Tau Protein in Cerebrospinal Fluid as an Aid in the Diagnosis of Alzheimer’s Disease*

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ABSTRACT

Neurofibrillary tangles and dystrophic neurites are characteristic pathological features found in the brains of Alzheimer’s disease (AD) patients. A major constituent of these lesions is the cytoskeletal protein tau. This study examined whether the measurement of tau in cerebral spinal fluid (CSF) has value in the diagnosis of AD.

Seventy-seven subjects were enrolled in this prospective study: These included AD (N = 24), Neurological Controls (dementing diseases/syndromes, N = 26), Normal Controls (N = 14), and Others (N = 13). CSF was obtained by lumbar puncture, and tau concentrations (pg/mL) were determined using a dual monoclonal antibody microplate immunoassay.

The mean tau value for AD subjects (1,430 ± 739) was significantly different from Neurological Control subjects (790 ± 579) (p < 0.001) and Normal Control subjects (816 ± 355) (p < 0.001). Tau values were elevated in two Neurological Control subjects, one with Binswanger’s disease (age 75) and one with depression (age 90). Tau values were also elevated in three Normal Control subjects; two were subjects with a family history of AD.

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Tau concentrations did not correlate significantly with age in AD subjects \((r = 0.05, p = 0.82)\) or in Normal Control subjects \((r = -0.49, p = 0.08)\). Tau also did not correlate with severity of cognitive impairment in AD subjects \((r = -0.03, p = 0.91)\) or duration of AD symptoms \((r = 0.16, p = 0.52)\).

Based on these results and others, CSF levels of tau protein may provide a useful biochemical marker to aid in the clinical diagnosis of AD.

**Introduction**

Differential diagnosis and treatment of dementia in the elderly is becoming increasingly important. The prevalence of dementia rises with age; estimates range as high as 10 percent of those over age 65.\(^1\) There are more than 60 causes of dementia, but Alzheimer's disease (AD) is by far the most common.\(^2\)

The clinical evaluation of patients with dementia is extensive and includes a medical history, physical and neurologic examination, neuropsychological testing, an interview with a spouse or caregiver, blood tests, and neuroimaging studies, as well as lumbar puncture, electroencephalogram (EEG), and other procedures if indicated. Diagnostic certainty increases if the patient can be observed over time as the disease progresses. Despite this complete workup, the diagnosis of AD is often inaccurate, and definitive diagnosis can only be determined postmortem.

The development of biochemical markers to aid in the diagnosis of AD would be beneficial if the diagnostic

<table>
<thead>
<tr>
<th>Diagnostic Group</th>
<th>Number of Subjects</th>
<th>Age (Years) Mean ± SD Median Range</th>
<th>Gender Male (N)</th>
<th>Female (N)</th>
<th>MMSE Score Mean ± SD Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD (^b)</td>
<td>24</td>
<td>73 ± 7</td>
<td>59 – 85</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Neurological controls (^c)</td>
<td>26</td>
<td>64 ± 13</td>
<td>65 30 – 90</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Normal controls</td>
<td>14</td>
<td>64 ± 18</td>
<td>73 30 – 79</td>
<td>5 9</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Other</td>
<td>13</td>
<td>50 ± 20</td>
<td>47 14 – 88</td>
<td>8 5</td>
<td>27 ± 7</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>64 ± 16</td>
<td>69 14 – 90</td>
<td>37</td>
<td>40</td>
</tr>
</tbody>
</table>

\(^a\) Mini-mental state examination.

\(^b\) Alzheimer's disease.

\(^c\) Neurological controls = dementing diseases/syndromes (vascular dementia, stroke, Binswanger's disease, transient ischemic attack, depression, Huntington's disease, Parkinson's disease, hydrocephalus, Parkinson's and alcohol related dementia, stroke and hydrocephalus, alcohol-related dementia and multiple sclerosis, and Pick's disease).
workup could be simplified, if the misdiagnosis rate of up to 30 percent could be lowered, or if the certainty of diagnosis could be increased earlier in the course of the disease. This becomes particularly crucial as new drug therapies are developed.

Neurofibrillary tangles (NFTs) are characteristic pathological features found within neurons in the brains of AD patients. These NFTs and their constituents, paired helical filaments (PHFs), consist largely of the abnormally phosphorylated cytoskeletal protein tau. This abnormal tau is found primarily in the cell body and dendrites. Tau in its normal state binds to and stabilizes microtubules in nerve axons. The immuno- histochemical staining density of neurofibrillary lesions correlates with disease severity. Previous studies have reported the presence of tau immunoreactivity in cerebrospinal fluid (CSF), and have also shown increased cerebrospinal fluid (CSF) tau concentrations in AD patients in comparison to elderly control patients. This study examined further whether or not the measurement of tau in CSF could have value in the diagnosis of AD.

