Alzheimer's Disease CSF Biomarkers

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Disclosures

- Dr. Bornhorst
  - None

- Dr. Algeciras
  - Advisory Boards
    - Roche Diagnostics
    - Fujirebio Diagnostics

Alzheimer’s Disease (AD) CSF Biomarkers

Brain

- Normal
- Alzheimer

CSF

- ↓ ~50% Aβ42
  - Brain amyloid deposit
- ↑ ~200% Total-Tau
- ↑ ~200% Phospho-Tau
  - Intensity of neurodegeneration
  - Tau pathology (?)

Figure 3: A representation of the basic biology of Alzheimer’s Disease, consisting of intracellular neurofibrillary tangles composed of hyperphosphorylated tau and extracellular amyloid plaques (depicted as Aβ plaques). Neurofibrillary tangles= Phospho-Tau

Amyloid plaques= Aβ42

Biological Definition of Alzheimer’s Disease

- **A/T/N system**
  - **A = Aβ pathology**
    - Measured by either amyloid PET or CSF Aβ42 or Aβ42/Aβ40
  - **T = tangle pathology**
    - Assessed by either tau PET or CSF P-tau
  - **N = neurodegeneration or neuronal injury**
    - Detected by either 18F-FDG-PET, structural MRI, or CSF T-tau

- Non-Alzheimer’s pathologic change
- Alzheimer’s pathologic change
- Alzheimer’s disease
- Alzheimer’s and suspected non-Alzheimer’s pathologic change
- Non-Alzheimer’s pathologic change

Mayo AD CSF Biomarkers Assay

- **Names:**
  - Alzheimer’s Disease Evaluation, CSF
  - Biogen Alzheimer’s Disease Eval, CSF
- **Tests:** Amyloid β-42 (Aβ42), total-Tau, and phosphorylated-Tau181
- **Manufacturer:** Roche Diagnostics
- **Sample collection:** CSF; low bind polypropylene tube
- **Report:** Individual markers plus pTau181/Aβ42 ratio
Mayo AD CSF Biomarkers Assay: Clinical Performance

- **Study population:**
  - Cognitively normal (N=416), individuals with mild cognitive impairment (n=69) and individuals with AD dementia (n=39)

- **Comparison:**
  - $^{11}$C Pittsburgh Compound B (PiB) PET imaging (amyloid PET).

### Agreement with β-amyloid PET

<table>
<thead>
<tr>
<th></th>
<th>Aβ42</th>
<th>t-Tau</th>
<th>p-Tau181</th>
<th>p-tau181/Aβ42 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC ROC</strong></td>
<td>0.89</td>
<td>0.78</td>
<td>0.89</td>
<td>0.97</td>
</tr>
</tbody>
</table>

### Agreement of pTau/Aβ42 ratio with β-amyloid PET

<table>
<thead>
<tr>
<th></th>
<th>Mayo</th>
<th>ADNI</th>
<th>BIOFINDER</th>
<th>Wash U</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>416</td>
<td>918</td>
<td>728</td>
<td>198</td>
</tr>
<tr>
<td><strong>Cut-point</strong></td>
<td>0.023</td>
<td>0.028</td>
<td>0.022</td>
<td>0.020</td>
</tr>
<tr>
<td><strong>Overall %</strong></td>
<td>92%</td>
<td>90%</td>
<td>90%</td>
<td>89%</td>
</tr>
<tr>
<td><strong>Positive %</strong></td>
<td>92%</td>
<td>88%</td>
<td>91%</td>
<td>92%</td>
</tr>
<tr>
<td><strong>Negative %</strong></td>
<td>92%</td>
<td>93%</td>
<td>89%</td>
<td>89%</td>
</tr>
</tbody>
</table>

*Roche Elecsys assays
Mayo AD assay report: NORMAL AD profile

Alzheimer's Disease Evaluation, CSF

p-Tau/Abeta42

0.012 ratio

AD Interpretation

The normal p-Tau/Abeta42 ratio is not consistent with the presence of pathological changes associated with Alzheimer's disease.

The p-Tau/Abeta42 ratio provides better concordance with amyloid Positron Emission Tomography (PET) imaging when compared to Abeta42, phospho-Tau and total-Tau individually. A cut-off of 0.023 provides optimal balance between NPA (negative % agreement) and PPA (positive % agreement) when compared to normal amyloid PET. A ratio of >0.023 has a 92% NPA with normal amyloid PET. A ratio of ≤0.023 has a 92% PPA with abnormal amyloid PET.

