Comparison of Cerebrospinal Fluid Levels of Tau and Aβ 1-42 in Alzheimer Disease and Frontotemporal Degeneration Using 2 Analytical Platforms

David J. Irwin, MD; Corey T. McMillan, PhD; Jon B. Toledo, MD; Steven E. Arnold, MD; Leslie M. Shaw, PhD; Li-San Wang, PhD; Viviana Van Deerlin, MD, PhD; Virginia M.-Y. Lee, PhD, MBA; John Q. Trojanowski, MD, PhD; Murray Grossman, MD

**Objective:** To use values of cerebrospinal fluid tau and β-amyloid obtained from 2 different analytical immunoassays to differentiate Alzheimer disease (AD) from frontotemporal lobar degeneration (FTLD).

**Design:** Cerebrospinal fluid values of total tau (T-tau) and β-amyloid 1-42 (Aβ 1-42) obtained using the Innotest enzyme-linked immunosorbent assay were transformed using a linear regression model to equivalent values obtained using the INNO-BIA AlzBio3 (xMAP; Luminex) assay. Cutoff values obtained from the xMAP assay were developed in a series of autopsy-confirmed cases and cross validated in another series of autopsy-confirmed cases using transformed enzyme-linked immunosorbent assay values to assess sensitivity and specificity for differentiating AD from FTLD.

**Setting:** Tertiary memory disorder clinics and neuro-pathologic and biomarker core centers.

**Participants:** Seventy-five samples from patients with cerebrospinal fluid data obtained from both assays were used for transformation of enzyme-linked immunosorbent assay values. Forty autopsy-confirmed cases (30 with AD and 10 with FTLD) were used to establish diagnostic cutoff values and then cross validated in a second sample set of 21 autopsy-confirmed cases (11 with AD and 10 with FTLD) with transformed enzyme-linked immunosorbent assay values.

**Main Outcome Measure:** Diagnostic accuracy using transformed biomarker values.

**Results:** Data obtained from both assays were highly correlated. The T-tau to Aβ 1-42 ratio had the highest correlation between measures (r = 0.928, P < .001) and high reliability of transformation (intraclass correlation coefficient = 0.89). A cutoff of 0.34 for the T-tau to Aβ 1-42 ratio had 90% and 100% sensitivity and 96.7% and 91% specificity to differentiate FTLD cases in the validation and cross-validation samples, respectively.

**Conclusions:** Values from 2 analytical platforms can be transformed into equivalent units, which can distinguish AD from FTLD more accurately than the clinical diagnosis.

neuritic pathology, using a combination of ELISA and xMAP technology. This approach allows for the differentiation of AD from FTLD, with high sensitivity and specificity.

**PARTICIPANTS**

Data from patients followed up at the Alzheimer Disease Center (ADC) or Frontotemporal Degeneration Center (FTDC) at the University of Pennsylvania were included for analysis. Enrollment criteria included a 20-gauge needle to collect about 20 mL of CSF in polypropylene tubes (Corning Life Sciences). Samples were centrifuged at 3000 rpm for 15 minutes at 4°C, aliquotted, and immediately stored at −80°C until analysis.

**METHODS**

**NEUROPATHOLOGIC DIAGNOSIS**

Autopsy was performed as previously described. Briefly, fresh brain and spinal cord tissue obtained at autopsy was fixed in neutral buffered formalin or 70% ethanol and 150 mmol of sodium chloride, embedded in paraffin blocks, and cut into 6-μm sections for microscopic analysis. Routine staining was performed on each case, including hematoxylin and eosin and the neuregulin-erbB2/neu receptor (neu) and the neu/ErbB-2 receptor (neu). Immunohistochemistry using well-characterized monoclonal antibodies (mAbs) specific for α-synuclein, tau, and TDP-43, which are found in characteristic inclusions seen in most neurodegenerative diseases. Microscopic diagnosis was made by an experienced neuropathologist (J.Q.T) using current neuropathologic diagnostic criteria for neurodegenerative diseases.

