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MINIREVIEW

An Ideal Biological Marker of Alzheimer´s Disease: Dream or Reality?

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Summary

Senile dementia of Alzheimer´s type (AD) is commonly characterized as a neurodegenerative disorder, which exhibits gradual changes of consciousness, loss of memory, perception and orientation as well as loss of personality and intellect. AD prevalence increases dramatically with age and is the fourth cause of death in Europe and in the USA. Currently, there are no available biological markers, which gives clinicians no other alternative than to rely upon clinical diagnosis by exclusion. There is no assay of objective *ante mortem* biochemical phenomena that relate to the pathophysiology of this disease. The pathophysiology of AD is connected with alterations in neurotransmission, plaque formation, cytoskeletal abnormalities and disturbances of calcium homeostasis. The search for a test, which is non-invasive, simple, cheap and user-friendly, should be directed at accessible body fluids. Only abnormalities replicated in large series across different laboratories fulfilling the criteria for a biological marker are likely to be of relevance in diagnosing AD. To date, only the combination of cerebrospinal fluid τ and Aβ42 most closely approximate an ideal biomarker of Alzheimer´s disease. A short review on the role of biological markers in AD on the basis of the literature, contemporary knowledge and our own recent findings are presented.

Key words

Alzheimer´s disease • Biological peripheral marker • β-amyloid • Protein τ • Review

Introduction

Alzheimer´s disease (AD) is the most common dementia among older persons. Almost unknown in the first half of the twentieth century, AD appears as a new epidemic threatening of human civilization in the next century. Its incidence, at present, doubles every five years. Social and economic consequences of this disease reach immense dimensions. It is therefore obvious that the pressure of medical and social factors have contributed to the intensive research in the neuropathological changes of the aging brain and to the search for a biological correlation with clinical manifestation of AD. With the public awareness of AD,

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more persons go to the doctor complaining of a failing memory. There is no doubt that nearly all people undergo a deterioration of memory late in life. On the basis of a one-year longitudinal study of more than 20, 500 adults, standardized to the US census population, 29 % met DSM-III-R diagnostic criteria for mental disorders (Regier *et al.* 1998). It seems difficult even for clinicians to identify dementia and to determine its severity. A decline of memory and logical thinking to an extent that interferes with everyday activities or a change in social behavior lead the clinician to diagnose dementia. The assessment of persons thought to have dementia is an undertaking of crucial importance for them and their families.

Many clinical decisions, especially diagnoses, are made on the basis of probability. Diagnosis of AD fits into this category: ”probable AD“ is generally used to improve the reliability of clinical diagnoses. Clinicians weigh the accuracy and validity of additional diagnostic tests, such as routine blood examinations, magnetic resonance imaging, computed tomography, functional brain imaging, and neuropsychological tests, to increase the probability of AD by excluding other disorders. Using contemporary diagnostic criteria, the *ante mortem* diagnosis of probable AD in centers specialized for AD is confirmed in 80 % to 90 % of the cases. It can not be excluded, however, that diagnostic accuracy is much lower in routine clinical practice outside the centers. Widespread screening using brain imaging is not really feasible since these techniques require highly expensive equipment and technical expertise. Clinicians therefore expect that identification of biological and biochemical markers will soon allow them to make more objective diagnoses.

The hope of proposed diagnostic tests for AD, such as the fingerprint test, the eye drop test, and the fibroblast test, claimed by their authors, and the associated publicity evoked by such claims, seem to be somewhat premature. The early and accurate diagnosis of AD thus falls short of the goal based on a series of subjective diagnostic criteria that rely heavily on the clinician´s experience or on the brain examination *post mortem*. The development of diagnostic criteria to avoid the misinterpretation and misapplication of various newly introduced tests is urgently needed.

Possible candidates for biological markers in AD

1. Brain imaging techniques in the search for biological markers of Alzheimer´s disease

Structural neuroimaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have demonstrated that rapid progression of atrophy in the temporo-parietal cortex is highly indicative of AD (Jobst *et al.* 1992). Volumetric measurement of specific brain structures by MRI revealed hippocampal atrophy (Laakso *et al.* 1995, Jack *et al.* 1999). However, this information alone is not sufficient to differentiate early AD from normal aging as hippocampal atrophy is commonly seen in normal aging (especially in persons aged over 75 years).

Functional imaging techniques such as positron emission tomography (PET) and single photon emission tomography (SPECT) were expected to play a useful role in the follow up of suspect patients, as being those that would contribute to early diagnosis. SPECT is simple to use and is less expensive than PET, but PET has the advantages of accuracy and depth of information possible. SPECT or PET can provide images of a variety of biochemical functions, such as cerebral blood flow and volume, cerebral glucose and oxygen metabolism, receptor mapping and amino acid incorporation rates into proteins.

Blind clinical PET evaluation indicates that AD patients can be differentiated from other dementias and cognitively intact persons (Powers *et al.* 1992). The mapping of glucose metabolism using fluoro-deoxyglucose by PET (FDG-PET) also in preclinical stages can differentiate AD from some dementias (for example frontal dementia or Levy-body dementia) and normal aging (Albin *et al.* 1996). Hypometabolism throughout the neocortical association areas (superior parietal cortex, the inferior parietal, superior temporal and prefrontal cortices) was reported in AD in many studies using PET (for review see Small *et al.* 1996). The rate of metabolic deficits correlated with the severity of cognitive deficits (Mazziota and Phelps 1986).

On the other hand, temporoparietal hypome-tabolism characteristic for AD could be seen with bilateral temporoparietal strokes (Foster 1998). Bilateral posterior temporoparietal hypoperfusion found in AD by

SPECT and confirmed by PET has a high specificity (80-95 %), but low sensitivity (40-80 %) to be able to act as the sole diagnostic marker (Claus *et al.* 1994, O'Mahony *et al.* 1994, Powers *et al.* 1992).

