

CRITERIA FOR PRECLINICAL ALZHEIMER'S DISEASE – June 2, 2010

Preclinical Alzheimer's Disease Workgroup

Reisa Sperling, Brigham and Women's Hospital (Chair)
Laurel Beckett, University of California, Davis
David Bennett, Rush University Medical Center
Suzanne Craft, VA Puget Sound Health Care System
Anne Fagan, Washington University, St Louis
Jeffrey Kaye, Oregon Health Science University
Tom Montine, University of Washington
Denise Park, University of Texas, Dallas
Eric Reiman, Banner Alzheimer's Institute
Eric Siemers, Eli Lilly and Company
Yaakov Stern, Columbia University
Kristine Yaffe, University of California, San Francisco

Adjunct Members:

Clifford Jack, Mayo Clinic, Rochester
Paul Aisen, University of California, San Diego

Towards defining the preclinical stage of Alzheimer's disease

Introduction

Converging evidence from both genetic at-risk cohorts and clinically normal older individuals suggests that the pathophysiological process of Alzheimer's disease (AD) begins years, if not decades, prior to the diagnosis of clinical dementia. Recent advances in neuroimaging, cerebrospinal fluid assays, and other biomarkers now provide the ability to detect evidence of AD pathologic changes *in vivo*. Emerging data in clinically normal older individuals suggest that biomarker evidence of amyloid- β accumulation is associated with functional and structural brain alterations, consistent with the patterns of abnormality seen in patients with mild cognitive impairment (MCI) and dementia due to AD. Furthermore, clinical cohort studies suggest that there may be very subtle cognitive alterations that are detectable years prior to meeting criteria for MCI, and that predict progression to clinical dementia consistent with AD. The long preclinical phase of AD provides a critical opportunity for potential intervention with disease-modifying therapy, if we are able to elucidate the link between the pathophysiological process of AD and the emergence of the clinical syndrome.

A recent report on the economic implications of the impending epidemic of AD, as the "baby boomer" generation ages, suggests that 13.5 million individuals will manifest clinical AD by the year 2050 (http://www.alz.org/alzheimers_disease_trajectory.asp). A hypothetical intervention that delayed the onset of AD by 5 years would result in a 57% reduction in the

number of AD patients, and reduce the projected Medicare costs of AD from \$627 to \$344 billion dollars. Screening and treatment programs instituted for other diseases such as cholesterol screening for cardiovascular and cerebrovascular disease, colonoscopy for colorectal cancer, and mammography for breast cancer have already been associated with a decrease in mortality due to these conditions. The current lifetime risk of AD for a 65 year old is estimated to be 10.5%. Recent computer models suggest that a screening instrument for AD pathology (with 90% sensitivity and specificity), and a treatment that slows progression by 50%, would reduce that risk to 5.7%.

Both laboratory work and recent disappointing clinical trial results raise the possibility that therapeutic interventions applied earlier in the pathophysiological process of AD would be more likely to achieve disease-modification. Studies with transgenic mouse models suggest that amyloid- β -modifying therapies may have limited impact once neuronal degeneration has begun. Several recent clinical trials in the stages of mild to moderate dementia have failed to demonstrate clinical benefit, even in the setting of biomarker or autopsy evidence of decreased amyloid- β burden. Although the field is already moving to earlier clinical trials at the stage of mild cognitive impairment (MCI) or prodromal AD, it is possible that similar to cardiac disease and cancer treatment, AD would be optimally treated prior to significant cognitive impairment, in the “presymptomatic” or “preclinical” stages of AD. Secondary prevention studies, which would treat “normal” individuals with evidence of occult AD pathology to delay the onset of clinical symptoms, are already in the planning stages. The overarching therapeutic objective of these preclinical studies would be to treat early pathologic processes (e.g. lower amyloid- β burden) in order to prevent subsequent neurodegeneration and eventual cognitive decline.

For these reasons, our working group sought to examine the evidence for a definable preclinical stage of AD, and to review the biomarker, epidemiological, and neuropsychological factors that best predict the risk of progression from “normal” to MCI and AD clinical dementia. To narrow the scope of our task, we chose to specifically focus on predictors of cognitive decline thought to be due to AD pathology. We did not address cognitive aging in the absence of recognized pathologic changes in the brain, nor cognitive decline due to other common age-related brain diseases, however, we clearly acknowledge that these brain diseases, in particular, cerebrovascular pathology, Lewy bodies, or other neurodegenerative processes may significantly influence clinical manifestations of AD and possibly its pathophysiology. Although there are likely life-long characteristics and mid-life risk factors that influence the likelihood of developing cognitive impairment in late-life, for feasibility in current studies, we chose to focus on the 10-year period prior to the emergence of cognitive impairment. We chose the term “preclinical AD” to refer to the full spectrum from completely asymptomatic individuals with biomarker evidence of AD pathologic change to individuals manifesting subtle cognitive decline but who do not yet meet accepted clinical criteria for MCI.

Furthermore, we propose operational research criteria for the study of preclinical AD. These criteria are intended to provide a common language to advance the scientific understanding of the preclinical stages of AD and a foundation for the evaluation of preclinical AD treatments. Additional data are clearly required, but we hope these criteria will enable researchers to further characterize the sequence of biological events over the course of preclinical AD, refine biomarker criteria that will best predict clinical outcome, and ultimately, aid in selecting appropriate populations for preclinical therapeutic intervention.

