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Dementia screening, biomarkers and protein misfolding

Implications for public health and diagnosis

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Misfolded proteins are at the core of many neurodegenerative diseases, nearly all of them associated with cognitive impairment. For example, Creutzfeldt-Jacob disease is associated with aggregation of prion protein,[1,2] Lewy body dementia and Parkinson disease with α-synuclein[3,4] and forms of frontotemporal dementia with tau, TDP43 and a host of other proteins.[5,6] Alzheimer disease (AD), the most common cause of dementia,[7] and its prodromal syndrome mild cognitive impairment (MCI)[8] are an increasing public health problem and a diagnostic challenge to many clinicians. AD is characterized pathologically by the accumulation of amyloid β-protein (Aβ)[9,10] as senile plaques and in the walls of blood vessels as amyloid angiopathy.[11,12] Additionally, there are accumulations of tau-protein as neurofibrillary tangles and dystrophic neurites.[11,12] Biological markers of AD and MCI can serve as in vivo diagnostic indicators of underlying pathology, particularly when clinical symptoms are mild[13-15] and are likely present years before the onset of clinical symptoms.[16-19] Research to discover and refine fluid and imaging biomarkers of protein aggregation has undergone a rapid evolution[20-22] and combined analysis of different modalities may further increase diagnostic sensitivity and specificity.[23-26] Multi-center trials are now investigating whether imaging and/or cerebrospinal fluid (CSF) biomarker candidates can be used as outcome measures for use in phase III clinical trials for AD.[27-29]

Key words: dementia, screening, biomarkers, amyloid, tau, Alzheimer disease, preclinical, presymptomatic

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Currently, the diagnosis of AD is based on exclusion of other forms of impairment with definitive diagnosis requiring autopsy confirmation.[30] Thus, there is a strong need to find easily measurable in vivo AD biomarkers that could facilitate early and accurate diagnosis[31] as well as prognostic data to assist in monitoring therapeutic efficacy.[32] Although biological markers such as MRI, PET scans and CSF increase the diagnostic likelihood that AD is present,[9,18-20,33,34] biomarkers are invasive, uncomfortable, expensive and may not be readily available to rural areas, underserved communities, underinsured individuals or developing countries, making them impractical for broad use. However, the lessons learned from biomarkers can be applied to increase the likelihood that clinicians will be able to detect disease at earlier stages in the form of dementia screening.

Public health may be best defined as the organized efforts of society to improve health, often framed in terms of primary, secondary and tertiary prevention. Prevention encompasses an understanding of causation, alteration of natural history of disease and understanding of pathophysiological mechanisms.[35] The clearest application of this from a public health perspective is in the setting of secondary prevention (i.e., screening)—early detection as a core element, coupled with treatments or preventative actions to reduce the burden of disease.[35] In this instance we seek to identify individuals in whom a disease has already begun and who may be experiencing very mild clinical symptoms but have not yet sought out medical

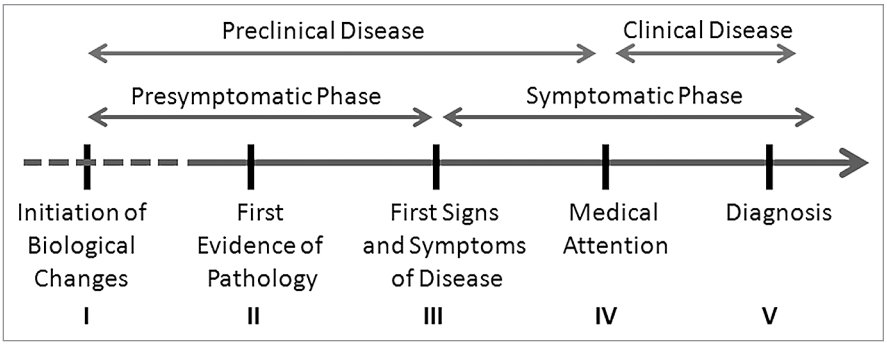

Figure 1. Model of the natural history of AD. Timeline from presumptive start of AD through patient diagnosis is plotted. The initiation of biological changes (stage I) marks the onset of disease and begins years to decades before any evidence is apparent (represented by dashed lines). At some point the first pathologic evidence of disease (stage II) begins and in theory can be detected with biomarkers such as CSF measurements of amyloid and tau or PET imaging with amyloid ligands. Subsequently, the first signs and symptoms of disease develop (stage III) followed by the patient seeking medical attention (stage IV) and finally a diagnosis is established (stage V). This timeline can be clustered into a presymptomatic phase (stages I–III) and a symptomatic phase (stages III–V). An alternative way to envision the disease spectrum is from the biological onset to the seeking of medical attention (stages I–IV) as the preclinical phase of disease with the clinical phase beginning with the initial clinical investigations into the cause of the patients' symptoms (stages IV and V). Stage III is the ideal time for dementia screening.

