



Short-interval reliability of blood-based biomarkers measured using the NULISAseq CNS disease panel

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Background

- Clinically relevant blood-based biomarkers (BBMs) capture only part of the complex pathophysiology and heterogeneity of Alzheimer's disease (AD). Recent advances enable simultaneous measurement of more than 100 CNS-relevant analytes across a broad dynamic range from a single plasma sample using the NULISA (NUcleic acid-Linked Immuno-Sandwich Assay) platform.
- Widespread implementation of BBMs in clinical practice and research requires a better understanding of factors that influence biomarker measurements, including pre-analytical factors and biological confounders.
- However, the short-term within-subject variability and test-retest reliability of many BBMs remains poorly characterised, especially on broad proteomic panels.

Aims

- Assess short-term (>3 months) within-subject variability and test-retest reliability of plasma biomarkers measured using low-plex (NULISAqpcr AD 5-Plex) and high-plex (NULISAseq CNS Disease Panel) immunoassay platforms in serial plasma samples collected from the same individuals.
- Determine whether the interval between serial blood collections influences within-subject variability.

Methods

Study population: 92 participants donated paired plasma samples.

Biomarker assays:

- Low-plex – NULISAqpcr AD 5-Plex assay, Alamar Biosciences (N=80)
- High-plex – NULISAseq CNS Disease Panel, Alamar Biosciences (N=92)

Analysis:

- Correlations between paired biomarker measurements was assessed using Spearman rho.
- Test-retest reliability was evaluated using intraclass correlation coefficient (ICC), calculated with a two-way random-effects model for absolute agreement and single measurements (ICC [2,1]).
- Within-subject variability was assessed using within-subject coefficient of variation (CV_w).
- Linear regression was used to assess whether longer inter-sample intervals were associated with greater CV_w, adjusting for age and sex (CV_w ~ inter-sample interval + age + sex).

Table 1: Participant demographics and clinical characteristics

Characteristic	Value, n=92
Age, years*	52, IQR 39–64
Female, n (%)	42 (45.7)
Clinical diagnosis, n (%)	
AD	22 (23.9)
Other neurodegenerative disease	8 (8.7)
ADAD pre-symptomatic mutation carriers	27 (29.3)
Controls	35 (38.0)

*Age is reported as median. ADAD = autosomal dominant AD. IQR = interquartile range.

Conclusions

- Core AD plasma biomarkers measured over short-intervals, during which no clinical progression would be expected, demonstrated excellent test-retest reliability, with high Spearman rho and ICC values and low within-subject variability.
- Other CNS-relevant biomarkers demonstrated lower test-retest reliability, including oligo and monomeric synuclein, suggesting greater susceptibility to biological variability, pre-analytical handling, or individual characteristics.
- NfL, amongst other BBMs, showed moderately increased CV_w over time. NfL is a dynamic biomarker that tracks neurodegeneration and some increases in variability, even over relatively short intervals, is therefore expected.

Future directions: Investigation of test-retest reliability in a more clinically heterogenous cohort, alongside assessment of other factors including fasting status and pre-analytical factors is required.

