

# Cerebrospinal Fluid Levels of $\beta$ -Amyloid 1-42, but Not of Tau, Are Fully Changed Already 5 to 10 Years Before the Onset of Alzheimer Dementia

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**Context:** Early detection of prodromal Alzheimer disease (AD) is important because new disease-modifying therapies are most likely to be effective when initiated during the early stages of disease.

**Objectives:** To assess the ability of the cerebrospinal fluid (CSF) biomarkers total tau (T-tau), phosphorylated tau (P-tau), and  $\beta$ -amyloid 1-42 (A $\beta$ 42) to predict future development of AD dementia within 9.2 years in patients with mild cognitive impairment (MCI) and to compare CSF biomarkers between early and late converters to AD.

**Design:** A clinical study with a median follow-up of 9.2 years (range, 4.1-11.8 years).

**Setting:** Memory disorder clinic.

**Patients:** A total of 137 patients with MCI who underwent lumbar puncture at baseline.

**Main Outcome Measure:** Conversion to AD dementia.

**Results:** During follow-up, 72 patients (53.7%) developed AD and 21 (15.7%) progressed to other forms of de-

mentia. At baseline, CSF A $\beta$ 42 levels were reduced and T-tau and P-tau levels were elevated in patients who converted to AD during follow-up compared with nonconverters ( $P < .001$ ). Baseline CSF A $\beta$ 42 levels were equally reduced in patients with MCI who converted to AD within 0 to 5 years (early converters) compared with those who converted between 5 and 10 years (late converters). However, CSF T-tau and P-tau levels were significantly higher in early converters vs late converters. A baseline A $\beta$ 42:P-tau ratio predicted the development of AD within 9.2 years with a sensitivity of 88%, specificity of 90%, positive predictive value of 91%, and negative predictive value of 86%.

**Conclusions:** Approximately 90% of patients with MCI and pathologic CSF biomarker levels at baseline develop AD within 9 to 10 years. Levels of A $\beta$ 42 are already fully decreased at least 5 to 10 years before conversion to AD dementia, whereas T-tau and P-tau seem to be later markers. These results provide direct support in humans for the hypothesis that altered A $\beta$  metabolism precedes tau-related pathology and neuronal degeneration.

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**A**LZHEIMER DISEASE (AD) IS the predominant cause of dementia and a major medical and socioeconomic problem worldwide. As populations get older, the prevalence of AD will increase substantially during the coming decades.<sup>1</sup> The pathologic characteristics of AD are senile plaques, consisting of aggregated  $\beta$ -amyloid (A $\beta$ ), and neurofibrillary tangles, containing hyperphosphorylated tau protein.<sup>1,2</sup> According to the amyloid cascade hypothesis, accumulation of the aggregation-prone 42-amino acid isoform of A $\beta$  (A $\beta$ 42) in the brain drives the neurodegenerative process in AD.<sup>1,2</sup> Accumula-

tion of A $\beta$  is believed to start decades before cognitive decline and might be detected by a reduction in cerebrospinal fluid (CSF) A $\beta$ 42 levels and elevated retention of positron emission tomography tracers for amyloid in the brain.<sup>3,4</sup> According to this theory, the initial, asymptomatic, phase is followed by neuronal dysfunction and neurodegeneration, which are reflected by increasing levels of CSF tau and regional cerebral atrophy visualized by magnetic resonance imaging.<sup>3,4</sup> However, direct evidence supporting this temporal sequence of events in humans affected by AD is still scarce.<sup>3</sup>

During the past few years, it has become more evident that disease-modifying

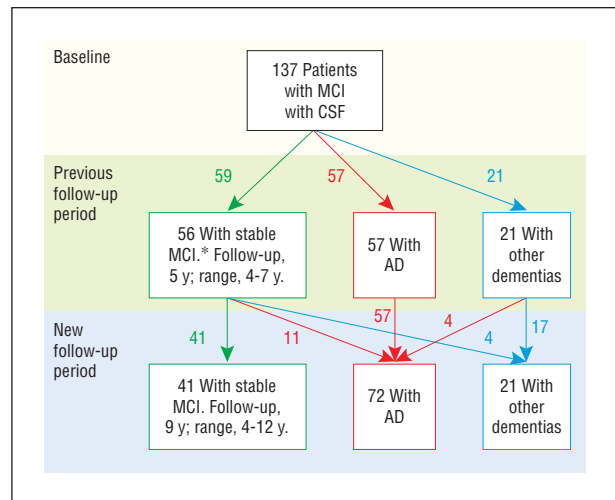
therapies, such as A $\beta$  immunotherapy, are more likely to be successful if initiated during the early stages of AD, when the neurodegeneration is not yet too severe.<sup>4,5</sup> Therefore, methods are urgently needed to accurately identify individuals affected by AD before they become demented.<sup>5,6</sup> The term *prodromal AD* denotes nondemented individuals with mild cognitive impairment (MCI) resulting from underlying AD pathology.<sup>7</sup> However, MCI is a heterogeneous syndrome, and only 30% to 60% of the patients are affected by prodromal AD, whereas the others have a benign form of cognitive impairment or another neurodegenerative illness.<sup>8</sup> Many studies<sup>9-18</sup> of patients with MCI have shown that abnormal baseline levels of CSF total tau (T-tau), phosphorylated tau at threonine 181 (P-tau), and A $\beta$ 42 are associated with subsequent conversion to AD dementia. However, these previous studies<sup>9-12,15-18</sup> have had short clinical follow-up of 1 to 3 years, except for 2 studies<sup>13,14</sup> with follow-up of 4 to 5 years. Given that AD is a slowly progressive disorder, it probably takes at least 10 years before most patients with prodromal AD develop dementia and can be diagnosed as having clinical AD.<sup>8</sup> Studies of MCI with short follow-up will, thus, underestimate the true prevalence of prodromal AD. Consequently, in such studies, the estimated specificity and positive predictive value will be falsely low for any biomarker that can detect AD pathologic features many years before conversion to dementia.

