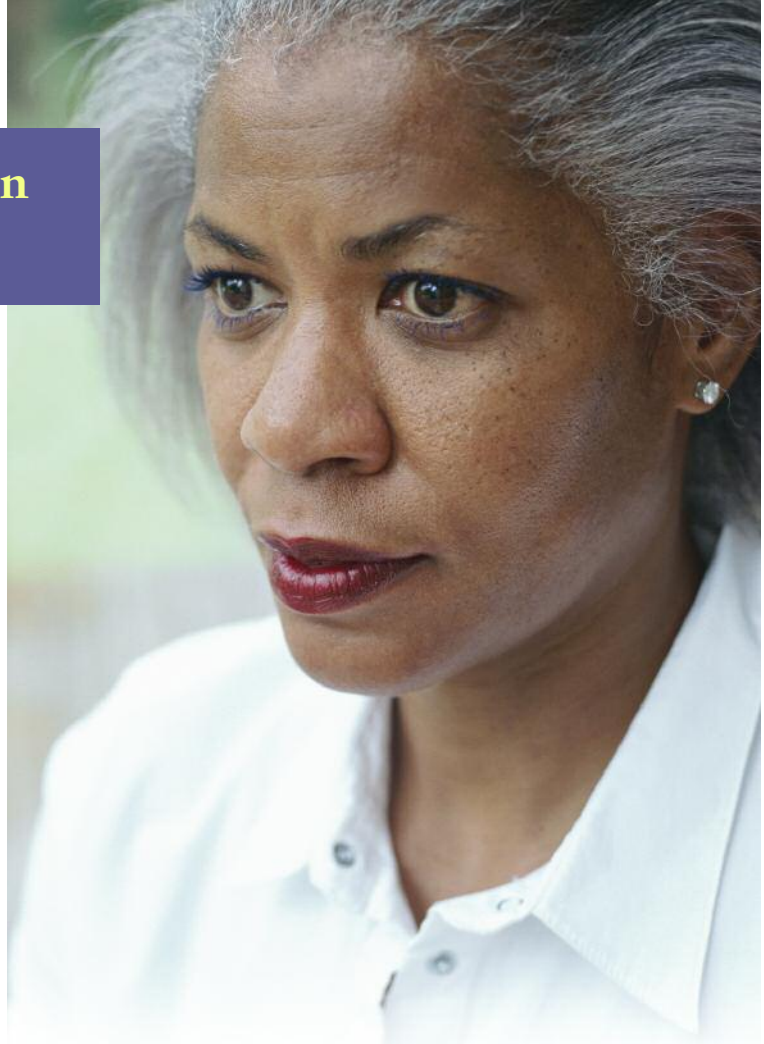


Biomarkers for Early Detection of Alzheimer Disease

Robert C. Barber, PhD

The existence of an effective biomarker for early detection of Alzheimer disease would facilitate improved diagnosis and stimulate therapeutic trials. Multidisciplinary clinical diagnosis of Alzheimer disease is time consuming and expensive and relies on experts who are rarely available outside of specialty clinics. Thus, many patients do not receive proper diagnosis until the disease has progressed beyond stages in which treatments are maximally effective. In the clinical trial setting, rapid, cost-effective screening of patients for Alzheimer disease is of paramount importance for the development of new treatments. Neuroimaging of cortical amyloid burden and volumetric changes in the brain and assessment of protein concentrations (eg, β -amyloid 1-42, total tau, phosphorylated tau) in cerebrospinal fluid are diagnostic tools that are not widely available. Known genetic markers do not provide sufficient discriminatory power between different forms of dementia to be useful in isolation. Recent studies using panels of biomarkers for diagnosis of Alzheimer disease or mild cognitive impairment have been promising, though no such studies have been cross-validated in independent samples of subjects. The ideal biomarker enabling early detection of Alzheimer disease has not yet been identified.

