Review Article

Alzheimer’s disease biomarkers in animal models: closing the translational gap

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Abstract: The rising prevalence of Alzheimer’s disease (AD) is rapidly becoming one of the largest health and economic challenges in the world. There is a growing need for the development and implementation of reliable biomarkers for AD that can be used to assist in diagnosis, inform disease progression, and monitor therapeutic efficacy. Preclinical models permit the evaluation of candidate biomarkers and assessment of pipeline agents before clinical trials are initiated and provide a translational opportunity to advance biomarker discovery. Fast and inexpensive data can be obtained from examination of peripheral markers, though they currently lack the sensitivity and consistency of imaging techniques such as MRI or PET. Plasma and cerebrospinal fluid (CSF) biomarkers in animal models can assist in development and implementation of similar approaches in clinical populations. These biomarkers may also be invaluable in decisions to advance a treatment to human testing. Longitudinal studies in AD models can determine initial presentation and progression of biomarkers that may also be used to evaluate disease-modifying efficacy of drugs. The refinement of biomarker approaches in preclinical systems will not only aid in drug development, but may facilitate diagnosis and disease monitoring in AD patients.

Keywords: Alzheimer’s disease, biomarkers, animal models, drug development

Introduction

Alzheimer’s disease (AD) represents a looming crisis as the number of victims continues to increase and limited treatments are available. There is a critical need for advances in the treatment of AD and in the tools used to detect cases early and monitor progression. Studies of AD populations show that biomarkers, including imaging approaches [1-3] and evaluation of cerebrospinal fluid (CSF) and blood [4-6], have value in diagnosis and tracking disease progression. Numerous candidate markers have been identified such as CSF or plasma levels of Beta-amyloid protein (Aβ) and phosphorylated tau (ptau); however, many lack the specificity necessary to be used diagnostically or do not correlate with clinical progression. An opportunity exists with the animal model systems that are employed in the study of AD mechanisms to examine potential biomarkers. Many of the investigations of mechanisms in AD also provide suitable targets for evaluation of novel treatment efficacy in both animal models and clinical populations. Investigation of potential biomarkers in animal model systems lends itself to examination of mechanisms underlying correspondence between central pathology and peripheral markers. Moreover, relationships in animal models can be explored between peripheral biomarkers and readily available neuropathology; these can be translated into human studies where biomarkers are accessible but neuropathology is often not.

Biomarkers can assist in monitoring the progression of AD and inform treatment approaches as the disease advances. Aβ targets, for example, may be more appropriate very early in the disease course and tau targets may be more relevant later in the disease. The complex evaluation and staging of treatment of AD requires assessment in animal models exhibiting core pathological features.

In this review we highlight several candidate biomarkers in AD that have been evaluated in preclinical animal models. Several potential
biomarkers have been suggested related to core disease pathologies (Aβ and tau), as well as those associated with AD risk factors (such as microglial and immune activation). We also discuss the need for greater evaluation of candidate biomarkers in preclinical systems in parallel with the investigations of brain pathology that is the central approach to animal models of AD.

Peripheral markers

The amyloid cascade hypothesis posits that early accumulation of Aβ leads to synaptic dysfunction, neurodegeneration, and cognitive deficits [7, 8]. Although substantial evidence exists to support this hypothesis, the mechanisms underlying the pathogenic contributions of Aβ remain elusive. Whether the deposition of Aβ into insoluble plaques or smaller, soluble species of Aβ drive neurodegeneration is unclear; however, recent evidence suggests Aβ oligomers (oAβ) may be more neurotoxic [9-11]. Early work with Tg2576 mice demonstrated a reduction in CSF and plasma levels of Aβ42 [12], findings which mirror AD patient data. In PDAPP mice, Aβ42 levels positively correlated with the abundance of plaques [13], contrary to what has been found in AD [14]. Canines represent a strong model for investigating disease-state mechanisms as they develop age-related Aβ deposition and cognitive deficits, which show similarities to human AD [15]. CSF Aβ42 and oAβ levels decreased with age and inversely correlated with plaque load, indicating these markers may reflect brain amyloidosis [16]. These observations mirror those in humans. In aged canines with mild cognitive impairment (MCI), plasma Aβ42 levels were increased compared to unimpaired or severely impaired dogs [17].

