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Total and Phosphorylated Tau Proteins: Evaluation as Core Biomarker Candidates in Frontotemporal Dementia

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Alzheimer's disease · Frontotemporal dementia · Differential diagnosis · Cerebrospinal fluid · Biomarker · Tau protein · Phosphorylated tau protein

Abstract
An ever increasing number of patients with neurodegenerative disorders calls for the evaluation of potential diagnostic markers that allow an early diagnosis and an early initiation of specific therapy. Clinical diagnosis of Alzheimer's disease (AD), the most common neurodegenerative disorder, reaches 80–90% accuracy upon autopsy in specialized clinical centers. Diagnosis of AD in early clinical or preclinical stages is far less accurate, as is the differential diagnosis between AD and other primary dementias, such as frontotemporal dementia (FTD). Microtubule-associated tau protein is abnormally phosphorylated in AD and aggregates as paired helical filaments in neurofibrillary tangles. Recently, immunoassays have been developed detecting tau phosphorylated at specific epitopes in cerebrospinal fluid (CSF). Four years of clinical research consistently demonstrate that CSF phosphorylated tau (p-tau) is highly increased in AD compared to healthy controls and may differentiate AD from its most relevant differential diagnoses. Tau phosphorylated at threonine 231 (p-tau$_{231}$) shows excellent differentiation between AD and FTD, whereas serine 181 (p-tau$_{181}$) enhances accurate differentiation between AD and dementia with Lewy bodies. Moreover, p-tau$_{231}$ levels decline with disease progression, correlating with cognitive performance at baseline. Total tau (t-tau) is regarded as a general marker of neurodegeneration for evaluation in future population-based studies. p-tau$_{231}$ and p-tau$_{181}$ yield excellent discrimination between AD and non-AD dementias including FTD, exceeding the differential diagnostic and prognostic accuracy of t-tau. Therefore, p-tau is a core biological marker candidate for future evaluation in large national and international multicenter networks.

Development of Biological Markers for Frontotemporal Dementia

The clinical entity of frontotemporal dementia (FTD) is believed to represent a wide range of neuropathological conditions. Most of the heterogeneous clinical presentations of FTD show pathological changes of the microtu-

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bule-associated tau protein. Tau gene mutations have been identified in familial FTD cases. Even in many sporadic FTD cases without detectable tau gene mutations, abnormal phosphorylation of microtubule-associated tau occurs as well.

Microtubule-associated tau protein is elevated in cerebrospinal fluid (CSF) in a wide range of acute or chronic conditions of neuron loss, like stroke, head trauma, Creutzfeldt-Jacob disease, and neurodegenerative disorders like FTD and AD. Therefore, tau protein in CSF is thought to be an unspecific marker of neuronal degeneration. It was expected that measurement of tau protein in CSF may help to distinguish early stages of FTD and AD from healthy aging. In contrast, the value of CSF tau protein for the distinction between FTD and other neurodegenerative disorders may be limited.

In the past 10 years, assays have been developed to detect specific abnormal phosphorylation sites of CSF tau, and have recently been applied to a range of neurodegenerative disorders. These assays had primarily been developed to capture the specific pattern of abnormal phosphorylation of tau protein in AD, but these newly established markers may be useful in the diagnosis of FTD as well. Because abnormal phosphorylation of tau proteins is a common pathway in the development of neurodegenerative disorders and different neurodegenerative disorders are believed to exhibit a distinctive pattern of tau protein phosphorylation sites, it was expected that measurement of specific phosphorylation epitopes of tau proteins in CSF may increase the accuracy of CSF tau to discriminate neurodegenerative disorders from healthy aging, but also to more accurately differentiate between different neurodegenerative disorders.

A future biomarker of FTD or any other neurodegenerative disorder will have to meet specific criteria. The ideal biomarker should detect a fundamental feature of neuropathology and be validated in neuropathologically confirmed cases; it should have a high diagnostic sensitivity and specificity, it should be reliable, reproducible, noninvasive, simple to perform, and inexpensive. Recommended steps to establish a biomarker include confirmation by at least two independent studies conducted by qualified investigators, with the results published in peer-reviewed journals [1]. Beyond these criteria for early and accurate diagnosis, the biomarker should also capture the beneficial effect of disease-modifying therapy. We need to be able to make accurate diagnoses early in the disease process and we need to be able to evaluate, effectively and inexpensively, whether treatments are working.

