Comparison of Analytical Platforms for Cerebrospinal Fluid Measures of β-Amyloid 1-42, Total tau, and P-tau181 for Identifying Alzheimer Disease Amyloid Plaque Pathology

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Background: Cerebrospinal fluid (CSF) biomarkers of Alzheimer disease (AD) are currently being considered for inclusion in revised diagnostic criteria for research and/or clinical purposes to increase the certainty of antemortem diagnosis.

Objective: To test whether CSF biomarker assays differ in their ability to identify true markers of underlying AD pathology (e.g., amyloid plaques and/or neurofibrillary tangles) in living individuals.

Design: We compared the performances of the 2 most commonly used platforms, INNOTEST enzyme-linked immunosorbent assay and INNO-BIA AlzBio3, for measurement of CSF β-amyloid (Aβ) and tau proteins to identify the presence of amyloid plaques in a research cohort (n=103). Values obtained for CSF Aβ1-42, total tau, and phosphorylated tau 181 (p-tau181) using the 2 assay platforms were compared with brain amyloid load as assessed by positron emission tomography using the amyloid imaging agent Pittsburgh compound B.

Setting: The Knight Alzheimer’s Disease Research Center at Washington University in St Louis, Missouri.

Subjects: Research volunteers who were cognitively normal or had mild to moderate AD dementia.

Results: The 2 assay platforms yielded different (approximately 2- to 6-fold) absolute values for the various analytes, but relative values were highly correlated. The CSF Aβ1-42 correlated inversely and tau and p-tau181 correlated positively with the amount of cortical Pittsburgh compound B binding, albeit to differing degrees. Both assays yielded similar patterns of CSF biomarker correlations with amyloid load. The ratios of total tau to Aβ1-42 and p-tau181 to Aβ1-42 outperformed any single analyte, including Aβ1-42, in discriminating individuals with vs without cortical amyloid.

Conclusions: The INNOTEST and INNO-BIA CSF platforms perform equally well in identifying individuals with underlying amyloid plaque pathology. Differences in absolute values, however, point to the need for assay-specific diagnostic cutoff values.


Alzheimer disease (AD), the most common cause of dementia in elderly persons, currently affects an estimated 35.6 million individuals worldwide, with numbers expected to triple and reach epidemic proportions by the year 2050 (http://www.alz.co.uk/research/statistics.html). Such an aggressive disease trajectory will have a devastating effect on patients, their caregivers, and health care resources as well as public health burden. Appreciation of the societal and economic implications of this projection and disappointing AD clinical trial results to date make it imperative that the AD research, clinical, and pharmaceutical communities work together to better identify those at the very earliest stages of the disease in concert with designing more effective clinical trial strategies. Converging evidence suggests that AD pathology (amyloid plaques and neurofibrillary tangles) begins to develop years, if not decades, before diagnosis of clinical dementia. This disease state, AD pathology prior to the development of dementia symptoms, has been termed preclinical or presymptomatic AD. With this in mind, the AD field is experiencing a paradigm shift in the way...
therapeutic strategies are viewed, from cure to prevention, with the ultimate goal of intervening very early in the disease process (perhaps even at the initial preclinical stage) to prevent neurodegeneration and its clinical sequela, dementia.

Although a definitive diagnosis still requires histopathologic evaluation of the brain at autopsy, AD currently is diagnosed clinically according to criteria published in 1984.4 These criteria (ie, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association) have been reliable for the diagnosis of probable AD, with mean reported sensitivities and specificities of 81% and 70%, respectively.5 However, research published during the past 25 years has led to a recent effort, cosponsored by the Alzheimer’s Association and the National Institute on Aging, to propose revision of these criteria to expand the scope of what is now considered to be AD (to more accurately reflect the full continuum of the disease) as well as incorporate newly discovered biomarkers of underlying AD pathology that would add to the certainty of antemortem diagnosis.