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More than 5 million individuals in the United States have Alzheimer disease, the most common form of age-related dementia.¹ Early symptoms of Alzheimer disease include memory impairment, disorientation, and executive dysfunction. As the disease progresses, cognitive impairment worsens, ultimately resulting in severe dementia and death. Histopathologic characteristics of Alzheimer disease found throughout the cortex include extracellular

plaques formed by aggregates of cleaved β -amyloid protein and intracellular neurofibrillary tangles comprised of tau proteins.²

Although a definitive diagnosis of Alzheimer disease is not possible until autopsy, research suggests that expert-based antemortem diagnoses are accurate.^{3,4} However, making such a diagnosis is not a simple task. A clinical diagnosis of Alzheimer disease is most accurate when based on a consensus opinion of a multidisciplinary panel, including such specialists as a neurologist, psychiatrist, and geriatrician.⁵ These experts typically have access to patients' clinical blood test results as well as to neuropsychologic and neuroimaging data.⁶ This consensus diagnostic methodology has been demonstrated as valid, though it is time consuming and expen-

sive, and it relies on several specialists who are rarely available outside of specialty clinics.

Research has demonstrated that most primary care physicians and other nonspecialty clinicians are not able to accurately identify Alzheimer disease in its early stages.^{7,8} Accordingly, many patients do not receive an expert evaluation and diagnosis until the disease has progressed well beyond the initial stages, when treatments are maximally effective.

Alzheimer disease is projected to grow substantially in prevalence during the coming decades, with the number of affected individuals expected to reach 7.7 million by 2030.⁹ This figure represents a more than a 50% increase from current prevalence rates.⁹ Estimates suggest that a new case of Alzheimer dis-

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ease develops in the United States every 71 seconds, and that by the mid-21st century, this rate of disease development will accelerate to a new case every 33 seconds.⁹ Alzheimer disease is the seventh leading cause of death in the United States and the fifth leading cause of death for Americans older than 65 years.⁹

As highlighted by recently released figures from the Alzheimer Association,¹⁰ Alzheimer disease poses a tremendous public health burden in terms of patient care, lost wages, and responsibilities of caregivers. This burden is projected to increase exponentially.

The current healthcare infrastructure in the United States is woefully inadequate to deal with Alzheimer disease projections, given that patient access to specialty clinics is limited outside of large medical centers, which are typically located only in heavily urbanized areas. These healthcare resources are already less accessible to various underserved populations (eg, citizens in rural areas and ethnic minorities). As the number of elderly individuals at risk for Alzheimer disease continues to grow, with a concomitant increase in Alzheimer disease diagnoses, the gap in healthcare access between adequately and inadequately served populations will widen unless many new specialty clinics are created and staffed.

For these reasons, research into biomarkers that have diagnostic and prognostic value in the early stages of Alzheimer disease—and that do not require specialty clinics for identification and interpretation—is of particular interest and importance.^{11,12}

Definition of Biomarker

In the broadest definition, a biomarker is any measurable biologic feature that can be used to diagnose or predict a physiologic or pathologic condition (ie, a phenotype). In the case of Alzheimer disease, biomarkers have been described using a range of mediums, including neuroimaging of β -amyloid protein deposition, magnetic resonance imaging (MRI) scans of brain volume, genotyping of genetic polymorphisms known to be associated with disease risk, and quantification of the abundance of specific proteins in the cerebrospinal fluid (CSF)

or in the blood (ie, plasma and serum). The remainder of this article addresses each of these biomarker classes—tests for which are summarized in *Figure 1*.

The ideal biomarker for Alzheimer disease would provide an indication of disease risk and rate of disease progression long before onset of symptoms. In addition, the ideal biomarker would be inexpensive to measure, with no need for specialty clinics or sophisticated analytical techniques. Finally, the ideal biomarker would be measurable in an easily accessible tissue of the patient.

Neuroimaging of Amyloid Deposition

The deposition of β -amyloid protein within cortical regions of the brain is a pathologic hallmark of Alzheimer disease that is believed to precede clinical symptoms by several years.¹³ This feature of the disease makes in vivo imaging of β -amyloid in the brain of particular interest for the identification of individuals at risk for, and in the early stages of, Alzheimer disease (*Figure 2*).

Quantification of amyloid deposition in the brain with neutrally charged derivatives of thioflavin-T was initially developed at the University of Pittsburgh School of Medicine in Pennsylvania.^{14,15} The marker selected for optimal amyloid detection—N-methyl-[¹¹C]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole—was named Pittsburgh Compound B (PiB) and is used in conjunction with positron emission tomography (PET) for in vivo identification of cortical amyloid burden.¹⁵ This technique is widely known as PiB PET imaging.