care. The objective of effective screening is to detect the disease earlier than it would have been detected with usual care. Recent healthcare reform (Accountable Care Act)[36] proposes a Personalized Prevention Plan including screening for cognitive disorders, reimbursable through Medicare. Thus tying knowledge about dementia screening with underlying biology of protein misfolding associated with neurodegenerative disease can have enormous implications.

A review of the natural history of dementia illustrates this point (**Fig. 1**). The timeline of disease from presumptive start to the patient demise is plotted. Stage I marks the biologic onset of disease; however this point often cannot be identified and may begin years to decades before any evidence is apparent (represented by dashed lines). As this stage is subclinical, it is difficult to study in humans but lends itself nicely to animal models. At some point in the progression of the biology, stage II begins heralding the first pathologic evidence of disease could be obtained—in the case of AD this could include CSF measurements of amyloid and tau[22,26,27] or PET imaging with amyloid ligands.[18,37] Subsequently, the first signs and symptoms of disease develop (stage III). Till this point, the disease process has been entirely presymptomatic. Beginning with the onset of symptoms, the patient may seek medical care (stage IV) and eventually be diagnosed (stage V). From stage III onwards, the patient enters the symptomatic phase of disease. From this point, the patient is typically treated with various pharmacologic and nonpharmacologic approaches towards some outcome. Another way to envision the disease spectrum is from the biological onset to the seeking of medical attention as the preclinical phase of disease with the clinical phase beginning with the initial clinical investigations into the cause of the patients' symptoms.

What is the value of thinking about disease in this fashion? Such models allow researchers and clinicians to model the approach to finding and applying new diagnostics and offering new interventions. From stage I to stage III, the patient is the presymptomatic, preclinical phase of disease. The only means of detection would be with a biological marker that reflected protein misfolding or some proxy marker of these events. Although longitudinal evidence of cognitive change exist from 1–3 years before clinical diagnosis, raw scores on neuropsychological testing during this time remains in the normal range.[38] After stage IV, the patient is in the symptomatic, clinical phase of disease. Testing here is centered on confirming the suspected diagnosis, correctly staging the disease and initiating the appropriate therapies. Basic scientific approaches focusing on the presymptomatic, preclinical phase and clinical care approaches focusing on the symptomatic, clinical phase are well established and will continue to benefit from additional research.

However, if we focus only on these two phases, an opportunity will be missed to make a decidedly important impact in the patient's well-being. From stage III to stage IV, the patient enters symptomatic, preclinical phase of disease; symptomatic because the patient or family is beginning to detect some aspect of change, but preclinical because these signs and symptoms have not yet been brought to medical attention. In the case of AD (and the other forms of dementia) this period may go for an extended length of time as patients, families and clinicians dismiss early cognitive symptoms as part of the normal aging process. Thus, the rationale for screening is that if we can identify disease earlier in its natural history than would ordinarily occur, intervention measures (those currently available and those that are being developed) would be more effective. Dementia screening therefore would be best suited to detect cognitive impairment at the beginning of disease signs (stage III), particularly if these screening measures reflect what is known about the symptomatic, clinical phase of disease and correlate with the pathologic changes occurring in the brain during the presymptomatic, preclinical phase of disease.

In a recent paper, we evaluated the relationship between several dementia screening tests and biomarkers of AD.[40] We tested whether a reliable and validated informant-based dementia screening test (the AD8)[41,42] correlates with changes in AD biomarkers and, if positive, screening with the AD8 clinically supports an AD clinical phenotype, superior to a commonly used performance-based screening tests including the Mini Mental State Exam (MMSE)[43] and the Short Blessed Test (SBT).[44] A total of 257 participants were evaluated, administered a comprehensive clinical and cognitive evaluation with the Clinical Dementia Rating scale (CDR)[45] used as the gold standard. Participants consented to and completed

