

Identifying and validating biomarkers for Alzheimer's disease

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The identification and validation of biomarkers for diagnosing Alzheimer's disease (AD) and other forms of dementia are increasingly important. To date, ELISA measurement of β -amyloid(1–42), total tau and phospho-tau-181 in cerebrospinal fluid (CSF) is the most advanced and accepted method to diagnose probable AD with high specificity and sensitivity. However, it is a great challenge to search for novel biomarkers in CSF and blood by using modern potent methods, such as microarrays and mass spectrometry, and to optimize the handling of samples (e.g. collection, transport, processing, and storage), as well as the interpretation using bioinformatics. It seems likely that only a combined analysis of several biomarkers will define a patient-specific signature to diagnose AD in the future.

Diagnosing Alzheimer's disease (AD) and other forms of dementia

AD is a severe neurodegenerative disorder of the brain that is characterized by loss of memory and cognitive decline. The majority of AD cases are sporadic (risk age >60 years), and <2.5% have a genetic disposition. It is estimated that in 2050, approximately 80 million people will suffer from AD worldwide. Thus, it is a great challenge to establish reliable surrogate markers to diagnose and monitor disease progression. Definitive diagnosis requires both clinical assessment of the disease and post-mortem verification of the AD pathology (plaques and tangles). A probable diagnosis of AD can be established with a confidence of >90%, based on clinical criteria, including medical history, physical examination, laboratory tests, neuroimaging and neuropsychological evaluation. Accurate, early diagnosis of AD is still difficult because early symptoms of the disease are shared by a variety of disorders, which reflects common neuropathological features. An ideal biomarker would distinguish AD from other types of dementia, such as mild cognitive impairment (MCI), or mixed forms of dementia, such as vascular dementia (VaD), frontotemporal lobe dementia (FTLD), or Lewy body dementia (LBD). This is important because treatment for these diseases might differ.

What is a biomarker?

A biological marker, or biomarker [1–5], is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention. A biomarker

can serve as an indicator of health (i.e. biomarker of ageing) and disease. The sensitivity, specificity and ease-of-use are the most important factors that ultimately define the diagnostic utility of a biomarker. Some biomarkers are more reasonably viewed as risk factors rather than true disease markers. In order for a diagnostic biomarker to be useful, certain criteria must be met (Box 1) [6–9].

Biomarkers for AD in cerebrospinal fluid (CSF)

Three biomarkers have been well-established and validated internationally to diagnose AD in CSF with ELISAs: β -amyloid(1–42) [$A\beta$ (1–42)], total tau and phospho-tau-181. It is now the consensus that only the combination of these three CSF biomarkers significantly increases the diagnostic validity for sporadic AD, which yields a combined sensitivity of >95% and a specificity of >85% [10–13].

$A\beta$ (1–42)

AD is characterized by extracellular $A\beta$ plaque depositions. $A\beta$ is cleaved from the large amyloid-precursor protein (APP) by secretases, and processing of amyloidogenic pathways produces a 42-amino-acid peptide [$A\beta$ (1–42)] that can aggregate in the brain under certain conditions (e.g. acidosis, metals). Analysis of CSF $A\beta$ (1–42) shows a highly significant reduction in AD patients compared to controls, with a cut-off of <500 pg/ml (Table 1). It has been suggested that reduced levels of $A\beta$ (1–42) in the CSF are caused by reduced clearance of $A\beta$ from the brain to the blood/CSF, as well as enhanced aggregation and plaque deposition in the brain. Changes in CSF $A\beta$ levels differ based on the disease (Table 2) [14–16]. For example, decreased $A\beta$ (1–38) levels correlate with FTLD, and $A\beta$ (1–37) levels with LBD [4]. CSF levels of shorter $A\beta$ (1–40) forms are unchanged or increased in AD. It has therefore been suggested that the $A\beta$ 42/ $A\beta$ 40 ratio can improve AD diagnosis, but others have not found such changes [17,18]. Novel detection methods allow measurement of $A\beta$ oligomers, which might improve the diagnostic specificity. As an example, surface-enhanced laser desorption/ionization-time-of-flight-mass spectrometry (SELDI-TOF-MS) (Table 3) has emerged as an ideal method for the simultaneous detection and quantitation of a variety of $A\beta$ peptide cleavage products [19].

