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Searching for the Biomarkers of Alzheimer's

The ability to detect this widespread but elusive condition through simple, noninvasive means has been a dream of neurologists for years. Can a landmark study make it a reality?

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Public awareness of Alzheimer's disease (AD) reached new heights in 2004 immediately following the death of Ronald Reagan, and the prominence of stem cell research in the recent presidential campaign has tantalized the public (though not researchers) with the prospect of an eventual "cure" for this debilitating condition. More Americans than ever before are acutely aware of the suffering that AD patients and their families endure, and are anxious for medical breakthroughs.

That has put greater emphasis on diagnosing and treating patients in the earliest stages of AD. Making this call, though, is often difficult. PET or MRI scanning can show the extent of damage, but there is debate about which methods to use and what to look for in addition to the high costs associated with each test. Neuropsychological tests can determine the extent of impairment, but they are rarely accurate methods of detecting the pathology of AD. At present, we have few options at our disposal.

Hopefully, though, we may soon find relevant biomarkers that will distinguish AD from vascular dementia, Parkinsonian dementia or related conditions and give us an early indication of its presence. In practice, this should be as non-invasive and as accurate as a pregnancy test or a blood sugar level to detect diabetes. Research has been intense and while no pregnancy test equivalent for the diagnosis of AD is available yet, there are several promising AD biomarkers, and some assays using these analytes are performed as part of studies conducted in AD research centers. However, a sweeping new initiative is currently underway to find better answers. In this article, we'll look at the extent of what is currently known about Alzheimer's biomarkers and the work being done to further our knowledge.

Defining the Diagnosis

The clinical diagnosis of AD is imprecise, although specialists at AD centers using consensus criteria can achieve an accuracy rate of almost 90 percent. Definite AD requires autopsy confirmation. Diagnostic accuracy is far lower at early and pre-symptomatic stages of AD when confusion with other dementias is common.[1-7] Although AD currently affects 4.5 million Americans, and is predicted to affect as many as 16 million Americans by 2050, this neurodegenerative dementia is the most devastating major human disorder for which there still is no effective long-term treatment.

However, clinical trials of promising new AD medications are lengthy, involve large numbers of subjects, are difficult to conduct, and are both risky and extremely expensive to implement. We've seen very modest progress in translating many of the spectacular laboratory advances in AD research over the past two decades[4] into meaningful therapeutic steps forward to significantly help affected patients, but the

dissection of pathways of neurodegeneration in AD through basic research studies is stimulating drug discovery efforts to find drugs that block steps in the formation of hallmark AD brain lesions such as senile plaques formed by amyloid-β (Aβ) filaments and neurofibrillary tangles, formed by tau fibrils, both of which are now known to represent different types of brain amyloid deposits.

Consensus meetings[1,3,7] have proposed that ideal AD biomarkers should have as many of the following attributes as possible:

(1) Detect a fundamental feature of AD neuropathology and brain degeneration.

(2) Be validated in autopsy-confirmed cases of the disease.

(3) Have a diagnostic sensitivity of >80 percent for detecting AD and a specificity of >80 percent for distinguishing AD from other dementias.

(4) The diagnostic laboratory tests or assays that are developed should be reliable, reproducible, non-invasive, simple to perform and interpret, as well as inexpensive.

Validating AD biomarkers and the tests based on them would require confirmation by at least two independent studies from qualified investigators, and these studies should be published in peer-reviewed journals. Finally, it also would be extremely useful for clinical trials of new AD therapies if the biomarker reflected the beneficial effect of disease-modifying therapy.

For those following the rapidly moving field of AD biomarker research, it is obvious that the quest to find the ideal AD biomarker or a panel of AD biomarkers has not yet culminated in success. Hence, the goal of finding the "pregnancy test equivalent" for diagnosing AD at its earliest stages or before it becomes overtly manifest remains an elusive one. Indeed, the lack of validated, robust and highly informative AD biomarkers

impedes the rapid, timely and cost-effective development of novel AD therapies as well as the assessment of potentially more effective treatments for AD through clinical trials.

