Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 3 (2016) 27-34

Alzheimer's & Dementia

Blood-Based Biomarkers

Comparing biological markers of Alzheimer's disease across blood fraction and platforms: Comparing apples to oranges

Sid E. O'Bryant[a,*], Simone Lista[b,c], Robert A. Rissman[d], Melissa Edwards[e], Fan Zhang[f], James Hall[a,g], Henrik Zetterberg[h,i], Simon Lovestone[j], Veer Gupta[k], Neill Graff-Radford[l], Ralph Martins[k], Andreas Jeromin[m], Stephen Waring[n,o], Esther Oh[p], Mitchel Kling[q], Laura D. Baker[r], Harald Hampel[b,c], for the ISTAART Blood Based Biomarker Professional Interest Area

[a]*Institute for Healthy Aging, Center for Alzheimer's & Neurodegenerative Disease Research, University of North Texas Health Science Center, TX, USA*
[b]*AXA Research Fund & UPMC Chair, Paris, France*
[c]*Sorbonne Universités, Université Pierre et Marie Curie, Paris 06, Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A) & Institut du Cerveau et de la Moelle épinière (ICM), Département de Neurologie, Hôpital de la Pitié-Salpétrière, Paris, France*
[d]*Alzheimer's Disease Cooperative Study, Department of Neurosciences, UCSD School of Medicine, La Jolla, CA, USA*
[e]*Department of Psychology, University of North Texas, Denton, TX, USA*
[f]*Department of Molecular and Medical Genetics, University of North Texas Health Science Center, Fort Worth, TX, USA*
[g]*Department of Psychiatry, University of North Texas Health Science Center, Fort Worth, TX, USA*
[h]*Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*
[i]*UCL Institute of Neurology, London, UK*
[j]*Department of Psychiatry, University of Oxford, Oxford, UK*
[k]*Center of Excellence for Alzheimer's Disease Research and Care, School of Medical Sciences, Faculty of Health, Engineering and Sciences, Edith Cowan University, Joondalup, WA, Australia*
[l]*Department of Neurology, Mayo Clinic, Jacksonville, FL, USA*
[m]*Quanterix Corp., Lexington, MA, USA*
[n]*Essentia Institute of Rural Health, Duluth, MN, USA*
[o]*Texas Alzheimer's Research and Care Consortium, TX, USA*
[p]*Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
[q]*Behavioral Health Service, Cpl. Michael J. Crescenz VA Medical Center and Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA*
[r]*Department of Medicine, Internal Medicine (Geriatrics), Wake Forest School of Medicine, Winston Salem, NC, USA*

Abstract

Introduction: This study investigated the comparability of potential Alzheimer's disease (AD) biomarkers across blood fractions and assay platforms.

Methods: Nonfasting serum and plasma samples from 300 participants (150 AD patients and 150 controls) were analyzed. Proteomic markers were obtained via electrochemiluminescence or Luminex technology. Comparisons were conducted via Pearson correlations. The relative importance of proteins within an AD diagnostic profile was examined using random forest importance plots.

Results: On the Meso Scale Discovery multiplex platform, 10 of the 21 markers shared >50% of the variance across blood fractions (serum amyloid A $R^2 = 0.99$, interleukin (IL)10 $R^2 = 0.95$, fatty acid-binding protein (FABP) $R^2 = 0.94$, I309 $R^2 = 0.94$, IL-5 $R^2 = 0.94$, IL-6 $R^2 = 0.94$, eotaxin3 $R^2 = 0.91$, IL-18 $R^2 = 0.87$, soluble tumor necrosis factor receptor 1 $R^2 = 0.85$, and pancreatic polypeptide $R^2 = 0.81$). When examining protein concentrations across platforms, only five markers

S.E.O. has multiple patients pending, submitted by the University of North Texas Health Science Center wherein he is an inventor and receives research grants from the National Institutes of Health, National Institute on Aging, award number R01AG039389 and P30AG12300. A.J. holds affiliations with Atlantic Biomarkers, LLC and reports no conflict of interests. S.L., R.A.R., M.E., F.Z., J.H., H.Z., S.L., V.G., N.G.-R., R.M., S.W., E.O., M.K., L.B., and H.H. report no conflicts of interests.

*Corresponding author. Tel.: +1-817-735-2961; Fax: +1-817-735-0611.

E-mail address: Sid.O'Bryant@unthsc.edu

http://dx.doi.org/10.1016/j.dadm.2015.12.003

shared >50% of the variance (beta 2 microglobulin $R^2 = 0.92$, IL-18 $R^2 = 0.80$, factor VII $R^2 = 0.78$, CRP $R^2 = 0.74$, and FABP $R^2 = 0.70$).

