

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Biomarkers in the diagnosis and management of Alzheimer's disease



Richard Wurtman*

Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139 USA

ARTICLE INFO

Keywords:

A-beta 1-42
Brain atrophy
Scanning
CSF
Plasma phospholipids

ABSTRACT

Traditionally Alzheimer's disease (AD) has been diagnosed and its course followed based on clinical observations and cognitive testing, and confirmed postmortem by demonstrating amyloid plaques and neurofibrillary tangles in the brain. But the growing recognition that the disease process is ongoing, damaging the brain long before clinical findings appear, has intensified a search for biomarkers that might allow its very early diagnosis and the objective assessment of its responses to putative treatments. At present at least eight biochemical measurements or scanning procedures are used as biomarkers, usually in panels, by neurologists and others. The biochemical measurements are principally of amyloid proteins and their A-beta precursors, or of tau proteins. Brain atrophy can be assessed by means of structural magnetic resonance imaging (sMRI), and decreased blood flow and metabolism can be estimated by functional magnetic resonance imaging (fMRI). [18F]fluorodeoxyglucose-positron emission tomography (FDG-PET) is used to measure the brain's energy utilization and to infer synaptic number. Impaired connectivity between brain regions is indicated by diffusion tensor imaging (DTI), while magnetic resonance spectroscopy (MRS) provides metabolic markers of diminished cell number. Additional proposed biomarkers utilize electroencephalography (EEG) and magnetoencephalography (MEG) for quantifying impairments in connectivity. Genetic analyses illustrate the heterogeneity of disease processes that can cause cognitive impairment syndromes. Recent observations awaiting confirmation suggest that levels of some plasma phospholipids can also be biomarkers of AD and that reductions in these levels can enable the accurate prediction that a cognitively normal individual will go on to develop MCI or AD within 2 years.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

For most of its history Alzheimer's disease (AD) was diagnosed solely on the basis of clinical observations – the age-related loss of memory functions, followed by additional cognitive deficits, and then frank dementia – and on two neuropathologic findings found post-mortem — senile plaques containing clumps of amyloid protein, and neurofibrillary tangles in neurons. Using clinical criteria and standardized behavioral tests, neurologists became expert in differentiating symptom-

atic AD patients from those with various other age-related illnesses such as vascular dementia, Lewy body disease, Parkinsonism with dementia, frontotemporal lobe degeneration, and primary progressive aphasia.

However it is now evident that neurochemical and neuropathologic evidence of active disease, even including reductions in the size and metabolic activity of brain regions involved in memory (e.g., left temporal structures; [1]), can precede AD's clinical manifestations by many years [2]. Hence utilizing behavioral symptoms alone to make the premortem diagnosis

* Tel.: +1 617 253 6731.

E-mail address: dick@mit.edu.

of AD risks failing to identify patients early in the course of their disease, when it may be most treatable. Moreover even well-established behavioral tests like the ADAS-Cog, which had been regarded as the "gold-standard" for AD diagnosis, are now recognized as sometimes giving false-negative results for patients with mild symptoms [3,4]. For these reasons there is now a consensus that objective biomarker data, which supplement clinical observations and behavioral measurements, are essential for diagnosing and following patients with early AD. Presently such data are derived principally from biochemical assays [5–9] or scanning methods [10–13], but they soon may also be based on genetic [14] or neurophysiologic analyses, as discussed below. Biomarker analysis might also enable the discovery of etiologic subtypes among patients with age-related memory impairment [ARMI] syndromes, for example those with disturbances of genes that are not specifically related to amyloid or tau, but which control the expression of other proteins like RbAp48, a histone-binding protein that modifies histone acylation. Levels of that protein, examined postmortem, were depressed with aging in human dentate gyrus, and transgenic mice with deficient RbAp48 exhibited hippocampus-dependent memory deficits similar to those characteristic of aged humans [14]. Such associations suggest additional therapeutic targets for discovering treatments that might help patients with a particular ARMI subtype.