To our knowledge, the study by Hansson et al,<sup>13</sup> with a median clinical follow-up of 5.2 years, is still the most extensive follow-up of a cohort of patients with MCI at baseline in which the diagnostic accuracy of biomarkers for prodromal AD has been studied. To further improve the clinical diagnostic accuracy and to elucidate the temporal aspects of the AD pathology in the prodromal stages, we performed an extended follow-up of the cohort from that study consisting of 137 patients with MCI at baseline. The levels of T-tau, P-tau, and A $\beta$ 42 were analyzed in baseline CSF samples. In the present study, patients with MCI at baseline subsequently either developed a certain type of dementia or were cognitively stable for a median of 9.2 years. The diagnostic accuracy of the CSF biomarkers for predicting AD within 9.2 years was assessed. Moreover, the baseline CSF biomarkers were compared between patients with prodromal AD who developed dementia within 0 to 5 years (early converters) and those who developed dementia between 5 and 10 years (late converters).

## METHODS

### INCLUSION OF PATIENTS AND PREVIOUS CLINICAL FOLLOW-UP

The inclusion of patients with MCI is described in the article by Hansson et al.<sup>13</sup> In brief, baseline CSF samples were acquired from 137 patients who at baseline fulfilled the criteria of MCI proposed by Petersen.<sup>8</sup> Patients with other causes of cognitive impairment, including subdural hematoma, brain tumor, central nervous system infection, schizophrenia, major depressive episode, and current alcohol abuse, were excluded. Other pathologic conditions were allowed to include a clinically relevant and heterogeneous population. In the previous study,<sup>13</sup> the 137 patients with MCI and CSF analysis at base-



**Figure 1.** The 137 patients with mild cognitive impairment (MCI) at baseline and the diagnostic outcomes after the previous and new (extended) follow-up periods. During clinical follow-up of the study, 41 patients remained cognitively stable after median follow-up of 9.2 years (range, 4.1-11.8 years), whereas 72 patients with MCI developed Alzheimer disease (AD) and 21 developed other types of dementia. \*Three patients with stable cognition at the previous follow-up were excluded because they died before sufficient follow-up (<4 years). Because of uncertainty about their cognitive stability, they were excluded from the study. CSF indicates cerebrospinal fluid.

line subsequently either developed a certain type of dementia (AD, n=57; other types of dementia, n=21) or were cognitively stable (n=56) over median follow-up of 5.2 years (range, 4.0-6.8 years). Three stable patients with MCI died before 4 years of follow-up. Because of uncertainty about their cognitive stability, they were excluded from the study.

### EXTENDED CLINICAL FOLLOW-UP

**Figure 1** depicts the total follow-up of the patients with MCI at baseline. The diagnosis of the patients with new clinical information since the previous follow-up was decided by a consensus group consisting of 4 physicians with a special interest in dementia disorders (P.B., L.M., Å.K.W., and O.H.). The consensus group was blinded to all the results obtained from CSF analysis and to any previous dementia diagnosis. Patients who received a diagnosis of AD had to meet the *DSM-III-R* criteria for dementia<sup>19</sup> and the criteria for probable AD defined by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.<sup>20</sup> Patients who were diagnosed as having vascular dementia (VaD) fulfilled the *DSM-III-R* criteria for dementia and the requirements for probable VaD by the National Institute of Neurological Disorders and Stroke Association-Internationale pour la Recherche et l'Enseignement en Neurosciences<sup>21</sup> or the recommendations of Erkinjuntti and coworkers<sup>22</sup> for VaD of the subcortical type. For patients who developed dementia with Lewy bodies or frontotemporal dementia, the consensus criteria by McKeith and collaborators<sup>23</sup> and McKhann and colleagues,<sup>24</sup> respectively, were used.

Of the 56 patients with MCI who were cognitively stable during the previously published follow-up period,<sup>13</sup> 11 developed AD dementia, 3 progressed to VaD, and 1 progressed to semantic dementia during the extended follow-up program (Figure 1). Moreover, the consensus group found that 4 of the patients who received a diagnosis of VaD during the previously published follow-up now exhibited symptoms consistent with subsequent development of significant AD co-

**Table 1. Demographic Data and Baseline Levels of Cerebrospinal Fluid Biomarkers of the 173 Included Study Patients<sup>a</sup>**

