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

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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; Lumines) 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 neuropathologic 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=.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.


Prevention of Underlying Neuropathology of Patients with Neurodegenerative Disease is Difficult in Clinical Practice Owing to the Vast Heterogeneity and Overlapping Clinical Presentations of These Disorders. This is exemplified by atypical presentations of Alzheimer disease (AD) mimicking the behavioral variant of frontotemporal degeneration (bvFTD),1-4 corticobasal syndrome,5 primary progressive aphasia,6 and other frontotemporal lobar degeneration (FTLD) spectrum disorders. Indeed, approximately 20% of clinically diagnosed patients with FTLD are diagnosed as having AD at autopsy.6 Conversely, FTLD spectrum pathology can present with an amnestic syndrome clinically resembling AD.7 With the emergence of disease-modifying treatments for neurodegenerative diseases, it will be of upmost importance to accurately identify the underlying neuropathology in these patients. Biomarkers of disease are crucial for this purpose, and new diagnostic criteria for AD8,9 and FTLD10,11 incorporate biofluid and neuroimaging biomarkers for research purposes.

Cerebrospinal fluid (CSF) values of the major constituents of AD pathology, tau and β-amyloid (Aβ), have been widely studied in patients with AD and mild cognitive impairment during the ongoing Alz-
heimer Disease Neuroimaging Initiative study, with higher levels of total tau (T-tau) and lower Aβ 1-42 values observed compared with control subjects.12,13 Using these measurements, our group has recently reported high sensitivity and specificity in differentiating AD from non-demented control subjects13 and predicting mild cognitive impairment conversion to AD.13,14 These biomarkers are less established in patients with FTLD, with some studies showing higher levels of CSF T-tau in FTLD compared with control subjects,17-23 while others find no difference24 or decreased levels in some FTLD subtypes.19 The observed CSF T-tau elevation in these reports is intermediate to the higher values observed in AD cases. Also, Aβ 1-42 has been reported at levels intermediate to control samples and the lower levels seen in AD21,22 or similar to control patient values.18,24 Using autopsy-confirmed cases, our group previously showed lower levels of total tau (T-tau) and lower Aβ 1-42 ratio in FTLD CSF compared with AD.25,26 Comparative studies are crucial to demonstrate that findings do not merely reflect the nonspecific presence of any central nervous system change. Nevertheless, reasons for these discrepancies most importantly include lack of autopsy-confirmed cases in a disease with considerable clinical heterogeneity. Other contributing factors include small patient numbers and variability in test center processing of samples.20,27 Two commercially available immunoassays measure these CSF analytes. Concentrations of tau and Aβ 1-42 obtained using the Innotest (enzyme-linked immunosorbent assay [ELISA]) compared with the INNO-BIA AlzBio3 (xMAP; Luminex) platform differ substantially; however, values from these 2 immunoassays are highly correlated,28,29 suggesting values from one platform can be transformed into equivalent units of the other. Combining these data is advantageous because it allows for increased sample sizes to fully use valuable research samples.

In this work, we use a linear regression model to transform values obtained from the ELISA method to equivalent units of tau and Aβ detected by the xMAP platform. Using these transformed data, we show that patients with autopsy-confirmed AD and FTLD can be differentiated with high sensitivity and specificity.

METHODS

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. Enzyme-linked immunosorbent assay and xMAP CSF values of T-tau, phosphorylated tau(p-tau), Aβ 1-42, and T-tau to Aβ 1-42 ratio, p-tau/Aβ, to Aβ 1-42 ratio, as well as the neuropathologic and genetic diagnoses were obtained from the integrated neurodegenerative disease database at the University of Pennsylvania.30 Ten autopsy-confirmed cases (FTDC) were previously reported using ELISA analysis only,25,26 and 36 autopsy cases (ADC) had previous xMAP values reported in an exploratory study of novel AD CSF biomarkers.31 Transformation of ELISA values was performed using data from 75 patients with available CSF biomarker data obtained from both methods. Different aliquots from the same initial CSF collection were used for these cases, with limitation to 1 freeze-thaw cycle in most instances. Five cases used in the transformation data set were also used in the autopsy-confirmed samples.