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Results

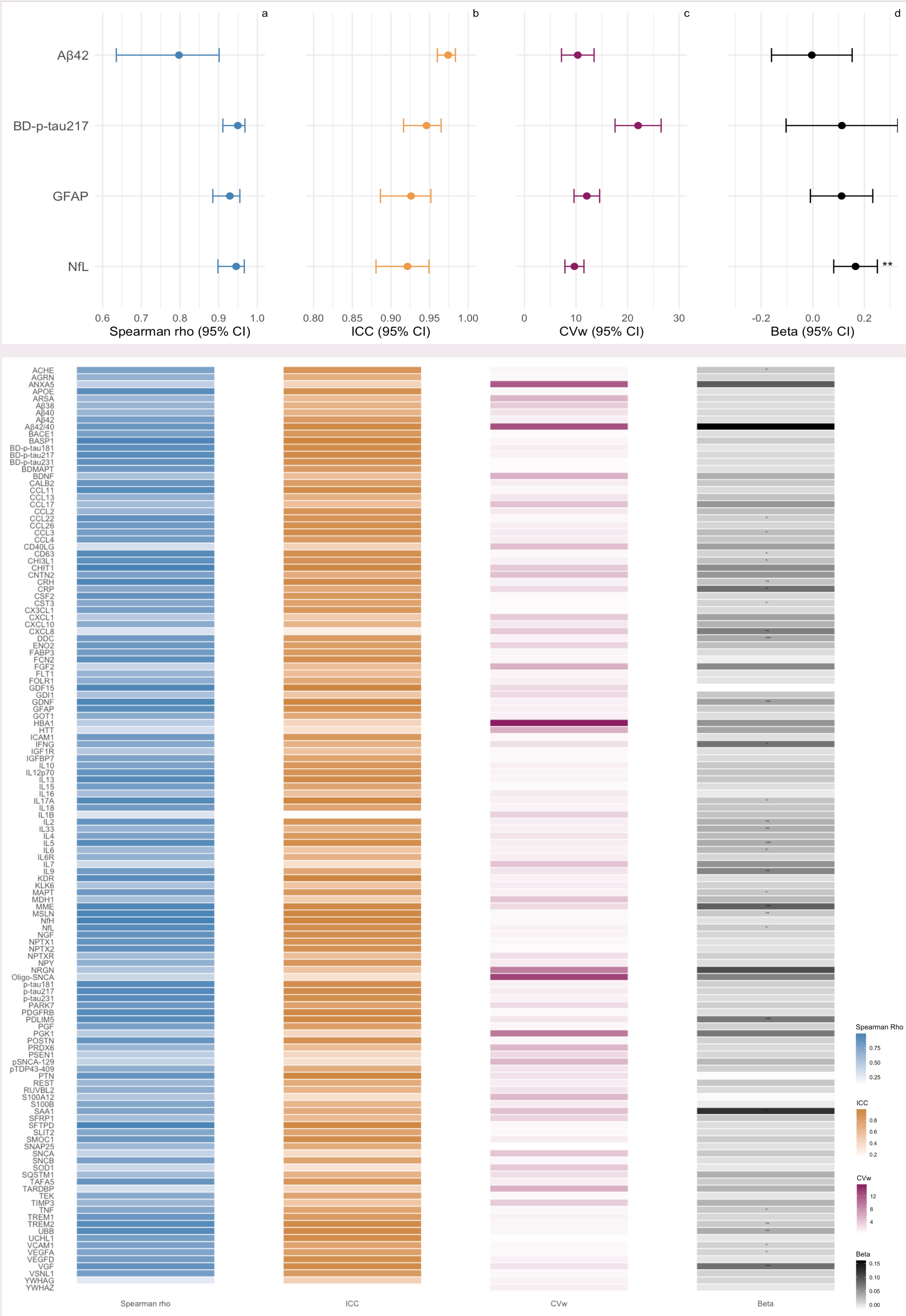


Figure 1: Test-retest reliability of core Alzheimer's disease plasma biomarkers measured using the NULISAqpcr AD 5-Plex immunoassay. Test-retest reliability for Aβ42, BD-p-tau217, GFAP and NfL was evaluated using (a) Spearman rho, (b) ICC, and (c) CV_w. Points represent estimated values and error bars indicate 95% confidence intervals (CIs). (d) Association between inter-sample interval and CV_w were estimated using linear regression, adjusting for age and sex. Points represent an individual biomarkers effect estimate (beta coefficients) and error bars indicate 95% CIs. False discovery rate (FDR) correction was applied to account for multiple comparisons. Adjusted p-values are not significant unless otherwise indicated; **p < 0.01.

Figure 2: Ranking of plasma biomarkers measured using the NULISAseq CNS Disease Panel. Biomarkers were ranked according to Spearman rho, ICC, and CV_w separately. For Spearman rho and ICC, darker colours indicate greater test-retest reliability. For CV_w, lighter colours indicate lower within-subject variability. Linear regression was used to assess whether longer inter-sample intervals were associated with greater CV_w, adjusting for age and sex. For beta coefficients, darker colours indicate larger effect estimates. False discovery rate (FDR) correction was applied to account for multiple comparisons. Adjusted p-values are shown; values are non-significant unless otherwise indicated; *p < 0.05, **p < 0.01, ***p < 0.001.

Core AD biomarkers (Aβ42, BD-p-tau217, GFAP, NfL and p-tau217) demonstrated high test-retest reliability with Spearman rho ≥ 0.79 and ICC ≥ 0.83 across the two immunoassay platforms.

Among the other CNS-relevant analytes assessed on the NULISAseq CNS Disease Panel, 68 showed strong correlations (Spearman rho ≥ 0.75) and 72 showed high reliability (ICC ≥ 0.75).

Within-subject variability was within expected bounds for nearly all analytes (CV_w < 20%), however, NfL measured using the NULISAqpcr AD 5-Plex assay showed a slight elevation (CV_w 22%).

After FDR correction, within-subject variability for 30 NULISAseq CNS Disease Panel biomarkers and 1 NULISAqpcr AD 5-Plex biomarker were significantly associated with longer inter-sample intervals. This included the core AD biomarker NfL.