Consistent Candidates

A number of compelling candidate AD biomarkers have surfaced in the past decade and some of the most promising ones are specific isoprostanes, tau, Aβ, sulfatide and homocysteine. However, there are a number of other very plausible analytes to consider as potential AD biomarkers, such as amyloid-β precursor proteins, apolipoprotein E (ApoE), α1-antichymotrypsin, interleukin-6 (IL-6) and IL-6 receptor complex proteins, C-reactive protein, C1q protein, etc.[1,3]

Indeed, there is intense interest in pursuing further research into the development of assays for these and other analytes that hold the promise of increasing the accuracy of the early and reliable diagnosis of AD as well as enhancing the ability to predict the progression from mild cognitive impairment (MCI) to AD, or providing insights into pathways influenced by potential AD treatments.[1,3] However, since no single AD biomarker is likely to serve all of these needs, it is important to recognize the likely need to develop a panel of AD biomarker assays that, in aggregate, provide the most informative diagnostic measures for the risk, onset and progression of AD.

Given the extensive research on AD biomarkers at this time, it is beyond the scope of this article to extensively summarize and assess all of the most current potential AD biomarkers. Instead, I will provide a brief overview of recent progress in the development of a several AD biomarkers (homocysteine, isoprostanes, sulfatide, tau, A-β) that are selected for consideration here because they are the initial focus of a new public/private AD research initiative called the Alzheimer's Disease Neuroimaging

Initiative (ADNI) that seeks to accelerate the pace for developing informative neuroimaging and chemical biomarkers of AD and the transition from MCI to early AD. This initiative is funded by the National institute on Aging (NIA) of the National Institutes of Health (NIH), several pharmaceutical companies and foundations in conjunction with the NIH Foundation, and the ADNI will have a profound impact on efforts to improve the diagnosis and therapy of AD.

The Promise of Homocysteine, Isoprotanes, Sulfatide, Tau and Aβ:

Consistent with the goals of the ADNI, the emphasis in the Biomarker Core of this UO1 is on biological sample collection, establishing a bank of biological fluids from the unique cohort of subjects followed in the ADNI, and conducting studies of selected AD biomarkers, *i.e.*, specific isoprostanes, tau, A-β, sulfatide and homocysteine. However, it should be emphasized that the ADNI will work with academic and industry partners to conduct studies funded by other sources on the samples collected in the ADNI. Thus, the biomarkers mentioned above will be the initial focus of studies in the ADNI and they were selected for high priority consideration based on a recent consensus of AD biomarker experts.[1,3] These biomarkers are briefly discussed below.

Homocysteine is a sulfur-containing amino acid, derived from the metabolism of methionine.[1,3] One of the first associations between homocysteine and AD came from a study comparing autopsied patients with AD versus controls. Homocysteine levels in the highest tertile were associated with a greater than fourfold increase in the relative risk of AD, while other studies showed that plasma homocysteine levels of >14 umol/L almost doubled the risk of AD. There are a variety of assays to measure homocysteine levels, including immuno-assays and HPLC-based methods which make it compelling to explore

the utility of measuring homocysteine levels in cerebrospinal fluid (CSF), plasma and urine to aid in the early diagnosis of AD.[1,3]

Growing evidence implicates oxidative/nitrative damage in the pathogenesis of AD, and specific isoprostanes (*i.e.*, 8,12-iso-iPF$_{2\alpha}$-VI) are elevated in urine, blood and CSF of AD patients. The values for these elevated levels correlate with memory impairments, CSF tau levels and the number of APOE4 alleles.[1,3] This suggests that 8,12-iso-iPF$_{2\alpha}$-VI is a useful AD biomarker. Isoprostane levels can be measured in CSF, blood, urine and brain using-HPLC/tandem mass spectrometry (MS) with electrospray ionization (ESI), and urine levels are expressed as ng per mg of creatinine while values in CSF, plasma and postmortem brain are normalized.[1,3] Additional studies will confirm and extend these findings in larger cohorts of MCI and AD patients as well as determine if 8,12-iso-iPF$_{2\alpha}$-VI will be an informative analyte for monitoring the response of AD patients to new therapies in clinical trials.

