

# Recent progresses on p-tau as a blood-based Alzheimer's disease biomarker

*Progressos recentes em p-tau como um biomarcador de doença de Alzheimer baseado no sangue*

*Progresos recientes en p-tau como biomarcador de la enfermedad de Alzheimer en sangre*

Danyelle Sadala<sup>1#</sup>, Vyctoria Ramos<sup>1#</sup>, Danielle dos Santos Maia Salheb de Oliveira<sup>2</sup>, Maria José da Silva Fernandes<sup>1</sup>, Marcia Regina Cominetti<sup>2</sup>

1.Universidade Federal de São Paulo, Department of Neurology and Neurosurgery, São Paulo-SP, Brazil.

2.Universidade Federal de São Carlos, Department of Gerontology, São Carlos-SP, Brazil.

#Both authors equally contributed to this work

## Resumo

**Introdução.** A hipótese da cascata amiloide propõe que as placas senis extracelulares - em grande parte compostas por peptídeos beta-amiloide (A $\beta$ ) agregados - são responsáveis pelos eventos que levam à morte neuronal que ocorre na doença de Alzheimer (DA). Por outro lado, a proteína tau hiperfosforilada (p-tau) e desestruturada é responsável pelos emaranhados neurofibrilares intracelulares, também comuns na DA. Os critérios de diagnóstico clínico para DA incluem testes dos biomarcadores A $\beta$  e p-tau no líquido cefalorraquidiano (LCR), além de medidas de neuroimagem, história clínica e testes psicométricos. No entanto, devido à sua natureza invasiva, efeitos colaterais e necessidade de pessoal treinado em ambiente hospitalar para sua coleta, os biomarcadores do LCR não são adequados para triagem em larga escala. Portanto, biomarcadores alternativos baseados no sangue estão sob intensa investigação.

**Objetivos.** Enfocar os avanços recentes em diferentes isoformas de p-tau como biomarcadores para DA baseados no sangue. **Método.** Revisão realizada por buscas nas bases de dados Medline/PubMed. **Resultados.** As isoformas 181 e 217 da p-tau representam moléculas acessíveis e escaláveis para triagem e diagnóstico de DA, principalmente devido à sua capacidade de diferenciar pacientes com a doença de participantes cognitivamente saudáveis. Esses resultados devem ser reproduzidos em coortes maiores e mais representativas da diversidade populacional. **Conclusões.** Essa revisão fornece uma exploração mais abrangente de p-tau sanguínea como um biomarcador molecular específico para DA, o que poderia contribuir não apenas para a triagem de pacientes pré-sintomáticos para ensaios clínicos, mas também para monitorar a progressão da doença e avaliar terapias modificadoras da doença.

**Unitermos.** Doença de Alzheimer; biomarcadores; sangue; plasma; p-tau; tau

## Abstract

**Introduction.** The amyloid cascade hypothesis proposes that extracellular senile plaques - largely composed of aggregated beta-amyloid (A $\beta$ ) peptides - are responsible for the events that lead to neuronal death that occurs in Alzheimer's disease (AD). On the other hand, the hyperphosphorylated (p-tau) and unstructured tau protein is responsible for intracellular neurofibrillary tangles, also common in AD. Clinical diagnostic criteria for AD include A $\beta$  and p-tau biomarker tests in cerebrospinal fluid (CSF), in addition to neuroimaging measures, clinical history, and psychometric tests. However, due to their invasive nature, side effects and need for trained personnel in a hospital environment for their collection, CSF biomarkers are not suitable for large-scale screening. Therefore, alternative blood-based biomarkers are under intense investigation. **Objective.** Focus on recent advances in different p-tau isoforms as blood-based AD biomarkers. **Method.** Review performed by searches in Medline/PubMed databases. **Results.** The p-tau isoforms 181 and 217 represent accessible and scalable molecules for screening and diagnosing AD, mainly due to their ability to differentiate patients with the disease from cognitively healthy participants. These results should be reproduced in

larger and more representative cohorts of population diversity. **Conclusions.** This review provides a more comprehensive exploration of blood p-tau as a specific molecular biomarker for AD, which could contribute not only to screening pre-symptomatic patients for clinical trials, but also to monitoring disease progression and evaluating modifying therapies. of the disease. **Keywords.** Alzheimer's disease; biomarkers; blood; plasma; p-tau; tau

---

## Resumen

**Introducción.** La hipótesis de la cascada de amiloide propone que las placas seniles extracelulares, en gran parte compuestas por péptidos beta-amiloides (A $\beta$ ) agregados, son responsables de los eventos que conducen a la muerte neuronal que ocurre en la enfermedad de Alzheimer (EA). Por otro lado, la proteína tau hiperfosforilada (p-tau) y no estructurada es responsable de los ovillos neurofibrilares intracelulares, también comunes en la EA. Los criterios de diagnóstico clínico para la EA incluyen pruebas de biomarcadores A $\beta$  y p-tau en líquido cefalorraquídeo (LCR), además de medidas de neuroimagen, historia clínica y pruebas psicométricas. Sin embargo, debido a su naturaleza invasiva, los efectos secundarios y la necesidad de personal capacitado en un entorno hospitalario para su recolección, los biomarcadores de LCR no son adecuados para el cribado a gran escala. Por lo tanto, se están investigando intensamente los biomarcadores alternativos basados en sangre. **Objetivo.** Concéntrate en los avances recientes en diferentes isoformas de p-tau como biomarcadores de EA en sangre. **Método.** Revisión realizada mediante búsquedas en bases de datos Medline/PubMed. **Resultados.** Las isoformas p-tau 181 y 217 representan moléculas accesibles y escalables para la detección y el diagnóstico de la EA, principalmente debido a su capacidad para diferenciar a los pacientes con la enfermedad de los participantes cognitivamente sanos. Estos resultados deben reproducirse en cohortes más grandes y representativas de diversidad de población. **Conclusiones.** Esta revisión proporciona una exploración más completa de la p-tau sanguínea como un biomarcador molecular específico para la EA, lo que podría contribuir no solo al cribado de pacientes presintomáticos para ensayos clínicos, sino también a monitorear la progresión de la enfermedad y evaluar las terapias modificadoras de la enfermedad.

