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Biomarkers of Alzheimer's Disease

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Abstract

Although a battery of neuropsychological tests are often used in making a clinical diagnosis of Alzheimer's disease (AD), definitive diagnosis still relies on pathological evaluation at autopsy. The identification of AD biomarkers may allow for a less invasive and more accurate diagnosis as well as serve as a predictor of future disease progression and treatment response. Importantly, biomarkers may also allow for the identification of individuals who are already developing the underlying pathology of AD such as plaques and tangles yet who are not yet demented, i.e. "preclinical" AD. Attempts to identify biomarkers have included fluid and imaging studies, with a number of candidate markers showing significant potential. More recently, better reagent availability and novel methods of assessment have further spurred the search for biomarkers of AD. This review will discuss promising fluid and imaging markers to date.

Keywords

Alzheimer's disease; amyloid- β ; biomarker; cerebrospinal fluid; neuroimaging; proteomics; tau

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder estimated to affect 5.1 million individuals in 2007 (Alzheimer's Association, 2007). The diagnosis of AD is largely based upon clinical assessment, with definitive diagnosis still requiring pathological evaluation at autopsy. The identification of biomarkers for AD would allow for a less invasive and more accurate diagnosis in the antemortem period. Additionally, biomarkers may facilitate early diagnosis, which is particularly difficult given that there are no signs or symptoms unique to AD. More importantly, they may allow for the identification of individuals with preclinical AD (those with AD neuropathology that do not yet display clinical symptoms) (Gomez-Isla et al., 1996; Hulette et al., 1998; Markesbery et al., 2006; Morris and Price, 2001; Price et al., 2001). Biomarkers may be instrumental not only in the diagnosis of disease cases, but may aid

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in following disease progression and response to treatment as well. Finally, biomarkers are key in advancing our understanding of the pathophysiology of AD, which in turn has important implications for patient diagnosis and treatment.

The identification of reliable biomarkers has been hindered by the fact that patient classification relies on clinical diagnosis which is not always accurate, especially at early stages of the disease. Requiring postmortem confirmation of disease diagnosis has been impractical for biomarker studies. Moreover, control groups are likely to contain individuals with preclinical AD. Limited patient sample size and lack of adjustment for covariates such as age, gender, ethnicity, and APOE genotype have restricted the application of results from some studies to the general population. In addition, protocols for sample collection, preparation, and analysis often vary widely between labs, thus contributing additional methodological variability. Adopting standardized protocols for clinical assessment, sample analysis, and statistical evaluation would help overcome many of these shortcomings. Given the multifactorial nature of the disease, it is unlikely that a single biomarker will meet the needs for clinical diagnosis, while a panel of biomarkers may offer the appropriate sensitivity, specificity, and positive and negative predictive values. These limitations notwithstanding, many potential biomarkers have been identified, the most promising of which are discussed below and in the accompanying Table. Where in the disease course these various candidate markers may be useful is shown in the Figure.

Fluid Biomarkers

APP—The postmortem pathological diagnosis of an AD brain relies on the presence of senile plaques and neurofibrillary tangles. These senile plaques are composed of β amyloid ($A\beta$), a proteolytic fragment of the Amyloid Precursor Protein (APP). If altered proteolytic processing of APP underlies AD, then measures of APP or its derivatives may serve as diagnostic markers. Indeed, early studies (Ghisso et al., 1989; Kitaguchi et al., 1990; Weidemann et al., 1989) observed increased levels of APP and/or its secreted forms in the cerebrospinal fluid (CSF) of AD individuals. However, later studies have reported decreased (Henriksson et al., 1991; Prior et al., 1991; Van Nostrand et al., 1992) or unchanged (Chong et al., 1990) levels. Several studies of AD patients have shown reduced CSF levels of sAPP α , the soluble product released following α -secretase cleavage of APP (Palmert et al., 1990; Sennvik et al., 2000; Van Nostrand et al., 1992). These inconsistent findings between studies do not currently support a consensus of CSF APP being a useful biomarker for AD.

$A\beta$ —APP is expressed in all tissues and undergoes cleavage by β -secretase to release the ectodomain (sAPP- β) and subsequent cleavage by γ -secretase to release $A\beta$ peptides of 38–43 amino acids (Evin and Weidemann, 2002). Because $A\beta$ 42 is the dominant component of the plaques seen in AD (Roher et al., 1993a), many groups have investigated the use of $A\beta$ 42, as well as the other $A\beta$ species, as a diagnostic tool. The amount of total $A\beta$ in CSF is not well correlated with the diagnosis of AD (Lannfelt et al., 1995; Southwick et al., 1996; van Gool et al., 1995). The majority of studies have demonstrated a decrease in CSF $A\beta$ 42 in AD patients (Andreasen et al., 1999a; Andreasen et al., 2001; Clark et al., 2003; Engelborghs et al., 2008; Fagan et al., 2007; Galasko et al., 1998; Hampel et al., 2004a; Hulstaert et al., 1999; Ida et al., 1996; Kanai et al., 1998; Kanamaru et al., 2000; Kapaki et al., 2001; Kapaki et al., 2003; Lewczuk et al., 2004; Mehta et al., 2000; Motter et al., 1995; Mulder et al., 2002; Otto et al., 2000; Riemenschneider et al., 2000; Rosler et al., 2001a; Sjogren et al., 2002; Sjogren et al., 2000; Skoog et al., 2003; Tamaoka et al., 1997; Vanderstichele et al., 2000); however, there have been a few reports of increased (Jensen et al., 1999) or unchanged (Csernansky et al., 2002; Fukuyama et al., 2000) CSF $A\beta$ 42. These discrepancies are likely due to differing methods for assaying samples and varying sizes and selection criteria of patient groups, including the usage of subjects at different points along the disease spectrum.

A number of studies have investigated CSF A β 42 levels in conjunction with those of tau, the primary protein component of neurofibrillary tangles. In perhaps the most comprehensive analysis of A β 42 and tau levels to date, Sunderland et al. (2003) assayed 131 AD patients and 72 controls, and performed a meta-analysis of 17 studies of CSF A β 42 levels and 34 studies of CSF tau levels. In their own patient cohort, they observed significantly lower mean levels of CSF A β 42 and higher CSF tau in AD compared to controls, but significant overlap between the groups. The results of the meta-analysis mimicked their findings, with an effect size, or difference in levels between AD and controls, of 1.53 for A β 42 and 1.31 for tau. Several interesting correlations were observed, with tau correlating with the age of the controls but not of the AD individuals, with gender for the AD group only, and with Clinical Dementia Rating (CDR) and Mini Mental State Examination (MMSE) scores, but not duration of illness. While the meta-analysis did not reveal correlations between CSF A β 42 and any score of dementia severity, age, or duration of illness, there have been studies reporting a negative correlation between A β 42 and dementia severity (Galasko et al., 1998; Jensen et al., 1999; Samuels et al., 1999) and *APOE* ϵ 4 dosage (Galasko et al., 1998).