The cost and lack of widespread availability of FDG-PET, the subjectivity of the interpretation of imaging studies and the variability of technical methods are the main obstacles for accepting brain imaging methods as diagnostic tests (Foster 1998).

2. Peripheral biological markers for AD

Recent fundamental research of the pathogenesis of AD has brought evidence that the main histopathological and functional characteristics of this disease are senile plaques and neurofibrillary tangles, alterations in neurotransmission and calcium homeostasis. The main cognitive deficits manifested in patients with AD are connected with the loss of cholinergic neurons resulting in lowered levels of acetylcholine and of choline acetyltranferase, an enzyme necessary for its synthesis. Inflammation and impairment in the action of free oxygen radicals are probably also involved in the pathogenesis of AD. The discovery of genes, which might be involved in the etiology of AD, have introduced new expectations for AD diagnosis.

2.1. β-amyloid

A key component of senile plaques in the brain is β-amyloid which exists in several forms produced from amyloid precursor protein (APP) by a series of proteolytic cleavages. A slightly shorter form Aβ40 is secreted more, but the longer form Aβ42, which is the predominant form of β-amyloid in AD brain, aggregates more readily (Wisniewski *et al.* 1997). It seems that genes called presenilins affect the production of neurotoxic β-amyloid (Sahara *et al.* 1996, Haass 1996).

Bush *et al.* (1990) found APP in blood platelets. It is not known whether Aβ42 is derived from an extracerebral source. It is a well-known fact that APP is an integral membrane glycoprotein encoded by a single gene on chromosome 21. A wide variety of APP-expressing cells have been shown to release β-amyloid (Lustig *et al.* 1994). Circulating blood platelets can be an important source of APP, which is contained in platelet α granules. The increased content of APP fragments was found in the blood of patients in middle and terminal stages of AD. Platelets could thus be a source of perivascular amyloid deposits (Davies *et al.* 1997). Any alterations in platelets or the cleavage of APP fragments will lead to a pathological accumulation of this protein. These findings support the hypothesis that changes in APP metabolism may occur in peripheral blood elements.

2.2. Proteins τ

Neurofibrillary tangles in the brains of AD patients contain abnormally phosphorylated protein τ (Mandelkow and Mandelkow 1998, Tolnay and Probst 1999). Proteins τ are microtubule-associated molecules which regulate the dynamics of the microtubule network, especially involved in axonal transport and neuronal plasticity. Proteins τ belong to a family of developmentally regulated isoforms generated by alternative splicing and phosphorylation. This generates several protein τ variants that interact with tubulin and other proteins. Therefore, proteins τ are influenced by many physiological regulations. In AD, all six adult isoforms of proteins τ can become maximally phosphorylated and can, bind to each other, rather than bind to microtubules, thus destabilizing the neuronal cytoskeleton (Khatoon *et al.* 1995). In addition to the modulation of microtubule stability, proteins τ are likely to play a role in signal transduction. A number of papers demonstrated that proteins τ interact with phospholipase C and phosphatidylinositol bisphosphate (Johnson and Hartigan 1998). There is a growing body of evidence that proteins τ interact with the actin cytoskeleton and thereby play a role in regulating cell shape, motility and microtubule-plasma interactions.

The hyperphosphorylation of τ proteins is catalyzed by various proteinkinases. For example proteinkinase I identified earlier is identical with 3β glycogensynthase kinase, proteinkinase II has a catalytic subunit identical with cyclin-dependent kinase 5; phosphorylation of τ proteins is also catalyzed by calcium-calmodulin-dependent proteinkinase II, phosphatidylinositol 3-kinase, etc. (Xiao *et al.* 1996, Imahori and Uchida 1997). Hence, it has been postulated that the disturbance in equilibrium between the activity of kinases and phosphatases is the reason for proteins τ hyperphosphorylation with its subsequent pathological consequences. Laboratory studies revealed that the increase in phosphatase activity, which catalyses protein τ dephosphorylation, might stop the neurofibrilar degeneration (Iqbal and Grundke-Iqbal 1996). Munoz-Montano *et al.* (1997) have proved that lithium, which inhibits the activity of 3-glycogensynthase kinase, causes the protein τ dephosphorylation in isolated neurons and rat brains *in vivo*.

Proteins τ are mostly expressed in the nervous system. The discovery of some novel roles for proteins τ

have revealed new and important roles of cytoskeletal proteins. Their degree of phosphorylation is a good marker of cell integrity. The search for peripheral markers of AD therefore suggests that pathological changes in cytoskeletal proteins may also become manifested in peripheral cells such as the blood elements (Strunecká *et al.* 2000).

2.3. Could alterations in calcium homeostasis serve as biological markers?

The calcium hypothesis of AD states that long-term slight elevation of cytosolic Ca^{2+} levels ($[Ca^{2+}]_i$) and/or disturbances in Ca^{2+} homeostasis are the cellular mechanisms underlying neuronal aging (Khachaturian *et al.* 1989, Khachaturian 1998). An alteration in calcium homeostasis could affect most of AD-related deficiencies (Řípová and Strunecká 1998). On the other hand, a key element of AD pathology, β-amyloid protein, has been shown to disrupt neuronal Ca^{2+} homeostasis (Mattson *et al.* 1993, Fraser *et al.* 1997).