**Palabras clave.** enfermedad de Alzheimer; biomarcadores; sangre; plasma; p-tau; tau

---

Trabalho desenvolvido para o curso "Neurociência em Pauta" ("Neuroscience at hand"), ministrado pelo Programa de Pós-Graduação em Neurologia/Neurociências da Escola Paulista de Medicina, Unifesp, São Paulo-SP, Brasil.

Conflito de interesse: não

Recebido em: 04/05/2021

Aceito em: 04/05/2021

Endereço de correspondência: Marcia Regina Cominetti. Department of Gerontology, Universidade Federal de São Carlos. Rod Washington Luis, km 235, São Carlos-SP, Brazil. CEP 13565-905. Phone: +55 16 33066663. E-mail: [mcominetti@ufscar.br](mailto:mcominetti@ufscar.br)

---

## INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common type of dementia worldwide, accounting for more than 60% of cases<sup>1</sup>. Currently, 46 million people are affected by dementia globally, with predictions beyond 131 million by 2050. As a result, the global cost of this disease is around US\$818 billion<sup>2</sup>.

AD patients have symptoms related to losses in different cognitive domains, such as memory, behavioral, language, mood, and movement changes, as well as physiological dysfunctions<sup>3</sup>. Different explanations were established to explain those symptoms, including the neurotransmitters, mitochondrial, neurovascular, inflammatory, diabetes, tau propagation and amyloid cascade hypotheses<sup>4</sup>. Of them, the amyloid cascade and tau hypotheses suggest that the main pathological hallmarks of AD are the extracellular senile plaques – primarily composed of amyloid- $\beta$  ( $A\beta$ ) peptides<sup>5</sup> – and the intracellular hyperphosphorylated neurofibrillary tangles (NFT) rich in tau protein<sup>6</sup>. Both structures, senile plaques and NFT, were observed to prompt cellular dysfunction and toxicity *in vitro* and *in vivo* leading to neuronal death, typical of AD<sup>7</sup>.

The cleavage of amyloid precursor protein (APP) through the amyloidogenic pathway produces senile plaques whose, according to the amyloid cascade hypothesis, is one of the AD causes<sup>8</sup>. On the other hand, the non-amyloidogenic cleavage of APP, carried out by  $\alpha$  and  $\gamma$ -secretases, avoids the  $A\beta$  formation.

Tau protein hyperphosphorylation results in another AD hallmark, the NFT. Tau is a major microtubule-associated protein (MAP) in neurons, whose principal role is related to the stabilization of microtubules, supporting cytoskeletal organization and axonal transport<sup>9</sup>. The interaction between tau and microtubules is dynamic and influences several cellular functions, such as neurite polarity and stability,

axonal transport of vesicles and organelles, and outgrowth, elongation, and guidance of axons. Moreover, tau is also involved in DNA protection, adult neurogenesis, synaptic plasticity, regulation of neuronal activity, and insulin signaling<sup>10</sup>.

The diagnosis of probable AD is based mainly on the clinical history and psychometric testing, with a requisite for disease pathology at *post-mortem* to confirm an exact conclusion<sup>11</sup>. However, this diagnostic framework was revised by the National Institute of Ageing-Alzheimer's Association (NIA-AA) in 2011 and now include cerebrospinal fluid (CSF) A $\beta$  and tau testing to support clinical criteria<sup>12</sup>, evidencing the importance of the biomarkers for AD diagnosis. Nevertheless, despite many advantages, these CSF-based AD biomarkers have some inconveniencies discussed in the next topic of this review. In this regard, several laboratories worldwide are studying biomarkers for AD, which derive from different sources, other than CSF, including saliva and urine, but particularly blood. In this sense, the recent findings on p-tau as a blood-based AD biomarker are the purpose of this narrative review, bringing the latest advances in the field.

### **CSF and image biomarkers for AD: their advantages and disadvantages**

CSF biomarkers have been incorporated into the clinical practice with successful results for AD diagnosis. Hopefully, in the future, when disease-modifying treatments for

common neurodegenerative diseases, including AD, become available, biomarkers may have a role to guide the treatments<sup>13</sup>. Three main CSF biomarkers for DA have been identified over the past twenty years: a 42-aminoacid form of A $\beta$  (A $\beta$ <sub>1-42</sub>), total tau (t-tau) and phosphorylated tau (p-tau). The typical profile for AD CSF biomarkers is low concentrations of A $\beta$ <sub>1-42</sub> due to cortical amyloid deposition, high concentrations of t-tau due to cortical neuronal loss and high p-tau concentrations, reflecting cortical tangle formation. In the mild cognitive impairment (MCI) phase of AD development, these biomarkers are also helpful and present with the same profile of AD patients: elevated t-tau and p-tau and reduced A $\beta$ <sub>1-42</sub>, although the statistical effect was less pronounced than in AD. For instance, a study showed that the decline in CSF A $\beta$ <sub>1-42</sub> levels precedes AD dementia by at least ten years<sup>14</sup>.