In addition to distinguishing AD from non-demented subjects, decreased levels of CSF A β 42 have been shown to be predictive of future dementia in MCI patients (Andreassen et al., 2003; Blennow and Hampel, 2003; Hampel et al., 2004a; Hansson et al., 2007; Hansson et al., 2006; Hansson et al., 2008; Herukka et al., 2005; Herukka et al., 2007; Riemenschneider et al., 2002a). Interestingly, significantly decreased CSF A β 42 has been observed in patients with very mild dementia (MMSE score of 25–28 or CDR 0.5) (Fagan et al., 2007; Riemenschneider et al., 2000), and levels have been reported to decrease from mild to more severe dementia (Jensen et al., 1999; Riemenschneider et al., 2000), suggesting that A β 42 may be useful in tracking the clinical course of patients.

It is important to consider whether a given biomarker makes sense in the context of the disease pathophysiology. Mouse models of AD have shown that CSF A β levels are related to the amount of plaque in the brain (DeMattos et al., 2002), and human studies have shown that increased neocortical and hippocampal plaque burden and cerebral amyloid angiopathy is highly associated with decreased A β 42 in postmortem CSF (Strozyk et al., 2003). These findings were furthered by Fagan and colleagues (Fagan et al., 2006; Fagan et al., 2007) who reported an inverse relationship between CSF A β 42 and in vivo plaque load using the amyloid imaging agent Pittsburgh Compound B (PIB) in living humans, supporting the authors' claim that plaques can function as "sinks" or "traps" of A β 42, thus decreasing the amount of A β 42 clearing the brain to the CSF. Other groups have likewise proposed this hypothesis (Motter et al., 1995; Samuels et al., 1999). Recent studies have shown that CSF A β 42 levels can identify PIB-positive individuals with near 100% sensitivity and greater than 80% specificity (Fagan and Holtzman, unpublished data).

One possible limitation of A β 42 for AD diagnosis is that decreased CSF levels have also been reported in Frontotemporal Dementia (FTD) (Hulstaert et al., 1999; Riemenschneider et al., 2002b; Sjogren et al., 2000), Creutzfeldt-Jakob disease (CJD) (Clark et al., 2003; Kapaki et al., 2001; Otto et al., 2000), Gerstmann-Straussler-Scheinker syndrome (Clark et al., 2003), 6 amyotrophic lateral sclerosis (Sjogren et al., 2002), multiple system atrophy (Holmberg et al., 2003), and dementia with Lewy bodies (DLB) (Clark et al., 2003; Kanamaru et al., 2000; Vanderstichele et al., 2000). While a number of studies have shown that CSF A β 40 is unchanged in AD (Fagan et al., 2007; Fukuyama et al., 2000; Lewczuk et al., 2004; Mehta et al., 2000; Shoji et al., 1998), the ratio of A β 42 to A β 40, rather than either marker alone, has been demonstrated to better distinguish AD subjects from controls or other dementias and to identify incipient AD in subjects with mild cognitive impairment (MCI) (Hansson et al., 2007; Kanai et al., 1998; Lewczuk et al., 2004; Shoji et al., 1998). The ratios of other markers such as tau / A β (Csernansky et al., 2002) tau / A β 42 (Csernansky et al., 2002; Fagan et al.,

2007; Kapaki et al., 2003; Li et al., 2007) and p-tau181 / A β 42 (Fagan et al., 2007; Maddalena et al., 2003) have similarly been used, and the CSF tau / A β 42 ratio has been shown to strongly predict future dementia in non-demented cohorts (Li et al., 2007).

While CSF is thought to more closely reflect what is happening in the brain, CSF is not as routinely obtained as blood. However, there has been little consensus among studies as to the relationship between plasma/serum A β and AD. Although Mehta et al., (2000) reported increased plasma A β 40 and Pesaresi et al., (2006) found decreased plasma A β 42 in AD, most groups have reported no difference in plasma/serum A β levels between sporadic AD and controls (A β 40 and A β 42 (Fukumoto et al., 2003; Kosaka et al., 1997; Tamaoka et al., 1996), A β 42 (Mehta et al., 2000; Vanderstichele et al., 2000)). In contrast, plasma A β 42 has been found to be increased (Kosaka et al., 1997; Scheuner et al., 1996) and A β 40 decreased (Kosaka et al., 1997) in individuals with autosomal dominant, disease-causing mutations (familial AD, FAD). Based on the findings of an early study showing that plasma A β 42 is elevated in presymptomatic FAD mutation carriers (Scheuner et al., 1996), a recent study investigated the levels of A β 42 in asymptomatic first-degree relatives of individuals with sporadic AD (Ertekin-Taner et al., 2008). As compared to controls, plasma A β 42 was found to be elevated in these subjects, irrespective of *APOE* ϵ 4 or FAD mutations. The difference between the A β 42 levels of the sporadic AD relatives and the controls was small, however (14.2 ± 0.6 and 12.3 ± 0.7 pM, respectively). It will be interesting to see in longitudinal studies whether these relatives with increased plasma A β 42 will go on to develop AD dementia.

Interestingly, several longitudinal studies have found that baseline plasma A β 42 levels were significantly higher in those cognitively normal individuals who later progressed to AD as compared to those who did not (Mayeux et al., 2003; Mayeux et al., 1999). Additionally, A β 42 levels were observed to decrease over time in these individuals, suggesting that while plasma A β 42 does not appear to be a suitable diagnostic marker for AD, it may be a marker for progression (Mayeux et al., 2003). Similarly, a case-cohort study originating from the prospective Rotterdam study found that increased plasma A β 40 at baseline was associated with an increased risk of AD as well as vascular dementia (VD) (van Oijen et al., 2006).

In a recent study however, any association between plasma A β 40 or A β 42 levels and progression from a normal to demented state was lost after adjusting for covariates such as age, cognitive status, cerebrovascular disease, *APOE* genotype, and kidney function (Lopez et al., 2008). A longitudinal study of MCI patients similarly found no correlation between plasma A β species and progression to AD (Hansson et al., 2008). This lack of association between plasma A β and AD is further supported by studies demonstrating that plasma A β 40 and A β 42 levels do not reflect brain A β or plaque levels (Fagan et al., 2006; Freeman et al., 2007) and that there is no correlation between plasma and CSF A β 42 or A β 40 (Mehta et al., 2001; Vanderstichele et al., 2000).

A number of anti-amyloid clinical trials have aimed at slowing or stopping the progression of AD by decreasing the production of A β 42, increasing its clearance, or reducing its aggregation. Based on animal findings that immunization with A β 42 resulted in a reduction of brain amyloid plaques (Janus et al., 2000; Morgan et al., 2000; Schenk et al., 1999), a phase II clinical trial (AN1792, Elan Pharmaceuticals) was undertaken to study its effects in humans. While this trial was cut short because of an increased incidence of meningoencephalitis (6%), a six-year follow up of a subset of the patients from the earlier phase I trial revealed a positive effect on A β load and plaque removal, but no effect on cognitive function, clinical outcomes, or long-term survival (Holmes et al., 2008). These findings would appear to cast doubt on the role of A β as a culprit in the cognitive decline characteristic of AD. The lack of correlation between amyloid load and dementia severity in clinicopathologic studies would also support this assertion (Arriagada et al., 1992; Bierer et al., 1995). It may be, however, that the

immunizations were given too late in the disease course, as the subjects already had mild to moderate dementia at the time of treatment. Studies have shown that brain accumulation of A β probably begins 10–20 years before clinical manifestations of the disease (Price and Morris, 1999) and can be imaged with a variety of compounds that can be visualized by PET (see below), and that this accumulation may drive the further accumulation of tau aggregates within vulnerable neurons (Lewis et al., 2001). If these demented patients already have substantial tau aggregation, it may be that the reduction of A β cannot reverse the tau-associated pathology and consequent cognitive impairment once the disease has progressed too far. However, this does not mean that A β is not promising as a candidate biomarker of AD. A repertoire of biomarkers that can serve as surrogates of underlying disease pathology would be crucial to our diagnosing of AD and following its progression and response to treatment. While it has been shown that CSF A β 42 reflects the presence of brain amyloid, the results from the A β 42 immunization trial suggests that tau is likely a better marker to follow for clinical disease progression and clinical outcomes. However, since A β load in the brain does not correlate with dementia severity (Arriagada et al., 1992; Bierer et al., 1995), and some degree of tangle pathology can exist in older individuals in the absence of dementia (Bouras et al., 1993; Haroutunian et al., 1999; Price et al., 1991), accurate diagnosis and prognosis of AD will most likely require a combination of these pathology-related biomarkers.

