ADNI 2: Third batch analyses of CSF biomarkers

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Summary
A total of 428 (275 BASELINE plus 153 paired 2 or 2.5 yr longitudinal CSF aliquot samples) never before thawed aliquots of ADNI GO and 2 CSF samples collected between 1/19/2012 and 10/30/2013, (collection dates provided in UPENNBIOMK7), plus 17 never before thawed randomly selected replicate aliquots were tested. All paired longitudinal samples were included in the same testing run on the same 96 well analytical plate. One or two of these “re-test” aliquots were included in each run subsequent to the first run. Each calibration standard sample, quality control sample and ADNI study subject sample were run in duplicate according to the manufacturer’s instructions. Each test result is the mean value of the duplicates. The attached “ADNI GO and 2 CSF” report provides details for the analyses including calibrator and quality control samples performance, test/re-test performance and the raw data for these analyses. The accompanying ADNI 2 2014 CSF Aβ1-42, t-tau and p-tau181 dataset in .csv file format (UPENNBIOMK7) provides the final set of results following rescaling (Transformation) based on 2012/13 BASELINE ADNI results, according to the procedure described below, for the 2014 ADNI GO and II CSF sample analyses. In addition, the UPENNBIOMK7 data file includes the RAW untransformed data.

Method
The xMAP Luminex platform and Innogenetics/Fujirebio AlzBio3 immunoassay kits were used following the SOP in place at the UPenn/ADNI Biomarker Laboratory, according to the kit manufacturer’s instructions and as described in previous publications (1-4). Analyses were performed in a series of 14 runs using a 96 well plate format, over the time period of April 8 through May 14, 2014. Acceptance criteria as documented in the UPenn/ADNI Biomarker Laboratory SOP were followed for these analyses.

Each of the 14 analytical runs met acceptance criteria for calibrator precision and accuracy (back calculated concentration result vs nominal concentration result) and quality control results were within stated limits (detailed data in “ADNI GO and 2 CSF report”). Individual sample results were acceptable in all cases except for a single p-tau181 result, where noted, and that was reported as “NA” in the .CSV file “UPENNBIOMK7”.

In order to assure cross-sectional comparability of results between these ADNI GO + ADNI 2 subject CSF samples and the earlier 2012/13 BASELINE CSF biomarker results, assessment of the concentrations of Aβ1-42, t-tau and p-tau181 were performed in a set of 147 never before thawed ADNI GO and II patient BASELINE CSF aliquots (“sister” pristine aliquots had previously been run in the 2012 or the 2013 batch runs and new pristine aliquots were included in the series of 153 longitudinal sample sets from ADNI GO and II subjects run in the 2014 batch.
run). Linear regression analyses (Passing-Babook) were performed for Aβ_{1-42}, t-tau and p-tau_{181} comparing RAW CSF concentration results obtained in 2014 with those obtained in the analyses performed in 2012/13 (referred in Figure 2 as “2013T”) transformed to 2007 BASELINE as described in UPENNBIOMK5 (see Figure 2 in this Methods report). Correlation results were obtained (R^2 values of 0.914, 0.959 and 0.763 for Aβ_{1-42}, t-tau and p-tau_{181}, respectively; for Aβ_{1-42} the slope value is 1.859 and y-intercept value is -61.1 pg/mL, for t-tau the slope value is 0.782 and y-intercept is 8.5 pg/mL and for p-tau_{181} the slope is 0.50 and y-intercept is 2.64 as summarized in Figure 2). The slope and intercept values were then used to bridge between the 2012/13 data and the current 2014 CSF concentrations. This was accomplished by solving the equation, X = (Y-b)/m (X is the rescaled (Transformed) 2012 or 2013 result; Y is the raw 2014 result; m is the slope of the regression analysis and b is the Y intercept value of the regression analysis summarized in Figure 2). For studies that use 2014 ADNI GO + ADNI 2 CSF biomarker concentration results, we recommend the use of the transformed results. As noted in the Summary the raw data can be found in the UPENNBIOMK7 dataset csv file and is also presented in the analytical report entitled “ADNI GO and 2 CSF report”.

Figure 1. Linear regression (Passing-Babook) analysis plots for 2014 CSF Aβ_{1-42}, t-tau and p-tau_{181} data rescaled [Transformed, (T)] to 2012/2013 baseline data vs the 2012/13 results for those samples, rescaled [Transformed, (T)] to the 2007 BASELINE dataset. The plot on the right side of each linear regression plot is a Bland-Altman bias plot of the %difference between the 2014(T) result and the corresponding 2013 (T) result.