However, regarding CSF AD biomarkers protocols, there is a diversity of analytical methods for antibody recognizing, with different epitopes of the same molecule, particularly for A $\beta$ <sub>1-42</sub> and p-tau<sup>15,16</sup>. Nevertheless, this multiplicity of assays can lead to different results and hamper the definition of cutoff concentration values (cut points) for each biomarker. Hence, exact cut points have not been given, although AD is associated with lower A $\beta$ <sub>1-42</sub> and higher t-tau and p-tau CSF levels than controls<sup>13</sup>. Moreover, there are other disadvantages of measuring AD biomarkers in CSF, as its collection is necessary to perform a lumbar puncture (LP), which has associated complications including local

discomfort, radicular pain, spinal hematoma, post-LP headache, and even meningitis. These side effects characteristically occur within five days of LP and typically resolves within one week with conservative management or within 48 h of definitive treatment, usually an epidural blood patch<sup>17</sup>. Nevertheless, CSF-based AD biomarkers represent a potent tool to diagnose and differentiate the disease from other types of dementia. A systematic review and meta-analysis comprising 15,699 patients with AD and 13,018 controls found that CSF A $\beta$ <sub>1-42</sub>, t-tau, p-tau, and neurofilament light protein (NFL) are biomarkers that, at least on a group level, robustly separate AD patients from controls. Importantly, for earlier disease detection, CSF A $\beta$ <sub>1-42</sub>, t-tau, and p-tau were also able to discriminate between MCI due to AD and stable MCI<sup>15,18</sup>.

Some neuroimage biomarkers such as positron emission tomography (PET) for amyloid plaques (amyloid-PET) and the energy consumption marker <sup>18</sup>F-deoxyglucose (FDG-PET) are helpful for early diagnosis, disease staging, and to predict clinical outcomes in AD<sup>19,20</sup>. FDG-PET scan measures regional glucose consumption, a direct indicator of neuronal and astrocytic activity. Therefore, it detects brain hypometabolism reflecting neuronal dysfunction in neurodegeneration<sup>19</sup>.

For AD, FDG-PET is helpful in the early diagnosis, showing a typical temporoparietal pattern of hypometabolism in individuals with MCI. Additionally, an abnormal FDG-PET during follow-up associates with a higher

risk of progressive cognitive deterioration. Moreover, this PET marker is useful for disease staging since hypometabolism patterns closely associate with cognitive deficits<sup>21</sup>. Despite its validity for clinical use, FDG-PET is not specific to the histopathological hallmarks of AD<sup>19</sup>.

On the other hand, amyloid-PET allows sensitive and specific non-invasive *in vivo* detection of amyloid plaques<sup>19</sup>. The Pittsburgh Compound-B (PiB), one of the amyloid tracers, often shows a 50-70% higher retention in AD cortex when compared with controls and is sensitive in predicting which MCI patient will develop AD<sup>20-25</sup>. This tracer, however, has <sup>11</sup>C as the radioisotope, giving PiB a half-life of only 20 minutes, which reduces its clinical application. <sup>18</sup>F-marked amyloid-PET tracers, although presenting a longer half live, are less specific than PiB<sup>21</sup>. Overall, amyloid imaging can increase diagnostic and change management in up to 60% of individuals<sup>19</sup>. However, it does not allow the differentiation between distinct A $\beta$ -positive disorders, which can show similar A $\beta$ -deposition patterns<sup>19</sup>.

Disadvantages of amyloid imaging include its inability to predict the early stages and the low availability to most patients, especially when there are no disease-modifying therapies for an early intervention. The main CSF AD biomarkers and neuroimage tools for AD diagnosis are presented in Table 1.

Table 1. Sensitivities and specificities of CSF and neuroimage techniques to correctly diagnose AD.

Biomarker	Specificity (%)	Sensitivity (%)	Reference
CSF t-tau	72	83	18
CSF A $\beta$ <sub>1-42</sub>	89	79	23
FDG-PET	74	76	24
Amyloid-PET	100	92	25

## Blood-based biomarkers for AD: their advantages and disadvantages

Plasma (or serum) biomarkers for AD have developed significantly over the years. Finding blood biomarkers for AD is needed since blood is more accessible than CSF and cheaper than PET. Furthermore, blood collection is already globally established, do not require sophisticated training by health-care professionals, neither new infrastructure to be created.

Although the blood-brain barrier (BBB) usually prevents brain antigens to reach the blood, this barrier is compromised in AD patients, increasing the levels of CNS markers in the peripheral blood<sup>15</sup>. The conventional blood biomarkers for AD are the same as CSF, including t-tau, p-tau, and A $\beta$ <sub>1-42</sub>. The first studies failed in identifying A $\beta$ <sub>1-42</sub> or A $\beta$ <sub>1-40</sub> differences between AD and control group. However, these studies have used enzyme-linked immunoassay (ELISA) as the method of detection<sup>26</sup>, which may not be sensitive enough to detect small amounts of such markers, given the complexity of blood plasma. Later, a meta-analysis



with 15,699 AD patients and 13,018 controls reinforced the lack of blood A $\beta$ <sub>1-42</sub> and A $\beta$ <sub>1-40</sub> while strongly linked plasma t-tau with AD. However, high levels of A $\beta$ <sub>1-42</sub> were associated with a faster cognitive decline among AD patients<sup>16</sup>. More recently, studies with single-molecule array (SIMOA) showed correlations between CSF and plasma biomarkers. Studies with these improved and more sensitive assays have revealed that plasma or serum measures have shown alterations in these AD biomarkers compared to cognitively healthy controls<sup>14,27</sup>.

