**Supplementary Material**

**Supplementary Figure 1.** Factors that influence the variability

**Supplementary Figure 2**

**Supplementary Table 1.**Diagnostic performance of CSF biomarkers when a shift in concentration was inducedto Aβ1-42 values in autopsy-confirmed AD patients and controls .

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Sensitivity (%)** | **Specificity (%)** | **Diagnostic accuracy (%)** |
| True data | 77.8 | 92.0 | 86.1 |
| +5% Aβ1-42; T-tau; P-tau181P | 77.8 | 92.0 | 86.1 |
| -5% Aβ1-42; T-tau; P-tau181P | 77.8 | 90.0 | 84.9 |
| +10% Aβ1-42; T-tau; P-tau181P | 76.4 | 92.0 | 85.5 |
| -10% Aβ1-42; T-tau; P-tau181P | 77.8 | 90.0 | 84.9 |
| +20% Aβ1-42; T-tau; P-tau181P | 75.0 | 93.0 | 85.5 |
| -20% Aβ1-42; T-tau; P-tau181P | 80.6 | 87.0 | 84.3 |
| +30% Aβ1-42; T-tau; P-tau181P | 75.0 | 93.0 | 85.5 |
| -30% Aβ1-42; T-tau; P-tau181P | 80.6 | 81.0 | 80.8 |
| +40% Aβ1-42; T-tau; P-tau181P | 63.9 | 95.0 | 82.0 |
| -40% Aβ1-42; T-tau; P-tau181P | 80.6 | 79.0 | 79.7 |

Aβ1-42, amyloid-β of 42 amino acids; T-tau, total tau protein; P-tau181P, tau phosphorylated at threonine 181

**Supplementary Table 2.**Diagnostic performance of CSF biomarkers when a shift in concentration was induced to T-tau values in autopsy-confirmed AD patients and controls.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Sensitivity (%)** | **Specificity (%)** | **Diagnostic accuracy (%)** |
| True data | 77.8 | 92.0 | 86.1 |
| Aβ1-42; -5% T-tau; P-tau181P | 77.8 | 93.0 | 86.6 |
| Aβ1-42; +5% T-tau; P-tau181P | 81.9 | 92.0 | 87.8 |
| Aβ1-42; -10% T-tau; P-tau181P | 76.4 | 93.0 | 86.1 |
| Aβ1-42; +10% T-tau; P-tau181P | 83.3 | 91.0 | 87.8 |
| Aβ1-42; -20% T-tau; P-tau181P | 72.2 | 93.0 | 84.3 |
| Aβ1-42; +20% T-tau; P-tau181P | 84.7 | 91.0 | 88.4 |
| Aβ1-42; -30% T-tau; P-tau181P | 72.2 | 93.0 | 84.3 |
| Aβ1-42; +30% T-tau; P-tau181P | 87.5 | 90.0 | 89.0 |
| Aβ1-42; -40% T-tau; P-tau181P | 69.4 | 93.0 | 83.1 |
| Aβ1-42; +40% T-tau; P-tau181P | 87.5 | 87.0 | 87.2 |

Aβ1-42, amyloid-β of 42 amino acids; T-tau, total tau protein; P-tau181P, tau phosphorylated at threonine 181

**Supplementary Table 3.**Diagnostic performance of CSF biomarkers when a shift in concentration was induced to P-tau181P values in autopsy-confirmed AD patients and controls.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Sensitivity (%)** | **Specificity (%)** | **Diagnostic accuracy (%)** |
| True data | 77.8 | 92.0 | 86.1 |
| Aβ1-42; T-tau; -5% P-tau181P | 77.8 | 92.0 | 86.1 |
| Aβ1-42; T-tau; +5% P-tau181P | 79.2 | 92.0 | 86.6 |
| Aβ1-42; T-tau; -10% P-tau181P | 77.8 | 92.0 | 86.1 |
| Aβ1-42; T-tau; +10% P-tau181P | 79.2 | 92.0 | 86.6 |
| Aβ1-42; T-tau; -20% P-tau181P | 76.4 | 92.0 | 85.5 |
| Aβ1-42; T-tau; +20% P-tau181P | 80.6 | 90.0 | 86.1 |
| Aβ1-42; T-tau; -30% P-tau181P | 76.4 | 92.0 | 85.5 |
| Aβ1-42; T-tau; +30% P-tau181P | 81.9 | 90.0 | 86.6 |
| Aβ1-42; T-tau; -40% P-tau181P | 76.4 | 92.0 | 85.5 |
| Aβ1-42; T-tau; +40% P-tau181P | 84.7 | 88.0 | 83.1 |

