Biomarkers in Alzheimer’s Disease—Perspectives for the Future

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Abstract
Alzheimer’s disease (AD) is characterized pathologically by the overproduction or excessive accumulation of amyloid beta protein leading to the deposition of diffuse and neuritic plaques. Hyperphosphorylation of tau protein promotes the accumulation of intracellular neurofibrillary tangles. These events comprise a pathological cascade in the AD brain that includes oxidation, inflammation, excitotoxicity, and apoptosis with activation of myriad associated intracellular pathways. Some of these neuropathological changes are detectable by biomarkers such as neuroimaging or serum or cerebrospinal fluid (CSF) measures. Magnetic resonance imaging (MRI) reveals cerebral atrophy, fluoroexoxyglucose positron emission tomography (FDG-PET) shows regional brain hypometabolism, amyloid imaging demonstrates fibrillar amyloid in plaques, and CSF measures reveal decreased levels of the amyloid protein and increased levels of total tau protein and hyperphosphorylated tau. These biomarkers provide the basis for identifying and characterizing asymptomatic pre-clinical AD pathology, minimally symptomatic prodromal AD, and AD type dementia. Biomarkers have an increasingly important role in AD care and research at all stages of disease, and promise to assist in the development of new AD therapies.

Keywords
Alzheimer’s disease, drug development, biomarkers, cerebrospinal fluid, amyloid imaging, fluoroexoxyglucose positron emission tomography, magnetic resonance imaging, target engagement, pharmaceutical industry, differential diagnosis, prognosis

There has been remarkable progress in developing biological markers (biomarkers) that provide insight into the progression of Alzheimer’s disease (AD). Biomarkers document the course of AD from structural, functional, molecular, and chemical perspectives, and promise to assist in the development and monitoring of new therapies. Biomarkers have become particularly important to AD drug development for their potential to establish efficacy in periods of time shorter than required to demonstrate clinical efficacy and to support a disease-modification claim when applying to the US Food and Drug Administration (FDA) as a disease-modifying treatment of AD.

This article describes the currently available biomarkers and their role in clinical practice and research, and discusses some of the anticipated applications of biomarkers for AD. Disease-course biomarkers and drug-activity biomarkers are emphasised. The use of biomarkers to monitor adverse events and other aspects of drug development are presented.

Definition of Biomarkers
A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.1 Biomarkers include neuroimaging, cerebrospinal fluid (CSF) measures, blood-based assessments, and, in some cases, urinary or salivary measures. The definition emphasizes several major aspects that must be considered when choosing and using biomarkers. A biomarker must be objectively measured; this implies that collection, storage, standards, and reference methodologies are available. The biomarker is an indicator of a biological process, disease, or therapy; the biomarker is a limited window on a process or response that is viewed as a guide to the disease processes ongoing in the brain or body.

In the case of AD, biomarkers provide documentation of brain atrophy, reduced brain metabolism, changed brain activation, deposition of fibrillar amyloid, alterations in CSF constituents, or alterations in serum measures.7 There are ‘trait’ and ‘state’ biomarkers for AD. Trait markers include genetic mutations involving the amyloid precursor protein (APP) and presenilin (PS) genes, as well as risk factor genes such as the
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Figure 1: Magnetic Resonance Imaging Map of Regional Cortical Thickness Showing the Areas of Difference Between Normal Elderly and Early Alzheimer’s Disease

Mini-Mental State Examination (MMSE) score >18. Left figures show the significant differences between the two groups, the right figures show the percent difference between the two groups; left and right hemispheres and lateral and medial hemispheres are shown. Figure courtesy of Apostolova L, Huang K, Babakchanian S, University of California, Los Angeles.

Figure 2: FDDNP Scan of an Individual with Prodromal Alzheimer’s Disease

An abnormal signal is shown in the temporal frontal, cingulate and brainstem regions.

Functional MRI demonstrates regional brain activation in response to specific cognitive tasks. It is most useful in patients with MCI or patients with mild AD; substantial patient cooperation is required for most activation tasks. Within this subgroup of patients, changes in functional MRI (fMRI) correlate with ADAS-cog scores.

