Progress regarding the context-of-use of tau as biomarker of Alzheimer's disease and other neurodegenerative diseases

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Abstract

Introduction Tau protein misfolding and accumulation in toxic species is a critical pathophysiological process of Alzheimer's Disease (AD) and other neurodegenerative disorders (NDDs). Tau biomarkers, namely cerebrospinal fluid (CSF) total-tau (t-tau), 181-phosphorylated tau (p-tau) and tau--PET tracers, have been recently embedded in the diagnostic criteria for AD. Nevertheless, the role of tau as a diagnostic and prognostic biomarker for other NDDs ~~still~~ remains controversial.

Areas covered We performed a systematical PubMed-based~~ly~~ review of~~ed~~ the most recent advances in tau-related biomarkers for NDDs. We focused on papers published ~~in the last five years~~from 2015 to 2020~~,~~ assessing the diagnostic or prognostic value of each biomarker.

Expert opinion The assessment of tau biomarkers in alternative easily accessible matrices, through the development of ultrasensitive techniques, represents the most significant perspective for AD-biomarker research. In NDDs, novel tau isoforms (~~eg~~.e.g., ~~217p-tau~~p-tau217) or proteolytic fragments (~~e.g.~~e.g., N-terminal fragments) may represent candidate diagnostic and prognostic biomarkers and may help monitoring disease progression. Protein misfolding amplification assays, allowing the identification of different tau strains (e.g. 3R- *vs.* 4R-tau) in CSF, may constitute a breakthrough for the in vivo stratification of NDDs. Tau-PET may help tracking the spatial-temporal evolution of tau pathophysiology in AD but its application outside the AD-spectrum deserves further studies.~~-~~

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Article highlights

- Tau protein misfolding and accumulation in toxic species and tangles is a critical pathophysiological process of Alzheimer's Disease (AD) and other neurodegenerative disorders (NDDs);
- Cerebrospinal fluid (CSF) total tau (t-tau) and phosphorylated tau (p-tau) are included in the current diagnostic criteria for AD; in primary tauopathies and in other NDDs (ALS-FTD spectrum and ~~synucleinopathies~~α-synucleinopathies) the role of tau as a diagnostic and prognostic biomarker is still controversial;
- The quantification of t-tau and p-tau and of emerging tau biomarkers in plasma by using ultrasensitive techniques are gaining momentum in the AD diagnostic workup;
- Novel tau fragments and tau isoforms detectable by means of ultrasensitive methods including Protein Misfolding Cyclic Amplification (PMCA) and Real-time quaking-induced conversion (RT-QuIC) may improve the in vivo stratification of NDDs;
- Tau-PET tracers are useful tools for the *in vivo* tracking of the spatial-temporal evolution of tau pathophysiology in AD but further studies are needed to define their proper context-of-use in other tauopathies.

1. Introduction

Tau is a natively unfolded microtubule-associated protein largely expressed in the central nervous system[1]. This protein plays an important role in microtubule polymerization, stabilization and remodeling, and is mainly expressed in the axons, where it also participates in regulating cellular transport processes[2,3]. Tau exists in 6 isoforms generated through the alternative splicing of the exons 2, 3 and 10 of the microtubule-associated protein tau (MAPT) gene[4]. The microtubule-binding domain (MBD) is encoded by the exon 10, whose alternative splicing produces 3 isoforms, each with 3 or 4 repeats, respectively called 3-repeat (3R) and 4-repeat (4R) tau[2]. Under pathological conditions, mutations in the MAPT gene along with a hyper-phosphorylation of tau may reduce the affinity of the MBD to the microtubules[5]. Accordingly, tau free-protein concentrations increase to a tipping point after which misfolded protein intermediates self-assembly into unwanted oligomers, fibrils, and neurofibrillary tangles (NFT)[5].

The neuronal and/or glial accumulation of aberrant tau aggregates represents the pathological hallmark of a broad spectrum of neurodegenerative disorders (NDDs) called tauopathies [6]. These include the so-called primary tauopathies, a group of NDDs, in which tau aggregates, including NFT, represents the main pathological hallmark ~~that drives the neurodegenerative process~~. Primary tauopathies can be classified into 3R-~~,~~ tauopathies, 4R-tauopathies and~~,~~ mixed 3R/4R tauopathies. The major clinical phenotypes belong to the spectrum of Progressive Supranuclear Palsy (PSP), Corticobasal Degeneration (CBD) and Frontotemporal Lobar Degeneration with tau deposits (FTLD-Tau) ~~Frontotemporal lobar Degeneration associated with MAPT mutations (FTLD-MAPT)~~ [2,6]. Secondary tauopathies are conditions resulting ~~by~~ from a complex interplay between tau and other misfolded proteins (e.g., amyloid-β - ~~(~~Aβ~~)~~) or related to alternative causes (e.g., autoimmune processes). Alzheimer's Disease (AD) represents the best characterized and more intensively studied NDDs and is classified as secondary tauopathy[2,7]. More recently, other forms of secondary tauopathies have been described including~~,~~ chronic traumatic encephalopathy (CTE) and the autoimmune~~,~~ anti-IgLON5 encephalitis [2]. Tau cytoplasmic inclusions have also been reported in ~~the Amyotrophic lateral sclerosis –~~ Frontotemporal Dementia (FTD) ~~(ALS-FTD) spectrum~~; up to 30% of patients with the behavioral variant of frontotemporal dementia (bvFTD) and the majority (approximately 90%) of patients with the non-fluent variant of Primary progressive aphasia (nfvPPA) show tau pathologic depositions [8]. Also in α-synucleinopathies, where aberrant α-synuclein aggregates underpin the neurodegenerative mechanisms, NFT are likely to cause neurodegeneration and contribute to the disease burden [9,10].