a variety of biomarker studies including MRI, amyloid imaging using the Pittsburgh Compound B (PiB)[37,46] and CSF studies of $A\beta_{42}$, tau and phosphorylated tau at Serine 181 ($p\text{-}tau_{181}$).[23,24] The sample had a mean age of 75.4 ± 7.3 years with 15.1 ± 3.2 years of education. The sample was 88.7% Caucasian and 45.5% male with a mean MMSE score of 27.2 ± 3.6. The formal diagnoses of the sample was 156 CDR 0 cognitively normal, 23 CDR 0.5 MCI, 53 CDR 0.5 very mild AD and 25 CDR 1 mild AD. Participants with positive AD8 scores (graded as a score of 2 or greater) exhibited the typical AD fluid biomarker phenotype characterized by significantly lower mean levels of CSF $A\beta_{42}$, greater CSF tau, $p\text{-}tau_{181}$ and the $tau(s)/A\beta_{42}$ ratios.[26,27] They also exhibited smaller temporal lobe volumes and increased mean cortical binding potential (MCBP) for PiB imaging similar to studies of individuals with AD.[18,19] These findings support that informant-based assessments may be superior to performance-based screening measures such as the MMSE or SBT in corresponding to underlying AD pathology, particularly at the earliest stages of decline. The use of a brief test such as the AD8 may improve strategies for detecting dementia in community settings where biomarkers may not be readily available and also may enrich clinical trial recruitment by increasing the likelihood that participants have underlying biomarker abnormalities.[40]

Table 1. Characteristics of nondemented CDR 0 individuals stratified by AD8 scores

Variable	AD8 <2	AD8 ≥2	p value
Clinical Characteristics			
Age, y	75.2 (7.1)	76.5 (8.4)	0.41
Education, y	15.4 (3.2)	15.9 (2.7)	0.47
Gender, % Men	42.1	36.4	0.45
ApoE status, % at least 1 e4 allele	25.8	34.4	0.08
Dementia Ratings			
CDR sum boxes	0.04 (0.13)	0.12 (0.22)	0.01
MMSE	28.6 (1.5)	29.2 (1.1)	0.07
SBT	2.4 (3.1)	2.3 (2.9)	0.82
AD8 Questions Endorsed "Yes," %			
Problems with judgment	12.9	72.0	<0.001
Reduced interest	0	4.0	0.02
Repeats	8.3	40.0	<0.001
Trouble with appliances	1.5	40.0	<0.001
Forgets month/year	0.8	0	0.66
Trouble with finances	0.8	16.0	0.002
Forgets appointments	2.3	28.0	<0.001
Daily problems with memory	20.0	66.7	0.008
Biomarkers			
MCBP, units	0.12 (0.23)	0.26 (0.39)	0.06
CSF $A\beta_{42}$, pg/ml	596.7 (267.9)	591.9 (249.9)	0.95
CSF tau, pg/ml	300.3 (171.5)	316.7 (155.0)	0.76
CSF $p\text{-}tau_{181}$, pg/ml	51.9 (24.0)	56.9 (22.6)	0.49

ApoE, apolipoprotein E; CDR, Clinical Dementia Rating; MMSE, Mini Mental State Exam; SBT, Short Blessed Test; MCBp, mean cortical binding potential; CSF, cerebrospinal fluid

To gain a better understanding of changes in biomarkers in the symptomatic, preclinical phase, a post hoc evaluation of the 156 individuals who were rated as CDR 0 no dementia at the time of their Gold Standard assessment was completed. Some of these nondemented individuals have abnormal AD biomarkers, but in the absence of performing lumbar punctures or PET scans, is it possible to detect evidence of change? AD8 scores for 132 individuals were less than 2; thus their screening test suggests no impairment (mean AD8 score = 0.30 ± 0.46). However 25 of these individuals had AD8 scores (≥2) suggesting impairment (mean AD8 score = 2.4 ± 0.91). Applying the model described in **Figure 1**, some of these individuals are hypothesized to be in the symptomatic, preclinical phase of disease. No difference in age, education, gender or brief performance tests (MMSE or SBT) were detected between groups (**Table 1**); however, the CDR sum of boxes[45] is increased in the individuals with higher AD8 scores supporting that informants were noticing and reporting changes in the participants cognitive function. A review of the individual AD8 questions that were first reported to change suggest that informants endorsement of subtle changes in memory (repeats questions, forgets appointments) and executive ability (trouble with judgment, appliances, finances) are valuable early signs. This is consistent with previous reports that changes in memory and judgment/problem solving CDR boxscores in nondemented individuals correlate with findings of AD pathology at autopsy.[17] Although biomarkers do not reach significance in this small sample, the direction of change in favor of "Alzheimerization" of this group suggests that some of these individuals may be in the symptomatic, preclinical phase of disease. More research with larger sample sizes and longitudinal follow-up is needed to confirm this hypothesis. It should be also noted that not all individuals with an AD8 score of 2 or greater have AD. The AD8 was designed to detect cognitive impairment from all causes, and as such, these mildly affected individuals may have other causes for their cognitive change such as depression, Lewy body dementia or vascular cognitive impairment.[41,42]