**BIOFLUID COLLECTION AND ANALYSIS**

Cerebrospinal fluid samples were obtained during routine diagnostic lumbar puncture, as previously described. In brief, lumbar puncture was performed at the L3-L4 lumbar space using a 20-gauge needle to collect about 20 mL of CSF in polypropylene tubes (Corning Life Sciences). Samples were centrifuged at 3000 rpm for 15 minutes at 4°C, aliquotted, and immediately stored at −80°C until analysis.

Samples were analyzed using the ELISA assay (Innotest; Innogenetics) or the Luminex xMAP platform (INNO-BIA Alz-
Bio3 for research-only reagents; Innogenetics) at the Center for Neurodegenerative Research (ELISA) and the biomarker core (xMAP) at the University of Pennsylvania, according to previous reports.13,25,26 Monoclonal capture and reporting antibodies used in the ELISA method for detection of T-tau and p-tau181 in CSF were AT120/HT7 and BT2, HT7/AT270, respectively. The ELISA values for Aβ1-42/HT7 (T-tau) and AT270 (p-tau181) were measured using an in-house ELISA method26 with the mAb BAN-50 as the capture and BC-05 as the reporting mAb. The xMAP platform used the capture MAb 4DTA3 (Aβ1-42), AT120 (T-Tau), and AT270 (p-tau181) bound to color-specific beads. The biomarker analytes were detected using the reporting Mabs 3D6 (Aβ1-42) and HT7 (T-tau and p-tau181).

### Table. Demographics of Study Patients

<table>
<thead>
<tr>
<th>Neuropathologic Sample 1, xMAP</th>
<th>Neuropathologic Sample 2, Transformed ELISA</th>
<th>P Value</th>
<th>Transformation Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>40</td>
<td>21</td>
<td>NA</td>
</tr>
<tr>
<td>Male/female, No.</td>
<td>19/21</td>
<td>13/8</td>
<td>.21</td>
</tr>
<tr>
<td>Neuropathologic diagnosis (No.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD (30), Aβ</td>
<td>AD (11), Aβ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTLD total (10), T-tau (5),</td>
<td>FTLD total (10), T-tau (5),</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FTLD-TDP (5),</td>
<td>FTLD-TDP (4),</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FTLD-DLDH (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical diagnosis (No.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD-p (25), PDD (1), bvFTD (2), CBS (1), MCI (1),</td>
<td>AD-p (1), bvFTD (2), hPPA (3), CBS (3), svPPA (2), bvFTD (5), ALSFTD (1), naPPA (3), CBS (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD-p (1), CBS (3), PSP (1), bvFTD (4), SD (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at onset, median (interquartile range), y</td>
<td>66.50 (57.00-71.75)</td>
<td>55.00 (51.00-66.50)</td>
<td>.008</td>
</tr>
<tr>
<td>Age at CSF collection, mean (SD), y</td>
<td>68.03 (7.70)</td>
<td>61.23 (8.62)</td>
<td>.003</td>
</tr>
<tr>
<td>Age at death, median (interquartile range), y</td>
<td>76.88 (69.88-80.41)</td>
<td>63.03 (55.78-74.18)</td>
<td>.001</td>
</tr>
<tr>
<td>Autopsy-CSF collection interval, mean (SD), mo</td>
<td>75.40 (32.37)</td>
<td>43.59 (35.85)</td>
<td>.001</td>
</tr>
<tr>
<td>Onset-CSF collection interval, mean (SD), mo</td>
<td>34.57 (25.28)</td>
<td>34.21 (21.36)</td>
<td>.96</td>
</tr>
</tbody>
</table>