Tau bBiomarkers ~~tracking tau pathology~~ are included in the diagnostic workup of AD according to the 2011 National Institute on Aging – Alzheimer's Association (NIA-AA), the 2014 International Working Group-2 (IWG-2) criteria and ~~to~~ the AT(N) classification system [11,12]. In 2018 the AT(N) scheme embed biomarkers of amyloid-β-related pathology (A), tau-pathology (T) and other markers of neurodegeneration (N) [13]. This classification includes ~~validated biomarkers to track tau pathology in AD such as~~ cerebrospinal fluid (CSF) total-tau (t-tau), ~~and 181-phosphorylated-tau (p-tau), all increased in AD individuals,~~ as biomarker~~s~~ of neurodegeneration (N) and 181-phosphorylated-tau (p-tau181 or simply p-tau), as biomarker of tau pathology [13]. These biomarkers are all increased in AD individuals. The AT(N) categorization for the first time added cerebral tau- positron emission tomography (PET) as core biomarker of AD. Indeed, the implementation of tau-PET tracers represents a recent step forward in the AD diagnostic workup [13].

Currently, the *ante-mortem* diagnosis of non-AD NDDs mainly relies on pure clinically based criteria. In this scenario, the diagnostic and prognostic value of tau protein in the spectrum of primary tauopathies remains unsatisfactory when measured with traditional approaches (e.g. ELISA) [14]. In fact, a biomarkers-based diagnosis is still an unmet need in NDDs and several methodological challenges should be addressed to overcome this limitation. In the last years, the use of ultrasensitive assays, the discovery of alternative tau byproducts in biofluids, and the development of innovative techniques, enabling the differentiation of tau strains, have represented potential breakthroughs for the *in vivo* dissection of tau pathology spectrum.

The validation of biomarkers tracking neurodegenerative processes and predicting pathophysiological progression at the earliest molecular/cellular changes is an unmet need for the early diagnosis, the prognostic evaluation and –for patients selection and follow-up in targeted-therapy~~ies~~ clinical trials ~~the monitoring of targeted-therapies in clinical trials~~ [15].

We aim to review the current state of the art on the use of tau protein as a biomarker for NDDs. First, we investigated recent advances of this fluid biomarker in the diagnostic and prognostic workup of AD and other NDDs. Secondly, we evaluated the contribution of cerebral PET-imaging in tracking tau pathology across the NDDs spectrum.

2. Literature search methods

The aim of the present work is to review the most recent advances on tau as a biomarker for neurodegenerative diseases. A systematic PubMed-based literature search was performed to select relevant papers assessing the diagnostic and/or prognostic value of plasma tau (both t-tau, p-tau, and other tau fragments) in AD and other NDDs, as evaluated through novel ultrasensitive

techniques and of CSF tau in other neurodegenerative diseases. We screened the papers using the following combination of keywords:

- for plasma tau in AD and other NDDs: ((("plasma" OR "blood" OR "serum") AND ("tau") AND ("Alzheimer" OR "Corticobasal degeneration" OR "Progressive Supranuclear Palsy" OR "Amyotrophic Lateral Sclerosis" OR "Frontotemporal Dementia" OR "Parkinson" OR "Multiple System Atrophy" OR "Lewy Body"));
- for CSF tau in NDDs (AD excluded): ((("CSF") AND ("tau") AND ("Corticobasal Degeneration" OR "Progressive Supranuclear Palsy" OR "Amyotrophic Lateral Sclerosis" OR "Frontotemporal Dementia" OR "Parkinson's Disease" OR "Multiple System Atrophy" Or "Dementia With Lewy Bodies")).

Only papers written in English, assessing the prognostic and/or diagnostic value of tau and published between 2015 and 2020, were included in the final analysis. A total number of 1480 and 297 articles for plasma tau and CSF tau respectively were initially screened. Fourteen papers on plasma tau and 14 on CSF tau were retained in the final analysis (Fig.2).

For each study, we analyzed the study population, the technique used for biomarkers detection, the diagnostic and/or prognostic performance of the investigated biomarker. Given the high heterogeneity of the papers included, we used the AuROC values as a concise measure of diagnostic accuracy to compare different studies. The diagnostic value of each marker to properly allocate patients to different diagnostic groups was identified when available and classified as follows: 'excellent' (area under ROC curve [AuROC] 0.90–1.00), 'good' (AuROC: 0.80–0.89), 'fair' (AuROC: 0.70–0.79), 'poor' (AuROC: 0.60–0.69), or 'fail' (i.e., no discriminatory capacity) (AuROC: 0.50–0.59) [16]. We used hazard ratios (HR) to compare the prognostic values. Possible comparisons across studies are only qualitative, and not based on statistical analysis.

3. Fluid biomarkers tracking tau-pathology: from conventional to ultrasensitive techniques

Fluid biomarkers represent promising tools for the in vivo tracking of early pathophysiological changes in NDDs. Their detection and quantification in different matrices and related technical issues are regarded as hot topics in biomarkers research[14].

Tau quantification in CSF represents the first and most widely validated approach for the in vivo ~~assessment~~ detection of tau protein ~~pathology~~. Tau, quantified in the CSF both as t~~otal~~-tau and ~~181-phosphorylated-tau (~~p-tau181~~)~~ currently belongs to the core AD biomarkers, along with amyloid-β_{42} ($A\beta_{42}$), and its assessment is based on traditional Enzyme-linked immunoabsorbent assay (ELISA) techniques [11–13]. Accordingly, several commercial kits are to date available for both clinical and research purposes~~, including the INNOTEST®hTAU Ag and the INNOTEST PHOSPHO-TAU~~

[17]. Even if broadly used and largely validated, standard tau quantification techniques present three main limitations: 1) they are unable to effectively quantify tau in "peripheral" matrices (e.g., plasma), where its concentration is significantly low [18], 2) they fail in detecting alternative tau species (e.g., p-~~p217-~~tau-217) 3) are ineffective in differentiating tau isoforms (e.g., 3R and 4R tau), seeds (e.g., tau fibrils), based on their tridimensional conformation [19].

Traditional methods show unsatisfactory and suboptimal power in differentiating patients with NDDs from healthy controls (HC) ~~individuals~~ when applied to biological matrices other than CSF (e.g., blood), where these molecules are expressed at femtomolar ranges instead of picomoles [20]. The quantification of tau in ~~unconventional~~ non-CSF substrates is challenging because the concentration of the analyte may not reflect primary neurodegenerative changes. ~~Unpredictable v~~Variations in tau concentrations could be due to peripheral proteolytic processes, metabolic clearance, the interaction with other proteins and modifications of the of brain blood barrier permeability[21].

