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Biomarkers in translational research of Alzheimer's Disease

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Abstract

The identification and characterization of amyloid-β (Aβ) and tau as the main pathological substrates of Alzheimer's disease (AD) has driven many efforts in search for suitable biomarkers for AD. In the last decade, research in this area has focused on developing a better understanding of the principles that govern protein deposition, mechanisms that link aggregation to toxicity and neuronal death, and a better understanding of protein dynamics in brain tissue, interstitial fluid and CSF. While Aβ and tau represent the two key pathological mediators of disease, other aspects of this multifaceted disease (e.g. oxidative stress, calcium-mediated toxicity, and neuroinflammation) are being unraveled, with the hope to develop a more comprehensive approach in exploring disease mechanisms. This has not only expanded possible areas for disease-modifying therapies, but has also allowed the introduction of novel, and potentially useful, fluid and radiological markers for the presence and progression of AD pathology. There is no doubt that the identification of several fluid and imaging biomarkers that can reliably detect the early stages of AD will have great implications in the design of clinical trials, in the selection of homogenous research populations, and in the assessment of disease outcomes. Markers with good diagnostic specificity will aid researchers in differentiating individuals with preclinical and probable AD from individuals who do not have AD pathology or have other dementing disorders. Markers that change with disease progression may offer utility in assessing the rates of disease progression and the efficacy of potential therapeutic agents on AD pathology. For both of these purposes, CSF Aβ42, amyloid imaging, and CSF tau appear to be very good markers of the presence of AD pathology as well as predictive or who will progress from MCI to AD. Volumetric MRI is also good at separating individuals with MCI and AD from controls and is predictive of who will progress from MCI to AD. Perhaps the most important role biomarkers will have, and the most needed at this time, lies in the identification of individuals who are cognitively normal, and yet have evidence of AD pathology (i.e. preclinical AD). Such individuals, it appears, can be identified with CSF Aβ42, amyloid imaging, and CSF tau. Such individuals are the most likely to benefit from future disease modifying/prevention therapies as they become available, and therefore represent the population in which the field can make the biggest therapeutic impact.

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Keywords

Alzheimer's disease; biomarkers; amyloid-β; tau; imaging; antecedent biomarkers; plaques

Introduction

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There is no doubt that the identification of several fluid and imaging biomarkers that can reliably detect the early stages of AD will have great implications in the design of clinical trials, in the selection of homogenous research populations, and in the assessment of disease outcomes. Markers with good diagnostic specificity will aid researchers in differentiating individuals with preclinical and probable AD from individuals who do not have AD pathology or have other dementing disorders. Markers that change with disease progression may offer utility in assessing the rates of disease progression and the efficacy of potential therapeutic agents on AD pathology. For both of these purposes, CSF Aβ42, amyloid imaging, and CSF tau appear to be very good markers of the presence of AD pathology as well as predictive or who will progress from MCI to AD. Volumetric MRI is also good at separating individuals with MCI and AD from controls and is predictive of who will progress from MCI to AD.

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AD pathology

The abnormal aggregation and deposition of two proteins in the brain, Aβ and tau, play key roles in AD pathogenesis. Aβ-containing amyloid plaques, dystrophic neurites, neurofibrillary tangles (NFT), and neuropil threads constitute the main pathological hallmarks of AD (Braak and Braak, 1991). Aβ peptides are 37–43 amino acids in length, 4 kDa in molecular weight, and are derived from the cleavage of a larger protein, the amyloid precursor protein (APP), by the action of β and γ secretases (Haass and Selkoe, 1993). The aggregation and deposition of Aβ in the extracellular space results in the formation of diffuse and neuritic plaques, while its deposition in the walls of small-to-medium arterioles and arteries in the meninges and cerebral parenchyma accounts for cerebral amyloid angiopathy (CAA) (Price et al., 2001b). Two different types of plaques have been identified. "Neuritic" plaques are mainly composed of extracellular deposits of Aβ in a β-pleated sheet

conformation along with degenerating neuronal processes (referred to as dystrophic neurites), astrocytes, and microglia (Benzing et al., 1993). Neuritic plaques are also referred to as "dense core" plaques. "Diffuse plaques", on the other hand, consist of Aβ deposits that contain little to no Aβ in fibrillar form and lack prominent neuritic or glial changes (Yamaguchi et al., 1988).

Tau is a cytoplasmic microtubule-associated protein involved in the assembly and stabilization of microtubules. Under normal conditions, both phosphorylated and dephosphorylated forms of tau are in equilibrium. However, the hyperphosphorylation and aggregation of tau is one of the earliest pathological changes in AD. Tau hyperphosphorylation and aggregation appears to interfere with tau's critical functions in the stabilization of microtubules (Maccioni et al., 2001), and also promotes its aggregation into paired helical filaments in the neuronal perikarya leading to the formation of neurofibrillary tangles, and in the neuronal dendrites leading to the formation of neuropil threads (Ballatore et al., 2007; Braak and Braak, 1988).

The temporal sequence of pathological changes in AD

There has been a large amount of evidence from human and animal studies that Aβ plays a crucial role in the pathogenesis of AD. The deposition of insoluble forms of Aβ in the form of amyloid plaques and possibly oligomers appears to be an early and pivotal event that ultimately culminates in axonal, synaptic, and neuronal loss as well as synaptic dysfunction. In addition, animal and human studies suggest that tau aggregation, which appears to begin independently during normal aging and in the early stages of AD, is further accelerated by the presence of concomitant amyloid pathology (Fagan AM et al., 2009; Price and Morris, 1999).

Although the knowledge of each of the individual pathological substrates of AD has suggested potential candidates of useful biomarkers of disease, insight into the temporal pattern and relative contributions of each of these processes in different stages of dementia severity is equally important. Early processes responsible for triggering and directly influencing secondary mechanisms of disease are likely to offer the best utility for early detection. The importance of early, and perhaps preclinical, detection cannot be overemphasized, as it allows the identification of individuals at a time when they are the most likely to benefit from potential disease-modifying therapies; that is, when the degree of irreversible loss is more likely to be preventable.

Several models utilizing molecular and pathological indicators of disease progression have been proposed in an attempt to shed light on the temporal pattern of biochemical and pathological changes in AD. Data from both human studies of familial AD and mouse models of AD support the notion that amyloid deposition is an early occurrence, the first signs of which arise many years prior to the appearance of clinical signs of cognitive impairment. In fact, amyloid deposition has been estimated to begin ~10–15 years prior to any clinically detectable signs of dementia, progress with time, and reach what many refer to as a "ceiling" or "plateau" effect (Price and Morris, 1999). There is ample evidence to suggest that Aβ accumulation achieves a high steady state by the early clinically evident stage of disease, with only little increase afterwards. In other words, by the time there is evidence of even very mild dementia (as sometimes termed mild cognitive impairment (MCI) or a clinical dementia rating [CDR] of 0.5), amyloid deposition has already reached or is close to reaching its peak (Gomez-Isla et al., 1996; Price and Morris, 1999).