Methods and Materials

SUBJECTS AND STUDY DESIGN

Between February 1993 and June 1994, 77 subjects were enrolled in this

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FIGURE 1. Distribution of CSF tau concentrations (pg/mL) for each of the diagnostic groups: Alzheimer's Disease (N = 24), Neurological Controls (N = 26), Normal Controls (N = 14), and Other (N = 13).
### TABLE II
Cerebral Spinal Fluid Tau and Total Protein

<table>
<thead>
<tr>
<th>Diagnostic Group</th>
<th>Tau (pg/mL) Mean ± SD</th>
<th>Total Protein (mg/dL) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD a</td>
<td>1430 ± 739</td>
<td>40.1 ± 13.0</td>
</tr>
<tr>
<td>Neurological controls b</td>
<td>790 ± 579</td>
<td>42.0 ± 17.8</td>
</tr>
<tr>
<td>Normal controls</td>
<td>816 ± 355</td>
<td>40.8 ± 19.0</td>
</tr>
<tr>
<td>Other</td>
<td>810 ± 441</td>
<td>65.2 ± 61.9</td>
</tr>
</tbody>
</table>

a Alzheimer’s disease.

b Neurological controls = dementing diseases/syndromes (vascular dementia, stroke, Binswanger’s disease, transient ischemic attack, depression, Huntington’s disease, Parkinson’s disease, hydrocephalus, Parkinson’s and alcohol related dementia, stroke and hydrocephalus, alcohol-related dementia and multiple sclerosis, and Pick’s disease).

Subjects were categorized into four groups: Alzheimer’s Disease (N = 24), Neurological Controls (N = 26), Normal Controls (N = 14), and Other (N = 13).

Alzheimer’s Disease was diagnosed as probable AD according to criteria established by the National Institute of Neurological and Communicative Disorders and Stroke—Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA).

Age of symptom onset and duration of illness were recorded.

The Neurological Control group consisted of subjects with dementing diseases or syndromes, or vascular diseases. This included vascular dementia (N = 3), stroke (N = 5), Binswanger’s disease (N = 3200, 2800, 2400).

![Figure 2](image-url)  
**Figure 2.** Relationship between tau concentration (pg/mL) and subject age: All subjects.
TABLE III
Relationship Between Tau Levels and Age, Mimi-mental State Examination, and Duration of Alzheimer’s Disease

<table>
<thead>
<tr>
<th>Correlation Coefficients</th>
<th>Statistical Significance</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAU vs. AGE:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fig. 2. All subjects</td>
<td></td>
<td>0.29</td>
<td>0.01 *</td>
</tr>
<tr>
<td>Fig. 3. AD b subjects</td>
<td></td>
<td>0.05</td>
<td>0.82</td>
</tr>
<tr>
<td>Neuronal control subjects</td>
<td></td>
<td>0.48</td>
<td>0.01 *</td>
</tr>
<tr>
<td>Fig. 4. Normal control subjects</td>
<td></td>
<td>-0.49</td>
<td>0.08</td>
</tr>
<tr>
<td>Other subjects</td>
<td></td>
<td>0.37</td>
<td>0.21</td>
</tr>
<tr>
<td>TAU vs. MMSE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All subjects</td>
<td></td>
<td>-0.28</td>
<td>0.03 *</td>
</tr>
<tr>
<td>Fig. 5. AD b subjects</td>
<td></td>
<td>-0.03</td>
<td>0.91</td>
</tr>
<tr>
<td>Neuronal control subjects</td>
<td></td>
<td>-0.20</td>
<td>0.44</td>
</tr>
<tr>
<td>Normal control subjects</td>
<td></td>
<td>-0.29</td>
<td>0.36</td>
</tr>
<tr>
<td>Other subjects</td>
<td></td>
<td>-0.18</td>
<td>0.65</td>
</tr>
<tr>
<td>TAU vs. Duration of AD b</td>
<td></td>
<td>0.16</td>
<td>0.52</td>
</tr>
</tbody>
</table>

* Statistically significant.

b Alzheimer’s disease.

c Neuronal controls = dementing diseases/syndromes (vascular dementia, stroke, Binswanger’s disease, transient ischemic attack, depression, Huntington’s disease, Parkinson’s disease, hydrocephalus, Parkinson’s and alcohol related dementia, stroke and hydrocephalus, alcohol-related dementia and multiple sclerosis, and Pick’s disease).

