Open Access Full Text Article

Alzheimer's disease and blood-based biomarkers – potential contexts of use

Martina Zvěřová[1,2]

[1]Department of Psychiatry, General University Hospital in Prague, Prague, Czech Republic; [2]First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Abstract: Alzheimer's disease (AD) is an irreversible, incurable, progressive neurodegenerative illness, where dementia symptoms gradually worsen over a number of years. The research of validated biomarkers for AD is essential to improve diagnosis and accelerate the development of new therapies. Biochemical markers including neuroimaging could facilitate diagnosis, predict AD progression from a pre-AD state of mild cognitive impairment, and be used to detect the efficacies of disease-modifying therapies. Established biomarkers of AD from cerebrospinal fluid and neuroimaging are highly accurate, but barriers to clinical implementation exist. The focus on blood-based AD biomarkers has grown exponentially during the past few decades. An ideal diagnostic test for AD should be noninvasive and easily applicable. Clinical cost-effectiveness also needs to be established.

Keywords: biomarker, Alzheimer's disease, neurodegeneration, cerebrospinal fluid, beta amyloid, tau protein

Introduction

Alzheimer's disease (AD) is becoming one of the most problematic and costly diseases for the society. AD is now viewed as a chronic and slowly progressive disorder with a long build of pathology that precedes symptoms by a decade or longer.[1] The number of those affected is expected to reach 115 million worldwide by 2050. It is the fifth leading cause of death for those over 65 years.[2,3] The most typical initial symptom is short-term memory impairment. However, there are also atypical clinical presentations of AD, for example, primary progressive aphasia or posterior cortical atrophy, and there are many other dementia-causing diseases that may be important differential diagnoses.[4]

There is no effective treatment capable of slowing down disease progression. With no known cure, it is crucial to look at what can be done to reduce the risk or delay the onset of developing the disease. AD with a progressive loss of cognitive abilities and daily life activities is devastating for those who acquire it, and can be equally devastating for the caregiver, whether that person is a professional or a family member. Treatment of AD requires an alliance with both the patient and the patient's family.[5]

AD has an annual health care cost similar to that of cardiovascular disease and more than that of cancer.[6] The burden on families and the health care system is expected to increase as baby boomers reach their golden years.[2] Medical diagnosis of AD is hard, particularly at the early stage of the disease, mainly because symptoms are often dismissed as normal consequences of aging. As a result of these facts, there is a growing need for the identification of a time-effective and cost-effective screening tool. It is a great challenge to search for novel biomarkers by using modern potent methods, such

Correspondence: Martina Zvěřová
First Faculty of Medicine, Charles University in Prague, Ke Karlovu 11, 121 08 Prague 2, Czech Republic
Tel +420 22 496 5317
Fax +420 22 492 3077
Email martina.zverova@vfn.cz

as microarrays and mass spectrometry, and to optimize the interpretation using bioinformatics.

Comprehensive research is being performed to identify more specific and more sensitive AD biomarkers that can not only accurately diagnose early-stage AD but also differentiate AD from non-AD dementias (vascular dementia, tauopathy, frontotemporal dementia, Lewy body dementia, etc.). These biomarkers should be able to assess the risk of AD in combination with other known risk factors, facilitate screening of potential therapeutic agents and their identification, track the prodromal stages of AD, guide therapeutic decision-making, and monitor therapeutic efficacy.[7,8]

Onset of AD occurs generally after 60 years of age but may span 8–10 years. It is the most common type of dementia in order of frequency, accounting for 60%–70% of all cases.[2,3] The vast majority of patients suffer from the sporadic form, which is subdivided into early-onset (under 65 years of age) and late-onset (95%–97% prevalence) forms. Familial AD (particularly, early-onset AD) is a rare form of Alzheimer's which comprises <5% of Alzheimer's cases and is caused by hereditary mutations.[9] To diagnose AD, extensive tests are required to eliminate all other possible causes. A clinical diagnosis of AD is usually based on medical records, physical and neurological examination, neuroimaging, laboratory tests (eg, thyroid and kidney function tests, assess vitamin B12, rule out syphilis, rule out metabolic problems, assess the levels of heavy metals and anemia), neuropsychological evaluation, and collateral history from relatives.[10]

The widely used neuropsychological tests, such as the Mini-Mental State Examination and the Addenbrooke's Cognitive Examination Revised (ACE-R) test, usually provide negative results in early AD, except for obvious severe cognitive impairment, which may not differ from that resulting from other disorders, including other types of dementia.

