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## Biological and genetic markers of sporadic Alzheimer's disease.

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## Abstract

With the development of new treatments, there is an increasing need for early diagnosis of sporadic Alzheimer's disease. Therefore, biological markers allowing positive diagnosis early in the course of the disease are highly desirable. Cerebrospinal fluid levels of protein tau were shown to be significantly increased in patients with Alzheimer's disease. Although sensitivity is high, poor specificity limits the diagnostic value of this marker. The same is true for the 42 amino acid isoform of beta-amyloid protein that is significantly decreased in cerebrospinal fluid of Alzheimer's disease patients. However, combining both markers could improve specificity at least allowing differentiation between Alzheimer's disease, normal ageing and depressive pseudodementia. Other biological markers such as cerebrospinal fluid levels of neurotransmitters, cytokines or superoxide dismutase were shown to have even less diagnostic value. The apolipoprotein epsilon 4 allele is a risk factor for Alzheimer's disease but not a diagnostic marker as many individuals who inherit epsilon 4 do not develop the disease. Till now, a single diagnostic marker allowing

discrimination between Alzheimer's disease and other dementias does not exist. Combined cerebrospinal fluid levels of beta-amyloid protein and tau protein might be used as a marker that helps discriminating Alzheimer's disease from normal ageing and depression.

**KEYWORDS:** alzheimer's disease, dementia, marker, neurochemistry, cerebrospinal fluid

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## Review

**Biological and Genetic Markers of  
Sporadic Alzheimer's Disease**

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With the development of new treatments, there is an increasing need for early diagnosis of sporadic Alzheimer's disease. Therefore, biological markers allowing positive diagnosis early in the course of the disease are highly desirable. Cerebrospinal fluid levels of protein tau were shown to be significantly increased in patients with Alzheimer's disease. Although sensitivity is high, poor specificity limits the diagnostic value of this marker. The same is true for the 42 amino acid isoform of  $\beta$ -amyloid protein that is significantly decreased in cerebrospinal fluid of Alzheimer's disease patients. However, combining both markers could improve specificity at least allowing differentiation between Alzheimer's disease, normal ageing and depressive pseudodementia. Other biological markers such as cerebrospinal fluid levels of neurotransmitters, cytokines or superoxide dismutase were shown to have even less diagnostic value. The apolipoprotein  $\epsilon 4$  allele is a risk factor for Alzheimer's disease but not a diagnostic marker as many individuals who inherit  $\epsilon 4$  do not develop the disease. Till now, a single diagnostic marker allowing discrimination between Alzheimer's disease and other dementias does not exist. Combined cerebrospinal fluid levels of  $\beta$ -amyloid protein and tau protein might be used as a marker that helps discriminating Alzheimer's disease from normal ageing and depression.

**Key words:** Alzheimer's disease, dementia, marker, neurochemistry, cerebrospinal fluid

**A**lzheimer's disease (AD) is the most frequent cause of dementia as it accounts for at least 60% of all dementias diagnosed [1]. With the development of new treatments of Alzheimer's disease, there is an increasing need and justification for early diagnosis. Diagnosis of sporadic AD is based on clinical exclusion criteria [2] and the required diagnostic work-up is time-consuming and expensive at best resulting in a diagnosis of probable AD. In specialised centres, a diagnostic accuracy of maximally 65-90% is obtained [3]. Most

studies evaluating accuracy rates are based on follow-up periods of several years so that a much lower diagnostic accuracy can be expected in the earliest stages of the disease. Besides, diagnosis is only definite on post-mortem neuropathological examination of the brain. Therefore, a marker allowing positive diagnosis of AD at an early stage of the disease is highly desirable.

According to the Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group [4], an ideal diagnostic marker would also allow predictive testing, monitoring of disease progression and measuring effects of (new potential) therapeutic compounds during treatment (trials). A diagnostic marker of AD should reflect a central

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pathogenic process of the disorder like degeneration of neurons and synapses or the development of typical lesions as senile plaques and neurofibrillary tangles. Markers should be validated in neuropathologically confirmed AD cases and should have a sensitivity of at least 80% for detecting AD and a specificity of at least 80% for distinguishing other dementias. Moreover, the biological marker should be present in body fluids that are easily accessible like urine, blood or cerebrospinal fluid (CSF). Because intercellular space of the central nervous system (CNS) is in direct contact with CSF, biochemical changes in CNS may be reflected in CSF that can easily be obtained by lumbar puncture.

As almost all AD patients suffer from the sporadic form and diagnosis is often difficult especially in early stages of the disease when symptoms can be vague, this work gives an overview of (possible) markers that can be helpful for diagnosing sporadic AD.

