Cerebrospinal Fluid Tau and β-Amyloid 42 Proteins Identify Alzheimer Disease in Subjects With Mild Cognitive Impairment

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Context: Cerebrospinal fluid tau protein and β-amyloid 42 (Aβ42) protein are altered even in very mild Alzheimer disease (AD). So far, few data exist for subjects with mild cognitive impairment (MCI).

Objective: To investigate the potential of cerebrospinal fluid tau and Aβ42 for predicting progression from MCI to AD in a longitudinal study of 28 patients with MCI who received follow-up for 18 months.

Design: An 18-month prospective study.

Setting: Clinical follow-up study of community-residing subjects with MCI.

Main Outcome Measures: Cerebrospinal fluid tau and Aβ42 concentrations were measured using enzyme-linked immunosorbent assay at baseline. The potential of both biomarkers was evaluated to predict the progression to dementia, the end point of this study, using multiple logistic regression analysis.

Results: Of 28 subjects with MCI, 12 progressed to dementia (2 to frontotemporal dementia; 10 to AD). Six subjects had progressive MCI, and 10 subjects showed stable MCI. Cerebrospinal fluid tau levels were significantly elevated in patients who progressed to probable AD (P = .002) and subjects with progressive MCI (P = .003) compared with subjects who had stable MCI. Cerebrospinal fluid Aβ42 levels were significantly lower in patients who progressed to probable AD (P = .007) and those with progressive MCI (P = .04) than in subjects with stable MCI. Logistic regression analysis identified elevated tau protein level as a predictor of cognitive deterioration (P = .02), whereas a delayed verbal recall score at baseline was significantly associated with the development of probable AD (P = .03).

Conclusion: Our results indicate that altered tau and Aβ42 concentrations may be detectable in subjects who are clinically diagnosed as having MCI but demonstrate the pathological changes of AD.

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MILD COGNITIVE impairment (MCI) is an etiologically heterogeneous syndrome characterized by memory performance below the age norm, otherwise unimpaired intellectual functioning, and preserved activities of daily living. A substantial proportion of patients with MCI later develop clinically diagnosable Alzheimer disease (AD). At autopsy, subjects with MCI show a broad spectrum of morphological brain changes including typical AD pathological characteristics. Therefore, MCI partly represents a pre dementia stage of AD. To maximize the benefit of therapeutic strategies that maintain cognitive and functional performance or delay the progression of the neurodegenerative process, it is essential to identify AD at the stage of MCI. Because the pattern of neuropsychological impairment in MCI is etiologically nonspecific, biochemical and neuroimaging markers will be required to establish the diagnosis so early in the course of the disease.

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Among the currently discussed biomarkers for AD, microtubule-associated tau protein and β-amyloid 42 (Aβ42) protein are the most promising. Both are closely associated with the histopathological hallmarks of AD. Increased neuronal production or decreased degradation of Aβ42 protein, a 42-amino acid peptide derived from the larger amyloid precursor protein by proteolytic cleavage, leads to the extensive formation of extracellularly located senile plaques in AD. Owing to the increased deposition in AD, cerebrospinal fluid (CSF) concentrations of Aβ42 are lower in patients with AD than in controls and in patients with other de-
Patients with memory complaints were recruited using a newspaper and television broadcast announcement; they came to the university memory clinic for further diagnostic evaluation. This study used 28 outpatients who were derived from a larger sample. They received a clinical diagnosis of MCI after a thorough evaluation at a university hospital memory clinic and agreed to participate in a follow-up protocol. The diagnostic work-up at baseline included psychiatric and neurologic evaluations, an informant interview, a cognitive examination using the CERAD neuropsychological battery, otherwise normal cognitive deficits, including vitamin B12 deficiency, folate deficiency, thyroid dysfunction, moderate or severe depression on the Geriatric Depression Scale, or significant cerebrovascular abnormalities (>2 lacunes in a strategic location, large or multiple cortical infarctions, or a severe white matter change).

The follow-up protocol consisted of annual visits to the clinic and telephone contact every 6 months, with additional clinical visits if worsening of symptoms was reported and an in-person examination of every subject at the 18-month follow-up time point. The follow-up visits were performed by trained physicians at the memory clinic who were blinded to the CSF results and APOE genotypes. Progression to dementia was assumed if impairment was present on the Bayer Activities of Daily Living Scale, the Clinical Dementia Rating was 1, and clinical signs of moderate or severe depression were absent. Patients with MCI who showed worsening of cognitive deficits on applied neuropsychological tests such as the CERAD neuropsychological battery, Mini-Mental State Examination, and clock drawing without meeting the diagnostic criteria of dementia were classified as having progressive MCI. Patients with MCI who retained their cognitive ability or showed minimal improvement were classified as having stable MCI. We also included 75 patients with probable AD and 30 cognitively healthy age-matched controls who underwent myelography as part of a neurosurgical work-up. Both groups have been described previously. The subjects with MCI are described in greater detail in Table 1. Patients who progressed to AD were significantly older than subjects who still had a diagnosis of MCI.