Neuroimaging is one of the advanced clinical methods for the confirmation of AD. Since 2007, the positron emission tomography (PET) scan combined with a volumetric magnetic resonance imaging and an invasive cerebrospinal fluid (CSF) protein analysis (Aβ, total-tau [T-tau], phospho-tau) has been accepted by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association as a suitable procedure for the diagnosis of AD.[11] Imaging biomarkers for AD include magnetic resonance imaging measurements of brain volume and neuronal connectivity and amyloid and tau PET to measure the amount of these protein deposits in the brain.[12] Amyloid-PET imaging (API) detects amyloid beta (Aβ) pathology early in the course of

AD with 90% sensitivity and 83% specificity for the diagnosis of AD.[12,13] [18F]Fluorodeoxyglucose-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, while magnetic resonance spectroscopy provides the metabolic markers of diminished cell number.[12] Unfortunately, the diagnostic methods including PET (still primarily a research technique, mostly inaccessible) are limited because they are invasive, have less sensitivity at the early stage, are expensive, or are time consuming.[14]

To date, a definitive diagnosis of AD can only be made with both a clinical diagnosis and a postmortem histopathologic examination of the brain since the presence of neurofibrillary tangles and senile plaques throughout the brain is not readily perceptible using current diagnostic technologies, making early diagnosis difficult and inaccurate.

Biological hypothesis of the disease

The complex etiology of the disease is not well understood, but published data indicate AD is more than just a neurodegenerative disease of the brain; it is a systemic disease with the symptoms in the peripheral tissues and blood caused by oxidative, metabolic, inflammatory, and biochemical processes.[8,15] The characteristic pathology of AD includes the extracellular neuritic plaques (composed of various Aβ peptides, including the 40- and 42-amino acid cleavage products [$A\beta_{40}$ and $A\beta_{42}$] of the amyloid precursor protein), the intracellular formation of neurofibrillary tangles (containing an abnormally phosphorylated form of tau protein), microglial activation, the loss of neuronal synapses, and pyramidal neurons in specific brain regions.[16] Several biological hypotheses trying to explain the cause of AD have been formulated: acetylcholine deficiency, Aβ overproduction and clearance, tau hypothesis, mitochondrial dysfunction and neuroenergetic hypothesis, brain-derived neurotrophic factor or nerve growth factor deficit, and others.[17,18]

One of the most accepted hypotheses, the amyloid cascade hypothesis, postulated that Aβ deposition in the form of senile plaques with changed structures causes cell loss, formation of neurofibrillary tangles, and dementia.[17] The revised amyloid cascade hypothesis supposes that a pathological overproduction of Aβ, not its deposition as plaque, causes the pathology of AD (neurofibrillary tangles and amyloid plaque formation, damage of blood–brain barrier, oxidative damage, impaired memory).[18] Brain pathologies accumulate predominantly not only in the medial temporal lobe but also elsewhere in the brain, and consist of synaptic

damage, neuronal loss, and neurogenesis defects, which in turn contribute to cognitive dysfunction.[19]

The major risk factor for sporadic AD development is aging. An individual's baseline risk is likely determined by inherited nuclear and mitochondrially encoded genes. Environmental factors, such as inflammatory processes, insulin resistance, hypertension, dyslipidemia, metabolic syndrome, and midlife obesity, likely modify this baseline risk.[15] The pathogenic process probably starts some decades (20–30 years) before the first clinical symptoms become apparent.[10]

An interesting and striking sign is that most neurodegenerative dementias show inclusions or aggregates of specific proteins in the brain extracellular matrix or within neurons or other cell types of the brain.[4] These disorders include, for instance, Parkinson's disease dementia and Lewy body disease with alfa synuclein inclusions, and frontotemporal dementia, where tau and/or TDP 43 may form inclusions and others.