### Protein tau

Tau protein is a normal human brain phosphoprotein that is located in neuronal axons where it binds to microtubules thus promoting microtubule assembly and stability. In AD brain, phosphorylated tau is the major component of paired helical filaments. Both tau and

phosphorylated tau can be detected in CSF.

Several studies have found increased CSF protein tau levels in AD [5, 6] with a reported sensitivity of at least 80% for most studies [6]. In a recently published large community-based study, increase in CSF-tau was found very early in the disease process, was stable over time and had low interindividual variation on repeated sampling [3]. Moreover, CSF-tau was shown to have a high sensitivity and specificity to differentiate AD patients from normal ageing and depression [3]. However, the use of CSF-tau as a diagnostic marker of AD is limited due to poor specificity as increased CSF-tau is found in stroke, vascular dementia, diffuse Lewy Body dementia, frontal lobe dementia and Creutzfeldt-Jakob disease as well [6-10]. Moreover, CSF levels of tau overlap between AD patients and controls. As CSF tau levels gradually increase with normal ageing [5], discriminative power of high CSF tau levels is higher in younger AD patients (age < 70 years) [11].

There now is evidence that phosphorylated tau is a more specific marker for AD allowing better discrimination between AD and controls [12]. Confirmation of these data by means of additional studies is required.

In conclusion, the diagnostic value of CSF-tau for AD is poor as high CSF-tau is not specific for AD. However, CSF-tau might be of help to differentiate AD

**Table 1** Overview of (possible) biological markers for AD with mean values for sensitivity and specificity

CSF Marker	AD vs. (studied population)	Sensitivity	Specificity
tau	normal ageing	82%	70%
	non-AD dementias and controls	85%	65%
BAP <sub>1-42</sub>	non-AD dementias and controls	85%	55%
tau and BAP <sub>1-42</sub>	non-AD dementias	85%	58%
	controls and non-AD dementias	85%	86%
ACh	early AD versus normal controls	low	moderately
	advanced AD versus normal controls	± high	high
other NT	normal controls	low	low
SST	normal controls	moderately	low
	controls with neurological diseases	high	
Cytokines	normal controls	low	low
SOD	normal controls	moderately	low
	controls with neurological diseases	high	
AA	normal controls	low	low

AA, amino acids; ACh, acetylcholine; AD, Alzheimer's disease; BAP<sub>1-42</sub>, 42 amino acid isoform of  $\beta$ -amyloid protein; CSF, cerebrospinal fluid; NT, neurotransmitters; SOD, superoxide dismutase; SST, somatostatin; tau, protein tau.

from normal ageing and depression especially in early stages of the disease when symptoms can be vague and diagnosis is often difficult.

### $\beta$ -Amyloid protein

$\beta$ -Amyloid protein (BAP) is generated continuously as a soluble protein during normal cellular metabolism and is secreted into the extracellular space allowing its detection in CSF. In AD, a portion of BAP aggregates and is incorporated into highly insoluble fibrils which are the main constituent of senile plaques. Of the different forms of BAP, the one containing 42 amino acids predominates in senile plaques [13].

Results of studies that determined total BAP levels in CSF of AD patients are contradictory as some revealed no change, slight increase or slight decrease [14]. However, different authors reported a statistically significant decrease of the 42 amino acid isoform of BAP (BAP<sub>1-42</sub>) in CSF of AD patients which might be explained by a decreased clearance of BAP<sub>1-42</sub> as it precipitates in senile plaques [14, 15]. Motter *et al.* reported a calculated sensitivity of 100% and a specificity of 63% for low BAP<sub>1-42</sub> as a marker for the presence of clinical AD [15]. Moreover, BAP<sub>1-42</sub> levels appeared to be stable for each patient suggesting that low levels of BAP<sub>1-42</sub> can be revealed in the earliest stages of the disease [14] although this needs to be confirmed. BAP<sub>1-42</sub> levels were not correlated with either severity or rate of progression of dementia [14]. Poor specificity limits the use of low BAP<sub>1-42</sub> as a diagnostic marker of AD because low CSF BAP<sub>1-42</sub> levels can also be found in other dementias [16].

### Neurotransmitters [17]

In recent years, extensive neurochemical research on AD was performed, trying to determine whether or not a primary neurochemical lesion characterises AD by analogy with the dopaminergic deficit of Parkinson's disease. The discovery of severe cholinergic deficits [18], occurring early in the course of the disease and correlating with cognitive impairment in AD [19], contributed to the development of relatively successful symptomatic treatment of AD-induced cognitive dysfunction with cholinesterase inhibitors. However, it now is obvious that several other neurotransmitter systems are involved in AD's neurochemistry although only a disappointing number of

neurochemical studies on biogenic amines in CSF of AD patients were published [17]. Small sample sizes are too often compromising the results so that conclusions are contradictory.

