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The Role of Biomarkers in Clinical Trials for Alzheimer Disease

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Abstract

Biomarkers are likely to be important in the study of Alzheimer disease (AD) for a variety of reasons. A clinical diagnosis of Alzheimer disease is inaccurate even among experienced investigators in about 10% to 15% of cases, and biomarkers might improve the accuracy of diagnosis. Importantly for the development of putative disease-modifying drugs for Alzheimer disease, biomarkers might also serve as indirect measures of disease severity. When used in this way, sample sizes of clinical trials might be reduced, and a change in biomarker could be considered supporting evidence of disease modification. This review summarizes a meeting of the Alzheimer's Association's Research Roundtable, during which existing and emerging biomarkers for AD were evaluated. Imaging biomarkers including volumetric magnetic resonance imaging and positron emission tomography assessing either glucose utilization or ligands binding to amyloid plaque are discussed. Additionally, biochemical biomarkers in blood or cerebrospinal fluid are assessed. Currently appropriate uses of biomarkers in the study of Alzheimer disease, and areas where additional work is needed, are discussed.

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Keywords

Alzheimer disease; amyloid beta; cerebrospinal fluid; clinical trials; cytokines; isoprostanes; positron emission tomography; tau; volumetric magnetic resonance imaging

INTRODUCTION

The development of new therapies for Alzheimer disease (AD) and other neurodegenerative conditions has become of increasing societal importance given our aging population and increasing longevity, combined with the fact that this disease typically begins late in life. Various biomarkers[1,2] can be used in a variety of ways to allow new therapies to be developed more quickly and to increase the probability of success in the pivotal trials ultimately needed to gain new drug approval by regulatory agencies. On November 11–12, 2004, a meeting of the Alzheimer's Association Research Roundtable was held with experts on biomarkers associated with AD. This report summarizes the information presented and discussed at that meeting. A discussion of the use of biomarkers in the diagnosis of pre-symptomatic AD was also held and will be reported separately.

Biomarkers may be applied to drug development for AD in a number of distinct ways. First, they may be applied as additional diagnostic measures in a population clinically identified as having AD. Sensitivity, specificity, and positive predictive value all must be considered for such an application.

One of the most important uses of biomarkers in drug development is as an indirect measure of disease severity. A number of points should be established for such use: the marker must have a scientific rationale (eg, tau in cerebrospinal fluid [CSF]), the biomarker should change with disease progression in longitudinal observational studies,[3] and the marker must be measurable and reproducible. Unlike typical diagnostic measures, when biomarkers are used for this purpose, high specificity is not required. These biomarkers can be of both scientific and regulatory value. Particularly in mid-phase trials, biomarkers can be used to identify appropriate dosage, improve safety assessments, demonstrate pharmacological activity, and identify preliminary evidence of efficacy.

ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE

The National Institute of Aging has initiated the Alzheimer's Disease Neuroimaging Initiative (ADNI), a large observational study of patients with AD, patients with mild cognitive impairment (MCI), and cognitively normal volunteers to assess longitudinal changes in AD biomarkers. Current trials of investigational treatments require large sample sizes and long treatment durations because cognitive measures do not easily reflect disease-modifying effects of treatment.

Many groups, including pharmaceutical companies, have great interest in using imaging and other biomarkers for treatment trials; however, current data are from many institutions using different methods and different subjects.

Outcome measures included in ADNI are based on previous studies assessing AD biomarkers. Volumetric magnetic resonance imaging (vMRI) will be a major focus of the study, based largely on data from patients with AD and MCI. These data suggest that MCI is a transitional state between normal and early diagnosable disease, and progression from MCI to AD is reflected by changes in brain volumes. Longitudinal studies of patients with diagnosed AD show greater rates of change in hippocampal and temporal horn volumes than are seen with normal aging, based on currently available data.[4] [18]fluoro-deoxy-glucose (FDG) positron

emission tomography (PET) will also be used in ADNI. For patients with AD, decline in glucose utilization as determined by FDG PET imaging is progressive, correlates with dementia severity, and predicts a histopathological diagnosis of AD.[1,5–7]

Based on these and other data, ADNI was established as a longitudinal, prospective naturalistic study of early AD, mild cognitive impairment, and normal aging. MRI, PET, biochemical biomarkers, and clinical data will be included in the final database, which will be placed in the public domain. ADNI's goals include the following: (1) identify the best biomarkers for early diagnosis; (2) identify the best biomarkers for following disease progression and monitoring treatment response; (3) develop surrogate endpoints for clinical trials; and (4) establish methods for the multisite acquisition, quality determinations, and processing of biomarker and clinical data. About two-thirds of the funding for the study is from the NIA, and the remaining one-third, via the NIH Foundation, is from the pharmaceutical industry, imaging equipment manufacturers, and nonprofit organizations. Additional information regarding the study can be found at the ADNI web site (http://www.loni.ucla.edu/ADNI/).

APPLICATIONS OF BIOMARKERS TO CLINICAL TRIAL DESIGNS

A key issue in the design of trials of investigational drugs for AD is the ability to distinguish between symptomatic effects of drugs and effects that are due to modification of the underlying disease process. A trial design that measures delay of an end point, such as the onset of disease, cannot distinguish between these two possibilities. Designs that may allow conclusions regarding disease modification include those incorporating a randomized start, randomized withdrawal, or a persistent difference in slope. Randomized start and randomized withdrawal designs are illustrated in Figure 1. The underlying assumption for both of these trial designs is that the group initially randomized to treatment has a slowing of underlying disease progression. Thus, using the randomized withdrawal design, even if a purely symptomatic effect of the drug were present, the treated group would not return to the same cognitive scores as the placebo group after drug withdrawal. Similarly, when active treatment is started in the placebo group using the randomized start design, the cognitive scores of the group originally assigned to placebo would not reach those of the group originally assigned to active treatment.