New evidence suggests that levels of sulfatide may be indicative of AD pathogenesis.[1] By screening with ESI/MS, sulfatide was identified as a potential AD biomarker of interest, and it decreases 93 percent in gray matter and 58 percent in white matter in MCI versus controls. Also, when normalized with phosphatidylinositol, CSF sulfatide distinguished non-demented individuals from those with very mild dementia with a sensitivity of 90 percent and a specificity of 100 percent. While preliminary, these exciting findings suggest the potential for sulfatide assays to be informative in the diagnosis of AD.

Tau and Aβ are components of the two neuropathological diagnostic hallmarks of AD (tangles and plaques, respectively), and they are the most frequently studied

candidate diagnostic AD biomarkers, best analyzed in CSF using extensively characterized ELISAs.[1,3] While thousands of living AD patients and normal as well as disease controls have been studied,[1,3] a recent examination of >100 subjects with autopsy-confirmed diagnoses showed that elevated CSF tau levels are associated with the presence of AD pathology and that CSF tau levels help discriminate AD from other dementing disorders.[2] On the other hand, while CSF Aβ levels are decreased in AD and are somewhat informative by themselves, CSF Aβ levels added little meaningful diagnostic value to measures of CSF tau.[2] Thus, these data underline the importance of postmortem follow-up studies of AD biomarkers and the potential greater utility of a panel of biomarkers rather than reliance on assays for a single analyte.

Taking the Initiative

On October 13, 2004, the NIA announced the launch of the ADNI, an exciting new research program to develop and improve methods for the diagnosis of AD and monitoring its progression as well as the transition from MCI to AD in order to reduce the time and cost of clinical trials (www.alzheimers.org/nianews/nianews70.html). To accomplish this daunting task, the ADNI will pursue five major goals:

(1) Develop standard AD neuroimaging methods for clinical trials.

(2) Improve methods for neuroimaging.

(3) Determine optimum methods for acquiring and processing brain images.

(4) Validate AD neuroimaging and biomarker findings from the ADNI.

(5) Provide a database for all ADNI findings that will be available to qualified scientific investigators for further data mining.

Since an effort like the ADNI is far too complicated, labor intensive and expensive for any one company or university to take on independently, the ADNI has been designed as a partnership among several entities including: the NIA and the National Institute of Biomedical Imaging and Bioengineering of the NIH, the Food and Drug Administration, investigators at academic health centers throughout the United States, private partners including pharmaceutical companies and the Institute for the Study of Aging all of whom have been brought together into a synergistic alliance of shared interests to develop better AD biomarkers by the Foundation for the NIH, with additional participation by the Alzheimer's Association.

The ADNI study is designed to enroll 800 people at 45-50 clinical sites with specialized expertise in AD at academic health centers throughout the United States over the course of five years and these individuals will include 200 normal elderly controls, 200 AD patients and 400 subjects with MCI. All subjects will undergo periodic brain imaging (*e.g.*, MRI, PET), and blood as well as urine samples will be collected from all subjects while CSF will be obtained from a subset of individuals so that studies of chemical AD biomarkers can be conducted.

While the analytes summarized above will be the initial focus of investigation, plans are in progress to expand the scope of these chemical biomarker studies in collaboration with industry partners and other investigators from academia. Clinical evaluations will also be done to allow researchers to correlate the imaging and biomarker data with neuropsychological and behavioral data. Thus, the ADNI is a one-of-a-kind study that offers the prospect of rapidly advancing efforts to develop better diagnostics

for AD in order to accelerate the pace of drug discovery and testing for this devastating dementia.

Still Looking for Signs

There is compelling evidence for the plausible diagnostic utility of a number of potentially informative AD biomarkers to improve the diagnosis of AD, especially in its early stages or even in the prodromal phase known as mild cognitive impairment. What we still lack at present, though, is the definite detectable signs that would meet our criteria for a reliable biomarker.

Indeed, public/private commitment to the ADNI underlines the importance and timeliness of identifying and validating informative AD biomarkers to facilitate efforts to translate laboratory advances in understanding mechanisms of AD brain degeneration. This study will hopefully yield information vital to the development of new AD therapies for our patients, vastly expanding our treatment options. It should also quicken future Alzheimer's research, since we would know what signs indicate favorable results.

citations are restricted to reviews and consensus reports wherein references to the primary literature may be found.

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