (0.76–0.88)	< 0.024	0.56	86	70
P-tau	0.74 (0.67–0.80)	> 51	0.40	46	94
Combinations of two continuous variables					
Aβ_{42xMAP} and T-tau/Aβ_{40}	–	< 209 and > 0.088	0.80	88	92
Aβ_{42xMAP} and T-tau	–	< 209 and > 62	0.77	89	88
Aβ_{42MSD}/Aβ_{40} and T-tau	–	< 0.069 and > 62	0.77	91	86
Aβ_{42xMAP}/P-tau and T-tau	–	< 6.6 and > 62	0.67	82	85

The variables are ordered by their diagnostic performance according to Youden's index.
Abbreviations: AUC, area under the ROC curve; Aβ_{42xMAP}, Aβ_{42} quantified with xMAP technology; Aβ_{42MSD}, Aβ_{42} quantified with MSD technology; T-tau, total tau; P-tau, phosphorylated tau.

subsequent development of AD in patients with MCI (Table 4). Also in the MCI cohort, none of the continuous biomarker variables resulted in significantly greater AUCs than that of Aβ_{42} alone when it was quantified with xMAP technology ($p > 0.05$) (Table 4). Corroborating earlier findings [11], the predictive values of Aβ_{42}/Aβ_{40} and Aβ_{42}/Aβ_{38} ratios were higher compared to that of Aβ_{42} alone ($p < 0.01$) (Table 4) when using the MSD technology to quantify Aβ in MCI patients. However, Aβ_{42} quantified with xMAP technology performed as well as the Aβ_{42}/Aβ_{40} (MSD) ratio.

Following recommendations in the STARD criteria, we applied the same cut-off levels previously established in the AD, control, and depression groups to the cohort with MCI patients.0 This approach resulted in sensitivity and specificity levels above 80% for several of the combinations of biomarkers. The highest Youden's index (0.70) was achieved using a combination of Aβ_{42} (< 209 ng/ml) and T-tau (> 62 ng/ml) (Table 4; Fig. 3). This combination could predict future development of AD in MCI patients with a sensitivity of 88%, specificity 82%, positive predictive val-

Table 4
The ability of CSF biomarkers to predict subsequent development of Alzheimer's disease in patients with mild cognitive impairment (MCI) when using the same cut offs as in Table 3

	AUC	Youden's index	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Biomarkers as continuous variables						
$A\beta_{42MSD}$/Tau	0.88 (0.82–0.93)	0.62	90	72	62	94
$A\beta_{42xMAP}$	0.84 (0.77–0.89)	0.61	90	71	60	94
$A\beta_{42xMAP}$/T-tau	0.88 (0.82–0.93)	0.61	90	71	60	94
$A\beta_{42xMAP}$/P-tau	0.87 (0.81–0.92)	0.59	87	72	61	92
$A\beta_{42MSD}/A\beta_{38}$	0.85 (0.79–0.91)	0.59	88	71	58	93
$A\beta_{42MSD}/A\beta_{40}$	0.86 (0.79–0.91)	0.56	85	71	56	92
T-tau/$A\beta_{40}$	0.84 (0.77–0.89)	0.56	83	73	60	90
$A\beta_{42xMAP}/A\beta_{40}$	0.84 (0.78–0.90)	0.51	86	65	54	90
T-tau	0.81 (0.74–0.87)	0.50	73	77	60	85
$A\beta_{42MSD}$/P-tau	0.84 (0.78–0.90)	0.48	87	61	52	90
$A\beta_{42MSD}$	0.73 (0.66–0.80)	0.38	67	71	50	83
P-tau	0.78 (0.71–0.84)	0.32	42	90	67	76
Combinations of two continuous variables						
$A\beta_{42xMAP}$ and T-tau	–	0.70	88	82	71	94
$A\beta_{42xMAP}$/P-tau and T-tau	–	0.66	87	79	66	92
$A\beta_{42MSD}/A\beta_{40}$ *and* T-tau	–	0.63	85	78	63	92
$A\beta_{42xMAP}$ *and* T-tau/Aβ40	–	0.62	81	81	68	90

The variables are ordered by their diagnostic performance according to Youden's index.
Abbreviations: AUC, area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value; $A\beta_{42xMAP}$, $A\beta_{42}$ quantified with xMAP technology; $A\beta_{42MSD}$, $A\beta_{42}$ quantified with MSD technology; T-tau, total tau; P-tau, phosphorylated tau.