	Controls (n=39)	Stable MCI (n=41)	MCI-AD (n=72)	MCI-Other (n=21)
Sex, M/F, No.	15/24	22/19	23/49 <sup>b,c</sup>	15/6 <sup>d</sup>
Age, mean (SD), y	72.4 (7.7)	61.9 (8.5) <sup>e</sup>	73.9 (5.8) <sup>f</sup>	71.1 (9.1) <sup>f</sup>
Carrier of any <i>APOE</i> $\epsilon$ 4 allele, No. (%)	10 (26)	19 (46)	53 (74) <sup>e,g,h</sup>	5 (24)
MMSE score at baseline, 0-30, mean (SD)	29.1 (1.0)	27.5 (2.0) <sup>e</sup>	26.9 (1.4) <sup>c,e</sup>	26.8 (1.2) <sup>e</sup>
Annual change in MMSE score, mean (SD)	0.07 (0.4)	0.1 (0.4)	-2.8 (2.1) <sup>e,f</sup>	-2.2 (2.2) <sup>e,f</sup>
Baseline T-tau, mean (SD), ng/L	326 (157)	297 (182)	737 (425) <sup>b,e,f</sup>	496 (511)
Baseline P-tau, mean (SD), ng/L	61.2 (17.3)	61.4 (14.0)	88.7 (29.9) <sup>e-g</sup>	60.1 (26.4)
Baseline A $\beta$ 42, mean (SD), ng/L	700 (182)	607 (165) <sup>i</sup>	337 (122) <sup>e-g</sup>	592 (128) <sup>i</sup>
Baseline A $\beta$ 42:P-tau ratio, mean (SD)	12.5 (4.7)	10.4 (3.5) <sup>i</sup>	4.3 (2.6) <sup>e-g</sup>	10.9 (3.4)

Abbreviations: A $\beta$ 42,  $\beta$ -amyloid 1-42; AD, Alzheimer disease; *APOE*  $\epsilon$ 4, apolipoprotein E  $\epsilon$ 4; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; P-tau, phosphorylated tau; T-tau, total tau.

<sup>a</sup>Stable MCI consists of patients with MCI with stable cognitive function during follow-up of 9.2 years (range, 4.1-11.8 years); MCI-AD, patients with MCI who developed AD during follow-up; and MCI-other, patients with MCI who developed other types of dementia during follow-up.

<sup>b</sup> $P < .008$  vs MCI-other.

<sup>c</sup> $P < .05$  vs stable MCI.

<sup>d</sup> $P < .05$  vs controls.

<sup>e</sup> $P < .001$  vs controls.

<sup>f</sup> $P < .001$  vs stable MCI.

<sup>g</sup> $P < .001$  vs MCI-other.

<sup>h</sup> $P < .008$  vs stable MCI.

<sup>i</sup> $P < .008$  vs controls.

pathology (gradual cognitive decline with cortical symptoms, including expressive dysphasia, dyspraxia, or visuospatial problems) during the extended follow-up. These 4 patients, therefore, received a diagnosis of mixed dementia (AD and VaD) and were included in the AD group in the statistical analyses presented in the "Results" section because CSF biomarkers are believed to detect AD pathologic features also in patients with vascular co-pathology. In general, the time from baseline to a certain type of dementia is given in units of 0.5 years based on clinical information acquired from the clinical interviews and examinations and from the medical records.

## COGNITIVELY HEALTHY CONTROLS

The control population consisted of 39 healthy volunteers who underwent lumbar puncture at baseline and were the same cohort as described by Hansson et al.<sup>13</sup> Inclusion criteria were absence of cognitive concerns or symptoms and preservation of general cognitive functioning. Exclusion criteria were active neurologic or psychiatric diseases. Other medical conditions that did not affect cognition were permitted.

This study was approved by the ethics committee at the University of Lund, Lund, Sweden, and followed the provisions of the Helsinki Declaration for controls and patients with MCI. The participants gave their informed consent (for research).

## CSF ANALYSIS

Cerebrospinal fluid was collected in polypropylene tubes, stored at  $-80^{\circ}\text{C}$ , and analyzed after clinical follow-up of the study was completed. The procedure followed the Alzheimer's Association flowchart for lumbar puncture and CSF sample processing.<sup>5</sup> The levels of T-tau, P<sub>181</sub>-tau<sub>181</sub>, and A $\beta$ 42 were determined as previously described by Hansson et al,<sup>13</sup> and the same baseline levels of CSF biomarkers are used in this article.

## STATISTICAL ANALYSIS

The statistical analyses were accomplished using a commercially available software program (SPSS for Windows, version 18.0.1; SPSS Inc/IBM) unless otherwise specified. To compare

demographic and CSF baseline data between groups, nonparametric Kruskal-Wallis tests were performed, followed by Mann-Whitney tests for continuous variables with correction for multiple comparisons (**Table 1**). The Pearson  $\chi^2$  test was used for dichotomous variables. The Spearman correlation coefficient was used for bivariate correlation analyses. Binary multivariate regression models (backward) were used to study the associations between CSF biomarkers and conversion to AD within 0 to 5 years of follow-up and 5 to 10 years of follow-up. The CSF biomarkers were analyzed separately, and the models were adjusted for potentially confounding factors, including age, sex, Mini-Mental State Examination (MMSE) total score, and apolipoprotein E  $\epsilon$ 4 (*APOE*  $\epsilon$ 4) carrier status (carriers of 0, 1, or 2 *APOE*  $\epsilon$ 4 alleles). Receiver operating characteristic curves were drawn by plotting the true-positive fraction (sensitivity) against the false-positive fraction (100% - specificity). The area under the curve (AUC) was calculated using a commercially available software program (MedCalc for Windows, version 11.1; MedCalc Software). The MedCalc software was also used to assess the statistical differences between the AUCs of the different biomarkers according to the method developed by Hanley and McNeil.<sup>25</sup> The Youden index [(sensitivity/100 + specificity/100) - 1] was also used to compare the accuracy of different combinations of biomarkers. To establish unbiased cutoff values in an independent and unsupervised way, without using information on the clinical diagnosis, we used a mixture model. Mixture modeling analysis was accomplished using R, version 2.12.0. Briefly, mixture modeling is a 2-step iterative procedure based on an expectation maximization algorithm, with the assumption that the biomarker data are a mix sampled from 2 different normal distributions. The model has previously been described in detail.<sup>26</sup>

A multivariate backward stepwise Cox regression model was used to estimate the impact of the baseline variables simultaneously (pathologic CSF, age, sex, MMSE total score, and *APOE*  $\epsilon$ 4 carrier status [carriers of 0, 1, or 2 *APOE*  $\epsilon$ 4 alleles]) on the conversion to AD in patients with MCI. Patients with MCI who did not develop AD dementia (ie, patients with stable MCI and patients with MCI who developed other dementias) were pooled together in the receiver operating characteristic analyses and the Cox regression model.