The detection and quantification of Aβ species in vivo has encountered difficulties, and questions have been raised about the feasibility of reliably measuring oligomers in CSF [18]. Specific ELISAs have been developed for the quantification of Aβ levels, which have been demonstrated to increase with age in the brains of transgenic (Tg) AD mice [18, 19]. However, depending on the specific antibodies utilized, oAβ has not been consistently detected in human CSF [18, 20]. Using flow cytometry, the increased presence of Aβ has been reported in the CSF of AD patients compared to controls [21]. The reliable quantification of Aβ species from biological fluids could serve as an important measure of therapeutic efficacy in preclinical models, underscoring the import of the development of a precise detection technique.

One of the principal enzymes responsible for amyloidogenesis, β-site amyloid precursor protein cleaving enzyme 1 (BACE1), has promise as a therapeutic target. Novel techniques for quantifying BACE1 levels from the CSF and plasma are emerging, permitting a primary measure of target engagement [22, 23]. Inhibitors of BACE1 decreased CSF and plasma levels of Aβ40 and Aβ42 in mice, guinea pigs, and non-human primates [23-26]. Levels of soluble amyloid precursor protein β (sAPPβ) are also decreased in plasma following BACE inhibition in rhesus monkeys [23], while mixed results have been observed from human AD plasma [23, 27]. An inhibitor of cathepsin B, one of the enzymes in the β-secretase complex along with BACE1 [28], reduced Aβ40 and Aβ42 in the CSF and plasma of guinea pigs [29]. Extending these findings and approaches to Tg models of AD could offer additional insight into therapeutic candidate efficacy and mechanisms of disease. BACE1 activity can be measured in human CSF permitting it to be used as a measure of target engagement by inhibitors of this enzyme.

Inhibition of gamma-secretase has been a target of therapeutic development despite initial high-profile failures [30]; more recent drugs have attempted to avoid Notch-related toxicity and side effects [31, 32]. Peripheral measurement of Aβ following administration of gamma-secretase modulators could provide a fast and reliable readout in addition to behavioral outcomes. Gamma-secretase modulators and inhibitors have been shown to reduce CSF and plasma Aβ levels in non-Tg rats and guinea pigs [33-35], reinforcing the utility of investigating peripheral biomarkers in animals and clinical trials. Using stable-isotope-labeling kinetics (SILK) methods, a gamma-secretase inhibitor was shown to reduce CSF Aβ production in rhesus monkeys without a subsequent rise in Aβ production [36]. Because gamma-secretase inhibitors lead to altered proteolysis of APP and the generation of shorter Aβ fragments (i.e. Aβ (1-15)), assays that target these isoforms may provide a reliable measure of drug activity, as has been demonstrated in canine CSF [37].
A number of immunotherapies have been developed and tested in AD models, most of which target amyloid pathology. Central immunotherapy may produce secondary, undesirable immune responses, and it is important to evaluate markers of inflammation as possible indicators of an adverse response. Plasma levels of interleukin (IL)-10, an anti-inflammatory cytokine, have been found to be increased following Aβ immunotherapy in Tg2576 mice [38, 39]. In contrast, T cell infusions specific for Aβ or administration of granulocyte colony stimulating factor (GM-CSF) reduced plasma levels of IL-4, TNF-α, and other cytokines [40, 41]. Inflammatory mechanisms and their peripheral markers can be further explored with parallel observations in humans and animals.

Isoprostanes, which reflect lipid peroxidation and oxidative stress [42], are elevated in plasma from Tg2576 mice in advance of plaque formation [43], suggesting isoprostane levels may have utility as a predictive biomarker.

Microgliosis is an important indicator of drug activity and a common pathological finding in AD [44]. Although microglial activity may be assessed with neuroimaging techniques, important information can be quickly and inexpensively obtained through peripheral measures. In rhesus monkey plasma, a non-viral Aβ vaccine did not alter chemokine (C-C motif) ligand 2 (CCL2) expression [45]. More studies may benefit from investigation of microglial markers in the future. However, care must be taken in the interpretation of immune responses from mice as recent evidence suggests they may not translate well to human inflammatory diseases [46].

