ORIGINAL ARTICLE

# Biological Markers in Alzheimer's Disease

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**ABSTRACT:** Biomarkers are required to improve our diagnostic sensitivity and specificity and to monitor the biological activity of the Alzheimer's disease (AD) in terms of the burden of neural involvement and the tempo of disease progression. Biomarkers will initially supplement our more traditional neuropsychological and imaging markers but may eventually evolve into useful surrogate endpoints in AD research. These markers may also provide important mechanistic clues to the pharmacological action of anti-dementia compounds. At this point, the combination of elevated cerebrospinal fluid phosphorylated TAU (CSF p-TAU) proteins and low CSF $A\beta_{1-42}$ are the only biomarkers with the sensitivity and specificity to serve as useful diagnostic biomarkers capable of distinguishing AD from other dementias in the early stages. Advances in non CSF tests is urgently required. Markers assessing the progression of disease do not necessarily require the same high disease specificity as diagnostic markers, but need to be sensitive to changes in disease state. At present, candidate markers fall under four main biological rationales: 1. Specific markers of AD neuropathology; 2. Non-specific markers of neural degeneration; 3. Markers of oxidative stress; 4. Markers of neural inflammation. It is foreseeable that a panel of such markers might prove advantageous. It will be important to develop "non-invasive " markers utilizing readily obtainable tissue samples such as serum or urine to monitor disease progression (or hopefully regression). Repeated sampling would allow for comparison with traditional neuropsychological and imaging measures. The assays themselves will need to be reproducible, reliable and relatively inexpensive. Unfortunately, these biomarkers are in the formative stages of testing and results at present are inconclusive. To facilitate biomarker development in the future it would be highly advantageous to begin to collect and store biological specimens as an adjunct to current research in AD.

**RÉSUMÉ: Les marqueurs biologiques dans la maladie d'Alzheimer.** Nous avons besoin de biomarqueurs pour améliorer la sensibilité et la spécificité du diagnostic et pour suivre l'activité biologique de la maladie d'Alzheimer (MA) en ce qui concerne le fardeau de l'atteinte neurologique et le rythme de progression de la maladie. Au début, les biomarqueurs serviront de supplément aux marqueurs traditionnels de neuropsychologie et d'imagerie, mais éventuellement ils pourraient devenir des critères d'évaluation de substitution dans la recherche sur la MA. Ces marqueurs pourraient également fournir des indices concernant les mécanismes d'action pharmacologique des médicaments anti-démence. Actuellement, la combinaison d'un taux élevé de protéines phosphorylées TAU dans le liquide céphalorachidien (LCR) et d'un taux bas d'Ab1-42 dans le LCR sont les seuls biomarqueurs qui ont une sensibilité et une spécificité permettant de les utiliser comme biomarqueurs diagnostiques capables de distinguer la MA des autres démences aux stades précoces. Il est urgent de développer des tests autres que les tests sur le LCR. Les marqueurs pour évaluer la progression de la maladie ne doivent pas nécessairement posséder une spécificité aussi élevée que les marqueurs diagnostiques, mais ils doivent être sensibles au changement au cours de la maladie. Actuellement, les marqueurs candidats se classent en quatre groupes principaux au point de vue biologique : 1. Des marqueurs spécifiques de la neuropathologie de la MA; 2. Des marqueurs non spécifiques de la dégénérescence neuronale; 3. Des marqueurs du stress oxydatif; 4. Des marqueurs de l'inflammation neuronale. Il est probable qu'une batterie de ces marqueurs pourra s'avérer utile. Il sera important de développer des marqueurs non effractifs utilisant des échantillons de tissus faciles à obtenir, comme du sérum ou de l'urine, pour surveiller la progression de la maladie ou même sa régression. Un échantillonnage sérié permettrait de les comparer aux mesures traditionnelles de neuropsychologie ou d'imagerie. Les analyses devront être reproductibles, fiables et relativement peu coûteuses. Malheureusement, ces biomarqueurs sont encore en évaluation et les résultats sont non concluants. Il serait très avantageux de commencer à récolter et à conserver des spécimens biologiques dans le cadre de la recherche actuelle sur la MA pour faciliter le développement de biomarqueurs dans l'avenir.