### FOLLOW-UP DIAGNOSES AND BASELINE BIOMARKER LEVELS

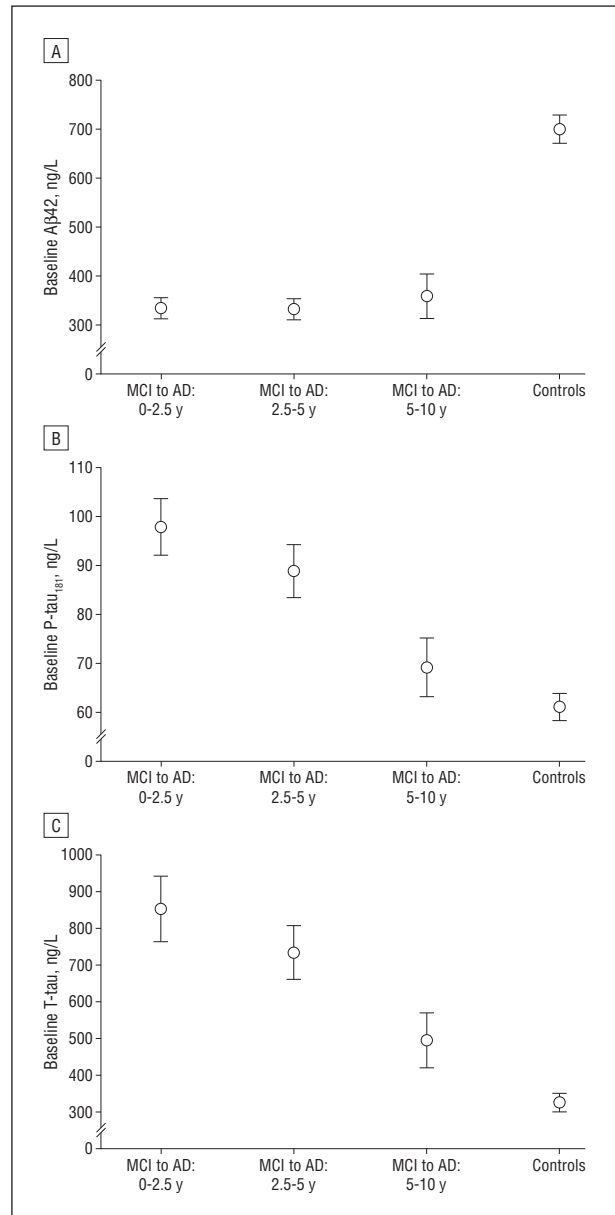
The total follow-up of the cohort of 137 patients with MCI at baseline and successful lumbar puncture and their diagnostic outcomes from the first<sup>13</sup> and the present (extended) follow-ups are given in Figure 1. During follow-up, 41 patients remained cognitively stable after median follow-up of 9.2 years (range, 4.1-11.8 years), and 72 patients with MCI developed AD dementia and 21 developed other types of dementia, including VaD (n=14), dementia with Lewy bodies (n=3), frontotemporal dementia (n=1), semantic dementia (n=2), and traumatic brain injury dementia (n=1).

The baseline levels of the biomarkers in the diagnostic groups are listed in Table 1. The frequency of APOE  $\epsilon 4$  carriers was higher in patients with MCI who subsequently developed AD than in controls and in patients with cognitively stable MCI ( $P < .01$ ) (Table 1). Patients with MCI who later developed AD had higher baseline levels of T-tau and P-tau and lower levels of A $\beta$ 42 than did controls ( $P < .001$ ), patients with cognitively stable MCI ( $P < .001$ ), and patients with MCI who developed non-AD dementias ( $P < .002$ ) (Table 1).

In the control group and in patients with MCI who progressed to AD during follow-up, no associations were found between biomarker levels and MMSE scores at baseline, sex, and age. The levels of A $\beta$ 42, T-tau, and P-tau did not differ significantly between carriers and noncarriers of APOE  $\epsilon 4$  in any of the diagnostic subgroups (eFigure 1; <http://www.archgenpsychiatry.com>). When we analyzed the carriers and noncarriers of APOE  $\epsilon 4$  separately, the levels of A $\beta$ 42, T-tau, and P-tau still differed significantly between patients with MCI who progressed to AD during follow-up and controls ( $P \leq .005$ ) and patients with cognitively stable MCI ( $P < .01$ ).

