CSF Biomarkers of Alzheimer’s Disease

Anne M. Fagan, PhD
Dept. of Neurology
Washington University in St. Louis
Disclosure Statement (2012)

Sources of Research Support

- National Institute on Aging
- Anonymous Foundation
- Charles F. and Joanne Knight Initiative
- Integrated Diagnostics

Consulting Relationships
None

Clinical Trials
None

Fees > $10,000
None

Stock Equity
None

Speaker’s Bureau
None

Editorial Boards
None

I own no stocks or equity in any biotech or pharmaceutical company
Acknowledgments

The Knight Alzheimer’s Disease Research Center at Washington University in St. Louis

- Randy Bateman (Wash U)
- Cliff Jack (Mayo Clinic)
- Bill Klunk (U Pitt)

*Our research participants*
Outline

- “Re”-Defining AD
- Role of biomarkers
- CSF biomarkers in AD
- Potential use of CSF markers in clinical trials
- Current and future challenges
Alzheimer’s Disease (AD) is a progressive neurodegenerative disorder that culminates in end-organ (brain) failure which manifests as dementia. 

...thus, AD refers to the neurodegenerative brain disorder regardless of clinical status.

AD can be conceptualized as having two major stages:

1) Preclinical (pre-symptomatic)
2) Symptomatic
   - Prodromal (incipient/MCI)
   - Dementia of the Alzheimer type
Prevalence of plaques compared to DAT suggests a “preclinical” stage of AD dementia.

DAT = dementia of the Alzheimer type

Sperling et al., 2011, Alzheimers Dement 7:280-92 (figure courtesy of Mark Mintun and John Morris)
### Clinicopathologic features of normal aging, “preclinical AD” and early stage DAT

<table>
<thead>
<tr>
<th></th>
<th>Normal Aging</th>
<th>Preclinical AD</th>
<th>Very Mild AD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plaques in neocortex</strong></td>
<td>None or a few diffuse plaques</td>
<td>Many plaques (diffuse &gt; neuritic)</td>
<td>Many plaques (diffuse &gt; neuritic)</td>
</tr>
<tr>
<td><strong>Tangles in entorhinal cortex &amp; hippocampus/CA1</strong></td>
<td>Few to many (increases w/age)</td>
<td>Many</td>
<td>Many</td>
</tr>
<tr>
<td><strong>Cell loss in entorhinal cortex &amp; hippocampus/CA1</strong></td>
<td>None</td>
<td>Little to none</td>
<td>Substantial (30%-60%)</td>
</tr>
<tr>
<td><strong>Clinical diagnosis</strong></td>
<td>Normal, CDR 0</td>
<td>Normal, CDR 0</td>
<td>Very mild dementia or MCI, CDR 0.5</td>
</tr>
<tr>
<td><strong>Pathological diagnosis</strong></td>
<td>Normal</td>
<td>AD</td>
<td>AD</td>
</tr>
</tbody>
</table>

**References:**

### Clinicopathologic features of normal aging, “preclinical AD” and early stage DAT

<table>
<thead>
<tr>
<th></th>
<th>Normal Aging</th>
<th>Preclinical AD</th>
<th>Very Mild AD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plaques</strong></td>
<td>None or a few diffuse plaques</td>
<td>Many plaques (diffuse &gt; neuritic)</td>
<td>Many plaques (diffuse &gt; neuritic)</td>
</tr>
<tr>
<td>in neocortex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tangles</strong></td>
<td>Few to many (increases w/age)</td>
<td>Many</td>
<td>Many</td>
</tr>
<tr>
<td>in entorhinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex &amp;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hippocampus/CA1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cell loss</strong></td>
<td>None</td>
<td>Little to none</td>
<td>Substantial (30%-60%)</td>
</tr>
<tr>
<td>in entorhinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cortex &amp;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hippocampus/CA1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td>Normal, CDR 0</td>
<td>Normal, CDR 0</td>
<td>Very mild dementia or MCI, CDR 0.5</td>
</tr>
<tr>
<td>diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pathological</strong></td>
<td>Normal</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Why is preclinical diagnosis and treatment important?