Redefining the earliest stages of Alzheimer's disease

The term "Alzheimer's disease" refers in some contexts to the neuropathological criteria for AD and in others to the clinical syndrome of progressive cognitive and behavioral impairment. As we move towards defining the earliest stages of AD, the dissociation between these two connotations of the term "Alzheimer's disease" becomes particularly salient. It has become increasingly clear that both the pathophysiological process and clinical symptomatology of AD are best conceptualized as a continuum or a trajectory and that these processes may evolve in parallel but temporally offset trajectories. In particular, emerging evidence suggests that there may be a time lag of a decade or more, between the beginning of the pathological cascade and the onset of clinically evident impairment. Our working group focused on defining this preclinical stage of AD, investigating the evidence that the pathophysiological process is progressing over this period, and determining the factors which best predict the emergence of clinical impairment at the stage of MCI.

The concept of a preclinical phase of disease should not be too foreign, as medical professionals readily acknowledge that cancer can be detected at the stage of "carcinoma *in situ*" and that hypercholesterolemia and atherosclerosis can result in narrowing of coronary arteries that is detectable prior to myocardial infarction. It is widely acknowledged that symptoms are not necessary to diagnose human disease. Organ failure, for example cirrhosis or chronic renal disease, can be suggested by blood tests (e.g., prothrombin time and liver chemistries, or creatinine) and then confirmed by tissue biopsy, in the absence of any symptoms. Effective treatment of the underlying disease (e.g. alcohol abuse, viral infection, inflammatory disease) can reduce or prevent the emergence of symptoms. Thus, we should be open to the idea that AD can be diagnosed preclinically by the presence of biomarker evidence of AD pathologic change, and that this and other biomarkers may eventually guide therapy prior to the onset of symptoms.

The difficulty in AD is that we have not yet established a firm link between the appearance of any specific biomarker and the subsequent emergence of clinical symptomatology. If we can, however, definitively determine the risk of developing AD and the temporal course of progression associated with occult pathology in individuals without dementia or MCI, we will open a crucial window of opportunity to intervene with disease-modifying therapy. While we hypothesize that the earliest detectable pathological change will be in the form of amyloid (A β) accumulation, it is possible that amyloid is necessary but not sufficient to produce the clinical AD syndrome, and that cognitive decline will occur only in the setting of synaptic dysfunction and/or additional neurodegeneration, including paired helical filament tau formation and neuronal loss. It also remains unknown whether there is a specific threshold of AD pathologic change, and/or specific anatomic location of AD pathology, and/or a specific combination of biomarker abnormalities that will best predict the emergence of clinical symptomatology. Evidence also suggests that additional factors, such as brain and cognitive reserve, and conversely, other age-related brain changes, such as white matter alterations and dopaminergic depletion, as well as other brain diseases, in particular cerebrovascular disease or presence of Lewy bodies, may modulate the relationship between the pathological and clinical manifestations of AD. We also recognize that a substantial subset of individuals can evidence all of the classic neuropathologic features of AD at autopsy but never express dementia during life. It remains unknown whether these individuals would have manifested clinical symptoms should they have lived longer. Recent advances in ante-mortem biomarkers

of AD pathologic change now allow us to test the hypothesis that these individuals are indeed in the preclinical stages of AD.

The clinical continuum of Alzheimer's disease

Three working groups were established by the NIA/Alzheimer's Association to address recent advances in AD. Two working groups focused on the stages of MCI and dementia due to underlying Alzheimer's disease, respectively. Our group was charged with developing recommendations for the study of individuals who have the underlying AD pathologic changes but do not meet accepted clinical criteria for dementia or MCI. It is likely that even this stage of the disease represents a continuum from completely asymptomatic to very subtle cognitive decline from baseline just prior to meeting criteria for MCI (see Figure 1).

Our group carefully considered a number of monikers to best capture this continuum, including "asymptomatic AD", "presymptomatic AD", "latent AD", and "preclinical AD". The term "preclinical" was felt to best encompass the continuum, from completely asymptomatic individuals with biomarker evidence suggestive of AD pathologic change to individuals demonstrating subtle decline, including subjective report of memory decline, and/or objective evidence of slight cognitive decline from their baseline, but not yet meeting standardized criteria for MCI. This latter group of individuals may be classified as "Not normal, not MCI" and may represent a group at high risk for cognitive decline, and would be included under the rubric of preclinical AD.

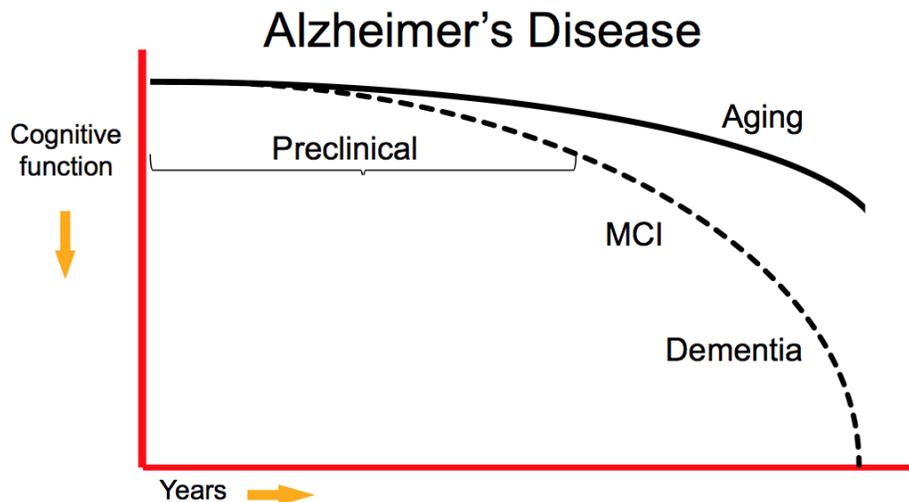


Figure 1. Model of the clinical trajectory of AD. The stage of preclinical AD precedes MCI and encompasses both asymptomatic individuals in whom the pathophysiological process has already begun but who are clinically indistinguishable from the profile of normal or "typical" aging, as well as individuals who have demonstrated subtle decline from their own baseline that exceeds that expected in typical aging, but would not yet meet criteria for MCI.