Tau and p-tau—The other pathognomic feature of AD brains, neurofibrillary tangles, is composed primarily of tau, a microtubule-associated protein which has similarly been extensively investigated as a biomarker. Many studies have demonstrated that CSF tau is increased in AD patients (Andreasen et al., 1999b; Andreasen et al., 2001; Andreasen et al., 1998; Arai et al., 1998; Arai et al., 1995; Arai et al., 1997; Blennow et al., 1995; Burger nee Buch et al., 1999; Csernansky et al., 2002; Fagan et al., 2007; Galasko et al., 1998; Golombowski et al., 1997; Green et al., 1999; Hampel et al., 2001; Hampel et al., 1999; Hock et al., 1995; Itoh et al., 2001; Jensen et al., 1995; Kahle et al., 2000; Kanai et al., 1998; Kanemaru et al., 2000; Kurz et al., 1998; Mecocci et al., 1998; Molina et al., 1999; Mori et al., 1995; Motter et al., 1995; Munroe et al., 1995; Nishimura et al., 1998; Rosler et al., 1996; Rosler et al., 2001a; Shoji et al., 1998; Shoji et al., 2002; Sjogren et al., 2002; Sjogren et al., 2000; Skoog et al., 1995; Tato et al., 1995; Vandermeeren et al., 1993; Vigo-Pelfrey et al., 1995).

In AD, tau undergoes abnormal hyperphosphorylation at many sites, and enzyme linked immunosorbent assays (ELISAs) have been developed to recognize various phosphorylated epitopes such as threonine 181 and 231 and serine 199, 235, 396, and 404 (Blennow and Hampel, 2003). As a result of this aberrant phosphorylation, tau is likely unable to bind and stabilize microtubules, possibly leading to axon degeneration (Mandelkow and Mandelkow, 1998). Thus, one possibility is that the increase in tau seen in AD CSF is due to the release of tau from degenerating neurons and its subsequent diffusion into the CSF (Mandelkow and Mandelkow, 1998). With the disturbance of the tau-microtubule binding equilibrium, there is a resulting increase in the cytosolic unbound levels of tau as well, and consequently an increased likelihood of protein misfolding and subsequent aggregation as neuropil threads in dystrophic neurites and as neurofibrillary tangles (Ballatore et al., 2007). While these observations suggest possible reasons for the increases in CSF tau level in AD, it is still unclear what is really happening in the human disease process.

Given that increased levels of CSF tau can be seen in other neurodegenerative disorders, in particular FTD, stroke, corticobasal degeneration, and CJD (Itoh et al., 2001), studies have begun looking specifically at phosphorylated forms of tau as diagnostic markers for AD. Hampel et al., (2004b) compared the accuracy of CSF p-tau231, p-tau181, and p-tau199 in discriminating AD from FTD, LBD, VD, and normal controls. They found that all three proteins were significantly increased in AD as compared to the other groups; however, the discriminative power of each differed, with p-tau231 providing for the greatest discrimination

between AD and non-AD, AD and controls, and AD and FTD. The combined use of the three p-tau markers did not provide further discrimination. Several studies have similarly shown that p-tau231 and p-tau199 can discriminate AD from other neurological disorders with sensitivities and specificities in the 80%–90% range (Buerger et al., 2002; Itoh et al., 2001; Kohnken et al., 2000).

While A β 42 and tau are specific markers of AD pathogenesis, a recent study has investigated the utility of a marker of neuronal death in the diagnosis of AD (Lee et al., 2008). Visinin-like protein 1 (VLP-1), a cytoplasmic calcium sensor protein that is thought to leak from damaged or dying neurons, was found to be significantly increased in the CSF of AD subjects compared to controls (Lee et al., 2008). Although VLP-1 is not specific to AD and indeed was originally studied in ischemic stroke subjects (Laterza et al., 2006), the combined use of A β 42, tau, p-tau, and VLP-1 resulted in increased diagnostic accuracy over any marker individually. Several studies have shown little correlation between amyloid plaque load and dementia severity (Arriagada et al., 1992; Bierer et al., 1995), thus VLP-1, in representing the end-result of the disease process, may provide a better reflection of the degree of dementia. Indeed, in this preliminary study, only VLP-1 and none of the other markers were found to correlate with MMSE (Lee et al., 2008). Clearly additional study of this molecule as a potential biomarker of cell death in AD is warranted.

Isoprostanes

Growing evidence suggests that oxidative damage may be important in the pathogenesis of AD. Isoprostanes, the end-products of lipid peroxidation, and in particular F2-isoprostanes, have been investigated in relation to AD. They have been found to be increased in the frontal and temporal cortex of AD compared to control and FTD brains, suggesting a specificity for AD (Pratico et al., 1998; Yao et al., 2003). Studies have shown F2-isoprostanes to be increased in postmortem ventricular CSF obtained from autopsy-verified AD cases (Montine et al., 1998; Montine et al., 1999b; Pratico et al., 1998), as well as in antemortem CSF from individuals diagnosed with AD dementia (Grossman et al., 2005; Montine et al., 1999a; Montine et al., 2001; Pratico et al., 2000; Pratico et al., 2002). CSF F2-isoprostanes have been shown to correlate with brain weight, degree of cortical atrophy, and Braak stage (Montine et al., 1999b), as well as dementia severity (Pratico et al., 2000). Several longitudinal studies have shown that over one and two year periods, CSF F2-isoprostanes increase in MCI and AD patients (de Leon et al., 2006; Quinn et al., 2004), and that baseline measurements could distinguish individuals that progress to MCI or AD from stable patients with 100% accuracy (de Leon et al., 2007). Moreover, the addition of isoprostane measurements to conventional memory testing or to quantitative MRI measurements resulted in increased diagnostic and prognostic power (de Leon et al., 2007), although confirmation awaits investigation in a larger number of subjects. Preclinical FAD mutation carriers have been shown to have increased CSF F2-isoprostanes as well, indicating that this marker may be suitable for both sporadic and familial AD (Ringman et al., 2008). Using a combined analysis of CSF A β 42, tau, and F2-isoprostanes, Montine et al., (2001) were able to diagnose AD with a sensitivity of 84% and specificity of 89%, while Grossman et al., (2005) were able to classify 88.5% of patients in accordance with their clinical or autopsy diagnosis using this same panel of markers.