Evaluation and establishment of diagnostic cutoff values for CSF analytes using the xMAP system was performed in a sample of 40 autopsy-confirmed cases (sample 1) with a neuropathologic diagnosis of AD or FTLD-spectrum disorders from the ADC. Cross validation of the diagnostic cutoff value was performed in a second sample set of 21 autopsy-confirmed cases from the FTDC (sample 2) using transformed ELISA values. To balance these groups, 5 FTLD cases (4 with known pathogenic mutations in the MAPT or PGRN genes, as the underlying neuropathology is universally FTLD-tau and FTLD-TAR DNA-binding protein [TDP], respectively)32 from the FTDC were included in sample 1 and 1 ADC AD case added to sample 2. Autopsy-confirmed cases of FTLD included the following neuropathologic diagnoses: FTLD with TDP-43 inclusions (n=4) and amyotrophic lateral sclerosis with FTLD (n=1)—collectively referred to as FTLD-TDP (n=9, including the non-deceased PGRN mutation cases [n=3])—as well as cortico-basal degeneration (n=5), progressive supranuclear palsy (n=2), and tangle-predominant senile dementia (n=2)—collectively referred to as FTLD-tau (n=10, including the nondeceased MAPT mutation case, n=1). One FTLD case did not contain significant TDP-43, tau, α-synuclein or FUS inclusions, and it was classified as dementia lacking distinctive histopathology.31 Thus, the autopsy-confirmed data set had roughly equal numbers of FTLD-TDP and FTLD-tau. All AD cases carried a primary neuropathologic diagnosis of high-probability AD.33 Demographic data were compared between groups using χ² tests for categorical variables and independent t tests or Mann-Whitney U tests for continuous variables, where appropriate (Table).34 Missing data included 3 cases in the transformation sample (age at onset) and 1 case in the transformation and sample 2 (age at CSF collection).

All procedures, including CSF fluid collection and autopsy, required informed consent and were performed in accordance with the rules of the institutional review board at the University of Pennsylvania.

NEUROPATHOLOGIC DIAGNOSIS

Autopsy was performed as previously described.8 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 amyloid-binding dye Thioflavin S, as well as 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.32,33,35

BIOFLUID COLLECTION AND ANALYSIS

Cerebrospinal fluid samples were obtained during routine diagnostic lumbar puncture, as previously described.25 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; Inogenetics) or the Luminex xMAP platform (INNO-BIA Alz-
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), a</td>
<td>AD (11), a</td>
<td>NA</td>
<td>AD (2), FTD-TDP(1), ND (72), b,c</td>
</tr>
<tr>
<td>FTLD total (10), FTLD-tau (5), b</td>
<td>FTLD total (10), FTLD-tau (5), d</td>
<td>FTLD-DLDH (1)</td>
<td></td>
</tr>
<tr>
<td>FTLD-TDP (5), c</td>
<td>FTLD-DLP (4), e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical diagnosis (No.)</td>
<td>AD-p (25), PDD (1), bvFTD (2), CBS (1), MCI (1), AD-p (1), CBS (3), PSP (1), bvFTD (4), SD (1)</td>
<td>AD-p (1), bvFTD (2), hPPA (3), CBS (3), sPPA (2), bvFTD (5), ALSDFTLD (1), nPPA (3), CBS(1)</td>
<td>NA</td>
</tr>
<tr>
<td>Age at onset, median (interquartile range), y</td>
<td>66.89 (60.97-71.75)</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-FTLD, 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, Nondeceased/no autopsy; PDD, Parkinson disease dementia; nPPA, nonfluent/agrammatic primary progressive aphasia; PSP, progressive supranuclear palsy; sPPA, semantic variant primary progressive aphasia; TDP, TAR DNA-binding protein.

aAD 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).
bContains 1 nondeceased MAPT mutation case (P301L).
cContains 3 nondeceased PGRN mutation cases (IVS36-16>G, IVS264+2T>C, and c.102delC).
dContains 1 case (PSP) with a secondary diagnosis of low-probability dementia with Lewy body disease.
eContains 2 cases (FTLD-TDP and ALS-FTLD) with a secondary diagnosis of low-probability AD.

Figure 1. Flowchart of the transformation (A) and validation and cross-validation (B) steps. ELISA indicates enzyme-linked immunosorbent assay; ROC, receiver operating characteristic.

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 19.0 (SPSS) and R version 2.13 (The R Foundation for Statistical Computing).

Sensitivity and specificity of the antemortem clinical diagnosis (FTLD spectrum or AD) were 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. A

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: (ln(value) × 1.313562) /1.040762) to convert ELISA values, which was used in subsequent analyses.

DIAGNOSTIC ACCURACY OF TRANSFORMED VALUES

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. 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 correlated and can be transformed by a conversion factor. 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. 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, MD, Susan Leight, Malgorzata Knapik-Czajka, 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. 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. T-ELISA = transformed data. 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.
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 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.

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.

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