Despite all the disadvantages mentioned above, Hampel *et al.*<sup>26</sup> found 1,039 studies of 196 possible blood-based AD biomarkers. Amongst the studies, 18% used A $\beta$  and tau markers, 19% searched genetic markers, 29% employed biomarker panels, and 34% were on markers related to inflammation, oxidative stress, DNA damage, mitochondrial dysfunction, and neuronal or microvascular injury<sup>26</sup>. Of these 196 biomarkers, some 'emerging targets' like axonal protein neurofilament light (NFL) and  $\beta$ -secretase 1 (BACE1) are essential to mention. Serum NFL levels correlate closely with CSF levels, demonstrating that its blood levels reflect CNS pathophysiology. Additionally, BACE1 activity in the plasma of patients with MCI and AD was increased compared with controls. Other groups propose using panels of blood-based biomarkers, which might better differentiate patients from healthy control groups than single markers, including a 21-protein panel for AD screening with a positive predictive value of 0.85 and a negative predictive

value of 0.94<sup>26</sup>. Overall, this high number of candidates highlight the importance of the theme and the intensive search carried out by different laboratories worldwide to find such biomarkers.

The blood-based biomarkers main inconvenience is their dilution in plasma. Besides, their degradation in the liver or directly by blood by proteases are also disadvantages of these biomarkers. Finally, specifically for plasma amyloid markers, their alteration by cardiovascular and cerebrovascular factors limits their diagnostic and predictive values<sup>14,27,28</sup>.

### **Tau and p-tau CSF biomarkers and their relationship with AD pathophysiology**

*Tauopathies* are neurodegenerative diseases characterized by the progressive accumulation of misfolded tau in the nervous system. In AD, misfolded and aggregated tau results in different neurodegenerative events, such as cytoskeleton and axonal transport disruption, calcium dysregulation, mitochondrial dysfunction, oxidative stress, neuroinflammation, microglia dysfunction, synaptic dysfunction and loss, altered neuronal activity, and neuronal loss<sup>10</sup>.

Four structurally and functionally distinct domains form tau. The (1) N-terminal projection domain regulates tau binding to microtubules and determines spacings between them<sup>29</sup>. The (2) central proline-rich domain is involved in cell signaling and binding to actin and tubulin<sup>30</sup>. There is also the

(3) microtubule-binding repeat domain (MTBD), whose primary function is binding to microtubule<sup>31</sup> and (4) the C-terminal projection domain that contributes to binding to tubulin and the plasma membrane<sup>30</sup>. The phosphorylation of specific amino acids in tau protein is essential for the molecule to exert its physiological function, and about 45 tau phosphorylation sites were described in AD brains<sup>10</sup>.

Tau protein is found inside the neurons, where stabilizes the microtubules and forms NFT on its hyperphosphorylated level. Furthermore, on AD, as on other tauopathies, abnormal hyperphosphorylation and aggregation of tau protein, which results in the NFT formation mainly in the hippocampus and entorhinal cortex, may trigger neuronal death. Tau sets a relationship with tubulin promoting its composition with microtubules and helping to stabilize its structure<sup>32</sup>. In the human brain, the tau pre-mRNA alternative splicing results in six molecular isoforms of the protein. These six tau isoforms differ in three (3R tau) or four (4R tau) microtubule-binding repeats (R) of 31-32 amino acids in the carboxy-terminal half and one (1N), two (2N), or zero (0N) amino-terminal inserts of 29 amino acids each; the extra repeat in 4R tau is the second repeat (R2) of 4R tau<sup>32</sup>.

NFT, together with senile plaques, comprises one of the main AD hallmarks, as already mentioned in this review. NFT isolation was first made in 1974 from frozen autopsied brains, identifying a ~50kDa protein as their main constituent<sup>33</sup>. In the following year, this molecule was

characterized as the tau protein from microtubules<sup>34</sup>, and more than ten years after, abnormally hyperphosphorylated tau (p-tau) was determined as the AD-associated protein in NTF<sup>35</sup> from neurons' cytosol, forming oligomers that inhibit microtubule assembly<sup>33</sup>. Several laboratories generated polyclonal and monoclonal, leading to the discovery of elevated levels of tangle immunoreactivity in CSF from AD patients<sup>36</sup>. Immunohistochemical staining of AD brain sections with anti-p-tau allowed identifying the six stages of neurofibrillary pathology, known as the 'Braak stages'<sup>37</sup>. Further, gene cloning and primary structure identification proved that alternative splicing led to the formation of six isoforms of tau in human brains<sup>38</sup> and the discovery that the expression of truncated tau isoforms produced NFT that generated neurocognitive impairment<sup>39</sup>.

Quantification of hyperphosphorylated and non-hyperphosphorylated pools of tau revealed that total tau (t-tau) levels were increased in the brains of AD people, with no significant differences of non-hyperphosphorylated tau levels between AD and normal aged brains<sup>40</sup>. These results revealed that affected neurons continue to synthesize tau to maintain their function in the presence of AD pathology.