Therefore, simple and practical biomarkers for AD are urgently required for an accurate diagnosis and to facilitate the development of disease-modifying interventions. The respective diagnostic and prognostic markers of AD are expected to improve patients' outcome significantly and to support the discovery of new treatment targets.

Biological markers

A biomarker (biological marker) is defined as

> a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. A biomarker is not an assessment of how an individual feels, functions, or survives.[20]

The proposed categories of biomarkers include susceptibility/risk biomarker, predictive biomarker, prognostic biomarker, monitoring biomarker, diagnostic biomarker, and pharmacodynamics/response biomarker. The most needed type of AD biomarkers to be applicable in short-term future includes those which could predict or indicate the likelihood of a successful treatment.[20] A major challenge is the detection of AD using minimally invasive or even noninvasive biomarkers from body fluids, such as plasma or serum. Plasma, platelets, and peripheral blood mononuclear cells are the key components of peripheral blood in studies of pathophysiology of neuropsychiatric diseases such as AD.[19,21] Problems in the identification of specific AD biomarkers are based, inter alia, on the fact that with increasing age, the incidence of various diseases increases, and it is, therefore, difficult to distinguish specific AD biomarkers. It is also important that the patient has not had any acute central nervous system disease at least 3–6 months before sampling of the fluid, as, for example, a head trauma, stroke, or meningitis may affect biomarker concentrations for this time window.[4]

Ideal AD biomarkers should meet the following criteria: 1) the ability to diagnose AD with high sensitivity and specificity, 2) the ability to recognize the initial stage of the disease and to monitor the progress of the disease, and 3) the ability to monitor the therapeutic effectiveness of administered drugs.

The most rigorous but the most solid definitions of "a diagnostic biomarker" in the field of neurodegenerative dementias, especially for AD, were those given by the National Institute on Aging and the Alzheimer Association consensus conferences.[22]

The following criteria should be fulfilled before accepting a biomarker as a valid one for AD: specificity (>85%; 100% identifies all individual free of the disease), sensitivity (>85%; 100% indicates that all patients are identified with the disease), prior probability (the background prevalence of the disease in the population tested), positive predictive value (>80%; refers to the percentage of people who are positive for the biomarker and have a definite disease at autopsy), and negative predictive value (percentage of people with a negative test and no disease at autopsy).[23]

With regard to AD problematics, most research in the AD space has focused on neuroimaging biomarker and CSF modalities, which will likely be the confirmatory diagnostic procedures.[8]

There are many potential contexts of use for AD biomarkers, for example, identification of AD risk, risk for progression from mild cognitive impairment to AD, disease monitoring, pharmacodynamics or treatment response monitoring, stratification into clinical trials, and so on.[8,24]

CSF biomarkers

CSF is a very useful fluid for AD diagnosis because it reflects, inter alia, metabolic processes in the brain owing to direct contact between CSF and the brain. Its diagnostic use is limited because of invasive collection by lumbar puncture.[25]

Currently existing diagnostic approaches are focused on the detection of the 42-amino acid isoform of A® (Aβ42) and the T-tau and phosphorylated tau (P-tau) levels in the CSF and in the brain. These analytes are established as the core AD CSF biomarkers reflecting the key aspects of pathogenesis of AD. Aβ42 can be measured by mass spectrometry or by antibody-dependent techniques (enzyme-linked

immunosorbent assay). Replicated and verified data show that AD patients have decreased CSF concentration of Aβ42, which reflects Aβ42 sequestration in senile plaques in the brain.

