Non-Invasive Novel Biomarkers to Predict Alzheimer's Risk

Enabling early intervention and facilitating the discovery of therapeutic targets, biomarkers have been an essential aspect of Alzheimer's research for years

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Since the National Institute on Aging and Alzheimer's Association issued its 2011 guidelines delineating separate diagnostic recommendations for preclinical, mild cognitive impairment, and dementia stages, Alzheimer's disease (AD) research has moved on. As a neurodegenerative syndrome, diagnosis initially hinged on the clinical picture, cognitive testing, and MRIs. More recently, however, this extremely complex disease continuum has come to be defined, primarily, by its biological manifestations.

Defining the disease by its biology enables more consistent characterisation and aids in understanding the disease and its mechanisms better (1). At the same time, it enables greater precision in clinical trials (1). With the revolution toward personalised and precision medicine, in the realm of AD, biomarkers are crucial. Biomarkers related to precise states and stages of the disease, at an individual level, are needed to provide the best patient management options, and are also needed as clinical trial endpoints to help further our understanding of this multifactorial disease.

Because of the intense search for effective biomarkers for AD and for central nervous system disease in general, the race toward validation of new biomarkers in these indications is fast-paced and exciting. This article discusses current biomarkers and non-invasive, investigational biomarkers for research and diagnosis of Alzheimer's disease, as well as clinical trial partner capabilities that may be helpful along the path to development.

Currently Established Alzheimer's Disease Biomarkers for Early Diagnosis and Clinical Trials

We now know that biological signs in AD appear 15 to 20 years before the first clinical symptoms, as levels of amyloid-β (Aβ) and tau peptides accumulate in the brain. These aggregates trigger intracellular signaling that leads to apoptosis.

The core cerebrospinal fluid (CSF) biomarkers of neuronal degeneration include:

- Beta-amyloid 42 protein (Aβ42)
- Total tau protein (t-tau)
- Phosphorylated tau protein (p-tau)

CSF levels of these three biomarkers can stage the disease with precision. Imaging studies are also used in AD diagnosis. Beta amyloid aggregation and cortical atrophy can be detected by MRI. Specialised positron emission tomography (PET) imaging with radiotracers allow visualisation of both tau-protein tangles and β-amyloid plaques in vivo (2).

In summary, a combined approach currently used in differential diagnosis to confirm AD and in evaluations during clinical trials may include:

- Clinical tests
- Cognitive tests
- Imaging
- Biomarkers in CSF

While these imaging and CSF biomarker studies are capable of detecting AD in the presymptomatic phase 15 to 20 years before the onset of symptoms, they are too expensive, invasive, and unavailable to be used as screening tests. By the time patients present, they are already cognitively impaired. While we do not yet have effective therapies, there is evidence that the institution of early lifestyle interventions can

prevent predisposed individuals from progressing to dementia (3-4). For screening and for clinical trial qualification, practical early screening tests are needed.

Developing Blood-Based Biomarkers for Early Diagnosis of Alzheimer's Disease

As yet, no routine clinical diagnostic techniques are available to confirm AD from blood samples. However, a large number of ongoing studies and recent publications focus on promising blood biomarkers for this purpose.

- P-tau 181: An ultrasensitive blood immunoassay demonstrated that plasma p-tau 181 levels correlated with the degree of tau and β-amyloid pathologies and differentiated AD from other neurodegenerative disorders (5). Several other studies awaiting publication concur
- β-amyloid 42/40: An immunoprecipitation and liquid chromatography-mass spectrometry assay showed that plasma Aβ42/Aβ40, especially combined with age and apolipoprotein E (APOE ε4) status (see below) accurately diagnosed brain amyloidosis (confirmed by PET), even in cognitively normal individuals (6)
- Aβ42 and t-tau: An immunomagnetic reduction assay in a small study of patients with mild cognitive impairment suggested that elevated plasma Aβ42 and t-tau levels are associated with later cognitive decline (7)
- Neurofilament light chain (NfL): Concentrations of plasma NfL were shown to correlate with degree of neurodegeneration in Aβ+ subjects, as shown by decreased fluorodeoxyglucose uptake in PET imaging (8)

Assays sensitive enough to detect these biomarkers at very low concentrations in the blood will likely soon aid in the clinical diagnosis of prodromal Alzheimer's disease through a simple blood test.