Cerebrospinal fluid (CSF) levels of β-amyloid 1-42 (Aβ1-42) and tau protein (the primary constituents of plaques and tangles, respectively) have been shown to have diagnostic utility for discriminating AD dementia cases from cognitively normal controls but with a wide range of reported sensitivities and specificities.6 Such discrepancies likely reflect (1) methodologic variables, including the clinical characteristics of the cohorts studied; (2) biological variables, such as possible misdiagnosis of cognitively impaired individuals (especially at the earliest clinical stages) and the inevitable inclusion of preclinical cases in the cognitively normal control group (estimated to be 25%-35% in these elderly cohorts)3; (3) the specific CSF collection and processing protocols used; and (4) the various CSF assays used. The advent of in vivo amyloid imaging techniques now permits evaluation of the extent to which these CSF measures are reflective of underlying AD pathology in living individuals. Several recent studies have demonstrated that low CSF Aβ1-42 is a good indicator of the presence of cortical amyloid.7,11 However, no one has reported a comparison of the head-to-head performance of the different CSF assays in identifying amyloid status within the same cohort. Because efforts are currently under way to standardize CSF collection, processing, and analytic protocols, we compared the performance of the 2 most commonly used commercial AD CSF assay platforms (INNOTEST enzyme-linked immunosorbent assay and INNO-BIA AlzBio3 xMAP technology; Innogenetics NV, Ghent, Belgium) for classifying amyloid status in a large cohort of research participants. Endorsement of a given assay platform for standardization purposes will depend on the extent to which the assay(s) truly marks underlying disease pathology.

### Table 1. Study Participant Demographics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
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<tbody>
<tr>
<td>Participants, No.</td>
<td>103a</td>
</tr>
<tr>
<td>Mean (SD) age at LP, y</td>
<td>67.8 (9.9)</td>
</tr>
<tr>
<td>Age range, y</td>
<td>46-89</td>
</tr>
<tr>
<td>Female, %</td>
<td>68</td>
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<tr>
<td>APOE genotype, % with α-ε4 allele</td>
<td>44</td>
</tr>
<tr>
<td>Median LP to PiB interval, d (IQR)</td>
<td>97 (35-236)</td>
</tr>
<tr>
<td>Mean (SD) LP to PiB scan interval, d</td>
<td>161.7 (166.5)</td>
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<tr>
<td>LP to PiB range, d</td>
<td>0-719</td>
</tr>
<tr>
<td>LP same d or prior to PiB, % (range, d)</td>
<td>80 (0-719)</td>
</tr>
<tr>
<td>LP after PiB, % (range, d)</td>
<td>20 (1-400)</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; LP, lumbar puncture; PiB, Pittsburgh compound B scan.

aClinical Dementia Rating (CDR) 0, n=89; CDR 0.5, n=11; CDR 1, n=1; CDR 2, n=2.

Table 1. Study Participant Demographics

Participants were community-dwelling research volunteers enrolled in longitudinal studies of healthy aging and dementia through the Knight Alzheimer’s Disease Research Center at Washington University in St Louis. Participants were in good general health, with no neurological, psychiatric, or systemic medical illnesses that could contribute importantly to dementia or medical contraindication to lumbar puncture (LP) for CSF collection or positron emission tomography (PET) with the amyloid imaging agent Pittsburgh compound B (PiB).14 One hundred three participants who had an LP and a PET PiB scan within 2 years of each other (between January 2004 and November 2008) were included in the analysis. The scan could be before or after the LP, but most subjects (80%) had LP prior to PET PiB scan (Table 1). The cohort included both individuals with dementia and cognitively normal participants (see later) to obtain a wide range of PiB and CSF biomarker levels. Although clinical status is not a variable of interest in the present study, cognitive status at the annual clinical assessment just prior to LP was determined in accordance with standard protocols and criteria.13,15 A Clinical Dementia Rating (CDR)15 of 0 indicates cognitively normal, whereas a CDR of 0.5, 1, or 2 indicates very mild, mild, or moderate dementia, respectively. All but 1 dementia diagnosis in this cohort was considered by clinicians to be due to AD; an etiologic classification of the remaining CDR 0.5 participant, who was diagnosed as having uncertain dementia.

All studies were approved by the Human Research Protection Office at Washington University, and written informed consent was obtained from all participants. Genotyping for apolipoprotein E (APOE) was performed by the Knight Alzheimer’s Disease Research Center Genetics Core.

### CSF COLLECTION AND PROCESSING

Cerebrospinal fluid (20-30 mL) was collected via standard sterile technique at 8:00 AM after overnight fasting, as described.7 The CSF (free of visible blood contamination) was obtained from the L4/L5 lumbar space using an atraumatic 22-gauge Sprotte spinal needle via gravity flow into a 50-mL polypropylene tube and put immediately on wet ice. Within 1 hour after collection, samples were gently inverted to avoid possible gradient effects, briefly centrifuged at low speed (2000g, 15 minutes, 4°C) to pellet any cellular debris, and aliquoted (500 µL) into polypropylene tubes prior to freezing at ~80°C.