Similarly, studies in non-demented elderly and in individuals with very early stages of AD suggest the presence of a significant degree of tau pathology with increased NFT densities in the hippocampus and entorhinal cortex (Price and Morris, 1999). That is, by the time

dementia just starts to become apparent clinically, both histopathological changes of AD have already been established with substantial numbers of both diffuse and neuritic plaques distributed widely throughout the cerebral cortex. In fact, even cases with the mildest stages of dementia (CDR 0.5) have a degree of plaque and tangle pathology that is often sufficient to meet the pathological criteria for a diagnosis of AD (Morris et al., 1991). Since these lesions are believed to accumulate relatively slowly, it seems likely that the disease process must begin at an even earlier stage. These findings have led to the important concept of "preclinical" AD in which Aβ deposition and tau accumulation are occurring in the absence of clinically detectable cognitive decline.

The other pathological hallmark of early AD is the presence of tau aggregates in the form of neurofibrillary tangles. There appear to be considerable differences in the spatial and temporal distribution of NFT and plaque pathology. At least a few NFTs can be seen in the brains of virtually all non-demented elderly with or without plaques, including vulnerable brain regions such as the entorhinal cortex and the hippocampus. Moreover, the rate of NFT formation exponentially increases with normal aging (Price and Morris, 1999). Some studies have suggested that tau pathology might even precede any signs of amyloid deposition by decades (Silverman et al., 1997). Despite the early appearance of neurofibrillary changes, their progression seems to be accelerated by the presence of concomitant amyloid pathology, and in the absence of plaques, neurofibrillary changes generally remain confined to the medial temporal lobe structures. There are several lines of evidence to support the presence of such an interaction between Aβ and tau in preclinical AD. Genetic studies have shown that single nucleotide polymorphisms in the tau gene are associated with increased tau levels in the CSF as well as with earlier onset of dementia of the Alzheimer's type, but only in individuals who have evidence of brain amyloid deposition (Kauwe et al., 2008). This presumed role for Aβ in inducing tau accumulation is supported by the observation that tau-containing dystrophic neurites tend to form around Aβ plaques (Ingelsson et al., 2004). In addition, animal studies show that Aβ accumulation appears to exacerbate tau accumulation (Gotz et al., 2001; Lewis et al., 2001).

While amyloid and tau pathology in the preclinical stages of AD are inevitably associated with some degree of neuronal, axonal and synaptic loss and neuronal injury, it is only after a threshold of neuronal loss has been reached in specific neocortical regions that the first clinical signs of dementia appear. Studies comparing tissue volumes and neuronal numbers in the entorhinal cortex and hippocampal field CA1 in healthy aging brain, preclinical AD, and very mild AD (MCI or CDR 0.5) indicate that tissue volumes and neuronal numbers are comparable in preclinical AD and nondemented elderly (Price et al., 2001a). On the other hand, both of these measures decrease substantially in cases with MCI or very mild AD.

Progressive neuronal and synpatic loss, and the progressive increase in NFT pathology on a background of already substantial Aβ accumulation, correlate with further cognitive decline and disease progression (Ingelsson et al., 2004). Decreased synaptic density, altered synaptic signaling and synaptic composition, while seen in the early stages of AD in association with amyloid pathology may have a more immediate effect on the severity and progression of dementia (Fiala et al., 2002). Many studies have shown that clinical progression correlates well with the extent of neuronal loss (Giannakopoulos et al., 2003; Gomez-Isla et al., 1997), with decreasing levels of synaptophysin (a synaptic marker), and with increasing numbers of NFTs in the hippocampus, entorhinal, and association cortices (Ingelsson et al., 2004).

In summary, preclinical AD is characterized by significant Aβ deposition and lesser degrees of tau aggregation, with minimal neuronal loss. The first clinically detectable stage of AD (CDR 0.5 or MCI) occurs when neuronal, axonal, and synaptic loss and dysfunction has reached a threshold, and is associated with further increases in tau and Aβ42 aggregation.

By the time patients have mild or moderate dementia (e.g. CDR 1 or 2), Aβ deposition has likely already peaked while tau aggregation, neuronal and synaptic loss, and inflammation continue through the more advanced stages of disease.

Potential roles for biomarkers in translational AD research

Identification of CSF and radiological biomarkers that can reliably detect the early stages of AD has been important in the design of clinical trials and in the accurate identification of research populations over the last decade. Markers with good diagnostic specificity aid researchers in differentiating individuals with probable AD from individuals whose cognitive impairment may be attributed to other causes. Markers that change with disease progression may offer utility in assessing rates of disease progression and the efficacy of potential therapeutics on AD pathology. This may be particularly true for biochemical or radiological markers of neuronal and synaptic loss; since the latter represents the final outcome of several pathological substrates. Biomarkers used to assess disease severity should be measurable, reproducible, and should demonstrate changes with disease progression in longitudinal studies. The use of such markers in the trials of disease-modifying therapies will help to identify the appropriate dosage, to measure drug efficacy in proof-of-concept trials, and to improve safety assessments. Although not without limitations, at least a few markers discussed herein (Table 1) may prove to be useful as secondary outcomes and to support disease-modification claims in phase 3 clinical trials (Thal et al., 2006).

CSF biomarkers

In the last few years, biomarker discovery research has successfully utilized genomics, proteomics, and metabolomics for the identification of several promising markers in the blood and the CSF of individuals with AD (Hu Y, 2007; Ray et al., 2007), including proteins involved in inflammation, oxidative stress, apolipoproteins, and markers of neurodegeneration (Maes et al., 2007). While promising, most of these markers have not yet been validated in more than one study and their ultimate usefulness awaits further studies. The majority of studies have focused on markers that directly reflect pathological substrates of AD, such as amyloid plaques and neurofibrillary tangles. However, the list of potential candidates is extensive and includes proteins involved in cytoskeletal maintenance, cellular trafficking, cellular stress response, redox homeostasis, transcription, and DNA repair (Maes et al., 2007).

Plasma and CSF levels of Aβ1-42 and Aβ1-40 have been among the first and perhaps most widely investigated markers. Several studies have suggested that baseline plasma levels of Aβ1-42 are higher in patients with AD, and that the plasma Aβ40/42 ratio predicts a high risk of progression to dementia in cognitively normal individuals (Mayeux et al., 2003; van Oijen et al., 2006). However, these results have not been reproduced in all studies, and the methods for Aβ detection and measurement have not been standardized across studies. The diagnostic utility of plasma Aβ has been further limited by its short half life in the plasma (on the order of 5–15 minutes), its presence in very low concentrations, and by the additional peripheral sources of Aβ production and clearance which can be influenced by confounding factors (such as renal function) [www.alzforum.org/res/enab/workshops/biomarkers.asp].

On other hand, since CSF is a compartment which is in direct contact with brain parenchyma, CSF biomarkers have shown more promise as reliable and early indicators of disease. The CSF biomarkers with the highest diagnostic accuracy are Aβ42, tau, and p-tau. In the following section, we will discuss the utility of each of these markers separately in the diagnosis and prognosis of AD.

CSF biomarkers in the early detection of dementia

CSF Aβ42 and Aβ40

There is strong evidence across many cross-sectional studies that CSF Aβ42 levels are reduced by about 50% in AD compared to controls, even in the early clinical stages of disease (Blennow et al., 2001). On the other hand, CSF Aβ40 levels are not different in individuals with AD compared to controls (Shoji et al., 1998). The decrease in CSF Aβ42 appears to precede amyloid retention as detected by amyloid imaging using compounds such as ^{11}C labeled Pittsburgh compound B (^{11}C-PIB), signifying what is perhaps the first evidence of AD pathology in cognitively normal individuals (Fagan AM et al., 2009; Fagan et al., 2006) (Figure [1]). While CSF Aβ40 does not differentiate individuals with AD from controls, CSF Aβ40 has recently been shown to be decreased in a subset of subjects with CAA (Verbeek et al., 2009). Aβ42 alone is less useful in differentiating AD from other dementias, since low levels have also been documented in patients with frontotemporal dementia (FTD), vascular dementia, and dementia with Lewy bodies (DLB). Recent studies have shown that in both cognitively normal subjects as well as in those with AD, CSF Aβ42 is a very sensitive and specific marker for brain amyloid deposition as assessed by ^{11}C-PIB PET imaging regardless of clinical diagnosis (Fagan AM et al., 2009; Fagan et al., 2006). It may be that Aβ 42 is low in other diseases because some of these patients also have brain amyloid deposition.