Novel techniques, developed and validated in the last five years, show better sensitivity in measuring tau levels than traditional methods by detecting up to subpicomolar and even subfemtomolar concentrations. These techniques mostly address the N-terminal rather than mid-region of tau. Single Molecule Array (Simoa), a fully-automated ELISA relying on antibody-coated magnetic beads, has been used to assess t-tau, p-tau [22–24] and N-terminal tau fragments [25] in blood samples of AD patients (Table 1). On the other hand, Electrochemiluminescence immunoassays (ECLIA) provide a quantification of t-tau [26], p-tau181 [27–29] and ~~p217-taup~~-tau217 [30,31] in patients with AD, with high accuracy even in low sample volumes (Table 1). An upgrade of standard mass spectrometry (MS), combining immunoprecipitation reactions with MS [32,33], tracks different phosphorylated tau isoforms like ~~p217-taup~~-tau217 and p-tau181 in plasma [33](Table 1). Also immunomagnetic reaction (IMR) – an antibody-mediated reaction generating changes in the magnetic fields that enable the quantification of the analyte [34,35] – detects t-tau [36–39] and p-tau181 [37,39]– concentrations with a greater sensitivity compared to traditional techniques, but with suboptimal specificity (Table 1).

However, these ultrasensitive techniques fail in discriminating different tridimensional conformations of tau (e.g., 3R vs 4R tau~~ seeds~~). Nevertheless, the selective identification of different tau seeds represents a critical issue to understand the pathophysiology of the primary tauopath~~iesy spectrum~~. Based also on the hypothesis of a prion-like transcellular propagation of tau, real-time quaking-induced conversion (RT-QuIC) and protein misfolded cycle amplification (PMCA) have been successfully applied in NDDs [40]. These techniques were initially developed for the detection of prion proteins in ~~Creutzfeld~~Creutzfeldt-Jakob disease. RT-QuIC uses

recombinant protein substrates undergoing shaking and incubation cycles to quantify seeding activity of prions (e.g. PrP) and prion-like proteins, such as α-synuclein (α-syn) and tau strains [41]. Similarly, in PMCA, seeding activity of substrates derived from brain homogenates or recombinant proteins is amplified by cycles of sonification, enabling a sensitive detection and a selective amplification of specific protein seeds [40].

More extensive research is necessary for the analytical and clinical validation of these approaches by clarifying advantages and drawbacks. Finally, an accurate qualification process, mostly based on clinical performance, will be required to define context(s)-of-use for pharmacological trials and clinical practice.

4. Advances in tau as a biomarker for Alzheimer's Disease: assessing novel matrices, techniques and targets

T-tau and p-tau proteins measured in CSF are core biomarkers of neurodegeneration in AD [11,12]. ~~However, their contribution in the identification of non-AD tauopathies is limited.~~ The finding of alternative tau isoforms specific for the different ~~tauopathies~~ clinical and pathological AD subtypes may represent a significant advancement, in parallel with the recent development of ultrasensitive techniques that allow the measurement of tau proteins in ~~the~~ blood.

To overcome several technical issues including the higher dilution of tau biomarkers in blood *vs.* CSF [42], several antibody-driven assays have been combined with different detection systems reaching subpicomolar and even subfemtomolar limits of detection (LOD) [43]. A metanalysis indicated that plasma t-tau is increased in AD [44]. A study performed with IMR techniques showed that plasma t-tau levels discriminated AD from controls with optimal accuracy (Table 2) [36]. By contrast, other studies conducted with Simoa and IMR reported higher plasma t-tau concentrations in AD or Mild Cognitive Impairment (MCI) patients compared to HC, but with consistent overlapping results [27,36,39,45,46]. Furthermore, plasma t-tau is not or only weakly correlated with CSF t-tau, p-tau in different studies [45,47–49]. An inverse association between t-tau and cortical thickness in AD-related areas has been reported [38,46]. Plasma t-tau was associated in AD, MCI and HC with a longitudinal decline of cognitive scores and cerebral 18FDG-uptake at PET imaging, increased ventricular volume, and a decrease of hippocampal volume [47,50]. Combining plasma t-tau into ratios (e.g., with plasma amyloid-β_{1-42}) increases the predictive value of cerebral tau accumulation, as assessed by PET, compared to t-tau alone [22]. On the other hand, plasma t-tau did not predict the amyloid-β ~~-~~status as assessed by cerebral amyloid-PET [27] or CSF [26] in AD, MCI, and HC. Intriguingly baseline plasma t-tau levels seem to be associated with atrophy of the basal forebrain cholinergic system (BFCS) in subjective memory

complainers, regardless of their amyloid-β-status [51], although no differences in plasma t-tau levels emerged between SCD and HC in a further study [48]. Finally, plasma t-tau levels were found to be associated with MAPT H1c haplotype in AD, MCI and HC, according to a genome-wide association study (GWAS) [52].

Full-length (FL) tau may account only for a small part of plasma tau proteins. Different proteolytic fragments of tau have been explored in plasma with ultrasensitive techniques so far. The levels of tau N1 fragments, measured with Simoa, are higher in AD and AD-MCI subjects than HC, showing good to optimal accuracy in discriminating HC from AD and MCI subjects, respectively [25] (Table 2). The actual eligibility of these fragments is still a matter of debate [53].

Conversely, pPlasma p-tau181 seems to be a ~~robust~~ more specific AD biomarker. Plasma p-tau181 concentrations assessed by Simoa, IMR and ECLIA-based techniques are higher in AD and MCI-AD compared to HCs [23,27–29,39]. Plasma p-tau181 distinguishes AD patients from non-AD dementias taken together [28] and from vascular dementia (VaD) with optimal accuracy [23], from FT~~L~~D with good to optimal accuracy [23,29] and from PSP, CBD and Parkinson's disease (PD) or Multiple System Atrophy (MSA) with good accuracy [23] (Table 2). Furthermore, plasma p-tau concentration is consistent with CSF p-tau levels in Aβ-PET positive individuals [28,29]. Plasma p-tau181 predicts the Aβ-PET status with good accuracy and is associated with both Aβ and tau PET positivity in MCI, AD, and HC [27] (Table 2). Moreover, in a cohort of AD, MCI and HC, plasma p-tau181 correlated with ~~the spreading of tauopathy~~ tau deposition within the brain [22].