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The rapid advances in Alzheimer disease (AD) biology and the forthcoming clinical trials with disease modifying therapies have heightened the urgency to develop sensitive and reliable biological markers to diagnose and monitor AD activity.

Diagnostic markers will be required to support early diagnosis and treatment of patients at risk for AD. Of equal significance are markers with the capacity to monitor the

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underlying biological burden of disease in terms of extent and intensity. These markers will eventually prove to be important surrogate outcome measures in clinical trials supplementing existing clinical and imaging data. Different markers will probably be required for each purpose.

### Diagnostic Markers

The majority of research has been focused on developing diagnostic markers of AD. These have centered on the core pathological biological proteiaceous products TAU and ß amyloid as measured in CSF.

### Total TAU

TAU proteins, the constituent proteins of the intra-neuronal neurofibrillary tangle, were first reported to be elevated in CSF in 1993.[1] These proteins probably are indicators of the intensity of neural degeneration. On average these proteins are 2-3 fold higher in AD CSF versus controls. Levels are increased very early in the disease process but levels do not correlate well with duration or intensity of disease.[2] As a diagnostic test in 2400 AD patients vs. 1250 controls the sensitivity is 82% while specificity is 88%.[3] Total TAU is not as accurate at distinguishing AD from Frontal Lobe Dementia (FTD) Vascular Dementia (VAD), Creutzfeld Jacob Disease or acute brain injury.[4]

### Phosphorylated TAU (P-TAU)

In AD-TAU proteins become abnormally phosphorylated and loose their ability to facilitate the assembly of tubulin monomers into microtubules. They then aggregate in neurofibrillary tangles.[5] Elisa assays for P-TAU have been developed at five phosphorylation sites.[6]

There is increasing evidence that CSF P-TAU discriminates adequately between AD, normals and other neurological disorders[7] notably FTD, Dementia with Lewy Bodies (DLB) and Vascular Dementia (VAD). P TAU does not rise with non-specific brain damage unlike T-TAU. Important early evidence suggests that P-TAU can distinguish patients with Minimal Cognitive Impairment (MCI) who will progress to Alzheimer's disease from those who do not.[8] P TAU elevates in CSF during the incipient stages of the disease then progressively declines as the disease progresses[9] and the decline is more pronounced with advancing impairment.

At present P TAU at various phosphorylation sites are promising biomarkers for AD diagnosis. In particular, their utility in early detection and differentiation from other dementing conditions will be important. The one major practical drawback is the difficulty with CSF as a sample medium.

### Amyloid $ß_{1-42}$

The major core protein deposited early in the senile plaque is $Aß_{1-42}$ ($Aß_{1-40}$ being seen in blood vessels). There is approximately a 50% reduction in $Aß_{1-42}$ in AD CSF vs. controls.[10] The reason for low levels of amyloid protein is not clear but hypothesized to be the result of sequestration of $Aß_{1-42}$ in brain tissue. The sensitivity and specificity is in the 85-90% range when comparing AD patients to normal controls. It is less clear that low $Aß_{1-42}$ distinguishes clearly between AD and other dementias, low levels being found in some patients with FTD, VAD and DLB.[11] Amyloid $ß_{1-42}$ in CSF may prove to be an important adjunctive marker in AD. There is poor correlation between $Aß_{1-42}$ levels and disease duration or severity.