The overarching therapeutic objective of “preclinical” treatment is to treat early pathologic processes (e.g., lower amyloid-β burden) in order to prevent subsequent neurodegeneration and eventual cognitive decline and dementia.
Late treatment...uncertain effect on dementia

Cognitive Function

NORMAL
Mildly Impaired
Demented

LATE TREATMENT
Normal aging
“Uncertain Outcome”
LATE Treatment
AD

Demented

Late treatment...uncertain effect on dementia
Early treatment...halt or delay established cognitive decline

Cognitive Function

Cognitively Normal
Mildly Impaired
Demented

TIME

EARLY TREATMENT

Normal aging

“Halt Progression”

“Delay Progression”

AD

Mildly impaired
Preclinical treatment...prevent cognitive decline

A PRECLINICAL TREATMENT

Normal aging “Prevention”

Cognitively normal
Potential Roles of CSF Biomarkers in the Clinical Setting*

**Diagnostic**: increase diagnostic certainty
Q? Who has dementia due to AD pathology?

**Prognostic**: predict cognitive decline
Q? Who, when, how fast?

**Theragnostic**: detect biochemical effects of treatment
Q? Target engagement?

*Research setting: understanding disease pathophysiology*
Established CSF biomarkers of AD

Aβ42  

Published Aβ42: sensitivity, 70-100%  
specificity, 40-90%

Published Tau: sensitivity, 40-85%  
specificity, 65-85%

Sunderland et al., 2003, JAMA 289:2094-103
Established CSF biomarkers of AD

Aβ42 plaques tangles

Published Aβ42:
sensitivity, 70-100%
specificity, 40-90%

Published Tau:
sensitivity, 40-85%
specificity, 65-85%

Sunderland et al., 2003, JAMA 289:2094-103
Do CSF biomarkers reflect underlying AD pathology?
Amyloid PET imaging agents as candidate biomarkers of AD

<table>
<thead>
<tr>
<th>Compound name</th>
<th>$K_i$ (nM, n=3)</th>
<th>Compound name</th>
<th>$K_i$ (nM, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAY 94-9172 (AV-1)</td>
<td>2.22 ± 0.54</td>
<td>AV-45</td>
<td>2.87 ± 0.17</td>
</tr>
<tr>
<td>AV-136</td>
<td>6.37 ± 3.75</td>
<td>AV-137</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>PIB</td>
<td>0.87 ± 0.18</td>
<td>BTA-1</td>
<td>1.28 ± 0.46</td>
</tr>
<tr>
<td>GE-067 (3'-F-PIB)</td>
<td>0.74 ± 0.38</td>
<td>AZD2184</td>
<td>1.70 ± 0.54</td>
</tr>
<tr>
<td>BF-170</td>
<td>428 ± 57</td>
<td>IMPY</td>
<td>1.29 ± 0.46</td>
</tr>
<tr>
<td>Thioflavin T</td>
<td>&gt;1,000</td>
<td>FDDNP</td>
<td>172 ± 18</td>
</tr>
<tr>
<td>IMSB</td>
<td>&gt;1,000</td>
<td>K-114</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>SB-13</td>
<td>3.18 ± 1.04</td>
<td>CG</td>
<td>&gt;1,000</td>
</tr>
</tbody>
</table>

Choi et al., 2009, J Nucl Med 50:1887-94

Klunk et al., 2004, Ann Neurol 55:306-19

Wong et al., 2010, J Nucl Med 51:913-20

$[^{11}C]$-PIB

$[^{18}F]$-AV-45
N=24

Slide animation courtesy of Bill Klunk

Fagan et al., 2006, Ann Neurol 59:512-19
Low CSF $\text{A}\beta_{42}$ is a marker of cortical amyloid as detected by PET PIB, even in the absence of cognitive symptoms (CDR 0)

Mixed Cognitive States

Cognitively Normal (CDR 0)

O CDR 0 (cognitively normal)

