Validation of biomarkers in the Alzheimer’s Disease Neuroimaging Initiative

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Disclosures

Grant support: ADNI 1, ADNI GO, ADNI 2, NIH/NIA;
Pfizer/UPenn rbm studies; PPMI, MJFox Research foundation

Contracts/consultant: Janssen Research & Development; Eisai Medical Research;
Bristol Myers Squibb; Saladax Biomedical; Baxter
Overview

• At least 40 studies (mostly single center small studies) have shown the diagnostic utility of CSF \(\text{A} \beta_{1-42}\) and tau measurement for AD detection-50% or more decrease in \(\text{A} \beta_{1-42}\) and ~2-3 fold increase in tau in comparison to age-matched normals.

• ELISA and Luminex xMAP multiplex immunoassays and Innogenetics reagents most commonly used methods, Mesoscale Diagnostics has introduced an immunoassay and others are in development

• Several studies show the predictive performance for \(\text{A} \beta_{1-42}\) and \(t\)-tau/ \(\text{A} \beta_{1-42}\) as predictors of progression from Cog Norm→MCI or MCI→AD (Hansson, 2006; Fagan, 2007; Shaw, 2009; Visser, 2009; Mattson, 2010).

• Studies from UWash, WashU, ADNI show that about a third of normal elderly have these changes but requires many years of time to observe conversion to MCI or early AD; relationship to changes in certain neuropsych. tests are significant.

• It is possible to obtain reproducible results within one laboratory, using one lot of manufacturer’s reagents, such that batched samples can be assayed with confidence in the results; appropriate qc materials are used to check performance continuously.

• A reference method is needed in order to create a reference material in CSF with accurate assignment of biomarker concentration. This would provide for manufacturers a common accepted reference CSF material to base their calibrator concentrations on. This will not guarantee comparable clinical performance across the analytical platforms/immunoassay systems—that requires clinical validation using appropriate populations and CSF samples.

• High level standardization requirements for sample collection, storage, handling, reagent manufacture, lab performance have been reported.

• An international CSF qc program sponsored by the Alz Association was established in 2010 that provides feedback to participating labs and should lead to improved practice worldwide (79 participating labs)
Qualification of the analytical and clinical performance of CSF $\text{A}\beta_{1-42}$, tau and $p$-tau$_{181p}$ in the ADNI study

1. Selection of CSF $\text{A}\beta_{1-42}$, tau, $p$-tau$_{181p}$ based on prior studies that showed their promise for AD detection & a consensus among experts in this field

2. **Pre-analytical factors for the lp & CSF handling**
   - Identify and control for pre-analytical variables
     - Time of day for lp - *morning following overnight fast*
     - Use of narrow gauge blunt (Sprotte) needle
     - Collection & aliquot tube type - *avoid PS and glass tubes & use PP tubes*
     - Transport temperature - *avoid storage at refrigerator temp*
     - # of freeze-thaw cycles - *minimize*
     - Time from collection to freezing - *minimize*
     - Sample id & annotation of details on each sample collection/processing history

3. **Analytical performance**
   - Assure stability of reproducibility of test performance
     - Follow consistently detailed method protocol
     - Within each run
     - Day to day
     - Among expert laboratories: interlab studies
     - From batch to batch of immunoassay reagents
     - AA-sponsored international CSF external blinded quality control program

4. **Clinical diagnostic performance**
   - Establish diagnostic and predictive performance using the qualified test method
     - Establish sensitivity & specificity in ADNI-independent CSF samples from autopsy-confirmed AD subjects
     - Use these diagnostic cutpoints to characterize AD CSF pathologic biomarker signatures in ADNI subjects
     - Assess the predictive performance of CSF biomarkers for cognitive decline
     - Evaluate predictive performance for MCI$\rightarrow$AD progressors
     - Characterize the longitudinal changes in CSF biomarker changes in a subset of ADNI CSF donors
     - Study multiple biomarker types in combination for optimal disease detection and progression
Immunoassays for AD CSF biomarkers

CSF Aβ₁-42, T-tau & P-tau methods:

• Pre-analytical, analytical and post-analytical sources of variability for commercially available methods have been extensively examined

• Current commercially available assays are high complexity, precision-based methods

• Reported clinical utility for CSF biomarkers is based on the precision-based methods

• The precision has been demonstrated in both intra-lab & inter-lab settings

• For the ADNI study we use the AlzBio3 xMAP immunoassay platform. Following analytical validation studies including a multicenter interlab investigation the clinical utility and cutpoints for CSF Aβ₁-42, t-tau and p-tau₁₈₁ were developed using an ADNI independent cohort with autopsy diagnosis of AD and pre-mortem CSFs, thereby establishing a link to AD pathology.