Models of the pathophysiological sequence of AD

In order to facilitate the discussion of the concept of a preclinical stage of AD, we propose a hypothetical model of the pathophysiological cascade of AD (see Figure 2). It is important to acknowledge that this model, although based on the prevailing evidence, may be incorrect, is certainly incomplete, and will evolve as additional laboratory and clinical studies

are completed. Indeed, this model should be viewed as an initial attempt to bring together multiple areas of research into our best estimate of a more coherent whole. Perhaps most importantly, the proposed model of AD views A β peptide accumulation as etiologic, a view driven by our deeper knowledge of autosomal dominant early-onset forms of AD and genetic polymorphisms, such as apolipoprotein ϵ 4 allele (*APOE*). However, we hasten to add that the etiology of late-onset AD is less clear and some investigators have proposed that synaptic, mitochondrial, metabolic, or neuronal cytoskeletal alterations may play an even earlier role than A β in the predisposition to late-onset AD.

The majority of currently proposed models of AD suggest that abnormal accumulation of amyloid- β_{42} , is an early event in the pathophysiologic cascade. There remains significant debate in the field as to whether abnormal processing or clearance of A β_{42} is in fact the primary inciting event in sporadic AD, and some investigators have suggested that sequestration of A β into fibrillar forms may be a protective mechanism. Nevertheless, all of the known autosomal dominant, early onset forms of AD are thought to be due, at least in part, to alterations in amyloid-precursor protein (*APP*) production or cleavage. Similarly, trisomy-21 invariably results in AD pathology in individuals who have three intact copies of the APP coding region located on chromosome 21. Finally, apolipoprotein E (*APOE*), the major genetic risk factor for late onset AD, has been implicated in amyloid trafficking and plaque clearance. Both autopsy and biomarker studies (see below) similarly suggest that A β_{42} accumulation increases with advanced aging, the greatest risk factor for developing AD.

Through mechanisms that remain to be elucidated, the accumulation of A β is postulated to lead to synaptic dysfunction, neurodegeneration, neurofibrillary tangle formation and eventually neuronal loss. It remains unknown whether this neurodegenerative process is due to direct synaptic toxicity from oligomeric forms of A β disruption of axonal trajectories from fibrillar forms of A β or a “second hit” that results in synaptic dysfunction and neuronal loss. It is clear that synaptic depletion, intracellular hyperphosphorylated forms of tau and neuronal loss invariably occur in AD, and at autopsy, these markers appear to correlate better than A β plaque load with clinical impairment. Although we present evidence below that the presence of markers of fibrillar A β accumulation is associated with markers of “downstream” pathologic change, including tau, neural dysfunction, glial activation, and neuronal loss and atrophy, it remains to be proven that A β accumulation is sufficient to incite the downstream pathological cascade of AD.

Epidemiological data suggest there are significant modulating factors that may alter the pace of the clinical expression of the disease, although evidence that these factors alter the underlying pathophysiologic process itself is less secure. Large cohort studies have implicated multiple health factors that may increase the risk for developing cognitive decline and dementia thought to be due to AD. In particular, vascular risk factors such as hypertension, hypercholesterolemia, and diabetes have been associated with an increased risk of dementia, and may contribute directly to the impact of AD pathology on the aging brain. Depressive symptomatology has also been linked to increased risk of manifesting MCI and AD, as well as chronic psychological distress. It also remains unclear whether there are specific environmental factors that may influence the progression of the pathophysiological sequence or the clinical expression of the pathology. On the positive side, there is some evidence that engagement in leisure activities, including cognitive, physical, and social activity, may be associated with decreased risk of MCI and AD.

The temporal lag between the appearance of pathology and the emergence of clinical symptomatology may also be altered by factors such as brain or cognitive reserve. The concept of reserve was originally invoked to provide an explanation for the observation that the degree of AD pathology at autopsy was not always associated with the clinical manifestation of cognitive impairment, and can be thought of as the ability to tolerate higher levels of brain pathology without exhibiting clinical symptomatology. Brain reserve refers to the capacity of the brain to withstand pathologic insult, perhaps due to greater synaptic density or larger number of healthy neurons, such that sufficient neural substrate remains to support normal function. In contrast, cognitive reserve is thought to represent the ability to engage alternate brain networks or cognitive strategies to cope with the effects of encroaching pathology. It is not clear, however, that the data support a sharp demarcation between these two constructs, as many factors, such as higher socio-economic status or engagement in cognitively stimulating activities, may contribute to both forms of reserve. Higher education and socio-economic status have been associated with lower age-adjusted incidence of AD diagnosis. Recent studies suggest that high reserve may primarily influence the capability of individuals to cope with their AD pathology for longer periods of time, and may be associated with rapid decline once an “inflection point” is reached and compensatory mechanisms begin to fail.