Because of the high prevalence of AD and the overlapping symptoms of AD and FTD, the decisive clinical use of any future FTD biomarker will be the separation of FTD from AD and other neurodegenerative disorders rather than the discrimination between FTD and healthy aging.

Tau Proteins

Normally, the 6 hyperphosphorylated brain tau isoforms (PHF-tau) are located mainly in axons, associated with the cytoskeleton and intracellular transport systems. Total tau (t-tau) and truncated forms of monomeric and phosphorylated tau (p-tau) can be measured in CSF. Using antibodies that detect all isoforms of tau proteins independent of phosphorylation or specific phosphorylation sites, ELISAs have been developed to measure t-tau and specifically phosphorylated CSF tau protein (p-tau) concentrations [2, 3].

Total Tau

The level of CSF tau probably reflects the general degree of neuronal degeneration and damage. This notion is supported by the finding that a marked transient increase in CSF tau is found after acute stroke, with a positive correlation between CSF tau and infarct size measured by CT [4]. Further, the degree of increase in CSF t-tau is higher in disorders with more extensive and/or rapid neuronal degeneration. A very marked increase is found in Creutzfeldt-Jacob disease, with very rapid degeneration [5] and a moderate increase is found in AD, with widespread degeneration [6, 7].

In FTD, increased tau levels compared to healthy aging have been found by some investigators [8–10], while not in other studies [7, 11]. Diagnostic sensitivity of tau protein classifying FTD patients and healthy subjects reached about 82–85% in those studies where tau protein was found elevated [12]. Tau levels in FTD compared to AD (fig. 1) have been found reduced, but the diagnostic value of tau for separation between FTD and AD is limited, with the sensitivity and specificity levels yielding 81 and 65%, respectively [13].

The most consistent and widely established finding is a statistically significant increase in CSF t-tau protein in AD [2, 3]. Applying appropriate statistical methods, such as multivariate discriminant analysis, combination of t-tau with other pathophysiologically relevant marker can-

didates may increase diagnostic accuracy compared to a single marker.

Because of its nature as an indicator of neuronal degeneration, elevated CSF t-tau is found in a wide proportion of cases with primary and secondary dementia disorders other than FTD or AD. In contrast, in patients with other types of dementias (e.g. alcoholic dementia), chronic neurological disorders (e.g. Parkinson's disease, progressive supranuclear palsy) and psychiatric disorders (e.g. depression), elevated CSF tau levels are found only in few cases [6, 10].

In summary, tau protein is of limited value in the discrimination of FTD from healthy aging and AD in some, but not all studies. One reason for the conflicting results in different studies may be the pathological heterogeneity of clinical cases with FTD. Tau levels may differ between different subtypes of FTD. Therefore, it is necessary to study clinical, and ideally pathological, subtypes of FTD to evaluate a potential use of tau proteins in the differential diagnosis of FTD.

Biochemical analyses of tau protein abnormalities in postmortem studies have been reported [14]. Fractionation of tau protein differentiates disorders with and without measurable amounts of insoluble tau. Further characterization of this abnormal insoluble tau differentiates cases with a predominance of tau with 3 microtubule-binding repeats, 4 microtubule-binding repeats, or a combination of both [15, 16]. One investigation has also suggested that some frontotemporal degenerative disorders have a marked reduction of all soluble fraction tau proteins with preserved levels of tau messenger RNA [17]. These results suggest that the analysis of soluble tau isoform may be useful in characterizing frontotemporal degenerative disorders.