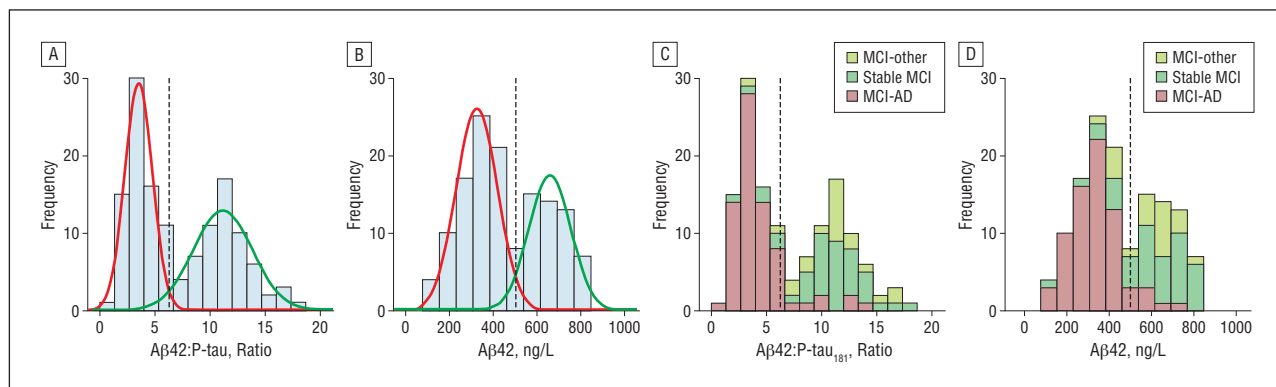
#### BIOMARKER LEVELS IN EARLY AND LATE CONVERTERS TO AD

The baseline levels of A $\beta$ 42 were equally reduced in patients with MCI who developed AD within 5 years of baseline (early converters) and those who developed AD between 5 and 10 years (late converters) (333 ng/L vs 359 ng/L,  $P > .86$ ), showing that patients who developed AD up to 10 years after the CSF tap had fully decreased levels of A $\beta$ 42 already at baseline (Figure 2A and eTable 1). On the contrary, T-tau levels were significantly higher at baseline in early converters vs late converters (786 ng/L vs 495 ng/L,  $P = .02$ ) (Figure 2B). Similarly, P-tau levels were significantly higher at baseline in early converters vs late converters (92.6 ng/L vs 69.2 ng/L,  $P = .009$ ) (Figure 2C). When performing multivariate regression models, we found that CSF A $\beta$ 42, T-tau, and P-tau were associated with conversion to AD within the first 5 years of baseline ( $P < .001$ ). However, only A $\beta$ 42 was associated with conversion to AD in the population observed for 5 to 10 years ( $P = .02$ ).



**Figure 2.** Mean baseline levels of cerebrospinal fluid  $\beta$ -amyloid 1-42 (A $\beta$ 42) (A), phosphorylated tau (P-tau) (B), and total tau (T-tau) (C) stratified into patients with mild cognitive impairment (MCI) who developed Alzheimer disease (AD) dementia within 0 to 2.5 years (n=28; median Mini-Mental State Examination [MMSE] score=26.5), 2.5 to 5 years (n=32; median MMSE score=27), and 5 to 10 years (n=12; median MMSE score=27). Biomarker levels in the cognitively healthy control group are also given. Levels of A $\beta$ 42 did not differ among any of the MCI-AD groups with different intervals to AD dementia. Levels of T-tau and P-tau were significantly lower in late converters (5-10 years) compared with very early converters (0-2.5 years) ( $P < .05$ ); however, no significant differences were found between very early converters (0-2.5 years) and the intermediate group (2.5-5.0 years). Error bars represent the SEM.

There were no significant differences between early and late converters regarding age, MMSE score at baseline, sex, and the presence of APOE  $\epsilon 4$  alleles ( $P > .05$ ). The lack of difference in MMSE scores between early and late converters might be explained by the fact that the MMSE is not a very sensitive test for early cognitive deficits in AD.



**Figure 3.** Mixture model classification for cerebrospinal fluid (CSF)  $\beta$ -amyloid 1-42 (A $\beta$ 42) to phosphorylated tau (P-tau) ratio (A) and CSF A $\beta$ 42 (B). Results are shown as histograms of observed biomarker levels overlaid with the 2 mixture distributions. The CSF A $\beta$ 42:P-tau ratios and the CSF A $\beta$ 42 levels exhibited a clear bimodal distribution. The dotted vertical lines represent the cutoff values established by the mixture model analysis. An A $\beta$ 42:P-tau ratio less than 6.16 (A) and an A $\beta$ 42 level less than 505 ng/L (B) were regarded as pathologic. C and D, The same histograms but including data revealing the clinical diagnostic outcome. AD indicates Alzheimer disease; MCI, mild cognitive impairment.

**Table 2.** Ability of Cerebrospinal Fluid Biomarkers to Predict Subsequent Development of Alzheimer Disease in Patients With Mild Cognitive Impairment Using the Diagnosis-Independent Cutoff Values From the Mixture Model Analysis

	Cutoff Value <sup>a</sup>	Youden Index	Sensitivity, %	Specificity, %	LR+	LR-	PPV, % <sup>b</sup>	NPV, % <sup>b</sup>
A $\beta$ 42	<505 ng/L	0.66	90	76	3.73	0.13	81	87
A $\beta$ 42:P-tau ratio	<6.16	0.78	88	90	9.02	0.14	91	86
A $\beta$ 42 + T-tau	<505 ng/L (A $\beta$ 42), >350 ng/L (T-tau)	0.72	82	90	8.44	0.20	91	81
A $\beta$ 42:P-tau ratio + T-tau	<6.16 (A $\beta$ 42:P-tau), >350 ng/L (T-tau)	0.75	82	94	12.60	0.19	94	82

Abbreviations: A $\beta$ 42,  $\beta$ -amyloid 1-42; LR+, positive likelihood ratio; LR-, negative likelihood ratio; NPV, negative predictive value; P-tau, phosphorylated tau; PPV, positive predictive value; T-tau, total tau.