It is not clear whether changes in $[Ca^{2+}]_i$ are the result or the cause of these pathogenic effects (Chen 1999). Although many experimental data have shown alterations of various Ca^{2+} regulatory mechanisms during aging and AD, the heterogeneity of the types of preparations and experimental techniques does not allow full confirmation of the Ca^{2+} hypothesis (Verkhratsky and Toescu 1999). Nevertheless, many experimental data have demonstrated alterations in cytosolic Ca^{2+} levels, calcium superficial binding, and calcium uptake in peripheral cells. Řípová *et al.* (1999, 2000) reported significant alterations in calcium homeostasis in blood platelets of patients with AD which were not observed during aging. These results further suggest the systemic aspects of AD and support the view that examination of peripheral cells may represent a useful approach in the study of pathological changes in AD. The possibility has not been excluded that disturbances in Ca^{2+} homeostasis may represent a common denominator for the various pathological hallmarks of AD. Despite the crucial role of the calcium signal in the pathogenesis of AD, the methods used for the study of calcium alterations are not yet accessible for routine clinical use.

2. 4. Genetics and biomarkers

It is difficult to understand the genetics of AD, as many of the more recent findings have not been unequivocally confirmed. Until recently, four loci have been identified as contributing to AD. The mutations in the βAPP gene on chromosome 21 (autosomal dominant, four mutations identified so far), and mutations on chromosomes 1 (presenilin 2, autosomal dominant, three mutations) and 14 (presenilin 1, autosomal dominant, 28 mutations), are sufficiently well established to be used as a basis for genetic screening in suspected cases of early-onset familial AD (FAD). These three genes are now known to account for some 30-40 % of early-onset FAD (Wilcock 1999). The theoretical implications of genetic tests in FAD is obvious, but what about subjects with sporadic forms of AD? Only a few cases with genetic mutations in early-onset sporadic AD have been reported (Naruse *et al.* 1991, Tanahashi *et al.* 1996) and no missence mutations in these genes have been found in any cases of late-onset sporadic AD (Tanzi *et al.* 1992, Tsuda *et al.* 1995).

The fourth gene, which had been identified and well established as contributing to AD, is on chromosome 19. Although this gene itself does not cause AD, it is considered a risk factor, which does not inevitably lead to the disease. Three common polymorphisms of apolipoprotein E gene known as alleles ε2, ε3 and ε4 (ε1 is very rare), are associated with late-onset familial and sporadic AD. The number of ApoE4 allele copies not only correlates with an earlier age of onset but also with an increased density of senile plaques in late-onset AD. ApoE4 genotype is clinically important although the ApoE genotype is not sufficiently reliable to be used as a diagnostic test. Carriers with the ApoE4 allele have a 1.5-3-fold increased risk of developing AD compared to the general population; inherence of two copies of the ApoE4 allele further increases this risk. However, it must be pointed out that the majority of ApoE4 individuals reach age 80 without evident cognitive impairment (Giacobini 1996, Hyman *et al.* 1996). ApoE status testing is not currently recommended as a means of predicting future risk of AD in asymptotic individuals, but this genotyping may be useful in improving the diagnosis in symptomatic subjects.

In conclusion, the actual genetic testing cannot fulfill the criteria of a biological marker (low sensitivity and specificity). At the clinical level, it may be important for some members of affected families and it may be useful for clinicians to confirm AD diagnosis. However, these new possibilities introduce numerous ethical, psychological and social problems.

2.5. Other possible biomarkers

The use of biomarkers in diagnosis presents a challenge. There is a huge and a still growing body of evidence from various research areas suggesting new AD biomarkers that are closely associated with the pathophysiology of the disease. These include oxidative stress and inflammation markers, neurotransmitters and related proteins (acetylcholine and enzymes of the cholinergic system; neuropeptides – somatostatin and neuropeptide Y; aminoacids – glutamate, aspartate, alanine, methionine), markers related to synaptic function (synaptophysin, synaptotagmin, chromatogranin A), destruction or synaptic degeneration markers (neuron-specific enolase, neuromodulin, growth-associated protein GAP-43), blood-brain barrier damage markers (CSF/serum albumin ratio), etc. (for review see Bancher *et al.* 1998). Phospholipid and fatty acid abnormalities were reported by other authors (Cuenod *et al.* 1995, Řípová *et al.* 1997, Kyle *et al.* 1999). The specificity and selectivity of all the above mentioned markers for AD remain open and will have to be investigated further. Some of the most interesting potential markers are outlined here.

Inflammation markers: Two major observations led scientists to study the role of inflammation in the development of AD: neurodegenerative changes in AD are accompanied by an inflammation reaction (on the other hand immunological reactions in the brain may play a role in neurodegenerative processes), and the risk of AD is reduced among users of antiinflammatory non-steroidal drugs. An acute phase response was observed in AD brain (increase in cytokine level – IL1 and IL6, expression of tumor necrosis factor, upregulation of the complement system). In the cerebrospinal fluid (CSF) more conflicting data have been reported (for review see Bancher *et al.* 1998). To date, no available data have supported the usefulness of these proteins in searching for biomarkers of AD.

Melanotransferrin (the iron-binding protein p97) has recently been proposed as a possible diagnostic marker of AD. It originates in the reactive microglia associated with dense senile plaques (Yamada *et al.* 1999) and was found elevated in the CSF and serum of AD patients (Kennard *et al.* 1996). This marker awaits validation on large numbers of patients in a multicenter study.

Glycosylation of acetylcholinesterase (AChE) as a diagnostic marker for AD was suggested (Saez-Valero *et al.* 1997). AChE activity was lower in the CSF of AD patients *post mortem* as compared to age-matched controls and other cases of dementia. There was a large overlap (40 %) between the groups. However, these authors demonstrated that glycosylated AChE was significantly elevated in AD. Thus lectin-binding analysis of CSF AChE could provide a diagnostic test which is 80 % sensitive and 97 % specific. This marker has to be replicated *ante mortem* and on a large sample of patients.

Tropicamide eye drop response was referred as a simple, fast, inexpensive and non-invasive neurobiological test for AD (Scinto *et al.* 1994). Marked hypersensitivity in the pupilar dilatation response to the cholinergic antagonist tropicamide has been proposed to demonstrate a central cholinergic deficit present in AD. However, these results were not confirmed by other authors who concluded that the papillary response is not a reliable and specific diagnostic test for AD (Growdon *et al.* 1997).