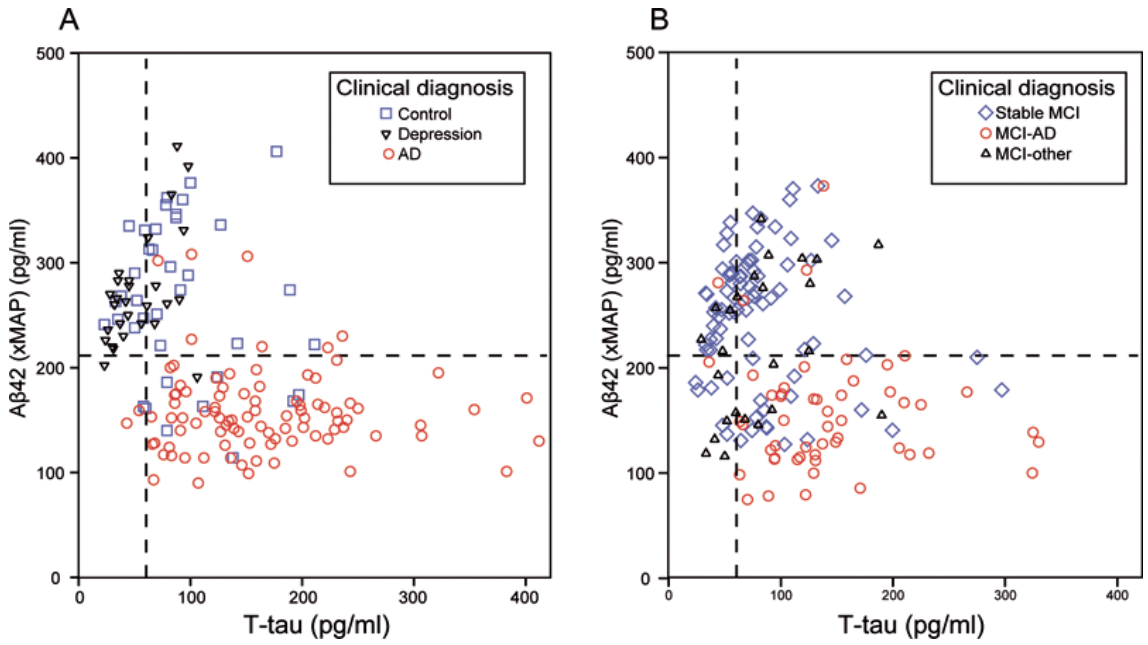


Fig. 3. Panel A depicts the scatter plot of CSF $A\beta_{42}$ and T-tau in AD patients, controls and patients with depressive disorder. Panel B shows the scatter plot of CSF $A\beta_{42}$ and T-tau in all patients with mild cognitive disorder, divided into those who did not progress to dementia (stable MCI), those who developed Alzheimer's disease (MCI-AD), and those who were diagnosed with other types of dementia (MCI-other) during follow-up. The horizontal hatched lines represent the cut-off value for $A\beta_{42}$ and the vertical hatched lines represent the cut-off for T-tau.

ue 71%, and negative predictive value 94% (Table 4; Fig. 3). Moreover, logistic regression analyses revealed that MCI patients with both low $A\beta_{42}$ and high T-tau levels had a substantially increased risk of developing AD (OR 20; 95% CI 6–58), even after adjustment for age, gender, baseline MMSE score, and *APOE ε4* carrier status. Furthermore, the risk factors were analyzed simultaneously using a multivariate backward

stepwise logistic regression model. In this stepwise model, pathological CSF (both low Aβ_{42} and high T-tau levels), and baseline MMSE score were significantly associated with progression to AD among MCI subjects, while the other risk factors (*APOE ε4* carrier status, age, and gender) did not contribute to the explanatory power of the model. As expected, the same results were obtained when using a multivariate *forward* stepwise logistic regression model (data not shown).

DISCUSSION

We found that several combinations of biomarkers exhibited sensitivity and specificity levels above 85% for differentiation of AD from controls and depressive disorder when optimal cut-offs were defined in the same diagnostic groups. Using the previously established cut-offs, a combination of Aβ_{42} and T-tau could predict future development of AD in MCI patients with a sensitivity of 88%, specificity 82%, positive predictive value 71%, and negative predictive value 94%. The MCI patients with both low Aβ_{42} and high tau levels had a substantially increased risk of developing AD (OR 20; 95% CI 6–58), even after adjustment for potentially confounding factors such as *APOE ε4* carrier status.