Currently the measurement of tau and phosphorylated tau (p-tau) from human or animal fluids is restricted to the CSF. Studies with Tg mice or rats have demonstrated elevated CSF p-tau and tau levels with age [47, 48]. Recent work has indicated promising data in the development of assays to reliably quantify ptau and tau from plasma and serum that would significantly advance the utility of blood-based markers [49].

**Imaging**

Imaging approaches in AD models have demonstrated strong correspondence with findings from AD populations, suggesting these techniques may have considerable translational utility. Imaging studies may complement behavioral and postmortem readouts in animals, especially in the evaluation of treatments designed to target different stages of pathological severity. Several imaging approaches have been employed in animal models of AD, including positron emission tomography (PET), magnetic resonance imaging (MRI), and magnetic resonance spectroscopy (MRS).

Fluorodeoxyglucose (FDG)-PET imaging shows consistent patterns in AD making it a suitable measure of drug efficacy in patients and animal models. Similar to AD, reduced FDG uptake has been observed in PDAPP mice [50-52], PSAPP mice [53], 3xTg-AD mice [54], and PLB1 mice [55]. However, null effects in other studies suggest the small size of mouse brains may limit the utility of FDG-microPET in mice [56, 57].

Radiolabeled amyloid imaging agents provide a measure of plaque load and may serve to longitudinally track changes in amyloidosis during clinical trials. Despite initial difficulty with uptake and retention of Pittsburgh compound B (PIB) in Tg AD mice [58, 59], recent studies indicate PSAPP mice display strong, age-related amyloid loads with PIB [60], while others have demonstrated that a high specific radioactivity facilitates PIB-microPET imaging in APP23 mice [61, 62]. Additional investigations have demonstrated utility in voxel-based analyses of plaque load using PIB in APP/PS1 double transgenic mice [63]. Work with monkeys shows that amyloid burden can be observed with PIB in multiple species [64]. An alternative radiotracer, 2-(1-{6-[18F-fluoroethyl](methyl) amino}-2-naphthyl)ethylidene)malononitrile (FDDNP), binds to plaques and tangles, displaying increased retention in aged macaques [64] and Tg AD rats [65], FDDNP binding to tangles has not been demonstrated in animal models; another ligand --- 18F-THK523 --- binds selectively to tangles in Tg mice expressing mutant tau [66].

A novel amyloid imaging probe recently approved by the US Food and Drug Administration (FDA) for use in PET imaging, florbetapir (Amyvid™), shows robust labeling in monkeys and PSAPP mice [67]. Another study with florbetapir revealed strong retention in the cortex, hippocampus, and striatum of PSAPP
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mice [68], areas rich in plaques. Each of these amyloid imaging agents could act as signpost markers of disease progression or determine Aβ immunotherapy efficacy. There is a consistent inverse correlation between CSF Aβ levels and brain amyloid load as demonstrated with amyloid imaging, indicating that the fluid marker is a good guide to this central aspect of AD.

The microglial probe 11C-(R)-PK11195 binds to the 18 kDa translocator protein (TSPO), also known as the peripheral benzodiazepine receptor, which reflects neuroinflammation [69]. PSAPP mice display progressively increased retention of this ligand, which mirrored the postmortem abundance of microglia [70]. Work with other TSPO probes suggests microgliosis may be more closely associated with tau pathology than amyloid pathology in mutant tau or APP mouse models, respectively [71].

MRI provides superior spatial resolution compared to PET, indicating it may be better suited to microimaging in Tg mice. Similar to AD populations, several Tg AD mouse lines have shown reduced volume of several brain structures including the hippocampus and cortex [72-77]. Other approaches to model AD also revealed brain atrophy with MRI, including aged rabbits chronically administered Aβ42 [78] and monkeys administered streptozotocin, a drug that disrupts insulin signaling and induces a diabetes phenotype and AD pathologies [79]. Because MRI-detected atrophy is progressive, a staging could be established which correlates with tau pathology or other markers of neurodegeneration and permits the evaluation of various stage-dependent therapeutics (i.e. MCI vs. advanced AD).