Failure to adhere to the sample collection instructions provided in the Lab Test Catalog may result in falsely reduced Abeta42 concentrations; potentially affecting subsequent interpretations as well as the p-Tau/Abeta42 ratio.

The pTau assay measures p-Tau181 (Tau phosphorylated at threonine 181), which has been shown to be a marker of AD pathology.

Abeta42

1098 pg/mL

Reference Value ≤1026

Total-Tau

151 pg/mL

Reference Value ≤238

Phospho-Tau(181P)

13.0 pg/mL

Reference Value ≤21.7

ADDITIONAL INFORMATION

The testing method is an electrochemiluminescence assay manufactured by Roche Diagnostics Inc. and performed on the Cobas system.

Values obtained with different assay methods or kits may be different and cannot be used interchangeably.

Received: 18 Nov 2021 10:57
Reported: 18 Nov 2021 10:57

Mayo AD assay report: ABNORMAL AD profile

Alzheimer's Disease Evaluation, CSF

p-Tau/Abeta42

0.161 ratio

AD Interpretation

The elevated p-Tau/Abeta42 ratio is consistent with the presence of pathological changes associated with Alzheimer's disease.

The p-Tau/Abeta42 ratio provides better concordance with amyloid Positron Emission Tomography (PET) imaging when compared to Abeta42, phospho-Tau and total-Tau individually. A cut-off of 0.023 provides optimal balance between NPA (negative % agreement) and PPA (positive % agreement) when compared to amyloid PET results. A p-Tau/Abeta42 ratio of >0.023 has a 92% NPA with normal amyloid PET. A ratio of ≥0.023 has a 92% PPA with abnormal amyloid PET.

Failure to adhere to the sample collection instructions provided in the Lab Test Catalog may result in falsely reduced Abeta42 concentrations; potentially affecting subsequent interpretations as well as the p-Tau/Abeta42 ratio.

The pTau assay measures p-Tau181 (Tau phosphorylated at threonine 181), which has been shown to be a marker of AD pathology.

Abeta42

519 pg/mL

Reference Value >1026

Total-Tau

682 pg/mL

Reference Value >238

Phospho-Tau(181P)

83.3 pg/mL

Reference Value >21.7

ADDITIONAL INFORMATION

The testing method is an electrochemiluminescence assay manufactured by Roche Diagnostics Inc. and performed on the Cobas system.

Values obtained with different assay methods or kits may be different and cannot be used interchangeably.

Received: 18 Nov 2021 10:57
Reported: 18 Nov 2021 10:58
Mayo AD assay report: UNABLE to Calculate Ratio

### Alzheimer's Disease Evaluation, CSF

<table>
<thead>
<tr>
<th>p-Tau/Abeta42</th>
<th>SDL</th>
<th>Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>≤0.023</td>
<td></td>
</tr>
</tbody>
</table>

*Unable to calculate the p-Tau/Abeta42 ratio because the measured Abeta42 concentration is above the assay measuring limit of 1700 pg/mL. The normal CSF concentration of Abeta42 present in this individual is not consistent with the presence of pathological changes associated with Alzheimer's disease. If there is a clinical suspicion of Alzheimer's disease consider repeating testing in 6-9 months.*

The p-Tau assay measures p-Tau181 (Tau phosphorylated at threonine 181), which has been shown to be a marker of AD pathology.

<table>
<thead>
<tr>
<th>Abeta42</th>
<th>SDL</th>
<th>Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1700 pg/mL</td>
<td>&gt;1026</td>
<td></td>
</tr>
</tbody>
</table>

Total-Tau: 213 pg/mL (Reference Value: ≤238)
Phospho-Tau(181P): 19.5 pg/mL (Reference Value: ≤21.7)

Mayo AD assay Sample report: Normal Ratio but abnormal Abeta42

### Alzheimer's Disease Evaluation, CSF

<table>
<thead>
<tr>
<th>p-Tau/Abeta42</th>
<th>SDL</th>
<th>Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>≤0.023</td>
<td></td>
</tr>
</tbody>
</table>

*The normal p-Tau/Abeta42 ratio is not consistent with the presence of pathological changes associated with Alzheimer's disease.*

However, the observed abnormally low Abeta42 concentration and lack of elevated p-Tau could indicate the early stages of an Alzheimer's disease process. Alternatively, concurrent decreased Abeta42 and the absence of elevated p-Tau concentration may also be associated with disorders of CSF dynamics, such as in normal pressure hydrocephalus (NPH), which is known to decrease protein levels in CSF. These results should be interpreted in combination with other clinical findings.