The normal function of tau is to bind to and stabilize tubulin multimers in neuronal axons. Abnormally phosphorylated and truncated tau proteins are the major components of neurofibrillary tangles in AD. AD patients have increased CSF tau concentrations, which correlate with tangle pathology in AD. The T-tau can be used as a general marker of neuroaxonal degeneration/injury in AD. AD patients have increased CSF T-tau concentrations, and the higher the increase, the more intense the neurodegenerative process.[4,22] T-tau sensitivity is >90% for AD and prodromal AD.[13,22]

A high diagnostic performance of the core AD CSF biomarkers has also been validated in studies that confirmed diagnosis by autopsy.[22] CSF neurofilament light chain protein (NFL) is another sensitive marker of neuronal injury in a variety of neurodegenerative conditions such as axonal degeneration.

CSF NFL concentration is increased in AD, mainly in patients with rapid disease progress. The synaptic protein neurogranin is a candidate CSF diagnostic biomarker. High CSF neurogranin is found in AD and prodromal AD, reflecting synaptic dysfunction or degeneration. It also predicts future cognitive decline and seems to be more specific for AD than, for example, T-tau.[26]

Blood-based biomarkers

First-line biomarkers are needed to fit the needs of the consistently growing aging segment of the world population.

The invasiveness to obtain CSF influences early diagnosis, monitoring of the disease, or the effectiveness of drug therapy negatively.[27] PET imaging is accessible in specialized centers, and it is very expensive.

Scientists have tried to discover molecular or cellular changes in blood associated with neurodegenerative diseases. Detection of AD by using minimally invasive or even noninvasive biomarkers from body fluids such as plasma or serum is very important. Some of the blood biomarkers appear to be just as diagnostically accurate as the CSF-based and genetic biomarkers, though further validation is warranted.[8] Various -omics (proteomic, lipidomics, metabolomics) methods have been used to identify blood-based biomarkers.[28] Current putative blood biomarkers of AD include Aβ-related proteins, proteins related to tau pathology, and additional factors involved in neuroinflammation, brain aging, cell death, and cerebrovascular dysfunction, including plasma melatonin, homocysteine, cortisol, and

prolactin levels.[19,28] Many recent studies have shown that biomolecules, such as creatine, 5-hydroxycytosine, serine, phospholipids, myo-inositol, glutamate, N-acetylaspartate, blood dehydroepiandrosterone, vary with the progression of AD – most of them even in other biofluids besides CSF.[7] First data from recent studies suggest associations of the concentrations of some plasma proteins (eg, interleukin 17, alfa2-macroglobulin, apolipoprotein A1, pancreatic polypeptide Y, IGM, clusterin) with amyloid burden in the brain.[29]

Recent published data have identified potential blood-based biomarkers (eg, neuronally derived exosome levels of phosphorylated tau, Aβ 1–42, neurogranin) that predict the risk for incident AD and the risk of progression, and discriminate between disorder and cognitively normal older adults.[8,9,30–32]

Brain-specific proteins reflect AD molecular mechanisms at much lower concentrations in the blood than in the CSF, and these very low levels must be (except for the requirements regarding analytical specificity) quantified within other proteins (eg, immunoglobulins, albumin). There are ultrasensitive measurement techniques (immuno-magnetic reduction and single-molecule array) that allow accurate analysis of blood-based biomarkers (tau levels in plasma, plasma NFL).[33,34] Both the abovementioned techniques have shown increased tau levels in plasma in AD; single-molecule array NFL assay has shown a marked increase in plasma NFL in AD and mild cognitive impairment patients as compared with controls. A diagnostic performance is comparable to the core AD CSF biomarkers.[21,32–34]

It is expected that suitable combinations of blood-based biomarkers, brain imaging, cognitive testing, and clinical data will provide a more complex diagnosis of AD or AD response to therapy over individual biomarkers.

Blood-based biomarkers have important advantages that are significant. The lack of cross-validation across academic laboratories, cohorts, methodologies, and industry laboratories remains an ongoing limitation. Further research and future longitudinal studies are needed to determine the cutoff for positivity and the specificity and sensitivity to identify AD.

