

Transthyretin Cerebrospinal Fluid Levels in Alzheimer's Disease – Is This a Biomarker for Disease Severity?

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Abstract

Alzheimer's disease (AD) is the most common cause of dementia. $A\beta_{1-42}$, $A\beta_{1-40}$, total tau and hyperphosphorylated tau have been reported to be suitable biomarkers in the diagnosis of AD. Additionally, transthyretin has been suggested as a biomarker as it has been shown to inhibit $A\beta$ aggregation and the formation of senile plaques. Aggregates of $A\beta_{1-42}$ are an important hallmark of the pathogenesis of AD. Patients with AD show significantly lower levels of transthyretin in the cerebrospinal fluid (CSF) compared with ageing controls or patients with other kinds of dementia. Furthermore, some data indicate that transthyretin levels reflect disease severity in AD. In conclusion, recent work demonstrates the potential of transthyretin as an additional biomarker in the neurochemical differential diagnosis of AD.

Keywords

Dementia, Alzheimer's disease (AD), cerebrospinal fluid (CSF), transthyretin (TTR), biomarker, disease severity, transthyretic, prognosis

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Alzheimer's disease (AD), described for the first time in 1906 by Alois Alzheimer, is a neurodegenerative disease and the most frequent cause of dementia worldwide. The major neuropathological hallmarks of the disease are loss of neurons and synapses, and senile plaques (extracellular aggregates primarily composed of β -amyloid; $A\beta$) and neurofibrillary tangles (aggregates of hyperphosphorylated forms of the microtubule-associated tau protein) throughout cortical and limbic regions of the brain.¹ Definite diagnosis still requires pathology according to these criteria; however, in recent years substantial progress has been made in the area of early clinical biomarker development. The use of cerebrospinal fluid (CSF) as a testing platform seems to be very promising because the CSF protein composition reflects the pathological processes of the brain and because it is easily accessible by lumbar puncture. Some proteins have been reported to meet the criteria for a biomarker, such as $A\beta_{1-42}$, $A\beta_{1-40}$, total tau and hyperphosphorylated tau (p-tau).²⁻⁴ Another series reported transthyretin (TTR) as a potential biomarker in AD. Whereas the aforementioned biomarkers have been studied extensively and were suggested to be included into clinical AD criteria, less information is available on TTR. This article focuses on the importance of TTR in AD, summarises the information on the potential involvement of TTR in AD pathogenesis and highlights its role in the differential diagnosis of dementia.

Transthyretin – Synthesis

TTR is a 55kDa plasma homotetrameric protein that was first discovered in 1942. In the CSF, it is the main iodothyronine-binding protein, transferring T_4 from the blood into the brain across the blood-choroid plexus barrier.⁵⁻⁷ Serum TTR is also involved in the transport of plasma retinol-binding protein complex to vitamin A.⁸

TTR is synthesised in the liver and the choroid plexus of the brain,^{9,10} and TTR messenger RNA (mRNA) has also been found in kidney cells.¹¹ In the CSF it is composed of two fractions: one originating in the blood (about 10%) and one from the choroid plexus.¹² Studies on the plexus fraction are limited, but they are extremely important and urgently needed, since both morphological and functional changes in the choroid plexus are known to occur in AD. The ratio of the concentration of TTR in CSF to the concentration in serum is 200 times in excess compared with albumin.¹³ The concentration of TTR in serum is a sensitive indicator for malnutrition and illness owing to a reduction in its production rate in combination with its very rapid rate of disappearance from the circulation: about 50% per day.¹⁴

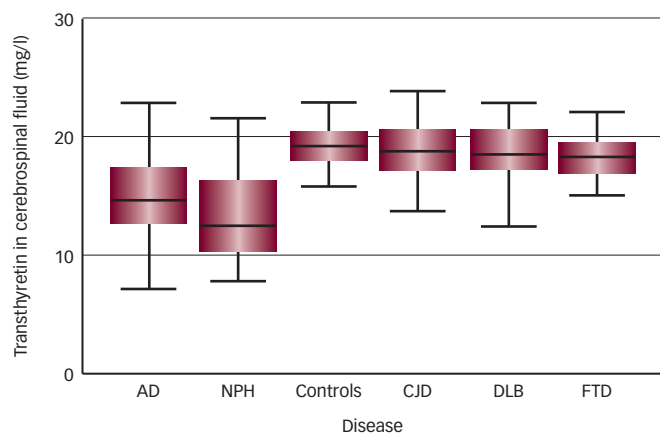
Transthyretin and Alzheimer's Disease Cerebrospinal Fluid Studies