This type of imaging has been reliably validated as an effective method for quantifying amyloid deposition within specific brain regions. It is especially useful and effective for discriminating between Alzheimer disease and other forms of dementia.¹⁶ Antiamyloid therapies, such as AN1792 vaccine or bapineuzumab antibody, that are designed to reduce amyloid accumulation involve the use of PiB PET imaging to monitor efficacy of treatment.¹⁷

The detection of increased amyloid burden with PiB PET imaging has been reported as a means of identifying individuals with mild cognitive impairment

- ☐ Positron emission tomography neuroimaging of β -amyloid protein deposition
- ☐ Magnetic resonance imaging of volume of hippocampus and other brain structures
- ☐ Genotyping of genetic polymorphisms (eg, epsilon 4 allele at apolipoprotein E locus) associated with risk
- ☐ Quantification of abundance of proteins (eg, β -amyloid 1-42, total tau, phosphorylated tau) in cerebrospinal fluid
- ☐ Quantification of abundance of various proteins in blood (ie, plasma and serum)

Figure 1. Tests for biomarker classes used in the diagnosis and prognosis of Alzheimer disease.

(MCI) who are at increased risk of progression to Alzheimer disease.^{17,18} In addition, increased amyloid deposition has been linked to reduced volume of the hippocampus and to episodic memory loss.¹⁹ However, a number of recent studies have documented high proportions of cognitively normal individuals with amyloid accumulation on the order of levels observed in patients with Alzheimer disease.^{20,21}

Furthermore, there is a logistical concern over using PiB PET imaging to analyze amyloid deposition. The short radioactive half-life (20 minutes) of the ¹¹C (carbon-11) label necessitates either the use of an on-site cyclotron or the rapid shipment of labeled substrate and very tight scheduling of patients to be imaged. This limitation has led to the search for more stable compounds that could be used in conjunction with PET imaging—such as compounds labeled with ¹⁸F (fluorine-18) rather than ¹¹C. The most recently identified of these compounds is ¹⁸F-AV-45—(E)-4-(2-(6-(2-(2-(¹⁸F)-fluoroethoxy)ethoxy)pyridin-3-yl)vinyl)-N-methyl benzenamine, though a number of other compounds exist, including ¹⁸F-FDDNP—[¹⁸F] 1,1-dicyano-2-[6-(dimethylamino)-2-naphthalenyl] propene.^{17,22,23} The half-life of ¹⁸F-AV-45 is on the order of 110 minutes, allowing for remote syn-