Table 1. Description of Patients at Baseline by Diagnosis After 18-Month Follow-up Period

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Alzheimer's Disease</th>
<th>Frontotemporal Dementia</th>
<th>Progressive Mild Cognitive Impairment</th>
<th>Stable Mild Cognitive Impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M:F</td>
<td>5:5</td>
<td>2:0</td>
<td>3:3</td>
<td>6:4</td>
</tr>
<tr>
<td>Age, y</td>
<td>75.2 ± 5.2</td>
<td>58.0 ± 63.0</td>
<td>27.1 ± 1.9</td>
<td>27.5 ± 1.1</td>
</tr>
<tr>
<td>MMSE score at baseline</td>
<td>27.6 ± 1.6</td>
<td>29.0 ± 29.0</td>
<td>25.3 ± 1.7</td>
<td>27.8 ± 1.2</td>
</tr>
<tr>
<td>MMSE score at follow-up</td>
<td>23.0 ± 1.9</td>
<td>27.0 ± 28.0</td>
<td>4.0 ± 1.1</td>
<td>3.8 ± 1.3</td>
</tr>
<tr>
<td>CERAD delayed verbal recall score at baseline</td>
<td>2.0 ± 1.3</td>
<td>5.0 ± 5.0</td>
<td>3.6 ± 1.8</td>
<td>4.7 ± 2.1</td>
</tr>
<tr>
<td>CERAD delayed recall score at follow-up</td>
<td>1.2 ± 1.5</td>
<td>2.0 ± 7.0</td>
<td>0.76 ± 0.9</td>
<td>0.89 ± 0.43</td>
</tr>
<tr>
<td>B-ADL score at baseline</td>
<td>1.22 ± 0.47</td>
<td>3.0 ± 2.5</td>
<td>3.0 ± 0.8</td>
<td>4.0 ± 2.6</td>
</tr>
<tr>
<td>GDS score at baseline</td>
<td>4.2 ± 1.9</td>
<td>3.0 ± 2.5</td>
<td>0.42</td>
<td>0.20</td>
</tr>
</tbody>
</table>

*Data concerning B-ADL scores are presented as the quotient of the sum of all questions divided by the number of questions.
†Data concerning B-ADL scores are presented as the quotient of the sum of all questions divided by the number of questions.

*Data are presented as mean ± SD unless otherwise indicated. MMSE indicates Mini-Mental State Examination; CERAD, Consortium to Establish a Registry for Alzheimer’s Disease; B-ADL, Bayer Activities of Daily Living Scale; and GDS, Geriatric Depression Scale.

To define the patient sample, we applied the Mayo Clinic MCI criteria for inclusion in combination with the exclusion terms used to characterize probable AD in the criteria of the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association. Patients were included if they had subjective memory complaints, memory performance of at least a 1.5 SD worse than the respective age norm (delayed verbal recall according to the CERAD neuropsychological battery), otherwise normal cognitive ability (normal verbal fluency, object naming, and visuoconstruction according to the CERAD neuropsychological battery and the clock-drawing test), normal results on the Bayer Activities of Daily Living Scale, and absence of dementia consistent with a Clinical Dementia Rating of 0.5, which was completed after interviewing the patient and informant. Patients were excluded if they showed evidence of any systemic disorder or other brain disease that could account for the memory deficit, including vitamin B12 deficiency, folate deficiency, thyroid dysfunction, moderate or severe depression on the Geriatric Depression Scale, or significant cerebrovascular abnormalities (>2 lacunes in a strategic location, large or multiple cortical infarctions, or a severe white matter change).
immunosorbet assay (Innogenetics, Zwijndrecht, Belgium) as described previously in greater detail.34,45

The DNA was obtained using a standard blood extraction kit (Quiagen, Hilden, Germany). APOE genotypes were determined by hybridization of the amplified biotinylated product with allele-specific oligonucleotides immobilized on membrane-based strips and subsequent chromogen detection (Innolipa APOE, Kit K-1038; Innogenetics). All statistical procedures were performed using SPSS statistical software (SPSS Inc, Chicago, Ill).