While natural history studies can be important in the initial analysis of clinical or biomarker measures, they also have limitations. In these studies, subjects generally do not meet the same inclusion and exclusion criteria as they would in a randomized clinical trial, and thus have greater comorbidities and may be on medications that would otherwise be excluded. These limitations may lead to overestimates of the rate of decline or event rate, thus leading to underpowered and failed clinical studies. As illustrated in Table 1, rate of change in cognitive scores can also change over time, with placebo-treated patients showing slower rates of decline in more recent studies.

Biomarkers may be used in phase 1 or 2 studies to show an effect of the investigational drug on its target. A potential caveat with this strategy is that some drugs require weeks to months to show such an effect (eg, antioxidants). In later phase studies, a biomarker may be used as a surrogate marker if the surrogate can be substituted for a clinical end point. In phase 2 studies, effects such as a reduction in CSF tau after 3 to 6 months or reduced isoprostanes in CSF can provide evidence of a biologic signal and may help with dose selection for phase 3. A potential caveat with this strategy is that biomarkers may be altered differently and may respond to treatment differently over the course of the disease. For phase 3 trials, primary outcome variables are likely to be clinical measures for the immediate future. Surrogate markers can be used in phase 3 trials to develop supportive evidence for efficacy and to support a claim of disease modification.

IMAGING TECHNIQUES USED IN THE STUDY OF CLINICALLY DIAGNOSED ALZHEIMER DISEASE

Changes in MR-based regional (hippocampus, entorhinal cortex, and corpus callosum) and global (whole brain and ventricles) brain volume measures have been demonstrated for patients with AD in a number of longitudinal studies.[3,4,8–20] These studies consistently show loss of brain volumes in AD patients that are at least twice the rate of loss seen in age-matched control subjects. Longitudinal studies of vMRI in AD patients are listed in Table 2.

Additionally, patients with MCI, compared with control subjects with stable cognitive scores, have greater rates of volume loss for most brain areas regardless of whether they converted to AD. Further, MCI patients who did convert to AD have greater rates of change than those who are cognitively stable.[21]

An important consideration for imaging and other biomarker studies is whether results obtained at a single site can be replicated when the same measures are applied in a multiple site trial. The vMRI results from a 52-week study of milameline using 38 sites have been reported.[21] Subjects were scanned at baseline and end point, and hippocampal and temporal horn volumes were obtained. Based on these results, sample size calculations for an AD trial designed to detect a 50% reduction in rate of progression would require 320 patients per arm based on change in ADAS-cog scores, compared with 21 patients per arm based on hippocampal volume and 54 patients per arm based on temporal horn volume. These estimates suggest that even considering the additional variability imposed by the use of multiple sites, vMRI can provide advantages in the number of subjects required in a clinical trial to demonstrate a statistically significant effect on a structural end point.

The fact that biomarker changes in observational studies do not always predict changes seen in therapeutic trials is illustrated by the apparent *decrease* in brain volumes seen in subjects actively immunized with $A\beta_{1-42}$ (AN1792).[22] Subjects who had measurable antibody titers in this trial also had improvements in cognitive scores using some instruments. While a number of potential explanations have been proposed for this surprising result (eg, loss of plaque volume or brain hydration), the dissociation between apparent response to active vaccination and expected change in brain volumes illustrates the need to examine biomarker changes in therapeutic as well as observational studies. The finding also illustrates the need for the further development and evaluation of additional promising therapeutic surrogates and the importance of considering the choice and number of putative surrogate end points to consider in a therapeutic trial, and the schedule in which these end points are assessed, such that the observed effect of a treatment on the end point(s) most likely reflects the treatment's effect on disease progression.

FDG PET studies reveal characteristic and progressive reductions in regional measurements of the cerebral metabolic rate for glucose (CMRgl) in patients with AD[5,6,23] and patients with MCI.[24–27] In patients with AD, CMRgl reductions in the posterior cingulate, parietal, temporal, and prefrontal cortex are correlated with dementia severity[6] and progression.[5] In a retrospective study of patients with mild to moderate dementia, the pattern of hypometabolism was about 94% sensitive and 73% specific in predicting subsequent clinical decline and the histopathological diagnosis of AD.[23] In patients with the diagnosis of amnestic MCI,[24,25] isolated memory impairment,[26] and nonamnestic MCI,[27] regional CMRgl reductions helped distinguish subsequent AD converters from nonconverters, but with some overlap between groups. In a longitudinal study of amnestic MCI,[25] the 1-year rate of CMRgl decline was greater in subsequent AD converters than in nonconverters.

Based on longitudinal CMRgl declines in AD patients, researchers have estimated the statistical power of FDG PET to detect the ability of a putative disease-modifying treatment to slow rates of regional CMRgl decline in randomized clinical trials.[5] The estimated number of AD patients per treatment arm needed to detect an effect with FDG PET (Table 3) is roughly comparable to that needed to detect an effect with MRI and almost one-tenth the number of patients needed using clinical end points, suggesting the promise of these imaging techniques in proof-of-concept trials.