Corroborating earlier data [10,14,16], the levels of Aβ_{42}, T-tau, and P-tau did not differ between individuals affected with AD in the MCI stage and those that were already demented, indicating that these biomarkers become substantially altered very early in the disease process of AD. Interestingly, there is evidence of changes of CSF tau and Aβ_{42} in the even earlier, pre-symptomatic phase of the AD [29–31]. Moreover, longitudinal studies with repeated CSF taps in patients with AD show that CSF biomarkers do not change substantially over time in demented AD patients [32–35], even though there might be a slight increase in T-tau levels over time [32]. These results strongly indicate that CSF tau and Aβ_{42}, like amyloid imaging, are diagnostic markers that change during the presymptomatic stages of the disease, and then are quite stable over time [5]. Accordingly, CSF biomarkers should primarily be used as diagnostic markers detecting the underlying disease state, and other methods, such as cognitive tests or measures of brain atrophy or regional cerebral blood flow, are needed to reflect the stage of the disease.

In the present study, the MCI patients who did not develop any dementia disorder were followed clinically for an average of 4.7 years (range 3.0–7.2), which is similar to our previously published cohort with MCI [10]. An even more extensive follow-up period of the cognitively stable MCI patients might increase the specificity (and positive predictive value) of CSF biomarkers further, since some of the included cognitively stable MCI patients with abnormal CSF might still develop AD in the future. We therefore intend to continue to follow this cohort of MCI patients over time.

According to the STARD recommendations [18], we established the cut-offs of each biomarkers or combination of biomarkers in an independent cohort and then applied them in the population with MCI. This was not the case in our previously published MCI study [10], and probably explains the somewhat lower predictive accuracy of CSF tau and Aβ_{42} in the present study. However, it is important to note that it is very difficult to use cut-off levels from other studies, because the absolute levels of T-tau, P-tau, and Aβ_{42} differ between studies. This is probably due to unsatisfactory standardization of biomarker levels between different laboratories and different handling of CSF samples before analysis [25]. This problem was highlighted in a recent multi-centre study showing quite large differences in CSF biomarker levels (especially Aβ_{42}) between clinical sites [16]. In spite of these problems, three multi-center studies, with average follow-up times of 1–3 years, have shown that CSF biomarkers are associated with future development of AD in cohorts with MCI [9,14,16].

The present study suggests that CSF biomarkers can stratify MCI patients into those with very low or high risk for future development of AD. Therefore, CSF biomarkers could be used to identify MCI patients who are at high risk of developing AD for therapeutic trials investigating new disease-modifying therapies. Using this approach, one would need to include a lower number of MCI patients in such treatment trials. In addition, beneficial effects of treatments are likely to be easier to detect in MCI cohorts with a very high prevalence of incipient AD. Finally, it could be considered more ethical to not include patients with a very low risk of AD in trials that could cause side effects.

It has recently been proposed that AD might be diagnosed before the patients fulfill the established clinical criteria of AD with the help of certain examinations, including CSF biomarkers, magnetic resonance imaging, and positron emission tomography [36]. However, the present study indicates that the positive predictive value (71%) of tau and Aβ_{42} for prediction of AD is still too low to be able to diagnose AD before the pa-

tients fulfill the clinical criteria. Even though the cognitively stable MCI patients were followed for almost an average of 5 years, it is still unclear if all MCI cases with abnormal CSF biomarkers will develop AD in the future. However, the positive predictive value of CSF biomarkers can be increased if they are combined with other diagnostic methods, such as measurements of regional cerebral blood flow [37]. Interestingly, the negative predictive value of tau and Aβ_{42} was found to be very high (94%) in the MCI cohort, indicating that MCI patients with normal CSF Aβ_{42} or tau levels do not run an increased risk of developing AD compared to the normal population. If used in the clinical practice, patients with normal CSF biomarkers might be reassured and they would probably not need an extended and costly clinical follow-up.

ACKNOWLEDGMENTS

We thank Ïsa Källén, Monica Christiansson, and Sara Hullberg at the Clinical Neurochemistry Laboratory, Mölndal, Sweden, for their skillful technical assistance. This study was supported by the Swedish Research Council (grant numbers: 14002 and 2006-6227); Alzheimer's Association (grant number NIRG-08-90356); the Royal Swedish Academy of Sciences and the Torsten and Ragnar Söderberg Foundation; Stiftelsen för Gamla Tjänarinnor; Skne county council's research and development foundation; The Swedish Society of Medicine; The Swedish Brain Power; The Trolle-Wachtmeister foundation; and The regional agreement on medical training and clinical research (ALF) between Skne County Council and Lund University.

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=454).

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