Fluorodeoxyglucose (FDG) positron emission tomography (PET) reveals reduced metabolism in the precuneus/posterior cingulate and in the parietal regions bilaterally in patients with AD and in many patients with MCI who progress to AD dementia. Glucose is metabolized primarily by synapses and reduced FDG activity implies a reduction in synaptic function. FDG PET correlates with cognitive measures including the ADAS-cog, and some investigators have observed correlations between higher CSF amyloid beta protein (Aβ) and greater PET metabolism, as well as poorer metabolism in patients with higher CSF total tau (t-tau) or hyperphosphorylated tau (p-tau).

FDG PET is useful in differentiating AD from non-AD type of dementia, such as frontotemporal dementia. Amyloid imaging is among the newest and most powerful biomarkers to emerge in AD diagnosis and drug development. The ligands used attach to fibrillar amyloid and the resulting scan demonstrates the presence of the type of amyloid found in neuritic plaques and some diffuse plaques. Soluble forms of amyloid such as the Aβ monomer or oligomer are not labelled. Several ligands have been developed for amyloid labelling including Pittsburgh Compound B (PIB), florbetaben, and AV-45. Amyloid imaging shows little change over the course of AD, which suggests that the total amyloid burden is relatively stable after the onset of the dementia phase of AD. There is limited correlation between Aβ burden as seen with amyloid imaging and cognitive function. FDDNP imaging differs from other amyloid ligands in attaching to both fibrillar Aβ and aggregated tau protein, providing an image of the regional distribution of neuritic plaques and neurofibrillary tangles (see Figure 2). Correlations are evident between FDDNP signaling and cognitive deterioration.

CSF Aβ42 (the 42 amino acid form of Aβ), t-tau protein, and p-tau protein undergo characteristic changes in the course of AD. Aβ42 declines whereas t-tau and p-tau levels increase. The ratio of decreased Aβ42 and increased tau is characteristic of AD, and when present in MCI, predicts progression to AD-type dementia. Once abnormal, CSF Aβ and tau levels remain relatively stable over the course of AD. Reduced Aβ42 is attributed to the deposition of amyloid in the fibrillar plaques with decreased availability of the Aβ monomer measured in the CSF. Reduced CSF Aβ42 levels correlate with brain Aβ levels as measured by amyloid imaging. Tau protein is a non-specific indicator of cell injury with discharge of tau protein into the CSF as cells die; neurofibrillary tangles comprise p-tau, and the appearance of this protein in the CSF reflects the death of tangle-bearing neurons.

No consensus has been reached on blood measures that assist in the diagnosis of AD. Aβ42 may be elevated in at-risk individuals and decline with disease onset, but the mean levels do not distinguish between populations of patients with AD and normal elderly subjects. Several panels of proteomic measures have been proposed as diagnostic of AD and await further verification.

Disease-course Biomarkers

The most well-studied biomarker for AD is structural imaging using magnetic resonance imaging (MRI). Measures useful in characterizing AD include hippocampal and medial temporal atrophy, whole brain atrophy, and ventricular enlargement. Hippocampal atrophy in normal aged persons predicts progression to dementia of the Alzheimer’s type, and hippocampal or medial temporal atrophy in patients with mild cognitive impairment (MCI) predicts progression to AD dementia. Hippocampal atrophy correlates with impairment of memory, but not in some studies with trial measures such as the Alzheimer Disease Assessment Scale—cognitive portion (ADAS-cog) or the Clinical Dementia Rating (CDR) Scale.

Whole brain atrophy and ventricular enlargement correlate with these more global assessments in most studies. Advanced analytic strategies allow the use of MRI to determine cortical thickness, a measure relevant to regional brain atrophy (see Figure 1).
Tracking the Course of Alzheimer’s Disease with Biomarkers

Normal elderly people consist of three groups of individuals: those who will never develop AD, those who will develop AD but do not yet have any evidence of the disease in the brain, and those who have the initial manifestations of AD in the brain but remain within the normal range of cognitive function. The second of these groups is typically characterized by risk factors such as ApoE 4 genotype, older age, female sex, mid-life hypertension, diabetes, hypercholesterolemia, and history of head trauma. The third group of normal elderly—with AD pathology—is recognizable only with biomarkers that demonstrate the presence of AD-type pathology in the brain.