In healthy elderly people, TTR levels increase with age.¹⁵ However, there was no correlation found between CSF production and CSF total protein concentration.¹⁶ By contrast, AD patients display significantly lower concentrations despite some overlap between AD patients and ageing controls. A potential explanation for this phenomenon is that AD is associated with the flattening of choroid plexus epithelial cells and with an increase in the diameter of the basal lamina.¹⁷ As both structures are involved in the production and filtration of CSF, it is likely that there is an association between morphological changes in the choroid plexus and a reduced TTR secretion rate.¹⁷

Pathology/Effect on $A\beta$ Aggregation

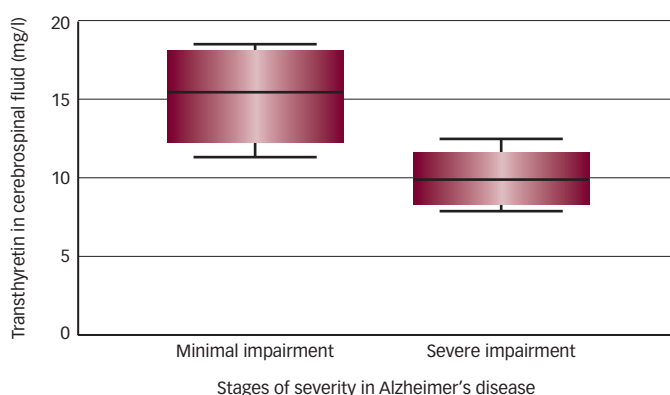
Aggregates of the amyloidogenic ($A\beta_{1-42}$) peptide play a major role in the pathogenesis of AD, although the precise mechanism is unclear.¹⁸

Figure 1: Transthyretin Levels in Cerebrospinal Fluid



AD = Alzheimer's disease; NPH = normal pressure hydrocephalus; CJD = Creutzfeldt-Jakob disease; DLB = dementia with Lewy bodies; FTD = frontotemporal dementia.

Figure 2: Transthyretin Levels in Different Stages of Alzheimer's Disease



TTR plays an important role in keeping intra-cerebral proteins such as amyloid fibrils in a soluble form, and some *in vitro* experiments have demonstrated that TTR inhibits A β aggregation and the formation of senile plaques.¹⁹⁻²⁴ Synthetic unlabelled A β was incubated with CSF, TTR or albumin and analysed later using Western blot techniques with anti-TTR and anti-Ac antibodies. TTR-A β complexes with an apparent molecular mass of 30kDa were observed under non-reducing conditions,¹⁹ which points towards high-affinity binding between those molecules. Transmission electron microscope (TEM) and light scattering analysis showed that TTR inhibited A β aggregation, and tryptophan (Trp) fluorescence quenching experiments also showed that full-length A β , but not non-aggregating A β fragments (A β_{1-11}) and A β_{12-28} quench TTR's Trp fluorescence.²⁵

In human CSF under physiological conditions, TTR forms soluble complexes by binding to A β protein. An important inhibition of A β aggregation is found at a molar ratio of 1:300, which suggests that TTR in CSF could be the major A β -binding protein and protects against A β deposition.^{19,20,26} As CSF concentrations of A β have been reported to increase with age,²⁹ the concomitant increase in TTR concentrations with age could be an important feature maintaining A β in soluble complexes. In AD, this physiological sequestration could be imperfect owing to inadequate concentrations of TTR or potentially some TTR modifications, the latter being possibly related to epithelial atrophy in the choroid plexus in patients with late-onset AD.¹⁵

Table 1: Transthyretin Concentrations in Alzheimer's Disease Patients Compared with Healthy Controls in Various Studies