Tau isoforms other than p-tau181 have been recently studied and showed a potential role in the differential diagnosis of AD from MCI and HC. Plasma p-tau217 was able to differentiate AD (clinically assessed) from non-AD patients and autopsy-confirmed AD from non-AD patients with slightly suboptimal accuracy [30] (Table 2). Plasma p-tau-217 levels predict the amyloid-β- status as assessed by amyloid-PET or CSF Aβ42/Aβ40 ratio with optimal accuracy across preclinical-AD, AD-MCI, moderate AD, non-AD-MCI and HC [33] (Table 2). Furthermore p-tau217 could discriminate amyloid-β positive and tau-PET negative participants from HC and ~~unrevealed its~~has a potential role as an early, preclinical biomarker, being able to assess tau increase before detectable tau aggregation. Both plasma p-tau217 and p-tau181 correlated with their CSF counterparts and were shown to be released from CNS rather than from peripheral sources, thus probably representing more reliable AD biomarkers ~~compared to~~than plasma t-tau and p-tau202 [33]. Moreover, the cerebral Aβ plaques and tau tangles accumulation ~~are~~ is independently associated with *ante-mortem* concentrations of plasma p-tau217 in an autopsy-confirmed cohort [54].

Recently, dynamic trajectories of subsequent site-specific phosphorylation of tau have been reported from preclinical to full-blown AD stages in CSF. While p-tau217 and p-tau181 increase as

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early as two decades before and tend to decline close to the symptomatic stage, the levels of t-tau and p-tau205 increase later (between 17-13 estimated years to symptom onset) and constantly increase along disease progression [55] Also p-tau231 assessed in CSF by using a mid-region target antibody increases in preclinical AD, largely before p-tau181 and p-tau217, when only subtle Aβ pathology is detectable [56]. These preliminary data suggest the potential role of alternative site-specific phosphorylated isoforms of CSF p-tau for the identification of preclinical AD and for the subsequent risk of phenoconversion. Dynamic changes of tau phosphorylation profile may also represent a potential useful biomarker to assess the efficacy of disease modifying therapies in clinical trials but a better understanding of these changes and a more extensive validation of these biomarkers and their potential context--of--use is needed. Furthermore, whether these changes are reflected in plasma and their timing is ana ~~question~~an issue that ~~still needs to~~should be assessed.

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5. The diagnostic and prognostic role of tau as a biomarker in 4R-tauopathies

Tauopathies are pathologically characterized by a predominant intracellular accumulation of hyperphosphorylated tau fibrils [1,2] Primary tauopathies can be divided into 3R-tauopathies, including Pick's Disease (PiD), and 4R-tauopathies like ~~Progressive Supranuclear Palsy (~~PSP~~),~~ ~~Corticobasal Degeneration (~~CBD~~)~~, Argyrophilic Grain Disease (AGD), Globular Glial Tauopathy (GGT). Mixed 3R- and 4R-tauopathies include Primary Age-Related Tauopathy (PART) and neurofibrillary tangles ~~(NFT)~~ dementia[2]. The genetic FTLD due to MAPT mutations may be related either to 3R-, 4R- or 3/4 R-tau pathological patterns [3](Figure 1). The clinical phenotype of these forms is highly heterogeneous and may not reflect the underlying pathology [14,57].

Currently, the diagnosis of 4R-tauopathies relies on the post-mortem detection of disease-specific patterns of abnormal tau aggregates [3]. Nevertheless, the in vivo diagnosis of these disorders is based on purely clinical-based criteria without reliable biomarkers to track the different tau pathologies [3,14,58].

The potential role of CSF tau concentration as a diagnostic and prognostic biomarker in 4R-tauopathies (especially PSP and corticobasal syndrome - C~~S~~BS) has been investigated by several studies since 1997, with conflicting results. The measurement of CSF t-tau and p-tau concentrations is helpful in the diagnostic workup of AD. Indeed the characteristic increase of tau species (t-tau or

p-tau) in the CSF is specific to AD pathology and lacks in non-AD dementias (including PSP and CBS) [59,60]. Further, CSF t-tau and p-tau do not distinguish PD from atypical parkinsonism with enough accuracy (in particular from PSP) [61]. Nevertheless these biomarkers when combined with others in CSF − α-synuclein, Aβ42, neurofilament (NFL) [62], soluble amyloid precursor protein α (sAPPα), soluble amyloid precursor protein β (sAPPβ), monocyte chemoattractant protein-1 (MCP-1), YKL-40 [63] − may discriminate PSP from PD individuals, as well as CBS from PD and AD/FTD though with conflicting results (Table 3). Similarly, p-tau and t-tau CSF concentrations do not differentiate idiopathic normal pressure hydrocephalus (iNPH), a well-known parkinsonian syndrome-mimic, from PSP [64] (Table 3). Nonetheless, the combination of t-tau, Aβ40 and MCP-1 differentiate iNPH from other neurodegenerative movement disorders taken as a whole (e.g. PD, MSA, PSP and CBD irrespective of their underlying pathology) with good accuracy (AuROC: 0.80) [65].

Within the tau-spectrum, a trend towards higher CSF tau (both t-tau and p-tau) levels has been reported in CBS compared to PSP and HCs [66–68]. Accordingly, Aerts and colleagues showed that t-tau and p-tau differentiated CBD from PSP with fair diagnostic accuracy (AuROC: 0.77 and 0.76, respectively) and that increased tau levels were associated with lower Mini Mental State Examination (MMSE) scores [69]. These data ~~could~~ suggest a ~~potential~~ role of tau biomarkers in the differential diagnosis of tauopathies and ~~a potential role~~ in the stratification of different phenotypes (e.g. motor-dominant *vs.* cognitive-dominant). However, these results should be taken with caution since AD-pathology underpins CBS in about 20% of cases, thus representing a potential bias for the correct interpretation of CSF tau levels. Additionally, these data ~~came~~ come from small samples, and ~~patients' selection relyare based on use old~~ obsolete diagnostic criteria ~~for patient selection~~ [58,70].