Total tau

The second hallmark of AD are intraneuronal inclusions of the microtubule-associated protein tau. In healthy controls, levels of total tau in the CSF increase with age [20]: <300 pg/ml (21–50 years), <450 pg/ml (51–70 years), and <500 pg/ml

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Box 1. Criteria for establishing a good biomarker for the diagnosis of dementia

- Reflect physiological aging processes
- Reflect basic pathophysiological processes of the brain
- React upon pharmacological intervention
- Display high sensitivity
- Display high specificity for the disease as compared with related disorders
- Allow measurements repeatedly over time
- Allow reproducibility in laboratories worldwide
- Should be measurable in noninvasive, easy-to-perform tests
- Should not cause harm to the individuals being assessed
- Tests should be inexpensive and rapid
- Samples should be stable to allow easy and cheap transport
- Easy collection of fluids not only in hospitals
- Changes should be at least twofold to allow differentiation of controls
- Define good cut-off values to distinguish diseases
- Data published in peer-reviewed journals
- Data reproduced by at least two independent researchers

Table 1. Internationally established biomarkers in CSF used to diagnose AD^a

Biomarker	Controls (pg/ml)	AD (pg/ml)
Aβ(1–42)	794±20	<500 [*]
Total tau	136±89 (21–50 years)	^b
	243±127 (51–70 years)	>450
	341±171 (>71 years)	>600 [*]
Phospho-tau-181	23±2	>60 [*]

^aData obtained using the Innogenetics single 96-well ELISA kits.

^bThis is not relevant for sporadic AD, because it is only for patients >60 years of age.

^{*}P<0.001

(>70 years). Total tau levels are significantly enhanced in AD patients as compared with age-matched control subjects with a cut off of >600 pg/ml (Table 1). Total tau levels are dramatically enhanced in Creutzfeldt–Jacob disease (CJD) (>3000 pg/ml). Tau levels might also be a prognostic marker with a good predictive validity for conversion from MCI to AD, because high CSF tau level has been found in 90% of MCI cases that later progressed to AD, but not in cases with stable MCI [10]. Observed changes in CSF total tau level for different diseases are shown in Table 2 [14].

Phosphorylated tau

Tau is markedly hyperphosphorylated (39 possible sites) in AD, which results in a lack of function and axonal transport dysfunction. The detection of tau phosphorylated at position 181 is significantly enhanced in AD compared to controls [21], with a cut-off of >60 pg/ml (Table 1). The

Table 2. Changes in the levels of established CSF biomarkers in different central nervous system diseases

Disease	Aβ(1–42)	Total tau	Phospho-tau-181
Acute stroke	–	↑(↑)	–
Alcohol dementia	–	–	–
AD	↓	↑	↑
CJD	↓↓	↑↑↑	–
Depression	–	–	–
FTLD	↓	↑	–
LBD	↓	↑	↑
Neuroinflammation	↓	–	–
Normal aging	–	–	–
Parkinson's disease	–	–	–
VaD	↓(↓)	↑	–

^aModified according to Ref. [14]: (–), no change; (↓), decrease; (↑), increase.

changes of CSF phospho-tau-181 levels in different diseases are given in Table 2 [14]. The analysis of other phosphorylated forms of tau (phospho-tau-199, -231, -235, -396 and -404) might offer significant improvements towards early diagnosis of AD [11]. Phospho-tau-231 and phospho-tau-181 can be used to distinguish AD from controls and FTLT, LBD, VaD and major depression [4,21]. Furthermore, the concentration of phospho-tau-231 has shown longitudinal decline from mild to moderate AD [21]. It is a high priority to develop specific and sensitive ELISAs for other site-specific phosphorylated tau isoforms to improve the diagnostic repertoire for AD.