Biological markers of Alzheimer´s disease in clinical practice

What is a meaning of biomarker for clinical use?

There are numerous reports supporting the possibility that protein τ or β-amyloid 42 in CSF are the most promising biomarkers for clinical use to aid in the diagnosis. However, lumbar puncture to obtain CSF samples is too invasive for use as a fully routine procedure. The search for a test which is non-invasive, simple, cheap and user-friendly should be directed at accessible body fluids. The finding of a biological peripheral marker for AD, even one based on an isolated pathological pathway, and not indicative of the basic etiology of AD, would be a significant progress.

The biochemical approach is based on the hypothesis that the causative disorder of AD may lurk at the level of cell physiology, altering various brain functions, such as recognition, memory, language and visuospatial skills. The identification of a biological peripheral marker would not only validate the presumptive clinical diagnosis but should also help to determine whether AD is a systemic disease or whether it is limited to the brain. According to the Consensus Report of the Working Group on "*Molecular and Biochemical Markers of Alzheimer´s Disease*" (1998), sponsored by the Reagan Research Institute of the Alzheimer´s Association and the National Institute on Aging, five different purposes of biomarker predictive testing were identified, namely improving and confirming diagnosis, monitoring disease progression (over time and/or after

pharmacological intervention), studying brain-behavior correlations and epidemiological screening.

An ideal biomarker for clinical use should fulfil the following criteria:

1. **To have a high specificity** (to be able to distinguish AD from other dementias, normal aging and other causes of cognitive impairment), specificity should exceed 80 %, the higher specificity the better to avoid false labeling of normal subjects;
2. **To be very sensitive** (enabling the identification of nearly every AD patient, with no false positive or negative results);
3. **To be able to detect AD early** in its course (or presymptomatically);
4. **To be useful in monitoring the progression** of the **disease** and pharmacological treatment;
5. **To be non-invasive or to be based on well accessible body fluids** (blood, urine, saliva); CSF tapping is not a simple routine procedure;
6. **To be inexpensive and simple to perform** as a routine procedure.

1. Protein τ and Aβ42: the most promising biomarkers for clinical use ?

1.1. Protein τ

Since proteins τ is normally an intracellular axonal protein, it is likely that increased CSF-τ level is associated with neurodegeneration. An increase of total CSF-τ level in AD was reported in numerous studies (for review see Vanmechelen 1998). The sensitivity of CSF-τ to identify AD is over 70-80 % and its specificity is also relatively high, although elevated CSF-τ level was detected in some studies in patients with frontal lobe dementia and vascular dementia (Blennow *et al.* 1995, Arai *et al.* 1997). Most follow-up studies in AD patients have revealed that protein τ level does not change during the course of the disease (Andreasen *et al.* 1998) and a clear correlation between CSF-τ level and the severity of dementia has not been well established (Munroe *et al.* 1995). The recent study of Blennow (2000) on a large sample of patients with mild cognitive impairment, who later progressed to AD (n = 480) has indicated that increased CSF-τ level might be an early marker. In this study, when CSF-τ measurement is combined with that of CSF-Aβ42, the sensitivity for the prediction of AD was 90 %, while its specificity was 80 %. The determination of CSF-τ level could also potentially serve for monitoring the effect of treatment. Although under treatment with galantamin and Aricept the level of CSF-τ did not change (as these drugs are symptomatological), treatment with GM1 ganglioside (Augustinsson *et al.* 1997) lowered the levels of CSF-τ in all patients.

The measurement of hyperphosphorylated protein τ (PHF-τ) in the CSF would theoretically reflect the formation of neurofibrillary tangles in the brain. A sandwich enzyme immunoassay has revealed that phosphorylated CSF-τ was higher in AD patients than in non-AD controls (Ishiguro *et al.* 1999). Discrimination between the two groups was clearer in PHF-τ than in total CSF-τ level. However, this method is not a routine procedure and there are some problems with overlapping. Consequently, this type of assay has to be developed further.

1.2. β-amyloid

β-amyloid in the CSF should reflect cerebral β-amyloid turnover. In AD patients, no differences in total β-amyloid were found in the CSF as compared to the controls (Wisniewski *et al.* 1989). Using an ELISA assay able to recognize the 42 amino acid form of β-amyloid (Aβ42) it was established that Aβ42 is decreased in the CSF of AD patients in many clinical trials (Motter *et al.* 1995, Tamaoka *et al.* 1997, Andreasen *et al.* 1999a). However, the sensitivity and the specificity of Aβ42 in CSF for the diagnosis of AD differ in many studies and are much lower than in the case of protein τ. Moreover, Lannsfelt *et al.* (2000) reported an increased Aβ42 in the early course of AD, which tends to decrease with the progression of the disease. The authors explain the discrepancies of some findings as being due to methodological differences. The β-amyloid signals could be reduced by albumin in CSF.

It is somewhat difficult to understand why the Aβ42 level is low in CSF of AD patients, while Aβ42 is high in the brain. It is possible that amyloid plaques in the brain act as a "black hole" and absorb extracellular Aβ42 in the brain, so that Aβ42 cannot reach the cerebrospinal fluid.