Despite its utility in the detection and differential diagnosis of dementia, CSF Aβ42 does not correlate well with disease duration or severity. This is consistent with results from ^{11}C-PIB studies showing the amyloid retention does not change appreciably with advanced disease or increased duration (Rowe et al., 2007), and further supports results from pathological studies of AD; amyloid pathology occurs very early in the disease process and has relatively stabilized by the time the first clinical signs of dementia appear.

Some limitations of CSF Aβ42 studies include the lack of standardization for Aβ42 quantification among different laboratories and assays, and the relatively small amount of data from longitudinal studies on normal individuals. Moreover, little is known about the influences of normal aging on CSF turnover and clearance. Another important consideration is the normal hour-to-hour or day-to-day variability of CSF Aβ40 and Aβ42 levels. In a study of 15 non-demented individuals, Bateman et al demonstrated the presence of significant variation in Aβ42 levels of 1.5–4 fold over 36 hours of serially sampled CSF via indwelling lumbar catheters (Bateman et al., 2007). These results are similar to what is observed in studies of the interstitial fluid of APP transgenic mice (Kang et al., 2009). These findings highlight a possible source of variability in Aβ42 levels among different studies and stress the need to control CSF sampling procedures such as timing and subject activities to minimize such variability.

T-Tau

Tau is the major protein component of intra-neuronal NFT and is elevated in the CSF in the majority of patients with AD. In addition to the presence of tau in neurofibrillary tangles, it has been shown that tau is released into the extracellular space as a result of neuronal injury, and therefore, may indicate the severity of the underlying neurodegeneration (Blennow, 2004b). Over 50 studies have demonstrated an increase in the concentration of total tau (t-tau) by approximately 2–3 fold in AD compared with non-demented elderly subjects (Blennow et al., 2001). Marked elevation of CSF tau differentiates AD from non-demented elderly with a sensitivity of 92% and a specificity of 89% (Sunderland et al., 2003). As mentioned previously, tau elevation seems to occur at the early stages of disease and in some cognitively normal individuals, where its levels correlate with the amount of amyloid

deposition, who may represent individuals with preclinical AD (Fagan AM et al., 2009). However, it is important to consider that tau elevation can be seen in other diseases, potentially limiting the utility of tau alone in the differential diagnosis of dementia (Arai et al., 1997). Tau, as a marker of neuronal injury, can be transiently increased after any acute brain injury (such as stroke or trauma) (Hesse et al., 2001). Moreover, tau levels seem to remain relatively stable throughout the disease process (Sunderland et al., 1999) and do not correlate with dementia severity. Age might affect the CSF levels of tau; however, studies have been conflicting regarding the direction and significance of such an effect (de Leon et al., 2007).

P-tau

Abnormal tau phosphorlyation is present in neurofibrillary tangles. Therefore, p-tau may be a more specific marker of tangle formation (Blennow and Hampel, 2003), and has been investigated as a marker of AD pathology. As many as 30 different phosphorylation sites of p-tau have been identified (Buee et al., 2000), and ELISAs (enzyme-linked immunoassays) have been developed for at least 5 of them. Studies examining the utility of different forms of p-tau in the early diagnosis of AD, and in the differentiation from other causes of dementia, have consistently shown that p-tau 181(Arai et al., 2000), p-tau 231-235, or p-tau 396-404 (Hu et al., 2002) offer equivalent and possibly better diagnostic utility for AD than total tau. Studies comparing the diagnostic performance of different phosphorylation sites (p-181, p-199, and p-231) suggest that all three assays are equally effective in differentiating AD from non-demented controls. P-tau 231 appears to provide diagnostic specificity for AD and improves the differentiation between AD and FTD (Buerger et al., 2002b), while there is evidence that p-tau 181 improves the differentiation between AD and DLB (Hampel et al., 2004). P-tau 396-404, and the ratio of p-tau 396-404/t-tau, but not tau alone, has been shown in one study to differentiate AD from vascular dementia (Hu et al., 2002). In contrast to t-tau, p-tau is not increased secondary to acute brain injury, further adding to its diagnostic specificity.

Combination of Aβ42 and tau

The combination of Aβ42 and tau provides good discriminative value for individuals with AD compared to healthy controls of the same age, with a sensitivity of 85% and a specificity of 86%. However, lower specificities are obtained when these ratios are used to differentiate AD from other dementia etiologies. Other studies have utilized the tau x Aβ40/42 ratio (referred to as the AD index) (sensitivity 69% and specificity 88%) (Shoji et al., 1998) or the combination Aβ42, Aβ40, and tau for the detection of AD with a high sensitivity and specificity (97% sensitivity and 75% specificity) (Welge et al., 2009).

CSF biomarkers in predicting progression from MCI to AD and from cognitively normal to MCI

Progression from MCI to AD

Several studies addressing the utility of CSF markers in predicting the risk of progression from MCI to AD suggest that increased levels of tau, p-tau, and decreased levels of Aβ42 are present in MCI (Brys et al., 2009), with a sensitivity that is comparable to that seen in more advanced AD. A recent population based study has shown that decreased CSF Aβ42 is present in asymptomatic elderly, who during a several year follow-up, were at higher risk to develop AD (Blennow, 2004a). Lower CSFA β42/40 ratios seem to indicate risk of progression to AD in individuals with very mild dementia (CDR0.5) (Brys et al., 2009). It has also been suggested that increased levels of CSF tau provide 90% sensitivity and 100% specificity in predicting the progression from MCI to AD (Arai H, 1997). These results were

confirmed by another study suggesting that CSF tau is markedly increased in individuals with MCI who later progressed to AD compared with those who showed no evidence of progression (Buerger et al., 2002a). P-tau also seems to distinguish the subgroup of individuals with very early dementia (MCI or CDR 0.5) who will progress to AD from those who will not (Brys et al., 2009). Some investigators have suggested that the use of p-tau231–235 results in equivalent (Brys et al., 2009), or even superior, accuracy to t-tau in predicting progression from MCI to AD.

The combination of Aβ42 and tau is also useful; the relative risk of progression from MCI to AD was increased in patients who had high tau, p-tau, and low Aβ42 at baseline with a 90% sensitivity and 100% specificity in one study (Arai H, 1997). An increased tau/Aβ42 ratio was seen in 90% of individuals with MCI who later progressed compared to 10% of those who did not in a large longitudinal study of MCI patients followed for 18 months (Riemenschneider et al., 2002). The combination of tau and the Aβ42/P-tau181 ratio strongly predicted progression of MCI into more advanced AD in a longitudinal study with average follow-up of 4–6 years (Hansson et al., 2006). The utility of the AD CSF profile (defined by decreased Aβ42 and increased tau) to detect progression from MCI to more advanced stages of AD was recently confirmed in a longitudinal study of 100 individuals with mild AD, 196 individuals with MCI, and 114 controls (Shaw et al., 2009) and in another large multicenter study (Mattsson et al., 2009). It has also been suggested that both markers predict not only conversion from MCI to AD, but also the rate of progression of cognitive decline as measured by the CDR sum of boxes and neuropsychological test scores (Snider et al., 2009).