Other CSF biomarkers

Despite strong efforts to characterize other potential biomarkers in CSF, several biomarkers have been tested in CSF that have displayed changes between AD and controls [4,22,23]. However, no other single biomarker has been validated to date for routine diagnosis, because the changes are very heterogeneous and low, and data have differed between laboratories. Cognitive decline is highly correlated with loss of the neurotransmitter acetylcholine in the cortex or the hippocampus. Degeneration of cholinergic neurons can be prevented by application of nerve growth factor (NGF), which has been found to be reproducibly increased in CSF of AD patients [22–24]; however, such low changes are not yet useful for establishing NGF as a biomarker. The use of mass spectrometry (MS) methods or different microarrays (Table 3) might rapidly increase the knowledge on disease-related changes to uncover novel biomarkers in CSF of AD patients.

Biomarkers in blood

The routine diagnosis of AD and mixed forms of dementia from CSF has several drawbacks: lumbar puncture and collection of CSF is an invasive treatment with potential side effects, and screening of patients is often difficult and follow-up analysis of the same patient over several years is problematic. Thus, there is a clear need to search for biomarkers in other body fluids (Box 2) to diagnose AD [13]. Although saliva or urine can be easily collected, blood analysis is the gold standard (Box 2); yet, it is still unknown how the concentration of analytes in the blood directly correlates with pathological changes in the brain, especially in AD. The search for blood biomarkers that correlate with AD should therefore begin with accepted CSF markers, such as Aβ and tau-related biomarkers, and further include factors involved in inflammation, protein aging and cell death, and cerebrovascular dysfunctions.

Aβ-related proteins

Blood plasma levels of Aβ are increased in familial AD and Down syndrome, but results are inconsistent with sporadic AD [4]. Studies have shown that plasma Aβ(1–42) and Aβ(1–40) levels can be elevated, reduced or even unchanged in AD versus control patients [4,14]. A significant increase in Aβ(1–42) plasma levels has been seen in women with MCI, but not in men, as compared with cognitively normal, age-matched subjects [25]. Recent longitudinal studies have shown that high plasma Aβ(1–42) levels are a risk factor for developing AD; however, there is

Table 3. Selected examples of methods to discover biomarkers for AD diagnosis

Analytical method	Description	Biomarker	Refs.
ELISA	Single 96-well assay	A β 42, total tau, phospho-tau-181 (single)	[9,10,12,22]
Multiplex Searchlight ELISAs (Aushon)	16 (chemiluminescence) or 24 (infrared) markers per single well in 96-well	16 signaling proteins	[37]
Filter-based array sandwich ELISA	'Capture' antibody is on a filter; requires detection antibody after antigen capture	18 signaling proteins	[36]
INNO-BIA AlzBio3 Luminex-based technology (Innogenetics)	Liquid bead arrays; xMAP	A β 42, total tau, phospho-tau-181 (multiplex)	[60]
Tissue array	Antigen-spotted microarray to detect auto-antibodies; consists of paraffin blocks with 1,000 separated tissues for multiplexed histological analysis	2,325 tissue specimens	[69]
Quantitative real-time RT-PCR	Amplification of DNA alone or coupled to immunoassays (immuno-PCR)	33 genes; multiple phosphorylated tau-epitopes	[62,64]
Liquid chromatography/electrospray ionisation MS		A β 40, A β 42	[66]
Capillary electrophoresis/MS	Rapid, 60-min analysis	1000 polypeptides	[68]
Ultrasensitive laser ablation inductively coupled plasma/MS		Trace elements and metal ions	[67]
Multiplex iTRAQ	Isobaric tagging for relative and absolute protein quantification with multi-dimensional chromatography and tandem MS	1,500 CSF proteins	[63]
Surface-enhanced laser desorption/ionization (SELDI)- or matrix-assisted laser desorption/ionization (MALDI)-MS	Time-of-flight (TOF) MS	Several A β species: A β 37, A β 36, A β 38, A β 40; several other biomarkers	[19,65, 74–77]
DNA/RNA chips, BioChips, GeneChips	Microspots on a matrix with a single, defined species of nucleic acids	Several thousand genes	[70–73]

agreement that this factor is not sensitive and specific for early diagnosis [26]. A decrease of serum A β (1–42) auto-antibodies has been found in AD [27], yet no correlation between CSF and plasma A β levels has been found. Antigen-spotted microarrays could help identify and validate AD-selective biomarker autoantibodies in blood and CSF. Such a rapid assay has been developed, which measures