Results have been less consistent in regards to peripheral F2-isoprostanes, with several studies reporting increased plasma levels (Pratico et al., 2000; Pratico et al., 2002), and others reporting no significant difference (Feillet-Coudray et al., 1999; Irizarry et al., 2007; Montine et al., 2000) in AD compared to controls. Similarly, urinary F2-isoprostanes have been reported to be increased (Pratico et al., 2000; Pratico et al., 2002; Tuppo et al., 2001) or unchanged (Bohnstedt et al., 2003; Montine et al., 2000). The discrepancies concerning peripheral F2-isoprostanes may be due to differences in patient selection criteria between studies, as smoking

and other conditions associated with oxidative stress, such as cardiovascular disease and diabetes, can significantly alter isoprostane levels (Flirski and Sobow, 2005).

Inflammatory markers

In addition to the classical pathological features of amyloid plaques and neurofibrillary tangles, AD brains display characteristics of inflammatory processes (Akiyama et al., 2000). One well investigated potential inflammatory marker of AD is α_1 -antichymotrypsin (ACT), a serine protease inhibitor that is colocalized with A β in senile/neuritic plaques (Abraham et al., 1988; Roher et al., 1993b; Shoji et al., 1991). Early studies of ACT yielded inconsistent results, however, with reports of increased ACT in AD serum (Brugge et al., 1992; Hinds et al., 1994; Lieberman et al., 1995; Matsubara et al., 1990) or CSF (Harigaya et al., 1995; Matsubara et al., 1990), along with reports of unchanged ACT in AD serum (Furby et al., 1991; Lanzrein et al., 1998; Pirttila et al., 1994) or CSF (Furby et al., 1991; Lanzrein et al., 1998; Pirttila et al., 1994). Four recent studies, however, have attempted to put this controversy to rest by measuring ACT levels in large groups of subjects or by including additional controls. In a study of 196 subjects, Licastro et al., (2000) observed increased plasma ACT in AD, and found that levels inversely correlated with cognitive performance. DeKosky et al., (2003) carried out a large study of 516 individuals, with AD subjects stratified by dementia severity, and similarly found that plasma and CSF ACT were increased, and that levels were negatively correlated with dementia severity. This study excluded those with systemic inflammatory diseases or those taking anti-inflammatory medications in an attempt to achieve as homogeneous a study population as possible. Additionally, plasma ACT was significantly increased in women compared to men, perhaps further explaining why previous studies which did not control for gender yielded inconsistent results. A proteomics approach, using gel electrophoresis and mass spectrometry, also identified ACT as being differentially expressed in AD versus controls, and findings were confirmed by ELISA validation in an independent sample set (Hu et al., 2007). In a 700+ subject case-cohort study within the Rotterdam Study, Engelhart et al., (2004) found that increased plasma ACT was associated with increased risk of dementia, AD, and VD. CSF ACT has also been found to be elevated in DLB, suggesting that it may be ineffective in distinguishing between these types of dementia (Nielsen et al., 2007).

The results of cytokine studies in AD have been highly inconsistent between groups. For example, in AD patients, CSF interleukin-6 (IL-6) has been found to be increased (Blum-Degen et al., 1995; Jia et al., 2005; Martinez et al., 2000; Rosler et al., 2001b), decreased (Yamada et al., 1995), or unchanged (Engelborghs et al., 1999; Hampel et al., 1997; Hasegawa et al., 2000; Lanzrein et al., 1998; Marz et al., 1997; Tarkowski et al., 1999). Plasma/serum IL-6 results have similarly been mixed (Angelis et al., 1998; Kalman et al., 1997; Lanzrein et al., 1998; Licastro et al., 2000; Maes et al., 1999; Tarkowski et al., 1999). Additionally, whereas Wada-Isoe et al., (2004) were able to discriminate VD from AD by CSF IL-6 levels, Jia et al., (2005) found no difference in levels between VD and AD. These inconsistencies have been mimicked in studies of IL-6 receptor, Gp130, IL-1 β , TNF- α , and Hp 2-1 (Teunissen et al., 2002). Moreover, most studies have either found no concentration differences or have yielded inconsistent results for additional cytokines such as IL-4, IL-8, IL-10, interferon-gamma, complement C1q, and TGF- β (Flirski and Sobow, 2005). These discrepancies between studies are likely due to several significant obstacles to the evaluation of cytokines in AD. Cytokine concentration can vary considerably over time, and can be influenced by an individual's genetic background, comorbid systemic inflammatory processes, usage of anti-inflammatory drugs, and exposure to environmental factors (Flirski and Sobow, 2005). Moreover, many studies use subjects with neurological diseases other than AD, such as Parkinson's disease (PD) or amyotrophic lateral sclerosis, as "controls."

Proteomics

A newer field of biomarker studies is moving away from the traditional approach of investigating levels of a single, or several, candidate biomarkers that have been implicated in the pathogenesis of AD, and is instead focusing on nonbiased profiling of human fluids in an attempt to discover novel biomarkers. As a result of improved mass spectrometry (MS) techniques, proteomics has emerged as a powerful tool for biomarker discovery. General methodologies in proteomic studies typically include protein preparation by two-dimensional gel electrophoresis (2-DE), liquid chromatography (LC), or protein-chip arrays, followed by MS or tandem MS and database searches to determine protein identity. Recent efforts to characterize the human CSF proteome have identified 2,594 proteins (Pan et al., 2007) and 563 peptide forms and 798 proteins (Zougman et al., 2008) using a combination of approaches. By comparing the differences in protein expression levels between AD and control CSF samples, a number of studies have identified potential diagnostic markers (Abdi et al., 2006; Carrette et al., 2003; Davidsson et al., 2002; Hu et al., 2007; Puchades et al., 2003; Selle et al., 2005; Zhang et al., 2005a). Additional studies have carried out similar analyses in samples with postmortem neuropathological confirmation of AD (Castano et al., 2006; Choe et al., 2002), with one study analyzing both antemortem and postmortem CSF from the same individuals (Finehout et al., 2006a).

An important concern of proteomics-based discovery, however, is that often candidate markers identified by a study are not confirmed in independent studies or by other more quantitative methods, indicating the present need for large validation studies and corroboration by alternative techniques. While some candidates have been identified in multiple studies, and furthermore have been implicated in AD pathogenesis, they unfortunately have not been consistently reported as increased or decreased in AD CSF. For example, β 2-microglobulin, the constant component of the class I major histocompatibility complex, has been identified as increased (Carrette et al., 2003; Davidsson et al., 2002; Hu et al., 2005; Simonsen et al., 2007; Zhang et al., 2005b) and decreased (Puchades et al., 2003) in AD CSF. Although the function of this protein is still unclear, it has been shown to accumulate as amyloid fibrils in dialysis patients (Gejyo et al., 1985; Gorevic et al., 1985). Similarly, transthyretin has been reported to be increased (Davidsson et al., 2002; Zhang et al., 2005b) and decreased (Castano et al., 2006; Puchades et al., 2003) in AD CSF. Transthyretin is thought to play a role in AD pathogenesis, as it can form complexes with A β 40 and A β 42, thus preventing A β aggregation (Schwarzman and Goldgaber, 1996; Schwarzman et al., 1994; Tsuzuki et al., 2000) and has been shown to negatively correlate with senile plaque abundance (Merched et al., 1998).