A variation on MRI, arterial spin labeling (ASL), is able to quantify differences in regional cerebral blood flow, which is typically reduced in AD [80-82]. Similarly, Tg mouse models of AD display cerebral hypoperfusion compared to wild-type (WT) mice during ASL-MRI [83, 84]. Combined with other imaging techniques or fluid biomarkers, ASL-MRI may assist in diagnosis and evaluation of clinical trial outcomes.

Using MRI, amyloid-specific contrast agents can visualize in vivo plaque load. A variety of approaches have been used to image plaques in Tg AD mice by coupling amyloid compounds to specific probes, including gadolinium to Aβ [85-89], 19F or 1H amyloidophilic agents [90, 91], and nanoparticle-based probes [92-94]. Although these compounds have not yet been tested in AD patients, their utility for measuring amyloid burden during testing of pipeline agents should be investigated. The enhanced spatial frequency associated with MRI makes it preferable to PET-based probes, especially for detecting subtle, region-specific differences. Plaques have also been visualized in AD mouse models without the help of a contrast agent because of their high iron content using high field intensity MRI [95-99]. Concerns about the consistency of interplaque metal content and non-specific arterial binding limit the application of this approach [99, 100].

Deficits in axonal transport have been discovered in Tg2576 and 3xTg-AD mice before Aβ and tau pathology with manganese-enhanced MRI (MEMRI) [101, 102]. Although it has not been demonstrated that these changes can be visualized in AD, this technique may prove useful for detecting early neuronal impairments in at-risk, aged individuals. Using ultra-high field diffusion tensor imaging (DTI), gray and white matter degeneration were observed in PSAPP mice [103], similar to what has been found in AD brains.

Proton magnetic resonance spectroscopy (1H-MRS) quantifies neurochemical biomarkers that are different in AD compared to age-matched controls [104, 105]. AD mouse models show a similar pattern of metabolite changes [106], with decreased levels of N-acetyl aspartate (NAA) and increased levels of taurine [107]. Other studies show increased ratios of myoinositol and decreased ratios of NAA levels compared to total creatine [108-112]. Because NAA levels likely reflect neuronal viability [113], 1H-MRS data may serve as an early marker for neurodegeneration or conversely, as a measure of the neuroprotective potential of therapeutic agents. Accordingly, chronic administration of non-steroidal anti-inflammatory drugs (NSAIDs) to aged PSAPP mice mitigated the decrease in NAA and glutamate levels, while reducing plaque burden [114].

Atrophy as measured by MRI and reduced metabolism as assessed by FDG-PET progress in the course of AD and correlate with cognitive decline. This contrasts with amyloid PET where the burden of pathology is relatively stable.
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Table 1. List of biomarkers examined in AD animal systems

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>Aβ42</td>
<td>[12, 13, 16, 17, 23-26, 29, 33-35]</td>
</tr>
<tr>
<td>Aβ oligomers</td>
<td>[16]</td>
</tr>
<tr>
<td>Prototibrillar Aβ42</td>
<td>[133, 134]</td>
</tr>
<tr>
<td>Soluble APPβ</td>
<td>[23]</td>
</tr>
<tr>
<td>Fragmented Aβ</td>
<td>[37]</td>
</tr>
<tr>
<td>Cytokines</td>
<td>[38-41]</td>
</tr>
<tr>
<td>Isoprostanes</td>
<td>[43]</td>
</tr>
<tr>
<td>Tau</td>
<td>[47]</td>
</tr>
<tr>
<td>Ptau</td>
<td>[48]</td>
</tr>
<tr>
<td>FDG-PET</td>
<td>[50-57]</td>
</tr>
<tr>
<td>Pittsburgh Compound B</td>
<td>[58-64]</td>
</tr>
<tr>
<td>FDDNP</td>
<td>[64, 65]</td>
</tr>
<tr>
<td>18F-THK523</td>
<td>[66]</td>
</tr>
<tr>
<td>Florbetapir</td>
<td>[67, 68]</td>
</tr>
<tr>
<td>Microglial probes</td>
<td>[70, 71]</td>
</tr>
<tr>
<td>MRI-based atrophy</td>
<td>[72-79]</td>
</tr>
<tr>
<td>ASL-MRI</td>
<td>[83, 84]</td>
</tr>
<tr>
<td>MRI amyloid contrast agents</td>
<td>[85-94]</td>
</tr>
<tr>
<td>MRI amyloid without contrast</td>
<td>[95-99]</td>
</tr>
<tr>
<td>Axonal transport via MEMRI</td>
<td>[101, 102]</td>
</tr>
<tr>
<td>DTI</td>
<td>[103]</td>
</tr>
<tr>
<td>1H-MRS metabolites</td>
<td>[106-112, 114]</td>
</tr>
<tr>
<td>Proteome</td>
<td>[129]</td>
</tr>
<tr>
<td>MicroRNAs</td>
<td>[132]</td>
</tr>
</tbody>
</table>