Hypothetical model of AD pathophysiological cascade

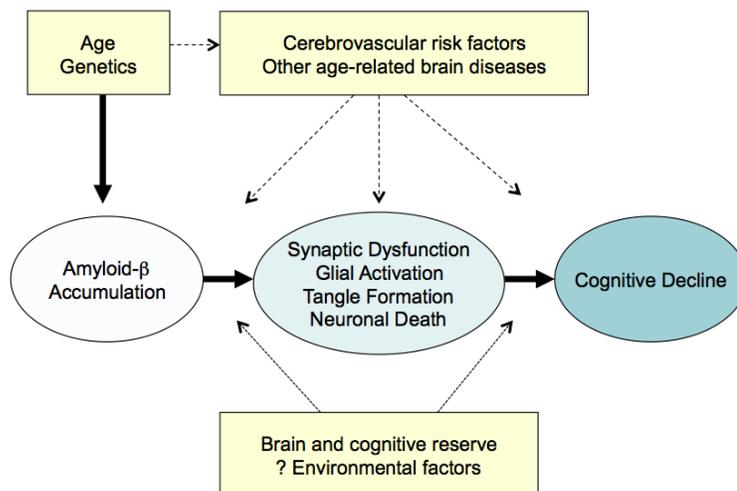


Figure 2: Hypothetical model of the pathophysiological sequence leading to cognitive impairment in AD. This model postulates that amyloid- β accumulation is an “upstream” event in the cascade that results synaptic dysfunction, which may lead directly to cognitive impairment and/or trigger “downstream” neurodegeneration and cell loss. Specific host factors, such as brain and cognitive reserve, or other brain diseases may mediate the response to amyloid toxicity and pace of progression towards the clinical manifestations of AD.

Biomarker model in the preclinical stage of Alzheimer’s disease

A biomarker model has recently been proposed in which the most widely validated biomarkers of AD pathology become abnormal and likewise reach a ceiling in an ordered manner (See Figure 3). This biomarker model parallels the hypothetical pathophysiological

sequence of AD, and is particularly relevant to tracking the preclinical stages of AD. Biomarkers of brain A β amyloidosis are reductions in CSF A β_{42} and increased amyloid PET tracer retention. Elevated CSF tau is not specific to AD and is thought to be a biomarker of tau-mediated neuronal injury. Decreased FDG uptake on PET in a characteristic temporal-parietal pattern is a biomarker of AD-related synaptic dysfunction. Brain atrophy on structural MRI in a characteristic pattern involving medial temporal, paralimbic and temporal-parietal isocortex is a biomarker of AD-related neurodegeneration.

This biomarker model has the following features. (1) A β amyloid plaque biomarkers become abnormal first and a substantial A β load accumulates prior to the appearance of clinical symptoms. Note that in this model, brain amyloid is necessary but not sufficient to produce the clinical symptoms of MCI and dementia. (2) Biomarkers of dysfunction, neuronal injury, and degeneration are dynamic later in the disease and, unlike amyloid biomarkers; the severity and change over time in these biomarkers do correlate with clinical symptoms. (3) Structural MRI is the last biomarker to become abnormal; however, MRI retains a closer relationship with cognitive performance later into the disease than other biomarkers. (4) None of the biomarkers are static; rates of change in each biomarker change over time and follow a non-linear time course, which is hypothesized to be sigmoid shaped. (5) Anatomic information from imaging biomarkers provides useful disease staging information in that the topography of disease-related imaging abnormalities changes in a characteristic manner with disease progression. (6) There is a lag phase of unknown duration between A β accumulation and clinical symptoms. Similar to the hypothetical pathophysiological model above, inter-subject differences in this time lag are likely due to differences in brain resiliency, cognitive reserve and the added contributions of co-existing pathologies.

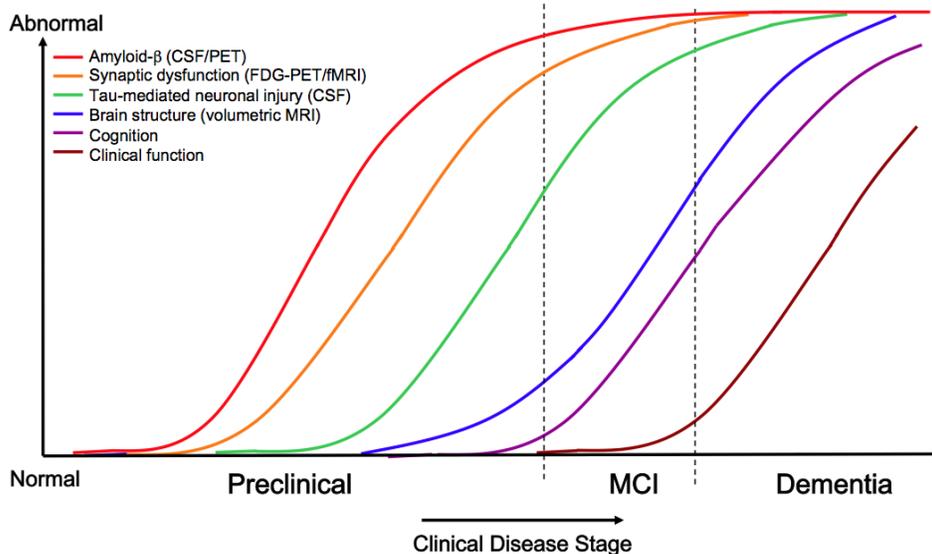


Figure 3: Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade, expanded in the preclinical phase. A β as identified by CSF A β_{42} assay or PET amyloid imaging. Synaptic dysfunction evidenced by FDG PET or functional MRI. Tau-mediated neuron injury by CSF tau or phospho-tau. Brain structure by structural MRI. Biomarkers change from maximally normal to maximally abnormal (y axis) as a function of disease stage (x axis). The temporal trajectory of two key indicators used to stage the disease

clinically, cognition and clinical function, are also illustrated. Figure adapted with permission from Cliff Jack.