Abbreviations: AD, Alzheimer disease; AD-p, probable AD; bvFTD, behavioral-variant frontotemporal degeneration; ALS-FTD, amyotrophic lateral sclerosis with frontotemporal degeneration; CBS, corticobasal syndrome; CSF, cerebrospinal fluid; DLDH, dementia lacking distinctive histopathology; ELISA, enzyme-linked immunosorbent assay; FTLD, frontotemporal lobar degeneration; hPPA, logopenic variant primary progressive aphasia; MCI, mild cognitive impairment; NA, not applicable; ND, Nondiagnosed/no autopsy; PDD, Parkinson disease dementia; naPPA, nonfluent/aggranular primary progressive aphasia; PSP, progressive supranuclear palsy; svPPA, semantic variant primary progressive aphasia; TDP, TAR DNA-binding protein.

a AD autopsy cases were all diagnosed with high-probability AD (6 cases from sample 1 and 3 cases from sample 2 also had a secondary diagnosis of dementia with Lewy bodies of low to intermediate probability).
b Contains 1 nondeceased MAPT mutation case (P301L).
c Contains 3 nondeceased PGRN mutation cases (IVS836-1G\rightarrowC, IVS264 + 2T\rightarrowC, and c.102delC).
d Contains 1 case (PSP) with a secondary diagnosis of low-probability dementia with Lewy body disease.
e Contains 2 cases (FTLD-TDP and ALS-FTLD) with a secondary diagnosis of low-probability AD.

#### STATISTICAL ANALYSIS

Percentage intra-assay coefficients of variation were calculated for both immunoassays using measurements from duplicate analysis from single runs (data missing for 1 case) and reported as mean and standard deviation.

The transformation, validation, and cross-validation steps are summarized in Figure 1. To transform the ELISA values to xMAP, a linear regression model was applied on the raw and natural log-transformed values of the training data set (n = 52). Then, the obtained formula was applied on ELISA values in the test data set (n = 23) and the intraclass correlation coefficient was measured. We selected the best transformation results (based on raw or natural log transformation) to select the transformation formula.

The diagnostic use of CSF biomarker levels in differentiating AD from FTLD cases was established in a separate sample set of autopsy-confirmed cases with available xMAP values (n = 40). A receiver operating characteristic curve analysis was performed for all analytes and assessed for optimal sensitivity and specificity for best test accuracy. The T-tau to Aβ1-42 ratio had the highest area under the curve compared with exploratory analyses assessing T-tau, p-tau181, Aβ1-42, and p-tau181 to Aβ1-42 ratio; thus, it was used in subsequent analysis. The diagnostic cutoff value of the T-tau to Aβ1-42 ratio obtained in the xMAP sample was applied to the transformed ELISA data in a separate cross-validation sample set (n = 21). Analyses were performed using SPSS version 19.0 (SPSS) and R version 2.13 (The R Foundation for Statistical Computing).32 Sensitivity and specificity of the antemortem clinical diagnosis (FTLD spectrum or AD) was calculated for comparison.
A clinical diagnosis of logopenic variant primary progressive aphasia (n = 3) was considered an accurate identification of AD pathology as most of these cases are atypical presentations of AD neuropathology.5

RESULTS

TRANSFORMATION OF ELISA VALUES

Mean (SD) coefficients of variation for ELISA and xMAP were: 5.3% (7.6%) and 4.9% (8.2%) for tau, respectively; 3.4% (7.6%) and 3.9% (4.3%) for p-tau, respectively; and 8.6% (6.7%) and 3.8% (5.2%) for Aβ 1-42, respectively. Seventy-five subjects with natural log-transformed CSF values from both ELISA and xMAP immunoassays were used for transformation of values (Table). This sample was divided randomly into training (n = 52) and test (n = 23) samples. Natural log-transformed data had the best correlation between the 2 immunoassays for most analytes, with CSF values of Aβ 1-42 (r = 0.819, P < .001), T-tau (r = 0.890, P < .001), p-tau181 (r = 0.779, P < .001), T-tau to Aβ 1-42 ratio (r = 0.928, P < .001), and p-tau181 to Aβ 1-42 ratio (r = 0.834, P < .001) (Figure 2A-E). When the regression model was used to transform data in the test sample, the intraclass correlation coefficients showed modest to high reliability, ranging from 0.63 to 0.89 (Figure 2A-E). The linear regression model for the T-tau to Aβ 1-42 ratio yielded the formula: \((\text{ln(value)} - 1.513562)/1.040762)\) to convert ELISA values, which was used in subsequent analyses.