More recently, PET imaging studies using radioligands that bind directly to β-amyloid plaques have been performed.[28,29] One of these ligands, Pittsburgh Compound-B (PIB), is a thioflavin derivative and appears to be relatively selective for β-amyloid plaques at the concentrations used for imaging studies. As shown in Figure 2, the binding of PIB to brain sections is highly correlated with total Aβ levels. Test/retest variability in clinical studies is less than 10% for most brain regions.[30]

Little longitudinal data have been accrued thus far for clinical PIB PET studies. A number of these studies have started recently or will begin in the near future. They will be crucial for determining sample size requirements for demonstrating a statistically significant effect of a given agent in clinical trials.

Newer imaging techniques may be available in the future that could be more sensitive to change or show qualitatively different effects of AD or effects of investigational drugs. For instance, automated algorithms are being used to deform MRIs into standard coordinates of a brain atlas, facilitating comparisons of the change in gray matter or white matter volume on a voxel-by-voxel basis.[31–33] These differences offer promise in the differential diagnosis, early detection, and tracking of AD and in the evaluation of putative disease slowing treatments.

A strategy for using existing methods to determine brain volumes could entail the use of serial MRI images for several months prior to the initiation of an investigational drug. Such an approach would compare rate of decline within a subject before and after drug treatment, thus improving statistical power and reducing sample size requirements to demonstrate a statistically significant effect of a given agent.

Magnetic resonance spectroscopy has been used to assess concentrations of N-acetyl asparate (NAA), creatine, and choline in the brain; Alzheimer disease and MCI are associated with reduced NAA concentrations.[34,35] While the ability to make biochemical measurements in brain parenchyma in vivo would be extremely valuable, these techniques currently suffer from lack of technical standardization and lack of correlation with clinical measures.

Pulsed arterial spin labeled (ASL) perfusion MRI may provide a means to determine regional cerebral blood flow without the use of radioactivity.[36–38] Regional differences in blood flow may help to distinguish AD from frontotemporal dementia and vascular dementia. Continuous ASL techniques are also being investigated and may benefit from higher magnet strengths (ie, at least 4 Tesla).

BIOCHEMICAL MEASURES USED TO ASSESS RATE OF ALZHEIMER DISEASE PROGRESSION

Biochemical biomarkers may be assessed in different matrices or compartments, including CSF, blood, and urine. Many of the same considerations given to imaging techniques also apply to these biochemical measures (eg, the validity and accuracy of the analytical method and the variability among multiple sites). More specific to biochemical measures are the need to

standardize sample-handling techniques and to standardize methods for obtaining and storing CSF.

Lumbar punctures and CSF analyses have been used routinely in the practice of neurology for decades, although with the advent of other diagnostic modalities, this procedure is now performed most frequently in research settings in the United States. Nevertheless, two large studies of lumbar punctures performed as part of an evaluation of possible AD biomarkers have shown that the procedure can be applied broadly and that it is well tolerated.[39,40] The only recorded complication was post-lumbar puncture headache. With the use of a small diameter needle (0.7 mm), the rate of mild headache (duration less than 1 day, not affecting daily life) was less than 4%, and the rate of moderate or severe headache (duration more than 1one day and/or affecting daily life) was less than 1%.

While the initial pathogenic events in AD are not known with certainty, biochemical markers of the disease can be considered as more proximal or upstream, compared with more distal or downstream events. As shown in Figure 3, a number of potential biomarkers can be measured that may be proximal or distal in the pathogenic process.

Each potential biomarker must have certain characteristics to be useful in multicenter trials. The assay must have excellent sensitivity and test/retest reliability. Sample handling requirements must be such that analyses have acceptable variability when samples are obtained at multiple sites. The biomarker analyte should reflect a key feature of AD pathology or a mechanism of disease. Finally, the pattern of change in the biomarker over time and variability of that change should be adequately described.

Aβ, and in particular $A\beta_{1–42}$, has been studied frequently as a biomarker for AD. CSF concentrations of $A\beta_{1–42}$ are reduced by 40% to 50%, whereas concentrations of $A\beta_{1–40}$ or "$A\beta_{total}$" (using an ELISA that does not distinguish C-terminal length) are similar to those of age-matched controls. CSF$A\beta_{1–42}$ does correlate to an extent with dementia severity; however, in most studies concentrations are stable over intervals as long as 12 months.[41]

Plasma concentrations of $A\beta_{1–42}$ do not correlate with those in CSF.[42] Longitudinal studies have not shown a consistent change in plasma Aβ over time in AD patients,[43] and cross-sectional differences between AD patients and controls that would allow plasma Aβ concentrations to be used as a diagnostic measure have not been identified.

Cerebrospinal fluid tau has also been studied as a potential biomarker in AD.[44] Elevations of 2- to 3-fold of CSF total tau (T-tau) levels in patients with AD have been demonstrated in cross-sectional studies. In longitudinal studies, weak correlations are present with changes in cognitive scores, and CSF T-tau levels remain stably elevated in AD over time intervals of 12 months or longer. Tau may be phosphorylated at various sites, and forms of CSF tau reflecting specific sites of phosphorylation (P-tau 181, 199, 231, 235, 396, and 404) have been studied.