$Aß_{1-42}$ can be measured in plasma although it is 100 fold lower in concentration. Levels are raised in familial and presenilin mutation patients but there is significant overlap in sporadic patients compared to controls.[12] Several cross-sectional studies and two longitudinal studies investigated plasma Abeta measures in Alzheimer disease patients and controls. Most studies but one have shown that plasma $Aß_{1-40}$ and $Aß_{1-42}$ levels are not different in Alzheimer disease and control groups, thus minimising its diagnostic usefulness.[13] There are suggestions that $Aß_{1-42}$ is elevated in serum in some patients several years prior to symptoms.[14] Subsequent studies have confirmed that there are higher levels of $Aß_{1-42}$ in patients who subsequently demented. Over a 3 year period their levels of $Aß_{1-42}$ subsequently declined. Serum $Aß_{1-42}$ will not be a biomarker for disease diagnosis due to lack of sensitivity and specificity but might be useful for predicting AD risk and useful in enhancing clinical trial selection.[15]

### Combined CSF $Aß_{1-42}$ and CSF Tau and Phospho Tau

As one would expect studies employing the combination of low CSF $Aß_{1-42}$ and elevated CSF TAU do provide good discriminative value for AD patients when compared to controls.[16] When AD patients are compared to other forms of dementia such as Lewy Body,Vascular and Fronto-Temporal the sensitivity and specificity is not as clear. One recent studyhowever reported a sensitivity of 72% and specificity of 93% when comparing AD to Fronto-Temporal dementia.[17]

### Biomarkers of Disease Intensity in Alzheimer's disease

There have been many other attempts to develop biomarkers that monitor disease activity.[18] While it would be ideal if they were disease specific there is no absolute necessity.

These markers have been developed along four basic biological rationales:

1. Alzheimer's disease specific pathologies
2. Non-specific markers of neural degeneration
3. Markers of neuroinflammation
4. Markers of excessive oxidation

### Markers of AD Specific Pathology

#### *Glycosylated acetylcholinestrease(Glyc-AChE) and butyryl-cholinestrase (Glyc-BuChE)*

Acetyl- and butryryl-cholinesterase are altered in AD brain. Unusual glycolated forms of these enzymes are increased in AD brain and CSF. Transgenic mouse models disclose a similar phenomenon.

Studies suggest that CSF levels are low in early disease but rise significantly as the disease progresses. The finding is not specific to AD but also seen in DLB, VaD and FTD. The progressive rise with disease progression suggests a value in ongoing disease monitoring but unlikely a diagnostic role.

## Non-specific Markers of Neuronal Degeneration

Neurofilament proteins and synaptic markers represent non-specific degenerative products of any neuropathological process. Their role would be in disease monitoring.

### *Neurofilament Proteins*

These are structural proteins seen predominantly within large calibre axons. In any disease in which axonal destruction plays a role these molecules will be released, and AD is no exception.[20] Recent studies have evaluated phosphorylated forms and suggest they may be more specific for AD and be useful markers of disease progression.

### *Synaptic Markers*

Synaptic loss is highly correlated to dementia severity. Utilizing isoelectrical focusing and western blot techniques synaptic constituents can be measured in concentrated CSF. Immunoreactive bands were detected against several constituent synaptic proteins; rab3a, synaptotagmin, growth associated protein (GAP-43), synaptosomal-associated protein (SNAP-25) and neurogranin. Development of assays to compare AD and control CSF is ongoing.[21]

## Markers of Neural Inflammation

Inflammation is an important component of the pathophysiology of AD. Several inflammatory markers have been assessed as potential biomarkers of AD progression. These include: C1q (the first component of compliment), the soluble interleukin-6-receptor-complex(IL-6,IL-6R,gp130),A1-Antichymotrypsyn (ACT) and Melanotransferrin (p97).

### *C1q*

C1q is the first component of compliment and is very significantly increased in AD brain compared to controls.[22] C1q binds TAU and Aß and is a potent facilitator of A_aggregation.[23] There are significant decreases in C1q in AD CSF compared to controls suggesting a sink phenomenon. The level of depression correlates with the degree of cognitive deficits.[24]

### *Soluble interleukin-6-receptor-complex (IL-6, IL-6R, gp130)*

Interleukin-6 (IL-6) has inflammatory and potentially a neuroregulatory function in the brain and is in high concentration in AD brain but not in normal aging brain. It infiltrates early plaques but disappears as plaques age and may play a role in neuritic transformation. In AD the levels sIL-6R and sgp130, the receptors for IL6, are decreased while sIL6 remains unchanged. The work on this cytokine is still preliminary.