2. Currently accepted biomarkers of Alzheimer's disease

Currently accepted biomarkers of AD include levels of brain chemicals related to amyloid or tau, and imaging-derived estimates of the size and metabolic activity of specific brain regions. As neurochemical evidence accumulated that two brain peptides, A-beta 1-42 and 1-40, derived from an amyloid precursor protein, could polymerize to form the amyloid proteins clumped within senile plaques, as well as oligomers which are toxic to neurons *in vitro*, it was proposed that measurements of the peptides might provide useful biomarkers for AD [15]. Similarly, measurements of CSF levels of the tau protein or its hyperphosphorylated derivatives found in neurofibrillary tangles were also proposed as candidate biomarkers for AD [5,8,16]. And since newly available imaging techniques demonstrated decreases in the size of the medial temporal lobe early in AD [7] and in the perfusion and metabolism of the temporoparietal cortex [10], these and other anatomic abnormalities also became candidate biomarkers.

At least eight particular measurements are now widely accepted as constituting probable biomarkers for AD [5–9,17] and more can be anticipated, as discussed below. Most neurologists currently utilize panels of two or more biomarkers as initially proposed by an NINCDS-ADRDA expert group [7]; an NIA-Alzheimer's Association Workgroup on Diagnostic Guidelines in AD [12]; and others [13]; plus clinical observations and behavioral tests, to diagnose AD. They may then follow the course of a patient's disease using the same panels, or just using individual biomarkers. (For example, it has been proposed that the evolution of MCI to AD can be tracked using neuropsychological testing plus a single biomarker: the ratio of CSF tau to A-beta [6]). One set of biomarkers involves measurements of Abeta 1-42 and A beta 1-40,

in CSF. The levels of Abeta 1-42 decline in AD patients [15], possibly reflecting utilization of the soluble peptide to form oligomers or amyloid protein, so its ratio to A beta 1-40 also declines. Amyloid levels in intact brain can also now be estimated, based on PET scanning after administration of isotopically labeled compounds (initially F18-florbetapir), which bind to the amyloid protein [11], as can levels of Tau protein, which binds the ligand 18 F-THK523 [18]. It should be noted that basing the diagnosis of AD on levels of Abeta peptides or their polymerized products in no way requires accepting the hypothesis that these compounds can cause the disease-related destruction of synapses or neurons: Several candidate drugs have been developed in the past few years which deplete the brain of amyloid, e.g. bapineuzumab [19] and solanezumab [20], however while the compounds do lower brain amyloid levels, none to date has clearly been shown to produce clinical improvement in AD patients [17].

Other scanning techniques that are sometimes used to assess biomarkers of AD include FDG-PET, an indicator of brain energy utilization and thus, presumably, of synaptic number; structural MRI for demonstrating atrophy of brain regions; fMRI for assessing regional blood flow and metabolism; and DTI (diffusion tensor imaging). Increases in brain diffusivity as shown by DTI reportedly correlate inversely with cognition and with connectivity between brain regions. As discussed below, connectivity can also be assessed using quantitative electroencephalography [21] and magnetoencephalography or by resting-state fMRI [22]. This latter method assesses the extent to which the BOLD (blood-oxygen-dependent) signals of spatially distant brain regions are correlated when subjects are focused on introspective activities and not sensory inputs, and the brain's "default network" is preferentially active.

Some additional measurements which may in the future become accepted as providing useful biomarkers of AD include MRS (magnetic resonance spectroscopy) for assessing levels of choline or N-acetylaspartate, or rates of phosphatide synthesis; CSF uridine levels [9] which may affect rates of synaptogenesis [23], and which are low in patients with AD; and plasma levels of various phospholipids, as described below.

3. Levels of specific plasma phospholipids, a potential biomarker of AD

Several laboratories have presented evidence that levels of certain phospholipids are subnormal in plasmas of AD patients [24,25]. Moreover these levels can also be depressed among cognitively normal people who go on to develop clinical mild cognitive impairment (MCI) and AD within 2–3 years [24]. The statistical correlation between the depressed plasma phospholipid levels and the subsequent appearance of MCI or AD is on the order of 90% [24].