Three species of p-tau (p-thr231, p-ser199, and p-thr181) have been examined in detail in cross-sectional studies.[45–49] All three species are elevated in the CSF of patients with AD, and concentrations of all three species appear to be linearly related. When assessed as diagnostic measures, these three measures have similar sensitivity, although p-thr231 may have somewhat greater specificity for AD versus other forms of dementia.[45] Interestingly, p-thr231 tau, as well as other forms, is elevated in MCI patients compared with control subjects, but longitudinal studies of AD patients show a progressive decline in concentration with disease progression.[50]

There are several studies in which the diagnostic performance of the combination of CSF T-tau and $A\beta_{1–42}$ has been evaluated.[44] In most but not all studies,[51] the sensitivity and

specificity for the combination of these two biomarkers have been slightly higher (89% and 90%, respectively) than for T-tau (81% and 91%, respectively) or $A\beta_{1–42}$ (86% and 89%, respectively) alone. Other combinations of CSF biomarkers have also resulted in slightly better diagnostic performance than the use of single markers. In a study on the combination of CSF p-tau181 and $A\beta_{1–42}$, the sensitivity was 86% at a specificity of 97%,[52] and in another study the combination of CSF T-tau and p-tau396/404 resulted in a sensitivity of 96% at a specificity of 100%.[53] Further studies examining the value of combinations of biomarkers in larger series of patients and controls, and in particular in MCI, are needed.

As with imaging measures, sample size calculations for clinical trials can be made using Aβ and tau measures. As shown in Table 4, samples sizes using biochemical measures are similar to those achieved with imaging and are smaller than sample sizes based on clinical cognitive measures.

Besides the pathologic hallmarks of the disease, which include amyloid plaques and neurofibrillary tangles, AD pathology is characterized by evidence of reactive-oxygen species (ROS)–mediated damage.[54] ROS are formed under normal conditions, and although they are chemically unstable and highly reactive, their levels are kept relatively low by efficient antioxidant systems including catalase, glutathione, uric acid, and vitamins E and C. However, in some situations their generation can exceed the endogenous capacity to destroy them. As a consequence, the oxidant versus the antioxidant balance is altered and oxidative damage is the final result.[55] Depending on the substrate attacked by ROS, oxidative damage will manifest as protein oxidation, DNA oxidation, or lipid peroxidation products, all of which have been described in AD brain (Fig. 4). In general, oxidative damage in the central nervous system predominantly manifests as lipid peroxidation because of its high content of polyunsaturated fatty acids that are easily susceptible to oxidation.[56]

Isoprostanes are members of a complex family of lipid oxidation products derived from an ROS-mediated attack on free or esterified fatty acids. One group of them, called F_2-isoprostanes (F_2-iPs) (Fig. 5), are present in detectable quantities in all normal biologic fluids and tissues. Assays for specific F_2-iPs isomers using gas chromatography/mass spectrometry have identified 8,12-*iso*-$iPF_{2\alpha}$-VI (IPF2A) to be the most abundant F_2-iP in human as well as in animals.[57]

IPF2A concentrations are elevated in brain, CSF, and plasma of AD patients compared with controls.[58,59] In cross-sectional studies, concentrations of IPF2A in CSF correlate directly with concentrations of total tau and inversely with $A\beta_{1–42}$ levels.[59] In patients with MCI, CSF concentrations of IPF2A are intermediate between those of AD patients and those of control subjects; interestingly, patients with MCI who progress to AD have higher concentrations than those who do not.[59] Recent investigations were conducted to determine whether the increase in this marker of lipid peroxidation is present in neurodegenerative diseases other than AD. For this reason, histopathologically confirmed AD was compared with frontotemporal dementia (FTD) subjects, a heterogeneous group of dementing disorders with neurodegeneration. Levels of IPF2A were found to be markedly elevated in postmortem AD brains compared with corresponding areas of FTD and control brain tissues.[60] This observation was also confirmed in CSF from living patients with clinical diagnosis of FTD.[61]

Longitudinal studies in MCI patients showed that CSF F_2-iPs levels were elevated at both baseline ($P < 0.001$) and follow-up ($P < 0.01$) compared with controls. This resulted in an overall classification accuracy of 88%, both at baseline and follow-up. Moreover, a significant longitudinal change was seen in the MCI patients relative to controls. The longitudinal change yielded an overall classification accuracy of 76%, and post hoc examination showed a significant isoprostane increase restricted to the MCI group (de Leon M, DeSanti S, Zinkowski

R, et al. Biomarkers for Alzheimer's disease improve early diagnosis. *Neurobiol Aging*. 2005 [in press]).

Many, including Alzheimer himself, have observed enlarged (more recently referred to as activated) microglia and astrocytes in brain of Alzheimer patients. A 1989 report provided the first evidence of a neuropathogenic role for two of the principal cytokines derived from activated microglia and astrocytes, viz., IL-1, a potent pro-inflammatory cytokine, and S100B, a potent neurotogenic cytokine: (1) overexpression was shown to be already dramatic in neonates, children, and young adults with Down's syndrome (a virtually certain risk for precocious development of AD)[62] and (2) a similar overexpression was demonstrated in end-stage AD.[63–65] The neuro-pathogenic role of these two cytokines has been further supported by findings that both S100B[66] and IL-1[67] regulate production of the β-amyloid precursor protein (βAPP) as well as reports that the number of activated astrocytes and microglia overexpressing these two cytokines are related to the progression of β-amyloid plaques.[68, 69] Overexpression of these cytokines has been implicated in the pathogenesis of AD by their demonstrated influences on the genesis and formation of the two principal features in AD, neuritic β-amyloid plaques and neurofibrillary tangles, as well as synaptic loss, and neuronal dysfunction and loss.[70–79] This strong evidence for cytokine involvement in such neurodegenerative processes, underscores the importance of astrocyte-derived and microglia-derived cytokines in the brain's innate immune system and its role pathogenesis. In addition to overexpression of IL-1 and S100B, expression of IL-6,[80] α_1-ACT,[81] and iNOS[82] are increased in AD brain. Moreover, there is evidence that expression of each is up-regulated by IL-1 and that expression of each is decreased by suppression of IL-1.[83–85] Findings such as these suggest that neuroinflammation, powered by glia-derived cytokines, drives a self-sustaining, self-amplifying cycle that leads to a vicious circle of inflammation and neuronal dysfunction and death. The way in which neuronal insults are implicated in AD neuropathogenesis is summarized in Figure 6.