Tau isoforms may help in differentiating tauopathies from other neuropathologies and to stratify the tau-spectrum. The quantification of alternative tau fragments compared to the classic ones detected by standard ELISA methods is promising [71]. Borroni and colleagues, showed that 33kDa-tau/55kDa-tau ratio discriminate PSP from HCs (AuROC: 0.90) and other NDDs (e.g. AD, CBD, FTD etc.) with ~~a~~ good to excellent diagnostic accuracy ~~ranging from good to excellent~~ (AuROC: 0.93 for AD, AuROC: 0.87 for CBD, AuROC: 0.86 for FTD) [68,72]. These authors reported a lower 33kDa-tau/55kDa-tau ratio, as assessed by semiquantitative immunoprecipitation, in patients with PSP compared to other tauopathies and α-synucleinopathies. They also showed a correlation of this ratio with brainstem atrophy and motor impairment in PSP [68,72]. Other groups did not replicate these results and further investigations are needed [73].

Available commercial kits currently quantify tau concentration targeting its central core region. More recently, alternative tau fragments detected using antibodies recognizing the N-terminal fragment τ12-BT2 has been assessed, showing an excellent diagnostic accuracy in differentiating PSP from AD [74](Table 3). Furthermore, a mass spectrometry-based study (~~(MS,~~ see table 1), identified 18 different CSF tau fragments with divergent patterns of expression in PSP compared to AD and to HCs [71].

In last year the application of RT-QuIC and PCMA technologies may isolate and quantify different tau strains (e.g. 4R and 3R tau) in CSF, thus representing an ideal tool to stratify *in vivo* 3R, 4R and mixed 3R/4R tauopathies [41].

Stepping aside from CSF, ~~only~~ a single study explored the potential discriminating value of plasma p-tau181 in tauopathies, using ultrasensitive detection techniques (Simoa). Plasma p-tau was able to differentiate PSP and CBS from AD with good diagnostic accuracy (Table 2), being associated with Aβ42 pathology assessed by cerebral amyloid-PET examinations [75]. The validation of tau-biomarkers in plasma in large longitudinal cohorts using ultrasensitive techniques is warranted.

Tau biomarkers may have a prognostic context-of-use in 4R-tauopathies. Constantinescu and colleagues showed that t-tau CSF levels may predict mortality in a cohort of PSP patients (see table 3 for the Hazard Ratio) [76]. Increased CSF t-tau levels at baseline are associated with a faster decline as measured by clinical progression scores like the Schwab and England ADL (SEADL); decreased baseline p-tau predicts low SEADL scores and rapid decrease in PSP Rating scale (PSPRS). The prognostic value of tau is increased when combining biomarkers into ratios (e.g., baseline p-tau, t-tau and NFL concentrations) [77]. Currently, the role of tau as prognostic biomarker in 4R-tauopathies is limited and should be clarified in longitudinal studies.

6. The diagnostic and prognostic role of tau in ALS-FTD spectrum

Frontotemporal dementia (FTD) and Amyotrophic lateral sclerosis (ALS) are NDDs with significantly clinical and pathological overlaps.

FTD is a heterogeneous cognitive disorder, recognizing multiple clinical and pathological phenotypes [78,79], related to the degeneration of frontal and temporal lobes [78]. Three main clinical variants of FTD have been described: the behavioral variant of FTD (bvFTD) [80], primary progressive aphasia (PPA) and the motor FTD syndromes (CBS and PSP) [81]. PPA can be further divided into a semantic variant (svPPA) and in a non-fluent variant (nfvPPA) [82]; the logopenic variant of PPA (lvPPA) is conversely considered an atypical AD variant rather than a proper FTD~~LD~~-syndrome [78]. On the other hand, three main pathological FTD phenotypes have been

described, namely FTD with TDP-43 aggregates (FTD-TDP), FTD with tau depositions (FTD-Tau) and FTD with FUS aggregates (FTD-FUS) [8]. It should be noticed that the correlation between the clinical and the pathological phenotype is not always predictable. While nfvPPA is associated in most cases with FTD-tau (85% of all cases) and svPPA with FTD-TDP43 pathology (90% of cases), bvFTD can be associated with both FTD-tau and FTD-TDP43 histological patterns [8].

ALS is progressive NDD characterized by motor symptoms related to the progressive degeneration of the upper (UMN) and lower motoneuron (LMN) [83]. ALS is strictly associated to TDP-43 pathology in about 95% of patients, alternative pathological inclusions (e.g. SOD-1 inclusions) may be reported in specific variants in a minority of cases [83].

The clinical and pathological boundaries between FTD and ALS are blurry and the current diagnostic criteria consider ALS and FTD as a complex clinical *continuum* and pathologic *spectrum* as well [84,85].

In fact, ALS patients show cognitive impairment in about 50% of cases, meeting current criteria for diagnosing dementia in 10-15% of cases [86], and behavioral impairment in up to 45-50% of patients baseline [87]. Conversely, FTD patients may present motor symptoms in approximately 30% of cases showing motor neuron dysfunction (12.5% of bvFTD patients) [88].

The assessment of Tau as a biomarker in the ALS-FTD spectrum is controversial and recent studies on CSF tau showed conflicting results.

Some authors observed a higher concentration of CSF t-tau in FTD patients compared to HC, distinguishing them with a good diagnostic accuracy [89–91], (see Table 3 for AUROC values). On the other hand, other investigations failed in detecting any significant difference [92–95]. These conflicting results may be due to the fact that FTD is a highly heterogeneous diagnostic frame, including different clinical and pathological phenotypes. Inconsistent results have been reported in ALS studies [91,93,96–100].

Regarding the differential diagnosis between FTD and AD, p/t-Tau, t-tau and its ratios with Aβ42 may be of clinical interest; FTD patients show lower levels of t-tau [91,94,95,101] and higher of p/t-Tau [94] when compared to AD patients. Moreover, the ratios between t-tau and Aβ42 are able to distinguish between FTD and AD patients [91,95,101].