### CSF BIOMARKER ASSESSMENT

The CSF samples were analyzed for Aβ1-42, total tau, and tau phosphorylated at tyrosine 181 (p-tau181) by plate-based enzyme-
linked immunosorbent assay (INNOTEST) by the Biomarker Core at Knight Alzheimer’s Disease Research Center according to the manufacturer’s instructions and by LumineX xMAP bead-based methods (INNO-BIA AlzBio3) by the Alzheimer’s Disease Neuroimaging Initiative Biomarker Core at the University of Pennsylvania, with performance characteristics and assay conditions as described. INNO-BIA reagents included monoclonal capture/detection antibodies 2F12/3D6 for Aβ1-42, AT120/HT7 and BT2 for total tau, and HT7/AT270 for p-tau181. INNO-BIA reagents included monoclonal capture/detection antibodies 4D7A3/3D6 for Aβ1-42, AT120/HT7 for total tau, and AT270/HT7 for p-tau181. Assays were performed by trained core technologists at each site who were blind to the clinical and PiB statuses of participants. For all biomarker measures, samples were continuously kept on ice and assays were performed in duplicate on sample aliquots after a single thaw following initial freezing.

IN VIVO AMYLOID IMAGING WITH PIb

All participants underwent in vivo amyloid imaging via PET PiB within 2 years of LP as described. The cerebellum was chosen as a region with very low specific PiB binding for use as a reference region, and Logan graphical analyses were performed to calculate the mean cortical PiB distribution volume (MCBP) for each participant. Mean cortical PiB was defined by averaging the distribution volumes for the prefrontal cortex, precuneus, lateral temporal cortex, and gyrus rectus. For certain analyses, amyloid positivity was defined by an MCBP cutoff of 0.18 based on prior studies.

STATISTICAL ANALYSIS

Statistical analyses were carried out using SAS statistical software (SAS Institute, Inc., Cary, North Carolina). The relationships between CSF analyte values obtained with each assay platform and between MCBP and the various CSF measures were evaluated using the Spearman ρ correlation coefficient (α = .05). Because different correlations were estimated from the same sample of individuals, these estimates were correlated. Comparative analyses of correlations were based on standard normal tests after applying Fisher ω-transformation to the correlated correlations. Receiver operating characteristic and area under the curve (AUC) analyses were used to assess the sensitivity and specificity of the various CSF measures to discriminate individuals with cortical amyloid deposition (PiB−) from those without (PiB+) using an MCBP cutoff of 0.18. Because the distributions of biomarker values were skewed, a logarithmic transformation was first used to approximate normality. Sensitivity was then estimated when specificity was fixed at 80% across biomarkers. Confidence intervals for AUCs were based on standard normal distributions. Because AUCs were estimated from the same set of individuals, comparative analysis of AUCs across biomarkers took into account the correlation between estimated AUCs and was based on standard normal tests.

RESULTS

Study participants who met the LP and PiB interval inclusion criteria included 89 who were cognitively normal (CDR 0), 11 with very mild dementia (CDR 0.5), 1 with mild dementia (CDR 1), and 2 with moderate dementia (CDR 2) (Table 1). Participants spanned a wide age range (46-89 years), with a mean (SD) age of 67.8 (9.9) years. Sixty-eight percent were female, and 44% had at least 1 APOE ε4 allele. The median interval between LP and PiB scan was 97 days (3.2 months). The shortest interval was 0 days and the longest was 719 days (1.97 years). Most participants (80%) had LP prior to PiB, including the 12 with the longest LP-PiB test intervals (range, 401-719 days).

According to data provided by the kit manufacturer, the intra-assay variability should be less than 6% with the INNOTEST and less than 4% with the INNO-BIA platform. In the present study, we observed mean (SD) coefficients of variation of 4.2% (3.8%), 4.5% (4.8%), and 1.7% (1.7%) for Aβ1-42, total tau, and p-tau181, respectively, using INNOTEST (Table 2). Similar coefficients of variation were observed for INNO-BIA, with 3.7% (2.8%), 3.6% (3.1%), and 3.9% (3.1%) for Aβ1-42, total tau, and p-tau181, respectively (Table 2).

