CSF Biomarkers and Incipient Alzheimer Disease in Patients With Mild Cognitive Impairment

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Context Small single-center studies have shown that cerebrospinal fluid (CSF) biomarkers may be useful to identify incipient Alzheimer disease (AD) in patients with mild cognitive impairment (MCI), but large-scale multicenter studies have not been conducted.

Objective To determine the diagnostic accuracy of CSF β-amyloid$_{1-42}$ (Aβ42), total tau protein (T-tau), and tau phosphorylated at position threonine 181 (P-tau) for predicting incipient AD in patients with MCI.

Design, Setting, and Participants The study had 2 parts: a cross-sectional study involving patients with AD and controls to identify cut points, followed by a prospective cohort study involving patients with MCI, conducted 1990-2007. A total of 750 individuals with MCI, 529 with AD, and 304 controls were recruited by 12 centers in Europe and the United States. Individuals with MCI were followed up for at least 2 years or until symptoms had progressed to clinical dementia.

Main Outcome Measures Sensitivity, specificity, positive and negative likelihood ratios (LRs) of CSF Aβ42, T-tau, and P-tau for identifying incipient AD.

Results During follow-up, 271 participants with MCI were diagnosed with AD and 59 with other dementias. The Aβ42 assay in particular had considerable intersite variability. Patients who developed AD had lower median Aβ42 (356; range, 96-1075 ng/L) and higher P-tau (81; range, 15-183 ng/L) and T-tau (582; range, 83-2174 ng/L) levels than MCI patients who did not develop AD during follow-up (579; range, 121-1420 ng/L for Aβ42; 53; range, 15-163 ng/L for P-tau; and 294; range, 31-2483 ng/L for T-tau, $P<.001$). The area under the receiver operating characteristic curve was 0.78 (95% confidence interval [CI], 0.75-0.82) for Aβ42, 0.76 (95% CI, 0.72-0.80) for P-tau, and 0.79 (95% CI, 0.76-0.83) for T-tau. Cut-offs with sensitivity set to 85% were defined in the AD and control groups and tested in the MCI group, where the combination of Aβ42/P-tau ratio and T-tau identified incipient AD with a sensitivity of 83% (95% CI, 78%-88%), specificity 72% (95% CI, 68%-76%), positive LR, 3.0 (95% CI, 2.5-3.4), and negative LR, 0.24 (95% CI, 0.21-0.28). The positive predictive value was 62% and the negative predictive value was 88%.

Conclusions This multicenter study found that CSF Aβ42, T-tau, and P-tau identify incipient AD with good accuracy, but less accurately than reported from single-center studies. Intersite assay variability highlights a need for standardization of analytical techniques and clinical procedures.

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Alzheimer disease (AD) is the most common cause of dementia, affecting more than 15 million individuals worldwide.[1] Pathological hallmarks of AD are neuronal intracellular neurofibrillary tangles consisting of the protein tau and extracellular deposits of synaptotoxic β-amyloid (Aβ) peptides in fibril structures.[2] Neuronal changes are present also in older individuals without dementia, and their development is thought to precede clinical symptoms by several years.[3]

See also p 436 and Patient Page.

The possibility that AD disease-modifying treatment with γ- and β-secretase inhibitors or vaccination regimens will be developed raise a need for methods enabling early diagnosis.[4,5] Treatments would need to be initiated very early in the disease process, before the neurodegenerative process is too severe. Much focus has thus been

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Table 1. Study Centers and Participants

Center	Dates of Inclusion	Dates of Analyses	Participants	No.
Amsterdam, the Netherlands	2000-2006	2000-2006	AD	89
			MCI	36
			Controls	16
The Netherlands and Greece	2003-2005	2008	MCI	34
Kuopio, Finland	1990-2004	2000-2005	MCI	141
			Controls	30
Göteborg, Sweden	1999-2006	2008	AD	36
			MCI	85
			Controls	51
Heidelberg, Germany	2000-2006	2008	MCI	44
Stockholm, Sweden	2002-2005	2008	AD	137
			MCI	113
			Controls	23
Linköping, Sweden	2007	2008	Controls	41
Malmö, Sweden	1999-2005	2005-2008	AD	159
			MCI	165
			Controls	39
Munich, Germany	1997-2006	2008	MCI	49
			Controls	48
New York, NY	1999-2006	2008	AD	7
			MCI	13
			Controls	42
Stavanger, Norway	2005-2007	2008	AD	20
Perugia, Italy	2002-2007	2008	AD	81
			MCI	70
			Controls	14

Abbreviations: AD, Alzheimer disease; MCI, mild cognitive impairment.

directed on patients with mild cognitive impairment (MCI), which is a syndrome characterized by cognitive impairment beyond the age-adjusted norm, but not severe enough to fulfill the criteria for dementia.[6] Many patients with MCI display the same morphological changes as AD patients, and the annual rate of AD diagnosis for patients with MCI is 10% to 15%.[7,8] Other individuals have a benign form of MCI and show no progression of symptoms, while some eventually develop other types of dementia.[9]