<table>
<thead>
<tr>
<th>Abeta42</th>
<th>SDL</th>
<th>Reference Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>526 pg/mL</td>
<td>&gt;1026</td>
<td></td>
</tr>
</tbody>
</table>

Total-Tau: 121 pg/mL (Reference Value: ≤238)
Phospho-Tau(181P): 11.5 pg/mL (Reference Value: ≤21.7)

Additional Information:
The testing method is an electrochemiluminescence assay manufactured by Roche Diagnostics Inc. and performed on the Cobas system.

Values obtained with different assay methods or kits may be different and cannot be used interchangeably.
Mayo AD assay Sample report: Normal Ratio but abnormal Abeta42

Alzheimer’s Disease Evaluation, CSF

What is the recommended next step if a patient’s p-Tau/Abeta 42 ratio is close to the cut-off of ≤0.023? What is recommended if there is high suspicion of AD but initial test results are normal?

✓ Consider other non-AD causes of the cognitive decline, rule out CSF dynamics issues, and repeat testing in about 6-9 months.

AD CSF biomarkers: Pre-analytical variables

AD testing components are relatively stable once in an appropriate collection sample container.

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>Refrigerated (preferred)</td>
<td>14 days</td>
</tr>
<tr>
<td></td>
<td>Frozen</td>
<td>30 days</td>
</tr>
<tr>
<td></td>
<td>Ambient</td>
<td>12 hours</td>
</tr>
</tbody>
</table>

However, multiple steps in the pre-analytical process may potentially differentially affect measured Abeta and Tau protein concentrations

Including:
Tube Material
Aliquot/Fill Volume
Absorption of Aβ CSF biomarker analysis: Tube Type effect

- Mostly relevant for Aβ than the tau proteins
  - Aβ peptides are hydrophobic (potentially sticky) peptides

- Potential for absorption to tube surface
  - This leads to falsely low Aβ42.
    - Consequently a falsely high pTau181/Aβ42 ratio
    - Potential increased clinical suspicion of AD

AD CSF biomarker analysis: Sample Tube effect

Aβ peptides showed major variations in concentration depending on tube type with best recovery in PP tubes.

For Aβ-42 peptide
Polypropylene (PP) has better recovery compared to Polystyrene (PS) tubes

PP tubes
Recovery of Aβ peptides

PS tubes
Recovery of Aβ peptides (72%)

P-Tau recovery was approximately 100% in both tube types
AD CSF biomarker analysis: Low bind tubes

Better recovery has been reported in low-bind polypropylene (PP) tubes versus polypropylene (PP) tubes

Recommended Sample Tubes
Preferred: Sarstedt Polypropylene Low-Bind tube (2.5mL), available upon request

Other acceptable CSF Polypropylene Tubes:
Sarstedt 72.703.600 (1.5 mL) or Sarstedt 72.694.600 (2 mL)

Note: Polystyrene collection tubes are not acceptable.
Exposure of CSF to polystyrene tubes may result in falsely low Abeta42 concentrations

Polypropylene Tube: Fill Volume

- The lower the relative fill volume the lower the recovery due to relatively larger surface area
- A 75% reduction in volume can result in 20-40% reduction in measured concentration

Example Short Sample Added Mayo Footnote:
The sample volume (1.0 mL) received in a 2.5 mL collection tube was less than the 50% required fill volume. A 2.5 mL polypropylene low binding tube (AD biomarker tube) is recommended for CSF collection. Failure to adhere to the sample collection instructions may result in falsely low Abeta42 concentration due to adhesion to the specimen tube.
Collection Recommendations\textsuperscript{6,8}

Alzheimer’s Association consensus protocol recommendation:
1. Perform lumbar puncture and discard the first 1 to 2 mL of CSF
2. Collect CSF directly into a low bind collection tube

Note: Use the drip method for CSF collection directly into a low-bind polypropylene (PP) tube. While the syringe pull method increases collection speed, the drip method reduces the risk of Abeta42 binding to the plastic of the syringe used

Mayo Recommendation:
Fill sample tube until at least 80% full

Sample report

![Alzheimer's Disease Evaluation, CSF](image_url)
Additional Info

• MCL Lab Test Catalog
  - https://www.mayocliniclabs.com/test-catalog/Overview/607273
• MCL Alzheimer’s disease landing page
  - https://news.mayocliniclabs.com/neurology/alzheimers-disease/

THANK YOU
References