Biomarker Evidence Linking AD pathology to Early Symptomatology

Several multi-center biomarker initiatives, including the Alzheimer’s Disease Neuroimaging Initiative (ADNI), and the Australian Imaging, Biomarker & Lifestyle Flagship Study of Ageing (AIBL), as well as major biomarker studies in preclinical populations at several academic center studies are ongoing. These studies have already provided preliminary evidence that biomarker abnormalities consistent with AD are detectable prior to the emergence of overt clinical symptomatology, and are predictive of subsequent cognitive decline. Many of the recent studies have focused on markers of amyloid- β , using either cerebrospinal fluid assays of $A\beta$ peptide or PET amyloid imaging with radioactive tracers that bind to fibrillar forms of $A\beta$. Both CSF and PET amyloid imaging studies suggest that a substantial proportion of clinically normal older individuals demonstrate evidence of $A\beta$ accumulation. The exact proportion of “amyloid-positive” normal individuals is dependent on the age and genetic background of the cohort, but ranges from approximately 20-40%, and is very consonant with large post-mortem series. Interestingly, the percentage of amyloid-positive normal individuals detected at a given age closely parallels the percentage of individuals diagnosed with AD dementia a decade later. These data support the hypothesis that there is a temporal lag between the appearance of detectable AD pathology and the emergence of clinical symptomatology.

Appearance of Plaques vs. Dementia

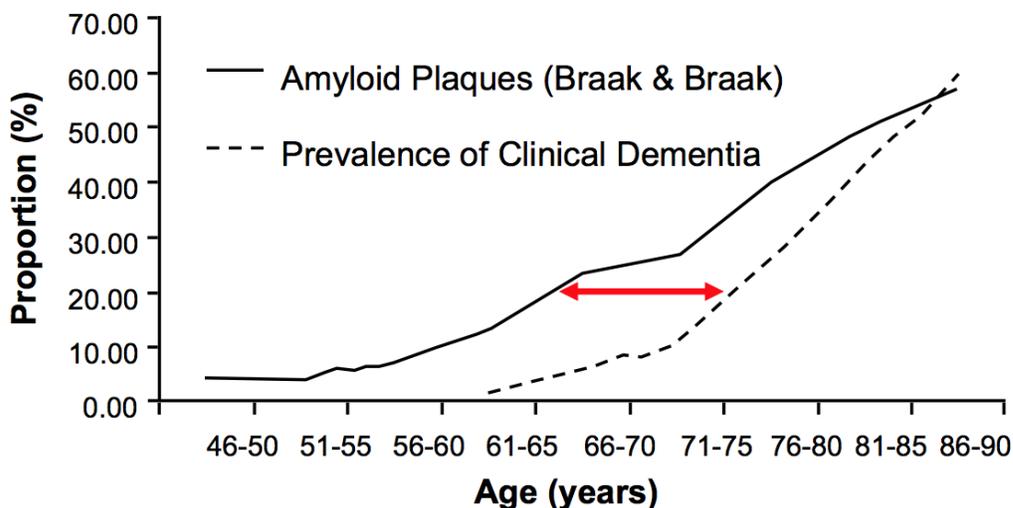


Figure 4: Evidence for a temporal lag of approximately a decade between the accumulation of amyloid- β (from large autopsy series) and the clinical syndrome of dementia (estimated prevalence from epidemiological studies). Figure courtesy of Mark Mintun and John Morris, Washington University.

Several groups have reported that cognitively normal older individuals with low CSF $A\beta_{1-42}$ or high PET amyloid binding demonstrate decreased brain volume and disruption of functional networks, consistent with the patterns seen in AD. There have been variable reports in the literature thus far, as to whether amyloid positive individuals demonstrate lower

neuropsychological test performance at the time of biomarker study, which may represent heterogeneity in where these individuals fall on the preclinical continuum and the degree of cognitive reserve in the cohorts. A few early studies have reported that amyloid positivity in clinically normal older individuals is associated with an increased risk of cognitive decline and progression to dementia. Studies focused on other biomarkers, including volumetric MRI, FDG-PET, and plasma A β in cohorts of clinically normal older individuals have also reported evidence that these markers are predictive of cognitive decline. Additional longitudinal studies are clearly needed to confirm these findings and to elucidate the combination of factors that best predict likelihood and rate of decline.

As a complement to longitudinal studies in the population at risk by virtue of age, researchers continue to detect and track the biological and cognitive changes associated with the predisposition to AD in cognitively normal people at differential genetic risk for AD, alone or in conjunction with other risk factors (such as a person's reported family history of the disease). To date, the best established genetic risk factors for AD include common variants of apolipoprotein E (*APOE*), the major late-onset AD susceptibility gene, uncommon early-onset AD-causing mutations in the presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), and amyloid precursor protein (*APP*) genes, and Trisomy 21 (Down syndrome). Biomarker studies in presymptomatic carriers of these genetic risk factors have revealed evidence of amyloid accumulation on CSF and PET amyloid imaging, FDG-PET hypometabolism, and brain atrophy that may precede symptoms by more than a decade.