Progression from cognitively normal to MCI

The increased ratio of tau/Aβ42 and p-tau/Aβ42 in normal individuals has been associated with an increased risk of conversion from normal to MCI in 2 recent studies. In a study by Fagan et al, ~70 % of those with a high ratio, compared to only 10% of those with a normal ratio, converted from normal to MCI over a 3 year period (Fagan et al., 2007). Li et al reported that over a follow up of 42 months, all subjects who converted to MCI had elevated tau/Aβ42 ratios, while no conversions occurred in the normal ratio group (Li et al., 2007). It appears that the subgroup of normal elderly with high ratios have already developed Aβ deposition and neurodegeneration, and therefore most likely represent a subset with a diagnosis of preclinical AD.

Other Potential Blood and CSF markers

Several other markers have been investigated including markers specific for AD pathology, nonspecific markers of neuronal injury, and markers of oxidative stress among others.

Detection and quantification of β-secretase (BACE1) in the CSF, one of the key enzymes responsible for the pathologic amyloidogenic cleavage of the amyloid precursor protein (APP), appears to be promising. BACE1 concentration and activity in the CSF is significantly increased in MCI subjects compared with healthy controls, particularly in subjects with the apolipoprotein E-4(ApoE-4) risk allele (Zhong et al., 2007).

The presence of altered glycolated forms of acetyl- and butyrl-cholinesterase in AD brains has been described in both human studies and animal models of AD (Saez-Valero et al., 2003). It has been postulated that the CSF levels of these two markers directly correlate with the degree of neuronal damage and increase progressively with disease progression (Bailey, 2007). Among promising markers of neuronal injury currently under study is visinin-like protein (VILIP-1), a neuron-specific calcium sensor protein, which is significantly increased in the CSF of AD compared to controls, and demonstrates strong correlations with CSF

levels of tau (Lee et al., 2008). Preliminary observations indicate that this marker correlates with dementia severity, whole brain atrophy, and atrophy of specific brain regions involved in AD such as the hippocampus, entorhinal cortex, fusiform gyrus, cingulate, and parahippocampal gyrus (R. Tarawneh, J.M. Lee, and D.M. Holtzman unpublished results).

Oxidative stress and lipid peroxidation may be important in the pathogenesis of AD (Markesbery and Carney, 1999). Isoprostane is a by-product of lipid peroxidation and increased levels of CSF isoprostane levels have been reported in cross sectional studies of AD and MCI. Neuropathological studies have suggested that neuronal oxidation correlates with the Braak staging of neurofibrillary pathology (Montine et al., 1999). Moreover, results from studies on individuals with MCI show that isoprostane levels increase longitudinally and predict progression to AD (de Leon et al., 2007). Although isoprostane levels are not specific and oxidative injury is involved in other neurodegenerative conditions, these studies suggest that CSF isoprostane levels might improve our ability to differentiate AD from other etiologies of dementia such as FTD (Montine et al., 2001).

Other markers of oxidative injury include 3-nitrotyrosine (3NT), the end product of the interaction of peroxynitrite with tyrosine residues. Peroxynitrite results from the interaction of NO with superoxide radicals, and 3NT is a marker of oxidative injury in AD (Tohgi et al., 1999). On the other hand, 8-OH deoxyguanine (OHDG) results from the interaction of oxygen free radicals with DNA and is also being investigated (Lovell et al., 1999). A variety of inflammatory markers and cytokines are being investigated though none have yet been validated in multiple studies.

The list of other putative markers under investigation is extensive and includes cholesterol metabolites (Fassbender et al., 2001), homocysteine (Thal et al., 2006), S100β (Griffin et al., 1998), S100A7 (Qin et al., 2009), ubiquitin, and markers of astrogliosis such as GFAP (Wallin et al., 1996) and glutamine synthetase (Takahashi et al., 2002) among others. Studies assessing the utility of these identified markers are still in progress, and large multicenter trials utilizing unbiased mass spectrometry techniques are currently underway.

In Vivo Amyloid Imaging

The introduction of positron emission tomography (PET) imaging using radiotracers such as ^{11}C labeled Pittsburgh compound B (^{11}C-PIB) ligand to image amyloid in the living brain has been one of the major landmarks of imaging as a diagnostic tool in AD. ^{11}C-PIB is a derivative of the thioflavin-T amyloid dye which binds with high affinity and high specificity to Aβ in a fibrillar form in neuritic plaques and CAA (Rowe et al., 2007). Robust ^{11}C-PIB binding in several brain regions has been demonstrated in human studies of AD, with a significant 1.5–2 fold increase of ^{11}C-PIB binding compared to controls (Klunk et al., 2004). ^{11}C-PIB binding correlates with the regional distribution of amyloid in the frontal, parietal and temporal cortex in postmortem cases, and corresponds to areas with reduced FDG-PET activity (Edison et al., 2007). In contrast, there do not seem to be appreciable differences in ^{11}C-PIB retention between AD and normal controls in areas of the brain not typically affected by amyloid deposition in AD. While initial ^{11}C-PIB load is predictive of disease progression in the next 2 years (Rowe et al., 2007), amyloid load does not change considerably with clinical disease progression (Engler et al., 2006). This is not surprising based on the notion that amyloid deposition most likely has peaked or is close to peaking and stabilized by the time cognitive deficits become clinically detectable.

Since amyloid deposition is known to precede clinical signs of dementia by many years, ^{11}C-PIB imaging may allow early detection of amyloid during preclinical AD. In fact, up to 30% of cognitively normal elderly demonstrate substantial ^{11}C-PIB retention in the cortex by their mid 70 s, similar in extent to the amounts observed in subjects who have

mild to moderate AD (Mintun et al., 2006). A recent paper by Wolk et al. reported a study in which patients with MCI who are amyloid-positive exhibit the amyloid-imaging phenotype of patients with mild to moderate AD as well as having a similar pattern of cognitive and structural changes albeit to a lesser extent (Wolk et al., 2009). These results suggest that ^{11}C-PIB positivity in MCI most likely indicates already advanced amyloid pathology of AD, and further attests to the ability of ^{11}C-PIB to detect a process that probably began 10–20 years prior to early clinical changes. Further longitudinal studies are needed to determine how well this imaging change predicts eventual progression to more significant cognitive impairment. Also, since ^{11}C-PIB does not detect tau pathology, it is possible that the inclusion of other markers, such as markers for tau accumulation, will be needed to accurately predict cognitive progression and dementia.

In addition to ^{11}C-PIB, another currently available ligand is the ^{18}F-2-(1-(6-[(2-fluoroethyl(methyl)amino]-2-naphthyl)ethylidene) malononitrile (^{18}F-FDDNP). Studies suggest that FDDNP binds to both amyloid plaques and neurofibrillary tangles in vitro. In one study of 28 individuals, global ^{18}F-FDDNP retention differentiated patients with MCI from AD or non-demented controls (Small et al., 2006). However, its use may be limited by its narrow range of binding, and further studies are needed to address its correlation with other CSF and imaging biomarkers of AD (DeKosky, 2008).