Fagan et al., 2006, Ann Neurol 59:512-19

Forsberg et al., 2008, Neurobiol Aging, 29:1456-65

Grimmer et al., 2009, Biol Psychiatry, 65:927-34

Jagust et al., 2009, Neurology 73:1193-99

Tolboom et al., 2009, J Nucl Med, 50:1464-70

Forsberg et al., 2010, Curr Alz Res, 7:56-66

N=189 CDR 0
Mean age 64 years

Fagan et al., 2009, EMBO Mol Med 1:317-80
Plasma levels of Aβ$_{40}$ and Aβ$_{42}$ are not related to cortical amyloid load as detected by PET PIB. 

Fagan et al., 2009, EMBO Mol Med 1:317-80

N=189 CDR 0
Mean age 64 years
Medial temporal lobe atrophy in AD


Vemuri et al., 2009, Neurology 73:287-93

*Atrophy measure = STAND
Lower CSF $\text{A}\beta_{42}$ is associated with smaller brain volume in cognitively normal (CDR 0) individuals but not those with very mild/mild AD dementia (CDR 0.5/1).

N=69 CDR 0
N=29 CDR 0.5/1
Mean age 72 years

Fagan et al., 2009, Ann Neurol 65:176-83
Higher CSF tau is associated with smaller brain volume in individuals with very mild/mild AD dementia (CDR 0.5/1) but not in cognitively normal individuals (CDR 0).

Fagan et al., 2009, Ann Neurol 65:176-83
Low levels of CSF $A\beta_{42}$ are correlated with volumetric reductions (over 1 year) in multiple brain regions in cognitively normal older individuals

N=71 CDR 0
Mean age 76 years

Fjell et al., 2010, Cereb Cortex 20:2069-79
Do CSF biomarkers reflect underlying AD pathology?

YES, EVEN WHEN INDIVIDUALS ARE COGNITIVELY NORMAL
Do CSF biomarkers reflect underlying AD pathology?

Do CSF biomarkers predict future cognitive decline?
The CSF tau(s)/Aβ<sub>42</sub> ratio predicts progression (yes vs. no) from MCI to AD dementia over 5 years.

Hansson et al., 2006, Lancet Neurol 5:228-34

N=134 MCI
Median age at LP ~72 years
**CSF Aβ_{42} predicts rate of cognitive decline (CDR-SB*) in individuals with very mild dementia/MCI (CDR 0.5)**

Of 49 very mild DAT/MCI subjects, the 10 with the lowest CSF Aβ_{42} progressed, whereas the 10 with the highest Aβ_{42} generally did not.

*Higher CDR-SB = worse performance

Abnormal CSF $\text{A}\beta_{42}$ and tau(s) predict increased rate of cognitive decline (CDR-SB*) in individuals with very mild AD/MCI (CDR 0.5)


*Higher CDR-SB = worse performance
The CSF tau/Aβ\textsubscript{42} ratio predicts progression (yes vs. no) from cognitively normal to MCI or AD dementia

Cognitively normal (CDR 0) → MCI/AD (CDR>0)

Graph showing the proportion remaining CDR 0 over time since baseline clinical assessment, with CSF tau/Aβ\textsubscript{42} ratio as a predictor. Upper 15% vs. lower 85% of values (HR 9.82; 95% CI: 3.16-21.28, p<0.0001)

N=164 CDR 0
Mean age at LP = 75 years

Fagan et al., 2007, Arch Neurol, 64:343-49
Li et al., 2007, Neurology, 69:631-39
Craig-Schapiro et al., 2010, Biol Psychiatry, 68:903-12
Tarawneh et al., 2011, Ann Neurol, 70:274-85
The ratio of CSF VILIP-1/Ab_42 predicts progression (yes vs. no) from cognitively normal (CDR 0) to MCI/very mild or mild AD dementia (CDR>0)

- VILIP-1 is a calcium-signaling protein produced by neurons. Levels in the CSF are elevated in response to acute stroke in animal models and in AD. Thus, VILIP-1 may be a marker of neurodegeneration.