Furthermore, p-tau presents higher specificity than other validated AD CSF biomarkers, t-tau and A $\beta$ , to characterize the NFT burden in AD, probably because, unlike t-tau, it is unaffected by possible comorbidities such as brain injury or stroke. Moreover, p-tau does not correlate with other potential tauopathies, such as frontotemporal lobar

degeneration, which is difficult to distinguish from AD clinically<sup>41</sup>. These findings provided the basis for investigating tau levels in CSF as an AD diagnostic biomarker<sup>42</sup>.

Following these researches, it was confirmed that CSF tau provides a valuable marker of tau pathology and can be used to monitor the efficacy of disease-modifying therapeutics<sup>42</sup>. Extensive evidence indicates increased CSF p-tau in patients with AD compared to controls<sup>16</sup> and correlates with cognitive impairment better than A $\beta$ -related biomarkers<sup>43,44</sup>, being useful for disease staging and as a prognostic biomarker<sup>45</sup>, accurately predicting progression from cognitively unimpaired to MCI and AD dementia<sup>46</sup>. Notably, CSF p-tau is an indicator of the preclinical and prodromal phases of the AD continuum, with an increase of p-tau concentrations detectable around 15 years before symptom onset<sup>47</sup>.

The aggregated p-tau in NFT can have hyperphosphorylation at different amino acid residues. In CFS, the most common tau phosphorylation site is threonine-181. This phosphorylation site is in the middle region of the protein, where most of the available p-tau antibody-based tests are directed<sup>48</sup>. Other phosphorylation sites, such as p-tau 217 and 231, increase early in the AD continuum's preclinical stage, probably in response to subtle pathology<sup>48</sup>. These discoveries stimulated research related to examining these biomarkers in fluids representing more accessible

collection, cost-effective and triage-suitable sources, such as blood.

### **Blood-based p-tau recent studies and their significance for the early diagnosis of AD**

Recent studies suggest that blood levels of p-tau isoforms have discriminative accuracy for AD and other neurodegenerative diseases<sup>49</sup>. Recently, new technologies such as SIMOA, cited previously in this review, have supported the detection of tau at very low concentrations (femtomolar) in the blood. Using SIMOA technology, several studies showed higher plasma tau levels in patients with neurological disorders compared to cognitively healthy controls. These results were promising, although they typically showed a relatively high degree of overlap in plasma tau concentrations between AD and controls and weak correlations of plasma tau with CSF total tau and p-tau levels in AD<sup>50</sup>.

So far, mainly two p-tau isoforms have been identified in blood as potential AD-related biomarkers: p-tau 181 and 217. Plasma p-tau 181 increases in preclinical AD<sup>48</sup> and further in the stages of MCI and AD, predicting and differentiating AD from other neurodegenerative disorders<sup>50-52</sup>. Moreover, there is growing evidence that plasmatic p-tau 181 can differentially diagnose AD from other neurodegenerative diseases, including frontotemporal dementia<sup>51</sup>.

In a longitudinal cohort study that included data from blood sampling performed for up to 8 years, plasma p-tau181 and NFL measurements from 1113 participants, including cognitively unimpaired, as well as patients with MCI and AD and also measures and FDG-PET or structural MRI scans showed that both p-tau181 and NFL were independently associated with cognition and neurodegeneration in brain regions typically affected in AD. Mediation analyses found that about 25% to 45% of plasma p-tau181 outcomes on cognition measures were mediated by the neuroimaging-derived markers of neurodegeneration, suggesting links between plasma p-tau181 and cognition, independent of these measures. Moreover, in this recent study, unlike NFL, plasma p-tau 181 was AD-specific, suggesting the potential of plasma p-tau 181 to monitor AD progression in clinical practice and treatment trials<sup>53</sup>.

Plasma p-tau 217 accurately predicted AD pathology in symptomatic stages and therefore can be considered a biomarker for discriminating AD from healthy control individuals and patients with other neurodegenerative diseases<sup>54,55</sup>. Notably, in a longitudinal cohort study of 490 individuals without dementia, plasma p-tau 217 levels were elevated in A $\beta$ -positive cognitively unimpaired participants before insoluble tau aggregates became detectable by tau-PET. In this same study, modeling approaches predicted that both plasma and CSF p-tau 217 increased before tau-PET in the entorhinal cortex, followed by more widespread cortical tau-PET changes<sup>54</sup>. Notably, a study in which p-tau 217 was

measured repeatedly for up to six years in A $\beta$ -positive but cognitively unimpaired people and prodromal A $\beta$ -positive MCI have shown increased p-tau 217 plasma levels, compared to A $\beta$ -negative cognitively unimpaired and A $\beta$ -negative MCI. MCI patients who later converted to AD had increased plasma p-tau 217, compared to other MCI patients. P-tau 217 did not change in A $\beta$ -negative participants or in patients with MCI who did not convert to AD. In addition, longitudinal increases in p-tau 217 correlated with longitudinal worsening of cognition and brain atrophy. In summary, these longitudinal data shows that plasma p-tau 217 increases during early AD and, therefore, can be a powerful tool to monitor disease progression<sup>56</sup>. All these discoveries indicate that, as well as CSF p-tau 181 and 217, plasmatic p-tau isoforms are useful AD biomarkers, mainly due to low invasiveness and cost. The main discoveries on plasmatic p-tau as AD biomarker are summarized in Table 2.