Results from phase 2 trials of the ^{18}F-labeled tracer ^{18}F-AV-45 in the detection of amyloid are promising and it has recently entered phase 3 development. ^{18}F-AV-45 has several unique characteristics that make it suitable for Aβ plaque imaging in the living human brain: excellent binding affinity, high selective Aβ plaque labeling, and excellent brain penetration and rapid kinetics in animal studies (Choi et al., 2009). Preliminary results from phase 2 studies suggest that ^{18}F-AV-45 is a sensitive marker for the presence of amyloid in cortical gray matter in elderly individuals, and can differentiate groups of subjects meeting standard diagnostic criteria for AD, MCI, and normal cognitive function (R. Sperling, 2009).

Structural Imaging Markers

Evidence from neuropathological studies of AD indicate that atrophy on structural magnetic resonance imaging (MRI) is a good surrogate of NFT formation and can capture the cumulative outcome of the neurodegenerative process; neuronal and synaptic loss, and loss of supporting cellular structures (Bobinski et al., 2000; Zarow et al., 2005). In other words, structural MRI offers an indirect marker of neuronal atrophy, the loss of brain tissue, a hallmark of the neurodegenerative pathology of AD.

MRI in early detection and predicting the progression of AD

Both quantitative and qualitative measures of the hippocampus have gained interest as indicators of very early dementia and as predictors of progression to more advanced stages of disease (Jack et al., 1998). Hippocampal volumes have been found to be an anatomical measurement to reliably differentiate individuals with very mild dementia (CDR 0.5 or MCI) from cognitively normal individuals in cross sectional studies (de Leon et al., 2007). The inclusion of the fusiform gyrus with hippocampal volumes further allows the differentiation of very early dementia (MCI) from more advanced stages (Convit et al., 2000). Automated measures of the thickness of the entorhinal cortex suggest high accuracy in differentiating AD from controls (Lerch et al., 2005).

In addition to their diagnostic accuracy, longitudinal studies suggest a benefit for MRI measures in predicting progression in AD. Decreased hippocampal volumes have been shown to predict progression to more severe stages of AD in individuals with MCI (de Leon et al., 1993). It has been suggested that changes in the entorhinal cortex of the hippocampal

formation often precede changes in the hippocampus, and consequently, may be even earlier markers of progression from cognitively normal to very early AD (MCI) and from MCI to AD (Jack et al., 1999). Other longitudinal studies also suggest that the rate of hippocampal atrophy is different in AD than in non-demented elderly; atrophy rates of 3% to 7% per annum were demonstrated in AD (Jack et al., 1998; Laakso et al., 2000), whereas healthy controls showed a maximum atrophy rate of 0.9% with old age (Raz et al., 2004).

The Alzheimer Disease Neuroimaging Initiative (ADNI) is a multicenter longitudinal study of MCI and AD which utilizes standardized methods in MRI data acquisition and processing across study sites (Jack et al., 2008; Petersen et al., 2010). A recent report by ADNI suggests that individuals with MCI who progress to AD demonstrate a very similar pattern of atrophic changes to the AD group up to a year before meeting the clinical criteria for AD. The degree of atrophy in the medial temporal lobe structures seems to be the best indicator of imminent progression to AD (Risacher et al., 2009). Other studies utilizing fluid-registered techniques to generate hippocampal and whole brain volumes in serial MRIs have also indicated different rates of hippocampal and whole brain atrophy between AD and controls. Moreover, these studies, suggest superiority of fluid registered techniques over manual measures in detecting such differences (Barnes et al., 2007).

Various automated methods have been developed to demonstrate change in brain structure and morphology in the last few years, in an effort to enhance efficiency and inter-rater reliability. Automated measurements which detect changes in whole brain volume over time are currently being employed as secondary end points in clinical treatment trials. Using this method, atrophy rates of approximately 2.5% reduction in whole brain volume over one year have been reported in AD, compared with only 0.4% to 0.9% in healthy controls (Hampel et al., 2008).

Voxel-based volumetry (VBM) appears to be more reliable in detecting atrophy of specific brain regions in AD. This method consistently shows reductions in the cortical gray matter in the region of the mediotemporal lobes and lateral temporal and parietal association areas in AD (Baron et al., 2001), and in the mediotemporal lobe and lateral association areas of the temporal and parietal lobes in MCI (Pennanen et al., 2005). Moreover, MCI individuals who are at high risk for conversion to AD demonstrate patterns of atrophy that resemble those of AD and are different from MCI individuals who do not progress (Chetelat et al., 2005). Atrophy of brain regions such as the mediotemporal, laterotemporal, and parietal association areas has been observed in genetically predisposed individuals, even years prior to the onset of clinical signs of cognitive impairment (Teipel et al., 2004).

Other imaging techniques that have been studied include deformation-based morphometry (DBM), which transforms the brain volumes at high resolution to a standard template brain, while preserving regional differences in gray matter volumes. Preliminary studies indicate that DBM can reliably differentiate AD from nondemented elderly, and predict progression from MCI to AD with 70–80% accuracy over a 1.5 year follow up period (Teipel et al., 2007). However, these findings will need to be validated in larger studies.

Previous longitudinal studies of structural MRI have concluded that MRI is not superior to CSF biomarkers in predicting future conversion to AD (Bouwman et al., 2007). On the other hand, recent longitudinal studies of cognitively normal individuals and individuals with early AD, which utilize automated rather than visual scoring systems, suggest that MRI may be a more sensitive predictor of decline in cognitive functions (as determined by the CDR-sum of boxes [CDR-SB]) than CSF biomarkers (Vemuri et al., 2009b). However, these results need replication from other groups, more subjects, and longer follow up periods to determine which methods, alone or together are the most accurate. While MRI and CSF

biomarkers are each independently associated with cognitive decline, their use in combination may provide an even higher utility in identifying the subset of individuals with very mild (CDR 0.5) or mild (CDR 1) AD who are at higher risk of progression to more advanced stages of disease (Hampel et al., 2008).

MRI as an indicator of disease severity

There is accumulating evidence that loss of cognitive function correlates well with measures of cortical atrophy on structural MRI (Ridha et al., 2008). This has been supported by pathological studies showing that MRI is a good surrogate of neurodegeneration, NFT formation, and an even better surrogate of neuronal and synaptic loss (Bobinski et al., 2000; Zarow et al., 2005). Based on this, some studies have suggested that MRI may in fact demonstrate a better correlation with cognitive function than CSF biomarkers in cross-sectional (Vemuri et al., 2009a) and longitudinal studies (Jack et al., 2005; Stoub et al., 2005). Rates of change on MRI, but not CSF tau, correlate with change in MMSE scores in longitudinal studies (Sluimer et al., 2008). There are several possible explanations for these findings. While T-tau is a marker of NFT pathology, there is evidence from pathological studies that NFT formation and neurodegeneration precede neuronal loss in areas such as the entorhinal cortex (Price et al., 2001a). Although T-tau levels reflect neuronal injury to some degree, MRI is a more direct reflection of the cumulative neuronal and axonal loss irrespective of underlying disease pathology (Vemuri et al., 2009b). Results of studies have been conflicting regarding the ability of validated CSF markers of AD such as tau and Aβ42 in measuring disease progression or dementia severity (Stefani et al., 2006; Sunderland et al., 1999), possibly due to the fact that each of these markers reflects one process, among many, that contribute to neurodegeneration in AD. On the other hand, by directly measuring the degree of neuronal, synaptic, and axonal loss, the use of MRI allows us to visualize the final outcome of several complex and highly intricate pathological processes that characterize AD. Another possible explanation for the better correlation of MRI with dementia severity, compared to CSF markers, is the relative stability of structural imaging, and the minimal degrees of physiological variation over short periods of time (Vemuri et al., 2009b). This in contrast to some fluid biomarkers, which as soluble proteins, undergo inevitable physiological, and sometimes even diurnal, variations in their tissue and fluid levels (de Leon et al., 2002).