Biochemical changes in the brain are reflected in the cerebrospinal fluid (CSF), and intense research efforts have been made to develop biomarkers for the central pathogenic processes in AD that can be used as diagnostic tools. Numerous studies have shown that AD patients display characteristic CSF changes with elevated levels of total tau (T-tau) protein and tau phosphorylated at threonine 181 (P-tau) and decreased levels of β-amyloid$_{1-42}$ (Aβ42).[1] Some studies have also shown that patients with MCI who have incipient AD display similar CSF changes.[10-20] Most of these studies, however, are small and conducted at single centers. Furthermore, principles used for establishing biomarker cutoffs as well as suggested cutoff levels vary considerably. We therefore undertook this multicenter study to assess the diagnostic accuracy of CSF Aβ42, T-tau, and P-tau in identifying incipient AD in a large heterogeneous group of patients with MCI.

METHODS

Study Population

The study was designed in accordance with the Standards for Reporting Diagnostic Accuracy (STARD) criteria.[21,22] Memory clinics at 12 centers were involved in the study (TABLE 1 lists center populations and abbreviations). Study participants were consecutive series of patients presenting with symptoms leading to a diagnosis of MCI or AD, together with healthy controls. Test results from AD patients and healthy controls were used in a cross-sectional study to define cutoffs for the index tests, which were then evaluated in a longitudinal prospective MCI cohort study.

CSF Sampling

All participants underwent lumbar puncture in the L3-4 or L4-5 interspace. No serious adverse events were reported. The samples were stored in polypropylene tubes and immediately frozen at –80°C or –70°C until analysis. At 2 centers, samples were stored temporarily on ice for 3 hours before freezing. Cross-examination of 10 samples, with 1 fraction frozen immediately and 1 stored on ice before freezing, showed no or small differences in biomarker levels from this variation in sample handling (Aβ42, R=0.75; T-tau, R=0.99; P-tau, R=0.99). All archived CSF samples were analyzed at the Clinical Neurochemistry Laboratory at Sahlgrenska University Hospital, Mölndal, Sweden, except for samples from centers from Amsterdam, the Netherlands; Kuopio, Finland; and Munich, Germany. A subset of samples from these centers was reanalyzed in 2008 in Mölndal to adjust for intercenter variation in analysis results. Weighting formulas were used if results from the 3 centers differed by more than 2 coefficients of variation from the results at Mölndal (eTable 1, available at http://www.jama.com). A portion of the data have been published before.[17,23,24]

Biochemical Procedures

Cerebrospinal fluid T-tau concentration was determined using a sandwich enzyme-linked immunosorbent assay ([ELISA] Innotest hTAU-Ag, Innogenetics, Ghent, Belgium) specifically constructed to measure all tau isoforms irrespective of phosphorylation status, as previously described.[25] Tau phosphory-

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lated at threonine 181 (P-tau181) was measured using a sandwich ELISA method (Innotest Phospho-Tau[181P]), as previously described.[26] Aβ142 levels were determined using a sandwich ELISA (Innotest β-amyloid[1-42]), specifically constructed to measure Aβ containing both the 1st and 42nd amino acids, as previously described.[27] Coefficients of variations for these assays are presented in eTable 2 (available at http://www.jama.com). For 2 centers (Malmö, Sweden and Göteborg, Sweden), CSF biomarkers were measured by the Luminex xMAP technology using the Inno-Bia AlzBio3 kit (Innogenetics, Zwijndrecht, Belgium) as previously described in detail.[28] Results were converted based on previously published conversion factors.[28] Experienced laboratory technicians who were blinded to clinical diagnosis and other clinical information performed the analyses. The biochemical procedures were the same at all laboratories.

Clinical Procedures

Patients evaluated at each of the centers for possible memory impairment, found to have AD or MCI, and consenting to participate in the studies were included. At inclusion, physicians specializing in cognitive disorders and blinded to the CSF results assessed all participants including a clinical history, examination, and cognitive testing with the Mini-Mental State Examination (MMSE). Laboratory evaluations included routine blood analysis and analysis of apolipoprotein E (*APOE*) genotype. Mild cognitive impairment was diagnosed according to the revised Petersen criteria.[29] These include a decline in memory, objectively verified by neuropsychological testing in combination with a precise history from the patient, proxy, or both, as suggested by Petersen,[30] and adjusted for age and education, or a decline in other cognitive domains, with none or minimal impairment of activities of daily living and not meeting criteria for dementia, as defined by *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition) (*DSM-IV*).[31] Since standard MCI criteria do not define optimal tests to establish the diagnosis,[30] cognitive testing was performed according to local memory clinic routines, using combinations of several tests. These included the Consortium to Establish a Registry for Alzheimer's Disease cognitive battery, Alzheimer's Disease Assessment Scale–Cognitive Subscale, Wechsler Adult Intelligence Scale–Revised, trail making test, verbal fluency test, learning trials, delayed recall tests, and clock drawing. Alzheimer disease was diagnosed using the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria.[32] Exclusion criteria were known causes of cognitive impairment, such as brain tumor, subdural hematoma, and ongoing alcohol abuse. Depressive symptoms and low plasma concentrations of vitamin B_{12} or folate were treated but did not lead to exclusion. The same was true for medical conditions not affecting cognition. Patients with MCI were followed up clinically with a minimum frequency of once a year until they were diagnosed with dementia or until they had been cognitively stable for at least 2 years. The follow-up visits were performed by physicians blinded to the CSF results. Criteria for AD at follow-up were the same as at baseline. A clinical diagnosis of AD in a patient with MCI defined the reference standard of the study (incipient AD). Patients fulfilling the requirements of National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherché et l'Enseignement en Neurosciences[33] for vascular dementia or the criteria established by Erkinjuntti et al[34] for subcortical vascular dementia were diagnosed as having vascular dementia. The criteria by McKeith et al[35] and Brun et al[36] were used for dementia with Lewy bodies and frontotemporal dementia, respectively.