Cognitively normal → MCI/AD

<table>
<thead>
<tr>
<th>Proportion Remaining CDR 0</th>
<th>Time Since Baseline Clinical Assessment, y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

Upper 15% vs. lower 85% of values
(HR 13.00; 95% CI: 4.38-30.90, p<0.0001)

N=164 CDR 0
Mean age at LP = 72 years

Lee et al., 2008, Clin Chem 54:1617-23

Tarawneh et al., 2011, Ann Neurol, 70:274-85
The ratio of CSF YKL-40/\(\text{A}\beta_42\) predicts progression (yes vs. no) from cognitively normal (CDR 0) to MCI/very mild or mild AD dementia (CDR\(>0\))

- In the brain, YKL-40 (aka, chitinase 3 like 1) is an astrocyte-derived glycoprotein that may play a role in neuroinflammation and/or remodeling. Mean levels in the CSF are increased in early stage AD.

\[ \text{YKL-40}/\text{A}\beta_42, \ p=0.0001 \]

(A) Cognitively normal → MCI/AD

(B) Upper tertile vs lower two tertiles

\( \text{HR} \ 3.35; \ 95\% \ CI: 1.42-7.90, \ p=0.0057 \)

N=174 CDR 0
Mean age at LP = 71 years

Craig-Schapiro et al., 2010, Biol Psychiatry, 68:903-12
Low levels of CSF Aβ42 are associated with more subtle cognitive decline in cognitively normal older individuals

- Baseline CSF Aβ42 levels correlate with reductions in MMSE over 8 year follow-up in cognitively normal elderly women (n=55)
  *Gustafson et al., 2007, J Neurol Neurosurg Psychiatry 78:461-64*

- Low levels of CSF Aβ42 at baseline are associated with future (3 yr) development of subjective memory impairment affecting quality of life (memQoL) in cognitively normal elders (n=57)
  *Stomrud et al., 2007, Dement Geriatr Cogn Disord 24:118-24*

- Low levels of CSF Aβ42 at follow-up (3-4 yrs) are associated with worse performance on ADAS-cog delayed recall and slower cognitive speed in cognitively normal elders (n=37)
  *Stomrud et al., 2010, Arch Neurol 67:217-23*

- BUT...Baseline CSF Aβ42 levels are not associated with rate of cognitive decline (CDR-SB) over 2 years in cognitively normal individuals (ADNI) (n=109)
  *Vemuri et al., 2009, Neurology 73:294-301*
Do CSF biomarkers reflect underlying AD pathology?

YES

Do CSF biomarkers predict future cognitive decline?
**Do CSF biomarkers reflect underlying AD pathology?**

YES

**Do CSF biomarkers predict future cognitive decline?**

YES...EVEN WHEN INDIVIDUALS ARE COGNITIVELY NORMAL
In sum, converging evidence demonstrates...

- There exists a “preclinical” stage of AD that likely spans ~10-15 years prior to dementia onset.
- The AD biomarker “signature” in CSF includes reductions in the level of Aβ$_{42}$ and increases in total tau and phosphorylated tau (p-tau).
- Changes in CSF measures are reflective of underlying disease pathologies (e.g., amyloid plaque load [amyloid imaging], neurodegeneration [MRI]).
- Certain biomarker changes can be detected in the preclinical (pre-symptomatic) stage (e.g., reduced Aβ$_{42}$, increased tau/Aβ$_{42}$).
- Presence of these pathologies and their biomarkers in the preclinical stage are not clinically benign, i.e., they are predictive of future cognitive decline.
Hypothetical model of dynamic biomarkers of AD with emphasis on the preclinical period

(Sperling et al., 2011, Alzheimers Dement 7:280-92)
(Modified from Jack et al., 2009, Brain 132:1355-65)
What are the “real world” clinical implications?