The Other group consisted of subjects with diseases or syndromes which would not be misdiagnosed as AD. This group served as a control to identify other conditions which might coexist with dementia and elevate the marker and thus confound marker result interpretation in medical practice. Included were subjects with meningitis, multiple sclerosis, encephalitis, Guillain-Barre syndrome, neuritis, brain tumor, psychosis, amyotrophic lateral sclerosis, chronic inflammatory demyelinating polyradiculoneuropathy, and congenital fusion of the cervical spine.

Each subject’s medical history was reviewed. A complete physical and neurological examination, including blood and urine laboratory testing, was performed. Neuroimaging studies, EEG, and other procedures were performed if indicated.

The Mini-Mental State Examination (MMSE) was administered to assess cognitive impairment; scores on this test range from 30 (normal) to 0 (severe impairment).

Approximately 5 mLs of CSF was obtained by lumbar puncture. The first 3 mLs were sent to the laboratory for cell counts, total protein, and other tests if indicated. The remaining CSF was immediately frozen at -80°C until assayed for tau.

Study personnel were blinded: Physicians did not have access to the individual subject marker values during the study, and laboratory personnel were unaware of subject diagnoses prior to assayng the samples.

ASSAY

Tau concentrations were determined from a tau standard curve using a dual monoclonal antibody microplate immunooassay. Microplates were prepared for use by coating with monoclonal antibody ZTGO85 (previously referred to as
16G7 in 10 mM Tris buffer, pH 7.8, overnight with rotation at room temperature. Plates were fixed by incubation for 1 hour with rotation with blocking buffer, 50 mM Tris, 150 mM NaCl, 0.25 percent casein, pH 7.5 with preservative. A second monoclonal antibody, ZTB083 (previously referred to as 16B5), was biotinylated with NHS-LC-biotin* according to the manufacturer's instructions. The assay was processed by adding 25 μL of sample or tau standard (baculovirus-derived recombinant 4-repeat tau, mass value-assigned)† to each well followed by 75 μL of biotinylated antibody and then rotated overnight at room temperature. All samples and standards were run in triplicate. Following incubation, the plates were washed 4 times with blocking buffer to which 0.05 percent Tween 20 had been added. One hundred μL of streptavidin-alkaline phosphatase conjugate (assay dilution at 1:50,000) was added to each well, incubated for 1 hour at room temperature with rotation, and then washed 3 times with blocking buffer to which 0.05 percent Tween 20 was added. Before addition of disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-tricyclo[3.3.1.1^{3,7}]decan}-4-yl)phenyl phosphate (AMPPD) substrate with emerald green enhancer,‡ the plate was washed once with diethanolamine buffer (0.5 percent (v/v) diethanolamine, 0.5 mM MgCl₂, 0.008 percent sodium azide, pH 10). Chemiluminescent signal was read using the Microlite 2 plate luminometer§ after 20 minutes substrate incubation.

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† Athena Neurosciences, South San Francisco, CA 94080.
‡ Tropix, Bedford, MA 01730.
§ Dynatech, Chantilly, VA 22021.
The monoclonal antibodies used in this assay to detect tau are not sensitive to phosphorylation state, and detect multiple isoforms in CSF and brain homogenates.

**Statistics**

The t-test or the Wilcoxon rank sums test was performed for data comparisons between subject groups, depending on whether or not the normality assumption was met. Linear regression and Pearson correlation analyses were performed for evaluation of tau versus age, MMSE, and duration of disease; p values are reported for $H_0: \rho = 0$.

**Results**

Subjects ranged in age from 14 to 90 years. Demographic and clinical characteristics are presented in table I. The majority of the subjects in the study were white (92 percent), 6 percent were black, and 2 percent Hispanic. Of the 24 AD subjects, the duration of disease ranged from 13 to 155 months (mean = 54 ± 35, median = 51).

Tau values (pg/mL) by group are plotted in figure 1. The mean tau value for AD subjects (1,430 ± 739) was significantly different from Neurological Control (790 ± 579) ($p < 0.001$) and Normal Control subjects (816 ± 355) ($p < 0.001$) (table II).