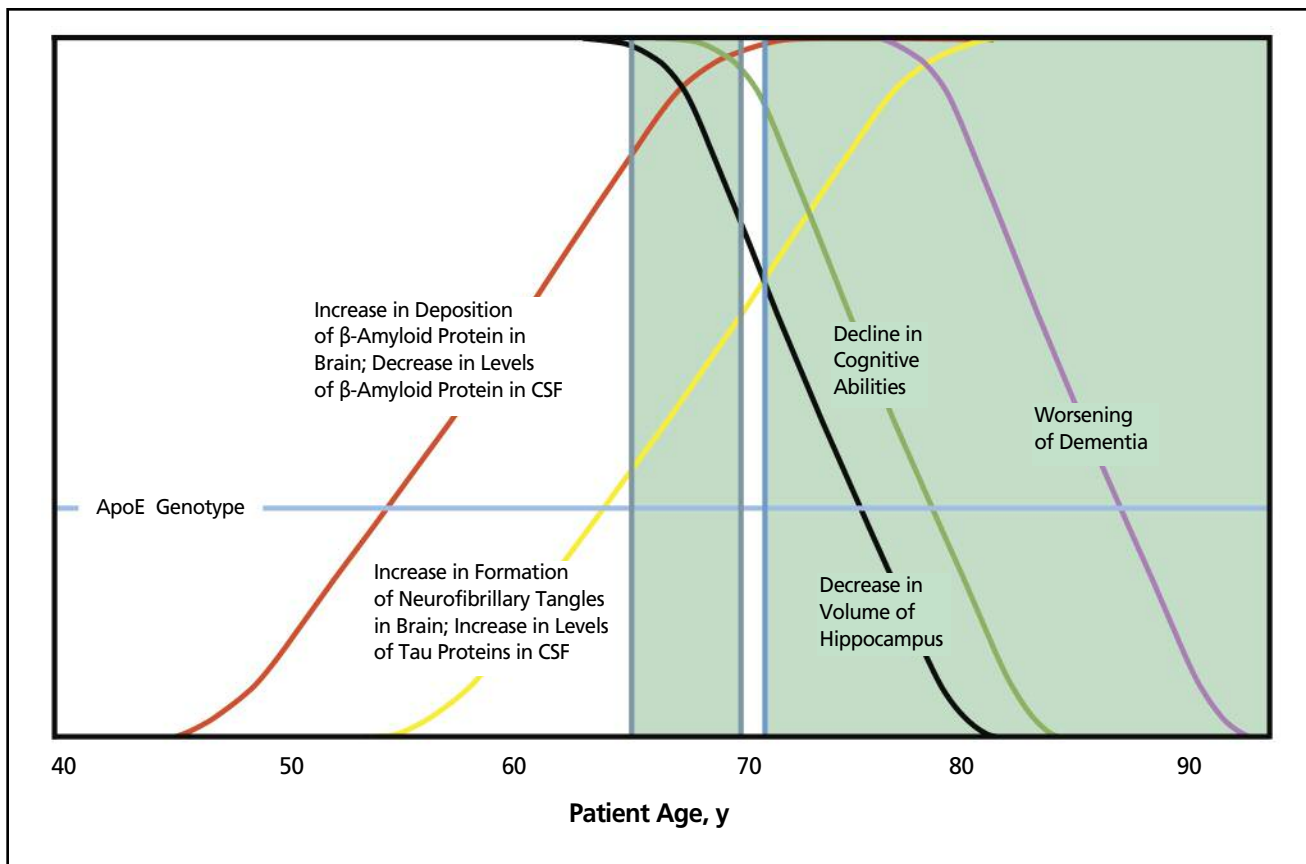


Figure 2. Timeline comparing positive findings on tests for biomarkers with typical progression of mild cognitive impairment (MCI) and Alzheimer disease in patients. The gap between MCI and Alzheimer disease represents the transition from MCI to Alzheimer disease. **Abbreviations:** ApoE, apolipoprotein E; CSF, cerebrospinal fluid.

thesis and shipment of the radio-labeled probe across moderate distances.

Magnetic Resonance Imaging

Magnetic resonance imaging is a widely available and relatively inexpensive technique for visualizing detailed internal brain structures and volumes. Unlike computed tomography, MRI does not require ionizing radiation for imaging. Rather, a powerful magnetic field is focused to align the nuclear magnetization of hydrogen atoms contained in water molecules throughout the brain. Radio frequencies are then used to systematically alter the alignment of these atoms, causing the hydrogen nuclei to produce a rotating magnetic field that is detected by a scanner. A number of high-resolution MRI-based methods for visualizing the structure of the whole brain, as well as specific brain regions, have been developed in recent years and adapted for the study of Alzheimer disease.²⁴

Atrophy of the hippocampus, the most widely accepted marker for early-stage Alzheimer disease (Figure 2), is readily detectable by high-resolution MRI.²⁴ In addition, hippocampal volume loss is predictive of conversion of MCI to Alzheimer disease, with an accuracy rate of approximately 80%.^{25,26} However, currently accepted methods for measuring volume changes in the hippocampus are labor- and time-intensive, making these methods appropriate only for patient stratification in research settings rather than for use as a clinical screening tool.²⁴

Measurements of various other brain regions and structures have been proposed as biomarkers for Alzheimer disease. These measurements, including volumetric analysis of the entorhinal cortex and calculation of cortical thickness, generally have the same drawbacks as hippocampal volumetric assessment. The main drawback is that accurate quantification of regional brain volumes is time- and labor-intensive. If this limi-

tation of MRI-based methods could be solved via automation of scan analysis, such methods are almost certain to become useful tools for the detection and monitoring of Alzheimer disease in patients.

An additional, experimental MRI-based technique has the potential to directly measure the decrease in neuronal density and loss of synaptic connections that occur with progression of Alzheimer disease. This method involves in vivo mapping of neuronal connections within the brain (ie, neural tractography) by way of diffusion tensor imaging (DTI), which uses MRI to measure nonrandom movement of water molecules. The movement of water molecules is greater lengthwise along neural tracts, relative to their movement across tract width—a phenomenon known as anisotropy. When the structure of an axon is disrupted, as occurs in traumatic brain injury, cancer, or neural inflammation, water moves more randomly through

the tissues, resulting in a reduction in anisotropy. In addition, barriers to water movement are affected by the destruction and disorganization of axons and synapses that characterize Alzheimer disease.²⁷

If a series of DTI scans are made over time, they would presumably allow for direct detection of neural changes associated with disease progression. The DTI technique is presently in early stages of development.

