Cerebrospinal Fluid Aβ and Tau Level Fluctuation in an Older Clinical Cohort

Abhay Moghekar, MB, BS; Joshua Goh, PhD; Ming Li, BA; Marilyn Albert, PhD; Richard J. O’Brien, MD, PhD

Objective: To determine whether cerebrospinal fluid (CSF) biomarkers for Alzheimer disease fluctuate significantly over time in a cohort of older, mildly symptomatic individuals.

Design: Biomarker validation in a clinical cohort.

Setting: University hospital inpatient unit.

Participants: Ten patients admitted for CSF drainage for diagnostic purposes.

Main Outcome Measures: The CSF levels of Aβ1-40, Aβ1-42, tau, and phosphorylated tau on threonine 181 (p-tau181) were measured every 6 hours for 24 or 36 hours.

Results: The mean coefficient of variation values for each biomarker assessed in our 10 patients were 5.5% (95% CI, 3.8%-10.0%) for Aβ1-42, 12.2% (9.0%-24.2%) for Aβ1-40, 8.2% (5.7%-15.1%) for total tau, and 11.9% (8.5%-23.0%) for p-tau181. These values are only slightly higher than the variability in the assay. In addition, no significant circadian fluctuation in any Alzheimer disease biomarker was observed given the limitations of our sampling frequency.

Conclusion: In a cohort of elderly patients, little fluctuation in the levels of important Alzheimer disease biomarkers in lumbar CSF is seen as a function of time.

Arch Neurol. 2012;69(2):246-250

Despite intensive research during the past 2 decades that has led to a better understanding of the pathogenesis of Alzheimer disease (AD), a therapy that alters its progression remains elusive. Several medications are available for symptomatic treatment of AD; however, none modifies the underlying evolution of the disease. Multiple trials of disease-modifying drugs in AD have failed thus far. One potential reason for this failure is the late stage in which these drugs are administered, a time at which neuronal injury may be irreversible. This has encouraged the identification of biomarkers for AD, which would facilitate early identification of the disease and in turn would enable trials of drug therapy when the course of the disease is still modifiable. Of all the biologically plausible biomarkers under study, only a few have been repeatedly identified by independent multicenter studies to be candidates that closely reflect the hallmark findings of the disease process. These include the serum apolipoprotein E allele status, positron emission tomographic imaging of brain amyloid, and the cerebrospinal fluid (CSF) levels of Aβ1-42, total tau, and phosphorylated tau on threonine 181 (p-tau181). The last 3 CSF biomarkers are particularly important, since they also have significant implications for disease-modifying therapy.

One obstacle to widespread use of these CSF biomarkers has been their diurnal variability seen in younger individuals. The fluctuations in CSF Aβ1-42 values, in particular, are as much as 3-fold during a 24-hour cycle. This variability poses a significant threat to the usefulness of CSF Aβ1-42 levels as a disease predictor, given that reductions of 30% to 40% can predict conversion from mild cognitive impairment to dementia. To examine this important issue further, we determined whether the levels of Aβ1-40, Aβ1-42, tau, and p-tau181 in lumbar CSF fluctuate in elderly patients.

METHODS

PARTICIPANTS

Ten patients suspected of having idiopathic normal pressure hydrocephalus (n=9) or pseudotumor cerebri (n=1) who were admitted to the hospital for intracranial pressure monitoring and extended CSF drainage as part of their routine clinical care were recruited. All patients provided consent to participate. Most had relatively modest cognitive problems associ-
ated with their suspected diagnosis (mean [SD] Mini-Mental State Examination score, 26.3 [2.9]; range, 20-30). All patients were monitored for at least 1 year after their initial evaluation. Clinical diagnoses of dementia and mild cognitive impairment were made by two of us (A.M. and R.J.O.) on the basis of informant history as well as cognitive testing,\textsuperscript{15} without knowledge of AD biomarker levels. A diagnosis of mild cognitive impairment required cognitive abnormalities documented by history and testing but no functional loss due to the abnormalities.\textsuperscript{16}

This study was approved by the Johns Hopkins Institutional Review Board.

**CSF COLLECTION**

All patients underwent insertion of a catheter into the lumbar subarachnoid space on the first day of hospitalization. After monitoring of intracranial pressure for 18 hours, drainage of CSF was initiated at noon the following day. Collection of CSF for analysis was commenced at 6 PM on the first day of drainage (the second hospital day). Forty milliliters of CSF was withdrawn from the lumbar catheter every 6 hours for 24 or 36 consecutive hours. The difference in collection duration was the result of investigator availability. The first 10 mL of CSF collected at each time point was discarded to eliminate CSF that had been in the subarachnoid space on the first day of hospitalization. After monitoring of intracranial pressure for 18 hours, drainage of CSF was initiated. The patient was ambulatory at least once each day and were served meals at 7 AM,\textsuperscript{1} 11 AM, and 6 PM.

