Cerebrospinal Fluid Biomarkers and Rate of Cognitive Decline in Very Mild Dementia of the Alzheimer Type

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Background: Cerebrospinal fluid (CSF) levels of Aβ peptide 1-42 (Aβ 42), tau, and phosphorylated tau (ptau) are potential biomarkers of Alzheimer disease.

Objective: To determine whether Aβ 42, tau, and ptau predict the rate of cognitive change in individuals with very mild dementia of the Alzheimer type (DAT).

Design: Retrospective analysis of CSF biomarkers and clinical data.

Setting: An academic Alzheimer disease research center.

Participants: Research volunteers in a longitudinal study of aging and cognition. Participants (n=49) had a clinical diagnosis of very mild DAT with a Clinical Dementia Rating (CDR) of 0.5 at the time of lumbar puncture. All the participants had at least 1 follow-up assessment (mean [SD] follow-up, 3.5 [1.8] years).

Main Outcome Measures: Baseline CSF levels of Aβ 42, Aβ 40, tau, and ptau at threonine 181 (ptau181) and the rate of dementia progression as measured using the CDR sum of boxes (CDR-SB) score and psychometric performance.

Results: The rate of dementia progression was significantly more rapid in individuals with lower baseline CSF Aβ 42 levels, higher tau or ptau181 levels, or high tau: Aβ 42 ratios. For example, the annual change in the CDR-SB score was 1.1 for the lowest 2 tertiles of Aβ 42 values and 0.3 for the highest tertile of Aβ 42 values.

Conclusions: In individuals with very mild DAT, lower CSF Aβ 42 levels, high tau or ptau181 levels, or high tau: Aβ 42 ratios quantitatively predict more rapid progression of cognitive deficits and dementia. Biomarkers of CSF may be useful prognostically and to identify individuals who are more likely to progress for participation in therapeutic clinical trials.

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The efficacy of treatments for Alzheimer disease (AD) will likely depend on accurately identifying individuals with underlying AD pathology (eg, plaques and tangles) early in the course of the disease. Although the clinical diagnosis of dementia of the Alzheimer type (DAT) is accurate in specialized centers, the sensitivity of diagnosis, particularly at milder stages of disease or with a single clinical evaluation, may be much lower.1,3 Because there is a growing emphasis on enrolling individuals with less cognitive impairment into clinical trials of putative anti-AD agents, methods are needed that will identify individuals with very mild DAT who are more likely to exhibit measurable cognitive decline during the study.

Disease-specific biomarkers, such as levels of Aβ peptide 1-42 (Aβ 42), tau, and phosphorylated tau (ptau) in cerebrospinal fluid (CSF), have reasonable levels of sensitivity and specificity for the diagnosis of DAT.4-6 Recent studies7,8 combining amyloid imaging using Pittsburgh Compound B with analysis of CSF biomarkers have shown that levels of CSF Aβ 42 can accurately separate individuals who have appreciable deposits of neocortical amyloid from those who do not. In a recent study, individuals diagnosed as having “mild cognitive impairment” (MCI9,10) who had “pathologic” concentrations of CSF tau or Aβ 42 had a 17.7-fold increased risk of progressing to diagnosed DAT during a 5-year period.11 The CSF Aβ 42: tau and Aβ 42: ptau ratios also identify cognitively healthy individuals who have a 4- to 5-fold increased risk of progressing to diagnosed DAT during a 5-year period.
risk of progressing to very mild DAT (Clinical Dementia Rating [CDR] of 0.5) within 3 to 4 years.\textsuperscript{,12} If CSF biomarkers reflect underlying pathophysiologic mechanisms that govern Aβ deposition and injury to neurons, levels of these biomarkers might correlate with the actual rate of cognitive decline in individuals with MCI or very mild dementia. We hypothesized that individuals with very mild DAT who had low CSF Aβ42 levels or high tau: Aβ42 ratios might undergo more rapid cognitive decline than mildly impaired individuals with higher CSF Aβ42 levels or lower tau: Aβ42 ratios. The ability of these CSF markers to predict rate of disease progression has implications for diagnosis and treatment of very mild DAT and for clinical trial design.