An important goal is to discover markers of conversion from a situation in which the brain can cope with a given level of insults and when the insults are sufficient to mildly impair brain function. Most of the studies cited above used brain tissue, but the need for studies of more accessible tissue has resulted in studies using CSF, some of which show tantalizing, but inconclusive, changes in α_1-ACT and IL-6.[2] Inflammatory markers have also been measured in serum, and early preliminary studies show that the relative levels of IL-1, IL-6, IL-8, IL-10, IL-12 and may be different in serum from patients who converted from control to MCI (Griffin et al, unpublished data). Measuring serum cytokine levels may serve as peripheral biomarkers of innate brain immune responses. At sufficient sensitivity, such biomarkers, although not likely to be specific for AD or other neurodegenerative condition, may be useful as peripheral indicators of progression of neural pathologies typical of AD. One way in which they might prove most useful is in testing the efficacy of therapeutic interventions. Not only the sensitivity but also the specificity of these putative biomarkers will need to be established in future trials.

PANEL DISCUSSION: BIOMARKERS USED IN THE STUDY OF CLINICALLY DIAGNOSED ALZHEIMER DISEASE

A panel (see Acknowledgments for participants) discussed a number of points related to the use of biomarkers for patients with AD. Several points of consensus were achieved, though a few points of disagreement were identified. Finally, a number of unmet needs for AD biomarker research were identified.

In the immediate future, biomarkers for AD are more likely to be used in clinical trials than in clinical practice. Use of CSF biomarkers as diagnostic measures in clinical practice is unlikely in the United States at this time; however, if a well-validated diagnostic marker and disease-

modifying treatments were available, patients may be referred from primary care physicians to neurologists for diagnostic lumbar punctures. Diagnostic biomarkers in clinical practice also are more important for patients with earlier disease.

Imaging biomarkers may have greater face validity and are more well developed, but more data are needed to improve the understanding of both imaging and biochemical biomarkers and their expected behavior after treatment with investigational drugs. Of various volumes measured using vMRI, whole brain and ventricular volumes appear most sensitive to change. Whether concentrations of $A\beta_{1-42}$ in CSF should increase or decrease with chronic dosing of a γ- or β-secretase inhibitor is unclear. The need for corrections in CSF concentrations based on ventricular volume has not been adequately assessed and more data are needed. More longitudinal studies with CSF measures are needed.

Biomarkers are likely to play an increasingly important role in the development of investigational drugs for AD. Lumbar punctures to obtain biochemical biomarkers can be performed safely, but may decrease recruitment rates in clinical trials. The choice of biomarker (s) in a clinical trial may depend on the mechanism of action of the investigational drug. There was not a clear consensus that a biomarker could be used as a primary outcome variable in a phase 2 clinical trial. Three potential uses for biomarkers in drug development were outlined: (1) for selection of homogeneous patient groups, (2) as an early indicator that an investigational drug is reaching its target and is having the intended effect, and (3) for indirect assessments of effects on disease progression. The validation of a biomarker as a surrogate marker for disease progression is likely to be iterative. Specifically, if a drug is identified that clearly changes clinical disease progression and that reduces or reverses an AD-specific biomarker, this would substantiate the use of such a biomarker for future therapeutic agents.

SUMMARY

Biomarkers are potentially very useful as tools in investigational drug trials of clinically diagnosed AD patients. Such markers could be used as indirect markers of disease severity, or might also be used as additional inclusion or exclusion criteria. When used as an indirect marker of disease severity, in almost all cases sample size estimates suggest that effects of putative disease-modifying drugs could be determined with fewer subjects using imaging or biochemical biomarkers than by using cognitive measures. Additional longitudinal multisite studies of these biomarkers, in particular FDG PET, amyloid-ligand PET, and CSF biochemical markers, would aid greatly in their applications to clinical trials. Such information will be obtained in a large observational study of patients with AD and MCI and elderly controls that will begin in mid-2005 (ADNI).

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Biomarkers for AD progression trials panelists: Neil Buckholtz, PhD, Leon Thal, MD, John Growden, MD, John Morris, MD, Martin Farlow, MD, David Knopman, MD, Mony De Leon, EdD, and Howard Fillit, MD. The support of the Alzheimer's Association and its Research Roundtable for this meeting is greatly appreciated. The assistance of Jay Lenn in the preparation of the manuscript is also greatly appreciated.

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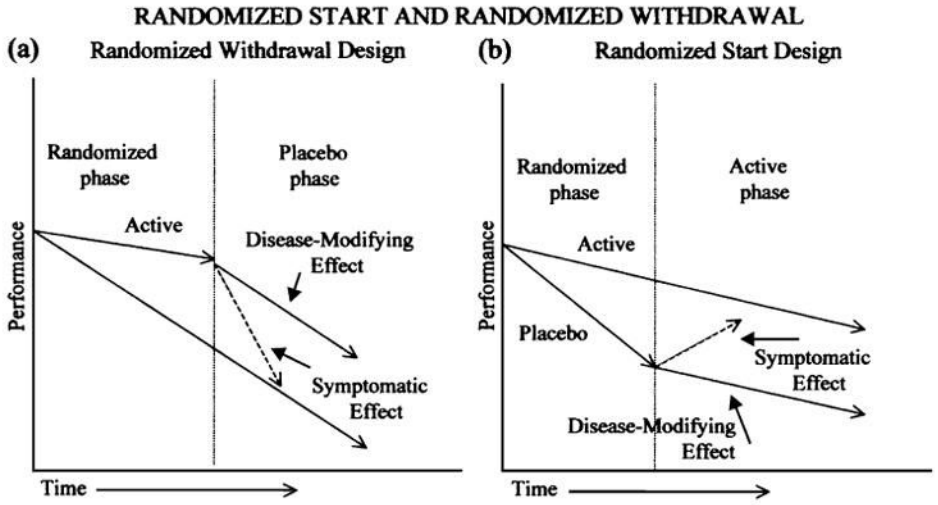

FIGURE 1.
Study design summaries for disease modification.