The CSF p-Tau and t-Tau ratio (p/t-Tau) is more interesting for the clinical workup. p/t-Tau was observed to be significantly lower in ALS patients [89,96–99] and in FTD patients [89,90,92,102], when compared to HC. Not surprisingly, ALS showed lower levels of CSF p/t-Tau than FTD phenotypes with a likely related tauopathy. This ratio was significantly lower in FTD variants with suspected TDP-related pathology (both patients with autopsy-proven TDP-related pathology and TDP-related mutation carriers) compared to FTD phenotypes with a likely underlying tauopathy

[89,90,92,94,102,103] (Table 3). Low p/t-Tau may therefore represent a marker of an underpinning TDP-43 pathology. From a pathophysiological point of view this could be due to a lower tau burden in TDP-related compared to tau-related FTLD variants. On the other hand, the different proportion of patients with concomitant ~~(~~MND~~)~~ and amyloid-β ~~beta~~ co-pathology in the two groups may contribute to these observed differences [102]. Furthermore p/t-Tau ratio seems to correlate with motor cortex thickness and seems to be associated with UMN involvement in ALS [99]. Accordingly ~~the potential role of~~ p-tau, t-tau ~~and~~or ~~of~~ their ratio may find a context-~~of~~-use in the ~~in~~ differentiation of~~ng~~ clinical endophenotypes within the ALS spectrum (e.g. preponderant UMN *vs.* LMN involvement) but ~~may deserve~~ further investigations are needed.

Different concentrations of CSF tau fragments other than classic tau analytes have been reported by some authors in TDP-related *vs.* tau-related FTLD [90]. This suggests that differential proteolytic processes may occur in different FTLD variants but this hypotesis and its implications for novel biomarkers research deserves further studies and a more extensive assessment [90].

As regards the role of CSF tau ad a prognostic biomarker, one study showed a potential prognostic value of baseline CSF t-tau in ALS, being a reduction of overall survival observed in individuals with higher CSF t-tau values (Table 3) [97]. Moreover p/t-tau ratio has been associated with survival in FTD [102] and disease progression in ALS [100].

Moving to surrogated progression biomarkers, a negative correlation between baseline CSF t-tau and the longitudinal increase of ALS Functional Rating Scale Revised (ALSFRS-R) score was described [100].~~; furthermore p/t-Tau ratio seems to correlate with motor cortex thickness and seems to be associated with UMN involvement in ALS [95]. Accordingly the potential role of p-tau, t-tau and of their ratio in differentiating clinical phenotypes within the ALS spectrum (e.g. preponderant UMN *vs.* LMN involvement) may deserve further investigations.~~

7. The diagnostic and prognostic role of tau in ~~alpha-synucleinopathies~~α-synucleinopathies

Parkinson's disease (PD), Dementia with Lewy Bodies (DLB) and Multiple system atrophy (MSA) belong to a group of NDDs collectively called ~~synucleinopathies~~α-synucleinopathies. They are pathologically characterized by abnormal deposition of α-synuclein ~~forming abnormal deposition~~ such as the (α-syn)-rich neuron intracytoplasmic inclusions (Lewy bodies, Lewy neuritis and glial cytoplasmic inclusions) or the oligodendrocytes cytoplasmic inclusions [104]. Nevertheless, other misfolded proteins have been recognized in degenerated neurons, including tau proteins [105] highlighting the necessity of a more complex pathophysiological model.

In contrast to tau, which seems to require cofactors, in vitro and in vivo studies demonstrated how α-syn is able to self-polymerize [106] [107]. Several pathophysiological

mechanisms have been proposed to explain the interaction between α-syn and tau. According to ~~a two-step model (initiation followed by propagation),~~different models α-syn can act~~s~~ as the "amyloidogenic seed" promoting tau aggregation with subsequent interaction between the two proteins enhancing ~~promoting tau aggregation, whereas α-syn and tau interact subsequently to promote~~ each other's fibrillization [108]~~.~~ or as the pathological chaperone ~~According to another model, α-syn represents the pathological chaperone~~ for tau fibrillization [109]. Whatever the mechanism, oligomeric forms of α-syn and tau co-exist in ~~synucleinopathies~~α-synucleinopathies and influence each other and perpetuate mutual aggregation, leading to hybrid oligomers' formation [9,110,111]. ~~Pathogenetic mutations of α-syn provide evidence about the role of α-syn in promoting the formation of tau inclusions in the human brain. PD patients with A53T mutation in the α-syn gene (SNCA) revealed abundant α-syn and tau inclusions [108]. Nonetheless, tau and α-syn inclusions have been also demonstrated in individuals with sporadic neurodegenerative disorders [104].~~

Interestingly, levels of CSF α-syn and p-tau are directly correlated in ~~synucleinopathies~~α-synucleinopathies. This could be because the accumulation of α-syn in PD brain could inhibit the release of p-tau in CSF through unknown mechanisms, supporting the relationship between α-syn and tau in ~~synucleinopathies~~α-synucleinopathies [112]**.** With regard to CSF, reduced levels of α-syn, t-tau, p-tau and ~~β-~~amyloid-β at disease onset have been found in PD compared to HC [112][113]. Furthermore, p-tau levels and p-/t-tau ratio increased over time after PD onset despite stable levels of t-tau/α-syn ratio. Patients exhibiting postural instability and gait impairment predominant phenotype had lower CSF p-tau and Aβ42 concentrations than those with tremor-dominant phenotype [112]. Male sex and SNCA polymorphism rs316181 were linked to increased levels of p-tau, while an increased t-tau/Aβ42 ratio was associated with REM behavior disorder [113]**.**

The diagnostic value of CSF tau biomarkers is higher when they are combined into ratios [112][114][115][113][115]**.** The p-tau/α-syn ratio (which is increased in PD compared to HC), showed the highest accuracy for PD diagnosis [115] (Table 3)**.** Roughly 75% of PD patients with a disease course longer than 10 years evolve to PD with dementia (PDD) [116]. CSF t-tau has been reported to be increased in PDD compared to HC, while p-tau did not show differences in the two groups [117]. Longitudinal studies in PD patients found neither a predictive value for the subsequent development of dementia for CSF t-tau and p-tau levels measured at PD onset [118], nor an association with dementia severity in PDD [117]. In contrast, plasma t-tau and p-tau181 levels measured through immunomagnetic reduction (IMR) were increased in DLB compared to HC, and reduced compared to PDD [37] (Table 2)**.**