The control population consisted of volunteers without cognitive symptoms (MMSE >25) and no active neurological or psychiatric disease. Volunteers were mainly recruited through advertisements or were spouses of patients. At some centers, volunteers were paid a small sum to participate. A small proportion of the volunteers were individuals referred to memory clinics due to subjective cognitive problems, but no objective cognitive impairment was present and no cognitive deterioration was seen during at least 1 year of follow-up. Cerebrospinal fluid sampling was planned and performed before the reference standard was established, making this a prospective study. All patients gave written informed consent to participate. In cases in which patients were judged unable to give informed consent, this was provided by their closest relative. The study was approved by the local ethics committees of the participating centers.

Statistical Analysis

Because the distribution of quantitative measures was significantly skewed, statistical tests were conducted using a nonparametric Kruskal-Wallis test followed by a Mann-Whitney *U* test for pairwise comparisons. The Spearman correlation coefficient was used for correlation analysis. Quantitative variables are presented as median (range). The area under the receiver operating characteristic curve was calculated for all biomarkers in patients with incipient AD vs all other patients with MCI. Cutoff levels for individual biomarkers identifying AD were calculated at the 85% sensitivity level, which has been suggested as a satisfactory level.[37] For multiple biomarkers, logistic regression analyses were conducted to derive analytical expressions for the risk of incipient AD, using CSF Aβ42, CSF T-tau, CSF P-tau, baseline MMSE score, and age as continuous variables, and sex and *APOE* genotype as nominal variables (see supplementary text for details).[38] The ratio of Aβ42 to P-tau was analyzed because previous studies have shown that it provides useful diagnostic information.[17] From the best model, a cutoff equation was constructed that obtained a preset sensitivity of 85% in patients with AD vs

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comparably aged controls. All cutoff points were first evaluated in patients with incipient AD vs controls, and in a final step within the MCI population only. Sensitivity, specificity, LRs, and predictive values were calculated. The positive likelihood ratio (LR) was sensitivity/(1 – specificity). The negative LR was (1 – sensitivity)/specificity. Confidence intervals for likelihood ratios were calculated as suggested by Deeks and Altman.[39] The positive predictive value was the ratio of true positives to all positive test results and the negative predictive value was the ratio of true negatives to all negative test results. The relative risk was calculated as the risk for incipient AD in patients with MCI with a pathological result on the cutoff equation divided by the risk in patients with MCI with a normal result on the cutoff equation. Power analysis was conducted as suggested by Altman.[40] Standardized differences were calculated using previously described biomarker data.[17] The power of the study exceeded 0.99, based on an expected effect size of a 2.5 increase in CSF T-tau, a 1.6 increase in CSF P-tau, and a 0.46 decrease in CSF Aβ42.[17] Statistical significance was determined at $P < .01$, corrected for multiple comparisons. All statistical calculations were performed using SPSS 15.0 (SPSS Inc, Chicago, Illinois).

RESULTS

A total of 750 individuals with MCI, 529 with AD, and 304 healthy controls were included in the study. Of the patients with MCI, 420 did not progress to dementia (stable MCI) when followed up for at least 2 years (median, 3; range, 2-11 years). During follow-up, 330 cases with MCI showed progression of cognitive symptoms to clinical dementia. Of these, 271 were diagnosed as having AD (ie, had incipient AD at baseline), and 59 with other types of dementia, including 28 with vascular dementia, 14 with dementia with Lewy bodies, 7 with frontotemporal dementia, and 10 with neurological diseases and dementia. In the MCI sample, the annual rate of AD diagnosis was around 11% in the first 4 study years. The median time to conversion was 24 months (range, 2-126 months) in AD, 30 months in vascular dementia (range, 6-77 months), 12 months in dementia with Lewy bodies (range, 7-52 months), 22 months in frontotemporal dementia (range, 6-37 months), and 36 months in other dementias (range, 24-60 months). Descriptive statistics on sex, age, MMSE, and *APOE* genotype are displayed in TABLE 2. Detailed demographic data on controls and MCI participants from different centers are displayed in eTables 3-5 (available at http://www.jama.com).

