Trials and triumphs in the development of a quantitative assay for amyloid-beta peptides

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Pathologically, Alzheimer’s disease is characterized by the presence of proteinaceous deposits in the brain including extracellular amyloid plaques and intracellular neurofibrillary tangles. While Alzheimer’s disease has a defined pathology on autopsy, in vivo diagnosis is challenging—particularly in early stages of disease when treatment opportunities are greatest, before there is significant functional disability. Toward the development of an in vivo diagnostic model, cerebrospinal fluid biomarkers amyloid-beta (Aβ) and tau proteins have been extensively studied and are now included in research diagnostic criteria. Amyloid plaques, the neuropathologic hallmark of AD, are composed of amyloid-β peptides, in particular a 42-residue isoform herein referred to as Aβ42. As Aβ42 peptides are sequestered into insoluble amyloid deposit in the brain, there is a corresponding decrease in the concentration of the soluble fraction of Aβ42 in the cerebrospinal fluid.

Until recently (1, 2), quantitative analysis of Aβ peptides in CSF had relied almost exclusively on the use of immunometric assays. In order to side step known immunoassay reagent issues, matrix effects, heterophile antibody interference, therapeutic antibody interference, and major adsorption losses (3, 4), we endeavored to develop a quantitative LC-MS/MS assay for Aβ40 and Aβ42 peptides. Due to the propensity of Aβ peptides to spontaneously aggregate and adhere to surfaces, we systematically characterized conditions and techniques that resulted in minimal adsorption and aggregation events.

Preliminary data from a method comparison (n=20), revealed Aβ42 concentrations from LC-MS/MS were greater than those reported by the Innogenetics ELISA (on average 2.5 fold
greater), similar to the relationship previously observed between UPLC-MS/MS and Luminex methods for Aβ42 (1). Using specimens from the biobank at the Clinic for Alzheimer’s Disease and Related Disorders, a case-control study is currently underway to assess the diagnostic performance of our mass spectrometric method.

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References