throughout the MCI and dementia phases of AD. This may make MRI and FDG-PET more relevant as outcome measures for clinical trials, especially for agents not targeting amyloid-related processes.

Future directions

Proteomic approaches allow the unbiased investigation of a multitude of fluid biomarkers simultaneously in order to increase diagnostic and prognostic accuracy, as well as identify novel targets. With advances in mass spectrometry (MS) and microarray techniques, many groups have recently undertaken proteomic examinations in the CSF and serum/plasma of AD and MCI patients. Although some results have been inconsistent, levels of several proteins have been found to differentiate AD compared to controls, including various apolipoproteins, α1-antitrypsin, and β2-microglobulin [115-121]. Proteomic analyses have been used to differentiate AD from other dementias [122] and predict progression of MCI to AD [123]. Although proteome-based studies of plasma and serum suggest AD may be sensitively characterized with this approach [124-128], few of these reports have been replicated or confirmed.

Proteomic analyses in animal systems are preliminary. A study in tau Tg mice investigated the proteome in blood plasma at a presymptomatic and symptomatic age. A few proteins were identified as potential biomarker candidates such as adenosine triphosphate (ATP) synthase and adenosine kinase [129]. Examining the proteome both in multiple preclinical models and AD could lead to the characterization of a biomarker panel specific to the disorder.

MicroRNAs (miRNAs) have also been implicated in AD pathogenesis and suggested as a putative biomarker [130, 131]. In non-Tg mice fed a high-fat diet, decreased expression of multiple miRNAs was observed in the serum [132]. Substantial translational work is required before miRNAs can be used in the clinic; however, the approach is advancing rapidly.

Alternative approaches have emerged recently that have considerable promise in investigations of cellular variations in core pathological markers. For example, attention is being directed towards the measurement and understanding of post-translational modifications of Aβ. Tg mice carrying the Arctic APP mutation provide an opportunity to study protofibrillar Aβ42; the development of protofibril-specific assays has permitted the quantification of CSF protofibrillar Aβ in these mice [133, 134].

Conclusions

The use of several animal model systems has provided invaluable data regarding AD pathologies and mechanisms involved in neurodegeneration. In this research, the primary focus has been on the examination of central tissues, and to a lesser extent on peripheral markers. For the investigations that have included peripheral measures, considerable progress has been made in the identification of needed biomarkers in AD. The literature documents the extensive effort that is underway in animal model systems to associate core pathological markers with less invasive and peripheral markers that may translate to the clinic readily (see Table 1). Given the progress that has been made, a greater emphasis in probing both central and peripheral tissues in multiple animal model systems would be advantageous. This
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approach may also greatly accelerate translational efforts to impact clinical research. Equally pressing is the need for more data from animal systems investigating biomarkers that can be directly translated to human biomarkers. Continued progress is needed in investigations of the same and overlapping markers in animal models and clinical populations to serve as a translational bridge between animal models systems and clinical populations [135]. Given the emphasis on multiple brain regions in histological analyses of AD animal models and the speed at which studies can be carried out, a directed effort of evaluating candidate biomarkers in parallel with central markers has tremendous potential to enlighten clinical AD biomarker approaches.

Disclosure of conflict of interest

Dr. Cummings has provided consultation to Abbott, Acadia, ADAMAS, Anavir, Avanir, Baxter, Bristol-Myers Squibb, Eisai, EnVivo, Forest, Genentech, GlaxoSmithKline, Grifols, Janssen, Lilly, Medtronic, Merck, Novartis, Pain Therapeutics, Pfizer, Prana, QR, Sanofi, Sonexa, Takeda, and Toyama pharmaceutical companies.

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