2. Improved discrimination of AD patients using a combination of markers in clinical practice

In a longitudinal study (Kanai *et al.* 1998), CSF examinations of AD patients revealed that such parameters as protein τ, Aβ42 and the ratio Aβ40/42, when estimated alone, did not meet all criteria for a biomarker (where specificity was high, sensitivity was

low and *vice versa*). Other authors (Andreasen *et al.* 1998, Hesse *et al.* 1998) confirmed these results. A combination of estimations of protein τ and Aβ42 may provide greater diagnostic accuracy than each individually. This was well documented by Hulstaert *et al.* (1999) in a large multicenter study in which eight European and two US university centers participated. Sandwich ELISA tests were used on site for measuring protein τ and Aβ42. Levels of Aβ42 in CSF were significantly lower in AD (n = 150) than in the controls (n = 100) and other neurological disorders (n = 84). Discrimination of AD from the controls and patients with other neurological disorders by the combined assessment of protein τ and Aβ42 was significantly improved. At 85% sensitivity, specificity of the combined test was 86 % compared with 55 % for Aβ42 alone and 65 % for protein τ alone. This combined test at 85 % sensitivity was 58 % specific for non-Alzheimer types of dementia (n=79). The authors concluded that the combined measurement of protein τ and Aβ42 meets the requirements for clinical use in discriminating Alzheimer´s disease from normal aging and specific neurological disorders. The use of these combined tests as predictors of AD development was reported by Andreasen *et al.* (1999b) in 16 patients with mild cognitive impairment (MCI) who had progressed to Alzheimer's disease 6-27 months later. At the beginning 14 out of 16 MCI patients had high CSF-τ and/or low CSF-Aβ42 levels. This indicates that these cerebrospinal fluid markers are abnormal before the onset of clinical signs of AD dementia. However, no other types of dementia and other neurological disorders in their preclinical stages were investigated in this study.

The discriminant power of combined CSF-τ and of the soluble interleukin-6 receptor complex (putative markers of neuroregulatory and inflammatory processes in the brain) in the diagnosis of AD was assayed on a group of 25 patients with mild and moderate AD and on a group of 19 age-matched healthy controls. The sensitivity was 92 % and specificity 90 % (Hampel *et al.* 1999). These data suggest that multivariate discriminant analysis of combined CSF-τ and soluble interleukin-6 receptor complex may add more certainty to the diagnosis of Alzheimer´s disease. However, the method will need to be extended to a larger group of subjects to assess its reliability. Moreover, the discriminant function of this method has to be validated by including other dementia disorders and confirmed by at least one other independent study.

Conclusions

Alzheimer´s disease is a heterogeneous disease from a clinical, genetic and pathological aspect. At present, it seems that more than one biological marker is needed to aid in the diagnosis of AD. In the context of the above mentioned six points on the definition of an ideal biological marker, at present a combination of CSF τ and Aβ42 most closely corresponds to an ideal biomarker of AD. A potential biological diagnostic marker has to be understood in the context of the basic clinical question. However, the complex of clinical, cognitive and behavioral impairment of AD patients is so varied, wide-ranging and subtle in early stages of the disease that some authors consider the expectation of ideal diagnostic biological markers to be an unachievable and unrealistic goal (Foster 1998). Nevertheless, the search for diagnostic markers could help in the therapeutic strategy of Alzheimer´s disease. Experience from AD therapy does not support the expectation that a "magic pill" will be found. AD provides an example demonstrating a failure of the highest levels of integration in the human brain. The pharmacological attempts to modify the failure of cognitive functions in AD patients have not been found to be fully effective.

In spite of this, the huge amount of laboratory and clinical observations should help in understanding the multifactorial aspects of the etiology and pathogenesis of AD in providing a better chance of preventing this most devastating neurodegenerative disease in man and in revealing new pathways for its treatment.

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References

ALBIN RL, MINOSHIMA S, D´AMATO CJ, FREY KA, KUHL DA, SIMA AAF: Fluoro-deoxyglucose positron emission tomography in diffuse Lewy body disease. *Neurology* **47**: 462-466, 1996.

ANDREASEN N, VANMECHELEN E, VAN DE VOORDE A, DAVIDSSON P, TARVONEN S, RAIHA I, SOURANDER L, WINBLAD B, BLENNOW K: Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer´s disease: a community based follow-up study. *J Neurol Neurosurg Psychiat* **64**: 298-305, 1998.

ANDREASEN N, HESSE C, DAVIDSSON P, MINTHON L, WALLIN A, WINBLAD B, VANDERSTICHELE H, VANMECHELEN E, BLENNOW K: Cerebrospinal fluid β-amyloid$_{(1-42)}$ in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch Neurol* **56**: 673-680, 1999a.

ANDREASEN N, MINTHON L, VANMECHELEN E, VANDERSTICHELE H, DAVIDSSON P, WINDBLAD B, BLENNOW K: Cerebrospinal fluid tau and Aβ42 as predictors of development of Alzheimer´s disease in patients with mild cognitive impairment. *Neurosci Lett* **273**: 5-8, 1999b.

ARAI H, MORIKAWA YI, HIGUCHI M, MATSUI T, CLARK MC, MIURA M: Cerebrospinal fluid tau levels in neurodegenerative diseases with distinct tau-related pathology. *Biochem Biophys Res Comunm* **236**: 262-264, 1997.

AUGUSTINSSON LE, BLENNOW K, BLOMSTRAND C, BRANE G, EKMAN R, FREDMAN P, KARLSSON I, KIHLGREN M, LEHMANN W, LEKMAN A, MANSSON JE, RAMSTROM I, WALLIN A, WIKKELSO C, GOTTFRIES CG, SVENNERHOLM L: Intracerebroventricular administration of GM1 ganglioside to presenile Alzheimer patients. *Dement Geriatr Cogn Disord* **8**: 26-33, 1997.

BANCHER C, JELLINGER K, WICHART I: Biological markers for the diagnosis of Alzheimer´s disease. *J Neural Transm* **53**: 185-197, 1998.

BLENNOW K: CSF biochemical markers for the early detection of AD. *Abstr 6th Int Stockholm/Springfield Symp on Advances in Alzheimer Therapy*, 2000, p 36.

BLENNOW K, WALLIN A, AGREN H, SPENGER C, SIEGFRIED J, VANMECHELEN E: Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer´s disease? *Mol Chem Neuropathol* **26**: 231-245, 1995.