1) Proposed revisions of NINCDS/ADRDA criteria for AD diagnosis
   a) expand the scope of AD (pre-symptomatic → MCI (AD) → AD dementia)
   b) include biomarkers
   c) distinguish between research criteria and clinical criteria
      Preclinical: *Sperling et al., 2011, Alzheimers Dement 7:280-92*
      MCI: *Albert et al., 2011, Alzheimers Dement 7:270-79*
      AD: *McKhann et al., 2011, Alzheimers Dement 7:263-69*

2) Paradigm shift from “cure” to “prevention”:
   *Intervene in **cognitively normal** individuals with preclinical AD to **prevent** neurodegeneration and symptomatic AD*

3) Potential use of biomarkers in design/evaluation of clinical trials
   a) target engagement
   b) inclusion criteria (confirm and enrich for presence of therapeutic target)
   c) surrogate outcome measures
      - amyloid = CSF Aβ_{42}, amyloid imaging
      - neurodegeneration = CSF tau(s), CSF VILIP-1, brain atrophy (MRI)
Current approach to Alzheimer’s disease (AD) clinical trials (a) and an alternative approach based on segmenting the population using biomarkers (b).

Adapted from Cummings, 2011, Curr Psychiatry Rep 13:437-42
# Potential use(s) of biomarkers in AD clinical trials

<table>
<thead>
<tr>
<th>BM TYPE</th>
<th>GOAL</th>
<th>PRACTICALITY</th>
<th>EXAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theragnostic</td>
<td>Prove target engagement</td>
<td>Drug choice</td>
<td>Secretase inhibitor: CSF $\alpha\beta$ synthesis/clearance (SILK)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Secretase modulator: CSF $\alpha\beta$ isoform synthesis (SILK)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anti-amyloid (e.g., antibody): CSF $\alpha\beta(42)$, amyloid imaging</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tau kinase inhibitor: CSF ptau</td>
</tr>
<tr>
<td>Diagnostic</td>
<td>Ensure AD pathology in subjects</td>
<td>Reduce subject</td>
<td>Amyloid: CSF $\alpha\beta_{42}$, amyloid imaging</td>
</tr>
<tr>
<td></td>
<td></td>
<td>number and</td>
<td>Neurofibrillary tangles/neurodegeneration: CVILIP-1, MRI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>heterogeneity</td>
<td></td>
</tr>
<tr>
<td>Prognostic</td>
<td>Define disease stage</td>
<td>Reduce trial</td>
<td>Proximity to clinical progression: CSF tau/$\alpha\beta_{42}$, CSF VILIP-1/$\alpha\beta_{42}$, combination of CSF, amyloid, MRI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>duration</td>
<td></td>
</tr>
<tr>
<td>Surrogate</td>
<td>Prove effect on downstream targets</td>
<td>Potentially</td>
<td>Neurodegeneration: CSF tau, CSF VILIP-1, MRI (e.g., in response to</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td>reduce trial</td>
<td>anti-amyloid therapies)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>duration</td>
<td></td>
</tr>
</tbody>
</table>
Stable-Isotope-Labeling Kinetics (SILK) method to assess the effect of a \( \gamma \)-secretase inhibitor on central A\( \beta \) production in humans

1. Infusion of \(^{13}\)C\(_6\)-leucine

2. In vivo labeling of new CNS proteins

3. ± drug

4. \( \gamma \)-secretase inhibitor (GSI)

5. CSF collection and quantification of labeled A\( \beta \) species over time

Adapted from Bateman et al., 2009, Ann Neurol 66:48-54
Production inhibitor model

Production Theoretical curve of Simulation Curve

Newly generated Aβ

Hour of Study

Placebo
25% inhibitor
50% inhibitor

Slide courtesy of Randy Bateman
The γ-secretase inhibitor LY 450139 enters then exits the brain rapidly (within 12 hrs) and reduces production (but not clearance) of CNS Aβ

Blue=placebo (100%)
Orange=100 mg (47%)
Green=140 mg (52%)
Red=280 mg (84%)

Bateman et al., 2009, Ann Neurol 66:48-54
Proposed stages of AD with potential prevention and treatment targets

Adapted from Sperling et al., 2011, Sci Transl Med 3:111cm33-111cm33

Clinical disease stage

Abnormal

1°
Primary Prevention
Delay onset of AD pathology
• Decrease Aβ_{42} production
• Prevent tangle formation

