Introduction
The ability to differentiate between Alzheimer’s disease (AD) and AD-related dementias is pivotal in the management and treatment of AD. A growing number of cerebrospinal fluid (CSF) biomarkers have recently been described as potential tools in the early detection and diagnosis of AD1. These biomarkers show promise for distinguishing between AD from dementias.

In this study, we describe the development and validation of a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay that quantifies A42, A40, and ApoE in CSF and qualitatively identifies ApoE isoforms. Utilizing the ratio of A42/A40, total levels of CSF ApoE, and the number of APOE e4 alleles, a risk assessment score was developed. Using this score, patients were categorized as low, average, or high risk of having AD.

Methods

**Assay Conditions**: Table 1 summarizes the assay conditions for A42, A40, and ApoE. Briefly, due to the propriety of both A40 and A42, to limit non-specific interactions with surfaces, all plastic consumables were treated with a complex non-human protein mixture to mitigate sample loss. After proteinic digestion, unique C-terminal portions of A40 and A42 were analyzed and quantified by LC-MS/MS. For ApoE genotyping (and ApoE genotyping) unique peptides correspond to the ApoE2 and ApoE4 isoforms, as well as 2 shared peptides between the ApoE2 and ApoE3 isoforms, and the ApoE3 and ApoE4 isoforms were targeted by LC-MS/MS (Table 1). A peptide concordance of ≥3 is summarized as a surrogate for total ApoE level.

**Sample Selection**: De-identified, clinically-diagnosed AD and non-AD CSF samples were supplied by the UC San Diego ADRC Neuropsychology and Brain Bank. Diagnosis of AD dementia, mild cognitive impairment (MCI), possible AD or undiagnosed AD was based on both imaging and clinical testing. A subset of 151 CSF samples was used as a training set to build a logistic regression model, and an additional 137 samples were used to validate the model. A separate cohort of 151 de-identified, blinded CSF samples, diagnosed as AD or non-AD (INNOTEST® A42; INNOTEST® A42, INNOTEST® Tau) was supplied by Athena Diagnostics for method comparison.

**Regression Modeling**: The results for A42/A40 ratio, ApoE4 allele count, and total ApoE (µg/mL) from the 151 subjects from the training set were used to fit a logistic regression model based on the clinical diagnosis.

**Results**: Among the 3 biomarkers associated with disease risk: a 0.05 increase in the A42/A40 ratio was associated with a 29% increase in the odds of AD (OR [95% CI], 1.28 [1.01-1.62]), an additional ApoE4 allele was associated with a 2-fold increase in the odds (OR [95% CI], 1.93 [1.00-3.73]), and a 1 µg/mL increase in total ApoE was associated with a 20% decrease in the odds (0.809 [0.65-0.99]).

**Conclusion**: We have devised a logistic regression model using biomarkers in CSF to stratify Alzheimer’s disease in patients with MCI or dementia.

References