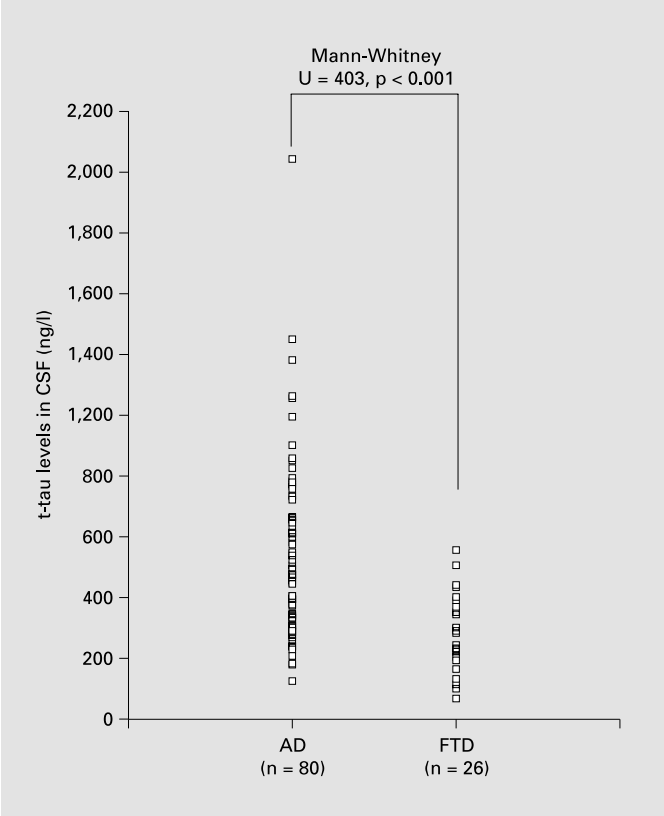

Fig. 1. CSF levels of t-tau in AD patients and FTD patients.

Phosphorylated Tau

Currently, promising efforts are under way to establish p-tau in CSF as a putative disease-specific biological marker for AD. Immunoassays have been developed to specifically detect tau at different epitopes, such as threonine 231 (p-tau$_{231}$), serine 199 (p-tau$_{199}$) and threonine 181 (p-tau$_{181}$) [2, 3]. Evidence from these studies indicates that quantification of tau phosphorylated at these specific sites may improve early detection, differential diagnosis and tracking of disease progression in AD. p-tau assays have been studied so far in several hundred AD patients and healthy subjects, as well as patient samples with other neurodegenerative disorders and vascular dementia over the past 4 years. In contrast, no assays have been developed to detect characteristic tau phosphorylation sites in FTD.

In a large-scale multicenter study, the levels of p-tau$_{231}$ were significantly increased in AD compared to FTD patients (fig. 2). Moreover, p-tau$_{231}$ compared to t-tau raised sensitivity levels in the discrimination of AD and FTD from 57.7 to 90.2% at a specificity level of 92.3% for both markers (fig. 3) [13]. In a multicenter study designed to provide evidence for the selection of p-tau$_{231}$, p-tau$_{181}$, and p-tau$_{199}$ bioassays for AD in larger-scale international dementia networks, p-tau$_{231}$ maximized group separation between FTD and AD compared with the other p-tau assays, reaching a sensitivity of 92%, when specificity was set at 85% [18]. For comparison, p-tau$_{181}$ and p-tau$_{199}$ reached sensitivity levels between 42 and 79% for the detection of FTD, when specificity was set at 85%.

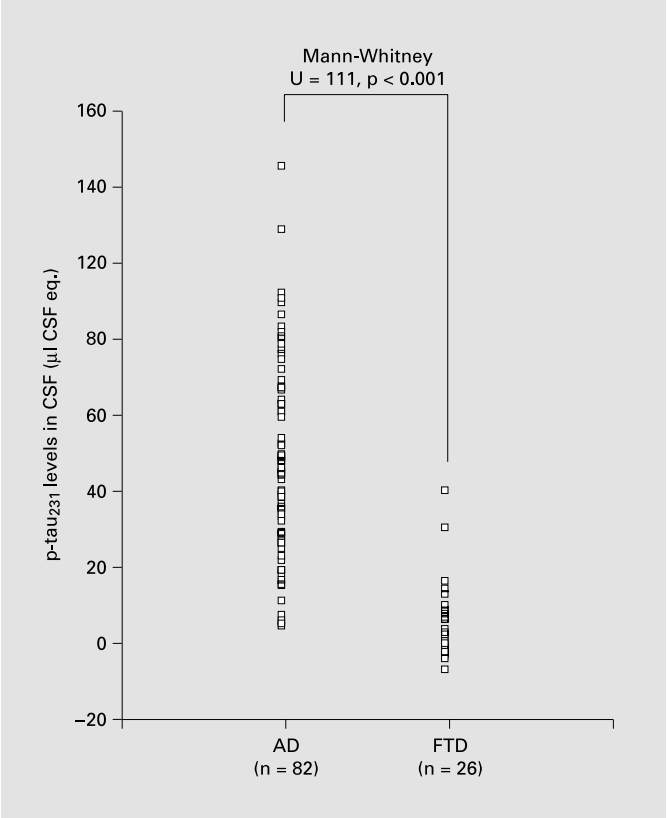

Fig. 2. CSF levels of p-tau$_{231}$ in AD patients and FTD patients.