**Acetylcholine.** A degeneration of the cholinergic nucleus basalis of Meynert characterizes AD [18]. Decreased synthesis of acetylcholine (ACh) in AD is reflected by unaltered concentrations of choline (Ch) and reduced concentrations of ACh in CSF of patients with AD [20]. A strong inverse correlation with dementia severity was reported for CSF ACh concentrations in AD patients [21]. However, diagnostic value of cholinergic markers is limited as cholinergic deficits are not detectable until relatively late in the course of the disease [22].

Besides these quantitative differences, an abnormal molecular form of acetylcholinesterase (AChE) in CSF of 83% of histologically diagnosed AD-patients was discovered [23]. As this abnormal molecular form was found in CSF of other dementias and many controls as well, lack of specificity limits its use as a diagnostic marker [24]. A recent study demonstrated that AChE is glycosylated differently in CSF of AD patients compared with controls and other dementias [25]. It was suggested that, seen the expected specificity, glycosylation of AChE might be a useful diagnostic marker for AD which has to be confirmed by future research.

**Catecholamines.** A neuronal cell loss in the noradrenergic locus coeruleus is reported in AD [26]. In younger AD patients, cell loss is even greater in the locus coeruleus than in the nucleus basalis of Meynert [27]. Martignoni *et al.* reported that concentration of norepinephrine (NA) is decreased in CSF of AD patients compared with controls [28]. Compared with moderately affected AD patients, patients suffering from severe AD had increased plasma and CSF NA levels [29]. One possible hypothesis explaining this phenomenon is an increased firing rate of the remaining locus coeruleus neurons in the advanced stages of AD [30].

Adrenaline (A) is the final product of the biosynthesis of catecholamines and the conversion of NA to A is specifically catalysed by the enzyme phenylalanine-N-methyltransferase. As a different locoregional distribution for NA and A in normal human CNS was reported and the ratio between NA and A is not the same for the whole cortex, it is generally accepted that the adrenergic neurotransmission is a functional system, distinct from the well-known noradrenergic system [31]. Although the

role and changes of the adrenergic system in AD have not yet been extensively studied, A was reported to be slightly decreased in all cortical and subcortical areas studied [32]. Solid results concerning the A content of CSF of AD patients are lacking. In normal ageing humans, CSF levels of A remain unchanged [33].

3-Methoxy-4-hydroxyphenylethylene glycol (MHPG), the major metabolite of both NA and A, is derived by the interaction of monoamine oxidase (MAO) and catechol-O-methyltransferase. Levels of MHPG are slightly increased in CSF and all cortical and subcortical areas of CNS of AD patients. An increased synaptic turn-over of A and NA is thought to be responsible for elevated MHPG/NA ratios [32]. In patients with severe AD, plasma and CSF MHPG levels were even higher compared with moderately affected individuals [29].

Several neurochemical studies on AD reported normal striatal levels of dopamine (DA), suggesting an intact nigrostriatal pathway [34]. However, a more recent publication using dopamine uptake sites as markers for dopaminergic neurites showed a 65% reduction of dopamine uptake sites in putamen of AD patients compared to normal controls [35]. This finding was supported by a decreased tyrosine hydroxylase immunoreactivity in the substantia nigra of AD patients [36]. Although these publications do not report whether or not the clinical picture of the investigated patients included extrapyramidal features, there now is evidence for involvement of the nigrostriatal projection in the neurochemistry of AD.

CSF concentrations of DA increase significantly with age in non-demented elderly [33]. According to Mann *et al.* [37], CSF concentrations of the dopamine metabolite homovanillic acid (HVA) do not distinguish AD patients from controls and are not correlated with the degree of cognitive deterioration [21]. More recent publications produced contradictory results as HVA was reported to be both increased and decreased in CSF of AD patients [17]. Levels of the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were lower in CSF of AD patients than in controls [38]. Patients with early onset AD had even lower levels of DOPAC and a higher HVA/DOPAC ratio compared with late onset AD patients [38].

In conclusion, sensitivity and specificity of catecholaminergic changes in CSF is too low for a possible role as a diagnostic marker in AD. Moreover, these changes tend to occur in more advanced stages of the disease.