BUSH AI, MARTINS RN, RUMBLE B, MOIT R, FULLER S, MILWARD E, CURRIE J, AMES D, WIEDMANN A, FISHER P, MULTHAUP G, BEYREUTHER K, MASTERS CL: The amyloid precursor protein of Alzheimer´s disease is released by human platelets. *J Biol Chem* **265**: 15977-15983, 1990.

CHEN M: Do the intracellular calcium states in Alzheimer´s disease need to be revisited? *J Neuropathol Exp Neurol* **58**: 310-311, 1999.

CLAUS JJ, VAN HARSKAMP F, BRETELER MMB, KRENNING EP, DE KONING Y, VAN DER CAMMEN TJM, HOFMAN A, HASAN D: The diagnostic value of SPECT with Tc 99m HMPAO in Alzheimer´s disease: a population-based study. *Neurology* **44**: 454-461, 1994.

CUENOD CA, KAPLAN DB, MICHOT JL, JEHENSON P, LEROY-WILLIG A, FORETTE F, SYROTA A, BOLLER F: Phospholipid abnormalities in early Alzheimer´s disease: in vivo phosphorus 31 magnetic resonance spectroscopy. *Arch Neurol* **52**: 89-94, 1995.

DAVIES TA, BILLINGSLEA A, JOHNSON R, GREENBERG S, ORTIZ M, LONG H, SGRO K, TIBBLES H, SEETOO K, RATHBUN W, SCHONHORN J, SIMONS ER: Stimulus responses and amyloid precursor protein processing in DAMI megacaryocytes. *J Lab Clin Med* **130**: 21-32, 1997.

FOSTER NL: The development of biological markers for the diagnosis of Alzheimer´s disease. *Neurobiol Aging* **19**: 127-129, 1998.

FRASER SP, SUH YH, DJAMGOZ MBA: Ionic effects of the Alzheimer´s disease beta-amyloid precursor protein and its metabolic fragments. *Trends Neurosci* **20**: 67-72, 1997.

GIACOBINI E: Closer to the truth about APOE-4. *Alzheimer Insights* **2**: 1-5, 1996.

GROWDON JH, GRAEFE K, TENNIS M, HAYDEN D, SCHOENFELD D, WRAY SH: Pupil dilatation to tropicamide is not specific for Alzheimer disease. *Arch Neurol* **54**: 841-844, 1997.

HAASS C: Presenilin genes: a major breakthrough in Alzheimer´s disease research. *The News* **45**: 1-11, 1996.

HAMPEL H, TEIPEL SJ, PADBERG F, HASLINGER A, RIEMENSCHNEIDER M, SCHWARZ MJ, KOTTER HU, SCHELOSKE M, BUCH K, STUBNER S, DUKOFF R, LASSER R, MULLER N, SUNDERLAND T,

RAPOPORT SI, MOLLER HJ: Discriminant power of combined cerebrospinal fluid τ protein and of the soluble interleukin-6 receptor complex in the diagnosis of Alzheimer´s disease. *Brain Res* **823**: 104-112, 1999.

HESSE C, MINTHON L, WALLIN A: Tau protein and β-amyloid$_{(1-42)}$ in cerebrospinal fluid from Alzheimer´s disease patients and controls. *Neurobiol Aging* **19**: S163, 1998.

HULSTAERT F, BLENNOW K, IVANOIU A, SCHOONDERWALDT HC, RIEMENSCHNEIDER M, DeDEYN PP, BANCHER C, CRAS P, WILTFANG J, MEHTA PD, IQBAL K, POTTEL H, VANMECHELEN E, VANDERSTICHELE H: Improved discrimination of AD patients using β-amyloid$_{(1-42)}$ and tau levels in CSF. *Neurology* **52**: 1555-1562, 1999.

HYMAN BT, GOMEZ-ISLA T, BRIGGS M, CHUNG H, NICHOLS S, KOHOUT F, WALLACE R: Apolipoprotein E and cognitive change in an elderly population. *Ann Neurol* **40**: 55-66, 1996.

IMAHORI K, UCHIDA T: Physiology and pathology of tau protein kinases in relation to Alzheimer's disease. *J Biochem Tokyo* **121**: 179-188, 1997.

IQBAL K, GRUNDKE-IQBAL I: Molecular mechanism of Alzheimer's neurofibrillary degeneration and therapeutic intervention. *Ann NY Acad Sci* **777**: 132-138, 1996.

ISHIGURO K, OHNO H, ARAI H, YAMAGUCHI H, URAKAMI K, PARK JM, SATO K, KOHNO H, IMAHORI K: Phosphorylated tau in human cerebrospinal fluid is a diagnostic marker for Alzheimer´s disease. *Neurosci Lett* **270**: 91-94, 1999.

JACK CR Jr, PETERSEN RC, XU YC, O´BRIEN PC, SMITH GE, IVNIK RJ, BOEVE BF, WARING SC, TANGALOS EG, KOKMEN E: Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology* **52**: 1397-1403, 1999.

JOBST KA, SMITH AD, SZATMARI M, MOLYNEUX A, ESIRI ME, KING E, SMITH A, JASKOWSKI A, McDONALD B, WALD N: Detection in life of confirmed Alzheimer´s disease using a simple measurement of medial temporal lobe atrophy by computed tomography. *Lancet* **340**: 1179-1183, 1992.

JOHNSON GVW, HARTIGAN JA: Tau protein in normal and Alzheimer´s disease brain: an update. *Alzheimer´s Dis Rev* **3**: 125-141, 1998.