Table 2. Sensitivities and specificities of blood p-tau 181 and 217 to correctly diagnose AD.

Phosphorylation site	Sensitivity (%)	Specificity (%)	Publication
p-tau 181	43.6 - 75.3	46.7 - 91.1	57
p-tau 217	83	93	55

## CONCLUSIONS

This review aimed to describe the recent advances on different p-tau isoforms as a blood-based biomarker for AD, provide a more comprehensive and detailed exploration of



these biomarkers, and contribute to the advance of the literature on the theme.

The results presented in this review support the two isoforms of plasma p-tau 181 and 217 have the potential to be considered simple, accessible, and scalable test for screening and AD diagnosis, mainly due to their ability to differentiate AD patients from cognitively healthy participants. Hence the advantages of more accurate and sensitive diagnostic tests for the different p-tau isoforms. These biomarkers are important for AD diagnosis and monitoring the disease progression and treatment, especially when added to CSF tau measures.

Taken together, the data presented here pointed out that, despite more research is needed to reproduce these results in large and distinct cohorts, the ability to receive diagnostic and prognostic information using the convenience of a blood test will completely change AD research, treatment, and care from what we have nowadays. Therefore, we emphasize the considerable ethical and social challenges that blood tests for AD will bring to the future, especially considering the absence of disease-modifying therapy for the disease.

## REFERENCES

- 1.Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, *et al*. Global prevalence of dementia: a Delphi consensus study. *Lancet* 2005;366:2112-7. [https://doi.org/10.1016/S0140-6736\(05\)67889-0](https://doi.org/10.1016/S0140-6736(05)67889-0)
- 2.Prince M, Comas-Herrera A, Knapp M, Guerchet M, Karagiannidou M. World Alzheimer Report 2018: the state of the art of dementia

- research: new frontiers. London: Alzheimer's Disease International; 2018. <https://www.alzint.org/u/WorldAlzheimerReport2018.pdf>
- 3.Wada-Isoe K, Kikuchi T, Umeda-Kameyama Y, Mori T, Akishita M, Nakamura Y, *et al*. ABC Dementia Scale Classifies Alzheimer's Disease Patients into Subgroups Characterized by Activities of Daily Living, Behavioral and Psychological Symptoms of Dementia, and Cognitive Function. *J Alzheimers Dis* 2020;73:383-92. <https://doi.org/10.3233/JAD-190767>
- 4.Liu PP, Xie Y, Meng XY, Kang JS. History and progress of hypotheses and clinical trials for Alzheimer's disease. *Signal Transduct Target Ther* 2019;4:29. <https://doi.org/10.1038/s41392-019-0063-8>
- 5.Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992;256:184-5. <https://doi.org/10.1126/science.1566067>
- 6.Maccioni RB, Farias G, Morales I, Navarrete L. The revitalized tau hypothesis on Alzheimer's disease. *Arch Med Res* 2010;41:226-31. <https://doi.org/10.1016/j.arcmed.2010.03.007>
- 7.Kametani F, Hasegawa M. Reconsideration of Amyloid Hypothesis and Tau Hypothesis in Alzheimer's Disease. *Front Neurosci* 2018;12:25. <https://doi.org/10.3389/fnins.2018.00025>
- 8.Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002;297:353-6. <https://doi.org/10.1126/science.1072994>
- 9.Barbier P, Zejneli O, Martinho M, Lasorsa A, Belle V, Smet-Nocca C, *et al*. Role of Tau as a Microtubule-Associated Protein: Structural and Functional Aspects. *Front Aging Neurosci* 2019;11:204. <https://doi.org/10.3389/fnagi.2019.00204>
- 10.Merezhko M, Uronen RL, Huttunen HJ. The Cell Biology of Tau Secretion. *Front Mol Neurosci* 2020;13:569818. <https://doi.org/10.3389/fnmol.2020.569818>
- 11.McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 1984;34:939-44. <https://doi.org/10.1212/wnl.34.7.939>
- 12.McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr., Kawas CH, *et al*. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:263-9. <https://doi.org/10.1016/j.jalz.2011.03.005>
- 13.Mattsson-Carlgrén N, Palmqvist S, Blennow K, Hansson O. Increasing the reproducibility of fluid biomarker studies in neurodegenerative studies. *Nat Commun* 2020;11:6252. <https://doi.org/10.1038/s41467-020-19957-6>
- 14.Rosen C, Hansson O, Blennow K, Zetterberg H. Fluid biomarkers in Alzheimer's disease - current concepts. *Mol Neurodegenerat* 2013;8:20. <https://doi.org/10.1186/1750-1326-8-20>