Aβ1-42, amyloid-β of 42 amino acids; T-tau, total tau protein; P-tau181P, tau phosphorylated at threonine 181

**Supplementary Table 4.**Diagnostic performance of CSF biomarkers for discriminating progressive from stable MCI patients when a shift in concentration was induced to Aβ1-42 values.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Sensitivity (%)** | **Specificity (%)** | **Diagnostic accuracy (%)** |
| True data | 68.1 | 79.0 | 72.9 |
| +5% Aβ1-42; T-tau; P-tau181P | 59.6 | 79.0 | 68.2 |
| -5% Aβ1-42; T-tau; P-tau181P | 68.1 | 73.7 | 70.6 |
| +10% Aβ1-42; T-tau; P-tau181P | 57.5 | 84.2 | 69.4 |
| -10% Aβ1-42; T-tau; P-tau181P | 72.3 | 73.7 | 72.9 |
| +20% Aβ1-42; T-tau; P-tau181P | 46.8 | 86.8 | 64.7 |
| -20% Aβ1-42; T-tau; P-tau181P | 76.6 | 68.4 | 72.9 |
| +30% Aβ1-42; T-tau; P-tau181P | 31.9 | 89.5 | 57.7 |
| -30% Aβ1-42; T-tau; P-tau181P | 76.6 | 65.8 | 71.8 |
| +40% Aβ1-42; T-tau; P-tau181P | 29.8 | 92.1 | 57.7 |
| -40% Aβ1-42; T-tau; P-tau181P | 76.6 | 63.2 | 70.6 |

Aβ1-42, amyloid-β of 42 amino acids; T-tau, total tau protein; P-tau181P, tau phosphorylated at threonine 181

**Supplementary Table 5.**Diagnostic performance of CSF biomarkers for discriminating progressive from stable MCI patients when a shift in concentration was induced to T-tau values.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Sensitivity (%)** | **Specificity (%)** | **Diagnostic accuracy (%)** |
| True data | 68.1 | 79.0 | 72.9 |
| Aβ1-42; -5% T-tau; P-tau181P | 68.1 | 81.6 | 74.1 |
| Aβ1-42; +5% T-tau; P-tau181P | 68.1 | 79.0 | 74.1 |
| Aβ1-42; -10% T-tau; P-tau181P | 68.1 | 81.6 | 74.1 |
| Aβ1-42; +10% T-tau; P-tau181P | 72.3 | 76.3 | 74.1 |
| Aβ1-42; -20% T-tau; P-tau181P | 68.1 | 81.6 | 74.1 |
| Aβ1-42; +20% T-tau; P-tau181P | 72.3 | 73.7 | 72.9 |
| Aβ1-42; -30% T-tau; P-tau181P | 68.1 | 84.2 | 75.3 |
| Aβ1-42; +30% T-tau; P-tau181P | 72.3 | 68.4 | 70.6 |
| Aβ1-42; -40% T-tau; P-tau181P | 66.0 | 84.2 | 74.1 |
| Aβ1-42; +40% T-tau; P-tau181P | 76.6 | 65.8 | 71.8 |

Aβ1-42, amyloid-β of 42 amino acids; T-tau, total tau protein; P-tau181P, tau phosphorylated at threonine 181