<sup>a</sup>The unbiased cutoff values were established after the mixture model analysis.

<sup>b</sup>The pretest probability for future Alzheimer disease dementia in patients with mild cognitive impairment at baseline was 0.54.

### PREDICTION OF AD WITHIN 9.2 YEARS IN PATIENTS WITH MCI

The AUCs for the different CSF biomarkers ranged from 0.81 to 0.91 when predicting future development of AD dementia within 9.2 years in patients with MCI. The predictive ability of A $\beta$ 42 (AUC, 0.91; 95% CI, 0.84-0.95), the A $\beta$ 42:P-tau ratio (AUC, 0.91; 95% CI, 0.84-0.95), and the A $\beta$ 42:T-tau ratio (AUC, 0.90; 95% CI, 0.84-0.95) were significantly greater than those of T-tau (AUC, 0.82; 95% CI, 0.74-0.88) and P-tau (AUC, 0.81; 95% CI, 0.74-0.88) ( $P < .05$ ). To avoid overestimation of the predictive accuracy of the biomarkers, a mixture model was used to establish accurate cutoff values for A $\beta$ 42 and the A $\beta$ 42:P-tau ratio. This is an independent and unsupervised way of establishing cutoff values because it does not use any clinical information.<sup>26</sup> The histograms in **Figure 3A** and **B** show the frequency in the MCI cohort of baseline CSF A $\beta$ 42:P-tau ratios and A $\beta$ 42 levels, respectively. These histograms reveal that 2 different populations seem to have existed among patients with MCI at baseline, indicating that the levels of these CSF biomarkers were a mixed sample originating from 2 different normal distributions (ie, 1 AD-like and 1 control-like population). The mixture model analysis revealed that a cutoff value of 6.16 for the A $\beta$ 42:P-tau ratio and 505 ng/L for A $\beta$ 42 could separate these 2 populations (Figure 3A and B and eFigure 2).

Using these unbiased cutoff values, the A $\beta$ 42:P-tau ratio could predict the future development of AD dementia

within 9.2 years with a sensitivity of 88%, a specificity of 90%, a positive predictive value of 91%, and a negative predictive value of 86% (Figure 3C and **Table 2**). This means that among patients with MCI at baseline, 91% of those with a pathologic A $\beta$ 42:P-tau ratio and 14% of those with a normal A $\beta$ 42:P-tau ratio progressed to AD dementia during follow-up. eTable 2 gives the demographic data of the true and false positives, as well as the true and false negatives, based on the A $\beta$ 42:P-tau ratio. The predictive ability of A $\beta$ 42 alone was somewhat less accurate, exhibiting a sensitivity of 90% and a specificity of 76% for the prediction of future AD (Figure 3D and Table 2). The higher specificity of the A $\beta$ 42:P-tau ratio compared with A $\beta$ 42 alone seemed to reflect the fact that the ratio less often misclassifies dementias other than AD as AD (Figure 3C and D). When defining pathologic CSF as a combination of a decreased A $\beta$ 42:P-tau ratio (<6.16) and an increased T-tau level (>350 ng/L), the positive predictive value increased from 91% to 94%, compared with using the A $\beta$ 42:P-tau ratio alone (Table 2). However, this combination of biomarkers resulted in a reduction in the negative predictive value from 86% to 82% because many patients with MCI who developed AD after 5 to 10 years had normal T-tau levels at baseline (Table 2).

Kaplan-Meier estimates for the ability of the A $\beta$ 42:P-tau ratio or the combination of T-tau and the A $\beta$ 42:P-tau ratio to predict the future development of AD using the unbiased cutoff values are depicted in **Figure 4**. Patients with MCI with a pathologic A $\beta$ 42:P-tau ratio ( $n=69$ )

exhibited an incidence of AD dementia of 27% per year compared with 2.2% per year in patients with a normal CSF A $\beta$ 42:P-tau ratio (n=65). When pathologic CSF was defined as a combination of T-tau (>350 ng/L) and A $\beta$ 42:P-tau ratio (<6.16), the incidence of AD dementia in patients with MCI who had pathologic CSF (n=63) was 30% per year compared with 2.9% per year in patients with normal CSF (n=71).

The risk factors were analyzed simultaneously using a multivariate backward stepwise Cox regression model. Pathologic CSF (A $\beta$ 42:P-tau ratio <6.16), age, and female sex were significantly associated with progression to AD in patients with MCI, and the other risk factors (APOE  $\epsilon$ 4 carrier status and MMSE score at baseline) did not contribute to the explanatory power of the model. The same results were obtained when defining pathologic CSF as an A $\beta$ 42:P-tau ratio of less than 6.16 combined with a T-tau level greater than 350 ng/L.