Role of structural imaging markers in translational research

In addition to their diagnostic and predictive utility, other potential roles for imaging markers include monitoring disease progression and response to therapy in clinical trials. Imaging markers with high diagnostic specificity will be very helpful in patient selection for clinical trials, by identifying a number of patients who might have other forms of dementia. Moreover, well-established imaging markers for AD such as hippocampal volumetry may allow risk stratification in MCI cohorts in treatment trials (Hampel et al., 2008). Structural markers that reflect rates of atrophy can be useful in monitoring disease progression and disease severity. MRI measures may be able to detect slowing in the rate of atrophy over time; as demonstration of disease modification in therapeutic trials. Volumetric measures of the hippocampus are already being employed as secondary end points in several pharmacologic trials, and in the near future may be approved as surrogate end points and secondary outcome variables in trials of potential disease-modifying therapies. It is important to note, however, that one of the possible limitations of the use of radiological markers as endpoints in clinical trials is the apparent discrepancy between rates of atrophy and clinical outcomes. This is perhaps best exemplified by the 3 yr study of individuals with MCI, in which the use of donepezil was associated with a slower rate of hippocampal atrophy on MRI (Hashimoto et al., 2005) despite no benefit in reducing the rate of progression from MCI to AD.

Functional Imaging Markers

FDG-PET(fluoro-deoxy-glucose positron emission tomography)

FDG-PET studies suggest the presence of characteristic and progressive reductions in regional measurements of the cerebral metabolic rate for glucose (CMRglc) in patients with AD and very mild dementia (or CDR 0.5) (Silverman et al., 2001). Individuals with AD demonstrate reductions in the posterior cingulate, parietal, temporal, and prefrontal cortex, which correlate with dementia severity and progression (Alexander et al., 2002; Minoshima et al., 1995). Furthermore, these changes can help predict the rate of cognitive decline in individuals with very mild dementia and accurately predict the presence of AD pathology (Silverman et al., 2001).

Even more interesting results have been found by studies exploring the utility of PET in detecting preclinical AD; particularly by measures of CMRglc reductions in the hippocampus and entorhinal cortex. Reductions in hippocampal glucose metabolism have been shown to predict decline to MCI in a group of cognitively normal individuals followed longitudinally (de Leon et al., 2001). Similar results were found in other longitudinal studies of cognitively normal individuals; individuals who progressed to dementia had significant reductions in glucose metabolism in the hippocampus and temporal neocortex compared to those who did not progress (Drzezga et al., 2003). Consistent with the knowledge that the entorhinal cortex (EC) is one of the earliest areas to be affected in AD, hypometabolism of the EC has also been shown to accurately predict decline to MCI or CDR 0.5 with a sensitivity of 83% and a specificity of 85% (de Leon et al., 2007). In a recent study by Jagust et al, FDG-PET seems to correlate well with CSF levels of Aβ42 and with measures of cognitive function (Jagust et al., 2009). To date, studies with FDG-PET that have been used to predict cognitive decline have been small compared to studies with CSF biomarkers and more subjects need to be studied with this technique to understand its ultimate value.

Over the last decade, FDG-PET has gained considerable interest as a research tool in dementia. The results of several studies attesting to the accuracy of FDG-PET in detecting early stages of AD have provided the basis for the current design and development of FDG-PET -based automatic diagnostic systems for AD. In fact, FDG-PET seems to be able to detect characteristic changes of AD (such as biparietal-temporal hypometabolism) in individuals with genetic susceptibility to AD in the absence of any clinical signs of cognitive impairment. Unlike CSF biomarkers, FDG-PET seems to correlate with disease progression and may, therefore, complement clinical evaluations in following disease course. Despite the correlation between regional metabolic changes and regional pathological changes in AD, the use of FDG-PET to identify subgroups of AD patients with different neuropsychological profiles has been limited by lack of validation from large pathological studies and somewhat conflicting results across studies.

As novel therapies for AD emerge, it will become essential to identify research tools that can be incorporated as secondary outcome measures in clinical therapeutic trials. A number of studies have correlated the response to cholinergic drugs with metabolic changes on FDG-PET (Bohnen et al., 2005). In addition, the effects of potential neurotherapeutic drugs on regional glucose metabolism have been used as an index for regional synaptic activity, and might therefore offer insight into therapeutic efficacy. FDG-PET paradigms that capture patterns of regional brain metabolism during the performance of a specific memory task (i.e. activation paradigms) might offer more information regarding specific drug effects on cognitive function.

Single Photon Emission Computed Tomography (SPECT), Magnetic Resonance Spectroscopy (MRS), and Perfusion MRI

SPECT has shown variable success in the prediction of progression from MCI to AD. It has been shown to be potentially useful in the early detection of AD (Jagust et al., 2001), and in monitoring disease progression. Cholinesterase inhibitors have been shown to reliably affect regional cerebral blood flow (rCBF) in group studies (Lojkowska et al., 2003). Magnetic resonance spectroscopy, which measures concentrations of N-acetyl-aspartate, creatine, and choline in the brain, has been investigated in AD and may improve early detection. Pulsed arterial spin labeled (ASL) perfusion MRI allows the assessment of cerebral blood flow with the use of radioactivity and preliminary studies suggest possible utility in differentiating AD from other dementia etiologies.

Functional MRI

Functional magnetic resonance imaging (fMRI) can be used to measure changes associated with brain activation during cognitive tasks with high resolution. Several studies have investigated brain activation changes as a potential marker for early AD; early changes in activation during memory tasks can precede and predict the occurrence of AD.

Moreover, insights into functional patterns of activation of different brain regions, or so called functional networks, have been gained from studies in cognitively normal individuals. Several studies utilizing functional MRI have identified what is often referred to “the default network”, a group of brain regions activated during several internally focused tasks such as remembering past events or imagining future events (Gusnard and Raichle, 2001). The neuroanatomical substrates of this network include the medial temporal lobe and hippocampus, medial frontal association area, the posterior cingulate cortex, the retrosplenial cortex, the inferior parietal cortex and the lateral temporal lobe (Buckner et al., 2005). Interestingly, these areas overlap significantly with the distribution of amyloid pathology, atrophy, and altered glucose metabolism in individuals with AD (Buckner et al., 2005). Recently, it has been shown that this network is disrupted in subjects with amyloid deposition, even in the preclinical period (Sheline et al., 2009; Sperling et al., 2009). These findings indicate that the levels of activity and metabolism in young age may be conducive to AD pathology in older age. This is consistent with our current knowledge of Aβ dynamics: CSF Aβ is dependent on synaptic activity and areas with higher synaptic activity may be more predisposed to amyloid deposition (Cirrito et al., 2005; Kamenetz et al., 2003).