15. Daneman R, Prat A. The blood-brain barrier. *Cold Spring Harbor Perspect Biol* 2015;7:a020412. <https://doi.org/10.1101/cshperspect.a020412>
16. Olsson B, Lautner R, Andreasson U, Ohrfelt A, Portelius E, Bjerke M, *et al.* CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol* 2016;15:673-84. [https://doi.org/10.1016/S1474-4422\(16\)00070-3](https://doi.org/10.1016/S1474-4422(16)00070-3)
17. Frisoni GB, Boccardi M, Barkhof F, Blennow K, Cappa S, Chiotis K, *et al.* Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. *Lancet Neurol* 2017;16:661-76. [https://doi.org/10.1016/S1474-4422\(17\)30159-X](https://doi.org/10.1016/S1474-4422(17)30159-X)
18. Wright BL, Lai JT, Sinclair AJ. Cerebrospinal fluid and lumbar puncture: a practical review. *J Neurol* 2012;259:1530-45. <https://doi.org/10.1007/s00415-012-6413-x>
19. Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, *et al.* CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* 2009;302:385-93. <https://doi.org/10.1001/jama.2009.1064>
20. Chetelat G, Arbizu J, Barthel H, Garibotto V, Law I, Morbelli S, *et al.* Amyloid-PET and (18)F-FDG-PET in the diagnostic investigation of Alzheimer's disease and other dementias. *Lancet Neurol* 2020;19:951-62. [https://doi.org/10.1016/S1474-4422\(20\)30314-8](https://doi.org/10.1016/S1474-4422(20)30314-8)
21. Ossenkoppele R, Rabinovici GD, Smith R, Cho H, Scholl M, Strandberg O, *et al.* Discriminative Accuracy of [18F]florbetapir Positron Emission Tomography for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA* 2018;320:1151-62. <https://doi.org/10.1001/jama.2018.12917>
22. Blennow K, Mattsson N, Scholl M, Hansson O, Zetterberg H. Amyloid biomarkers in Alzheimer's disease. *Trends Pharmacol Sci* 2015;36:297-309. <https://doi.org/10.1016/j.tips.2015.03.002>
23. Zhang S, Han D, Tan X, Feng J, Guo Y, Ding Y. Diagnostic accuracy of 18 F-FDG and 11 C-PIB-PET for prediction of short-term conversion to Alzheimer's disease in subjects with mild cognitive impairment. *Int J Clin Pract* 2012;66:185-98. <https://doi.org/10.1111/j.1742-1241.2011.02845.x>
24. Ewers M, Mattsson N, Minthon L, Molinuevo JL, Antonell A, Popp J, *et al.* CSF biomarkers for the differential diagnosis of Alzheimer's disease: A large-scale international multicenter study. *Alzheimers Dement* 2015;11:1306-15. <https://doi.org/10.1016/j.jalz.2014.12.006>
25. Nadebaum DP, Krishnadas N, Poon AM, Kalff V, Lichtenstein M, Villemagne VL, *et al.* A head-to-head comparison of cerebral blood flow SPECT and (18) F-FDG PET in the diagnosis of Alzheimer's Disease. *Intern Med J* 2020:1-23. <https://doi.org/10.1111/imj.14890>
26. Clark CM, Pontecorvo MJ, Beach TG, Bedell BJ, Coleman RE, Doraiswamy PM, *et al.* Cerebral PET with florbetapir compared with neuropathology at autopsy for detection of neuritic amyloid-beta plaques: a prospective cohort study. *Lancet Neurol* 2012;11:669-78. [https://doi.org/10.1016/S1474-4422\(12\)70142-4](https://doi.org/10.1016/S1474-4422(12)70142-4)

27. Hampel H, O'Bryant SE, Molinuevo JL, Zetterberg H, Masters CL, Lista S, *et al.* Blood-based biomarkers for Alzheimer disease: mapping the road to the clinic. *Nat Rev Neurol* 2018;14:639-52. <https://doi.org/10.1038/s41582-018-0079-7>
28. De Meyer S, Schaevebeke JM, Verberk IMW, Gille B, De Schaepdryver M, Lockett ES, *et al.* Comparison of ELISA- and SIMOA-based quantification of plasma Aβ ratios for early detection of cerebral amyloidosis. *Alzheimers Res Ther* 2020;12:162. <https://doi.org/10.1186/s13195-020-00728-w>
29. Derisbourg M, Leghay C, Chiappetta G, Fernandez-Gomez FJ, Laurent C, Demeyer D, *et al.* Role of the Tau N-terminal region in microtubule stabilization revealed by new endogenous truncated forms. *Scientific Rep* 2015;5:9659. <https://doi.org/10.1038/srep09659>
30. Arendt T, Stieler JT, Holzer M. Tau and tauopathies. *Brain Res Bull* 2016;126:238-92. <https://doi.org/10.1016/j.brainresbull.2016.08.018>
31. Guo T, Noble W, Hanger DP. Roles of tau protein in health and disease. *Acta Neuropathol* 2017;133:665-704. <https://doi.org/10.1007/s00401-017-1707-9>
32. Iqbal K, Liu F, Gong CX, Grundke-Iqbal I. Tau in Alzheimer disease and related tauopathies. *Curr Alzheimer Res* 2010;7:656-64. <https://doi.org/10.2174/156720510793611592>
33. Iqbal K, Liu F, Gong CX. Tau and neurodegenerative disease: the story so far. *Nature reviews Neurology* 2016;12:15-27. <https://doi.org/10.1038/nrneurol.2015.225>
34. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW. A protein factor essential for microtubule assembly. *Proceed Nat Acad Sci USA* 1975;72:1858-62. <https://doi.org/10.1073/pnas.72.5.1858>
35. Kosik KS, Joachim CL, Selkoe DJ. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. *Proceed Nat Acad Sci USA* 1986;83:4044-8. <https://doi.org/10.1073/pnas.83.11.4044>
36. Mehta PD, Thal L, Wisniewski HM, Grundke-Iqbal I, Iqbal K. Paired helical filament antigen in CSF. *Lancet* 1985;2:35. [https://doi.org/10.1016/s0140-6736\(85\)90074-1](https://doi.org/10.1016/s0140-6736(85)90074-1)
37. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991;82:239-59. <https://doi.org/10.1007/BF00308809>
38. Goedert M, Spillantini MG, Potier MC, Ulrich J, Crowther RA. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *Embo J* 1989;8:393-9. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC400819/pdf/emboj00126-0065.pdf>
39. Zilka N, Filipcik P, Koson P, Fialova L, Skrabana R, Zilkova M, *et al.* Truncated tau from sporadic Alzheimer's disease suffices to drive