DLB is the second most common cause of dementia after Alzheimer's disease (AD). DLB belongs to the spectrum of ~~synucleinopathies~~α-synucleinopathies, nevertheless, a mixed neuropathology with amyloid-β plaques and tau pathology is present in nearly 40% of patients (α-syn+AD) and are associated with an increased rate of dementia and a decreased survival time [119]. Although previous studies reported increased levels of t-tau in DLB compared to HC [120], these findings were not replicated in more recent studies [121]. The use of different diagnostic criteria and populations may in part explain these inconsistent results. In general, CSF t-tau and p-tau levels are lower in DLB than AD [120], suggesting that levels of p-tau in CSF could represent a valuable marker for the differential diagnosis between these two NDDs [123]. On the other hand, t-tau levels are higher in DLB in comparison to PD and PDD, supporting the hypothesis that limbic and cortical Lewy body pathology is the main and specific pathologic correlate of dementia in PD [124]. In a neuropathological study, the regional distribution of tau and amyloid-β is different in α-syn+AD patients and AD, with a greater proportion of tau in the temporal neocortex for DLB patients and in frontal neocortex in AD. Moreover, the severity of tau burden was correlated with worse *ante-mortem* cognitive performances in DLB individuals [125]**.** Tau pathology has also been correlated to the pattern of brain atrophy in MRI. Indeed, increased CSF t-tau levels were associated with high global atrophy scores [126] and elevated CSF p-tau with a more selective posterior atrophy [127]. CSF ratios t-tau/Aβ42 and p-tau/Aβ42 recently proved to predict the presence of AD co-pathology in DLB, with higher levels of both ratios in α-syn+AD brains when compared to α-syn-AD brains, while no correlation was found analyzing single biomarkers. Besides, higher CSF t-tau/Aβ42 and lower CSF Aβ42 levels were associated with a higher burden of neocortical α-syn [121].

MSA is a NDD characterized by autonomic failure associated with a poorly levodopa-responsive parkinsonian syndrome or cerebellar ataxia, thus defining two main phenotypes respectively identified as MSA-P and MSA-C [128,129]. Differential diagnosis between PD and MSA could be challenging because of the clinical overlap in the early stages and a reliable biomarker for the differential diagnosis of MSA from PD yet represents an unmet need. ~~Reduced CSF levels α-syn in MSA patients compared to HC have been reported in most studies, while results on blood samples were inconclusive [111]. Also~~D~~, d~~ata on CSF biomarkers in MSA are conflicting~~on tau are conflicting~~. In some studies, CSF t-tau concentration was higher in MSA than PD patients or HC [130][131]**,** whereas others reported a reduction of its level [132]. By contrast, p-tau concentration was similar in MSA and HC [130][132]. Conflicting data have been published on the p-tau/t-tau ratio, reported either higher [130][132] or lower [133][134] in MSA compared to PD. The combination of p-tau181, t-tau and DJ1 (a protein related to PD pathogenesis whose function is still unknown in MSA) proved high sensitivity and specificity (respectively 82% and 81%) to

discriminate MSA from PD [131], as well as the p-tau/Aβ42 ratio (specificity 71%, sensibility 93%), which is significantly increased in MSA [134] (Table 3). Similarly, the AD index (CSF Aβ40/42 ratio × t-tau) and the ratio t-tau/α-syn were higher in MSA than HC, while no significant differences were found comparing PD and HC [130]. Finally, no CSF biomarkers proved to be reliable for the differential diagnosis between MSA-C and MSA-P [114].

Fluid biomarkers for ~~synucleinopathies~~α-synucleinopathies were recently investigated also in blood. Red blood cells (RBC) seem to be a preferential niche for misfolded proteins [135] and oligomeric α-syn was described in RBC of patients with PD [136]. Analysis of RBC in PD patients showed ~~reduced levels of α-syn and~~ increased levels of phospho-tau (p-tau) in PD compared to HC [110]. Interestingly, RBC t-tau concentration was inversely correlated to MMSE scores both in the general PD cohort and in PD drug naïve patients, suggesting a central role of tau pathology in promoting cognitive decline in PD [110]. Plasma t-tau and p-tau were higher in MSA compared to HC when measured through IMR [137][37], while levels of p-tau measured through Simoa did not differ between MSA and HC [24].

8. Tau PET in Tauopathies

In the field of neurodegenerative dementias, in vivo imaging with radioligands designed to detect each underlying proteinopathy aims to find a gold standard biological marker. Amyloid-β imaging has been incorporated into AD diagnostic criteria since 2007 [138]. However, cerebral amyloid-PET uptake did not correlate with cognitive performance nor neurodegeneration in AD. Biomarkers of tau pathology may be closely related to neuronal injury and changes in cognition. Tau-PET will support the investigation of the spatial-temporal evolution of tau pathophysiology in AD and other NDD as well as the clinical validation of fluid biomarkers. Therefore, research effort is focusing on the development of tau-~~selective positron emission tomography (~~PET~~)~~ radiotracers in order to reveal underlying tau deposits in a broad range of tauopathies, such as AD, FTD, PSP. A significant number of tau PET tracers have been synthesized so far, but many of those lack sufficient specificity and selectivity [139,140]. The main difficulties to develop these tracers are related to [141]: 1) lipophilicity, which is necessary to cross the blood-brain barrier and the cell membrane (tau is located both intra and extracellularly); by contrast, excessive lipophilicity could lead to unspecific binding; 2) affinity for tau, relevant to obtain high selectivity for tau molecule and overcoming the high Aβ concentrations interfering with tau-ligand binding, but requiring prolonged scanning time to reach the steady-state; 3) different conformations of tau aggregates and different tau isoforms [142], making it challenging to develop a unique tau radiotracer for all types of tauopathies. Based on both in vitro and in vivo results, three families of radiotracers were

initially synthesized: 1) the THK family (THK117, its (S)-enantiomer THK5317, and THK5351; 2) 18F-AV1451, also known as T807 or Flortaucipir; 3) PBB3. The so-called first-generation tau tracers finally showed a lack of specificity, with relevant off-target binding to monoamine oxidase B (MAO-B) for the HK family and Flortaucipir [143], neuromelanin for Flortaucipir [144], and Aβ-plaques and α-syn for PBB3 [145,146]. Hence, the second generation of tau PET tracers has been developed: RO948, PI2620, JNJ311, MK6240, PM-PBB3 and AM-PBB3 [147]. However, the most widely studied agent is [18F]AV-1451 (Flortaucipir). It was the first approved tau PET tracer for estimation of the density and distribution of aggregated tau neurofibrillary tangles in adults with cognitive impairment and suspected AD [148].