In conclusion, structural and functional imaging markers can be useful in the early detection of AD. They may offer utility in monitoring and predicting disease progression as well as providing insight into networks that are involved early in the disease process, including the preclinical phases of disease. It is important to consider current limitations such as lack of validation in large studies of some methods. Functional MRI and amyloid imaging offer great promise for future studies. The ability to accurately image aggregated tau or neurofibrillary tangles would be a major advance in the field.

Electrophysiological studies: Electroencephalography (EEG) and Event-related Potentials (ERP)

There has been increasing interest lately in electrophysiological studies for the detection of early stages of AD. With recent technological advances in data acquisition and processing, it is now possible to collect electrophysiological data from up to 256 leads, generate 3 dimensional topographical images, and perform source localization analyses. EEG is a noninvasive and relatively cost-effective technique, adding to its appeal as a potential marker in AD.

These technological advances have stimulated a series of studies addressing the potential utility of quantitative EEG (qEEG) and event-related potentials (ERPS) in the early identification of individuals with mild and very mild AD, and those individuals who are at high risk of progression to more advanced stages of disease.

EEG in early detection of very mild and mild dementia

There appear to be differences in the low-frequency power spectra, theta and delta power spectra, in individuals with MCI compared to normal controls (Jackson and Snyder, 2008). A few studies have shown that delta power of the centrotemporal and posterior fields is reduced in MCI compared to normal (Liddell et al., 2007), and that the delta power directly correlates with immediate memory recall in these individuals (Grunwald et al., 2001). Increased theta activity has also been documented in very early dementia, and appears to differentiate those individuals from cognitively normal elderly. In addition, when compared to cognitively normal elderly, individuals with MCI show decreased alpha power. However, individuals with MCI had a smaller decrease in low range alpha power (8–10.5 Hz) on picture memory tasks when compared to controls (net result is higher low range alpha in MCI) (Grunwald et al., 2001). Since an increase in low range alpha power is associated with poor memory performance, these findings suggests low range alpha power may be a sensitive marker of cognitive impairment in MCI. Decreased beta power has also been described in individuals with mild AD.

The P 600 is an ERP measure of episodic memory which has been investigated in AD. Results of these studies suggest that both individuals with very mild and mild AD demonstrate decreased P600 response to word repetition (Olichney et al., 2002). Furthermore, the P600 repetition effect amplitude correlates with several measures of declarative verbal memory (Olichney et al., 2002). N 400 is thought to be an indicator of semantic comprehension and is generated by several brain regions including the anterior fusiform gyrus, medial temporal lobe and parahippocampus; areas known to be involved in early AD. Changes in the latency and amplitude of the P300 and N400 responses have been described in AD (Gironell et al., 2005; Olichney et al., 2002).

Predicting the progression from MCI to AD

In one study, EEG theta power was increased in patients with very mild dementia who progressed to AD compared to those who did not progress with a predictive accuracy of 90% (Prichep et al., 2006). Another study identified significantly stronger EEG sources for delta (temporal), theta (parietal, occipital, and temporal), and alpha 1 (central, parietal, occipital, temporal, and limbic areas) rhythms for patients with very mild dementia who progressed to AD during a one year follow up (Rossini et al., 2006). Furthermore, P300 latency has been suggested to predict progression to AD (Gironell et al., 2005). Functional connections between different brain regions can be measured by coherence; a linear measure of the correlations between two signals as a function of frequency. Low frontoparietal coherence has been proposed as another predictor of progression to AD in individuals with very mild dementia (Pogarell et al., 2005).

Conclusion and future directions

There have great advances in the identification of several CSF and radiological markers for AD over the last decade. CSF Aβ42 and tau as well as amyloid imaging reflect the main pathological hallmarks of AD, and these measurements combined with accurate volumetric assessments by structural MRI are all proving to be useful as diagnostic and prognostic markers (Table 1). This is particularly important given the ability of these markers to detect the earliest stages of disease, even prior to the onset of clinical symptoms. However, AD is a

multifaceted disease and several pathological substrates, besides plaques and tangles, contribute to its progression. These are likely to include inflammation, oxidative stress, and excitotoxicity. Therefore, biomarkers that allow the detection and monitoring of these processes will complement current validated markers of disease, and may enhance our ability to detect disease in the presymptomatic stages. One particular advantage for radiological markers is their ability to directly measure neuronal damage, the cumulative end product of the many pathological processes of AD. Therefore, structural MRI measures and possibly FDG-PET scans correlate more closely with disease progression and with clinical measures of dementia severity. Results from recent fMRI studies at rest and during cognitive tasks are particularly promising as prognostic markers. Other CSF markers of neuronal injury may offer similar utility to MRI as diagnostic and prognostic markers, and are being investigated.

There is no doubt that the identification of preclinical markers will have a significant impact on the design and outcome of clinical trials, and will allow the identification of individuals who will benefit from disease modifying therapies as they become available. While each of these markers offers certain utility in diagnosis or prognosis, it is very likely that no single marker will be able to capture all aspects of this disease, and that a combination of markers will eventually prove to be the most useful.

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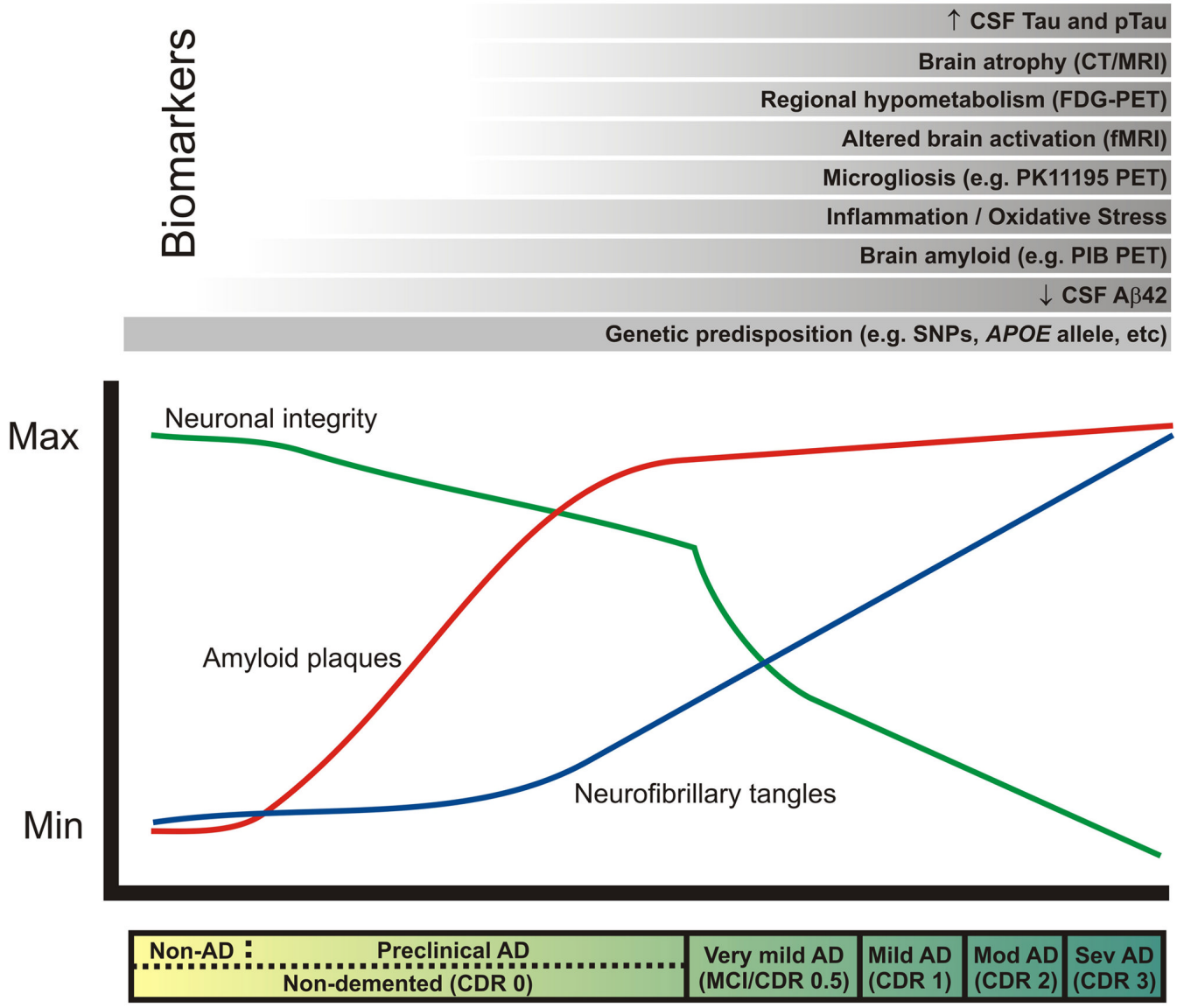