Biomarker Levels

Data on either of CSF Aβ42, CSF T-tau, or CSF P-tau were missing in 19 AD patients, 1 control, and 1 MCI patient. Comparisons between diagnostic groups were complicated by intercenter assay differences. Substantial differences in CSF Aβ42 levels were seen, while differences in T-tau and P-tau were much smaller. In centers for which the mean biomarker level in controls differed by more than 2 coefficients of variation from the overall mean in the control group, values for all participants from that center were normalized to the overall mean (see eTable 6 for normalization factors). Because only patients with AD were enrolled at the Stavanger, Norway, center, and only patients with MCI were enrolled at the centers in the Netherlands, Greece, and Heidelberg, Germany, the procedure was performed for those centers using only patients with AD or MCI, respec-

Table 2. Demographic Data for the Total Study Population

Group	No. of Patients	No. of Men/Women	Age, y[a]	*APOE* ε4, No. (%) Heterozygote	*APOE* ε4, No. (%) Homozygote	MMSE Score at Baseline[a]
Controls	304	142/162	67 (44-91)	47 (25)	4 (2)	29 (26-30)
AD	529	192/337[b]	71 (43-89)[c]	214 (54)[c]	78 (20)[c]	22 (2-30)[c]
MCI						
All	750	341/409	69 (43-89)[d]	267 (43)[c,e]	59 (10)[c,d]	27 (16-30)[c,d]
Stable	420	209/211[d]	68 (43-83)[d]	134 (39)[c,d]	15 (4)[d]	28 (17-30)[c,d]
Incipient AD	271	100/171[f]	72 (49-85)[c,g]	118 (53)[c,f]	43 (19)[c,g]	27 (16-30)[c,d,g]
All other MCI	59	32/27[e]	69 (46-89)	15 (33)[e,h]	1 (2)[e,h]	27 (19-30)[c,d]
Vascular dementia	28	18/10[c,h]	74 (55-89)[b,f]	7 (26)[e,i]	1 (4)	27 (23-30)[c]
Dementia with Lewy bodies	14	9/5	72 (61-81)	4 (40)	0	27 (20-30)[c]
Frontotemporal dementia	7	2/5	63 (46-78)	0	0	27 (25-27)[c]
Other	10	3/7	65 (49-79)	4 (57)	0	28 (19-29)[b]

Abbreviations: AD, Alzheimer disease; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination.
[a] Data presented as median (range).
[b] $P < .01$ vs controls.
[c] $P < .001$ vs controls.
[d] $P < .001$ vs AD.
[e] $P < .01$ vs AD.
[f] $P < .01$ vs stable MCI.
[g] $P < .001$ vs stable MCI.
[h] $P < .01$ vs incipient AD.
[i] $P < .001$ vs incipient AD.

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tively. The relative differences in CSF biomarker levels between the diagnostic groups in each center were generally consistent, supporting normalization and making participant selection an unlikely explanation for the intercenter differences. There were no indeterminate results and no restrictions were given to outliers.

Patients with MCI who had incipient AD had higher CSF levels of T-tau and P-tau and lower levels of Aβ42 compared with healthy controls, stable MCI cases, and MCI cases with other dementias (TABLE 3). However, after analyzing the other dementias by subgroup, Aβ42 levels in MCI patients diagnosed with dementia with Lewy bodies did not differ significantly from those in patients with MCI and incipient AD (Table 3).

P-tau correlated strongly with T-tau in all study groups (R=0.77 to 0.88; $P \leq .001$). Aβ42 correlated with T-tau (R=0.16, P=.004) and P-tau (R=0.27, $P<.001$) in controls, and with T-tau in stable MCI (R=–0.16, $P<.001$). In controls, age correlated with T-tau (R=0.22) and P-tau levels (R=0.23, $P<.001$). In patients with stable MCI, age correlated with Aβ42 (R=–0.23), T-tau (R=0.32), and P-tau (R=0.22) ($P<.001$). No correlations were found between age and the biomarkers in patients with AD or incipient AD. Baseline MMSE did not correlate with biomarker levels in controls or patients with AD (P=.10-.97). In patients with MCI, baseline MMSE correlated with Aβ42 (R=0.20, $P<.001$), P-tau (R=–0.23, $P<.001$), and T-tau (R=–0.24, $P<.001$). *APOE* ε4 carriers had a lower median Aβ42 than noncarriers in controls (543 ng/L [range, 315-958 ng/L] vs 682 ng/L [range, 182-1214 ng/L], $P<.001$), stable MCI (479 ng/L [range, 121-1210 ng/L] vs 659 ng/L [range, 125-1420 ng/L], $P<.001$), and incipient AD (344 ng/L [96-930] vs 402 ng/L [range, 108-1075 ng/L], $P<.001$). In patients with stable MCI, *APOE* ε4 also correlated significantly with higher median levels of T-tau (339 ng/L [range, 71-1050 ng/L] vs 284 ng/L [range, 31-1195 ng/L], P=.001) and P-tau (61 ng/L [range, 21-133 ng/L] vs 53 ng/L [range, 20-163 ng/L], P=.003), and in controls to higher levels of T-tau (320 ng/L [range, 55-915 ng/L] vs 268 ng/L [range,42-846 ng/L], P=.006).