DIAGNOSTIC ACCURACY OF TRANSFORMED VALUES

Receiver operating characteristic curve analysis using xMAP values from a cohort of autopsy-confirmed cases (20 with AD and 10 with FTLD) showed the highest diagnostic accuracy using the T-tau to Aβ 1-42 ratio (area under the curve = 0.989, sensitivity = 90%, and specificity = 96.7% for best test accuracy) (Figure 3). Using the cutoff value of 0.34 (ln value = −1.078), we correctly identified 29 of 30 patients with AD and 9 of 10 patients with FTLD (90% sensitivity and 96.7% specificity) and outperformed the clinical diagnosis (86.7% sensitivity and 66.7% specificity) (Figure 4). This T-tau to Aβ 1-42 ratio value was then used for cross validation in the transformed ELISA set, with added sensitivity and specificity to the clinical diagnosis. The individual cases misclassified by our system reveal 1 nondeceased genetic (PGRN) FTLD case and 2 AD cases, both of whom had no comorbid neuropathologic findings and, interestingly, had atypical clinical presentations of logopenic variant primary progressive aphasia and bvFTD. Since the PGRN case carries a known pathogenic mutation (c.102delC), it certainly will contain TDP-43 pathology at autopsy; however, comorbid AD pathology cannot be ruled out. The age of this patient at the time of CSF collection was 68 years, indicating the possibility of age-associated Aβ amyloidosis, which could influence the T-tau to Aβ 1-42 ratio. Indeed, another FTLD case that was very close to the diagnostic threshold but correctly identified in the transformed data set (T-tau:Aβ 1-42 ratio = 0.29) had a neuropathologic diagnosis of corticobasal degeneration pathology with comorbid Aβ amyloidosis (Consortium to Establish a Registry for Alzheimer Disease plaque score C). The

We have confirmed our previous data showing a lower CSF T-tau to Aβ 1-42 ratio in FTLD compared with AD in a much larger autopsy-confirmed sample.22,26 In addition, we demonstrate that CSF biomarker analysis can be compared directly between the ELISA and xMAP analytical platforms. The transformed data were highly sensitive and specific in correctly differentiating autopsy-confirmed cases of AD from FTLD in a clinically demented sample, with added sensitivity and specificity to the clinical diagnosis.

COMMENT

We have confirmed our previous data showing a lower CSF T-tau to Aβ 1-42 ratio in FTLD compared with AD in a much larger autopsy-confirmed sample.22,26 In addition, we demonstrate that CSF biomarker analysis can be compared directly between the ELISA and xMAP analytical platforms. The transformed data were highly sensitive and specific in correctly differentiating autopsy-confirmed cases of AD from FTLD in a clinically demented sample, with added sensitivity and specificity to the clinical diagnosis.

These findings complement previous work showing that AD biomarkers obtained from these 2 immunoassays are highly correlated28,29,38 and can be transformed by a conversion factor.29 Others have suggested that values obtained from these platforms cannot be converted owing to a high coefficient of variation for the xMAP to ELISA ratio of raw biomarker values.39 Recent work from our group has shown effective transformation of ELISA biomarker data into equivalent xMAP values in differentiating AD from normal control subjects (Li-San Wang, PhD, Yuk Yee Leung, PhD, Shu-Kai Chang, ME, Susan Leighton, Malgorzata Knapi-Kazjka, Young Baek, Leslie M. Shaw, PhD, Virginia M.-Y. Lee, PhD, John Q. Trojanowski, MD, PhD, Christopher M. Clark, MD, unpublished data, 2011). The linear regression model used in that study was similar to our formula here, extending the generalizability of such a transformation method. Moreover, our report extends this approach to a comparative study and provides autopsy-confirmed validation. Further validation of this method is exemplified by previous work showing an equivalent ability of the T-tau to Aβ 1-42 ratio values independently obtained from both platforms to distinguish patients with evidence of in vivo amyloidosis.29 Thus, the T-tau to Aβ 1-42 ratio values obtained from these 2 assays have comparable diagnostic accuracy for AD neuropathology, despite differing absolute values.

