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Global standardization measurement of cerebral spinal fluid for Alzheimer's disease: An update from the Alzheimer's Association Global Biomarkers Consortium

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Abstract Recognizing that international collaboration is critical for the acceleration of biomarker standardization efforts and the efficient development of improved diagnosis and therapy, the Alzheimer's Association created the Global Biomarkers Standardization Consortium (GBSC) in 2010. The consortium brings together representatives of academic centers, industry, and the regulatory community with the common goal of developing internationally accepted common reference standards and reference methods for the assessment of cerebrospinal fluid (CSF) amyloid β42 (Aβ42) and tau biomarkers. Such standards are essential to ensure that analytical measurements are reproducible and consistent across multiple laboratories and across multiple kit manufacturers. Analytical harmonization for CSF Aβ42 and tau will help reduce confusion in the AD community regarding the absolute values associated with the clinical interpretation of CSF biomarker results and enable worldwide comparison of CSF biomarker results across AD clinical studies.

Keywords: Alzheimer's disease; Biomarkers; Cerebral spinal fluid; Early diagnosis; Standardization; Assay development; Precompetetive

1. Quality control to reduce measurement variability

Biomarkers have become increasingly important in research, clinical trials, and clinical practice, particularly in light of revised diagnostic criteria published in 2011, which recognized that Alzheimer's disease (AD) pathology is reflected in the cerebrospinal fluid (CSF) as a decrease in amyloid β42 (Aβ42) and an increase in total tau (T-tau) and phosphorylated tau (P-tau) [1]. However, variability across studies, laboratories, and assays limits the usefulness of biomarkers to outside expert centers [2–4]. Variability in the accuracy of measurements can be introduced at multiple points, such as in the production of assay materials and test kits, during sample collection and storage, at the testing laboratory as a result of operator and instrumentation

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differences, and in the process of data collection, entry, and calculation. With this in mind, the Alzheimer's Association launched an external quality control (QC) program in 2009 to establish standardized protocols for biomarker assessment and as an external proficiency testing program of CSF analyses among participating laboratories [5]. Investigators involved in the QC program have also undertaken efforts to establish reference measurement procedures [6]. These international collaborations build on the Alzheimer's Disease Neuroimaging Initiative (ADNI), which was established in 2004 to develop uniform standards and protocols for acquisition of neuroimaging and biomarker data and has since been expanded to become a worldwide project [7].

QC samples prepared at the Clinical Neurochemistry Laboratory at the Mölndal campus of the University of Göteborg, Sweden, are shipped to participating labs around the world that run assays and report back data to Mölndal. The program now includes 85 labs in over 20 countries, with new labs joining continuously. Each round consists of three samples, two samples unique to that round and one that returns in each round for longitudinal monitoring. The program is open to any lab using commercially available assays for Aβ or tau. The majority of the laboratories currently in the program use INNOTEST® enzyme-linked immunoassay (ELISA) test kits, although some use Luminex Alzbio3 xMAP® or early versions of the Meso Scale Discovery (MSD) kits. Data are analyzed to assess variability among labs. Each lab receives a report showing how the values they obtained rank compared with other labs as well as data on the longitudinal sample. By summarizing data collected from all labs, outliers can be easily identified. Laboratories are then alerted to potential discrepancies and urged to review their procedures. The program also detects batch variability, if present.

Seven rounds had been completed and analyzed by the time of the Vancouver AAIC 2012 meeting. An analysis of the first two rounds showed large between-laboratory variation [8]. An analysis of data from rounds 3–8 is underway and an article is in preparation. Overall variability (CV) for ELISA and xMAP assays in the first seven rounds has been about 25% for Aβ42 and between 15% and 20% for T-tau and P-tau. A reasonable target interlaboratory CV is 10%–15%, so there is still room for improvement.

Variance component analysis has enabled investigation of the causes of variability. Preliminary data suggest that Aβ42 measurement is more highly affected by between-batch differences, whereas T-tau and P-tau measurements are more highly affected by between-lab differences. The investigators have also shown that labs with more experience and large turnover have smaller variability compared with other labs, suggesting that experience can reduce variability, particularly for Aβ42. Further investigation will continue to pinpoint error sources and alert outliers and kit producers so that they can improve procedures and methods.

The QC program only provides monitoring, and in the long run interventions will be needed to reduce variability. These include implementation of standard operating procedures, certified reference materials and methods [6], and novel assays for fully automated analysis.

2. Engaging industry partners

Developing robust assays with common reference standards and reference methods will require the engagement of industry partners that are developing new diagnostic tools, not only for CSF biomarkers but for genetic markers, new imaging ligands, and other imaging biologicals. Assay manufacturers are responsible for assay-related parameters that introduce variability into biomarker assessments, including the production, QC, and validation of reagents and kits, as well as compliance with regulatory requirements. Four of these companies were represented at the Vancouver meeting: Meso Scale Discovery (MSD), Innogenetics, Saladax Biomedical, and Roche Diagnostics.

Meso Scale Discovery has developed and validated immunoassays for T-tau and Aβ42, with full analytical validation assessing precision accuracy, matrix tolerance, and matrix interference upon dilution, as well as clinical validation indicating that the kits provide good clinical performance in differentiating patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD) from controls. The company has also initiated a multisite validation study with released lots and has established a training, certification, and proficiency program to ensure minimal variability across sites. They have also encouraged all labs using their kits to join the QC program. Ongoing work also includes validation of a multiplex assay as well as development of biomarker assays for traumatic brain injury and Parkinson's disease using markers that are currently being used only for research.

Innogenetics, part of the Fujirebio group, has a neuroassay portfolio that includes single-analyte ELISAs (INNOTEST) for T-tau, P-tau, and Aβ42, as well as multiple-analyte xMAP (INNO-BIA) and genetic assays (LiPA) in development. In the USA, the assays are for research use only (RUO). In addition to participating in the Alzheimer's Association's external QC program, Innogenetics, like most manufacturers, has its own internal QC program intended to guarantee quality and consistency. They are now running a feasibility study to design and develop run validation control (RVC) samples and ready-to-use (RTU) calibrators for each of the INNOTEST assays. RVCs will enable validation of test runs and alignment with lab accreditation requirements; RTU calibrators will ensure ease of use and reduction of interrun and intralab variation. These feasibility studies have helped in the identification of a suitable and stable matrix and reduced variability on CSF sample concentrations over different runs. They also confirmed that the use of GuHCl in the buffer had a negative impact on recovery of Aβ42.

Saladax Biomedical, in partnership with Bristol-Myers Squibb and Johnson & Johnson Ortho Clinical Diagnostics,