Cognitive Studies

Despite the clear potential of biomarkers for diagnosis of early Alzheimer's disease, it is important not to lose sight of the potential that behavioral markers hold for the disorder. Tests developed by both neuropsychological and cognitive aging researchers have provided evidence that normal aging is accompanied by declines in speed of information processing, executive function (working memory, task switching, inhibitory function) and reasoning. Studies that have conducted assessments of cognitive function at multiple time points prior to dementia have also consistently shown a long period of gradual cognitive decline in episodic memory as well as non-memory domains progressing up to a decade before onset of dementia. Importantly, in studies that have modeled the curve of cognitive change, the preclinical trajectory suggests not only a long and slow rate of presymptomatic change, but also a period of acceleration of performance decrement that may begin several years before MCI onset. Recent studies also suggest that self-report of subtle cognitive decline, even in the absence of significant objective impairment on testing, may portend future decline in older individuals. Despite the existence of multiple studies spanning thousands of participants, the promise of both subjective and objective cognitive measures for assessing risk of progression to AD has not yet been fully realized. It is likely that measured change in cognition over time will be more sensitive than any one-time measure. Additional longitudinal studies of older individuals, perhaps combining biomarkers with measures sensitive to detecting very subtle cognitive decline, are clearly needed.

Caveats

Although the afore-mentioned studies provide compelling evidence that the presence of AD pathology in "normal" older individuals is associated with brain

alterations consistent with AD, and that specific factors may accurately predict those individuals who at a higher risk of progression to meet MCI and dementia criteria, it is important to note several potential confounding issues in the majority of these studies. It is likely that many of these studies suffer from cohort biases. In particular, the biomarker and cognitive studies are likely not representative of the general older population, as they are typically “samples of convenience”, that is volunteer cohorts who tend to come from highly educated and socio-economic status backgrounds. Older individuals who are willing to participate in such intensive studies may also represent the “volunteer gene”, and may be more actively engaged than the typical aging population. Conversely, these cohorts may include subjects who self-select for this research due to subjective concerns about their own memory function or positive family history, as reflected by the high rate of *APOE* $\epsilon 4$ carriers in some of these cohorts.

It is also important to note that although these biomarkers have revolutionized the field of early AD, these markers are merely “proxies” for the underlying pathology and may not fully reflect the biological process in the living brain. For example, both CSF and PET amyloid imaging markers are estimates of the fibrillar forms of $A\beta$ and may not provide information about oligomeric forms, which may be the relevant species for synaptic toxicity. Similarly, our proxy measurements for synaptic dysfunction, such as functional MRI or FDG-PET are indirect measurements of neural function. Other markers of neurodegeneration such as CSF tau and volumetric MRI are not specific to the AD process. Finally, it is important to acknowledge that the relationship between biomarkers and cognition may vary significantly across age and genetic cohorts.

Draft Operational Research Criteria for Preclinical AD

In order to facilitate future studies, we propose draft operational research criteria to define preclinical study cohorts for use in 1) longitudinal natural history studies to determine whether the presence of $A\beta$ markers, either in isolation or in combination with additional markers of neurodegeneration, is predictive of cognitive decline in clinically normal older individuals, and 2) clinical trials of potential disease modifying agents to investigate effects on biomarker progression and/or the emergence of clinical symptoms.

We emphasize that these criteria are not intended to serve as diagnostic criteria for clinical purposes. Use of these criteria in the clinical setting are currently unwarranted because many individuals who satisfy the proposed criteria may not develop the clinical features of AD in their lifetime and use of this information in this context could be associated with unwarranted concern, as there is currently insufficient information to relate preclinical biomarker evidence of AD to subsequent rates of clinical progression with sufficient certainty.

These criteria are based on the postulate that AD is characterized by a sequence of biological events that begins far in advance of clinical dementia. Based on current evidence from both genetic at-risk and older cohort studies, we put forth the hypothesis that $A\beta$ accumulation, or the stage of cerebral amyloidosis, is one of the earliest measurable stages of AD, and occurs prior to any evidence of cognitive symptomatology. We postulate that the presence of biomarker “positivity” for $A\beta$, alone or in combination with other AD biomarkers, may have implications for the subsequent course of AD and the responsiveness to treatments targeting different stages of the disease.

Recognizing that the preclinical stages of AD represent a continuum, we further suggest

the following preclinical staging criteria, which may prove useful in defining study cohorts to test specific hypothesis. Research cohorts could be selected on the basis of these staging criteria, to optimize the ability to ascertain the specific outcomes important for a given type (e.g. natural history or treatment trial) and duration of the study. Evidence of “downstream” biomarkers or subtle cognitive symptoms may increase the likelihood of rapid emergence of cognitive symptomatology and clinical decline to MCI/prodromal AD within several years, eventually permitting us to distinguish earlier and later preclinical stages of AD. The presence of one or more of these additional biomarkers would indicate that individuals are already experiencing early neurodegeneration, and as such, it is possible that amyloid-modifying therapies may be less efficacious once the downstream pathology is set in motion. There are specific circumstances, however, such as pharmaceutical industry trials that may desire a cognitive or clinical endpoint, rather than solely biomarker outcomes. In these cases, it may be advantageous to enrich the study population with individuals in late preclinical stages of AD, who would be most likely to rapidly decline and manifest MCI within a short time period. We recognize that these stages will likely require further modification as new findings emerge, and the feasibility of delineating these stages in recruiting clinical research cohorts remains unclear. These criteria are intended to facilitate the standardized collection of new data to generate some of those findings.