Many studies have formulated panels of proteins for the discrimination of AD from normal cohorts. For example, using 2-DE and tandem MS, Finehout et al., (2006b) formulated a panel of 23 protein spots that differentiated AD from non-AD with a sensitivity and specificity of 94% and a predictive error rate of 5.9%; the application of this same panel to a validation cohort yielded only slightly lower values. Moreover, panels derived from proteomic studies have been shown to differentiate AD, PD, and DBL with high accuracy (Abdi et al., 2006) and to distinguish MCI individuals who progress to AD from those who do not (Simonsen et al., 2007). Interestingly, the fragment signature of four CSF A β species (A β 1–16, A β 1–33, A β 1–39, and A β 1–42) as analyzed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight MS (MALDI-TOF-MS) has been shown to discriminate AD with a sensitivity of 89%, specificity of 83%, and accuracy of 86% (Portelius et al., 2006). Finally, the few proteomic studies of serum (German et al., 2007; Lopez et al., 2005) and of plasma (Hye et al., 2006; Ray et al., 2007) have similarly yielded markers to distinguish AD from controls, as well as to predict progression from MCI to AD (Ray et al., 2007), although verification in independent cohorts is needed.

Imaging Biomarkers

Neuroimaging techniques have increasingly been used to detect brain changes associated with AD, and thus have potential as markers of disease progression, monitors of therapeutic effects, and predictors of future dementia prior to symptoms.

Structural

Computed tomography (CT) and Magnetic Resonance Imaging (MRI)

Neuropathological studies have documented an abundance of neurofibrillary tangles (Braak and Braak, 1991; Schmitt et al., 2000) and significant neuronal loss in the hippocampus and entorhinal cortex of AD patients (Gomez-Isla et al., 1996; Kordower et al., 2001; Price et al., 2001; West, 1993; West et al., 1994); therefore, these areas have frequently been targeted by imaging techniques. Atrophy of medial temporal regions, the area where AD pathology is seen early in the disease, has been observed by CT (De Leon et al., 1997; Jobst et al., 1992), but MRI has more recently surpassed CT in AD studies due to its greater accuracy, manipulability, and precision (Frisoni, 2001; Scheltens et al., 2002). Meta-analysis confirms the ability of MRI to distinguish AD subjects from normal controls, with volumetric studies of the medial temporal lobe and hippocampus having a sensitivity of 78–94% and specificity of 60–100% (Bosscher and Scheltens, 2001). In addition to discriminating AD from control cases in cross-sectional studies, measurements of the banks of the superior temporal sulcus, the anterior cingulate, and the entorhinal cortex have been shown to discriminate with high (93%) accuracy MCI patients who later convert to AD from those who do not (Killiany et al., 2000). Similar predictive power has been reported for entorhinal cortex and hippocampal volumes (de Toledo-Morrell et al., 2004; Tapiola et al., 2008). Longitudinal studies have demonstrated that the rate of whole brain atrophy increases in early AD, from two (Fotenos et al., 2005) to five times (Thompson et al., 2003) that observed in age-matched non-demented controls. In addition, a recent study reported that the rate of ventricular volume expansion could predict future MCI in non-demented cohorts followed for up to 15 years, and that this rate further accelerated years prior to the diagnosis of MCI, suggesting this measurement may also be useful in preclinical diagnosis (Carlson et al., 2008). MRI has also revealed patterns of atrophy that differ between AD, FTLN, and DLB subjects, suggesting its use in the discrimination of these dementias (Barnes et al., 2007; Barnes et al., 2006; Whitwell and Jack, 2005; Whitwell et al., 2007).

Blood Flow and Metabolism

Functional magnetic resonance imaging (fMRI)

Functional MRI studies have revealed abnormalities in brain activation in AD, such as decreased activation of entorhinal cortex (Dickerson et al., 2005; Kato et al., 2001), supramarginal gyrus, prefrontal regions, anterior inferior temporal lobe (Kato et al., 2001), medial temporal lobe (Machulda et al., 2003; Rombouts et al., 2000), and hippocampal regions (Celone et al., 2006; Dickerson et al., 2005; Sperling et al., 2003), and increased activation of medial parietal cortex and posterior cingulate (Sperling et al., 2003). Results in MCI patients have been less consistent, with reports of decreased (Machulda et al., 2003) and increased (Dickerson et al., 2004; Kircher et al., 2007) medial temporal lobe activation. Additional studies have reported decreased activation in posterior cingulate, frontal cortex, hippocampus, and cerebellum (Johnson et al., 2006; Petrella et al., 2006). However, other studies have reported increased hippocampal activation in MCI cohorts (Celone et al., 2006; Dickerson et al., 2005; Hamalainen et al., 2007). Such disparities are likely due to differences in the clinical group assessment, the tasks patients are asked to perform (e.g. modality, underlying mechanism, etc.) and how the data are analyzed. It has been proposed that an increased or altered area of activation seen early in MCI may represent a compensatory recruitment for the neuronal loss and degeneration that is occurring, while in later stages of MCI and in AD this

activation is not possible (Dickerson and Sperling, 2008). For a recent review on fMRI studies of the medial temporal lobe memory system in AD and MCI, see (Dickerson and Sperling, 2008)

Positron emission tomography (PET)

PET has been employed in many AD studies to examine regional cerebral metabolism using ^{18}F -2-deoxy-2-fluoro-D-glucose as a marker (CMRglc using FDG-PET). Observed changes in AD brains include decreased metabolism in temporoparietal (Hoffman et al., 2000; Sakamoto et al., 2002), posterior cingulate (Minoshima et al., 1997; Nestor et al., 2003), hippocampal complex, medial thalamic regions, and mamillary bodies (Nestor et al., 2003). A number of studies have shown that decreased CMRglc in similar areas—temporoparietal, entorhinal, and posterior cingulate—is indicative of who will progress from MCI to AD (Arnaiz et al., 2001; Chetelat et al., 2005; de Leon et al., 2001; Drzezga et al., 2005; Mosconi et al., 2004). Additionally, PET studies using H_2^{15}O to measure regional cerebral blood perfusion, and hence cerebral activation, have shown that AD and MCI brains have patterns of activation that differ significantly from normal controls when performing the same task (Becker et al., 1996; Grady et al., 2001; Moulin et al., 2007; Woodard et al., 1998).

Single photon emission computed tomography (SPECT)

Many SPECT studies have demonstrated decreased regional cerebral perfusion in the temporoparietal cortex in AD compared to normal controls (Burns et al., 1989; Harris et al., 1998; Hunter et al., 1989; Jobst et al., 1992; Leys et al., 1989; Montaldi et al., 1990; Perani et al., 1988). SPECT has also been used to accurately discriminate MCI patients who convert to AD from nonconverters and normal controls by measuring perfusion increases in cerebellum and frontal lobe, decreases in parietal lobe (Huang et al., 2007; Huang et al., 2003), posterior cingulate (Borroni et al., 2006; Hirao et al., 2005; Huang et al., 2002; Kogure et al., 2000), and precuneus (Borroni et al., 2006; Hirao et al., 2005; Kogure et al., 2000). Importantly, in a study of 70 autopsy-verified dementia patients and 14 control patients, Jagust et al., (2001) found the accuracy of premortem clinical AD diagnosis was enhanced using SPECT. Furthermore, antemortem SPECT changes have been shown to correlate well with postmortem Braak staging within the same individuals, and the areas displaying perfusion defects appeared to evolve with disease progression (Bradley et al., 2002).