The first recognized change to occur in AD is the reduction of Aβ42 levels in the CSF; this is followed by positive amyloid imaging and medial temporal lobe atrophy. The biomarker changes predict the progression from AD pathology only to prodromal AD. In prodromal AD, cognitive changes occur, especially impairment of episodic memory, progression from AD pathology only to prodromal AD. In prodomal AD, cognitive changes occur, especially impairment of episodic memory, and MRI atrophy progresses. With progression to AD-type dementia, cognition declines further and involves multiple cognitive domains. FDG PET demonstrates more widespread hypometabolism typically including the frontal lobes. MRI reveals diffuse cortical atrophy and ventricular enlargement. Amyloid imaging and CSF measures remain stably abnormal. Figure 3 shows the relationship of biomarkers to the course of AD.

Drug-activity Biomarkers

Drug development is facilitated by employing a combination of biomarkers, including assessments documenting a direct effect of the drug on the biological target and biomarkers demonstrating an effect of the treatment on disease course. Progress is being made in developing disease-course biomarkers through collaborative efforts such as the AD Neuroimaging Initiative (ADNI). Few drug-activity biomarkers have been identified. One promising approach is stable isotope labeled kinetics (SILK) in which an indwelling spinal fluid catheter is used to make serial measures of Aβ production and clearance. A decrease in Aβ production has been shown with gamma-secretase inhibition. Similarly, serum Aβ declines with gamma-secretase inhibition, suggesting that this measure may be responsive to drug treatment, although not a discriminating diagnostic measure. Aβ imaging may provide an opportunity to demonstrate reduced accumulation of Aβ in prevention trials (discussed below) or to measure disaggregation or removal of Aβ in trials of agents that interrupt protein aggregation or enhance removal. Bapineuzumab, a monoclonal antibody targeting Aβ, was shown to decrease brain Aβ burden as measured by PIB PET imaging.

A wide variety of biomarkers for AD have been proposed that relate to many cellular signaling and disease pathways. Linking effects of agents with specific mechanisms of action to these pathway-dependent biomarkers offers an opportunity to develop drug-activity biomarkers critical to advancing AD drug development. Assessment of these biomarkers should begin in the pre-clinical phases of drug development to allow progressive understanding of collection, banking, measurement, and dose relationships prior to application in human drug trials. Putative disease-modifying agents should be advanced to clinical testing only if a plausible biomarker is available.

Toxicity Biomarkers

Biomarkers have a crucial role in detecting toxicity in drug development programs and clinical trials. Toxicity biomarkers include liver function tests, electrocardiograms, and other laboratory assessments, as well as biomarkers to evaluate toxicities specific to AD patients; for example, immunotherapies have been associated with cerebral vasogenic edema, which is routinely monitored in immunotherapy trials with MRI.
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Biomarkers in Alzheimer’s Disease Clinical Trials

Figure 4 summarizes the potential diverse roles played by biomarkers in clinical trials.

Phase I trials are first-in-human trials that involve cohorts of 10–12 individuals given single increasing doses of the test drug and then multiple doses of the candidate agent for up to two weeks of therapy. Phase I trials are designed to demonstrate the safety and tolerability of the therapy. Most phase I trials involve healthy volunteers; demonstration of efficacy is not a goal of these studies. Biomarkers in phase I focus on safety. Drug-activity biomarkers may be integrated into some phase I trials; the SILK technique can be used with healthy volunteers to document effects on AP production and clearance.38,39

Phase II AD trials typically involve symptomatic AD patients. Phase IIa studies seek to demonstrate proof of principle (POP) or proof of concept (POC) and phase IIb studies are intended to establish one or two doses to advance to phase III. POC studies are intended to establish clinical efficacy. This represents a major challenge, as showing efficacy requires an adequately powered study for a duration of at least six months for a symptomatic agent and 12–24 months for a disease-modifying agent.40 POP trials using biomarkers represent a plausible alternative to collecting clinical information, but this approach involves substantial risk as no biomarker has been shown to predict clinical outcome. Biomarker measures are much less variable than clinical outcomes, and demonstrating a drug-placebo difference theoretically requires many fewer patients. Jack and co-workers41 estimated, on the basis of a sample from a clinical trial of mild to moderate AD patients, that a one-year trial would need 21 patients per arm to demonstrate a 50% reduction in hippocampal atrophy and 54 patients per arm to show a 50% difference in temporal horn enlargement (90% power). This compares with 320 to show a similar drug-placebo difference on the ADAS-cog. Such projections may be optimistic as they depend on measuring cerebral atrophy and most drugs would affect atrophy only as a secondary effect with uncertain relationships to the drug’s primary mechanism of action. The power analyses however demonstrate the marked differences between biomarkers and clinical outcomes in showing drug effects. In the future it may be possible to substitute biomarker measures for clinical outcomes in phase II, and such measures may be used now as major secondary outcomes to build understanding of their use in trials, their sensitivity to drug effects, and their potential application in phase III.