**METHODS**

**PARTICIPANTS AND CLINICAL ASSESSMENTS**

Participants were a subset of individuals enrolled in longitudinal studies of healthy aging and dementia at the Washington University Alzheimer’s Disease Research Center (WU-ADRC). All participants enrolled in the longitudinal studies were at least 60 years old at enrollment and were in good general health. All agreed to undergo lumbar puncture (LP); historically, 72% of participants enrolled complete LP and 78% return for annual follow-up. Individuals were selected for analysis herein if they had a diagnosis of DAT with a CDR\textsuperscript{13} of 0.5 (very mild impairment) at the time of LP and had at least 1 follow-up clinical assessment after LP. Participants underwent annual assessments that included assignment of CDR and a 1.5-hour psychometric test battery.\textsuperscript{14} The CDR, an assessment of the presence or absence of dementia and of dementia severity, is based on semistructured interviews with the individual and a collateral source. The CDR and diagnosis were determined independently of psychometric test results. The CDR sum of boxes (CDR-SB) score is a more quantitative representation of the CDR.\textsuperscript{15} Demographic features, health history, language function, medications, and depressive features were also assessed. Participants underwent a neurologic examination and had blood samples collected for apolipoprotein E genotyping.

The psychometric test battery included measures of episodic memory (Forward and Backward Digit Span, Associate Memory subscale of the Wechsler Memory Scale [WMS], and the Benton Visual Retention Test), executive function (digit span measures from the WMS, a word fluency test, and the WMS Mental Control subtest), and speeded visuospatial measures (Wechsler Adult Intelligence Scale block design and digit symbol and Trail-Making Test A). The general psychometric composite score\textsuperscript{16} used was prorated based on the other tests used to generate the original composite score because of changes in the psychometric test battery across the study period. Better cognitive functioning is indicated by lower scores on the CDR-SB and by higher scores on the psychometric composite.

All clinical diagnoses, including DAT and depression, were made in accordance with standard criteria.\textsuperscript{17} At the WU-ADRC, a CDR of 0.5 originally denoted individuals whose mental state was “neither clearly demented nor healthy;”\textsuperscript{18} the criteria for MCI also include a CDR of 0.5 to indicate the absence of clear dementia. With experience, the WU-ADRC has successfully identified the subset of individuals for whom the MCI is caused by underlying AD as subsequently determined by progression to greater stages of dementia severity and by histopathologic confirmation of AD.\textsuperscript{19,20} The CDR 0.5 designation in the WU-ADRC now denotes very mild dementia; in all the individuals with a CDR of 0.5 included herein, the clinical diagnosis was DAT. Some of these individuals would be considered to have MCI at other medical centers, but many were insufficiently impaired to meet the criteria for MCI and might be designated as “pre-MCI.”\textsuperscript{14} For comparison, we applied revised criteria for MCI\textsuperscript{21} to identify individuals with a CDR of 0.3 and DAT who scored 1-5 SDs or more below the mean of a comparison group of nondemented individuals on a measure of episodic memory (the Associate Memory subscale of the WMS). Studies were approved by the institutional review board at Washington University, and informed consent was obtained from all the participants.

**CSF COLLECTION AND ANALYSIS**

All individuals underwent LP for the collection of CSF using a standard procedure.\textsuperscript{1} The CSF samples were analyzed for total tau, ptau at threonine 181 (ptau181), and Aβ42 by means of a commercial enzyme-linked immunosorbent assay (Innotest; Innogenetics NV, Ghent, Belgium) as previously described.\textsuperscript{7} The Aβ40 was analyzed using an enzyme-linked immunosorbent assay as previously reported.\textsuperscript{21}