Box 2. Tissue and liquids to screen for biomarkers: advantages and disadvantages

Biomarkers can be discovered from different human tissues or liquids. The analysis of postmortem brain tissue is necessary to verify AD by immunohistochemical analysis of plaques (A β) and tangles (tau). Postmortem analysis or, alternatively, brain biopsies might also allow screening for general pathological changes in the AD brain, but are not useful for routine biomarker analysis. CSF is a very useful fluid for AD diagnosis, because it reflects metabolic processes in the brain owing to direct contact between the brain and CSF. Its diagnostic use is only limited because of invasive collection by lumbar puncture.

Plasma/serum measurements are the gold standard in clinics, because they are minimally invasive, as compared with CSF, and therefore easily collected and processed. For AD biomarker discovery, the use of plasma is still limited, because changes are very small and heterogeneous and plasma/serum data reflect a broad spectrum of changes; not all necessarily related to AD. A major advantage of blood samples is that patients can be followed up and screened over several years. The analysis of blood cells (e.g. peripheral blood mononuclear cells, lymphocytes, monocytes or platelets) might be more restricted to specific AD-related pathologies. The disadvantage is the more complex and time-consuming processing of blood cells, especially when culturing blood cells under sterile conditions. The collection of other fluids (e.g. saliva, urine, fibroblasts, or eye secretions) is fast, cheap and noninvasive; however, the use of these fluids requires very sensitive methods to detect low-level proteins and the correlation to AD pathologies is unclear.

the difference between dissociated sera and the corresponding non-dissociated sera [28].

There are several reasons why plasma levels of A β (1–42) are unstable: (i) A β expression is influenced by medications [29]; (ii) A β binds to other proteins and thus becomes trapped; (iii) A β levels in blood fluctuate over time and among individuals, and might differ in mild, early and late AD; and (iv) blood platelets contain high amounts of A β , which directly affects plasma levels. Platelets express APP and the secretase machinery, and mainly process A β (1–40), which plays a role in platelet aggregation. In platelets of AD patients, three subtypes of APP (106, 110 and 130 kDa) have been found [30,31]. AD patients show enhanced processing of the 130-kDa APP subtype, which results in a mean APP form ratio – [130 kDa subtype]/[(106 kDa subtype) + (110 kDa subtype)] – of 0.35 ± 0.18 , whereas controls have a mean ratio of >0.83 . A cut-off level of >0.6 results in a sensitivity of 83% and a specificity of 71% when diagnosing AD [30,31].

Autophagic degradation of intracellular components via the lysosomal pathway is abnormal in AD. Macrophages and monocytes of AD patients are generally poorly phagocytic for A β , whereas cells of control patients robustly internalize A β [32]. Phagocytosis of A β can be tested using *in vitro* differentiated monocytes/macrophages exposed to fluorescent A β [32]. Monocytes can be detected by immunofluorescent staining with anti-CD68 and visualized in flow cytometric tests (e.g. fluorescence-activated cell sorting) or by immunohistochemistry [32].

Enzymes related to tau pathology

Tau is a brain-specific protein, and altered function of protein kinases and phosphatases has been implicated

in tau pathology [21]. A number of kinases contribute to tau hyperphosphorylation, including glycogen synthase kinase-3 (GSK-3), cyclin-dependent kinase 5, and microtubule affinity-regulating kinase; conversely, protein phosphatase 2A dephosphorylates tau. It has been reported that GSK-3 is significantly increased in white blood cells in AD and MCI patients, as compared with healthy subjects [33]. This correlation notwithstanding, reduced GSK-3 levels in peripheral-blood mononuclear cells have also been noted in AD patients [34], which indicates high variability. Protein kinase C (PKC) appears to be altered in fibroblasts, lymphocytes, and red blood cells of AD patients [35], which supports the view that PKC conformation in peripheral cells could be an early predictive marker for AD. At present, tau-related biomarkers in the periphery do not seem to be useful for AD diagnosis.