• Multiple independent studies on other populations provide substantial support for the link between CSF Aβ₁-42, t-tau and p-tau₁₈₁ and AD pathology.

• Consensus reference standards & methods for an accuracy-based approach are not available, but a collaborative effort within the AA sponsored GBSC is underway to develop a reference method (an mrmHPLC/MSMS method) to be used to assign values of Aβ₁-42 to CSF pool-based standard reference materials (SRMs).

• Harmonization of methods is an important goal

• The clinical utility of accuracy-based methods has not been established
ADNI 1 MCI subjects who progressed to AD or reverted to cognitively normal

Orange are ADNI 1 MCI subjects who progressed to clinical AD by month 12, yellow are ADNI 1 MCI subjects who progressed to AD between 12 and 36 months and sea-green are subjects who “reverted” back to cognitively normal. The bar chart frequency plot is the distribution of ADNI 1 MCI (BASELINE diagnosis) subjects’ Aβ_{1-42} concentrations at BASELINE.

Progressors
144±37 pg/mL, 12 mo
145±42 pg/mL, 12-36 mo

“reverters”
229±56 pg/mL
CSF Aβ$_{1-42}$ is Strongly Correlated to Plaque Counts in autopsied brains and Plaque Burden by PiB testing

Pittsburgh compound-B labeled positron emission tomography; SUVR = standard uptake value ratio

Efforts underway to improve standardization

- ADNI
- Alz Assn: International qc program; Global Biomarker Standardization Consortium
- CAMD[Coalition Against Major Diseases Biomarker Working Group]
- UPenn/Wash U collaboration on ELISA/xMAP/PiB relationships (Anne Fagan, David Holtzman, John Morris, John Trojanowski)
- UPenn ADRC studies in neurodegenerative diseases with autopsy diagnosis: xMAP/ELISA data integration (Jon Toledo, David Irwin, John Trojanowski)
- Collaborative studies between labs for xMAP immunoassay:
  - With Mayo Clinic (Ron Petersen & Roy Dyer)
  - With Japan ADNI (Hiroyuki Arai, Ryosun Kuwano & Takeshi Iwatsubo)
- ABSI: Innogenetics-sponsored workgroup on standardization guidelines [Alzheimer’s Biomarkers Standardization Initiative]
ADNI 1: Within Lab Analytical Precision Across Days & Lots

Shaw et al 2011 Acta Neuropathologica

Adaptive biomarker core laboratory

Innogenetics
INNO-BIA
AlzBio3 xMAP

Average Test/re-test (%CV)

- $A\beta_{1-42}$: 5.7%
- t-tau: 5.6%
- p-tau$_{181}$: 11.5%

$A\beta_{1-42}$ Bland-Altman Bias Plot

$A\beta_{1-42}$ Test/Re-test Correlation
ADNI GO & ADNI 2: first batch analyses of CSF biomarkers (1st quarter 2012)

- All ADNI GO + ADNI 2 CSFs (through 2/21/2012)
- N=467 [390 Baseline + 77 follow-up]; in addition, 28 replicates
- This set of CSFs is enriched with EMCI subjects samples, smaller numbers of new LMCI, early AD and cognitively normal subjects
- AlzBio 3 immunoassay (Fujirebio/Innogenetics) for Aβ_{1-42}, t-tau, p-tau_{181}
- Assessed 2012 vs 2007 using 12 CSFs (ADNI 1 BASELINE) across conc. range for lot to lot performance prior to use
- ~5% random samples re-tested
- Inclusion of 2 new CSF pools, 1 (cog normal), 1 (clin AD) & 2 aqueous qc’s
- Detailed quality control review underway; blinded to diagnoses until data lock shortly
- Report on analytical performance in Melbourne
- Report results at the ADNI meeting in New Orleans
CSF analyses for ADNI GO & ADNI 2 (1st quarter 2012)

• Continued assessments of analytical performance of the AlzBio3 xMAP immunoassay system
  – Precision performance
    • Calibrator reproducibility
    • Run-to-run precision of new abnormal and normal CSF pools
    • Test/re-test precision for randomly selected ~5%
  – Concentration accuracy with ADNI 2007 as basis for comparison of kit lot as source of variability. The checking of lot to lot variation would benefit from the availability of a standard reference material (SRM) to use in place of the valuable replicate CSF aliquots