Discussion: The current findings highlight the importance of considering blood fractions and assay platforms when searching for AD relevant biomarkers.

Keywords: Alzheimer's disease; Blood; Serum; Plasma; Biomarker discovery; Multiplex assay platform; Meso Scale Discovery; Rules Based Medicine; Proteins; Preanalytic processing; Standardization; Diagnostics

1. Introduction

Despite tremendous scientific advancements, there remains a significant concern regarding the lack of reproducibility of research findings [1–4] with most believing that "at least 50%" of academic findings will not be replicable within industry laboratories [4]. In fact, the National Institutes of Health recently highlighted this problem and outlined a plan to address the issue [2]. In recent years, there has been an explosion in the search for blood-based biomarkers related to Alzheimer's disease (AD) for a variety of functions, such as detection, diagnosis, risk estimation, as well as clinical trial enrichment, stratification, and treatment response. However, this work has not been immune to the problem of replicability as conflicting findings are commonplace in the field. In an effort to generate consistent methods and protocols to increase replicability and move the field of blood-based biomarkers for AD forward, the international collaboration of the blood-based biomarker professional interest area (BBB-PIA) of the Alzheimer's Association's International Society to Advance Alzheimer's Research and Treatment was formed, which has published consensus statements regarding the current state of the field along with most of the immediate research needs [5,6]. More recently, the BBB-PIA published the first ever consensus-based guidelines for preanalytic processing for blood-based AD biomarker research [7]. The purpose of the present study was to examine two potential sources contributing to failures to replicate in the blood-based biomarker field of AD, (1) blood fraction (i.e., serum vs. plasma) and (2) analytic platform. These initiatives have been of paramount importance and additional topics require careful consideration.

A major concern for blood-based AD biomarker studies is the selection of the most suitable blood fraction. The type of blood fraction is important not only for the abundance of specific analytes but also for the role of additives such as heparin, citrate, or ethylenediaminetetraacetic acid (EDTA), which can significantly impact both stability and detectability of biomarkers [8,9]. However, to date, there remains little consistency in the type of blood fraction assayed across studies. One of the most extensively studied plasma-based biomarkers is amyloid β (Aβ), which is one of the hallmarks of AD pathology investigated at autopsy and is a well-validated marker of AD in cerebrospinal fluid samples. Work by Watt et al. [10], however, highlights many of the issues regarding plasma Aβ studies. Although some markers appear to be robust in both serum and plasma (e.g., C-reactive protein), other markers appear to be more robust in one fraction over the other. For example, EDTA inhibits many proteases, which may preserve many proteins better than serum; however, EDTA can interfere with some mass spectrometry assays. Recent reviews on the topic highlight the variability in blood-fraction selection as a major contributor to inconsistent findings in blood-based biomarker studies [11,12]. On the one hand, several markers have been found to be significant across multiple studies and cohorts, despite different blood fractions used (e.g., pancreatic polypeptide [PPY] and C-reactive protein [CRP]) [13–16]. Few studies, however, have directly compared plasma to serum-based findings in AD. When examining the association between serum- and plasma-based proteomics in the Texas Alzheimer's Research & Care Consortium (TARCC; available at http://www.txalzresearch.org/), a total of 40 proteins (from >100 candidate proteins) were highly correlated across blood fractions ($R^2 \geq 0.75$; ≥56% shared variance of proteins) [17]. In another study using the TARCC and Alzheimer's Disease Neuroimaging Initiative (ADNI) data, only 11 proteins (from >100) were highly correlated across serum and plasma ($R^2 \geq 0.75$) and significantly associated ($P < .05$) with AD status (CRP, adiponectin, PPY, fatty acid-binding protein [FABP], interleukin 18 [IL-18], beta 2 microglobulin [β2M], tenascin C [TNC], I309, factor VII [FVII], soluble vascular cell adhesion molecule-1 [sVCAM-1], and monocyte chemoattractant protein-1). The serum-plasma biomarker algorithm yielded an area under the curve (AUC) = 0.88 across cohorts [18]. These data suggest that some markers are consistent across blood fraction and may be useful for diagnostic purposes; however, others are likely less comparable despite statistically significant correlations.

Another key issue for blood-based AD biomarker studies is the selection of the most appropriate assay platform. Many cohorts have used the Myriad Rules Based Medicine (Myriad RBM) platform (e.g., ADNI, TARCC, and the Australian Imaging, Biomarker & Lifestyle Flagship Study of Aging) [13,14,16,18]; however, many other approaches have been used, including the Meso Scale Discovery