A research lab with a track record of innovative and early-stage implementation of non-invasive biomarker techniques is most likely to offer drug developers the requisite services for successful translation from bench to market. Standard and esoteric testing; experts with both practical and extensive, collaborative research experience; and special divisions devoted to validation and translation of new biomarkers from bench to market, are all key for successful biomarker, and companion, diagnostic development to aid in clinical trials.

Genetics Presents Additional Biomarkers for AD Diagnosis and Research

Understanding as much about a disease as possible can yield additional predictive biomarkers and uncover therapeutic opportunities by identifying disease causes, mechanisms, and typical progression. Recently, though AD is largely sporadic, genomic studies across large cohorts have identified several genetic associations for the disease. Biomarker changes in these heritable forms of AD are generally similar to those for sporadic AD (9).

For example, an autosomal-dominant, early-onset form of AD features a genetic mutation that impacts signaling at the neuronal level. Specifically, affected genes code for presynaptic vesicle protein synaptotagmin and presenilin, a catalyst in amyloid protein precursor (APP) conversion to amyloid β-protein (10). This insight makes these proteins biomarker candidates for AD in general.

As another genetic biomarker example for AD, different APOE polymorphs ε2, ε3, and ε4 are associated with varying concentrations of CSF Aβ42. The APOE genotype affects a patient's risk factor for developing AD. Patients with the ε4 allele demonstrate earlier decreases in CSF Aβ42 concentrations, corresponding with earlier amyloid deposition. The increased risk is greatest in homozygous ε4 carriers, but is also elevated in heterozygous ε4 carriers (11).

Genetics-driven biomarker discovery for AD is ongoing, with a recent genome-wide meta-analysis confirming 20 known high-risk loci and identifying five new ones, paving the way for future investigations (12). Involved pathways were related to immunity, lipid metabolism, tau binding proteins, and APP metabolism, mirroring the complex nature of Alzheimer's disease.

Multiomics Approach to Biomarker Discovery

No single biomarker is likely to function as the signature for as complex a disease as AD. Characterising the disease fully may require incorporation, not only of genomics factors, but also transcriptomics factors, proteomics factors, lipidomics, and metabolomics factors, all working in tandem. A team with a high degree of skill and an expert technical platform are required to integrate all the separate components of metabolic survey research into a complete and clinically relevant profile.

CNS Biomarkers for Other Diseases

When cognitive and clinical tests are inconclusive, differential diagnosis among neurodegenerative diseases can be a challenge. Special biomarkers can help in these cases as well. For example, in the absence of biomarkers for AD, elevation in special biomarker studies of CSF protein 40-3-3 can suggest rapidly progressive dementias from cerebral injury or prion diseases, such as Creutzfeldt-Jacob (CJD). To confirm a diagnosis of CJD, cerebrospinal fluid real-time quaking-induced conversion is a highly sensitive and specific test for the presence of protease-resistant prion protein (13).

Future Diagnostic Profiling, Research Tools, and Therapeutic Developments

Through the combination of metabolomics, genomics, and proteomics, biomarker profiles of patients predisposed to develop AD can be created. At the same time, clinical research diagnostics can be developed to better elucidate the dysfunctional pathways leading to AD brain pathology, along with corresponding therapeutic targets.

In the future, gene therapies – possibly RNA interference techniques – may be devised to decrease the level of amyloid precursor protein gene expression in these patients to prevent or delay the onset of Down's symptoms.

Non-Invasive Biomarkers for Alzheimer's Disease Will Help with Prevention Soon and Therapies Later

With the ageing global population, decreasing the incidence of Alzheimer's disease is a current research priority. In the near future, novel, non-invasive AD biomarkers will likely be available for prevention screening and to assist with clinical research. This blood-based biomarker testing may include quantification of plasma tau, β-amyloid, and neurofilament concentrations.

Effective development of biomarkers that enable precision medicine and personalised medicine requires true expertise in a gamut of specialties from proteomics, to genetics, to metabolomics. It also requires experienced assistance in biomarker validation and planning.

Biomarker studies and multiomics profiling will provide a better understanding of Alzheimer's disease, help discover new therapeutic agents, and allow preventive interventions to be implemented well before the onset of this widespread and devastating disease.

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