• Planned analyses include: Cross sectional comparisons of the EMCI cohort with each of the following cohorts: LMCI, AD, NC biomarker data; characteristics of the data distributions, eg, presence of bimodal distribution; using pathologically based cutpoints estimate the number of EMCI subjects with AD-like biomarker profiles as compared to LMCI subjects; check for progression to AD in EMCI subjects

**Aβ_{1-42}**

- Intercept = 8.623
- Slope = 1.031
- \( r^2 = 0.953 \)
- Mean Error = 13.67
- Mean Absolute Error = 17.13
- RMSE = 18.91

**t-tau**

- Intercept = 4.502
- Slope = 0.8127
- \( r^2 = 0.975 \)
- Mean Error = -13.75
- Mean Absolute Error = 13.75
- RMSE = 18.47
Detailed monitoring of each analytical run

UPenn ADNI Biomarker Laboratory Check Form for AlzBio3 assays

| Experiment:  |  |
| Plate #: | Performer: |
| QC used for analyses: |  |

**Day 1**

1. About 30 min before assay take out the Beads, Conjugate 1 and Diluent and let them reach RT.
   
   Start time: **PM**  
   Temperature: **9°C**

2. About 30 min before assay take out wash solution 25x and put it for 30 min into water bath 37°C.

   Start time: **PM**  
   Temperature: **9°C**

3. After use put reagents back in to +4°C (max.120 min after taking out).

   Time:

4. Approx. 20 min before assay take out standards, controls and samples and let them reach RT.

   Standards Start time: **PM**  
   Temperature: **4°C**

   Samples Start time: **PM**  
   Temperature: **9°C**

5. **Dilute Wash Solution 25x**:

   Wash sol. 25x **mL**  
   Distilled water **mL**

6. Prepare polypropylene tubes for working solutions of Beads and Conjugate 1

7. Vortex Coated beads and sonicate 3 min and vortex once more

8. Dilute coated beads 100x with Diluent (immediately cover polypropylene tube with diluted beads with aluminum foil) and vortex solution

   Coated beads 100x **mL**  
   Diluent **mL**

9. Prepare Conjugate 1 working solution: Dilute Conjugate 1 with Diluent 100x and vortex solution

   Conjugate 1 100x **mL**  
   Diluent **mL**

10. Vortex thawed Standards, Controls, and samples

11. Prepare basins for working solutions

12. Transfer 225µL of diluted wash buffer into each well of the plate, aspirate the wells using vacuum manifold

13. Aspirate the filter plate by using vacuum manifold

14. Transfer 100µL of beads to the wells of filter plate

15. Aspirate the filter plate by using vacuum manifold

16. Add 25µL of Conjugate 1 working solution to the wells. Proceed to the next step immediately.

17. Add 75µL of Diluent (blank), standards, controls and samples to the wells. Cover the plate with aluminum foil.

18. After use put Samples, STs and Cons back into -80°C (max.60 min after taking out)
Calibrator stability

MFI vs nominal concentration

\[ \text{A}\beta_{1-42} \]

N=14

MFI data for calibrators

Calibrator #2

Calibrator #4

Precision data for back-calculated calib concs

<table>
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<tr>
<th>Analyte</th>
<th>Sample</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
<th>5% CI</th>
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<td>212.4</td>
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<td>0.7841</td>
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<td>33.07</td>
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<td>0.465</td>
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<td>17.68</td>
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<td>2.57</td>
<td>10.71</td>
<td>11.71</td>
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<td>3.648</td>
<td>0.2192</td>
<td>6.01</td>
<td>3.334</td>
<td>3.944</td>
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</table>
Analytical precision within-lab 2012

Abnormal CSF pool

Aβ<sub>1-42</sub>

p-tau<sub>181</sub>

Normal CSF pool

Aβ<sub>1-42</sub>

t-tau

Aβ<sub>1-42</sub> test/retest correlation

Aβ<sub>1-42</sub> Bland-Altman bias plot

ADNI Core Laboratory
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Fujirebio/Innogenetics
INNO-BIA
AlzBio3 xMAP
---

Average Test / re-test (%CV)

<table>
<thead>
<tr>
<th></th>
<th>Aβ&lt;sub&gt;42&lt;/sub&gt;</th>
<th>t-tau</th>
<th>p-tau&lt;sub&gt;181&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6.4%</td>
<td>5.4%</td>
<td>7.5%</td>
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</table>

Concentration [pg/mL]

