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EDITORIAL

The iterative process of fluid biomarker development and validation in Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative dementia for which disease-modifying treatments, likely to be the most effective in early disease stages, are now being developed at a rapid pace.[1] AD-related pathologies, key among which are extracellular amyloid beta (Aβ) plaques, intra-neuronal tau tangles, and neurodegeneration, as well as astrocytic and microglial activation, are evident in the brain decades before symptom onset; it is increasingly recognized that a pre-symptomatic phase, whereby pathologies accumulate years before symptom onset, is a common feature of most neurodegenerative diseases.[2]

As the field moves toward treating ever earlier, biomarkers that can determine the onset, profile, and intensity of neurodegeneration-related brain changes in individual patients are required for diagnosis, prognosis, and for use in clinical trials, both as inclusion and outcome measures. While reliable cerebrospinal fluid (CSF) and imaging biomarkers for AD-related pathologies, validated against neuropathology and in clinical and population-based longitudinal cohorts, have been available for some time, the field has recently been spurred by the development of blood-based biomarkers.

A large number of papers during the past few years show that the ratio of 42–40 amino acid-long amyloid beta (Aβ42/Aβ40) in plasma is a reproducible (albeit not always robust) biomarker for cerebral Aβ pathology, that plasma concentrations of phosphorylated tau (p-tau) forms reflect AD-type tau pathophysiology and amyloid burden, that plasma or serum neurofilament light (NfL) concentration reflects neuroaxonal degeneration (onset and intensity), and that plasma or serum glial fibrillary acidic protein (GFAP) concentration reflects astrocytic activation (a typical feature of Aβ-laden brain tissue, but not specific).[3] Given the large amount of corroborative data on these novel biomarkers, and the availability of easy-to-use fully automated clinical chemistry tests to measure them, several of these biomarkers are expected to be adopted in clinical practice in the next few years.[4]

Nevertheless, there are several areas in which further research is needed. First, studies evaluating blood biomarker performance have been conducted in settings wherein the study populations were relatively healthy, apart from dementing disorders. For example, presence of unstable somatic disease (such as heart disease, diabetes, and kidney disease) is often an exclusion criterion for research studies, and patients diagnosed with brain diseases other than those under study are almost always excluded. Nevertheless, we know that aging can be accompanied by the development of several illnesses. Improved understanding of the factors that may influence the diagnostic performance of blood biomarkers is thus highly important. It is also important to dissect if presence of multiple illnesses is confounding interpretation, or whether relationships between AD biomarkers and multiple comorbidities reflect real effects on the pathophysiological process the biomarker is intended to measure. This is not trivial.

Second, there is a dearth of studies in population-representative samples, including racial and ethnically diverse populations, wherein the performance of blood biomarkers has been evaluated. Most blood biomarker studies have been conducted in selected cohorts that are largely White and lacking inclusion of participants identified by the National Institutes of Health to be underrepresented in biomedical research, including Black or African American participants and Hispanic or Latino participants. Research cohorts are also often from geographically restricted regions, and the number of blood biomarker studies performed in North America and European countries is much higher than the number of studies performed in, for example, Asian, South American, or African countries. Furthermore, few blood biomarker studies that include racially and ethnically diverse participants have considered the social factors that lead to disparities in health outcomes among racialized and minoritized individuals.[5]

In the current issue of DADM, two papers that investigate important aspects of these issues have been published. Berry et al. examined the influence of liver cirrhosis and biomarker evidence of kidney dysfunction on blood concentrations of AD-related biomarkers.[6] Windon et al. tested whether there are differences in plasma and CSF biomarkers among participants in the multi-site Alzheimer's Disease Neuroimaging Initiative (ADNI) study when stratified by race and ethnicity, and controlling for factors that included measured sociodemographic variables and history of cardiovascular disease.[7]

Considering the role of confounding health conditions, Berry et al. found that presence of liver and kidney disease has the potential to complicate the interpretation of blood biomarker measurements in older adults. Both hepatic and renal diseases may influence blood

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composition. For example, when considering the role of the liver, most plasma proteins are synthesized by hepatocytes, which bind and carry targeted biomarkers of interest. The sample diluents of most immunoassay protocols are optimized to minimize this type of binding, making the target analyte available to the assay antibodies, but for some proteins, for example, sticky Aβ, this may be difficult. The liver and reticuloendothelial system are also involved in clearance of proteins from the blood, which may contribute to the variability in blood biomarker concentrations. Renal clearance, on the other hand, is a significant route of elimination of water-soluble small molecules but not proteins (the molecular weight cut-off for glomerular filtration is 30–50 kDa, and the proximal tubules actively reabsorb proteins smaller than that), and thus (mild) kidney dysfunction contributes minimally to blood protein concentrations. However, kidney disease with proteinuria could impact blood biomarker concentrations in other ways, for example via plasma protein loss, which may decrease measured levels of biomarkers for neurodegeneration-related processes. Furthermore, both hepatic and renal dysfunction (as well as other conditions, such as type 2 diabetes) may result in peripheral neuropathy that can increase biomarkers of neurodegeneration in blood. Severe liver or kidney disease can also contribute to central neurotoxicity,[8,9] which could be accompanied by increased concentrations of neurodegeneration biomarkers. Taken together, the potential implications for confounding of AD blood biomarker results by these conditions is a concern, particularly as these biomarkers transition to clinical use in older adults with multiple conditions that may impact biomarker interpretation.