Inflammatory markers

In the brain of an AD patient, neuroinflammatory processes occur, including activation of microglia and expression of proinflammatory cytokines, which probably contribute to neuronal cell death. A recent promising study has shown that the combination of 18 selected biomarkers (chemokines, cytokines, growth factors and binding proteins) in plasma might allow the diagnosis of AD [36] and MCI with nearly 90% accuracy. These 18 biomarkers have been selected from 120 signalling proteins by sandwich ELISAs (Table 3). Recently, 16 of these 18 biomarkers have been tested using a similar Multiplex Searchlight ELISA system (Aushon: www.aushon.com) (Table 3), but similar changes could not be confirmed, and furthermore, a lower sensitivity/specificity of 60% was demonstrated [37]. Similarly, the use of Luminex-based technology (Table 3) yielded a diagnostic accuracy of only 70% [38]. The Luminex method differs from conventional ELISAs in the way that the capture antibody is attached to 5.6- μ m polystyrene beads, which are internally dyed with fluorophores of different intensities, and can be detected using dual laser systems for classification and quantification.

Other disease-related markers

Failure of the ubiquitin–proteasome pathway function has been linked to A β toxicity, and indeed ubiquitin levels are elevated in AD [39]. It is well known that ubiquitin levels are increased several-fold in the cerebral cortex and CSF of patients with AD. This increase strongly correlates with the degree of neurofibrillary changes in the tissue [40,41]. In CD45⁺ T lymphocytes, the ubiquitin–proteasome pathway has been found to be reduced during ageing [40]. We have recently shown that in peripheral blood mononuclear cells, the enzymes for proteasomal activity (conjugating and activating enzymes E1 and E2) are changed [42].

Cellular senescence is a stress response phenomenon that results in functional changes, such as telomere shortening [43]. In peripheral blood cells of AD and VaD patients, shorter telomeres than those in age-matched controls have been discovered [44–46]. Recently, it has been reported that fibroblasts from sporadic AD patients specifically express an anomalous and detectable conformational state of the senescence marker p53 (mutant-like p53) that allows for differentiation of AD subjects [47].

Peripheral blood cells of AD patients are also more sensitive to apoptosis [48–50]. Increased apoptosis has been noted in CD4⁺ T cells and natural killer cells of AD patients, accompanied by enhanced expression of the anti-apoptotic protein B-cell lymphoma 2 (Bcl-2), the antioxidant enzyme superoxide dismutase 1 (SOD1), and different subtypes of caspases [51,52].

In AD, damage of the cerebrovascular system, including the blood–brain barrier and neurovascular unit, results in a loss of energy supply to the brain, accompanied by silent strokes; thus, measurement of biomarkers associated with cerebrovascular damage could provide sensitive instruments for the diagnosis of AD. It is well-established that high concentrations of C-reactive protein (CRP) are predictive of cognitive decline and dementia [53]. Furthermore, increased plasma homocysteine in combination with decreased folate and vitamin B12 are biomarkers for VaD, and might discriminate between AD and controls [54]. The analysis of vasodilators or vasoconstrictors (e.g. pro-adrenomedullin, pro-atrial natriuretic peptide, C-terminal endothelin 1 precursor fragment) or cell adhesion molecules [vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, ICAM-3, and P-selectin] might lead to improved diagnosis of AD [55]. Increased plasma levels of VCAM-1 and ICAM-1 have been observed in AD patients [55,56].

Searching for patient-specific, multi-biomarker profile signatures

The principal goal is to discover biomarkers, with the ultimate objective of identifying differentially expressed proteins between diseased and healthy controls. Novel and fast high-throughput systems dramatically enhance the analysis of thousands of proteins and genes (Table 3) with very low volumes. It is becoming clear that the consideration of a single biomarker might not be potent enough to improve diagnostic specificity. Thus, it is essential to develop methods to measure several biomarkers together in a single well or on a biochip to create an accurate prognostic profile.