Author	Disease	Biomarker/Methods
Gloeckner et al., 2008 ³⁰	Controls (n=19) AD (n=23) CJD (n=18) DLB (n=23) FTD (n=10) NPH (n=13)	Decreased concentration of TTR in AD and NPH in comparison with controls, CJD, DLB and FTD
Abdi et al., 2006 ³⁴	Group of AD (n=10) versus 10 control group (n=10) pooled samples Group of DLB (n=5) versus 10 control group (n=10) pooled samples	TTR precursor: ratio=1.08, no change Quantitative proteomic iTRAQ TTR precursor: ratio=1.55 Quantitative proteomic iTRAQ
Biroccio et al., 2006 ³⁵	Group of (n=39) AD versus control group (n=27) individual samples	Oxidised forms of TTR are significantly less abundant in the AD group
Castaño et al., 2006 ³¹	Group of AD (n=43) versus AD; control group (n=43) individual samples	Decreased concentration of TTR in using 2D gel electrophoresis and <i>post mortem</i> CSF
Puchades et al., 2003 ³⁶	Group of AD (n=7) versus control group (n=7) pooled samples	Decreased concentration of TTR in AD, quantitative proteomic
Davidsson et al., 2002 ³⁷	Group of AD (n=15) versus control group (n=12) individual samples	Increased concentration of TTR in AD, quantitative proteomic
Riisøen et al., 1988 ¹³	Group of AD (n=24) versus control group (n=47) individual samples	Decreased concentration of TTR in AD
Merched et al., 1998 ²²	Group of AD (n=20) control group (n=10) versus individual samples	Decreased concentration of TTR in AD

AD = Alzheimer's disease; TTR = transthyretin; NPH = normal pressure hydrocephalus; CJD = Creutzfeldt-Jakob disease; DLB = dementia with Lewy bodies; FTD = frontotemporal dementia; iTRAQ = isobaric tag for relative and absolute quantitation; CSF = cerebrospinal fluid.

In Vivo and In Vitro Experiments

In 2008, Buxbaum et al. obtained *in vitro* evidence of direct protein-protein interaction between TTR and A β aggregates. These findings suggest that TTR is protective because of its capacity to bind toxic or pre-toxic A β aggregates in both the intracellular and extracellular environment in a chaperone-like manner. Older APP 23 transgenic mice showed better cognitive function carrying multiple copies of human wild-type TTR gene. Subsequent experiments confirmed this effect. Expressing the APP 23 gene in the absence of mTTR, these mice showed immunohistochemical evidence of increased A β deposition relative to APP 23 animals with an active endogenous TTR gene. This study also demonstrated that both hTTR and mTTR bind A β_{1-40} and A β_{1-42} .¹⁸

Transthyretin as a Biomarker for Alzheimer's Disease

Most of the literature described reduced levels of TTR in patients with AD. TTR levels in the CSF of patients with AD are shown to be

reduced in comparison with patients without dementia.^{13,22,27} Other studies have reported decreased TTR concentrations in the CSF of patients with depression and normal pressure hydrocephalus (NPH).^{28,30} Although hampered by an artificial study design, similar results were achieved for *post mortem* CSF in AD.³¹ In other neurodegenerative diseases, normal concentrations of TTR are detected.³⁰ Table 1 summarises the data available on TTR from CSF studies. TTR levels are significantly reduced in the CSF of patients with AD and NPH compared with controls and other dementia, and TTR CSF values in AD did not differ significantly from those in NPH patients (see Figure 1).³⁰ TTR levels did not correlate with tau, A β ₁₋₄₀ or A β ₁₋₄₂ CSF.³⁰

Several attempts have been made to identify parameters that might reflect disease severity in AD. Studies on this subject are limited and contradictory results have been obtained so far. Both positive and negative associations have been reported. Increased levels of p-tau and tau were reported to reflect disease severity in AD in a study that analysed the abundance of symptoms in AD and low levels of A β ₁₋₄₂. The investigators found a higher risk of early death in AD in those with low A β ₁₋₄₂, and the follow-up patients who died had significantly lower levels of A β ₁₋₄₂ and significantly higher concentrations of tau.³² By contrast, Haense et al.³³ found no associations between tau and p-tau with dementia severity in AD. In

another study that analysed tau, A β ₁₋₄₂ and TTR in the same population, clear differences between biomarkers were elucidated. Whereas no association of dementia severity with tau and A β ₁₋₄₂ levels were detected, this was clearly observed for TTR: patients with severe AD showed a significantly lower TTR concentration (p=0.05) compared with mild cognitive impairment or mild dementia. At a cut-off of 15mg/l both stages could be discriminated, with a sensitivity of 91% and a specificity of 75% (Youden=0.66). Such an effect was not seen in patients with NPH (see Figure 2).³⁰ In summary, TTR levels have been demonstrated to be selectively decreased in AD compared with other dementias. In addition, some data indicate that levels reflect disease severity in AD, which makes this protein an interesting biomarker for AD diagnosis. Studies on early AD stages are lacking so far, and more must follow. ■

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