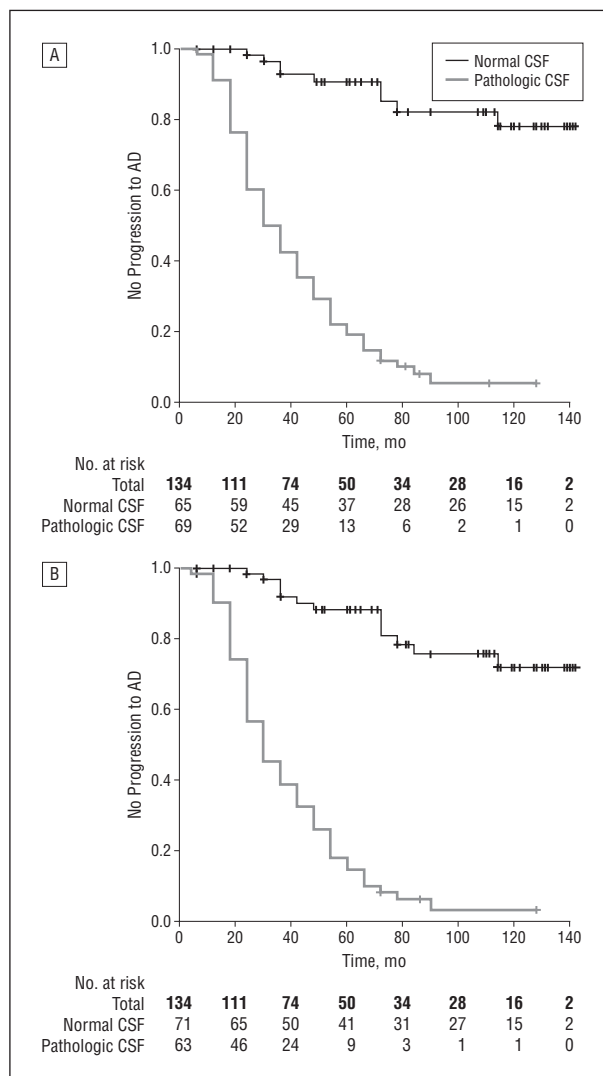
### COMMENT

We found that pathologic CSF biomarkers in patients with MCI are highly predictive of future AD dementia. A low baseline A $\beta$ 42:P-tau ratio predicted the future development of AD dementia within 9.2 years with a positive predictive value of 91% and a negative predictive value of 86%. Moreover, we found that A $\beta$ 42 levels are already fully altered at least 5 to 10 years before conversion to AD dementia, whereas T-tau and P-tau seem to be later markers.

### DIAGNOSTIC FOLLOW-UP

A major strength of this study is that it is by far the longest clinical follow-up of patients with MCI at baseline.<sup>9-18</sup> The patients with MCI either developed a certain type of dementia during follow-up or remained cognitively stable for a median of 9.2 years. Since the previous follow-up of 5.2 years have finished,<sup>13</sup> additionally 15 patients progressed to clinical dementia during the extended follow-up program. In the previous study,<sup>13</sup> with median follow-up of 5.2 years, this group was misclassified as having stable MCI, a fact that supports the need for extended follow-up of more than 5 years in studies evaluating the ability of biomarkers to identify prodromal AD. Consequently, studies with shorter clinical follow-up will underestimate the positive predictive value and the specificity of biomarkers. Furthermore, long-term clinical follow-up also increases the accuracy of the clinical diagnoses because the course of the symptoms can be assessed in a more reliable way. However, although the patients with cognitively stable MCI in the present study were observed for a median of 9.2 years, clinical follow-up ranged from 4 to 12 years. Consequently, a few patients with prodromal AD might be present in the subgroup of cognitively stable patients with stable MCI as a group have slightly lower levels of A $\beta$ 42 compared with controls.

Another advantage of the present study was the detailed diagnostic workup. Although most patients who developed dementia during follow-up received a diagnosis of AD, a subset developed other dementia disor-



**Figure 4.** Kaplan-Meier estimates of the rate of progression to Alzheimer disease (AD) in patients with mild cognitive impairment with either normal or pathologic cerebrospinal fluid (CSF) biomarker levels at baseline. A, Pathologic CSF was defined as a  $\beta$ -amyloid 1-42 (A $\beta$ 42) to phosphorylated tau (P-tau) ratio less than 6.16. B, Pathologic CSF was defined as a combination of total tau less than 350 ng/L and an A $\beta$ 42:P-tau ratio less than 6.16. Numbers at risk are the number of patients with mild cognitive impairment at each time point who had not developed any type of dementia and for whom clinical follow-up was still ongoing.

ders, including VaD, dementia with Lewy bodies, frontotemporal dementia, and semantic dementia. This is in line with postmortem studies showing that a significant subset of patients with MCI exhibit neuropathologic features associated with those forms of non-AD dementias.<sup>27-29</sup> The lack of neuropathologic data to support the clinical diagnoses is a limitation of the present study. These data are difficult to obtain in studies including patients with MCI in a consecutive manner because the life expectancy from baseline usually exceeds 10 years.

### TEMPORAL ORDER OF THE PATHOLOGIC PROCESSES

It has recently been suggested that altered A $\beta$  metabolism precedes tau-associated pathologic features and neu-

rodegeneration during the development of AD, although there is still limited evidence of this relationship in human studies.<sup>3,4</sup> However, the results of the present study might provide the first direct evidence for this hypothesis. We found that the levels of A $\beta$ 42 in CSF, which reflect brain A $\beta$  deposition,<sup>5</sup> were already fully altered 5 to 10 years before the development of AD dementia, which was not the case for P-tau and T-tau, which reflect the hyperphosphorylation state of tau and neurodegeneration, respectively (Figure 2).<sup>5</sup> Thus, A $\beta$ 42 levels probably decrease very early during the pathogenesis of AD and reach plateau levels long before dementia develops, maybe even during presymptomatic stages, that is, before any cognitive symptoms are recognized by the affected individual.<sup>30,31</sup> In contrast, the finding that late converters (5-10 years) have more normal P-tau and T-tau levels than do early converters (0-5 years) indicates that these biomarkers probably increase gradually over a 5- to 10-year period before conversion to AD dementia. These results are in agreement with earlier findings showing that patients with AD dementia exhibit stable levels of A $\beta$ 42 over time,<sup>32,33</sup> whereas the P-tau and T-tau levels tend to increase gradually over the years.<sup>32-34</sup> The present results indicate that CSF A $\beta$ 42, similar to positron emission tomography amyloid imaging, is a valid diagnostic marker that changes during the very early stages of the disease and then is stable over time when symptoms have developed. Although CSF P-tau and T-tau can be regarded as diagnostic biomarkers to some extent, they might also reflect disease progression during the early symptomatic phase, together with measurements of brain atrophy, cerebral blood flow reductions, and cognitive performance.<sup>3,4</sup>