Conclusion

Early diagnosis of neurodegenerative diseases such as AD represents an important clinical need supporting in-time treatment. Simple and practical biomarkers with high sensitivity and specificity for AD are urgently required for an accurate diagnosis and to facilitate the development of disease-modifying interventions. There are many potential contexts of use for AD biomarkers, for example, identification of AD

risk, risk for progression from mild cognitive impairment to AD, disease monitoring, pharmacodynamics or treatment response monitoring, stratification into clinical trials, and so on. New biomarkers based on biochemical data, proteomic data, and metabolomic analysis of plasma and other constituents of the blood or tissues are expected to be found in the field of active biomolecules. Imaging biomarkers (PET imaging of amyloid and tau aggregates) and CSF markers (measurement of amyloid and tau) are very helpful to identify AD pathophysiology, but are unlikely to be a routine diagnostic tool: PET technique is only accessible in specialized departments and is very expensive; lumbar puncture may be regarded as invasive, complicated, and time consuming by many physicians. Results from recent studies have shown that plasma NFL has a diagnostic performance comparable to the core AD CSF biomarkers and have predicted future cognitive decline.

The hope is to diagnose Alzheimer's disease before the symptoms start. Future treatments could then target the disease in its earliest stages before irreversible brain damage or mental decline occurs.

The development of marker panels is in its early stages and requires further substantial preclinical and clinical validation. It seems likely that only a combined analysis of several biomarkers will define a patient-specific signature to diagnose AD in the future. The respective diagnostic and prognostic markers of AD are expected to improve patients' outcome significantly and to support the discovery of new treatment targets.

Compared with neuroimaging or collection of CSF, it is important for blood-based biomarkers to be cost-effective and time consuming.

Acknowledgment

Disclosure

The author reports no conflicts of interest in this work.