Tau values were elevated in two Neurological Control subjects, one with Binswanger’s disease (age 75) and one with depression (age 90). The patient with Binswanger’s disease was hospitalized at the time of lumbar puncture, was on numerous sedative medications, and has not yet received a complete mental
status examination to determine the likelihood of AD as a possible diagnosis. Tau values were also elevated in three Normal Control subjects; two were subjects with a family history of AD.

There were no differences in CSF total protein concentration in the AD versus the Neurological Control subjects \( p = 0.94 \) or in the AD versus the Normal Control subjects \( p = 0.93 \) (Table 2).

Tau concentrations increased slightly with subject age, when all subjects were included in the analysis (figure 2, table III). This same trend was seen in the AD subjects, but was not statistically significant (figure 3, table III). Normal control subjects showed a slight decrease in tau with subject age, but this also was not statistically significant (figure 4, table III).

Tau showed a slight increase with severity of cognitive impairment as measured by MMSE \((30-28 = \text{Normal}, 0 = \text{Most severely impaired})\), when all subjects were analyzed (table III), but tau did not correlate with MMSE in AD subjects (figure 5) or other subject groups when analyzed independently (table III).

Discussion

Mean CSF tau concentrations in AD subjects are significantly higher than the mean concentrations seen in normal control subjects and in neurological control subjects with dementing diseases or syndromes. These results are consistent with those reported by Vigo-Pelfrey et al.\(^6\) Only 8 percent \((2/26)\) of the neurological control subjects showed elevated tau val-
ues. Clinical antemortem accuracy for diagnosis of AD is approximately 70 percent to 80 percent; thus, it is possible that these patients may have had coexisting undiagnosed AD. Three of the normal control subjects had slightly elevated tau levels; two were subjects with a family history of AD. Additional long-term follow-up of these subjects is required to determine if these elevated values indicate actual false positives or alternatively a future risk for development of AD.

Overlap of tau concentration is seen between groups, as has been reported by others.\(^5,6\) The AD group in particular showed a wide distribution of results. Alzheimer's Disease may consist of a heterogeneous group of diseases, of which only a subset shows elevated tau values. Previous studies have shown that significant numbers of patients diagnosed with AD lacked neocortical tangles.\(^4,8,9\) High concentrations of tau would therefore not necessarily be expected in these patients. In addition, one AD subtype, the Lewy body variant, may account for 30 percent or more of all AD cases.\(^10\) This variant shows subcortical and cortical Lewy bodies in autopsy series, with amyloid plaques but few neurofibrillary tangles.\(^11\) Unfortunately, this group is currently difficult to diagnose clinically; thus, many of the AD patients may appear as "false negatives" owing to current clinical limitations. Efforts are underway to standardize the clinical diagnostic criteria for Lewy body patients.\(^12\)

Other subtypes of AD have also been suggested, possibly determined by such characteristics as aphasia, anxiety, psychosis, depression, age of onset, ApoE genotype, family history, rate of cognitive

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**Figure 6.** Relationship between tau concentration (pg/mL) and duration of disease (months): AD subjects.
decline, gender, education, or other factors. Alternatively, these features may be seen as disease characteristics that emerge at different states of a single disease. Markers such as tau may correlate with various subtypes or with various states of disease progression, but further research is required in this area.

Tau concentration does not appear to be strongly correlated with patient age. A slight decrease is seen in normal controls, and a slight increase is seen in AD patients. This increase is also observed with duration of disease in the AD patients, although this relationship is weak. Thus, any apparent age-related rise in tau may actually be reflecting a disease severity-related rise.

Tau and MMSE scores showed no correlation in this study. The MMSE test may not be sensitive enough to detect very early, mild cognitive impairment, and score variability may be relatively wide, masking any correlation between tau and cognitive impairment. A more comprehensive neuropsychological battery of tests might detect mild dementia or specific areas of cognition which may show a correlation with this marker as the disease progresses.

The results of this study are in agreement with the findings of Vandermeeren et al and Vigo-Pelfrey et al, who found increased CSF tau concentrations in AD patients. Although the clinical findings are consistent, the absolute values for tau concentrations reported in these papers vary owing to differences in calibration, depending upon the source of the tau standards (recombinant vs natural).

Tau protein might provide a useful biochemical marker to aid in the clinical diagnosis of AD. An elevated CSF tau value would provide the first "positive" marker for this common and fatal disease, allowing early family counseling and planning, as well as early therapeutic intervention as drugs for the treatment of AD are developed.

Acknowledgments

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References


