

Tau in biofluids - relation to pathology, imaging and clinical features

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Abstract

Tau is a microtubule-binding protein that is important for the stability of neuronal axons. It is normally expressed within neurons and is also secreted into the brain interstitial fluid that communicates freely with cerebrospinal fluid (CSF) and, in a more restricted manner, blood via the glymphatic clearance system of the brain. In Alzheimer's disease (AD), neuroaxonal degeneration results in increased release of tau from neurons. Further, tau is truncated and phosphorylated, which leads to aggregation of tau in neurofibrillary tangles of the proximal axoplasm. Neuroaxonal degeneration and tangle formation are reflected by increased concentrations of total tau (T-tau, measured using assays that detect most forms of tau) and phospho-tau (P-tau, measured using assays with antibodies specific to phosphorylated forms of tau). In AD CSF, both T-tau and P-tau concentrations are increased. In stroke and other CNS disorders with neuroaxonal injury but without tangles, T-tau is selectively increased, whilst P-tau concentration often stays normal. In tauopathies (diseases with both neurodegeneration and neurofibrillary tangles) other than AD, CSF T-tau and P-tau concentrations are typically unaltered, which is a puzzling result that warrants further investigation. In the current review, I discuss the association of T-tau and P-tau concentrations in body fluids with neuropathological changes, imaging findings and clinical features in AD and other CNS diseases.

Background

In 1986, one year after the sequencing of amyloid β ($A\beta$) in senile plaques in Alzheimer's disease (AD) brains [1], data were published showing that neurofibrillary tangles (the other major neuropathological hallmark of AD) are composed of abnormally phosphorylated and truncated forms of tau [2, 3]. Tau is a microtubule-binding axonal protein that promotes microtubule assembly and stability [4]. Abnormal phosphorylation and truncation of tau may lead to disassembly of microtubules and impaired axonal transport with compromised neuronal function and tau aggregation into paired helical filaments and neurofibrillary tangles [5]. Apart from executing important intracellular functions, tau is normally secreted from neurons into the brain interstitial fluid [6, 7]. This fluid communicates freely with cerebrospinal fluid (CSF) and, in a more restricted manner that is regulated by the glymphatic clearance system of the brain, with blood, where tau may be enzymatically degraded and cleared from the body by unknown mechanisms. Several methods to measure total tau (T-tau) and phospho-tau (P-tau) in CSF have been developed. They are reviewed in detail below and their utility in differential diagnostics of neurodegenerative diseases is summarized in Table 1.

CSF T-tau

The first CSF T-tau assay was published in 1993 [8]. This assay was a sandwich ELISA in which a monoclonal antibody against the mid-domain of tau was combined with a polyclonal anti-tau antiserum. Two years later, the first assay based on three mid-region monoclonal antibodies that recognise all tau isoforms irrespective of phosphorylation state was published [9]. AD patients displayed clearly increased T-tau concentrations in their CSF [8, 9], a finding that has been replicated in hundreds of papers, using different assays, in many different clinical contexts [10]. In response to acute brain injury, CSF T-tau concentrations are dynamic; they increase during the first few days following the injury and stay elevated for some weeks until they normalise [11, 12]. This has led to the view that increased CSF T-tau concentration reflects ongoing axonal injury or degeneration, which in turn may indicate disease intensity. In AD-type neurodegeneration, CSF T-tau concentrations are stably increased over many years [13].

CSF P-tau

The first CSF assay for P-tau, the form of tau that is thought to represent neurofibrillary tangles, was published in 1995 [9]. Since then, P-tau assays for different forms of phosphorylated tau protein have been examined [14]. They correlate well and associate with

AD in a similar manner [14]. There are at present only 3 conditions in addition to AD in which elevated CSF P-tau levels have been reported, none of which is an important differential diagnosis of AD: (i) in term and pre-term newborns, possibly reflecting physiological tau phosphorylation in brain development [15], (ii) in herpes encephalitis [16], and (iii) in superficial CNS siderosis [17, 18].