KANAI M, MATSUBARA E, ISOE K, URAKAMI K, NAKASHIMA K, ARAI H, SASAKI H, ABE K, IWATSUBO T, KOSAKA T, WATANABE M, TOMIDOKORO Y, SHIZUKA M, MIZUSHIMA K, NAKAMURA T, IGETA Y, IKEDA Y, AMARI M, KAWARABAYASHI T, ISHIGURO K, HARIGAYA Y, WAKABAYASHI K, OKAMOTO K, HIRAI S, SHOJI M: Longitudinal study of cerebrospinal fluid levels of tau, A beta$_{1-40}$, and A beta$_{1-42(43)}$ in Alzheimer's disease: a study in Japan. *Ann Neurol* **44**: 17-26, 1998.

KENNARD ML, FELDMAN H, YAMADA T, JEFFERIES WA: Serum levels of the iron binding protein p97 are elevated in Alzheimer´s disease. *Nat Med* **2**: 1230-1235, 1996.

KHACHATURIAN ZS, COTMAN CW, PETTERGREW JW (eds): *Calcium, Membranes, Aging, and Alzheimer´s Disease*. New York, New York Academy of Sciences, 1989, 292 p (*Ann N Y Acad Sci* vol. 568).

KHACHATURIAN ZS: An overview of Alzheimer´s disease research. *Am J Med* **104**: 26S-31S, 1998.

KHATOON S, GRUNDKE-IQBAL I, IQBAL K: Guanosine triphosphate binding to beta-subunit of tubulin in Alzheimer's disease brain: role of microtubule-associated protein tau. *J Neurochem* **64**: 777-787, 1995.

KYLE DJ, SCHAEFER E, PATTON G, BEISER A: Low serum docosahexaenoic acid is a significant risk factor for Alzheimer´s dementia. *Lipids* **34** (Suppl): S245, 1999.

LAAKSO MP, SOININEN H, PARTANEN K, HELKALA EL, HARTIKAINEN P, VAINIO P, HALLIKAINEN M, HANNINEN T, RIEKKINEN PJ Sr: Volumes of hippocampus, amygdala and frontal lobes in the MRI-based diagnosis of early Alzheimer´s disease: correlation with memory functions. *J Neural Transm Parkinson´s Dis Dementia Sect.* **9**: 73-86, 1995.

LANNSFELT L, FORSELL C, NASLUND J, NILSBERTF C: Genetics and pathophysiology of Alzheimer´s disease. *Abstr 6th Int Stockholm/Springfield Symp on Advances in Alzheimer Therapy,* 2000, p 98.

LUSTIG E, KOHAN S, FAMULARI AL, DOMINGUEZ RO, SERRA JA: Peripheral markers and diagnostic criteria in Alzheimer´s disease: critical evaluation. *Rev Neurosci* **5**: 213-225, 1994.

MANDELKOW EM, MANDELKOW E: Tau in Alzheimer´s disease. *Trends Cell Biol* **8**: 425-427, 1998.

MATTSON MP, BARGER SW, CHENG B, LIEBERBURG I, SMITH-SWINTOSKY VL, RYDEL RE: Beta-amyloid precursor protein metabolites and loss of neuronal Ca^{2+} homeostasis in Alzheimer´s disease. *Trends Neurosci* **16**: 409-414, 1993.

MAZZIOTTA JC, PHELPS ME: Positron emission tomography studies of the brain. In: *Positron Emission Tomography and Autoradiography: Principles and Applications for the Brain and Heart.* M PHELPS, J MAZZIOTTA, H SCHELBERT (eds), Raven Press, New York, 1986, pp 493-579.

MOTTER R, VIGO-PELFREY C, KHOLODENKO D, BARBOUR R, JOHNSON-WOOD K, GALASKO D, CHANG L, MILLER B, CLARK C, GREEN R, OLSON D, SOUTHWICK P, WOLFERT R, MUNROE B, LIEBERBURG I, SEUBERT P, SCHENK D: Reduction of β-amyloid peptide-42 in the cerebrospinal fluid of patients with Alzheimer´s disease. *Ann Neurol* **38**: 643-648, 1995.

MUNOZ-MONTANO JR, MORENO FJ, AVILA J, DIAZ-NIDO J: Lithium inhibits Alzheimer's disease-like tau protein phosphorylation in neurons. *FEBS Lett* **411**: 183-188, 1997.

MUNROE WA, SOUTHWICK PC, CHANG L, SCHARRE DW, ECHOLS CL Jr, FU PC, WHALEY JM, WOLFERT RL: Tau protein in cerebrospinal fluid as an aid in the diagnosis of Alzheimer disease. *Ann Clin Lab Sci* **25**: 207-217, 1995.

NARUSE S, IGARASHI S, KOBYASHI H, AOKI K, INUZUKI I, KANEKO K, SHIMIZU T, IHARA K, KOJIMA T, MIYATAKE T, TSUJI S: Missense mutation (Val->Ile] in exon 17 of the amyloid precursor protein gene in Japanese Alzheimer disease. *Lancet* **337**: 978-979, 1991.

O´MAHONY D, COFFEY J, MURPHY J, O´HARE N, HAMILTON D, FREYNE P, WALSH JB, COAKLEY D: The discriminant value of semiquantitative SPECT data in mild Alzheimer´s disease. *J Nucl Med* **35**: 1450-1455, 1994.

POWERS WJ, PERLMUTLER JS, VIDEEN TO, HERSCOVITCH P, GRIFFETH IK, ROYAL HD, SIEGEL BA, MORRIS JC, BERG L: Blinded clinical evaluation of positron emission tomography for diagnosis of probable Alzheimer´s disease. *Neurology* **42**: 765-770, 1992.

REGIER DA, KAELBER CT, RAE DS, FARMER ME, KNAUPER B, KESSLER RC, NORQUIST GS: Limitations of diagnostic criteria and assessment instruments for mental disorders. Implications for research and policy. *Arch Gen Psychiatry* **55**: 109-115, 1998.