Concentration [pg/mL]
Analytical Methods: Intra-lab & Inter-lab precision

ADNI-sponsored Round Robin
Shaw et al 2011 Acta Neuropathologica
Automated sample processing for plasma Aβ\textsubscript{1-42/1-40} INNO-BIA
Plasma Aβ forms immunoassay

Figurski M, et al, Alz and Dem, in press
CSF biomarkers in AD, FTLD and mixed pathologies

Use of clinical diagnosis underestimated biomarkers’ accuracy and was less accurate than the biomarkers

<table>
<thead>
<tr>
<th>Biomarkers based on neuropathological diagnosis (AD vs. FTLD)</th>
<th>Biomarkers based on clinical diagnosis (AD vs. FTLD)</th>
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<tbody>
<tr>
<td>AUC</td>
<td>0.98</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100.0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>87.5%</td>
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</tbody>
</table>

In addition to the improved diagnostic accuracy using CSF biomarkers, this study shows that there is a significant number of subjects with a “mixed” pathology, an important issue to consider when using biomarkers to enrich AD treatment trials. Toledo, et al, Acta Neuropathologica, in press.
Comparison of biomarkers and ratios for AD vs FTLD

These data are from patients followed at the Alzheimer’s Disease Center of Frontotemporal Disease Center at the Perelman School of Medicine, University of Pennsylvania.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Area under the curve (AUC)</th>
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<tr>
<td>Aβ₁₋₄₂</td>
<td>0.874</td>
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<tr>
<td>t-tau</td>
<td>0.941</td>
</tr>
<tr>
<td>p-tau</td>
<td>0.889</td>
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<tr>
<td>t-tau: Aβ₁₋₄₂ ratio</td>
<td>0.989</td>
</tr>
<tr>
<td>p-tau: Aβ₁₋₄₂ ratio</td>
<td>0.956</td>
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</table>

Figure 3. Receiver operating characteristic curve analysis of xMAP analyte values in an autopsy-confirmed sample (neuropathological sample 1). The t-tau: Aβ₁₋₄₂ ratio had the highest area under the curve at the optimal diagnostic cut-point of 0.34.
Candidate reference method for $A\beta_{1-42}$

- Development of an mrmHPLC/tandem mass spectrometry direct sample preparation methodology for quantification of $A\beta_{1-42}$.
- The method uses high concentration of guanidine HCl to denature all species of $A\beta_{1-42}$ (monomer, dimer, higher oligomers, complexes with other proteins?, aggregates?)
- A group of investigators is pursuing this collaboratively [Erin Chambers(Waters Co), Rand Jenkins(PPD), Les Shaw(UPenn) and Kaj Blennow(Goteborg) as an AA/GBSC-sponsored effort to develop a candidate reference method. This would have several spin-off benefits including use for assigning accuracy-based values to CSF pools.
- At least 4”lab years” of work has been put into this project collectively but the group is optimistic about the possibility of having a viable assay ready for testing.
- The clinical performance of this type of methodology is unknown.
ADNI biomarker studies

- ADNI/PPSB/FNIH RBM study in 328 baseline CSF samples completed, data uploaded, 2 abstracts submitted to AAIC from this group and others outside the group as well
- Merck/ADNI BACE analyses in ADNI BL CSF samples completed and scheduled for upload on the ADNI web site by mid-April
- mrmMass spectrometry pilot on a limited number of ADNI 1 BASELINE CSFs scheduled to get underway soon; if successful, will then proceed with analyses of ADNI 1 BL CSFs.
- Continuation of standardization efforts including close collaboration with our Japan ADNI colleagues to achieve harmonization between Japan ADNI and US ADNI—a 3 way interlab study is underway involving Japan ADNI, USADNI and Fujirebio/Innogenetics.
Published or in press studies using the RBM platform in various study populations:


It takes a great team effort!

John Q Trojanowski
Virginia M-Y Lee
Chris Clark
Steve Arnold
Hugo Vanderstichele
Magdalena Korecka
Margaret Knapik-Czajka
Magdalena Brylska
Teresa Waligorska
Michal Figurski
Ravi Patel
Leona Fields
Sarah Pan
William Hu
Ju Hee Kang
Jon Toledo
Anne Fagan
Uwe Christians
Kaj Blennow
Henrik Zetterberg
Holly Soares
Adam Simon
Robert Dean
Eric Siemers
Piotr Lewczuk
William Potter
Rand Jenkins
Erin Chambers

Supported by the NIH/NIA and families of our patients

ADNI investigators include: (complete listing available at www.loni.ucla.edu\ADNI\Collaboration\ADNI_Manuscript_Citations.pdf).