To determine the potential impact of liver cirrhosis and kidney dysfunction on blood biomarker concentrations, Berry et al. measured plasma biomarkers including p-tau (phosphorylated at amino acid 181), NfL protein, and total tau in 135 individuals with liver cirrhosis and 22 healthy controls. They found highly elevated biomarker levels (2- to 4-fold) in the cirrhosis group—which included individuals with autoimmune hepatitis and non-alcoholic fatty liver disease—and moderate correlations between these biomarkers and creatinine levels (an indicator of renal function). The magnitude of the biomarker elevation in cirrhosis was similar to the magnitude of change seen in neurodegenerative diseases, although the authors point out that the results should be interpreted with caution because the tube types were not standardized. They further dissected the plasma biomarker findings in cirrhosis with additional liver function tests and concluded that most of the effect was likely related to altered albumin–biomarker binding interactions in the cirrhosis patients. The correlation with creatinine suggests that renal dysfunction may be a clinically relevant confounder for blood biomarkers of AD and neurodegeneration, which corroborates earlier data,[10,11] but more studies on the underlying mechanism are needed.

With regard to examining blood biomarkers among diverse research participants, Windon et al. leveraged fluid biomarker data collected in the ADNI study.[7] They examined CSF Aβ42, total tau, and p-tau181 concentrations and plasma p-tau181 and NfL concentrations in 47 Black participants matched to 141 Non-Hispanic White participants and 43 Latino participants matched to 129 Non-Hispanic White participants. While the overall racial and ethnic diversity of ADNI is limited—with Black and Latino participants included here likely representing 10% of ADNI participants with fluid biomarkers and magnetic resonance imaging—a strength of leveraging data from a multi-site study such as ADNI is the greater geographic diversity compared to single-site cohort studies. Furthermore, Windon et al. controlled for measured sociodemographic factors and some comorbidity (cardiovascular disease). In contrast to prior studies, which have reported differences between Black individuals and White individuals in concentrations of plasma tau and NfL biomarkers,[12] as well as longitudinal Aβ42/Aβ40 ratio and tau concentration changes in CSF,[13] Windon et al. found no differences between racial and ethnic groups when controlling for measured covariates. The contrast to other published studies suggests that it is important to consider the underlying reasons why differences in biomarker levels have been previously found, including possible differences in recruitment strategies, or different distributions of structural inequity-linked social factors that may drive biomarker findings. One limitation is that while the investigators were able to consider racial and ethnic diversity in their study, ADNI is not a population-based study, and additional blood biomarker validation studies in population-representative samples are needed. Moving forward, biomarker studies in diverse cohorts should consider the impact of social determinants of health on measured outcomes, as disparities in income, education, employment, housing quality, and structural inequities driven by factors such as racism and discrimination contribute to greater disease burden disproportionately across population groups. This includes higher rates of dementia, as well as greater incidence of comorbid conditions, that may impact blood biomarker interpretation.

In clinical chemistry, new tests are often discovered and validated in relatively homogeneous populations with very clear diagnoses and strict exclusion criteria to minimize noise and show the potential of a biomarker. However, when the test is more widely applied, its diagnostic performance typically drops, and sources of variability need to be identified to improve performance. For example, one of the first myocardial infarction biomarkers used clinically was creatine kinase (CK), but the non-specificity of the assay against skeletal muscle injury confounded interpretation. This was solved when assays for the "myocardial band" isoform of CK (CK-MB) were developed, followed by eventual adoption of superior cardiac troponin-based assays. Likewise, alkaline phosphatase (ALP) tests, which were previously non-specific, were made tissue specific by the generation of antibodies that capture ALP isoforms with differential expression patterns in liver, kidney, and bone, providing more relevant clinical information. It is likely that similar developments will occur in the AD biomarker pipeline. For example, given the challenges in interpreting blood-based assays for central nervous system (CNS) diseases, assay development is under way for tests that differentiate central from peripheral nervous system (PNS) tau (unpublished data). Likewise, among the neurofilaments, NfL is expressed in both central and peripheral axons, which may explain the noisier results found in populations with a high prevalence of type

2 diabetes mellitus and peripheral neuropathy.[14] To address this issue, it may be necessary to measure both CNS-specific (α-internexin) and PNS-specific (peripherin) neurofilaments in addition to (or replacing) NfL. As the field advances, it is likely that a panel of blood biomarkers for AD and related disorders could be developed that facilitates clinical interpretation, accompanied by education on the caveats and confounders that may impact biomarker measurements. These approaches may solve some (but not all) problems identified in real-world clinical populations and improve biomarker usage and interpretation in clinical settings, in which patients may have more than one disease.

Biomarker development and validation (analytical and clinical) are iterative processes; while AD has lagged behind other fields in developing easily accessible, affordable, and meaningful biomarkers, the field is now entering a phase in which blood biomarkers will find widespread use in both research as well as the clinic. Determining the confounders to interpretation across population-generalizable cohorts, as well as the boundaries of where, why, and how the biomarkers are implemented, is needed as soon as possible.

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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

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