ŘÍPOVÁ D, STRUNECKÁ A: Phosphoinositide signalling system: a new enticing pathway to therapy of Alzheimer´s disease? *Homeostasis* **38**: 157-162, 1998.

ŘÍPOVÁ D, NĚMCOVÁ V, HÖSCHL C, FALES E, MAJER E, STRUNECKÁ A: Lipid composition of different brain regions in patients with Alzheimer´s disease and multi-infarct dementia. In: *Progress in Alzheimer´s and Parkinson´s Diseases.* A FISHER, I HANIN, M YOSHIDA (eds), Plenum Press, New York, London, 1997, pp 301-307.

ŘÍPOVÁ D, PLATILOVÁ V, STRUNECKÁ A, JIRÁK R: Calcium homeostasis and its disturbance during aging and in Alzheimer´s disease**.** *Physiol Res* **48:** S112, 1999.

ŘÍPOVÁ D, PLATILOVÁ V, STRUNECKÁ A, JIRÁK R, HÖSCHL C: Cytosolic calcium alterations in platelets of patients with early stages of Alzheimer´s disease. *Neurobiol Aging* **21**: 729-734, 2000.

SAEZ-VALERO J, SBERNA G, MCLEAN CA, MASTERS CL, SMALL DH: Glycosylation of acetylcholinesterase as diagnostic marker for Alzheimer´s disease. *Lancet* **350**: 929, 1997.

SAHARA N, YAHAGI YI, TAKAGI H, KONDO T, OKOCHI M, USAMI M, SHIRASAWA T, MORI H: Identification and characterization of presenilin I-467, I-463 and I-374. *FEBS Lett* **381**: 7-11, 1996.

SMALL GW, KOMO S, LA RUE A, SAXENA S, PHELPS ME, MAZZIOTA JC, SAUNDERS AM, HAINES JL, PERICAK-VANCE MA, ROSES AD: Early detection of Alzheimer´s disease by combining apolipoprotein E and neuroimaging. *Ann N Y Acad Sci* **802**: 70-78, 1996.

SCINTO LFM, DAFFNER KR, DRESSLER D, RANSIL BI, RENTZ D, WEINTRAUB S, MESULAM M, POTTER H: A potential noninvasive neurobiological test for Alzheimer´s disease. *Science* **266**: 1051-1054, 1994.

STRUNECKÁ A, JUŘEK V, PALEČEK J, ŘÍPOVÁ D, JIRÁK R: Cytoskeleton and aluminium in Alzheimer´s disease: an important link? *Physiol Res* **49**: P37, 2000.

TAMAOKA A, SAWAMURA N, FUKUSHIMA T, SHOJI S, MATSUBARA E, SHOJI M, HIRAI S, FURIYA Y, ENDOH M, MORI H: Amyloid β protein 42(43) in cerebrospinal fluid of patients with Alzheimer´s disease. *J Neurol Sci* **148**: 41-45, 1997.

TANAHASHI N, KAWAKATSU S, KANEKO N, YAMANAKA H, TAKAHASHI K, TABIRA T: Sequence-analysis of presenilin 1 gene mutation in Japanese Alzheimer disease patients. *Neurosci Lett* **218**: 139-141, 1996.

TANZI RE, VAULA G, ROMANO D, MORTILLA M, HUANG T, TUPLER R, WASCO W, ST.GEORGE-HYSLOP P: Assessment of amyloid β protein gene mutations in a large set of familial and sporadic Alzheimer disease cases. *Am J Hum Genet* **51**: 273-282, 1992.

THE RONALD AND NANCY REAGAN INSTITUTE OF THE ALZHEIMER´S ASSOCIATION AND THE NATIONAL INSTITUTE ON AGING WORKING GROUP: Consensus Report of the Working Group on "Molecular and Biochemical Markers of Alzheimer´s Disease." *Neurobiol Aging* **19**: 109-116, 1998.

TOLNAY M, PROBST A: Review: tau protein pathology in Alzheimer´s disease and related disorders. *Neuropathol Appl Neurobiol* **25**: 171-187, 1999.

TSUDA T, CHI H, LIANG Y, ROGAEV EI, POLLEN D, FREEDMAN M, DUARA R, ST.GEORGE-HYSLOP P: Failure to detect missense mutations in the S182 gene in a series of late onset Alzheimer disease cases. *Neurosci Lett* **201**: 188-190, 1995.

VANMECHELEN E: Tau protein in the diagnosis of old-age dementia. *Arch Gerontol Geriatr* Suppl 6: 519-524, 1998.

VERKHRATSKY A, TOESCU EC: The role of calcium in aging. *Physiol Res* **48**: S20, 1999.

WILCOCK G: Clinical interpretation and application of genetic tests in Alzheimer´s disease. *Alzheimer Insights* **5**: 10-11, 1999.

WISNIEWSKI HM, MEHTA PD, KIM KS, MERZ GS: Cerebrospinal fluid based laboratory test for Alzheimer´s disease. In: *Research and Perspectives in Alzheimer Disease. Biological Markers of Alzheimer´s Disease,* Y CHRISTEN (ed), Springer, Berlin, 1989, pp 23-29.

WISNIEWSKI T, GHISO J, FRANGIONE B: Biology of beta amyloid in Alzheimer´s disease. *Neurobiol Dis* **4**: 313-328, 1997.

XIAO J, PERRY G, TRONCOSO J, MONTEITO MJ: Alpha-calcium-calmodulin-dependent kinase II is associated with paired helical filaments of Alzheimer's disease. *J Neuropathol Exp Neurol* **55**: 954-63, 1996.

YAMADA T, TSUIJOKA Y, TAGUCHI J, TAKAHASHI M, TSUBOI Y, MOROO I, YANG J, JEFFERIES WA: Melanotransferrin is produced by senile plaque-associated reactive microglia in Alzheimer´s disease. *Brain Res* **845**: 1-5, 1999.

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