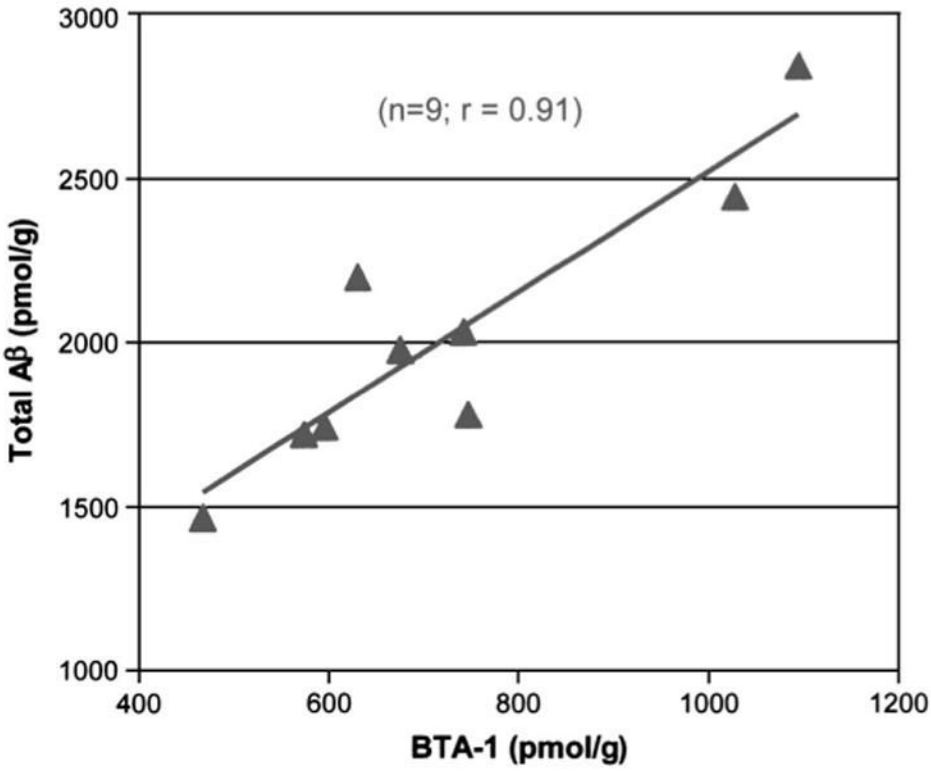

FIGURE 2.
Correlation between PIB-derivative binding and β-amyloid levels in brain slices.[28]

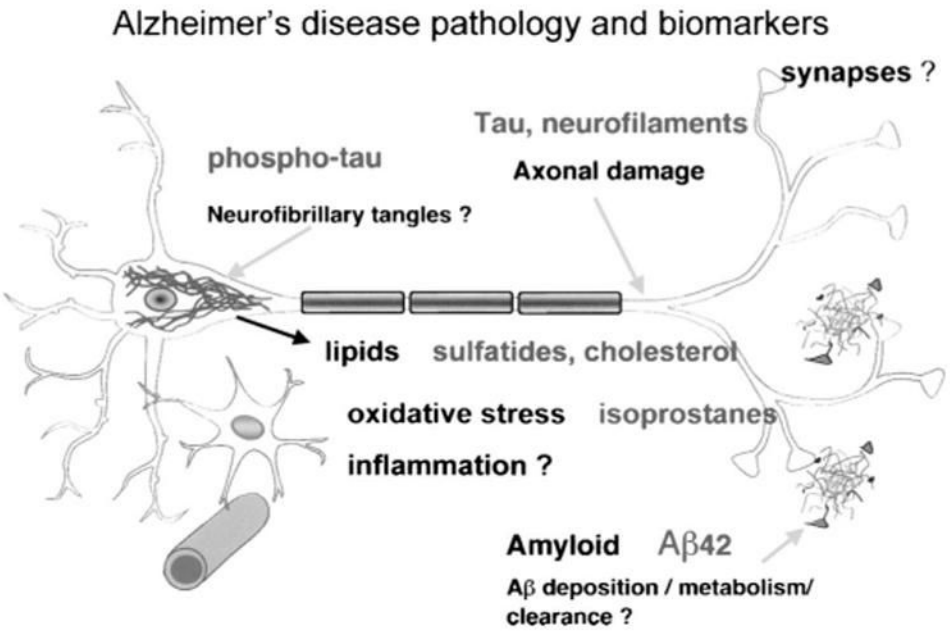

FIGURE 3.

Potential biochemical biomarkers in AD. Question marks indicate processes or anatomic areas that may be proximal in the disease process. Possible biomarkers that can be considered given these postulated disease processes include tau, phospho-tau, sulfatides, cholesterol, isoprostanes, and Aβ42.