Table 3. Concentrations of Aβ42, Total Tau (T-Tau), and Phosphorylated Tau (P-Tau) in Cerebrospinal Fluid Obtained at Enrollment[a]

Group	No. of Patients	Aβ42, ng/L	T-Tau, ng/L	P-Tau, ng/L
Controls	304	675 (182-1897)	280 (42-915)	51 (16-156)
AD	529	370 (85-1354)[b]	559 (85-2782)[b]	82 (17-279)[b]
MCI				
All	750	467 (96-1420)[b,c]	380 (31-2483)[b,c]	61 (15-183)[b,c]
Stable	420	589 (121-1420)[b,c]	298 (31-1580)[c]	54 (15-163)[c]
Incipient AD	271	356 (96-1075)[b,d]	582 (83-2174)[b,d]	81 (15-183)[b,d]
All other MCI	59	487 (158-857)[b,c,e,f]	275 (40-2483)[c,e]	47 (22-163)[c,e]
Vascular dementia	28	512 (190-825)[b,c,e]	319 (86-2483)[c,e]	51 (24-163)[c,e]
Dementia with Lewy bodies	14	427 (199-654)[b,g,f]	329 (40-1010)[g,h]	55 (25-125)[g,h]
Frontotemporal dementia	7	600 (366-857)[g,h]	275 (237-347)[c,e]	45 (41-58)[c,h]
Other	10	585 (158-760)	149 (58-828)[c,e]	39 (22-81)[c,e]
Stable MCI plus all other MCI cases	479	579 (121-1420)[b,c,e]	294 (31-2483)[c,e]	53 (15-163)[c,e]

Abbreviations: AD, Alzheimer disease; MCI, mild cognitive impairment.
[a] Data presented as median (range), data from normalization model.
[b] $P<.001$ vs controls.
[c] $P<.001$ vs AD.
[d] $P<.001$ vs stable MCI.
[e] $P<.001$ vs incipient AD.
[f] $P<.01$ vs stable MCI.
[g] $P<.01$ vs AD.
[h] $P<.01$ vs incipient AD.

Biomarkers Predicting Incipient AD

The frequency of incipient AD in patients with MCI by biomarker level was examined in pairwise combinations of T-tau quintiles and Aβ42/P-tau ratio quintiles. Among MCI patients with biomarker values in the fifth quintile of T-tau plus the first quintile of Aβ42/P-tau ratio, a high proportion were patients with incipient AD compared with patients with values in the opposite quintiles (FIGURE 1).

Following recommendations in the STARD criteria, we established cutoff levels for individual biomarkers in all AD patients vs all controls, with sensitivity for the index test set at 85%. Positive CSF T-tau and P-tau test results were defined as values above the cutoff (≥320 ng/L and ≥52 ng/L, respectively), and positive CSF Aβ42 as values below the cutoff (≤482 ng/L). When these cutoffs were applied to CSF levels of MCI patients to determine how well they predicted who developed incipient AD, Aβ42 had a sensitivity of 79% (215 of 271; 95% CI, 74%-84%), a specificity of 65% (321 of 479; 95% CI, 61%-69%), a positive LR of 2.3 (95% CI, 2.0-2.6), and a negative LR of 0.32 (95% CI, 0.28-0.36). P-tau had a sensitivity of 84% (227 of 270; 95% CI, 80%-88%), a specificity of 47% (225 of 479; 95% CI, 42%-52%), a positive LR of 1.6 (95% CI, 1.4-1.8), and a negative LR of 0.34 (95% CI, 0.31-0.37). T-tau had a sensitivity of 86% (232 of 271; 95% CI, 82%-90%), a specificity of 56% (268 of 479, 95% CI, 51%-61%), a positive LR of 1.9 (95% CI, 1.7-2.2), and a negative LR of 0.26 (95% CI, 0.23-0.29). The area under the receiver operating characteristic curve was 0.78 (95% CI, 0.75-0.82) for Aβ42; 0.76 (95% CI, 0.72-0.80) for P-tau; and 0.79 (95% CI, 0.76-0.83) for T-tau.

