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Simultaneous analysis of cerebrospinal fluid biomarkers using microsphere-based xMAP multiplex technology for early detection of Alzheimer's disease

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ABSTRACT

The xMAP-Luminex multiplex platform for measurement of Alzheimer's disease (AD) cerebrospinal fluid (CSF) biomarkers using Innogenetics AlzBio3 immunoassay reagents that are for research use only has been shown to be an effective tool for early detection of an AD-like biomarker signature based on concentrations of CSF $A\beta_{1-42}$, t-tau and p-tau_{181}. Among the several advantages of the xMAP-Luminex platform for AD CSF biomarkers are: a wide dynamic range of ready-to-use calibrators, time savings for the simultaneous analyses of three biomarkers in one analytical run, reduction of human error, potential of reduced cost of reagents, and a modest reduction of sample volume as compared to conventional enzyme-linked immunosorbant assay (ELISA) methodology. Recent clinical studies support the use of CSF $A\beta_{1-42}$, t-tau and p-tau_{181} measurement using the xMAP-Luminex platform for the early detection of AD pathology in cognitively normal individuals, and for prediction of progression to AD dementia in subjects with mild cognitive impairment (MCI). Studies that have shown the prediction of risk for progression to AD dementia by MCI patients provide the basis for the use of CSF $A\beta_{1-42}$, t-tau and p-tau_{181} testing to assign risk for progression in patients enrolled in therapeutic trials. Furthermore emerging study data suggest that these pathologic changes occur in cognitively normal subjects 20 or more years before the onset of clinically detectable memory changes thus providing an objective measurement for use in the assessment of treatment effects in primary treatment trials. However, numerous previous ELISA and Luminex-based multiplex studies reported a wide range of absolute values of CSF $A\beta_{1-42}$, t-tau and p-tau_{181} indicative of substantial inter-laboratory variability as well as varying degrees of intra-laboratory imprecision. In order to address these issues a recent inter-laboratory investigation that included a common set of CSF pool aliquots from controls as well as AD patients over a range of normal and pathological $A\beta_{1-42}$, t-tau and p-tau_{181} values as well as agreed-on standard operating procedures (SOPs) assessed the reproducibility of the multiplex methodology and Innogenetics AlzBio3 immunoassay reagents. This study showed within-center precision values of 5% to a little more than 10% and good inter-laboratory %CV values (10–20%). There are several likely factors influencing the variability of CSF $A\beta_{1-42}$, t-tau and p-tau_{181} measurements. In this review, we describe the pre-analytical, analytical and post-analytical sources of variability including sources inherent to kits, and describe procedures to decrease the variability. A CSF AD biomarker Quality Control program has been established and funded by the Alzheimer Association, and global efforts are underway to further define optimal pre-analytical SOPs and best practices for the methodologies available or in development including plans for production of a standard reference material that could provide for a common standard against which manufacturers of immunoassay kits would assign calibration standard values.

1. Introduction

Across different laboratories (research, clinical, and pharmaceutical) involved in Alzheimer's disease (AD) studies, there is a growing need for rapid, accurate, sensitive, reproducible, multiplexed and cost-effective measurement methods for key biomarkers. Extracellular Aβ amyloid plaques, axonal degeneration and intraneuronal neurofibrillary tangles formed by pathological tau are the major pathologic hallmarks that define dementia as being due to AD. These pathologic features are highly associated with three cerebrospinal fluid (CSF) biomarkers, amyloid β(1–42) ($A\beta_{1-42}$), total tau (t-tau), and tau phosphorlyated in the 181

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threonine position (p-tau$_{181}$), respectively [1–4]. Considering the pathogenesis of AD, accumulation of Aβ_{1-42} caused by abnormal processing of amyloid precursor protein in the brain might be an initial driver of AD pathology [5–7]. There is increasing evidence that simultaneous, and perhaps even earlier, tau pathology appears independently in the brainstem, especially the locus coeruleus followed later by the transentorhinal cortex and then progressing to the hippocampal region, amygdala and the neocortex [8,9]. Following the increase in Aβ_{1-42} load in the brain, neurodegeneration slowly progresses over many years through the intracellular accumulation of hyperphosphorylated tau, neuronal loss and dementia.

Of the many diverse causes of dementia, that due to AD is most common. Dementia with Lewy bodies (DLB), frontotemporal degeneration (FTD), vascular dementia, dementia with Parkinson's disease, are other forms of this class of neurological disorder. Moreover, a significant proportion of dementia patients have mixed pathology [10–12]. Due to the heterogeneity of dementia pathology and complexity of AD pathogenesis, therefore, multiple biomarkers with high sensitivity and specificity to detect AD are needed. In addition, numerous studies have shown that Aβ amyloid load in the brain correlates negatively with CSF Aβ_{142} concentration, while CSF t-tau levels reflect the intensity of neuronal and axonal degeneration and brain damage [1–4,13–15]. High concentrations of CSF t-tau have also been associated with fast progression from mild cognitive impairment (MCI) to AD, and rapid progression of dementia in AD patients [16,17]. Phosphorylated tau in CSF is helpful for the differentiation of AD from other types of dementia [18–20]. Therefore, the measurement of one CSF biomarker alone is not sufficient to discriminate MCI and AD patients from age matched cognitively normal individuals, from patients with other dementias or to provide reliable prediction of risk for progression from either a cognitively normal state or MCI to dementia. For example, as compared to use of a single CSF biomarker for discrimination of AD from healthy control (t-tau with sensitivity of 69.6% and specificity of 92.3%; Aβ_{1-42} with a sensitivity of 96.4% and specificity of 76.9%), the combination of two biomarkers, as the ratio t-tau/Aβ_{1-42}, showed balanced sensitivity (85.7%) and specificity (84.6%) [21]. Furthermore, the combination of CSF biomarkers (e.g., t-tau and Aβ_{1-42}) showed excellent prediction of MCI to AD conversion (positive predictive value of 81% and negative predictive value of 96%) with high sensitivity (95%) and specificity (83%) [22].