Operational Research Criteria for Defining Preclinical AD

1. Biomarker evidence of amyloid- β accumulation (Stage 1 = asymptomatic cerebral amyloidosis)
 - a. Elevated tracer retention on PET amyloid imaging and/or low A β_{42} on CSF assay
2. Biomarker evidence of synaptic dysfunction and or early neurodegeneration (Stage 2 = evidence of amyloid positivity + presence of one or more additional AD markers)
 - a. Elevated CSF tau or phospho-tau
 - b. Hypometabolism in an AD-like pattern (i.e. posterior cingulate, precuneus, and/or temporo-parietal cortices) on FDG-PET
 - c. Cortical thinning/grey matter loss in AD-like anatomic distribution (i.e. lateral and medial parietal, posterior cingulate and lateral temporal cortices) and/or hippocampal atrophy on volumetric MRI
3. Evidence of subtle cognitive decline, but does not meet criteria for MCI or dementia (Stage 3 = amyloid positivity + markers of neurodegeneration + very early cognitive symptoms)
 - a. Demonstrated cognitive decline over time on standard cognitive tests, but not meeting criteria for MCI
 - b. Subtle impairment on challenging cognitive tests, particularly accounting for level of innate ability or cognitive reserve but not meeting criteria for MCI

Stage 1. The stage of asymptomatic cerebral amyloidosis

These individuals have biomarker evidence of A β accumulation on CSF A β ₄₂ assays and/or on PET amyloid imaging, but no detectable evidence of additional brain alterations suggestive of neurodegeneration or subtle cognitive or behavioral symptomatology. As above, we note that the currently available CSF and PET imaging biomarkers of A β primarily provide evidence of amyloid accumulation and deposition of fibrillar forms of amyloid. Although limited, current data suggest that soluble or oligomeric forms of A β are likely in equilibrium with plaques, which may serve as reservoirs for soluble forms, but it remains unknown whether there is an identifiable pre-plaque stage in which only soluble forms of A β are present. There are also emerging data from genetic-risk cohorts that suggest early synaptic changes may be present prior to evidence of amyloid accumulation using currently available amyloid markers. As laboratory data increasingly suggest that oligomeric forms of amyloid may be critical in the pathological cascade, and there is ongoing work to develop CSF and plasma assays for oligomeric forms of A β Thus it may be possible in the future to detect a stage of disease that precedes Stage 1.

The standards for determining “amyloid-positivity” are still evolving (see next section). Although recent work suggests there may be a for CSF A β ₄₂ cut-off value that is predictive of progression from MCI to AD dementia, it is unknown whether a similar threshold will be most accurate in individuals with normal or near normal cognition. Similarly, using PET imaging techniques, it remains unknown whether a summary numeric threshold within an aggregate cortical region or within specific anatomic region will provide most useful predictive value. Recent data suggest that although CSF A β ₄₂ is strongly inversely correlated with quantitative PET amyloid imaging measures (distribution value ratio DVR or standardized uptake value SUVR), there are some individuals who demonstrate decreased CSF A β ₄₂ who would not be considered amyloid positive on PET scans. It remains unclear whether this finding reflects different thresholds employed across these techniques or if decreased CSF A β ₄₂ is an earlier marker of accumulation.

Stage 2. Amyloid positivity + evidence of synaptic dysfunction and/or early neurodegeneration

These individuals have evidence of amyloid positivity and presence of one or more markers of “downstream” AD pathologic change. The markers include synaptic dysfunction detectable on FDG-PET, network dysfunction detectable on functional MRI, tau-mediated neuronal injury detectable on CSF measures of tau or phospho-tau and/or neuronal loss, as suggested by cortical or hippocampal atrophy detectable on volumetric MRI. Emerging evidence suggests that biomarker evidence of A β accumulation is often associated with markers of dysfunction and neurodegeneration in a distributed network of brain regions, including posteromedial cortices (precuneus, posterior cingulate, and retrosplenial cortices), lateral parietal, lateral temporal, and medial temporal regions, including the hippocampus and entorhinal cortices. Although these studies have demonstrated that, on average, amyloid positive individuals demonstrate significantly greater abnormalities on these markers compared to amyloid negative individuals, there is significant inter-subject variability. We hypothesize that amyloid-positive individuals with evidence of early neurodegeneration are farther down the trajectory, and in later stages of preclinical AD. It remains unclear whether it will be feasible to detect differences among these other AD biomarkers, but there is some evidence that early synaptic dysfunction, as assessed by functional imaging techniques such as FDG-PET and functional MRI, may be detectable prior to volumetric loss.

Stage 3. Amyloid positivity + evidence of neurodegeneration + subtle cognitive decline

We postulate that individuals with biomarker evidence of amyloid accumulation, early neurodegeneration, and evidence of subtle cognitive decline are in the last stage of preclinical AD, and are approaching the border zone with accepted MCI criteria. These individuals may demonstrate evidence of decline from their own baseline (particularly if putative proxies of cognitive reserve are taken into consideration), even if they still perform within the “normal” range on standard cognitive measures. There is emerging evidence that more sensitive measures, particularly with challenging episodic memory measures, may detect very subtle cognitive impairment. It remains unclear whether self-complaint of memory decline will be a useful predictor of progression, but it is possible that the combination of biomarkers and subjective assessment of subtle change will prove useful.