Genetic Markers

Genetically, Alzheimer disease is heterogeneous and complex, with age of onset being one of the most evident dichotomies between forms of the disease. Familial (ie, early-onset) Alzheimer disease is inherited in a Mendelian manner, with more than 160 highly penetrant, but rare, mutations having been described in three genes—the genes that code for amyloid precursor protein, presenilin 1, and presenilin 2.²⁸ Familial Alzheimer disease accounts for less than 1% of the Alzheimer disease burden.²⁹

Other than differences in age at onset and inheritance pattern, familial Alzheimer disease is clinically similar to the more common late-onset form of the disease. Late-onset Alzheimer disease is genetically and etiologically heterogeneous, with myriad genes and environmental factors having been implicated in disease risk and progression rate.

The strongest and most reliably replicated genetic association for increased risk of late-onset Alzheimer disease involves the epsilon 4 ($\epsilon 4$) allele at the apolipoprotein E (ApoE) locus.³⁰⁻³³ The ApoE gene, located on chromosome 19, encodes a protein responsible for lipid transport. Three major alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) are present at this location; they can be distinguished by a pair of cytosine-thymine polymorphisms (rs7412, rs429358). Carriers of the $\epsilon 4$ allele have increased amyloid deposition,³⁴ and recent research has demonstrated adverse effects of the $\epsilon 4$ allele on memory and executive function.³⁵

Because the allelic designation is determined by a pair of polymorphisms rather than a single nucleotide substitution, genotyping of the ApoE locus is not entirely straightforward. However, a

family physician needs only to collect a small sample of blood or a cheek swab, which can be mailed to a number of commercial molecular laboratories for DNA (deoxyribonucleic acid) isolation and ApoE genotype determination.

Although the association between the ApoE $\epsilon 4$ allele and Alzheimer disease is strong and well-accepted, the risk of Alzheimer disease for carriers of the $\epsilon 4$ allele is far from 100%. A large body of literature has confirmed that carrying the ApoE $\epsilon 4$ allele lowers overall age at onset for Alzheimer disease,^{28,29} though the ApoE genotype does not by itself have any specific implications for the individual carrier. Therefore, the main usefulness of the ApoE $\epsilon 4$ genotype as a biomarker for Alzheimer disease is to add accuracy to a test as part of a biomarker panel.

Cerebrospinal Fluid Markers

The concentrations of several proteins in CSF have been associated with increased risk for Alzheimer disease and conversion of MCI to Alzheimer disease. Chief among these proteins are a cleaved portion of the β -amyloid protein composed of amino acids 1-42 (ie, β -amyloid protein 1-42), total tau protein, and phosphorylated tau protein.³⁶⁻⁴² In the case of β -amyloid protein 1-42, the correlation between protein concentration and Alzheimer disease is negative—that is, increased risk for disease is associated with a lower concentration of β -amyloid protein 1-42 in CSF (Figure 2).³⁷⁻³⁹ Presumably, decreased CSF β -amyloid protein 1-42 is brought about by sequestration of the protein inside amyloid plaques located throughout the cortex.

The opposite is true for the relationships between Alzheimer disease and tau proteins, which are positively correlated with risk (Figure 2).³⁶ Elevated levels of total tau and phosphorylated tau in CSF are associated with increased risk of disease. In addition, although decreased CSF β -amyloid protein 1-42 levels are typically observed in patients several years before clinical symptoms and cognitive decline, increased concentrations of total and phosphorylated tau in CSF occur later in the course of disease and are more closely aligned with the onset of disease symptoms. In a

recent study, De Meyer and colleagues⁴² detected an Alzheimer disease signature in the composite levels of β -amyloid 1-42, total tau, and phosphorylated tau proteins in the CSF of patients enrolled in the Alzheimer Disease Neuroimaging Initiative who were diagnosed as having MCI.

