Development of Two Multiplexed Immunoassays for Study of Nine Blood-Based Biomarkers of Alzheimer’s Disease

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Abstract

Objective: To develop multiplexed immunoassays that measure candidate biomarkers for Alzheimer’s disease (AD) in blood.

Background: Blood-based biomarkers provide a cost-effective means to screen for AD at the population level. Circulating alpha-2-macroglobulin (A2M), beta-2-microglobulin (B2M), factor VIII (FVIII), von Willebrand factor (vWF), interleukin-18 (IL-18), fetuin acid binding protein 3 (FABP3), thrombospondin (TSP), and I-309 are proteins that have been identified as candidate AD biomarkers. We describe 2 multiplexed immunoassay panels that measure these candidate biomarkers in human plasma and serum.

Methods: Multiplex panels were developed according to fit-for-purpose assay development and validation principles. The panels include a matrix, sensitivity, specificity, and matrix tolerance. Protein levels were measured in EDTA plasma and serum samples from patients with and without AD.

Results: The panels were validated based on biomarker abundance in human plasma and serum. A low abundance panel (2-fold dilution) included TPO, FABP3, PPY, IL-18, and 3-L09, and a high abundance panel (4,000-fold dilution) included A2M, B2M, FVII, and TNC. All biomarkers were detectable in plasma and serum at their respective dilutions with minimal levels of non-specific binding. For both panels, dilutional linearity and spike recovery were within 80-120% of the expected values with the exception of PPY. Differences in analyte levels between AD and non-AD samples were protein-dependent.

Conclusions: These multiplex panels provide reproducible results across multiple blood-based matrices. They support efforts to study blood-based biomarkers of AD and may accommodate inclusion of additional novel AD biomarkers.

Methods

Two multiplexed immunoassay panels were developed and optimized for electrochemiluminescence detection. Analytes were stratified into 2 panels based on common dilution factors that allowed us to detect all analytes in each panel (3-4 fold dilution for Panel 1 and 40-50 fold dilution for Panel 2). Analyte specificity was assessed by measuring samples spiked with blended calibrators in assays using individual detection antibodies. Dilutional linearity and spike recovery tests were conducted to assess matrix interference. Matched EDTA plasma and serum samples from patients diagnosed with AD and from patients without AD were evaluated using both panels.

Assay Specificity

Individual assays are specific to the targeted analytes. Signal and calculated cross-reactivity are presented below.

Panel 1: Dynamic Range and Sensitivity

Panel 1: % Cross-reactivity

Panel 2: Dynamic Range and Sensitivity

Panel 2: % Cross-reactivity

Panel 1: Sample Quantification

Panel 2: Sample Quantification

Conclusion

Two multiplexed immunoassay panels were developed and tested for suitability to measure candidate biomarkers of AD in blood. The panels were shown to have reproducible performance demonstrated by precision of calibration curves, and assay results were quantified in multiple tissue-derivates matrices. This study suggests that biomarker levels may differ between EDTA plasma and serum; this should be further explored with a larger sample set. These panels will support ongoing efforts to study blood-based biomarkers of AD and may be further expanded to accommodate additional novel AD biomarkers.