**Serotonin.** The serotonergic nucleus raphe dorsalis also shows evidence of degeneration in AD [39]. Concentrations of conjugated and free serotonin (5-HT) and of the serotonergic precursors tryptophan and 5-hydroxytryptophan were reported to be significantly reduced in CSF of AD patients whereas CSF levels of the serotonergic metabolite 5-hydroxyindoleacetic acid (5-HIAA) remain unchanged suggesting increased serotonergic catabolism in AD [40]. On the other hand, normal ageing causes a statistically significant increase of CSF-levels of 5-hydroxytryptophan (but not of tryptophan) and not significantly elevated concentrations of 5-HIAA [33].

Again, limited specificity and sensitivity does not allow the use of serotonergic markers in diagnosis of AD.

### Cytokines

Substantial evidence suggests that senile plaque formation in AD is accompanied by an acute-phase response in the CNS [41]. Plaque-associated activated glia and glia-derived cytokines could lead to chronic, self-propagating, cytokine-mediated molecular and cellular reactions that are thought to be important pathogenic factors in the progression of neuropathological changes of AD [42].

The hypothesis that senile plaque formation in AD is associated with immune-mediated inflammatory reactions in the CNS is supported by several histopathological studies. Activated microglia containing immunoreactive interleukin-1 (IL-1) are increased sixfold in brains of patients with AD compared to brains from age-matched controls [43] and are associated with neuritic plaques [44]. The pattern of IL-1 expression in various brain regions of patients with AD correlates with neuritic plaque distribution [45]. IL-1 immunoreactive microglia are most abundant in neuritic plaques without dense  $\beta$ -amyloid cores, are less prominent constituents of mature neuritic plaques and are absent in burnt-out plaques which might suggest that microglial-dependent and IL-1-induced inflammatory processes play a role in amyloid plaque formation in AD [42, 44]. Moreover, IL-6 immunoreactivity is present in and around amyloid deposits, a phenomenon restricted to the CNS of AD patients [46].

It has been suggested that CSF cytokines might be adequate biological markers for AD possibly allowing monitoring of biological effects of anti-inflammatory treatment which is subject of several ongoing clinical trials

[47]. However, studies regarding inflammatory changes in CSF of AD patients produced conflicting results [48]. We therefore set up a prospective study to assess CSF levels of IL-1 $\beta$ , IL-6, IL-10, IL-12, soluble IL-2 receptor, neopterin, interferon- $\gamma$  and tumor necrosis factor- $\alpha$  in a large series of AD patients and age- and sex-matched controls but did not reveal any statistically significant changes compared to controls [48]. Apparently, immune-mediated inflammatory changes found in CNS by histopathological studies are not reflected in CSF of patients with AD. Probably, cytokine production appears very localised in the CNS, not allowing representative detection in CSF. Our findings suggest that the studied inflammatory parameters in CSF should not be considered as diagnostic markers for AD and do not allow monitoring of biological effects of anti-inflammatory treatment.

### Superoxide dismutase

Among several theories that have been proposed to identify the etiologic factors of AD, the one relying on oxidative stress resulting from free radicals has gained support [49]. Free radical action has been reported to play an important role in the ageing brain and in age-related degenerative processes of the CNS [50]. One of the main enzymes that provide protection against cellular damage due to oxygen radicals in human cells is superoxide dismutase (SOD). It transforms superoxide in hydrogenperoxide.

Several studies reporting on SOD activity in erythrocytes (RBC) and fibroblasts of patients suffering from AD could not reveal significant differences compared to controls [49]. Perrin *et al.* [51] and De Lustig *et al.* [52] on the other hand, found increased RBC CuZn-SOD activities in AD patients compared to controls whereas plasma SOD activities did not alter significantly. Zemlan *et al.* [53] found increased SOD activity in fibroblasts obtained from patients with AD and Down syndrome and suggested that the formation of paired helical filaments might be free-radical mediated.

Only few data on CSF SOD activity are available. Several authors reported that CSF SOD activity increases with age [54, 55] while this age-dependent increase was not found in AD patients [54]. In AD patients, CSF SOD activity was reported to be significantly lower compared to controls and compared to patients with other neurological diseases [49, 54]. CSF

SOD activity did not significantly correlated with dementia severity as expressed by MMSE score [49].

In conclusion, nor CSF, serum or fibroblast SOD activities were shown to be reliable biological markers of AD.

### Amino acids

CSF studies on amino acids in AD have produced most contestable results, which is partially explicable by small sample sizes and differences in sample preparation (deproteinisation). Degrell *et al.* [56] showed significantly higher alanine, methionine, phenylalanine and tyrosine CSF levels in senile compared to presenile AD patients. Levels of glycine, alanine, methionine and ornithine were increased compared to age-matched controls [56]. Whereas CSF glutamate concentrations were significantly reduced in AD patients, aspartate was found to be significantly increased [57]. After Degrell *et al.* [56] however, glutamate concentrations were unchanged compared to controls. CSF gamma-aminobutyric acid was reported to be decreased in AD patients compared to controls [58].