The final index test was an equation for the combination of Aβ42:P-tau ratio (y) and T-tau (x), with cutoffs constructed in the training set of all patients with AD vs all controls, and sensitivity for AD set at greater than 85% based on logistic regression analysis ($y=3.694+0.0105x$, FIGURE 2, panel A). This equation was evaluated in MCI patients with incipient AD vs controls in a first step (Figure 2, panel B) and in MCI patients only in a final step

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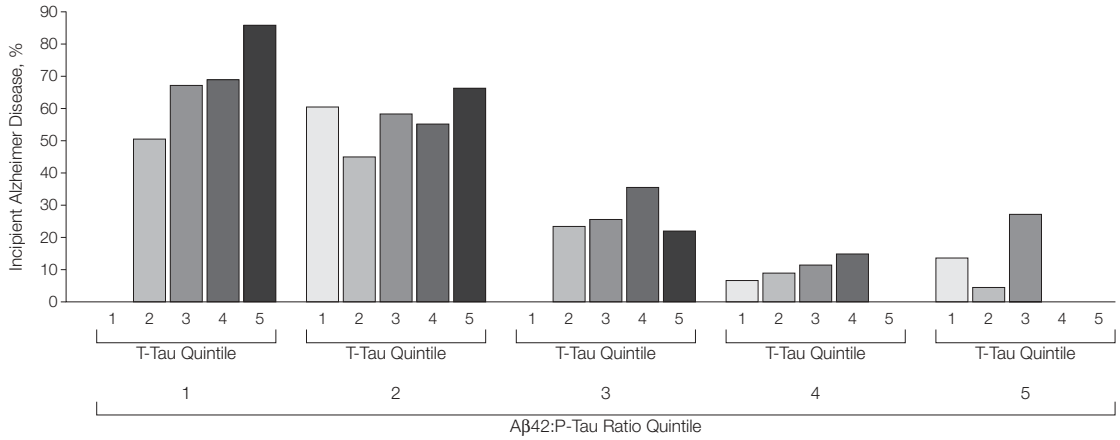
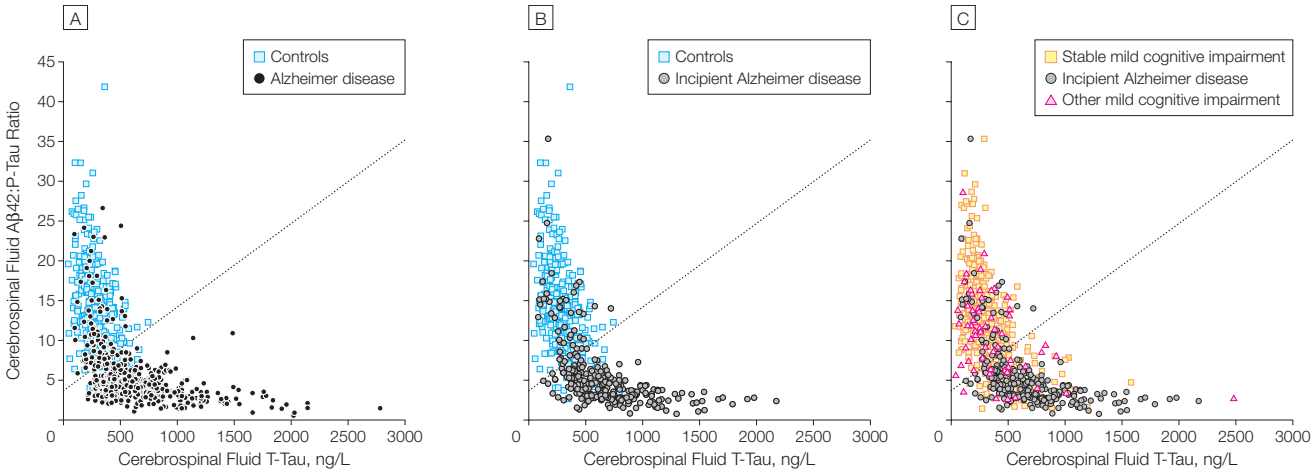
(Figure 2, panel C). As shown in earlier studies, the predictive value of the biomarkers combined was greater than the predictive value of any individual biomarker. In comparing patients with MCI and incipient AD with controls, the cutoff equation achieved a sensitivity of 83% (223 of 270, 95% CI, 78%-88%), a specificity of 88% (266 of 303, 95% CI, 84%-92%), a positive LR of 7.0 (95% CI, 5.7-8.5), and a negative LR of 0.17 (95% CI 0.14-0.21). When applied to all MCI patients only, the specificity was 72% (345/479, 95% CI, 68%-76%), the positive LR was 3.0 (95% CI, 2.5-3.4), the negative LR was 0.24 (95% CI, 0.21-0.28), the positive predictive value was 62%, and the negative predictive value was 88%. The relative risk for incipient AD in MCI patients with a positive result on this equation was 5.2 (95% CI, 3.9-6.9). FIGURE 3 is a flow diagram of the evaluation of the cutoff equation for patients with MCI. Because some of the MCI patients were followed up for much longer than 2 years, we also evaluated the specificity of the equation for patients with stable MCI using different lengths of follow-up. No significant differences were seen in specificity comparing the 213 patients with MCI with up to 36 months of follow-up (the median follow-up time in stable MCI [specificity, 73%; 95% CI, 67%-79%]), the 207 who were followed up for more than 36 months (specificity, 72%; 95% CI, 68%-76%), or the 105 who were followed up for more than 56 months (the 75th percentile [specificity, 74%; 95% CI, 66%-82%]). When testing the equation for

Figure 1. Percentage of Patients With MCI Who Developed Alzheimer Disease by Quintiles of CSF T-Tau and CSF Aβ42/P-Tau Ratio



Quintiles with less than 5 cases are excluded from the graph. CSF indicates cerebrospinal fluid.