Data concerning tau and Aβ42 proteins were presented as medians with the 25th and 75th percentiles because values were not normally distributed. Wilcoxon and Kruskal-Wallis tests were applied to 2-group and 3-group comparisons, respectively. Group differences in APOE allele frequencies were calculated using the 2-tailed Fisher exact test. To identify factors that contribute to progression, we performed multiple logistic regression analysis comparing subjects with progressive MCI (subjects who progressed to AD and subjects showing progressive MCI) with subjects showing stable MCI, and subjects who progressed to AD with subjects showing progressive MCI, using the covariates of tau and Aβ42 protein concentrations, age, sex, APOE genotype, and scores on the Mini-Mental State Examination, delayed verbal recall, and Bayer Activities of Daily Living tests.

### RESULTS

The 18-month follow-up data are available for all 28 patients. Of these, 12 (42.9%) progressed to dementia (2 [7.1%] to frontotemporal degeneration [FTD] according to consensus criteria;83 and 10 [35.7%] to AD), whereas 16 (57.1%) were still diagnosed as having MCI. Assuming that the conversion rate is constant with regard to time,28 the annual progression rate to AD was calculated at 23.8%. However, cognitive performance declined in 6 of 16 subjects with MCI (progressive MCI) and remained unchanged in 10 subjects with this condition (stable MCI). Subjects who progressed from MCI to AD were significantly older (mean±SD age, 75.2±5.2 years) and had lower scores on the CERAD delayed verbal recall test (mean±SD score, 2.0±1.3) than subjects still diagnosed as having MCI (mean±SD age, 66.2±9.2 years; P=.02; mean±SD score, 3.8±1.3; P=.003) (Table 1). No significant differences between groups were observed on the Bayer Activities of Daily Living or Geriatric Depression Scale tests.

Patients who progressed from MCI to AD had significantly higher median CSF tau protein concentrations at baseline than subjects with stable MCI (P=.002) and controls (P<.001) (Table 2). Patients with progressive MCI also showed significantly higher CSF tau concentrations at baseline than subjects with stable MCI (P=.003) and controls (P<.001). No statistically significant differences in CSF tau concentration were found among patients who progressed from MCI to AD, subjects with progressive MCI, and a historical group of patients with clinically diagnosed AD (Figure 1).

Patients who progressed to AD had significantly lower CSF Aβ42 protein concentrations at baseline than patients with stable MCI (P=.007) and controls (P<.001) (Table 2) but did not differ in this regard from subjects with progressive MCI and patients with AD. In patients with progressive MCI, CSF Aβ42 concentrations were also significantly lower than in subjects with stable MCI (P=.04) and controls (P<.001) (Figure 2).

Considering both biochemical markers simultaneously and using a cutoff line (Aβ42 = 240 + 1.18 × tau) derived from a recent study,9 (90%) of 10 subjects who progressed to AD and 5 (83%) of 6 subjects with progressive MCI showed the pathological CSF marker pattern of AD, whereas 9 of 10 subjects with stable MCI showed low tau and high Aβ42 concentrations (Figure 3). Thus, the application of both markers to identify subjects with the highest risk of conversion from MCI to AD yielded a sensitivity and specificity of 90%.

The ε4 allele frequency was considerably higher in subjects who progressed to AD (0.5) and subjects with progressive MCI (0.42) compared with those who had stable MCI (0.2). However, because of the small sample size, these differences failed to reach a significant level. In addition, both subjects who progressed from MCI to FTD were homozygous for the ε3 allele.

When comparing patients who retained their cognitive ability with those who showed a progression of cognitive symptoms (excluding patients who developed FTD), tau was the only differentiating variable according to logistic regression analysis (P=.02). In the 16 patients who showed a worsening of cognitive impairment (excluding patients who developed FTD), the CERAD delayed verbal recall score at baseline was the only significant variable indicating the progression to probable AD (P=.03), whereas chronological age showed a nonsignificant trend (P=.09).