**STATISTICAL ANALYSES**

Associations between each of the CSF biomarkers at the time of LP and years of education and age were tested using Pearson product moment correlations; tests for independent samples were used to determine whether mean biomarker values differed by sex or by the presence of at least 1 APOE4 allele. General linear models (PROC GLM; SAS Institute Inc, Cary, North Carolina) were used to test whether there was a significant association between each of the CSF biomarkers and having a depression diagnosis while adjusting for the effects of age, sex, and education. We used mixed linear models (PROC MIXED; SAS Institute Inc) to determine whether there was a relationship between the slope of the CDR-SB score and time after the LP as a function of biomarker values after controlling for age, sex, and education. Similar analyses were conducted to examine biomarker-related differences in the slope of the psychometric composite scores after the LP.

**RESULTS**

**DEMOGRAPHIC AND BIOMARKER VALUES AT BASELINE ASSESSMENT IN INDIVIDUALS WITH VERY MILD DAT**

Forty-nine participants with a CDR of 0.5 and DAT underwent LP and had at least 1 follow-up clinical assessment. Follow-up varied because enrollment was ongoing. Demographic variables at the baseline assessment (before LP) are given in Table 1, and CSF biomarker values are given in Table 2. More than half of these participants performed better than the cutoff score for MCI on episodic memory performance and can be considered to be pre-MCI.\textsuperscript{14} Twenty-nine of these participants were included in the data set of Fagan et al.\textsuperscript{8} There were no significant correlations between the biomarkers and age, years of education, the CDR-SB score, or the psychometric composite score at the time of LP (see eTable; http://www.archneurol.com). Individuals with 1 or more APOE4 alleles had lower mean CSF...
Aβ42 levels than did those without an APOE4 allele (304.86 vs 418.42 pg/mL, *P* = .006). Individuals who had been diagnosed as having depression or mild mood disorder had significantly higher CSF Aβ42 levels than did those with no depression diagnosis (least squares means, 600.4 vs 364.0 pg/mL, *P* = .001 after adjustment for sex, age, and education).

### CORRELATION OF BIOMARKER VALUES WITH SUBSEQUENT CHANGE IN PSYCHOMETRIC COMPOSITE SCORES

We performed a similar analysis to compare the rate of change in the psychometric composite scores with the CSF biomarker values, again dividing the cohort into tertiles based on the distribution of the biomarker values for illustrative purposes. Individuals with lower Aβ42 values exhibited a more rapid rate of decline in the psychometric composite score after LP than did individuals with higher levels (*P* = .03). The slope of change was −0.6 points per year for the lowest tertile (CSF Aβ42 < 319 pg/mL), −0.5 points per year for the middle tertile (CSF Aβ42, 319-411.2 pg/mL), and −0.06 points per year for the highest tertile (CSF Aβ42 > 411.2 pg/mL). There was a faster rate of decline in the psychometric composite score for those with higher values of tau (*P* = .05), ptau181 (*P* = .04), and the ratio measures (*P* = .03) ([Figure 3](#)). Like the CDR-SB score, the slope of the psychometric composite score was not significantly associated with CSF Aβ40 values (*P* = .16).

### USE OF A “CUTOFF” VALUE TO IDENTIFY INDIVIDUALS WITH VERY MILD DAT LIKELY TO HAVE MORE RAPID COGNITIVE DECLINE

The utility of CSF biomarkers in clinical practice will require practical guidelines for interpretation of individual results. For example, we tested the ability of a CSF Aβ42 value of 411 pg/mL or less to predict more rapid disease progression. These values encompass the lower 2 tertiles of CSF Aβ42 values, and previous studies suggest that such individuals will uniformly demonstrate increased cortical binding of Pittsburgh Compound B [9] consistent with deposition of amyloid in the brain. 