Figure 1. Biomarkers and Alzheimer's disease: proposed changes in biomarkers in relation to time course of pathological and clinical stages

The clinical stages of Alzheimer's disease (AD), marked by progressive dementia described as very mild/mild cognitive impairment (MCI), 'mild', 'moderate' and 'severe', correspond to clinical dementia rating (CDR) scores of 0.5, 1, 2, and 3, respectively (see bar at bottom). These stages are associated with abundant amyloid plaques (red line), the gradual accumulation of NFTs (blue line) and synaptic and neuronal loss in certain brain regions (green line). In the preclinical stage of Alzheimer's disease, $A\beta_{42}$ peptide forms amyloid plaques in the brains of non-demented individuals (CDR 0) for approximately 10–15 years, and damages neuronal processes and synapses. Eventually, dramatic neuronal losses occur in association with the onset of dementia. Alzheimer's disease biomarker research seeks to measure changes in the structure and function of the brain (for example atrophy, regional activity changes and hypometabolism, amyloid-plaque and NFT formation, microgliosis, inflammation and oxidative stress) that might be useful for diagnosis and prognosis during this preclinical phase of the disease, before irreversible neuronal loss occurs. These changes can be measured by radiological imaging modalities (for example computed tomography

(CT), magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), and positron-emission tomography (PET) with various imaging contrast agents) and/or by biochemical examination of cerebrospinal fluid (CSF). The most promising biomarker candidates at present are listed (gray bars at top) chronologically, according to the earliest stage of the pathological process at which they seem to show utility. A reduced concentration of $A\beta_{42}$ in cerebrospinal-fluid may provide the earliest definitive evidence of Alzheimer's pathology in the brain. Genetic variations (for example single nucleotide polymorphisms (SNPs)) may also be considered biomarkers that allow the earliest possible estimation of risk. PIB, Pittsburgh compound B. Figure reprinted with permission, from Perrin RJ, Fagan AM, Holtzman DM. 2009 Nature, 461:916–922.

Table 1

Levels of Evidence for AD biomarkers.

Marker type	Diagnosis of AD versus controls	Diagnosis of MCI versus controls	Prognosis: predicting progression from MCI to AD	Prognosis: predicting progression from cognitively normal to MCI or AD
CSF biomarkers	Primary studies - Sufficient evidence of a direct relationship[1*]	Primary studies - Sufficient evidence of a direct relationship[1**]	Primary studies - Sufficient evidence of a direct relationship[1***]	Primary studies - Sufficient evidence of a direct relationship[1]
Volumetric MRI	Primary studies - Sufficient evidence of a direct relationship[1***]	Primary studies - Sufficient evidence of a direct relationship[1***]	Primary studies - Sufficient evidence of a direct relationship[1]	Primary studies - Sufficient evidence of a direct relationship[1]
Functional MRI	Primary studies – Limited/ Suggestive Evidence of an Association[3]	Primary studies – Limited/ Suggestive Evidence of an Association[3]	Primary studies – Inadequate/Insufficient Evidence to Determine Whether an Association Exists[4]	Primary studies – Inadequate/Insufficient Evidence to Determine Whether an Association Exists[4]
FDG-PET	Primary studies – Sufficient evidence of a direct relationship[1]	Primary studies – Sufficient evidence of a direct relationship[1]	Primary studies – Sufficient evidence of a direct relationship[1]	Primary studies – Limited/ Suggestive Evidence of an Association[3¶]
PET-PIB	Primary studies – Sufficient evidence of a direct relationship[1]	Primary studies – Sufficient evidence of a direct relationship[1]	Primary studies – Sufficient evidence of a direct relationship[1]	Primary studies- Inadequate/Insufficient Evidence to Determine Whether an Association Exists[4¥]
Standard EEG	Primary studies - Limited/ Suggestive Evidence of an Association[3€]	Primary studies – Limited/ Suggestive Evidence of an Association[3€]	Primary studies – Inadequate/Insufficient Evidence to Determine Whether an Association Exists[4†]	Primary studies Inadequate/Insufficient Evidence to Determine Whether an Association Exists[4†]

Levels of evidence are based on Categories of Association established and used by the Institute of Medicine for association between a biomarker and a specific health outcome (Committee on Health Effects Associated with Exposures During the Gulf War. Institute of Medicine, 2000):

[1] Sufficient Evidence of a Direct Relationship: Evidence fulfills the guidelines for sufficient evidence of an association, is supported by experimental data in humans and animals, and satisfies several of the guidelines used to assess causality: strength of association, dose–response relationship, consistency of association, and a temporal relationship.

[2] Sufficient Evidence of an Association: Evidence of association is sufficient to conclude that there is a positive consistent association, in human studies in which chance and bias, including confounding, could be excluded with reasonable confidence. It differs from category 1 because of the lack of experimental data in humans or animals that support the relationship.

[3] Limited/Suggestive Evidence of an Association: Evidence is suggestive of an association between a biomarker and a specific health outcome in human studies, but the body of evidence is limited by the inability to exclude chance and bias, including confounding, with confidence.

[4] Inadequate/Insufficient Evidence to Determine Whether an Association Exists: Evidence is of insufficient quantity, quality, or consistency to permit a conclusion regarding the existence of an association between exposure to a specific agent and a specific health outcome in humans.

* There are over 50 studies (including cross-sectional and longitudinal studies) for CSF tau, over 16 studies including longitudinal studies for CSF Aβ42, and over 5 studies including longitudinal studies for the combination of tau and Aβ42.

** Several studies indicate that CSF biomarkers can detect very mild dementia (MCI) with a sensitivity that is similar to that of more advanced AD.

*** There are several studies including studies with neuropathological confirmation of diagnoses.

¶ Evidence is limited to a small number of studies with methodological variations.

¥ Insufficient evidence: there are a limited number of studies.

€ Inconclusive evidence: studies have shown conflicting results with methodological variations. The majority of studies suggest utility in differentiating AD or MCI from controls.

† Insufficient evidence: there are only a few prospective studies with limited sample size populations.