### *Melanotransferrin*

Melanotransferrin (p97) is a protein with a role in iron transport in the brain. It also is found in neuroglia surrounding AD plaques. Levels are elevated in both CSF and serum in AD.[25] Several methodological issues have arisen with the assays which require clarification in order to assess this molecule as a potential biomarker.[26,27]

### *Alpha 1 Antichymotrypsin (ACT)*

Alpha 1 Antichymotrypsin, a serine protease inhibitor, is elevated in AD brain and is related to the inflammatory cascade. It is present in senile plaques. There have been conflicting reports regarding the levels in serum and CSF.[28-30] It may increase with the severity of dementia.[31]

## Markers of Excessive Oxidation

Oxidative stress occurs in a variety of neuro-degenerative diseases including AD.[32] It remains unclear whether this is a pathogenic process or a downstream effect of these diseases. Markers of Lipid and nucleic acid oxidation/peroxidation; isoprostanes, 8OH2 deoxyguanosine, and protein nitration, 3-nitrotyrosine have been assessed.

### *Isoprostanes*

8,12-iso-iPF$_{2}$-VI is a sensitive marker for lipid peroxidation in AD patients. Studies have demonstrated elevated levels in urine, blood and CSF in both humans and mouse models.[33] The levels correlate well with cognitive changes and this is one of the more informative new markers at present undergoing evaluation.

### *3-Nitrotyrosine*

3-nitrotyrosine (3NT) is formed as a result of nitric oxide reacting with superoxide radicals to form a peroxynitrite. Peroxynitrite in turn reacts with tyrosine to produce 3NT. The levels of 3NT are 6 –fold higher in CSF in AD vs. controls.[34] Levels increase as cognitive status declines. This marker also appears promising but further longitudinal studies are required.

### *8OH 2 Deoxyguanosine*

8OH 2 deoxyguanosine (OHDG) is elevated in brain tissue and CSF in several degenerative conditions including AD, Parkinson's and Huntington's diseases.[35] 8OHDG is thought to represent the attack of free radicals on DNA. It can also be measured in urine. The assay is complex and reliability is still being assessed and longitudinal studies will be required.

### *Other Markers*

Several other lines of inquiry have also been suggested. Metabolites of cholesterol may be interest as there does appear to be a relationship between cholesterol metabolism and amyloid deposition.[36] Recent trials have suggested that lipid lowering agents may ameliorate the course of AD.[37]

Sulfatide, another lipid, is reported reduced in the CSF of AD and MCI patients when compared to controls. One small study demonstrated good separation between the two groups early in the disease.[38] Further studies are warranted.

Several markers of astrogliosis have been assessed including glial fibrillary acidic protein (GFAP), which has proven non-specific in CSF.[39] More promising are levels of glutamine synthetase, an astrocytic enzyme, involved in ammonia detoxification. There are recent encouraging results that serum levels are elevated in AD.[40] Clear separation from controls was found. These results require confirmation.



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## Conclusions

Biomarkers are not adequately developed for general clinical practice or as clinical trial surrogate outcome markers. Further research is required. When disease-modifying treatments become available, biomarkers may prove to be the most effective means of early or predictive diagnosis in the incipient stages of disease and also a mechanism to monitor treatment effects.

This is an important phase of research in Alzheimer's disease in which large longitudinal clinical trials assessing disease-modifying interventions are underway. The biological fluids of these cohorts of well-characterized patients will be an extraordinary resource for future biomarker research. Strategies to appropriately consent patients, collect and store samples and link the appropriate demographic and clinical profiles should be developed, a tissue bank.

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