CSF tau – relation to pathology

In AD and normal pressure hydrocephalus, CSF P-tau concentration correlates with neurofibrillary tangle pathology [19, 20]. The major outstanding research question regarding P-tau is why other tauopathies, like frontotemporal dementias and progressive supranuclear palsy, do not show increased CSF P-tau concentration, at least not as systematically as AD does. It is possible that these disorders show disease-specific tau phosphorylation, or that tau is processed or truncated in a way that is not recognised by currently available assays, which is an area in need of further research.

CSF tau – relation to imaging

One limitation with CSF T-tau and P-tau is that lumbar CSF concentrations will never give information on the anatomic location of the pathology. Nevertheless, it has been shown that CSF T-tau concentration correlates with structural imaging measures of hippocampal atrophy [21] and grey matter degeneration [22], which is logical given the high expression of tau in thin unmyelinated axons of the cortex [23]. Additionally, rapid progress is being made on new positron emission tomography (PET) tracers for studying tau pathology in living humans. The results so far show that CSF and PET tau markers correlate [4]. In classical AD, the two may be more or less interchangeable, but in atypical forms of AD, tau PET may be superior. This was recently illustrated in a case report that described a young patient with progressive short term memory impairment and florid sensory symptoms [24]. CSF A β 42 was abnormal and 18F-flutemetamol PET showed a distribution of amyloid- β fibrils similar to sporadic AD. CSF T-tau and P-tau concentrations were normal, but the pattern of tau pathology, revealed using 18F-AV1451 PET, showed higher uptake in posterior cingulate, precuneus, parietal and occipital cortices, as compared to late-onset sporadic AD. Further, tau pathology exhibited a very clear inverse relationship with 18F-fluorodeoxyglucose metabolism, indicating neuronal hypometabolism in regions affected by tau aggregates. Genetic testing revealed a mutation of the presenilin-1 gene (Thr116Asn), previously described in a Danish family with familial AD

[25]. Tau PET is currently being examined in regards to its usefulness to detect tauopathies other than AD, where CSF tau markers currently fail.

In clinical trials of disease-modifying drug candidates against AD, CSF and PET tau markers may provide complementary information. Tau PET may be used to detect changes over months to years in tau pathology, whilst CSF T-tau and P-tau may be used to monitor changes in the neuronal release pattern of these molecules over weeks to months. This reasoning is presently hypothetical and waits formal testing in clinical trials.

CSF tau – relation to clinical features

CSF T-tau and P-tau are markers of disease intensity in AD; the higher concentrations, the more rapid clinical disease progression [26, 27]. The most extreme neurodegenerative condition we are aware of, Creutzfeldt-Jakob disease, is associated with very pronounced CSF T-tau elevations that are often orders of magnitude higher than what is typically seen in AD [28].

Plasma tau

Standard ELISAs for T-tau were recently transferred onto the Single molecule array (Simoa) platform, which allows for the ultrasensitive measurement of tau in blood [29]. There is so far no assay for P-tau in plasma. Plasma T-tau concentrations correlate poorly with CSF [30], but in acute hypoxic brain injury, a biphasic release of tau into the bloodstream was observed with a first peak occurring during the first few hours post-injury and a second broad peak occurring after a few more days; these increases were predictive of outcome [29]. In the dementia stage of AD, plasma tau concentrations are slightly increased compared with cognitively normal control individuals, but not as clearly as in CSF [30], which is a well-replicated finding [10]. In a study on concussed professional ice hockey players, plasma T-tau concentration at 1 hour post-injury was increased compared with preseason concentrations and predicted return-to-play time with high accuracy [31]. In another study of 34 patients with varying degree of traumatic brain injury (TBI), plasma tau concentrations in samples collected within 24 hours post-injury showed high accuracy to differentiate complicated mild TBI from controls (area under the curve 0.901) [32]. Recently, Olivera *et al.* reported increased mean plasma tau concentration in military personnel with a history of self-reported TBI compared with controls without such a history [33]. Additionally, post-concussive symptoms correlated with plasma tau concentration in the TBI group, suggesting that the increase, although

variable, might be of pathophysiological relevance [33]. The expression of tau is brain-enriched, but tau is also detectable at both mRNA and protein level in salivary glands and kidney (<http://www.proteinatlas.org/ENSG00000186868-MAPT/tissue>). This is an important potential confounder that may help explain the poor correlation of plasma with CSF tau. The half-life of tau also appears to be much shorter (hours) in plasma [29] than in CSF (weeks) [11].