Cerebral perfusion defects in AD have also been assessed by PET and MR, yielding hypoperfusion patterns similar to those obtained with SPECT. Contrast-based MR imaging methods have shown decreased temporoparietal perfusion in AD (Bozzao et al., 2001; Gonzalez et al., 1995; Harris et al., 1998), while arterial spin-labeling perfusion MR imaging (ASL-MRI) has shown decreases in areas including temporoparietal, frontal, and posterior cingulate cortices (Alsop et al., 2000; Johnson et al., 2005). ASL-MRI has also revealed decreased perfusion in MCI (Johnson et al., 2005) and distinct patterns of hypoperfusion that differ between FTD and AD (Du et al., 2006). Since ASL-MRI does not use ionizing radiation, radioactive isotopes, or contrast injection, and allows for a structural MRI to be performed during the same imaging session, this technique may be particularly attractive for clinical applications.

Molecular

Definitive diagnosis of AD relies on the presence of amyloid plaques and neurofibrillary tangles assessed at biopsy or, more commonly, at autopsy. Recent studies, however, have aimed at developing compounds for the *in vivo* imaging of brain amyloid, neurofibrillary tangles, and activated microglia. These imaging markers would allow for earlier diagnosis, as AD pathology

precedes the onset of dementia symptoms by many years, and for the monitoring of disease progression and treatment efficacy.

Amyloid

Five amyloid PET ligands have been tested in AD patients and have yielded promising results.

FDDNP

The first probe for imaging amyloid plaques that was used in living patients was 2-(1-{6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile, or [F-18]FDDNP (Barrio et al., 1999). This label has been shown to bind to neurofibrillary tangles *in vivo* (Shoghi-Jadid et al., 2002) and prion plaques *ex vivo* as well (Bresjanac et al., 2003). An early AD case study using [F-18]FDDNP (Agdeppa et al., 2001) was followed by a study of 16 subjects showing that the relative residence time (RRT) of [F-18]FDDNP was highest in hippocampus-amygdala-entorhinal regions-- areas most dense in plaques and tangles (Shoghi-Jadid et al., 2002). Additionally, [F-18]FDDNP RRT was strongly correlated with performance on memory tests and MMSE scores (Shoghi-Jadid et al., 2002). A later study of 83 subjects found that retention in MCI individuals was intermediate between that of normal controls and AD, and that FDDNP-PET was better able to discriminate the three groups than FDG-PET or volumetric MRI (Small et al., 2006).

PIB

Arguably the most successful of the imaging amyloid agents has been ¹¹C-labelled Pittsburgh compound B, or PIB, (2-[4'-(methylamino)phenyl]-6-hydrobenzothiazole) (Mathis et al., 2003). In AD, PIB retention is increased in the frontal, parietal, temporal, and occipital cortices and striatum, and studies have consistently shown that nearly all patients diagnosed with Alzheimer's dementia test PIB-positive (Klunk et al., 2004; Pike et al., 2007; Rabinovici et al., 2007; Rowe et al., 2007). Additionally, the difference in ligand binding in AD versus controls is significantly more robust with PIB than with FDDNP (Kemppainen et al., 2006; Klunk et al., 2004; Price et al., 2005; Small et al., 2006). PIB binding correlates well with rates of cerebral atrophy in AD (Archer et al., 2006) and with reductions in CSF Aβ42 (Fagan et al., 2006; Fagan et al., 2007; Forsberg et al., 2007). PIB retention is also inversely correlated with cerebral glucose metabolism as determined by FDG-PET (Klunk et al., 2004), and is strongly related to the degree of memory impairment in MCI and AD (Engler et al., 2006; Forsberg et al., 2007; Pike et al., 2007). Interestingly, a longitudinal study of early AD patients taking cholinesterase inhibitors and/or the NMDA antagonist memantine, found that PIB retention did not change over a two-year follow-up, although cortical rCMRGlC decreased (Engler et al., 2006). This suggests that amyloid burden reaches a maximum early in the course of the disease, and indeed, several studies have found that certain MCI individuals have PIB uptake in the AD range (Jack et al., 2008; Kemppainen et al., 2007; Pike et al., 2007; Rowe et al., 2007). Importantly, studies have also shown PIB uptake in a proportion of non-demented elderly controls (Fagan et al., 2006; Jack et al., 2008; Klunk et al., 2007; Lopresti et al., 2005; Mintun et al., 2006; Pike et al., 2007; Rowe et al., 2007), consistent with the known presence of AD pathology in a subset of cognitively normal elders as reported in clinicopathological studies (Hulette et al., 1998; Price and Morris, 1999). These subjects presumably have preclinical AD, but longitudinal studies are needed to test this hypothesis before we can conclude that brain amyloid has adequate sensitivity and specificity to be considered a viable biomarker of AD. PIB retention is not observed in frontotemporal lobar degeneration (FTLD) (Rabinovici et al., 2007), or more specifically in its two syndromes FTD (Rowe et al., 2007) and semantic dementia (Drzezga et al., 2008). PIB imaging has also been shown to detect cerebral amyloid angiopathy (Bacsikai et al., 2007; Johnson et al., 2007). Together these studies suggest that PIB imaging may be suitable for confirming the diagnosis

of AD in symptomatic cases as well as for identifying individuals in the pre-symptomatic (preclinical) stages of the disease.

¹⁸F-BAY94-9172, ¹¹C-SB-13, ¹¹C-BF-227

¹⁸F-BAY94-9172, a stilbene derivative with structural similarity to PIB, has been shown to bind in a nearly identical pattern to that of ¹¹C-PIB, and to provide a similar effect size and accuracy in discriminating AD from controls and FTLD (Rowe et al., 2008). The roughly six-fold longer half-life of ¹⁸F over ¹¹C may allow for its easier integration into clinical settings (Rowe et al., 2008). Another stilbene derivative, ¹¹C-SB-13, has shown a binding performance similar to that of ¹¹C-PIB in a preliminary study of a small group of patients (Verhoeff et al., 2004). Finally, ¹¹C-BF-227 is a benzoxazole derivative that has been shown to label cerebral amyloid in a pattern distinct from that of ¹¹C-PIB (Kudo et al., 2007). The study's authors suggest that the difference in cortical distribution of the two amyloid labeling agents can be explained by the preferential binding of BF-227 to more dense amyloid, thus increasing retention in temporoparieto-occipital cortex where neuritic (dense core) plaques are most abundant (Kudo et al., 2007).