**STATISTICAL ANALYSIS**

To detect the presence of significant fluctuations in biomarker levels across participants, a repeated-measures analysis of variance was performed using time (up to 6 time points for each of the 10 patients) as the independent variable, with Greenhouse-Geisser adjustment for nonsphericity. To evaluate overall variation of the biomarkers, within-subject CVs during the sampling period were computed.\textsuperscript{18} The 95% CIs for these CVs provide information on the range of expected variation in the population (95% of the time) given the data from this sample, and these were determined by assuming a noncentral T distribution.\textsuperscript{19} Similar results were obtained assuming a normal distribution. To compare different groups (eg, young vs elderly patients and low vs high CSF Aβ1-42), we used an independent-samples t test.

There were 6 men and 4 women in the study, with a mean age of 72 years (Table 1). The age of the patients spanned 5 decades, from 38 years to 87 years. Six patients demonstrated improvement in gait following drainage, indicating that they had a high likelihood for idiopathic normal pressure hydrocephalus, although the condition of 1 patient (patient 5) deteriorated after placement of a permanent ventriculo-peritoneal shunt; she is presumed to have a neurodegenerative disorder. Patient 4, who had elevated CSF pressure and headaches, had pseudotumor cerebri diagnosed (she was not receiving acetazolamide at the time of the drainage). The other 3 patients

### Table 1. Patient Characteristics\textsuperscript{a}

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Initial MMSE Score</th>
<th>Short-term Response to Drainage</th>
<th>Gait/Cognition Response to Permanent CSF Shunt</th>
<th>Cognitive Assessment 1 y After Initial Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/83</td>
<td>27</td>
<td>Improved gait</td>
<td>Awaiting shunt</td>
<td>Normal</td>
</tr>
<tr>
<td>2/F/70</td>
<td>27</td>
<td>No improvement</td>
<td>NA</td>
<td>Dementia</td>
</tr>
<tr>
<td>3/M/84</td>
<td>26</td>
<td>Improved gait</td>
<td>Improvement</td>
<td>Normal</td>
</tr>
<tr>
<td>4/F/38</td>
<td>30</td>
<td>Improved headache</td>
<td>NA</td>
<td>Normal</td>
</tr>
<tr>
<td>5/F/87</td>
<td>26</td>
<td>Improved gait</td>
<td>Deterioration</td>
<td>Dementia</td>
</tr>
<tr>
<td>6/M/78</td>
<td>29</td>
<td>No improvement</td>
<td>NA</td>
<td>MCI</td>
</tr>
<tr>
<td>7/F/62</td>
<td>27</td>
<td>Improved gait</td>
<td>Improvement</td>
<td>Normal</td>
</tr>
<tr>
<td>8/M/78</td>
<td>28</td>
<td>No improvement</td>
<td>NA</td>
<td>Dementia</td>
</tr>
<tr>
<td>9/M/75</td>
<td>23</td>
<td>Improved gait</td>
<td>Improvement</td>
<td>MCI</td>
</tr>
<tr>
<td>10/M/64</td>
<td>20</td>
<td>Improved gait</td>
<td>Improvement</td>
<td>Dementia</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebrospinal fluid; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; NA, not applicable.

\textsuperscript{a}Patient 1 had not had a shunt placed because of recurrent meningitis. Patient 4 received a diagnosis of pseudotumor cerebri after the results of pressure monitoring, response to drainage, and subsequent improvement while receiving acetazolamide. No definitive diagnosis was made for patients 2, 6, and 8.
had an undiagnosed neurodegenerative process associated with cognitive impairment and gait disorder. The CSF was sampled during 24 hours in 7 patients and during 36 hours in 3 patients. The levels of A\textsubscript{β1-42}, A\textsubscript{β1-40}, total tau, and p-tau\textsubscript{181}, although significantly different between the patients, did not fluctuate appreciably over time within any of them (Figure). The mean CVs were 5.5% (95% CI, 3.8%-10.0%) for A\textsubscript{β1-42}, 12.2% (9.0%-24.2%) for A\textsubscript{β1-40}, 8.2% (5.7%-15.1%) for total tau, and 11.9% (8.5%-24.2%) for p-tau\textsubscript{181}. Thus, the CV in the population is expected to fall within these CIs 95% of the time given these sample data. Each of these is slightly above the variability of the assay. Significant fluctuations in A\textsubscript{β1-42} did not occur in the patients with the highest CSF A\textsubscript{β1-42} levels (ie, those at least risk for coexistent AD pathologic characteristics) as well as in those with the lowest CSF A\textsubscript{β1-42} levels (ie, those at highest risk for coexistent AD pathologic characteristics). The mean (SD) CV for A\textsubscript{β1-42} in patients with the 4 highest mean values of A\textsubscript{β1-42} (536 [33] pg/mL) was 4.7% (1.2%). This value was not significantly different from that observed in the 4 patients with the lowest mean A\textsubscript{β1-42} values (346 [43] pg/mL; CV 6.5% [3.3%]; P < .001 for means and P = .32 for CV). Moreover, the mean CV for A\textsubscript{β1-42} in the 3 youngest subjects in our cohort (mean age, 54.6 [14.0] years; CV 5.6% [3.0%]) is similar to that in the 3 oldest patients (mean age, 84.6 [2.0] years; CV 3.8% [2.3%]; P = .01 for age and P = .41 for CV). When concentrations of individual CSF analytes at different times were pooled across all 10 participants, there was no evidence of a circadian fluctuation (Table 2). In addition, the 6 AM value (when all patients had been fasting for at least 10 hours) did not differ significantly from any of the other values determined when the patients were able to eat (Table 2).