**Supplementary Table 6.**Diagnostic performance of CSF biomarkers for discriminating progressive from stable MCI patients when a shift in concentration was induced to P-tau181P values.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Sensitivity (%)** | **Specificity (%)** | **Diagnostic accuracy (%)** |
| True data | 68.1 | 79.0 | 72.9 |
| Aβ1-42; T-tau; -5% P-tau181P | 66.0 | 81.6 | 72.9 |
| Aβ1-42; T-tau; +5% P-tau181P | 72.3 | 76.3 | 74.1 |
| Aβ1-42; T-tau; -10% P-tau181P | 66.0 | 81.6 | 72.9 |
| Aβ1-42; T-tau; +10% P-tau181P | 72.3 | 76.3 | 74.1 |
| Aβ1-42; T-tau; -20% P-tau181P | 66.0 | 81.6 | 72.9 |
| Aβ1-42; T-tau; +20% P-tau181P | 76.6 | 73.7 | 75.3 |
| Aβ1-42; T-tau; -30% P-tau181P | 66.0 | 81.6 | 72.9 |
| Aβ1-42; T-tau; +30% P-tau181P | 76.6 | 65.8 | 71.8 |
| Aβ1-42; T-tau; -40% P-tau181P | 66.0 | 81.6 | 72.9 |
| Aβ1-42; T-tau; +40% P-tau181P | 78.7 | 60.5 | 70.6 |

Aβ1-42, amyloid-β of 42 amino acids; T-tau, total tau protein; P-tau181P, tau phosphorylated at threonine 181

**Supplementary Table 7.**Change in diagnostic classification of individual subjects based on combinations of CSF biomarkers (Aβ1-42, T-tau, and P-tau181P) using the IWG-2 criteria.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | -5% (n) | -10% (n) | -20% (n) | -30% (n) | -40% (n) | 5% (n) | 10% (n) | 20% (n) | 30% (n) | 40% (n) | Total changed profile (n) | Unchanged abnormal profile (n) | Unchanged normal profile (n) |
| **Aβ1-42** | AD dementia | - | - | 2\* | - | - | - | 1^ | 1^ | 3^ | 5^ | 12 | 47 | 13 |
|  | Controls | 2\* | - | 3\* | 6\* | 2\* | - | - | 1^ | - | 2^ | 16 | 5 | 79 |
|  | MCI progressive | - | 2\* | 2\* | - | - | 4^ | 1^ | 5^ | 7^ | 1^ | 22 | 14 | 11 |
|  | MCI stable | 2\* | - | 2\* | 1\* | 1\* | - | 2^ | 1^ | 1^ | 1^ | 11 | 3 | 24 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **T-tau** | AD dementia | - | - | 4^ | - | 2^ | 3\* | 1\* | 1\* | 2\* | - | 13 | 50 | 9 |
|  | Controls | 1^ | - | - | - | - | - | 1\* | - | 1\* | 3\* | 6 | 7 | 87 |
|  | MCI progressive | 1^ | - | - | - | - | 1\* | 1\* | - | - | 2\* | 5 | 31 | 11 |
|  | MCI stable | 1^ | - | - | 1^ | - | - | 1\* | 1\* | 2\* | 1\* | 7 | 6 | 25 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **P-tau181P** | AD dementia | - | - | 1^ | - | - | 1\* | - | 1\* | 1\* | 2\* | 6 | 55 | 11 |
|  | Controls | - | - | - | - | - | - | - | 2\* | - | 2\* | 4 | 8 | 88 |
|  | MCI progressive | 1^ | - | - | - | - | 2\* | - | 2\* | - | 1\* | 6 | 31 | 10 |
|  | MCI stable | 1^ | - | - | - | - | 1\* | 1\* | - | 3\* | 1\* | 7 | 7 | 24 |

AD, Alzheimer’s disease; MCI, mild cognitive impairment. Classification of CSF AD biomarker profiles was subdivided into three groups: subjects who remained having an abnormal biomarker profile (unchanged abnormal profile), subject who remained having a normal biomarker profile (unchanged normal profile), and subjects who changed of diagnostic classification (completely changed diagnostic classification). Change in biomarker classification if concentration shifts were induced (from ±5% to ±40%) per biomarker and per disease category for each individual: \*from normal to abnormal and ^from abnormal to normal