#### CSF BIOMARKERS AS DIAGNOSTIC TOOLS IN DRUG TRIALS AND IN CLINICAL PRACTICE

New disease-modifying therapies are more likely to be effective when initiated in the prodementia (or even better in the presymptomatic) phase of AD, when the neurodegeneration is not too widespread. However, this approach requires methods to detect prodromal AD with high accuracy.<sup>5,6</sup> The present study strongly supports the hypothesis that CSF biomarkers can identify MCI cases with a very high risk of subsequent AD dementia. Especially, the combination of the A $\beta$ 42:P-tau ratio with T-tau exhibits a very high positive predictive value (94%) (Table 2). Therefore, patients with MCI with pathologic CSF biomarkers could be selected for clinical trials of new disease-modifying therapies.<sup>5,6</sup> Using this approach, fewer patients with MCI would be needed, and the potential beneficial effects of treatments would be easier to detect in such treatment trials because most of the included patients would be affected by AD pathology. Furthermore, exclusion of patients with MCI with normal CSF profiles would prevent most patients with MCI without an underlying AD pathology from being exposed to potential harmful adverse effects in such trials.

The emergence of symptomatic treatments for patients with AD (eg, acetylcholinesterase inhibitors) and the general awareness of the disease has highlighted the clinical need for diagnostic markers for prodromal AD.<sup>1</sup> New diagnostic research criteria for AD have been proposed re-

cently.<sup>7</sup> According to these criteria, AD can be diagnosed at a prodementia (prodromal) stage supported by diagnostic biomarkers, such as CSF tau and A $\beta$ 42; magnetic resonance imaging–detected medial temporal atrophy; and amyloid positron emission tomography tracer retention.<sup>7</sup> However, to be used in the clinical practice of memory disorder clinics, the positive predictive values of such diagnostic methods (or combinations of methods) need to be very high (preferably >95%). The results of the present study suggest that the positive predictive values of these CSF biomarkers for the detection of prodromal AD in patients with MCI are approximately 90%. Consequently, approximately 10% of patients with MCI with pathologic levels of CSF biomarkers at baseline will not develop AD within 9.2 years. Because AD is a disorder with severe consequences, such common misclassification is undesirable. Therefore, CSF biomarkers can be only one piece of the puzzle in the identification of prodromal AD. Multimodal use of CSF biomarkers together with thorough clinical assessment and brain imaging will optimize the diagnostic accuracy. For example, the positive predictive value of CSF biomarkers can be increased if they are combined with other diagnostic methods, such as measurements of brain atrophy and regional cerebral blood flow reductions.<sup>35-37</sup> Evaluation of the optimal combinations of biomarkers for the identification of prodromal AD in patients with MCI, and of their interaction, needs to be performed in large MCI cohorts with extensive follow-up.<sup>4,7</sup>

Note that the incidence of AD dementia in patients with MCI with normal A $\beta$ 42:P-tau ratios was only 2.2% over 10 years in the present study, which is similar to that observed in cognitively healthy age-matched individuals.<sup>38</sup> When used in clinical workup along with a structured clinical assessment, patients with MCI with normal CSF biomarkers might be reassured and would most likely not need extensive and costly follow-up. For this use, biomarkers with a high negative predictive value are preferred, such as the A $\beta$ 42:P-tau ratio in the present study (negative predictive value, 86%) (Table 2).

There are still no established cutoff values for CSF biomarkers that can be applied in a global manner. The lack of external control programs has resulted in unsatisfactory standardization of biomarker measurements between different laboratories.<sup>39</sup> Therefore, a global external control program has been established, funded by the Alzheimer's Association.<sup>5</sup> In the present MCI cohort, we applied a mixture model analysis for the levels of A $\beta$ 42 and A $\beta$ 42:P-tau ratios to establish natural cutoff values. Compared with optimized cutoff values (when the value representing the highest sensitivity and specificity is chosen), the cutoff estimation based on mixture modeling is independent of clinical diagnosis. Therefore, overestimation of the predictive accuracy of the investigated biomarkers could be avoided.

In conclusion, taken together, approximately 90% of patients with MCI and pathologic CSF biomarkers at baseline will develop AD within 9.2 years. Therefore, these markers can identify individuals at high risk for future AD at least 5 to 10 years before conversion to dementia. Hopefully, new therapies that can retard or even halt progression of the disease will soon be available. Together with an early and accurate diagnosis, such therapies could

be initiated before neuronal degeneration is too widespread and patients are already demented. Moreover, the present data provide support in humans for the hypothesis that altered A $\beta$  metabolism precedes neurofibrillary tangle formation and neuronal degeneration because A $\beta$ 42 levels, but not P-tau and T-tau levels, are already fully altered in the CSF at least 5 to 10 years before conversion to AD dementia. However, further longitudinal biomarker studies of nondemented individuals with extended clinical follow-up are needed to provide more details about the temporal changes in CSF biomarkers in the prodromal phase of AD.

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