To explore this further, changes in AD biomarkers (CSF $A\beta_{42}$, Tau and PiB-PET) were plotted against the age of the participant (**Fig. 2**). Previous research suggest that biomarker changes are more commonly seen in older populations[47] and increasing age is the greatest risk factor for developing AD.[7] AD8 scores of 0 or 1 (no impairment) are depicted

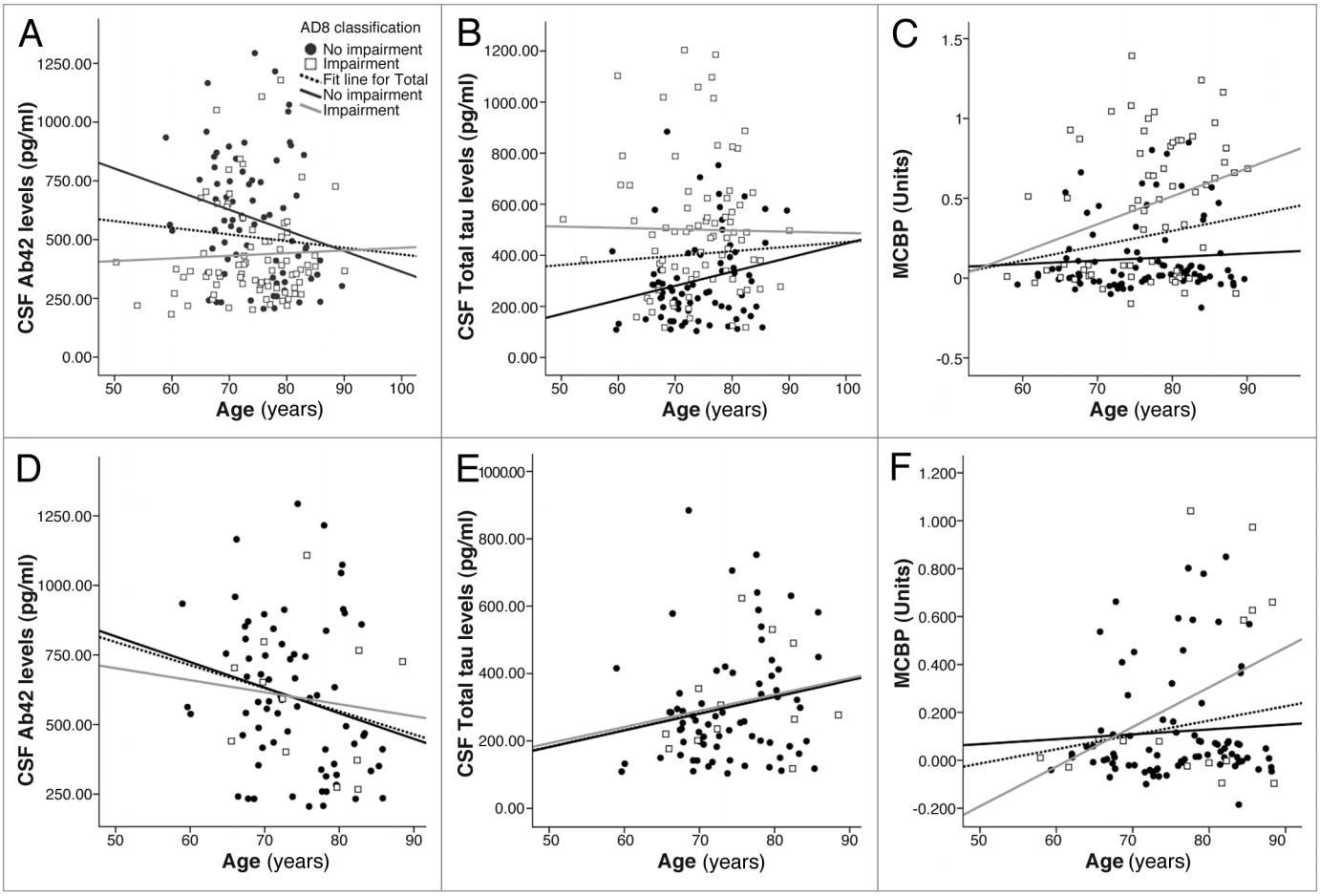

Figure 2. Changes in AD biomarkers by age and AD8 scores. AD biomarkers are plots as a function of age (x-axis) and AD8 scores. AD8 scores of 0 or 1 (no impairment) are depicted as filled circles while AD8 scores of 2 or greater (impairment) are depicted as open squares. Regression lines are plotted for the entire cohort (dashed black line) and for each subset (black for AD8 no impairment; gray for AD8 impairment). The top row (A–C) represents biomarker profiles for the entire cohort (n = 257) divided by their AD8 scores. With age, there are changes in biomarkers with decreasing CSF $A\beta_{42}$ (A), increasing CSF Tau (B) and increased PiB-PET binding potential (C). The effect of age on CSF biomarkers is most marked in the AD8 no impairment group (black line) while changes in PiB binding is seen only in the AD8 impaired group (gray line). The bottom row (D–F) represents biomarker profiles for the individuals rated CDR 0 no dementia (n = 156), 25 of whom had AD8 scores in the impaired range. Similar age-related changes in CSF $A\beta_{42}$ and PiB binding are seen with CSF $A\beta_{42}$ having the greatest rate of decline in the AD8 no impairment group and PiB binding having the greatest rate of change in the AD8 impairment group. Increases in CSF Tau are seen as a function of age regardless of group.

as filled circles while AD8 scores of 2 or greater (impairment) are depicted as open squares. Regression lines are plotted for the entire cohort (dashed black line) and for each subset (black for AD8 no impairment; gray for AD8 Impairment). The top row (Parts A–C) represents biomarker profiles for the entire sample of 257 individuals divided by their AD8 scores. With age, there are changes in biomarkers with decreasing CSF $A\beta_{42}$ (A), increasing CSF Tau (B) and increased PiB-PET binding potential (C). The effect of age on CSF biomarkers is most marked in the AD8 No Impairment group (black line) while changes in PiB binding is seen only in the AD8 Impaired group (gray line). The second row in **Figure 2** (Parts D–F) represents biomarker