The individual cases misclassified by our system reveal 1 nondeceased genetic (PGRN) FTLD case and 2 AD cases, both of whom had no comorbid neuropathologic findings and, interestingly, had atypical clinical presentations of logopenic variant primary progressive aphasia and bvFTD. Since the PGRN case carries a known pathogenic mutation (c.102delC), it certainly will contain TDP-43 pathology at autopsy; however, comorbid AD pathology cannot be ruled out. The age of this patient at the time of CSF collection was 68 years, indicating the possibility of age-associated Aβ amyloidosis, which could influence the T-tau to Aβ 1-42 ratio. Indeed, another FTLD case that was very close to the diagnostic threshold but correctly identified in the transformed data set (T-tau:Aβ 1-42 ratio = 0.29) had a neuropathologic diagnosis of corticobasal degeneration pathology with comorbid Aβ amyloidosis (Consortium to Establish a Registry for Alzheimer Disease plaque score C). The
Figure 2. Transformation of cerebrospinal fluid analytes into equivalent values between platforms. Shown are plots of raw and natural log transformed values of Aβ1-42 (A), total tau (T-tau) (B), phosphorylated tau181 (p-tau181) (C), T-tau to Aβ1-42 ratio (D), and p-tau181 to Aβ1-42 ratio (E) obtained with enzyme-linked immunosorbent assay (ELISA) and xMAP. ICC indicates intraclass correlation coefficient.
close-to-diagnostic-threshold elevated ratio in this case is most likely owing to the relative lower value of Aβ 1-42 (ELISA value of 321.94 pg/mL), suggesting that FTLD cases with significant comorbid AD pathology may have values of tau and Aβ 1-42 that are more typical of AD, which can complicate clinical interpretation of CSF biomarker analysis in living patients. Since most FTLD cases are relatively young, this reduces the likelihood of age-associated amyloidosis. Using in vivo amyloid imaging or other modalities may help improve diagnostic accuracy of mixed-pathology cases.

Limitations to this study include lack of autopsy-confirmed nondemented control subjects and other neurodegenerative dementias because study of mixed dementia groups may be more applicable to clinical practice; however, this represents a diagnostic challenge beyond the scope of this work. We have shown previously that CSF levels of these biomarkers cannot accurately differentiate FTLD cases from nondemented control patients, although the recent availability of clinical criteria for bvFTD and primary progressive aphasia reduces the likelihood that individuals with an FTLD spectrum clinical disorder will be confused with healthy adults. Additionally, patients with nonprogressive, non-neurodegenerative illnesses with cognitive/behavioral symptoms resembling FTLD (phenocopy syndrome) can be accurately distinguished from patients with underlying FTLD-spectrum neuropathology by serial clinical evaluations.