### COMMENT

In this ongoing study, we evaluated whether the CSF tau and Aβ42 proteins could potentially identify AD pathological characteristics at the stage of MCI. We found significantly higher tau and significantly decreased Aβ42 levels in 10 subjects who progressed from MCI to AD during an 18-month period, compared with 10 patients who had stable MCI and 2 patients who progressed to FTD. We observed the same pattern of significantly elevated
CSF tau and decreased Aβ42 concentrations in 6 subjects who showed progressive MCI but were still diagnosed as having this condition at follow-up. Although patients with MCI need to receive further follow-up for us to determine whether or not they will develop the typical clinical syndrome of AD, we assume that most subjects with progressive MCI may have the neurological features of AD because the CSF marker profile and ε4 allele frequency are identical for the 2 conditions. Simple fluctuation of cognitive test scores in these patients seems unlikely; all patients with progressive MCI showed a decline of their cognitive abilities in several areas. Because of these considerations and in agreement with the concept of MCI, we believe that the term progressive MCI as used in this article does not describe an independent progressive disorder but rather a stage of MCI closer to AD.

The reason for cognitive impairment in patients with stable MCI is not clear. However, these patients had significant deficits in memory tasks (≥1.5 SD worse than the age norm) but no symptoms on the Geriatric Depression Scale, so we assume that this impairment is not the result of anxiety or depression.

Despite the small sample size and limited follow-up data in this study, it is obvious that MCI is an etiologically heterogeneous syndrome. Therefore, MCI represents a transition stage both for patients with a primary degenerative disease and for those who may recover (eg, major depression or metabolic deficits). In other patients, MCI may delineate the final stage. In light of current and upcoming therapies aimed at slowing the progression of AD, it is of particular interest to identify those subjects with a primary degenerative type of MCI who will be at highest risk for progression to AD.
In our sample, multiple logistic regression analysis identified tau as a single variable that predicted progression in subjects either showing progressive MCI or who progressed to probable AD. This result is consistent with the view that tau release reflects neuronal degeneration and identifies it even at a predementia stage. The fact that CSF tau protein concentrations did not differ significantly among patients with progressive MCI, subjects with MCI who progressed to probable AD, those with very mild AD, or patients with mild to moderate AD confirms our previous findings and supports the theory that elevated tau level is a trait marker of AD independent of the clinical expression of the neurodegenerative process. Assuming that the tau concentration in the CSF reflects the rate of neuronal damage and thereby the activity of the neurodegenerative process, our observation is consistent with the view that the neurodegenerative process commences before the onset of MCI and continues at a constant rate during an extended phase of the disease. In both subjects with FTD, however, tau concentrations were not elevated at baseline. The reason CSF tau levels were not elevated in these patients remains to be investigated. A possible explanation is that the neurodegenerative process in patients with FTD may be restricted to distinct brain areas that do not affect the ventricles or lumbar CSF at this early stage of the disease. In contrast, the localization of the neurodegenerative process in subjects with MCI who progressed to AD may involve structures surrounding the ventricles, such as the hippocampus, in addition to other cortical areas.

The CERAD delayed verbal recall score at baseline was the only variable that discriminated between patients who progressed to probable AD and those who showed progressive MCI. A possible explanation is that disease progression may be more advanced in patients with lower delayed verbal recall scores, so these subjects may progress faster to clinically diagnosable AD than those with higher scores. In addition, the significantly older ages of these patients may further contribute to the progression. The predictive value of the delayed recall score identified in this study corresponds to the results obtained by other authors. The effect of age is consistent with epidemiological studies showing that increased age is a major risk factor for the development of AD, and was also present in a recent MCI study.

Because of the small sample size after stratifying for APOE4 genotype, we were not able to determine the effect of this genotype as a prognostic indicator. However, the ε4 allele frequency was considerably higher in subjects who progressed to AD as compared with those who had stable MCI. Although our results are complicated by the different designs between this study and 2 recent ones measuring CSF markers in subjects with MCI, the values obtained for sensitivity and specificity to predict progression to AD were comparable in all 3. Both recent studies, however, excluded subjects if they had stable MCI or progressed to forms of dementia other than AD. Thus, our annual conversion rate to AD (23.8%) was considerably lower than that in both studies. Compared with community-based studies, our conversion rate was relatively high but was within the magnitude of other studies, whose rate ranged from 1% to 25% per year. The relatively high conversion rate of this study may be caused by our focus on subjects with a primary degenerative type of MCI and exclusion of most other MCI-causing diseases. For further validation of these biomarkers and their utility to predict progression to AD among subjects with MCI, community-based studies are required.

In conclusion, the results of this ongoing study suggest that the CSF biochemical marker profile typical for AD—elevated tau and decreased Aβ42 levels—may often be present at a predementia stage of the disease. Therefore, our study provides preliminary evidence that CSF markers may have high predictive power to identify subjects with MCI who have the greatest risk of progressing to clinically diagnosable AD. Shifting the diagnostic threshold to the predementia stage is of paramount importance with respect to future disease-modifying treatment strategies.

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REFERENCES