**Comment**

Multiple studies in the past decade, including the most recent from the Alzheimer’s Disease Neuroimaging Initiative, have suggested that CSF levels of A\textsubscript{β1-42}, total tau, or p-tau\textsubscript{181} could serve as biomarkers for AD risk. The International Working Group for New Research Criteria for the Diagnosis of Alzheimer’s Disease has recommended incorporating levels of these biomarkers into routine clinical practice. Indeed, new criteria for AD and mild cognitive impairment have been proposed that rely in part on measurements of levels of these biomarkers in the CSF.

There are many uncertainties regarding the validity of these biomarkers to assess the risk of AD. First, several studies have shown significant laboratory-to-laboratory variability in CSF levels of A\textsubscript{β1-42}, total tau, or p-tau\textsubscript{181}, even when using the same reagents and samples. Second, CSF levels of A\textsubscript{β1-40} and A\textsubscript{β1-42} have been shown to fluctuate significantly in young volunteers (2- to 3-fold for A\textsubscript{β1-42}) during 24 hours in a partially noncircadian rhythm. If this also occurs in older impaired individuals (ie, the ones most likely to receive...
this test), this variability could prevent recognition of the underlying disease (30%-40% lower levels of CSF Aβ1-42 in individuals at risk for AD). Given that the reported variability in CSF Aβ values was not completely explained by circadian factors, standardizing the time of CSF collection would not necessarily correct this problem.

The lack of significant fluctuations in any of the biomarkers we assayed in our study of older, cognitively impaired individuals shows promise for the real-world application of CSF AD biomarkers, as these people are most likely to undergo testing for these biomarkers. Moreover, it is reassuring that changes in the levels of these biomarkers in a clinically relevant cohort imply a disease- or age-relevant variation rather than fluctuations resulting from preanalytical variables such as time of collection.

Table 2. Circadian Fluctuations in CSF Alzheimer Disease Biomarkers in 10 Patients

<table>
<thead>
<tr>
<th>Biomarker Level</th>
<th>6 PM</th>
<th>12 AM</th>
<th>6 AMb</th>
<th>12 PMa</th>
<th>P Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ1-40</td>
<td>5515 (2058)</td>
<td>6023 (2280)</td>
<td>5752 (2254)</td>
<td>5871 (2244)</td>
<td>.21</td>
</tr>
<tr>
<td>Aβ1-42</td>
<td>443 (110)</td>
<td>447 (91)</td>
<td>437 (92)</td>
<td>429 (105)</td>
<td>.50</td>
</tr>
<tr>
<td>Total tau</td>
<td>78.6 (25.4)</td>
<td>81.3 (29.1)</td>
<td>80.9 (29.3)</td>
<td>78.5 (29.7)</td>
<td>.72</td>
</tr>
<tr>
<td>p-tau181</td>
<td>27.0 (7.8)</td>
<td>28.1 (6.5)</td>
<td>26.4 (7.1)</td>
<td>28.1 (9.2)</td>
<td>.66</td>
</tr>
</tbody>
</table>

Abbreviations: CSF, cerebrospinal fluid; p-tau181, phosphorylated tau on threonine 181.

a All patients were fasting at 6 AM.
b Determined by repeated-measures analysis of variance.

Accepted for Publication: April 11, 2011.
Correspondence: Richard J. O’Brien, MD, PhD, Department of Neurology, The Johns Hopkins University School of Medicine, Mason Lord Center Tower Suite 5100, Johns Hopkins Bayview Medical Center, Baltimore, MD 21224 (robrien@jhmi.edu).

Author Contributions: Study concept and design: O’Brien. Acquisition of data: Moghekar and Li. Analysis and interpretation of data: Moghekar, Goh, and Albert. Drafting of the manuscript: Moghekar, Li, and O’Brien. Critical revision of the manuscript for important intellectual content: Goh, Albert, and O’Brien. Statistical analysis: Goh. Obtained funding: Albert and O’Brien. Administrative, technical, and material support: Moghekar and Li.

Financial Disclosure: None reported.

Funding/Support: This study was supported by grants P50 AG05146 and U01 AG033655 from the National Institute on Aging, by the Burroughs Wellcome Fund for Translational Research, and by the Intramural Research Program, National Institute on Aging, National Institutes of Health.

REFERENCES