Until recently, probable AD was usually diagnosed according to the clinical criteria of the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) established in 1984 [23]. Using these criteria, the mean reported sensitivities and specificities for clinical diagnosis of probable AD, neurologists skilled at memory disorder diagnoses, are 81% (range 49–100%) and 70% (range 47–100%), respectively [10]. The relatively low specificity of clinical diagnosis reflects the many features of AD shared with non-AD dementias [24–26]. To date, all AD clinical trials have used clinical assessment as a diagnostic tool to enroll patients. However, when clinical symptoms are apparent, it may be too late to reverse associated extensive neuronal degeneration. In addition, it is difficult to assess the rates of progression from mild clinical symptoms in MCI subjects to AD dementia or which MCI subjects may revert to a cognitively normal state or remain stable with MCI using clinical assessment alone. There is no doubt that new drugs with disease-modifying activity should be developed, however, clinical outcomes of many clinical trials for these drugs in AD patients enrolled by clinical assessment have not shown positive results [27]. Therefore, it is essential to develop tools for the discrimination of earlier stages of AD (MCI – prodromal AD, and/or cognitively normal with plaque burden – preclinical AD) from normal free of plaque burden and other forms of dementia with high sensitivity and specificity. These efforts also might increase the homogeneity of enrolled patients in clinical trials, thereby decreasing the number of subjects enrolled in clinical trials and accelerating the development of new treatments with disease-modifying activity.

Biologic markers of AD should have a sensitivity >80% for detecting AD and specificity >80% for discriminating other forms of dementia [28]. Measurement of Aβ_{1-42} alone has been shown in numerous studies to be a sensitive test for discrimination of AD vs. cognitively normal. However, best discrimination of AD from other neurodegenerative diseases is achieved using a combination of Aβ_{1-42}, t-tau and/or p-tau$_{181}$ [18,22,29–34]. Thus, the measurement of Aβ_{1-42} and the tau proteins with the addition of apolipoprotein E (ApopE) genotype can improve CSF biomarker performance [21]. To this end, a multiplex (xMAP) technology-based method for simultaneous measurement of Aβ_{1-42}, t-tau and p-tau$_{181}$ in human CSF was developed. The prototype multiparametric bead-based assay principle which was further developed into the xMAP-Luminex technology platform was first described by Gordon and McDade [35]. Using this platform, Olsson et al. were the first to demonstrate the clinical utility and very good precision over time for the multiplexed bead-based assay for quantification of AD biomarkers in human CSF [36]. Using this multiplexed immunoassay of AD CSF Aβ_{1-42}, t-tau and p-tau$_{181}$, Hansson et al. reported one of the earliest studies that documented sensitive and specific detection of AD-like biomarker concentrations in MCI patients who subsequently progressed to AD dementia [22]. In addition to the results showing better or equal analytical and clinical performance of the xMAP multiplex immunoassay system using the Luminex platform and Innogenetics research use only INNO-BIA AlzBio3 immunoassay kits (multiplex platform for AD CSF biomarkers) compared to enzyme-linked immunosorbant assay (ELISA) methodology [4,36,37], multi-analyte testing provides a wide dynamic range of ready-to-use calibrators, time savings by simultaneous analyses of three biomarkers in one analytical run, reduction of human error, potential of reduced cost of reagents, and a modest consumption of sample volume (Table 1) [38]. However, there are several challenges that remain for optimal performance of the multiplex platform for AD CSF Aβ_{1-42}, t-tau and p-tau$_{181}$. In this review, we describe several characteristics and advantages of this multiplex immunoassay platform for AD CSF biomarkers and diagnostic utilities for AD detection, discuss sources of variability observed in the results obtained by this assay system, and finally provide current and future efforts to decrease the variability and establish a standard reference material that can promote comparability of concentration values achieved across different immunoassays.

2. xMAP multiplex immunoassay system using the Luminex platform and Innogenetics INNO-BIA AlzBio3 immunoassay to analyze AD CSF biomarkers

2.1. xMAP technology

The xMAP Luminex technology is a flow cytometric method that allows simultaneous detection of several analytes on different sets of microspheres in a single well. Each set of microspheres has embedded a precise concentration ratio of red- and infrared fluorochromes, resulting in unique spectral identities. This allows flow cytometric discrimination of mixed microsphere sets. The multiplex bead array assays use fluorescence as a reporter system. This immunoassay design provides a wider detection range (3–4 logs) of concentration than equivalent ELISA (1–2 logs) [36]. The dynamic range for the final assay format is largely dependent on the intended use of product. The selection of a very broad range of calibrator concentrations, even outside the clinical range, can