Need for additional study

We propose a general framework with biomarker criteria for the study of the preclinical phase of AD, however, more work is needed to clarify the optimal CSF assays, PET or MRI analytic techniques, and in particular the specific thresholds needed to meet these criteria. There are significant challenges in implementing standardized biomarker “cut-off” values across centers, studies, and countries. Work to standardize and validate both fluid-based and imaging biomarker thresholds is ongoing in multiple academic and pharmaceutical industry laboratories, and in several multi-center initiatives. These criteria will need to be validated in large multi-center natural history studies, or as provisional criteria for the planning of preventative clinical trials. For instance, it will be important to establish the test-retest and cross-center reliability of biomarker measurements, further characterize the sequence of biomarker changes, and the extent to which these biomarkers predict subsequent clinical decline or clinical benefit.

It remains unclear whether it is meaningful or feasible to make the distinction between amyloid as a risk factor for developing clinical AD versus amyloid accumulation as the earliest detectable stage of AD. The threshold for amyloid-positivity may also evolve over time, as it may be most useful to retrospectively define a “cut-off” on the basis of predictive value. It may be most feasible to select research cohorts for large studies solely on the basis of amyloid-positivity on CSF or PET amyloid imaging, and to utilize additional measures for post-hoc analyses to determine additional predictive value.

Although recent advances in biomarkers have revolutionized our ability to detect evidence of early AD pathology, there is still a need for novel biomarker development. In particular, although the current amyloid biomarkers provide evidence of A β deposition, an *in vivo* marker of oligomeric forms of A β would be of great value. Imaging markers of intraneuronal pathology, including specific markers of tangles and alpha-synuclein, are also needed. In addition, more sensitive biomarkers that can detect early synaptic dysfunction and neural network disruption may be useful to track early response to amyloid-lowering therapies, prior to the stage of neuronal loss. Finally, we may be able to use the currently available biomarkers as a new “gold standard” to re-evaluate simple blood and urine markers that were discarded on the basis of excessive overlap between clinically normal and AD patients. The significant proportion of clinically normal individuals who are amyloid-positive on both CSF and PET imaging may have confounded prior studies attempted to differentiate “normal” controls from AD.

Similarly, additional work is required to identify and validate neuropsychological measures to detect the earliest clinical manifestations of AD. We need to develop sensitive measures in multiple cognitive domains that will reveal early synaptic dysfunction in neural networks vulnerable to early AD pathology. We also need to develop measures of very early function change in other domains, including social interaction, mood, psychomotor aspects of function, and decision making. These measures would allow us to better link the pathology to the emergence of clinical symptomatology, and may be particularly useful to monitor response to potential disease-modifying therapies in these very early stages.

The proposed criteria apply primarily to individuals at-risk by virtue of advanced age, as inclusion criteria for trials in autosomal dominant mutation carriers and homozygous *APOE* ϵ 4 carriers will likely be defined primarily on genetic status. Trials in genetic-risk populations might utilize the above criteria to stage individuals within the preclinical phase of AD. In genetic-risk cohorts, it may even be possible to detect an even earlier stage of presymptomatic AD, prior to the point when there is already detectable cerebral amyloidosis. Several FDG-PET and fMRI studies have suggested that evidence of synaptic dysfunction may be present in young and middle-aged *APOE* ϵ 4 carriers, and there may be other biological alterations that are present prior to significant deposition of fibrillar forms of amyloid that would be preferentially responsive to presymptomatic intervention.

Finally, in parallel with further development of novel bio, cognitive, and behavioral markers, we need to begin large longitudinal studies with existing markers as soon as possible, including planning for interventional trials for preclinical populations. There are a number of burgeoning efforts to design and conduct clinical trials in both genetic at-risk and amyloid positive older individuals, including the Dominantly Inherited Alzheimer Network (DIAN study of familial AD), the Alzheimer Prevention Initiative, and Anti-Amyloid Treatment in Asymptomatic AD (A4) trial being considered by the Alzheimer's Disease Cooperative Study (ADCS).

The definitive studies to determine whether asymptomatic individuals with A β accumulation are indeed destined to develop AD dementia, and whether intervention with potential disease-modifying therapies in the preclinical stages will prevent cognitive decline are likely to take more than a decade to fully accomplish. Thus, we must move quickly to test the postulates put forth above, and adjust our models and study designs as new data become available. It is entirely possible that promising drugs will fail to impact the clinical course of AD at the stage of dementia or even MCI, when the neurodegenerative process is well entrenched, but may be efficacious at the earliest stages of the pathophysiological process, prior to the onset of symptoms. As potential biologically active treatments may be associated with small but significant risk of adverse side-effects, we will need to determine whether we can predict the emergence of cognitive symptoms with sufficient certainty to appropriately weigh the risk/benefit ratios. It is clear that many questions remain to be answered, and that there may be additional factors which will influence the probability of developing clinical AD. However, the considerable progress made over the past two decades enables a strategic path forward to test these hypotheses, move the field towards earlier intervention, and ultimately towards the prevention of cognitive decline associated with AD pathology.