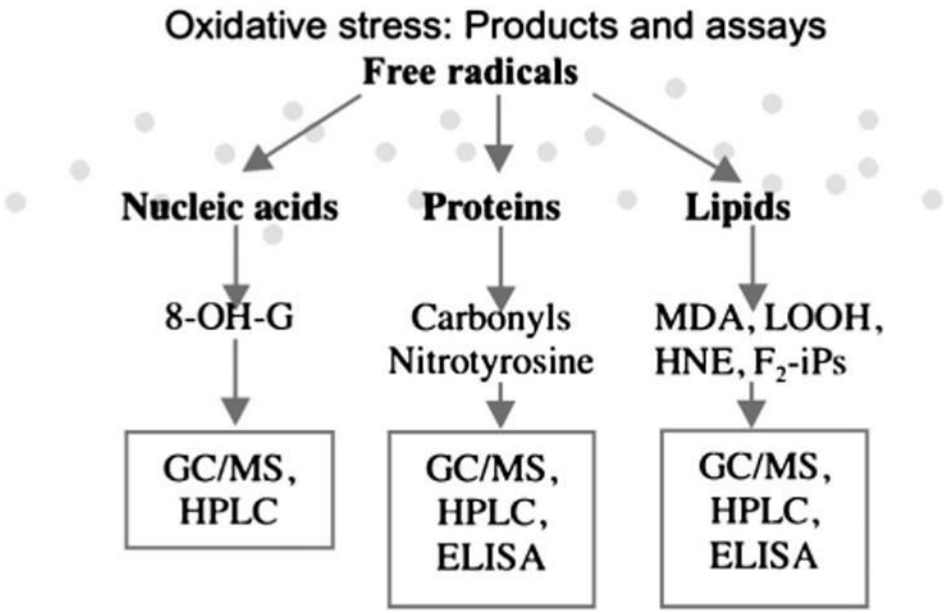

FIGURE 4.

Products of reactive oxygen species–dependent attack of different substrates (nucleic acid, protein, lipid) and relative most employed analytical methods (GC/MS: gas chromatography/mass spectrometry; HPLC: high performance liquid chromatography; ELISA: enzyme-linked immuno-assay; 8-OH-G: 8-hydroxyguanosine; MDA: malondialdehyde; LOOH: lipid hydroperoxide; HNE: 4-hydroxynonenal).

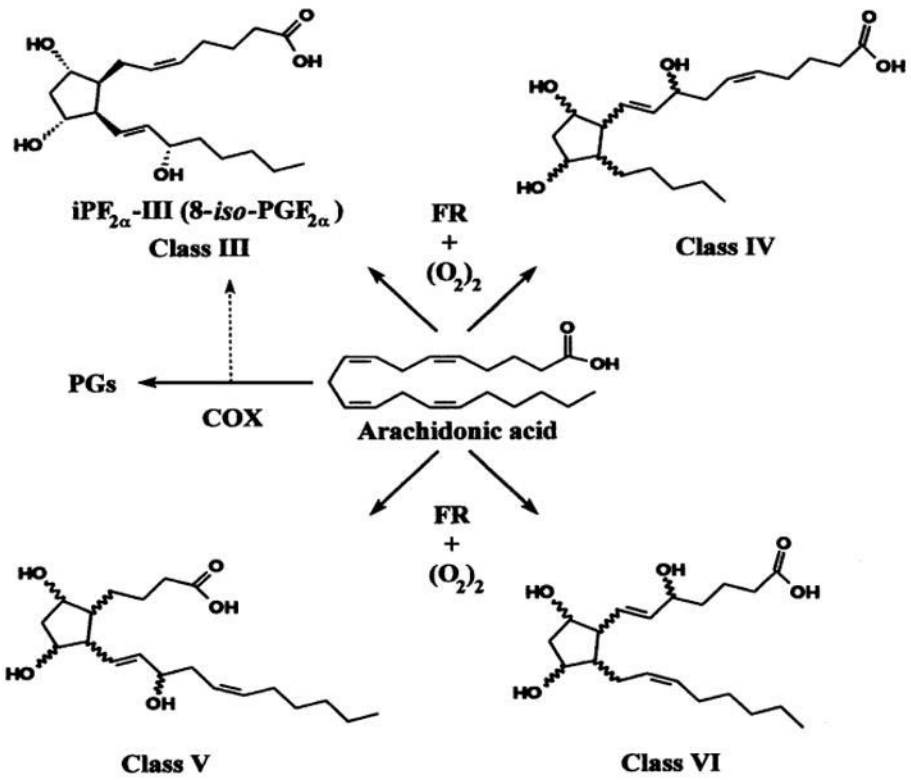

FIGURE 5.
The four classes of F_2-isoprostane deriving from the ROS-mediated oxidation of arachidonic acid.[86]