In the Mapstone et al. study [24], 525 community-dwelling participants aged 70 or older were followed for 5 years. Tests of memory performance and other cognitive abilities were performed at entry into the study and periodically thereafter, and plasma samples – taken at entry and after the test scores of some subjects (N = 28) had been converted to those characteristic of MCI/AD – were assayed for putative metabolomic and

lipidomic markers. The high predictive value of this set of plasma constituents suggests that its measurement might provide an effective and relatively inexpensive biomarker for identifying and possibly treating MCI/AD patients early in the course of their disease.

The relation, if any, between phosphatidylcholine (PC) molecules in plasma and brain is obscure. However the biochemical pathways of PC synthesis and metabolism are similar in brain and peripheral organs. Moreover levels of brain PC, like those of the seven special plasma PCs, are reduced in AD brain, while those of PC's main metabolite, glycerophosphocholine (GPC), are increased [26], suggesting accelerated PC breakdown. Phosphatides, particularly PC, are both the principal components of all cell membranes, including synaptic membranes, and constituents the blood plasma. Individual PC molecules are highly heterogeneous, since each of their two constituent fatty acids may contain zero double bonds (e.g. palmitic [16:0] or stearic [18:0] acids); a single double bond (oleic acid [18:1]) or two or more double bonds (e.g., the omega-6 fatty acid arachidonic acid [20:4] or the omega-3 fatty acid docosahexaenoic acid (DHA [22:6])). Most PC is synthesized via the *Kennedy Cycle*, from uridine (the circulating precursor for brain UTP and CTP [27]; fatty acids (incorporated first into diacylglycerol); and choline [23]. (Some PC can also be formed from the sequential methylation of another phosphatide, phosphatidylethanolamine (PE), the turnover of which is also increased in AD brain [26]). PC is metabolized principally by deacylation, which sequentially releases its two fatty acids, forming first lysophosphatidylcholine and then glycerophosphocholine (GPC).

It will be interesting to determine whether a treatment that normalizes plasma levels of the ten phospholipids that predict the development of MCI/AD also affects the clinical onset and course of these diseases.

Conflict of interest

Dr Wurtman's university, the Massachusetts Institute of Technology, owns United States and foreign patents related to the ability of uridine, DHA, and choline to enhance synaptogenesis by accelerating the production of synaptic membrane. These patents are licensed to the Nutricia Company, and Dr Wurtman serves as a scientific advisor to Nutricia. All of the research in Dr Wurtman's laboratory described in this article was supported by the National Institutes of Health, and without corporate funding.