#### Table 1. Demographic Characteristics of the 49 Study Participants With a CDR of 0.5 and DAT at the Assessment Before LP

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at LP, mean (SD), y</td>
<td>73.8 (10.0)</td>
</tr>
<tr>
<td>Sex, M:F, No.</td>
<td>36:19</td>
</tr>
<tr>
<td>CDR-SB score, mean (SD)</td>
<td>2.4 (1.2)</td>
</tr>
<tr>
<td>Education, mean (SD), y</td>
<td>15.2 (3.1)</td>
</tr>
<tr>
<td>MMSE score, mean (SD)</td>
<td>26.4 (2.9)</td>
</tr>
</tbody>
</table>

Abbreviations: CDR, Clinical Dementia Rating; CDR-SB, CDR sum of boxes; DAT, dementia of the Alzheimer type; LP, lumbar puncture; MMSE, Mini-Mental State Examination.

#### Table 2. Baseline CSF Biomarker Values for Mildly Impaired Individuals Included in the Analysis and for a Cohort of Nondemented Individuals

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>CDR of 0.5, DAT (n=49)</th>
<th>CDR of 0, Nondemented (n=90)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ42</td>
<td>434.1 (211.5)</td>
<td>567 (207)</td>
</tr>
<tr>
<td>Aβ40</td>
<td>9672.1 (3397.72)</td>
<td>9758 (3827)</td>
</tr>
<tr>
<td>tau</td>
<td>564.9 (302.5)</td>
<td>342 (175)</td>
</tr>
<tr>
<td>ptau181</td>
<td>85.9 (45.4)</td>
<td>62 (26)</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ42, Aβ peptide 1-42; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; DAT, dementia of the Alzheimer type; ptau181, phosphorylated tau at threonine 181.

*These values are for individuals without impairment, from an earlier study by Fagan et al. [4] and are included for comparison.

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**Figure 1**

**Figure 2**

**Figure 3**

[9] A CSF Aβ42 level of 411 pg/mL or less predicted a significant change in the CDR-SB score.
COMMENT

The main finding of this study is that baseline levels of the AD-related CSF biomarkers Aβ42, tau, and ptau181 and the tau:Aβ42 ratio quantitatively predict the rate of cognitive decline across time in individuals with very mild dementia. This study differs importantly from earlier studies in that we show that levels of biomarkers are strongly predictive of the actual rate of decline rather than with a dichotomous assessment of conversion/no conversion from mild impairment to diagnosed DAT. These findings are consistent with those from previous studies showing that CSF levels of Aβ42, tau, and ptau can be used to predict the likelihood that individuals without dementia will develop MCI or very mild dementia and with studies showing that biomarkers predict progression from MCI to DAT. The only published studies correlating AD-related CSF biomarkers with rate of cognitive decline showed that increased levels of 3 different ptau epitopes (ptau181, ptau231, and ptau199) correlated with a decline in the Mini-Mental State Examination score in individuals with MCI observed for 1 year.

The present study differs from those linking CSF biomarkers to “conversion” from MCI to DAT because although some of these individuals would be diagnosed as having MCI at other centers, most diagnosed as having very mild DAT at the WU-ADRC did not have sufficient impairment on objective memory testing to meet the MCI criteria. Their mean Mini-Mental State Examination scores were similar to those of individuals with MCI in other studies. The mild impairment, slow disease progression, and higher levels of CSF Aβ42 in some individuals raise the possibility that some of these individuals do not have underlying AD pathology. Clinical diagnosis is subsequently confirmed on neuropathologic examination approximately 90% of the time at the WU-ADRC, even at such mild levels of impairment. In one series, in individuals diagnosed as having mild DAT (CDR of 0.5) who did not meet the MCI criteria, at autopsy, 43 of 47 had AD, 1 had corticobasal degeneration, and 3 had healthy brains. In the present study, 5 of 16 individuals in the highest tertile for CSF Aβ42 had Aβ42 levels greater than 715 pg/mL, the highest level reported to date in autopsy-confirmed AD, which makes it unlikely that these individuals have underlying AD pathology. The CSF biomarker levels may accurately identify the 10% of individuals clinically diagnosed as having mild DAT who do not have underlying AD pathology, but pathologic studies are required to test this hypothesis. Most individuals in the highest tertile (11 of 16) had CSF Aβ42 levels less than 715 pg/mL, a finding consistent with possible AD pathology. Although the rate of progression in these individuals (0.3 boxes per year) is slow, such slow progression has been observed in individuals with autopsy-confirmed AD. For example, at the WU-ADRC a recent individual with autopsy-confirmed AD had no increase in the CDR-SB score for the first 2 years after LP; the CSF Aβ42 level was 457 pg/mL. Progression may not be linear throughout the course of disease and may be slower at milder stages of dementia.