The role of biomarkers in phase III differs from that in earlier stages of drug development. Phase III trials are confirmatory trials involving large numbers of AD patients and designed to demonstrate efficacy of the doses intended for use after marketing. Clinical data are the primary outcomes of phase III; a drug-placebo difference must be demonstrated in two well-conducted trials on a measure of cognitive function (usually the ADAS-cog) and a global or functional measure. Biomarkers included in phase III are supportive of the clinical data and can be used to apply for labeling relevant to disease-modification.42 To support a disease-modification claim, the biomarker and the clinical data must be correlated. This correlation supports the hypothesis that the biomarker effects and the clinical effects are mediated by the same mechanism.

Prevention of AD is the ultimate desirable goal of drug development for AD. Recognition of patients at risk for the disease combined with effective therapy could result in prevention of AD or delay of serious cognitive decline until death occurs from competitive late-life mortality. Patients included in prevention trials are by definition without cognitive abnormalities and cannot be identified by clinical assessment. Elderly patients appropriate for prevention trials who are cognitively normal but harbor the earliest change of AD can be identified by documenting low levels of CSF Aβ42 or by demonstrating an abnormal burden of brain amyloid with amyloid imaging. In this type of prevention trial, biomarkers represent the only methods for identifying trial participants. These trials could have a drug-placebo difference in cognitive decline, functional decline, reaching a disease milestone (e.g. meet criteria for mild cognitive impairment), or a biomarker as the trial outcome.

When no treatment is available for a given condition, the disorder has disastrous consequences (as is the case of AD), and there is a biomarker that is reasonably likely on the basis of epidemiologic, therapeutic, and pathophysiologic evidence to predict clinical outcome, a drug can be approved based on findings from on an unvalidated biomarker.43 This provides a way forward in AD drug development as there are no validated biomarkers available to predict clinical response. Drugs approved with unvalidated markers must provide meaningful therapeutic benefit and be superior to existing products. Post-marketing studies to establish the predictive relationship of the biomarker to the clinical outcome may be required. An unvalidated marker intended for use in a trial must go through a fit-for-purpose approval process with the FDA. With these caveats, it is conceivable that AD trials – including prevention trials – can be performed with an unvalidated but well-supported biomarker as the primary outcome.

Corporate decision-making can be greatly facilitated by biomarkers. Compounds can be prioritized and optimized on the basis of biomarker data. Biomarkers may suggest stopping a program with well-documented biological adverse events. Cycle times should eventually be shortened and development costs reduced by biomarkers. These benefits will become more obvious as databases of biomarker data are constructed, multiple compounds are assessed with the same marker, and markers for multiple pathways are identified. This will allow incremental advances in linking biomarkers to clinical outcomes.

Future Applications of Biomarkers

In the future, biomarkers may have a much greater role in medical decision-making. Detection of asymptomatic individuals would be entirely biomarker-dependent and the availability of markers of early disease such as amyloid imaging would benefit early recognition and diagnosis. Once identified, biomarkers may play a role in guiding therapy. Amyloid imaging, tau imaging, and alpha synuclein imaging would provide insight into the protein-misfolding patterns present in the brain and would facilitate differential diagnosis. Intervention would be guided by the predominant protein or protein ensemble. Microglial imaging may help identify the inflammatory component of the illness and suggest avenues for therapy.44 Serum or CSF measures may provide additional informative data to guide the therapeutic choice or dose.
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Biomarkers may assist in identifying subpopulations of AD patients responsive to specific therapies. Bateman et al. reported that carriers of the APOE-ε4 allele are more likely to have a better response to the drug galantamine than non-carriers, while carriers of the APOE-ε2 allele may have a worse response. Another study found that carriers of the APOE-ε3 allele were more likely to respond to donepezil than non-carriers.

The use of biomarkers in clinical trials has increased over the past decade, with the number of clinical trials using biomarkers as endpoints increasing from 18% in 2002 to 38% in 2010. This trend is likely to continue as the importance of early identification and intervention in AD continues to grow.


2. Cummings JL, Biomarkers in Alzheimer’s disease drug development, Alzheimer’s Disease and Dementia, 2010; in press.