We first assessed the relationship between the analyte values obtained with the 2 platforms. Although the absolute values for Aβ1-42, total tau, and p-tau181 obtained with the 2 platforms were different, the values correlated well with each other (Aβ1-42 r = 0.7715, P < .001; total tau r = 0.9446, P < .001; p-tau181 r = 0.8017, P < .001) (Figure 1A-C), as did the ratios of total tau to Aβ1-42 (r = 0.8746; P < .001) and p-tau181 to Aβ1-42 (r = 0.8586; P < .001) (Figure 1D and E).

Given that a low level of CSF Aβ1-42 has been shown by several groups to be a marker of brain amyloid (as assessed by PET PiB), we next compared head-to-head the amount of cortical PiB binding (MCBP) with CSF Aβ1-42, as determined by the 2 platforms. Overall, increased MCBP, indicating the presence of cortical amyloid, was associated with low levels of CSF Aβ1-42, as determined by both assays (Figure 2A and B). The MCBP was positively correlated with levels of CSF total tau and p-tau181 (Figure 2C-F) as well as the tau to Aβ1-42 and p-tau181 to Aβ1-42 ratios (Figure 2G-J). When comparing PiB CSF correlation coefficients between the 2 assay platforms, the correlation between MCBP and INNO-BIA Aβ1-42 (r = -0.7068) was stronger than that of MCBP and INNOTEST Aβ1-42 (r = -0.5673; P < .001); INNOTEST total tau (r = 0.5961) was slightly stronger than that of INNO-BIA total tau (r = 0.5546; P = .004); INNO-BIA p-tau181 (r = 0.6417) was stronger than that of INNOTEST p-tau181 (r = 0.5326; P = .005); and INNO-BIA p-tau181 to Aβ1-42 (r = 0.7477) was stronger than that of INNOTEST p-tau181 to Aβ1-42 (r = 0.6784; P < .001), whereas the correlation between MCBP and the ratio of

<table>
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<th>Analyte</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Median (IQR)</th>
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</thead>
<tbody>
<tr>
<td>Aβ1-42</td>
<td>0-16.1</td>
<td>4.2 (3.8)</td>
<td>3.7 (1.45-5.05)</td>
</tr>
<tr>
<td>Total tau</td>
<td>0-20.2</td>
<td>4.5 (4.8)</td>
<td>2.5 (0.9-6.25)</td>
</tr>
<tr>
<td>p-tau181</td>
<td>0-10.7</td>
<td>1.7 (1.7)</td>
<td>1.2 (0.7-2.35)</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ1-42, β-amyloid 1-42; IQ, interquartile range; P-tau181, tau phosphorylated at tyrosine 181.
The ratios of tau(s) to Aβ1-42 obtained with both assay platforms outperformed each single analyte (including Aβ1-42) in discriminating PiB+ from PiB- individuals, reaching high levels of sensitivity. Together these data demonstrate that the 2 assay platforms both performed well in identifying individuals with underlying amyloid plaque pathology in living individuals. Each of the assays exhibited high levels of intra-assay reliability, with mean coefficients of variability of less than 5% for INNOTEST and less than 4% for INNO-BIA. The 2 methods yielded different (approximately 2- to 6-fold) absolute values for the various analytes but the relative values were highly correlated (r values from 0.77 to 0.94), consistent with previous articles.28,34 Also consistent with previous studies using individual assays,7,12,30 the various CSF analytes were found to correlate with cortical PiB binding, albeit to differing degrees. Given the increase in literature supporting the diagnostic and prognostic potential of AD biomarkers,26 inclusion of biomarker data for use in disease diagnosis for research and/or clinical purposes is currently being proposed.27 However, biomarker validation and standardization must be fully demonstrated before such measures will be considered acceptable for individual patient diagnosis in the clinical care setting. To this end, we compared the performances of the 2 most commonly used commercial assays for CSF biomarkers of AD in identifying PiB+ amyloid plaque pathology in living individuals. The 2 methods yielded different (approximately 2-to-6 fold) absolute values for the various analytes but the relative values were highly correlated (r values from 0.77 to 0.94), consistent with previous articles.28,34 Also consistent with previous studies using individual assays,7,12,30 the various CSF analytes were found to correlate with cortical PiB binding, albeit to differing degrees.
plaque pathology, especially the tau(s) to Aβ1-42 ratios, and support a strong relationship between amyloid and tau pathologies in AD.