REFERENCES

- [1] De Meyer G, Shapiro F, Vanderstichele H, et al. Diagnosis-independent Alzheimer disease biomarker signature in cognitively normal elderly people. *Arch Neurol* 2010;67(8):949–56.
- [2] Bernard C, Helmer C, Dilharreguy B, et al. Time course of brain volume changes in the preclinical phase of Alzheimer's disease. *Alzheimers Dement* 2014;10:143–51.
- [3] Hobart J, Cano S, Posner H, et al. Putting the Alzheimer's cognitive test to the test I: traditional psychometric methods. *Alzheimers Dement* 2013;9(1):1–6.
- [4] Posner HB, Cano S, Carrillo MC, et al. Establishing the psychometric underpinnings of cognition measures for clinical trials of Alzheimer's disease and its precursors: a new approach. *Alzheimers Dement* 2013;9(1):S56–60.
- [5] deJong D, Kremer BPH, Olde Rikkert MGM, et al. Current state and future directions of neurochemical biomarkers for Alzheimer's disease. *Clin Chem Lab Med* 1992;45(11):1421–34.
- [6] Ewers M, Walsh C, Trojanowski J, et al. Prediction of conversion from mild cognitive impairment to Alzheimer's disease dementia based upon biomarkers and neuropsychological test performance. *Neurobiol Aging* 2012;33:1203–14.
- [7] Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol* 2007;6:734–46.
- [8] Mattson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* 2009;202(4):385–93.
- [9] Czech C, Berndt P, Busch K, et al. Metabolite profiling of Alzheimer's disease cerebrospinal fluid. *PLoS ONE* 2012;7(2):e31501. <http://dx.doi.org/10.1371/journal.pone.0031501>.
- [10] Jagust WJ, Bandy D, Chen K, et al. The Alzheimer's Disease Neuroimaging Initiative positron emission tomography core. *Alzheimers Dement* 2010;6:221–9.
- [11] Clark CM, Schneider JA, Bedell BJ, et al. Use of florbetapir-PET for imaging beta-amyloid pathology. *JAMA* 2011;305:275–83.
- [12] McKhann GM, Knopman DS, Chertkow H, et al. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:263–9.
- [13] Hampel H, Wilcock G, Androeu S, et al. Biomarkers for Alzheimer's disease therapeutic trials. *Prog Neurobiol* 2011;95(4):579–93.
- [14] Pavlopoulos E, Jones S, Kosmidis S, et al. Molecular mechanism for age-related memory loss: the histone-binding protein RbAp48. *Sci Transl Med* 2013. <http://dx.doi.org/10.1126/scitranslmed.3006373>.
- [15] Motter R, Vigo-Pelfrey C, Kholodenko D, et al. Reduction of beta-amyloid peptide42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol* 1995;38:643–8.
- [16] Hu YY, He SS, Wang XC, et al. Elevated levels of phosphorylated neurofilament proteins in cerebrospinal fluid of Alzheimer's disease patients. *Neurosci Lett* 2002;320:156–60.
- [17] Gandy S, DeKosky S. Toward the treatment and prevention of Alzheimer's disease: rational strategies and recent progress. *Annu Rev Med* 2013;64:367–83.
- [18] Fedoro-Tavoletti MT, Okamura N, Furumoto S, et al. 18 F-TH523: a novel in vivo tau imaging ligand for Alzheimer's disease. *Brain* 2011;134:1089–100.
- [19] Salloway S, Sperling R, Fox NC, et al. Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med* 2014;370:322–33.
- [20] Doody RS, Thomas RG, Farlow M, et al. Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N Engl J Med* 2014;370:311–21.
- [21] de Waal H, Stam CJ, Lansbergen MM, et al. The effect of souvenaid on functional brain network organisation in patients with mild Alzheimer's disease: a randomised controlled study. *PLoS One* 2014. <http://dx.doi.org/10.1371/journal.pone.0086558>.
- [22] Gomez-Ramirez J, Wu J. Network-based biomarkers in Alzheimer's disease: review and future directions. *Front Aging Neurosci* 2014;1. <http://dx.doi.org/10.3389/fnagi.2014.00012/>.
- [23] Wurtman RJ, Ulus IH, Cansev M, et al. Synaptic proteins and phospholipids are increased in gerbil brain by administering uridine plus docosahexaenoic acid orally. *Brain Res* 2006;1088:83–92.

-
- [24] Mapstone M, Cheema AK, Fiandaca MS, et al. Plasma phospholipids identify antecedent memory impairment in older adults. *Nat Med* 2014. <http://dx.doi.org/10.1038/nm.3466>.
- [25] Whiley L, Sen A, Heaton HJ, et al. Evidence of altered phosphatidylcholine metabolism in Alzheimer's disease. *Neurobiol Aging* 2014;35:271–8.
- [26] Nitsch RM, Blusztajn JK, Pittas AG, et al. Evidence for a membrane defect in Alzheimer disease brain. *Proc Natl Acad Sci* 1992;89:1671–5.
- [27] Wurtman RJ, Regan M, Ulus I, et al. Effect of oral CDP-choline on plasma choline and uridine levels in humans. *Biochem Pharmacol* 2000;60:989–92.