Figure 2. Scatterplot of Cerebrospinal Fluid (CSF) Aβ42:P-Tau Ratio and CSF T-Tau in Patients With Alzheimer Disease and Controls



The reference line shows the cutoff equation derived for sensitivity of 85%. A, The scatterplot of CSF of Aβ42:P-Tau Ratio and CSF T-tau in AD patients and controls. B, The scatterplot of cerebrospinal fluid Aβ42:P-tau ratio and cerebrospinal fluid T-tau of patients with mild cognitive impairment who developed Alzheimer disease and controls. C, The scatterplot of cerebrospinal fluid Aβ42:P-tau ratio and cerebrospinal fluid T-tau in all patients with mild cognitive disorder, by those who did not progress to dementia, those who developed Alzheimer disease, and those who were diagnosed with other types of dementia. See interactive figure at http://www.jama.com.

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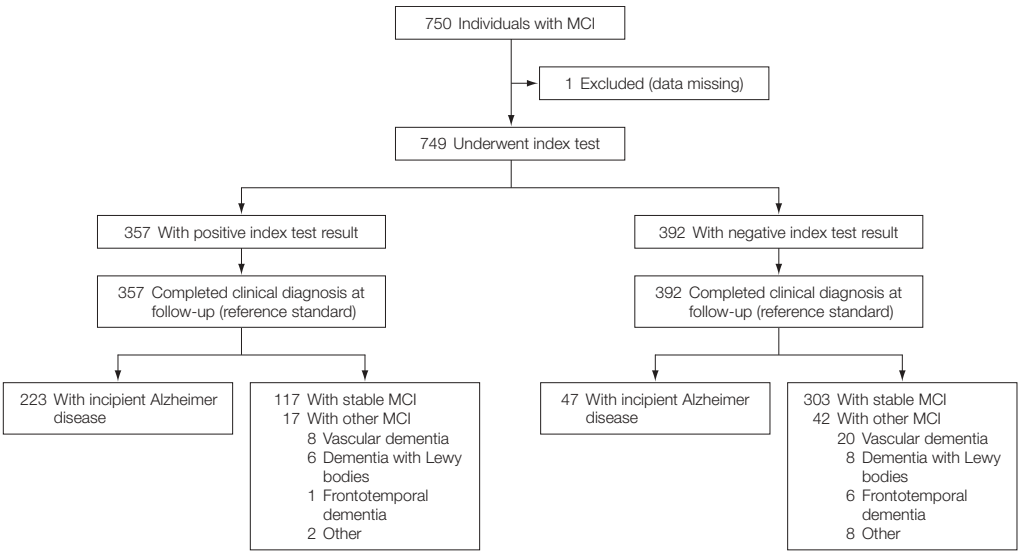
Figure 3. Flow Diagram Demonstrating Evaluation of the Diagnostic Test



First, the index test was established in 510 patients with Alzheimer disease at baseline and 303 controls. This resulted in the cutoff equation Aβ42/P-tau >3.694 + 0.0105 × T-tau. The index test was then applied to patients with mild cognitive impairment (MCI) to determine its ability to predict Alzheimer disease, the reference standard.

patients with MCI who had incipient AD vs those who had developed specific other dementias, the specificity varied between 57% and 86% for the different follow-up diagnoses (TABLE 4).

Table 4. Diagnostic Accuracy of the Cutoff Equation for Aβ42:P-Tau Ratio and T-Tau for Excluding AD, Derived in Patients With Alzheimer Disease and Controls and Applied to Patients With Mild Cognitive Impairment Who Did Not Develop Alzheimer Disease[a]

Group	No. of Patients	Specificity, % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Stable MCI	420	72 (68-76)	3.0 (2.5-3.4)	0.24 (0.21-0.28)
Vascular dementia	28	71 (55-88)	2.9 (1.6-5.2)	0.24 (0.14-0.44)
Dementia with Lewy bodies	14	57 (31-83)	1.9 (1.1-3.5)	0.31 (0.17-0.56)
Frontotemporal dementia	7	86 (60-100)	5.8 (0.94-35.5)	0.20 (0.03-1.24)
Other	10	80 (55-100)	4.1 (1.2-14.3)	0.22 (0.06-0.75)

Abbreviations: AD, Alzheimer disease; CI, confidence interval; LR, likelihood ratio; MCI, mild cognitive impairment.
[a]The sensitivity for incipient AD was 83% (78%-88%). The specificity for all other MCI cases was 71% (60%-83%).