Microglia

Inflammatory mechanisms may play an important role in the neurodegeneration of AD, as various inflammatory mediators have been reported in AD brains, and epidemiological studies have reported altered AD risk with the use of anti-inflammatory drugs. Indeed, activated microglia closely associate with neuritic plaques (Sheng et al., 1997), and A β and APP species can act as microglial activators (Barger and Harmon, 1997; Combs et al., 1999; Rogers and Lue, 2001). Upon microglial activation, expression of the peripheral benzodiazepine receptor (PBR) is up-regulated, providing a marker for inflammatory processes. A PET study measuring microglial activation with [¹¹C](R)-PK11195, a ligand for the PBR binding site, found increased binding in the entorhinal, temporoparietal, and cingulate cortices in AD, and a similar pattern in an MCI individual (Cagnin et al., 2001). A 1-2 year follow-up with MRI revealed that the areas of highest [¹¹C](R)-PK11195 binding had the greatest rates of atrophy (Cagnin et al., 2001). The authors point to differences in the acquisition and analysis of PET data and their use of the higher affinity R-enantiomer of the ligand as reasons why their results differed from those of an initial study that did not detect any differences between AD and non-demented patients (Groom et al., 1995). Using [¹²³I]iodo-PK11195 as a SPECT ligand, Versijpt et al., (2003) observed significantly increased binding in the frontal and right mesotemporal regions of AD patients, and a correlation between ligand binding and performance on tasks of cognition. An additional PET ligand for PBR, [¹⁸F] FE-DAA1106 (Zhang et al., 2004), has also been shown to be useful in assessing glial activation in *in vivo* AD mouse models (Maeda et al., 2007). To our knowledge, this compound has not yet been tested in humans. While microglial activation is not specific to AD, indeed PK11195 binding has been studied in glial neoplasms, ischemic stroke, and multiple sclerosis among other diseases, the unique pattern of ligand binding within AD brains along with the concomitant use of other more specific markers of AD may prove useful to diagnosis.

Conclusions

The field of AD biomarkers has experienced a renewed level of enthusiasm due to better availability of reagents and novel methods for the assessment of a variety of fluid and imaging measures. CSF A β 42 and tau have stood the test of time, proving particularly promising as potential predictors of cognitive decline in individuals with very mild cognitive impairment as well as future dementia in non-demented cohorts. Further, low levels of CSF A β 42 are an excellent marker for the presence of neocortical amyloid deposition, in the presence or absence of dementia. Whether brain amyloid invariably leads to subsequent dementia in AD is not

known at present but is currently being studied. More rigorous investigation of fluid markers of inflammation, oxidative damage, and neuronal death are clearly warranted. Plasma and serum analytes have been notoriously difficult to interrogate but results from recent array-based panels are promising. Development of “molecular” imaging agents for the detection of AD pathologies (amyloid, tangles, activated microglia) has propelled the imaging field forward. It is likely that panels of markers and multiple biomarker modalities, especially combinations of fluid and imaging measures, will be required. Not only may these biomarkers eventually be useful as diagnostic tools in the clinic but also, in the more immediate future, for the design and evaluation of clinical trials of disease-modifying therapies by helping to reduce sample size, reduce trial duration, and evaluate treatment efficacy.

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Figure 1.

Hypothesized relationship between the timecourse of changes in various biomarkers in relation to the neuropathology and clinical changes of Alzheimer's disease.

Table
Select candidate fluid and imaging biomarkers of AD

Fluid	Biomarker	Observations	References
CSF	A β 42	<ol style="list-style-type: none"> 1 Decreased in AD 2 Decreased in subjects with brain amyloid deposition 3 Predictive of conversion from MCI to AD 	<ol style="list-style-type: none"> 1 (Andreasen et al., 1999a; Andreasen et al., 2001; Clark et al., 2003; Engelborghs et al., 2008; Fagan et al., 2007; Galasko et al., 1998; Hampel et al., 2004; Hulstaert et al., 1999; Ida et al., 1996; Kanai et al., 1998; Kanemaru et al., 2000; Kapaki et al., 2001; Kapaki et al., 2003; Lewczuk et al., 2004; Mehta et al., 2000; Motter et al., 1995; Mulder et al., 2002; Otto et al., 2000; Riemschneider et al., 2000; Rosler et al., 2001a; Sjogren et al., 2002; Sjogren et al., 2000; Skoog et al., 2003; Sunderland et al., 2003; Tamaoka et al., 1997; Vanderstichele et al., 2000) 2 (Fagan et al., 2006; Fagan et al., 2007) 3 (Andreasen et al., 2003; Hampel et al., 2004; Hansson et al., 2007; Hansson et al., 2006a; Hansson et al., 2008; Herukka et al., 2005; Herukka et al., 2007; Riemschneider et al., 2002)
Plasma and/or serum	A β 42	<ol style="list-style-type: none"> 1 Mostly no difference in AD 2 Mixed results for prediction of conversion from normal or MCI to AD 3 Increased in FAD 	<ol style="list-style-type: none"> 1 (Fagan et al., 2007; Fukumoto et al., 2003; Kosaka et al., 1997; Mehta et al., 2000; Pesaresi et al., 2006; Tamaoka et al., 1996; Vanderstichele et al., 2000) 2 (Ertekin-Taner et al., 2008; Hansson et al., 2008; Lopez et al., 2008; Mayeux et al., 2003; Mayeux et al., 1999) 3 (Kosaka et al., 1997; Scheuner et al., 1996)
CSF	A β 40	No difference in AD	(Fagan et al., 2007; Lewczuk et al., 2004; Mehta et al., 2000; Tamaoka et al., 1997)
Plasma and/or serum	A β 40	<ol style="list-style-type: none"> 1 Mostly no difference in AD 2 Mixed results for prediction of conversion from normal or MCI to AD 3 Decreased in FAD 	<ol style="list-style-type: none"> 1 (Fagan et al., 2007; Fukumoto et al., 2003; Kosaka et al., 1997; Mehta et al., 2000; Tamaoka et al., 1996) 2 (Hansson et al., 2008; Lopez et al., 2008; van Oijen et al., 2006) 3 (Kosaka et al., 1997)
CSF	Ratio of A β species (A β 40 and A β 42)	<ol style="list-style-type: none"> 1 Discriminates AD from normals 2 Predictive of conversion from MCI to AD 	<ol style="list-style-type: none"> 1 (Fagan et al., 2007; Kanai et al., 1998; Lewczuk et al., 2004; Shoji et al., 1998) 2 (Hansson et al., 2007)
CSF	Tau	Increased in AD	(Andreasen et al., 1999b; Andreasen et al., 2001; Andreasen et al., 1998; Arai et al., 1998; Arai et al., 1995; Arai et al., 1997; Blennow et al., 1995; Burger nee Buch et al., 1999; Csernansky et al., 2002; Fagan et al., 2007; Galasko et al., 1998; Golombowski et al., 1997; Green et al., 1999; Hampel et al., 2001; Hampel et al., 1999; Hock et al., 1995; Itoh et al., 2001; Jensen et al., 1995; Kahle et al., 2000; Kanai et al., 1998; Kanemaru et al., 2000; Kapaki et al., 2001; Kurz et al., 1998; Mecocci et al., 1998; Molina et al., 1999; Mori et al., 1995; Motter et al., 1995; Munroe et al., 1995; Nishimura et al., 1998; Rosler et al., 1996; Rosler et al., 2001a; Shoji et al., 1998; Shoji et al., 2002; Sjogren et al., 2002; Sjogren et al., 2000; Skoog et al., 1995; Sunderland et al., 2003; Tato et al., 1995; Vandermeeren et al., 1993; Vigo-Pelfrey et al., 1995)
CSF	p-tau231, p-tau181, and p-tau199	<ol style="list-style-type: none"> 1 Increased in AD 2 p-tau231 predicts conversion from MCI to AD 	<ol style="list-style-type: none"> 1 (Buerger et al., 2002; Fagan et al., 2007; Hampel et al., 2004b; Itoh et al., 2001; Kohnken et al., 2000) 2 (Buerger et al., 2002)
CSF	Ratio of tau species to A β 42	<ol style="list-style-type: none"> 1 Increased in AD 	<ol style="list-style-type: none"> 1 (Csernansky et al., 2002; Fagan et al., 2007; Kapaki et al., 2003; Maddalena et al., 2003) 2 (Fagan et al., 2007; Li et al., 2007)