Novel fluid biomarkers for tau

Tau proteins constitute a family of six isoforms ranging from 352-441 amino acids in size. The longest isoform in the CNS has four repeats (R1, R2, R3 and R4) and two inserts (441 amino acids in total), whereas the shortest isoform has three repeats (R1, R3 and R4) and no insert (352 amino acids in total) [34]. All of the six tau isoforms are present in an often hyperphosphorylated state in paired helical filaments from AD. In addition, there are endogenous fragments of tau; these fragments often lack different parts of the C-terminus [35]. A few years ago, ultrasensitive assays specific for 4R-tau and 3R-tau were developed [36]. Selective decreases of 4R-tau in CSF of progressive nuclear palsy and AD patients compared with controls, and lower 4R-tau levels in AD compared with Parkinson's disease dementia were reported, which was a promising result, but the assay has been difficult to standardize and the results are in need of replication. Recently, Lewczuk and colleagues reported increased CSF concentration of non-phosphorylated tau using a novel tau assay that specifically measures tau not phosphorylated at amino acids 175, 181 or 231 [37]. Using a new mass spectrometry workflow to enhance the detection of phosphoproteome components of AD brain tissue in CSF and enable the quantification of these analytes, over 75% coverage of full-length (2N4R) tau was detected in the CSF with 47 phosphopeptides covering 31 different phosphorylation sites. In a pilot study of a few AD and control CSF samples, 11 tau phosphopeptides were up-regulated by at least 40%, along with an overall increase in tau levels in the CSF of AD patients relative to controls; a few phosphopeptides were down-regulated [38]. Using another mass spectrometry approach, tau peptides along the entire molecule were detected in CSF and the peptide abundance pattern was similar in progressive supranuclear palsy and dementia with Lewy bodies but distinct in AD [39 [ENREF 39](#)]. These data emphasise the complexity of tau as a biomarker and warrant further investigation.

Standardization of fluid biomarker assays for tau

There is as yet no certified reference method or material for the most established AD biomarkers T-tau and P-tau, but such work is currently ongoing in the International Federation of Clinical Chemistry and Laboratory Medicine working group on CSF proteins. A candidate reference method for CSF T-tau has been published [40].

Conclusions

CSF T-tau and P-tau are markers of AD-type neurodegeneration and tangle formation. Other tauopathies show mostly normal CSF tau protein concentrations, potentially due to release of other tau forms that the current assays fail to detect. One hypothesis regarding the reason for the AD-specific increase in CSF T-tau and P-tau concentrations is that neuronal CSF tau protein secretion may be induced by alzheimerogenic factors such as A β 42. Tau is a complicated biomolecule with several isoforms and fragments. More tau markers are to be expected in the near future in the light of new mass spectrometric findings. CSF and PET tau markers seem to provide complementary information on AD pathology and should as much as possible be analysed in parallel on patients in different stages of the disease, as well as in atypical forms of AD and in other tauopathies, to increase our knowledge on how to best interpret the two measures.

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Table 1. CSF total- and phospho-tau concentrations in neurodegenerative diseases and common differential diagnoses

Disease	Total-tau concentration	Phospho-tau concentration
Alzheimer's disease	Medium to strong increase	Medium to strong increase
Vascular dementia	Normal to strong increase	Normal
Frontotemporal dementia	Normal to slight increase	Normal
Dementia with Lewy bodies	Normal to slight increase	Normal
Parkinson's disease	Normal	Normal
Atypical parkinsonian disorders	Normal to slight increase	Normal
Creutzfeldt-Jakob's disease	Very strong increase	Normal
Stable mild cognitive impairment	Normal	Normal
Normal aging	Normal	Normal
Depression	Normal	Normal