- neurofibrillary degeneration in vivo. *FEBS Lett* 2006;580:3582-8. <https://doi.org/10.1016/j.febslet.2006.05.029>
40. Khatoon S, Grundke-Iqbal I, Iqbal K. Brain levels of microtubule-associated protein tau are elevated in Alzheimer's disease: a radioimmuno-slot-blot assay for nanograms of the protein. *J Neurochem* 1992;59:750-3. <https://doi.org/10.1111/j.1471-4159.1992.tb09432.x>
41. Rabinovici GD, Miller BL. Frontotemporal lobar degeneration: epidemiology, pathophysiology, diagnosis and management. *CNS Drugs* 2010;24:375-98. <https://doi.org/10.2165/11533100-000000000-00000>
42. Vandermeeren M, Mercken M, Vanmechelen E, Six J, van de Voorde A, Martin JJ, *et al.* Detection of tau proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme-linked immunosorbent assay. *J Neurochem* 1993;61:1828-34. <https://doi.org/10.1111/j.1471-4159.1993.tb09823.x>
43. Aschenbrenner AJ, Gordon BA, Benzinger TLS, Morris JC, Hassenstab JJ. Influence of tau PET, amyloid PET, and hippocampal volume on cognition in Alzheimer disease. *Neurology* 2018;91:e859-66. <https://doi.org/10.1212/WNL.0000000000006075>
44. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol* 2010;6:131-44. <https://doi.org/10.1038/nrneurol.2010.4>
45. Schindler SE, Li Y, Todd KW, Herries EM, Henson RL, Gray JD, *et al.* Emerging cerebrospinal fluid biomarkers in autosomal dominant Alzheimer's disease. *Alzheimers Dement* 2019;15:655-65. <https://doi.org/10.1016/j.jalz.2018.12.019>
46. Ferreira D, Rivero-Santana A, Perestelo-Perez L, Westman E, Wahlund LO, Sarria A, *et al.* Improving CSF Biomarkers' Performance for Predicting Progression from Mild Cognitive Impairment to Alzheimer's Disease by Considering Different Confounding Factors: A Meta-Analysis. *Front Aging Neurosci* 2014;6:287. <https://doi.org/10.3389/fnagi.2014.00287>
47. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, *et al.* Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 2012;367:795-804. <https://doi.org/10.1056/NEJMoa1202753>
48. Suarez-Calvet M, Karikari TK, Ashton NJ, Lantero Rodriguez J, Mila-Aloma M, Gispert JD, *et al.* Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in Aβ pathology are detected. *EMBO Mol Med* 2020;12:e12921. <https://doi.org/10.15252/emmm.202012921>
49. Shen XN, Li JQ, Wang HF, Li HQ, Huang YY, Yang YX, *et al.* Plasma amyloid, tau, and neurodegeneration biomarker profiles predict Alzheimer's disease pathology and clinical progression in older adults without dementia. *Alzheimers Dement (Amst)* 2020;12:e12104. <https://doi.org/10.1002/dad2.12104>

50. Fossati S, Ramos Cejudo J, Debure L, Pirraglia E, Sone JY, Li Y, *et al.* Plasma tau complements CSF tau and P-tau in the diagnosis of Alzheimer's disease. *Alzheimers Dement (Amst)* 2019;11:483-92. <https://doi.org/10.1016/j.dadm.2019.05.001>
51. Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, *et al.* Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol* 2020;19:422-33. [https://doi.org/10.1016/S1474-4422\(20\)30071-5](https://doi.org/10.1016/S1474-4422(20)30071-5)
52. Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, *et al.* Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement* 2018;14:989-97. <https://doi.org/10.1016/j.jalz.2018.02.013>
53. Moscoso A, Grothe MJ, Ashton NJ, Karikari TK, Lantero Rodriguez J, Snellman A, *et al.* Longitudinal Associations of Blood Phosphorylated Tau181 and Neurofilament Light Chain With Neurodegeneration in Alzheimer Disease. *JAMA Neurol* 2021;78:396-406. <https://doi.org/10.1001/jamaneurol.2020.4986>
54. Janelidze S, Berron D, Smith R, Strandberg O, Proctor NK, Dage JL, *et al.* Associations of Plasma Phospho-Tau217 Levels With Tau Positron Emission Tomography in Early Alzheimer Disease. *JAMA Neurol* 2020;78:149-56. <https://doi.org/10.1001/jamaneurol.2020.4201>
55. Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, *et al.* Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA* 2020;324:772-81. <https://doi.org/10.1001/jama.2020.12134>
56. Mattsson-Carlsson N, Janelidze S, Palmqvist S, Cullen N, Svenningsson AL, Strandberg O, *et al.* Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain J Neurol* 2020;143:3234-41. <https://doi.org/10.1093/brain/awaa286>
57. Rizzi L, Maria Portal M, Batista CEA, Missiaggia L, Roriz-Cruz M. CSF Aβ1-42, but not p-Tau181, differentiates aMCI from SCI. *Brain Res* 2018;1678:27-31. <https://doi.org/10.1016/j.brainres.2017.10.008>