DNA and RNA microarrays have led to the identification of multiple genes that appear in early stages of AD [5]; however, DNA/RNA microarrays or transcriptomics (Table 3) alone probably provide only a limited view of the biological process. The combination of genomics and proteomics has rapidly gained interest, and such interdisciplinary research will markedly further biomarker discovery [57]. Thus, research needs to develop simple, inexpensive and rapid tools to measure several biomarkers at the same time. As an example, a combination of the following biomarkers on a single Multiplex Sandwich ELISA Microarray is a possibility: five-plex of macrophage inflammatory proteins-1 δ and -4 (MIP1 δ and MIP4), regulated upon activation normal T-cell (RANTES) [23], tumor necrosis factor-alpha (TNF α) [37] and midregional pro-atrial natriuretic peptide (MR-proANP) [55] in plasma; a three-plex of ICAM-3, P-selectin and CD14 in monocyte extracts [56]; and a novel four-plex ELISA of selective APP 130-, 110- and 106-kDa proteins, including actin as a control from platelet extracts [31]. A combination of such a 12-plex array could have the potential to improve AD diagnosis from blood. The development of a patient-specific chip array could allow us to define specific,

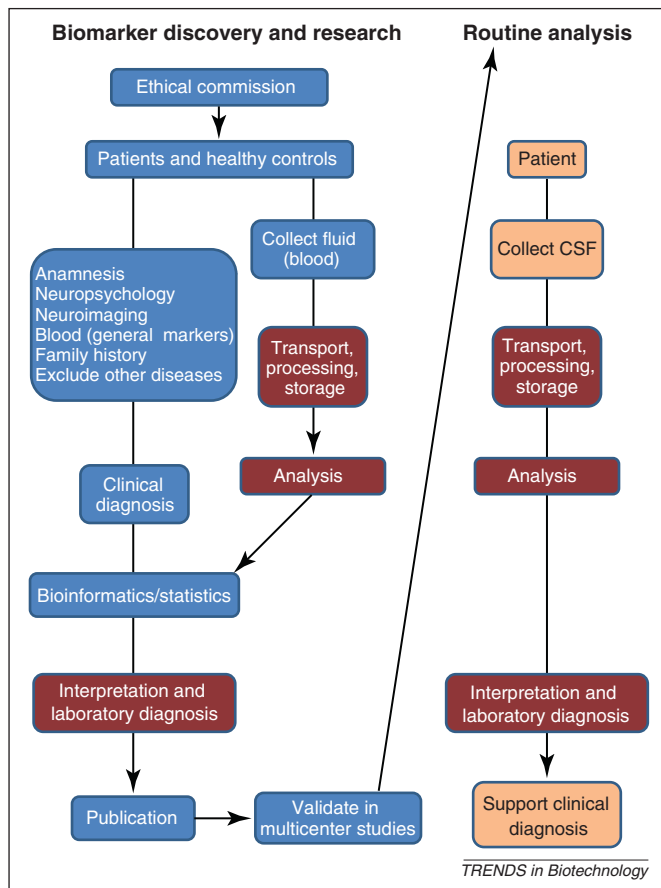


Figure 1. The principal steps necessary for AD biomarker discovery (left flowchart) and for routine analysis of CSF samples (right flowchart). Similar steps are shown in red boxes. Only a good clinical diagnosis is the basis for statistical analysis of biomarker discovery. To date, laboratory diagnosis only supports the clinical diagnosis, which cannot be regarded as a stand-alone diagnostic tool.

multi-biomarker profile signatures, which could even act as a prognosticator for AD.

Future challenges and criteria for a good diagnostic assay

Routine analysis of CSF samples (Figure 1) is well-established worldwide; however, a common consensus [58] on several issues is still missing, and thus the interpretation and support of clinical diagnosis is still not unique. It is essential to define routine procedures for: (i) collection, transport, processing and storage of samples; (ii) analysis; and (iii) interpretation (diagnosis) and cut-off values.