References

1. Galasko D. Expanding the repertoire of biomarkers for Alzheimer's disease: targeted and non-targeted approaches. *Front Neurol*. 2015;6:256.
2. WUSTL. Washington University of St. Luis. Available from: http://neuroscienceresearch.wustl.edu/News.aspx. Accessed May 11, 2017.
3. Alzheimer's Association. A future without Alzheimer's; 2017. Available from: http://www.alz.org/research/overview.asp. Accessed May, 2017.
4. Zetterberg H. Applying fluid biomarkers to Alzheimer's disease. *Am J Physiol Cell Physiol*. 2017;313(1):C3–C10.
5. World Health Organization, International Alzheimer's Disease. Dementia: a public health priority. WHO; 2012. Available from: http://www.who.int/mental_health/publications/dementia_report_2012.
6. Hurd MD, Martorell P, Delavande A, Mullen KJ, Langa KM. Monetary costs of dementia in the United States. *N Engl J Med*. 2013;368(14):1326–1334.
7. Fiandaca MS, Zhong X, Cheema AK, et al. Plasma 24-metabolite panel predicts preclinical transition to clinical stages of Alzheimer's disease. *Front Neurol*. 2015;6:237.
8. O'Bryant SE, Mielke MM, Rissman RA, et al. Blood-based biomarkers in Alzheimer disease: current state of the science and a novel collaborative paradigm for advancing from discovery to clinic. *Alzheimers Dement*. 2017;13(1):45–58.
9. Panegyres PK, Chen HY. Differences between early and late onset Alzheimer's disease. *Am J Neurodegener Dis*. 2013;2(4):300–306.
10. Chintamaneni M, Bhaskar M. Biomarkers in Alzheimer's disease: a review. *ISRN Pharmacol*. 2012;2012:984786–6.
11. 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(8):734–746.
12. Carswell CJ, Win Z, Muckle K, et al. Clinical utility of amyloid PET imaging with (18)F-florbetapir: a retrospective study of 100 patients. *J Neurol Neurosurg Psychiatry*. 2018;89(3):294–299.
13. Rabinovici GD, Rosen HJ, Alkalay A, et al. Amyloid vs FDG-PET in the differential diagnosis of AD and FTLD. *Neurology*. 2011;77(23):2034–2042.
14. Su Y, Blazey TM, Owen CJ, et al. Correction: quantitative amyloid imaging in autosomal dominant Alzheimer's disease: results from the DIAN study group. *PLoS One*. 2016;11(9):e0163669.
15. Morris JK, Honea RA, Vidoni ED, Swerdlow RH, Burns JM. Is Alzheimer's disease a systemic disease? *Biochim Biophys Acta*. 2014;1842(9):1340–1349.
16. Wurtman R. Biomarkers in the diagnosis and management of Alzheimer's disease. *Metabolism*. 2015;64(3 Suppl 1):S47–S50.
17. Selkoe DJ, Hardy J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med*. 2016;8(6):595–608.
18. Takahashi RH, Nagao T, Gouras GK. Plaque formation and the intraneuronal accumulation of β-amyloid in Alzheimer's disease. *Pathol Int*. 2017;67(4):185–193.
19. Pláteník J, Fišar Z, Buchal R, et al. GSK3β, CREB, and BDNF in peripheral blood of patients with Alzheimer's disease and depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;50:83–93.
20. Martins-de-Souza D. Biomarkers for psychiatric disorders: where are we standing? *Dis Markers*. 2013;35(1):1–2.
21. Maes OC, Schipper HM, Chertkow HM, Wang E. Methodology for discovery of Alzheimer's disease blood-based biomarkers. *J Gerontol A Biol Sci Med Sci*. 2009;64(6):636–645.
22. Blennow K. A review of fluid biomarkers for Alzheimer's disease: moving from CSF to blood. *Neurol Ther*. 2017;6(Suppl 1):15–24.
23. Lewczuk P, Riederer P, O'Bryant SE, et al. Cerebrospinal fluid and blood biomarkers for neurodegenerative dementias: an update of the consensus of the task force on biological markers in psychiatry of the world federation of societies of biological psychiatry. *World J Biol Psychiatry*. 2018;19(4):244–328.
24. Khan TK, Alkon DL. Peripheral biomarkers of Alzheimer's disease. *J Alzheimers Dis*. 2015;44(3):729–744.
25. Humpel C. Identifying and validating biomarkers for Alzheimer's disease. *Trends Biotechnol*. 2011;29(1):26–32.
26. Kvartsberg H, Duits FH, Ingelsson M, et al. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. *Alzheimers Dement*. 2015;11(10):1180–1190.
27. Folch J, Petrov D, Ettcheto M, et al. Current research therapeutic strategies for Alzheimer's disease treatment. *Neural Plast*. 2016;2016:1–15.
28. Heneka MT, Carson MJ, El Khoury J, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol*. 2015;14(4):388–405.

29. Voyle N, Baker D, Burnham SC, et al. Blood protein markers of neocortical amyloid-β burden: a candidate study using SOMAscan technology. *J Alzheimers Dis*. 2015;46(4):947–961.

30. Huynh RA, Mohan C. Alzheimer's disease: biomarkers in the genome, blood, and cerebrospinal fluid. *Front Neurol*. 2017;8:102.

31. Nicolas G, Wallon D, Charbonnier C, et al. Screening of dementia genes by whole-exome sequencing in early-onset Alzheimer disease: input and lessons. *Eur J Hum Genet*. 2016;24(5):710–716.

32. Foo JN, Liu JJ, Tan EK. Whole-genome and whole-exome sequencing in neurological diseases. *Nat Rev Neurol*. 2012;8(9):508–517.

33. Mattsson N, Zetterberg H, Janelidze S, et al. Plasma tau in Alzheimer disease. *Neurology*. 2016;87(17):1827–1835.

34. Mattsson N, Andreasson U, Zetterberg H, Blennow K. Alzheimer's disease neuroimaging I. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. *JAMA Neurol*. 2017;74:557–566.