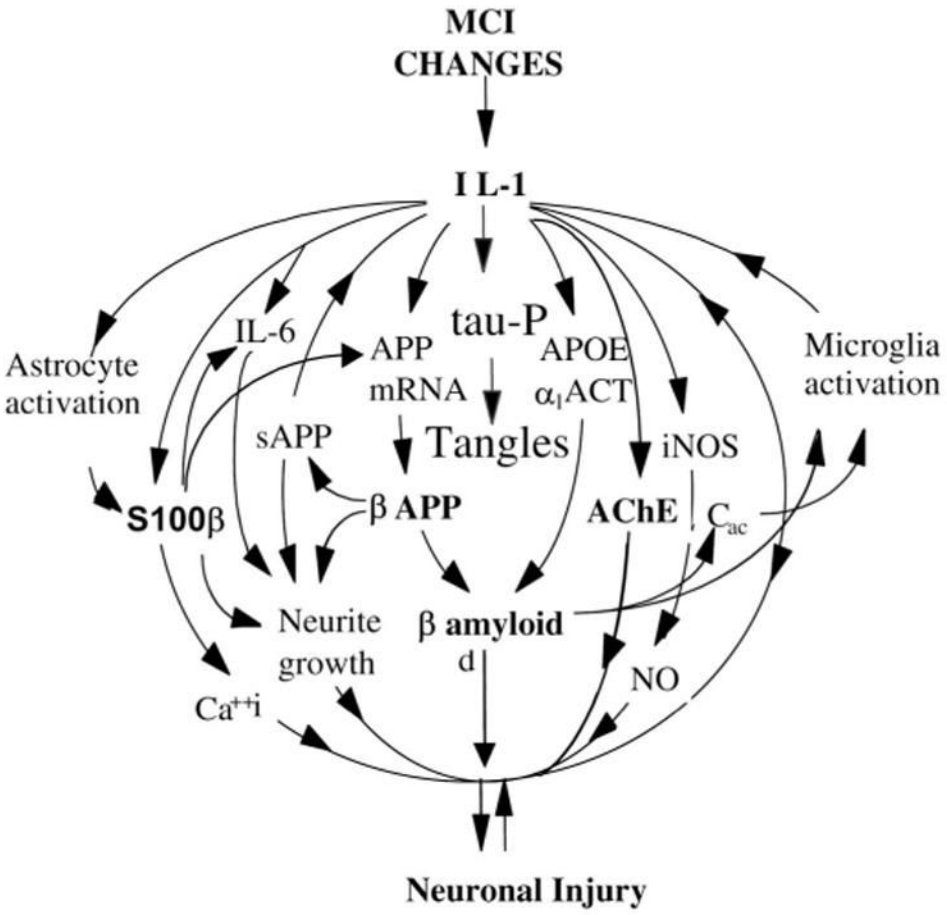

FIGURE 6.

Inflammatory changes implicated in Alzheimer disease (AD). This conceptual scheme of the cytokine cycle illustrates the relationship of glial activation and inflammatory cytokine overexpression to neurodegenerative events in AD, with the effect of overexpression of IL-1 in the brain. IL-1 = interleukin-1; APOE = Apolipoprotein E; ACT = antichymotrypsin; sAPP = secreted amyloid precursor; iNOS = nitric oxide synthase; Cac = activated complement; MCI = mild cognitive impairment.

TABLE 1

Rate of Decline in Alzheimer's Disease Assessment Scale–Cognition (ADAS-cog) Scores in Five Studies[87–91]

Study	N	Baseline MMSE	Mean ± SD
Thal et al, 1996	211	19.6	7.0 ± 7.8
Thal et al, 2000	102	20.6	7.5 ± 8.
Aisen et al, 2000	69	22	6.3 ± 6.4
Aisen et al, 2003	111	20.8	5.7 ± 8.2
Reines et al, 2004	346	21	5.4

Each value in the far right column is the annual rate of decline in ADAS-cog score. MMSE, Mini-Mental State Examination.

TABLE 2

Longitudinal Volumetric Magnetic Resonance Imaging Studies in Alzheimer Disease[8–11,14–20,92]

Source	Region	N (Control/Alzheimer Disease [AD])
Kaye et al, 1997	Hippocampi Parahippocampal gyri Temporal lobes*	18/12 (preclinical AD)
Jack et al, 1998, 2004	Intracranial volume Hippocampi* Entorhinal cortex* Temporal horns* Whole brain* Ventricle*	24/24 and 55/64
Fox et al, 2000	Whole brain*	18/18
Laakso et al, 2000	Hippocampi	8/27
Teipel et al, 2002	Corpus callosum*	10/21
Bradley et al, 2002	Whole brain* Ventricle* Ventricle/brain ratio*	32/5
Wang et al, 2002	Cerebrum* Lateral ventricles* Temporal lobes*	14/14
Du et al, 2003, 2004	Entorhinal cortex* Hippocampus*	23/21 and 25/21
Schott et al, 2003	Entorhinal cortex* Hippocampus* Temporal lobe* Brain*	20/5 (presymptomatic AD)
Thompson et al, 2004	Hippocampus* Ventricle*	14/12

*There were statistically significant differences between patients with Alzheimer disease and controls.

TABLE 3

Sample Size Estimates Using FDG PET for Alzheimer Disease Trials[5] Number of Alzheimer Disease Patients Per Treatment Group Needed to Detect an Effect With 80% Power in 1 Year

	Treatment Effect			
	20%	**30%**	**40%**	**50%**
Frontal	85	38	22	14
Parietal	217	97	55	36
Temporal	266	119	68	44
Cingulate	343	153	87	57
Combined	62	28	16	10

$P = 0.01$ (two-tailed).

No adjustment for normal aging effects or subject attrition.

Adapted from Alexander et al, 2002.

TABLE 4

Sample Size Calculations Based on Tau and $A\beta_{42}$[7,41,93–96]

Marker	Study	Number of Subjects Needed Per Group
CSF total tau in AD	Andreasen et al, 1999 Moriearty et al, 1999	40
CSF Aβ42 in controls	Andreasen et al, 1999 Galasko et al, 1998 Prince et al, 2004	16
CSF Aβ42 in AD	Andreasen et al, 1999 Moriearty et al, 1999 Simons et al, 2002	36
Plasma Aβ	Hoglund et al, 2004	27

Assumptions: Two-arm, drug versus placebo; $\alpha = 0.05$, $\beta = 0.80$; 25% effect size. AD, Alzheimer disease; CSF, cerebrospinal fluid.