The relationship between CSF Aβ42 and the Aβ42 pools in the brain, both soluble and in plaques, is likely complex and may change during the course of dis-
ease, but there is substantial evidence that once CSF levels of Aβ42 are low, they remain stable for several years in unimpaired and impaired individuals.33-37 The idea that changes in Aβ homeostasis, including the decrease in CSF Aβ42 levels, precede clinically detectable cognitive decline in late-onset AD by at least several years and perhaps by 10 to 15 years is supported by the correlation between CSF Aβ42 levels and the presence of cortical amyloid deposition even in cognitively healthy individuals and by the finding that an increased ratio of tau:Aβ42 is predictive of short-term decline from normal to very mild dementia.7,8,12 The recent study by Sluimer et al,38 which shows that change in levels of CSF biomarkers with time in mildly impaired individuals did not correlate with cognitive change as quantified by Mini-Mental State Examination scores,
supports the idea that biomarker levels remain stable across time even after the onset of impairment. These findings support a model in which, in individuals destined to develop AD, CSF Aβ42 levels decrease from normal to a new steady state before any symptoms of cognitive impairment develop. This decrease might be triggered by deposition of Aβ plaques in some brain regions. The present findings suggest that this new “set point” for Aβ42 will correlate with the rate of disease progression once impairment is present.

Although the number of participants in this study was relatively small, the results suggest that CSF biomarkers might be useful as entry criteria for clinical trials of disease-modifying therapies for MCI and very mild DAT. Limiting enrollment to individuals with CSF Aβ42 values below a certain cutoff point might ameliorate the difficulties...
caused by lack of disease progression in some individuals during the trial. For example, in this study, individuals with CSF Aβ42 values of 411 pg/mL or less progressed at a rate of 1.11 boxes per year, with a variance of 0.49, whereas the unselected group of all individuals with a CDR of 0.5 progressed more slowly, at a rate of 0.78 boxes per year, with a variance of 0.70. Using these group characteristics, we calculated how many participants would be needed to power a hypothetical clinical trial, assuming a 2-armed study (1:1 treatment vs placebo). If all individuals with a diagnosis of very mild dementia and a CDR of 0.5 were enrolled, 354 participants would be needed to detect a 50% treatment effect on the CDR-SB score after 1.5 years using a standard normal test at a significance level of 5%, whereas less than half as many participants (n = 154) would be needed if CSF Aβ42 levels less than 411 pg/mL were included as an inclusion/exclusion criterion to select participants.39 These findings are likely to have important implications for reducing the number of participants needed to show an effect in clinical trials for very mild DAT and MCI and, ultimately, to assist in making treatment decisions as more invasive and potentially harmful disease-modifying treatments for AD become available.

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Author Contributions: Dr Snider had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Snider, Fagan, Grant, Morris, and Holtzman. Acquisition of data: Fagan, Shah, Morris, and Holtzman. Analysis and interpretation of data: Snider, Fagan, Roe, Grant, Xiong, and Holtzman. Drafting of the manuscript: Snider, Shah, and Holtzman. Critical revision of the manuscript for important intellectual content: Snider, Fagan, Roe, Grant, Xiong, and Holtzman. Statistical analysis: Roe and Xiong. Obtained funding: Morris and Holtzman. Administrative, technical, and material support: Grant, Morris, and Holtzman. Study supervision: Snider, Fagan, Grant, and Holtzman.

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Additional Information: The eTable is available at http://www.archneurol.com.

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REFERENCES