COMMENT

We determined in a large multicenter study that the CSF biomarkers Aβ42, T-tau, and P-tau can be used to predict with good accuracy which MCI patients will develop AD, as previously found in smaller studies.[10-20] This multicenter collaboration avoids several of the risks of biases associated with single-center studies by having included substantially more patients than previous studies. Cerebrospinal fluid biomarker changes were found to be significantly associated with incipient AD. However, the considerable intercenter variations in assays and patient assessments described point to a need for standardization of sample handling as well as of clinical assessments. Although each memory clinic center followed up its cohorts prospectively and used established clinical criteria, a limitation of the present study is the lack of fully harmonized study protocols for all centers, which might account for some of the intercenter variations that we observed. Cutoff levels for the CSF biomarkers were established in an independent sample of AD and control cases. These cutoffs were then applied to the MCI group to determine the accuracy of the biomarkers to predict incipient AD. This procedure follows the recommendations in the STARD criteria to minimize potential test review bias, ie, distortion of the diagnostic accuracy caused by establishing a cutoff for the index test (CSF biomarkers) directly on the reference standard (clinical status in the MCI cohort).[22]

The specificity of the combined biomarkers was somewhat lower than found earlier.[14,17] This may be partly attributed to the relatively short follow-up period in the present study; thus, longer follow-up is needed to verify the benign nature of stable MCI.[17] However, smaller studies performed at single centers are also likely to have a narrow spectrum of patients and controls, which risks overestimating diagnostic accuracy.[41] As mentioned above, we found large intercenter variations in biomarker levels caused either by variations in preanalytical sample handling or by genuine differences in biomarker levels related to patient characteristics. Although the coefficients of variation for the assays are low and in the range of what is found for other immunoassays, the between-day variation for the ELISAs could also add to

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the variation in biomarker levels between centers. In sum, these differences emphasize the need for standardizing the sample handling protocols as well as the clinical evaluations of the patients.

Deriving cutoffs from the population under study is another risk for overestimating diagnostic accuracy.[42] To avoid this, some earlier studies have applied externally established cutoffs, such as those provided by Riemenschneider et al.[41,43] However, within-assay and intercenter variations make this an unpredictable strategy. To steer clear of these problems, we derived cutoffs from controls and patients with AD from multiple centers, although we analyzed them in the same setting as patients with MCI. Previous studies have found cutoff levels of Aβ42 from 452 to 661 ng/L and T-tau from 300 to 478 ng/L.[12,13,17] The cutoff levels found in this study were within those ranges.

Mild cognitive impairment is a heterogeneous condition with several possible outcomes. In our study, memory impairment resolved in at least 31 (4%) patients with MCI during follow-up. In population-based studies this figure is significantly higher, possibly due to a bias for more severe cases of patients referred to a memory clinic.[44] There were no correlations between biomarker levels and time to AD diagnosis in MCI patients (Aβ42: $R=-0.044$, $P=.43$; P-tau: $R=0.009$, $P=.87$; T-tau: $R=-0.008$, $P=.88$) . However, because the total MCI disease duration is unknown, such correlation analyses are difficult. This remains a problem for all studies including those enrolling patients with MCI at baseline.

Another problem is the uncertainty of the reference standard. Neuropathologically, there is a large overlap between vascular dementia, AD, and dementia with Lewy bodies.[8] Recently, it has been suggested that the clinical AD criteria should be complemented by including CSF or imaging biomarkers.[45] In this study, patients with clinical evidence of vascular pathology in addition to AD were classified as AD (83 patients [16%] with AD at baseline, and at least 17 patients [6%] with incipient AD). These did not differ in biomarker levels from AD patients without signs of vascular involvement. Fourteen MCI patients had incipient dementia with Lewy bodies, with CSF Aβ42 levels between those of incipient AD and stable MCI.

As expected, *APOE* ε4 genotype was an independent risk factor for patients with incipient AD, but stable MCI patients also had higher prevalence of *APOE* ε4 than controls, and these *APOE* ε4–positive MCI patients had biomarkers more similar to an AD pattern. It is not unlikely that some of these individuals would have developed AD with a longer follow-up. In a previous study we found that *APOE* ε4 carriers with severe and moderate episodic memory impairment had lower Aβ42 and higher T-tau and P-tau than non–*APOE* ε4 carriers with similar episodic memory impairment.[46]

Because AD is a deleterious condition, a diagnostic test underlying decisions of treatment or follow-up should have a high sensitivity. The biomarkers CSF Aβ42, T-tau, and P-tau had a sensitivity of 83% in this multicenter study. However, the precise cutoffs presented herein are not immediately applicable in all memory clinics, considering the normalizations performed in the study. It is also important to note that if these biomarkers are to be used throughout the world, external control programs that help laboratories harmonize their measurements with each other will be essential. Using CSF Aβ42, T-tau, and P-tau in memory clinics will result in some false-positive cases, as well as false-negative cases, and the biomarkers may therefore be useful primarily as screening tools, selecting individuals for a detailed further clinical follow-up. Furthermore, they may be useful in enriching study populations for clinical trials of future disease-modifying AD treatments. Until such treatments become available, however, these tests are not generally appropriate for routine clinical use because it is not currently possible to alter the development of AD.

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