Fluid	Biomarker	Observations	References
		2 Predictive of conversion from normal to MCI or AD 3 Predictive of conversion from MCI to AD	3 (Hansson et al., 2006b)
CSF	Isoprostanes	1 Increased in postmortem and antemortem AD CSF 2 Predictive of conversion from normal to MCI or AD 3 Increased in preclinical FAD mutation carriers	1 (Grossman et al., 2005; Montine et al., 1999a; Montine et al., 1998; Montine et al., 2001; Montine et al., 1999b; Pratico et al., 2000; Pratico et al., 2002; Pratico et al., 1998) 2 (de Leon et al., 2007) 3 (Ringman et al., 2008)
Plasma	Isoprostanes	Results mixed, showing increase or no change in AD	(Feillet-Coudray et al., 1999; Irizarry et al., 2007; Montine et al., 2000; Pratico et al., 2000; Pratico et al., 2002)
Urine	Isoprostanes	Results mixed, showing increase or no change in AD	(Bohnstedt et al., 2003; Montine et al., 2000; Pratico et al., 2000; Pratico et al., 2002; Tuppo et al., 2001)
CSF	α_1 -antichymotrypsin	Results mixed, showing increase or no change in AD	(DeKosky et al., 2003; Furby et al., 1991; Harigaya et al., 1995; Hu et al., 2007; Lanzrein et al., 1998; Matsubara et al., 1990; Pirttila et al., 1994)
Plasma and/or serum	α_1 -antichymotrypsin	1 Results mixed, showing increase or no change in AD 2 Predictive of AD risk	1 (Brugge et al., 1992; DeKosky et al., 2003; Furby et al., 1991; Hinds et al., 1994; Lanzrein et al., 1998; Licastro et al., 2000; Lieberman et al., 1995; Matsubara et al., 1990; Pirttila et al., 1994) 2 (Engelhart et al., 2004)
CSF	Interleukin-6	Results mixed	(Blum-Degen et al., 1995; Engelborghs et al., 1999; Hampel et al., 1997; Hasegawa et al., 2000; Jia et al., 2005; Lanzrein et al., 1998; Martinez et al., 2000; Marz et al., 1997; Rosler et al., 2001b; Tarkowski et al., 1999; Yamada et al., 1995)
Plasma	Interleukin-6	Results mixed, showing increase or no change in AD	(Angelis et al., 1998; Kalman et al., 1997; Lanzrein et al., 1998; Licastro et al., 2000; Maes et al., 1999; Tarkowski et al., 1999)
CSF and plasma	Various markers of inflammation	Results mixed	For reviews see (Flirski and Sobow, 2005; Teunissen et al., 2002)
Imaging Modality		Observations	References
CT and MRI		1 Regional atrophy in AD 2 Whole brain atrophy in AD 3 Predictive of conversion from MCI to AD 4 Predictive of conversion from normal to MCI	1 (Bosscher and Scheltens, 2001; de Leon et al., 1997; Jobst et al., 1992) 2 (Fotenos et al., 2005; Thompson et al., 2003) 3 (deToledo-Morrell et al., 2004; Killiany et al., 2000; Tapiola et al., 2008) 4 (Carlson et al., 2008)
fMRI		1 Altered activation in AD 2 Altered activation in MCI	1 (Celone et al., 2006; Dickerson et al., 2005; Kato et al., 2001; Machulda et al., 2003; Rombouts et al., 2000; Sperling et al., 2003) 2 (Celone et al., 2006; Dickerson et al., 2004; Dickerson et al., 2005; Hamalainen et al., 2007; Johnson et al., 2006; Kircher et al., 2007; Machulda et al., 2003; Petrella et al., 2006)
FDG-PET		1 Regional hypometabolism in AD 2 Predictive of conversion from MCI to AD	1 (Hoffman et al., 2000; Minoshima et al., 1997; Nestor et al., 2003; Sakamoto et al., 2002) 2 (Arnaiz et al., 2001; Chetelat et al., 2005; de Leon et al., 2001; Drzezga et al., 2005; Mosconi et al., 2004)
H ₂ ¹⁵ O-PET		Altered activation in AD	(Becker et al., 1996; Grady et al., 2001; Moulin et al., 2007; Woodard et al., 1998).

Fluid	Biomarker	Observations	References
SPECT		<ol style="list-style-type: none"> 1 Altered regional cerebral perfusion in AD 2 Predictive of conversion from MCI to AD 	<ol style="list-style-type: none"> 1 (Burns et al., 1989; Harris et al., 1998; Hunter et al., 1989; Jobst et al., 1992; Leys et al., 1989; Montaldi et al., 1990; Perani et al., 1988) 2 (Borroni et al., 2006; Hirao et al., 2005; Huang et al., 2007; Huang et al., 2003; Huang et al., 2002; Kogure et al., 2000)
ASL-MRI and contrast- based MRI		Regional hypoperfusion in AD	(Alsop et al., 2000; Bozzao et al., 2001; Du et al., 2006; Gonzalez et al., 1995; Harris et al., 1998; Johnson et al., 2005)
FDDNP-PET		Increased retention in AD and MCI brain	(Agdeppa et al., 2001; Shoghi-Jadid et al., 2002; Small et al., 2006)
PIB-PET		<ol style="list-style-type: none"> 1 Increased retention in AD brain 2 Increased retention in a subset of cognitively normal controls 3 Detects cerebral amyloid angiopathy 	<ol style="list-style-type: none"> 1 (Klunk et al., 2004; Pike et al., 2007; Rabinovici et al., 2007; Rowe et al., 2007) 2 (Fagan et al., 2006; Jack et al., 2008; Klunk et al., 2007; Lopresti et al., 2005; Mintun et al., 2006; Pike et al., 2007; Rowe et al., 2007) 3 (Bacskai et al., 2007; Johnson et al., 2007)
Other PET amyloid imaging agents: ¹⁸ F-BAY94-9172, ¹¹ C-SB- 13, ¹¹ C-BF-227		Increased retention in AD brain	(Kudo et al., 2007; Rowe et al., 2008; Verhoeff et al., 2004)
PET markers of microglial activation: [¹¹ C](R)-PK11195 and [¹²⁵ I]iodo-PK11195		Increased retention in AD and MCI brain	(Cagnin et al., 2001; Versijpt et al., 2003)

AD, indicates clinical diagnosis of dementia believed to be Alzheimer's disease, not necessarily autopsy confirmed AD cases.

FAD, Familial Alzheimer's disease

MCI, mild cognitive impairment