A major strength of this study is the use of autopsy-confirmed cases in the validation and cross-validation steps (Jon B. Toledo, MD, Johannes Brettschneider, MD, Murray Grossman MD, PhD, Steven E. Arnold, MD, William T. Hu, MD, PhD, Sharon X. Xie, PhD, Virginia M.-Y. Lee, PhD, Leslie M. Shaw, PhD, John Q. Trojanowski, MD, PhD, unpublished data, 2011). Indeed, the importance of autopsy-confirmed samples in FTLD biomarker research is highlighted here, as the diagnostic accuracy outperformed the clinical diagnosis in both centers. Because sample 2 was derived mainly from the FTDC, most AD cases had atypical clinical syndromes (ie, corticobasal syndrome, bvFTD, and semantic variant of primary progressive aphasia), with resultant lower clinical diagnostic sensitivity for AD pathology. This discrepancy in clinical presentations of AD pathology between samples should not influence our findings, as these cases do not have a CSF biomarker signature that would alter the T-tau to Aβ 1-42 ratio; however, it does exem-
plify the vast heterogeneity and diagnostic challenges of this clinical spectrum of disease and underlines the usefulness of CSF biomarkers to distinguish FTLD from atypical presentations of AD.

The transformed ELISA sample had an earlier age at onset (P = .008), CSF collection (P = .003), and death (P = .001) compared with the xMAP sample as well as a shorter interval between CSF collection and autopsy (P = .001) (Table). This is most likely owing to most typical amnestic AD cases being in the xMAP sample, which would be expected to have a longer duration of illness compared with FTLD-spectrum diseases. The annual variation in AD CSF biomarkers is small for patients with AD after the onset of dementia, while the longitudinal profile of these biomarkers in FTLD is less clear; there was no significant difference between groups in the interval from reported onset of dementia to CSF collection (P = .96), thus these differences in demographics between groups should have minimal influence on CSF analyte levels.

There is significant use in combining values obtained from these analytical platforms, as obtaining CSF samples from patients is invasive and may be limited in size for multiple analyses. In addition, samples from longitudinally followed up autopsy-confirmed cases are extremely valuable research tools. Combining data sets from these 2 methods helps conserve these biofluid samples and expands available sample sizes for future studies. Previous studies have shown that developing a universal AD CSF biomarker diagnostic cutoff value for use between centers is very difficult owing to multiple sources of variability within and between laboratories that need to be harmonized, limiting the immediate clinical application of CSF analysis in dementia diagnosis; however, our data support the combined use of these immunoassay platforms in a research setting. Of note, the data were obtained from 2 different laboratories within 1 institution with acceptable intra-assay variability.

That said, this study emphasizes the continuing need to standardize all aspects of biomarker methods and research protocols so that data from different centers can be compared worldwide. This will greatly facilitate understanding the pathobiology of biomarker changes and define best practices for applying biomarker technologies, especially in the context of AD clinical trials that increasingly are carried out on a global scale.

With these caveats in mind, our work provides a method for maximizing use of valuable research samples and reinforces the use of AD biomarker profiles, specifically the T-tau to Aβ 1-42 ratio, in an autopsy-confirmed sample differentiating FTLD from AD. These findings further highlight the need for FTLD-specific biomarkers and the potential value of a multimodal approach combining clinical, neuroimaging, and biofluid biomarkers to increase antemortem diagnostic accuracy for neurodegenerative diseases in clinical practice.

Accepted for Publication: January 9, 2012.
Published Online: April 9, 2012. doi:10.1001/archneur.2012.26
Correspondence: Murray Grossman, MD, Hospital of the University of Pennsylvania—Perelman School of Medi-
cine, 3400 Spruce St, Philadelphia, PA 19104 (mgrossma@mail.med.upenn.edu).


Financial Disclosure: Dr Arnold has served as a board member for the Cowan Group, Eli Lilly, and Bristol-Myers Squibb. He has also served as a consultant for the Philadelphia District Attorney’s Office and Bonner Kernan Treback and Crocatta LLP.

Funding/Support: This study was supported by grants P30AG010124-20, P01 AG017586, R01 NS44266, R01 AG15116, P01 AG32953, and P01 NS53488 from the National Institutes of Health and grants from the Wyncote Foundation. Dr Irwin’s work is supported by training grant T32-AG000255 from the National Institutes of Health, and Dr Toledo’s work is supported by a grant from the Alfonso Martin Escudero Foundation.

REFERENCES