Despite differences in the general assay platform, the specific Aβ antibodies used, and the different absolute values generated by the 2 assays, the 2 assays performed equally
well in discriminating amyloid-positive from amyloid-negative individuals (AUC = 0.89 and 0.93, respectively; P = .26). This was the case in the full cohort (of mixed CDRs) as well as in the subcohort of CDR 0 cases (data not shown), providing further support that CSF Aβ1-42 measures are able to identify amyloid-positive cases even in the earliest (preclinical) stage of the disease. However, the fact that the 2 assay platforms generated different absolute values (although positively correlated) poses a challenge for biomarker standardization efforts as far as being able to define universal cutoff values to identify groups with or without underlying disease pathology. The similarity in performance of the two Aβ1-42 assays precludes endorsement of one assay platform over the other. Other factors, such as test sample volume requirements, reproducibility between laboratories, and overall assay cost, will likely have to be considered when making protocol standardization recommendations.

Similar to the findings observed for CSF Aβ1-42, CSF total tau and p-tau181, measures obtained by both assays correlated significantly with the amount of cortical PiB binding, consistent with previous studies using 1 assay platform. Although the strengths of the correlations between amyloid load and the various CSF analytes differed between the 2 assay platforms, these differences were not consistent; certain analyte/platform combinations correlated better with cortical amyloid load than other combinations. The ratios of tau(s) to Aβ1-42 obtained with both platforms exhibited the strongest correlation with PiB binding compared with the single analytes. This was also reflected in the greatest AUCs obtained for the ratios (0.92–0.98 for the ratios compared with 0.65–0.89 for the single analytes) in identifying amyloid-positive vs amyloid-negative groups. The CSF tau(s) to Aβ1-42 ratios have also been shown to predict future cognitive decline in cognitively normal individuals and those with mild cognitive impairment or very mild dementia. Together, these data suggest a relationship between the development of amyloid plaque and tangle pathologies and neurodegeneration in AD that eventually leads to dementia. Although the pattern of elevated CSF tau only in the presence of substantial amyloid load suggests that Aβ1-42 aggregation is driving subsequent tangle formation and/or neurodegeneration, analysis of within-subject longitudinal clinical, CSF, and amyloid (and eventually neurofibrillary tangle) imaging results will be required to determine the precise timing of the development of these pathologies during the course of the disease.

Establishment of a link between fluid biomarker profiles and underlying disease pathology is a necessary requisite for proposing their usefulness in disease diagnosis and, eventually, therapeutic intervention. All published AD therapeutic trials to date have used clinical criteria (ie, mild cognitive impairment or mild to moderate AD dementia) for patient enrollment despite the fact that, by the time any clinical symptoms are apparent, significant AD pathology has already developed, including substantial synaptic and neuronal loss that will likely be unable to be reversed. Therefore, it is perhaps not surprising that no proposed disease-modifying treatment has resulted in a positive clinical outcome. Given that various CSF measures are reflective of underlying amyloid pathology and neurodegeneration, even when cognitive symptoms are absent, these biomarkers may be useful in the design and
evaluation of more appropriate prevention trials. Such markers could be used for patient selection (eg, high CSF tau to AB1-42 ratio for identifying individuals with cortical amyloid and neurodegeneration), thus helping to reduce enrollment requirements and trial duration. These markers could also be used to monitor response to therapy, especially for therapies designed to directly influence disease pathology itself. The ultimate goal, of course, will be to use biomarkers to assist in making treatment decisions once such disease-modifying treatments for AD become available.

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Author Contributions: Dr Fagan had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Fagan, Vanderstichele, Trojanowski, and Holtzman. Acquisition of data: Fagan, Vanderstichele, Mintun, and Morris. Analysis and interpretation of data: Fagan, Shaw, Xiong, Vanderstichele, Mintun, Coart, and Holtzman. Drafting of the manuscript: Fagan and Trojanowski. Critical revision of the manuscript for important intellectual content: Shaw, Xiong, Vanderstichele, Mintun, Trojanowski, Coart, Morris, and Holtzman. Statistical analysis: Xiong and Coart. Obtained funding: Fagan, Mintun, Morris, and Holtzman. Administrative, technical, and material support: Fagan, Shaw, Vanderstichele, Trojanowski, and Morris. Study supervision: Fagan and Holtzman.

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REFERENCES