Seen the discordant results and limited specificity of reported changes, no single amino acid can be selected as a possible biologic marker of AD.

### Neuropeptides

Besides its function as neuroendocrine release inhibiting factor of growth hormone, thyrotropin and prolactin, somatostatin (SST) might play a role of neuromodulator or neurotransmitter as its receptors are widely and heterogeneously distributed in the human CNS [59].

A degeneration of somatostatinergic interneurons, a decreased SST-like immunoreactivity and decreased SST concentrations in AD brain were reported [17]. CSF measurements of SST are believed to be representative of CNS extracellular fluid concentrations [59]. Most publications consistently report decreased SST levels in CSF of AD patients [17] but overlapping of individual values with those obtained from normal control and neurologic control subjects limits the use of CSF SST as diagnostic marker of AD [60].

Davies *et al.* [61] found neuropeptide Y (NPY) levels to be decreased in both CSF and frontal and temporal cortex of AD patients. Reduced CSF NPY concentrations were reported to correlate with disease

duration [62].

### Apolipoprotein E

In most families with early-onset autosomal dominant AD, mutations in 3 genes - presenilin 1 on chromosome 14, presenilin 2 on chromosome 1 and amyloid precursor protein on chromosome 21 - fulfil the criteria of diagnostic markers [63]. However, these markers do not allow monitoring of disease progression and measuring effects of therapeutic compounds [4].

The  $\epsilon 4$  allele of apolipoprotein E (apo E) is 3 to 4 times more common in sporadic AD than in non-demented elderly [64]. However, the  $\epsilon 4$  allele is only a risk factor but not a diagnostic marker for AD as many individuals who inherit  $\epsilon 4$  do not develop AD. Consequently, specificity and sensitivity of the apo E genotype is too low to be used as a diagnostic marker [65]. Although the pathogenic mechanism of apo E  $\epsilon 4$  in AD is still unknown, apoE has been shown to bind to BAP *in vitro* [66]. Nevertheless, the CSF BAP<sub>1-42</sub> levels are not different in AD patients carrying the  $\epsilon 4$  allele compared to AD patients without the  $\epsilon 4$  allele [14]. On the other hand, the apo E  $\epsilon 2$  appears to protect against AD. Although controversial, some other genetic factors such as  $\alpha$ -antichymotrypsin and HLA A2 are thought to modify the risk of developing AD [4]. In patients with late-onset sporadic AD, the presenilin-1 distributions were similar compared to sex- and age-matched controls [67]. Plasma apoE concentrations were similar in patients and controls as well [67].

### Other possible markers

Several other substances have been proposed as candidate-markers for AD based on changes in CSF of AD patients compared to controls. However, most require independent confirmation. Moreover, these substances have not been determined in large populations of AD patients and controls so that specificity and sensitivity are unknown.

Neuronal thread proteins (NTP) are a family of molecules that are expressed in the brain. In a postmortem study, brains from AD patients contained significantly more NTP immunoreactivity compared to normal controls and patients with other neurological diseases [68]. CSF studies revealed increased levels of NTP in AD patients [69]. These changes correlated

with progression of dementia and with neuronal degeneration. Additional studies are needed to confirm these findings and to determine specificity and sensitivity.

### Combination of different markers

Discriminative power can be improved by combining different markers. A promising combination exists of CSF BAP<sub>1-42</sub> and CSF tau levels [15, 70, 71]. An international multicenter trial evaluating a combined assessment on a large population of AD, other dementias and normal controls, revealed a specificity of 86% when sensitivity was set at 85% [71]. However, specificity of the combined assessment is lower discriminating AD of other dementias. Reliable data of combined assessment to discriminate AD from MCI are lacking so far. Nevertheless, sensitivity and specificity of combined CSF tau and BAP<sub>1-42</sub> assessment is high enough for discriminating AD of normal ageing and depressive pseudodementia.

### Conclusions

Till now, markers directed toward neuropathological features of AD seem the most promising (Table 1). However, a single marker allowing early diagnosis of AD does not exist. Sensitivity and specificity of a combined assessment of CSF levels of BAP<sub>1-42</sub> and tau protein seems high enough to be used as a diagnostic marker in clinical routine to discriminate AD from normal ageing and depression. In these circumstances, diagnostic accuracy may improve if information from (para)clinical investigations is combined with biological markers. Further research allowing better discrimination between AD and other dementias is indispensable.

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