Some evidence suggests that CSF levels of total and phosphorylated tau protein, as well as ratios of β -amyloid protein 1-42 to other β -amyloid isomers (eg, β -amyloid protein 1-38) and to tau protein, can be used to discriminate between Alzheimer disease and other forms of dementia (eg, vascular or frontotemporal dementia).⁴³⁻⁴⁶ In particular, CSF levels of tau proteins that are phosphorylated at serine 181 or threonine 231 have been shown to improve the diagnostic ability of total tau to differentiate between Alzheimer disease and other forms of dementia.⁴⁷⁻⁴⁹ However, a recent study conducted in Sweden found that measurements of total tau and β -amyloid protein 1-42 could not be used to reliably distinguish patients with Alzheimer disease from normal controls.⁵⁰ Although the sample size in the study from Sweden was relatively small, 3 of 8 patients diagnosed as having Alzheimer disease had levels of total tau and β -amyloid protein 1-42 that were consistent with levels in cognitively normal controls.

Blood Markers

Some researchers have suggested that blood-based screeners should be the first step in the diagnostic process for Alzheimer disease, to be followed by neuroimaging of the brain or CSF protein assessments.⁵¹ However, this approach remains out of reach for clinical use because of the lack of an accurate blood-based assessment device. Although great advancements have occurred in the development and validation of imaging and CSF biomarkers for Alzheimer disease, less momentum has occurred in the area of blood-based biomarkers.

In 2007, Ray and colleagues⁵² assessed a large number of plasma proteins in an effort to identify a profile of multiple biomarkers that was indicative of Alzheimer disease. These efforts yielded a panel of 18 proteins that were

effective at distinguishing patients with Alzheimer disease from control individuals. The overall classification accuracy for the resulting algorithm was 90%. The algorithm also accurately identified 81% of patients who had MCI that progressed to Alzheimer disease within a 2-to-6-year follow-up period.⁵²

In 2010, O'Bryant and colleagues in the Texas Alzheimer Research Consortium⁵³ constructed an algorithm using differences in serum protein concentrations derived from a large group of individuals, including patients diagnosed as having Alzheimer disease and cognitively normal individuals. The authors analyzed 121 proteins related to inflammation, cytoskeletal remodeling, and cell signaling, as well as growth factors, hormones, and other proteins—in combination with age, sex, years of education, and ApoE genotype. The analysis allowed the authors to generate an algorithm that was highly accurate at identifying cognitive status. The model had an overall diagnostic sensitivity and specificity of 94% and 84%, respectively, and an overall accuracy of 95% for detecting Alzheimer disease.⁵³ However, the ability of O'Bryant et al⁵³ to predict conversion of MCI to Alzheimer disease or disease progression rates among patients was not reported.

A caveat to all blood-based biomarker studies reported to date is that none of the studies have been cross-validated with independent samples of subjects. Nor have the blood-based biomarker studies been tested to determine their ability to distinguish Alzheimer disease from other forms of dementia.

Future Use of Biomarkers for Alzheimer Disease

The development of a rapid, cost-effective means of providing routine screening of elderly patients for Alzheimer disease is of paramount importance. Although advanced neuroimaging techniques and assessment of protein concentrations (in particular, β -amyloid 1-42, total tau, and phosphorylated tau) in CSF are accurate diagnostic tools, these technologies are not widely available. In contrast, the development of a blood-based biomarker or biomarker

panel test would provide a screening method that could be performed in nearly any clinical setting, resulting in increased access to proper care for patients with Alzheimer disease.

As is standard practice with many currently implemented medical diagnostic tests, a blood test could be used as an initial screen to indicate the need for follow-up referral to neuroimaging or CSF analysis⁵¹—thus increasing the efficiency and accuracy of Alzheimer disease diagnoses. Such a staged methodology would reduce patient burden on specialty clinics and expand access to accurate Alzheimer disease diagnoses throughout the community healthcare setting. Such an advantage would become increasingly apparent if the burden of Alzheimer disease increases during the coming decades, as is predicted.⁹

If a screening test was available that could reliably detect Alzheimer disease in its earliest stages and that could predict progression of the disease, this screening instrument could become standard care for elderly patients at their annual medical evaluations in primary care settings.

Finally, a major hurdle to therapeutic trials for Alzheimer disease is rapid and effective screening of patients. In the trial setting, screen failures pose a substantial financial burden because current screening methods are expensive and time-intensive. The provision of an effective screening device for Alzheimer disease would greatly facilitate improved scientific knowledge by directly stimulating therapeutic trials.

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