2°
Secondary prevention
Delay onset of cognitive impairment in individuals with evidence of pathology
• Decrease accumulated Aβ burden
• Decrease neurodegeneration with anti-tau or neuroprotective agents

3°
Tertiary prevention and treatment
Delay onset or progression of dementia
• Neuroprotection-prevent neuronal loss
• Enhance function of remaining neurons
• Neurotransmitter repletion

No pathology
Preclinical
MCI
Dementia
**Current challenges and potential future directions**

- Establish the **reliability and validity** of biomarkers (individual and panels) for diagnostic (sensitivity/specificity) and prognostic purposes
- Define biomarker **cut-off values** (standardization efforts)
- Better define the **sequence** of biomarker changes and the extent to which they predict subsequent cognitive decline
- Need for **novel** biomarker development (esp. Aβ oligomers, imaging for intra-neuronal pathologies, e.g., tangles, α-synuclein inclusions)
- Need more sensitive biomarkers that can detect early **synaptic dysfunction** and neural network disruption (e.g., BOLD imaging)
- Begin/continue large longitudinal studies with existing markers ASAP, including planning for **prevention trials** in preclinical populations, e.g., Dominantly Inherited Alzheimer Network [DIAN], Alzheimer Prevention Initiative [API], Anti-Amyloid Treatment in Asymptomatic AD [A4])
Quality control and standardization efforts for AD fluid biomarkers

<table>
<thead>
<tr>
<th>PRE-ANALYTIC FACTORS</th>
<th>ANALYTIC PERFORMANCE</th>
<th>CLINICAL DIAGNOSTIC PERFORMANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Define and control</td>
<td>Assure stability and reproducibility</td>
<td>Analyte validation between studies</td>
</tr>
<tr>
<td>Time of sample collection</td>
<td>Assay platform</td>
<td>Sensitivity/specificity (autopsy confirmed)</td>
</tr>
<tr>
<td>Fasting state (Y/N)</td>
<td>Assay QC</td>
<td>Define cut-off values</td>
</tr>
<tr>
<td>LP needle type (atraumatic Sprotte vs cutting)</td>
<td>Reagent lot-to-lot variability</td>
<td>Longitudinal change in biomarkers within individuals over time</td>
</tr>
<tr>
<td>Plastic tubes (propylene vs polystyrene)</td>
<td>Laboratory expertise</td>
<td></td>
</tr>
<tr>
<td># freeze/thaw cycles</td>
<td>Within-run/between-run reliability</td>
<td></td>
</tr>
<tr>
<td>Interval between sample collection/freezing</td>
<td>Within-lab/between lab reliability</td>
<td></td>
</tr>
<tr>
<td>Centrifugation (Y/N)</td>
<td>Equipment QC</td>
<td></td>
</tr>
</tbody>
</table>
INNOTEST CSF values are ~2-5-fold higher than INNO-BIA (xMAP) values

Fagan et al., 2011, Arch Neurol 68:1137-44
The CSF tau(s)/Aβ$_{42}$ ratios are better indicators of PIB-positivity than the individual markers alone.
Recent data from the QC Program for AD CSF Biomarkers

INNOTEST ELISA

**Aβ42**

CV = 24%

Mean: 275 pg/mL
SD: 67 pg/mL
CV: 24%

**Tau**

CV = 20%

Mean: 666 pg/mL
SD: 132 pg/mL
CV: 20%

**P-tau181**

CV = 12%

Mean: 111 pg/mL
SD: 13 pg/mL
CV: 12%

AlzBio3 (xMAP)

**Aβ42**

CV = 24%

Mean: 326 pg/mL
SD: 74 pg/mL
CV: 24%

**Tau**

CV = 22%

Mean: 211 pg/mL
SD: 46 pg/mL
CV: 22%

**P-tau181**

CV = 28%

Mean: 65 pg/mL
SD: 18 pg/mL
CV: 28%

Mattsson et al., unpublished observations