profiles for the 156 individuals who were rated as CDR 0 no dementia at the time of their Gold Standard, 25 of whom had AD8 scores in the impaired range. Some of these individuals are hypothesized to be in the symptomatic, preclinical phase of AD. Similar age-related changes in CSF $A\beta_{42}$ and PiB binding are seen with CSF $A\beta_{42}$ having the greatest rate of decline in the AD8 no impairment group and PiB binding having the greatest rate of change in the AD8 impairment group. Increases in CSF Tau are seen as a function of age regardless of group.

While a number of interpretations are possible from this type of data, if one considers the model of disease in **Figure 1** it appears that CSF changes in $A\beta_{42}$ and Tau precede PiB binding changes in the presymptomatic, preclinical phase of disease consistent with previous attempts at modeling AD.[25] Even with sensitive measurements, this phase is unlikely to be detected without some biological evaluation. At the start of the symptomatic, preclinical phase of AD, PiB binding increases and this may be detected by careful evaluation of the patient and a knowledgeable informant with a validated dementia screening instrument such as the AD8. As patients move into

the symptomatic, clinical phase of disease, biomarkers are markedly abnormal as is most cognitive testing permitting careful staging and prognostication.

AD and related disorders will become a public health crisis and a severe burden on Medicare in the next two decades unless actions are taken to (1) develop disease modifying medications,[48] (2) provide clinicians with valid and reliable measures to detect disease at the earliest possible stage and (3) reimburse clinicians for their time to do so. While this perspective does not address development of new therapeutics, it should be clear that regardless of what healthcare reform in the US eventually looks like,[1] dementia screening is a viable means to detect early disease as it enters its symptomatic phase. Dementia screening with the AD8 offers the additional benefit of corresponding highly with underlying disease biology of AD that includes alteration of protein conformation, protein misfolding and eventual aggregation of these misfolded proteins as plaques and tangles.

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Drs. James E. Galvin, Catherine M. Roe and John C. Morris hold the copyright for the AD8.

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