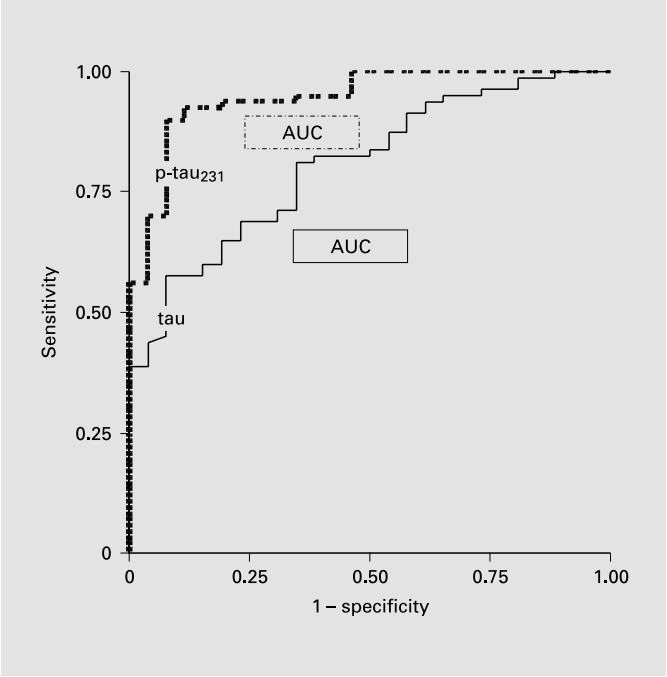

Fig. 3. Receiver operating characteristics (ROC) curve analysis for the discrimination of 80 AD and 26 FTD patients by t-tau and p-tau$_{231}$. The area under the ROC curve (AUC) represents the probability to correctly allocate a patient into the AD or FTD group, given the subject's CSF t-tau or p-tau$_{231}$ concentration. AUC was significantly higher for p-tau$_{231}$ than for t-tau ($p < 0.05$).

Conclusions

The utility of t-tau in the differentiation of FTD from healthy aging is not yet clear. The heterogeneity of clinical FTD with respect to the underlying tau pathology very likely contributes to the conflicting findings on CSF tau elevations in FTD. Future studies may reveal certain pathological subtypes of FTD which can be distinguished from healthy subjects by the measurement of t-tau or tau phosphorylation epitopes. This would bear relevance both for the diagnosis of FTD and for the prognosis and treatment of different subtypes of FTD.

The separation of FTD and AD by t-tau is not sufficient, with sensitivity and specificity levels far below 80%. Much more promising in this respect is the detection of specific tau phosphorylation epitopes in CSF. The most extensively studied epitopes are p-tau$_{231}$ and p-tau$_{181}$. For p-tau$_{231}$, recent multicenter studies suggest a correct allocation of FTD and AD patients with an accuracy above 80%. The role of p-tau as a biomarker for FTD, however, appears to be limited to the distinction from AD. The separation of FTD from healthy aging and other types of dementias is far below a correct classification rate of 80%. Recent postmortem examinations have suggested a specific fingerprint of tau phosphorylation and fractionation in FTD that may prove useful as a biomarker of FTD in the future.

The development of a biological marker for the diagnosis of disease can be conceptualized in three subsequent steps [19]. The first step is the technical and methodological development of the marker, including the establishment of assay variance, test-retest variability, and the technical feasibility of the marker. The second step is the evaluation of the marker in selected patient samples. This step serves to estimate sensitivity and specificity of a marker. The third step is the evaluation of the marker in a clinical setting, where the population is not selected according to the known clinical status, but according to the indication of the diagnostic procedure in clinical routine.

The outlined development of CSF biomarkers for AD can serve as a model which provides guidelines showing what still has to be achieved in the development of biomarkers for FTD. Additionally, a specific expression pattern of tau proteins may serve in the future to discriminate different subtypes of neuropathology underlying the clinical entity of FTD that may differ in prognosis and response to treatment.

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