Aβ_{1-42}:
t-tau:

![Graph showing t-tau relationship]

- Intercept: -0.286 (±2.757 - 2.537)
- Slope: 1.003 (±0.963 - 1.044)
- $R^2 = 0.95$
- Mean Error: -0.50%
- Mean Absolute Error: 8.67%
- RMSE: 10.96%

p-tau181:

![Graph showing p-tau181 relationship]

- Intercept: -0.8432 (±1.327 - 1.436)
- Slope: 1.025 (±0.955 - 1.164)
- $R^2 = 0.763$
- Mean Error: 2.63%
- Mean Absolute Error: 15.95%
- RMSE: 23.91%
In Figure 1 Aβ1-42 2013 (T), t-tau 2013 (T) and p-tau181 2013 (T) data are ADNI 2012/2013 BASELINE Transformed CSF results on 147 subjects, using the Research Use Only Innogenetics AlzBio3 xMAP immunoassay. Abeta 2014 (T) are never before analyzed replicate CSF aliquots (continuously stored at -80°C) from the 147 subjects whose values were rescaled(Transformed, T) as discussed in the paragraph above. The analyses done in 2012/2013, done as one batch in 2012 or 2013 and transformed as described in the document “ADNI GO and ADNI 2 First batch analyses of CSF biomarkers: revised anchoring to 2007 ADNI 1 BASELINE dataset procedure”, included ADNI 1 BASELINE pristine CSF samples. The analyses done in 2012 and 2013 were done in each case as one batch (with different lots of reagents and calibrators, in each instance, than the lot# used in the 2007 analyses), using Fujirebio/Innogenetics AlzBio3 xMAP immunoassay reagents. Shaded areas are the 95% CI for the regression fit line. R² values are from least squares analyses of the data. Slope and intercept values determined using Passing-Bablok linear regression. The P-B linear regression parameters for each biomarker for the 2014 (T) vs 2013 (T) results are summarized in Table 1 below.

Table 1. P-B linear regression equation parameters defined by anchoring 2014 analyses of one hundred & forty-seven (147) ADNI GO/2 CSFs to 2012/13 BASELINE data (Fig 1). Ninety-five % confidence intervals are provided for the slope and intercept values.

<table>
<thead>
<tr>
<th></th>
<th>Aβ1-42</th>
<th>t-tau</th>
<th>p-tau181</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-B reg eqn</td>
<td>Y = 0.99X + 1.95</td>
<td>Y = 1.003X - 0.29</td>
<td>Y = 1.02X - 0.84</td>
</tr>
<tr>
<td>Y intercept</td>
<td>1.95(-6.2 - 10.2)</td>
<td>-0.29(-2.8 - 2.5)</td>
<td>-0.84(-3.3 - 1.4)</td>
</tr>
<tr>
<td>Slope</td>
<td>0.987(0.94 - 1.04)</td>
<td>1.003(0.96 - 1.04)</td>
<td>1.02(0.96 - 1.10)</td>
</tr>
<tr>
<td>r²</td>
<td>0.914</td>
<td>0.96</td>
<td>0.763</td>
</tr>
</tbody>
</table>
Figure 2. Passing-Bablok linear regression analysis plots of  for comparing RAW CSF concentration results obtained in 2014 with those obtained in the analyses performed in 2012/13 (referred to as “2013T”) transformed to 2007 BASELINE as described in “ADNI GO and ADNI 2 First batch analyses of CSF biomarkers: revised anchoring to 2007 ADNI 1 BASELINE dataset procedure”). The linear regression equations derived from these analyses for each of the 3 biomarkers were used to rescale or Transform the RAW 2014 dataset to the 2012/13 data sets BASELINE data that had been rescaled or Transformed to the 2007 BASELINE dataset (see Figure 1 for the comparisons, by linear regression analyses, of the Aβ1-42, t-tau and p-tau181 2014T data with the 2012/2013T data.
t-tau:

\[ \text{Intercept} = 2.354 [1.959 - 2.751] \]
\[ \text{Slope} = 0.459 [0.416 - 0.501] \]
\[ \text{LS}^2 = 0.763 \]
\[ \text{Mean Error} = 40.12\% \]
\[ \text{Mean Absolute Error} = 40.22\% \]
\[ \text{RMSE} = 42.06\% \]

Bland-Altman plot

p-tau\textsubscript{181}:

\[ \text{Intercept} = 3.531 [3.212 - 3.851] \]
\[ \text{Slope} = 0.493 [0.477 - 0.509] \]
\[ \text{LS}^2 = 0.991 \]
\[ \text{Mean Error} = -9.67\% \]
\[ \text{Mean Absolute Error} = 12.56\% \]
\[ \text{RMSE} = 15.00\% \]

Bland-Altman plot
Recommendation for cross sectional studies of ADNI GO and ADNI 2.
For cross sectional studies involving the ADNI GO and ADNI 2 study subjects, at BASELINE, we recommend combining all BASELINE results reported in UPENNBIOMK5; UPENNBIOMK6 and UPENNBIOMK7.

References


About The Authors

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