Handling of samples (collection, transport, processing and storage)

It is crucially important to search not only for the biomarkers themselves, but also for biomarkers that are stable. Often, samples are collected by medical doctors outside hospitals and without a laboratory. They do not have the time to process or capability to freeze samples. Thus, it is highly desirable to identify biomarkers with long half-lives. RNAs are very unstable; therefore, these criteria largely exclude the use of RNA chips, unless RNA stabilizers are accessible. It also needs to be considered that several other parameters can influence the stability of a protein, such as repeated freeze/thaw cycles or long-term

storage at room temperature. Even measurements of proteins in serum or plasma with or without EDTA, heparin, or citrate can produce variable results for the same biomarker. Worldwide multicenter studies are necessary to compare the diagnostic accuracy in different laboratories [59].

Analysis

Analytical methods are often limited by cost. Commercial ELISAs (e.g. Innogenetics: www.innogenetics.be) for A β , tau and phospho-tau-181 are very expensive. We have calculated that a 96-well plate ELISA costs approximately €900, and three kits are typically needed for quantifying A β , total tau and phospho-tau-181. Analysis is performed in duplicate, and includes a standard curve. In this situation, the estimated cost is approximately €68 per patient, excluding personnel and laboratory expendables. Cheaper diagnostic methods that cost around €10 per patient are warranted. Towards this goal, it is necessary to develop a multiplexed ELISA system to measure all three biomarkers concurrently in a single well. The INNO-BIA AlzBio3 assay (Innogenetics) already allows simultaneous quantification of all three biomarkers using xMAP Luminex technology [60].

Another limitation is the analysis of lowly and highly expressed proteins in the same well. If we want to measure low-level proteins, then a dilution of 1:2 in the assay might be required; however, a high-level protein cannot be quantified at such a low dilution in the same well, because it reaches saturation. Therein lies the need for two separate assays, which increases the cost associated with diagnosis.

Interpretation and cut-off values

Finally, it is important to provide good interpretation of the data to support clinical diagnosis. In our experience, a medical doctor without a background in laboratory experimentation and analysis cannot interpret raw data. Thus, standardized testing and international cut-off values will be important.

Validating novel biomarkers in AD diagnosis

In the scientific search for biomarkers (Figure 1), several additional criteria must be met and standardized: (i) healthy controls; (ii) clinical diagnosis and verification; and (iii) publication and validation by multicenter studies. First, it is important to collect healthy controls who are age-matched and have a similar lifestyle, sex and education. This is extremely difficult to achieve, and might explain the high variety in the data. The 'nun-study' [61] has tried to overcome this problem by analyzing a well-defined cohort of people.

Second, in the search for a biomarker, it is important to have a perfect clinical diagnosis. A medical doctor must collect all criteria for enabling a correct diagnosis (including anamnesis, family history, general blood markers, neuropsychology, and neuroimaging, excluding other diseases). Secure diagnosis of AD can only be defined by postmortem brain analysis. It is worth noting that, to date, CSF diagnosis only supports the clinical (not postmortem) diagnosis.

Lastly, the data must be reproduced by others to validate the biomarkers, thus the scientific data must be

published in well-known, peer-reviewed journals. At least two independent research studies worldwide should confirm the results. Global initiatives, multicenter studies, and consensus protocols of analysis are of crucial importance. In this way, there will be evidence that the novel biomarkers are (or are not) useful in therapeutic studies of AD and other forms of dementia.

Summary

At present, only the analysis of A β (1-42), total tau and phospho-tau-181 in CSF allows reliable, sensitive and specific diagnosis of AD, but not of other forms of dementia. Unfortunately, the use of CSF biomarkers is limited because of invasive collection methods. Efforts are underway to discover reliable blood biomarkers. To date, it seems probable that only the combination of several biomarkers derived from blood will be successful to define a patient-specific signature. Early, fast and cheap diagnosis from body fluids using modern, ultrasensitive analytical methods (e.g. microarrays or MS) will become extremely important in the future to differentiate AD from other forms of dementia, and to gauge therapeutic relevance.

Acknowledgements

The work on biomarker identification was supported by the Austrian Science Funds (Translational Project L429-B05).

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