8.1 Aging

First-generation tracers demonstrated retention of uptake confined to the medial temporal lobe in normal cognitively elderly, in accordance with pathological data [149], likely reflecting an age-related process of tau-deposition [150]. Accumulation of tau in the temporal cortex, without an associated significant Aβ burden, may suggest a promoting effect of tau in mild amnestic deficits; hippocampal atrophy or a PART related pathology in such subjects.

8.2 Alzheimer's Disease

There is now convincing evidence that cortical tau binding at PET imaging reflects tau accumulation reported from histopathological studies in AD brains [149]. 18F-AV1451 demonstrated high affinity in vitro for paired helical filaments (PHF) of 3R/4R tau isoforms reported in AD brains [151,152]. A recent study in 82 individuals with or without dementia showed a high concordance between the visual reading of 18F-AV1451 PET scans and staging of cortical neurofibrillary tangles, reinforcing the concept that Flortaucipir effectively reflects pathological changes of AD [153]. Similar results were replicated in vivo, with 18F-AV1451 demonstrating to accurately differentiate clinically diagnosed AD from HC and other NDDs [154]. Higher levels of tau tracer retention in the inferior lateral temporal region, but also in the posterior cingulate and lateral parietal regions, provided the best discrimination areas between AD patients and HC [155–158]. Other studies comparing ~~NCF~~ cognitively normal subjects with MCI patients showed differences in binding restricted to medial temporal regions (parahippocampal and entorhinal cortex) [159,160], as well as lateral temporal and parietal areas when examining only amyloid-β-positive MCI individuals [158]. Furthermore, some studies reported greater retention of 18F-AV1451 in the cortex of younger compared to older AD patients, with a cut off of 75 years-old [161], and in early-onset compared to late-onset AD

patients with a cut off of 65 years-old [160], as similarly reported in previous post-mortem studies [162]. Moreover, neocortical Flortaucipir retention was found in preclinical AD cases and rarely in amyloid-β negative cases [161,163].

The extent of tau accumulation intimately correlates with the severity of MCI due to AD and AD dementia, providing an objective index for disease severity. The few available longitudinal tau PET studies in AD demonstrated a tracer retention related to disease duration [164–167]. However, results from the largest studies, which compared cognitively unimpaired Aβ–negative and Aβ–positive individuals, MCI and AD dementia patients, are conflicting. Cho and colleagues studied 107 participants (45 Aβ-negative cognitively unimpaired, 7 Aβ-positive cognitively unimpaired, 31 MCI, and 24 AD dementia) who completed both 18F-flortaucipir and 18F-florbetaben at baseline and who were followed up for 2 years. The authors found a predominant tau accumulation in the medial and basal temporal cortices in MCI and in the lateral temporal cortices in AD dementia [167], thus supporting a progressive tau accumulation pattern in line with the Braak model [149]. Conversely, Jack and colleagues evaluated the longitudinal change in tau PET signal during a one-year follow-up in a group of 126 individuals (59 cognitively unimpaired Aβ-negative, 37 cognitively unimpaired Aβ-positive, and 30 cognitively impaired Aβ-positive). They demonstrated a tau accumulation in the Aβ-positive clinically unimpaired group in the medial temporal lobe and in the medial parietal areas, including posterior cingulate cortex. This suggests that initial accumulation of tau aggregates in AD may not be restricted to the medial temporal lobes as implied by Braak staging [165]. Overall these evidence proved that longitudinal tau PET may be an useful biomarker in clinical trials to monitor the effect of disease-modifying therapies tailored to reduce tau as well as cerebral amyloid-β plaque burden [168]. In AD increased 18F-AV1451 uptake is strongly co-localized with hypometabolic regions and has been associated with worse performance on various cognitive domains in regionally specific patterns [169]. Accordingly, 18F-AV1451 distribution may predict the clinical variants of AD suggesting on-site neurotoxicity provoked by tau aggregates in posterior cortical atrophy, logopenic variant of primary progressive aphasia, or behavioral/dysexecutive variant of AD [169–172]. The main ~~dare~~ challenge for tau tracers remains the capability to detect preclinical / prodromal / early AD stages. Importantly, the sensitivity for an early diagnosis is limited by the fact that in the early AD stages (e.g. MCI) neurofibrillary tangles are only present in deep brain regions. In more advanced AD stages 18F-AV1451 discriminate mild-to-moderate AD patients from HC [154].

Currently, there are not conclusive studies on second-generation tau tracers in AD patients and we do not have any head-to-head comparison between them. Available studies suggest that they may

be more useful in identifying earlier Braak stages. They also show substantially less non-specific binding or higher affinities to primary tauopathies [173]. These agents include [18F]MK-6240 (Merck & Co), PM-PBB3 (APRINOIA Therapeutics), F18-PI-2620 (Life Sciences) (50) and RO-948 (Roche). RO-948 tracer showed similar patterns of cerebral uptake then 18F-AV1451 [174]. Moreover, RO-948 showed a greater stability, a higher retention in the medial temporal lobe and lower intracerebral "off-target" binding than Flortaucipir [175,176]. In AD subjects, PET images of F18-PI-2620 showed a significantly higher uptake than control subjects in temporal lobe, parietal and cingulate cortex. Importantly, in non-demented control subjects it showed robust initial brain uptake and fast washout from the brain, as well as no age dependency. This last finding suggests that F18-PI-2620 could improve the discrimination between non-demented control and AD subjects in elderly. Moreover, excellent test-retest variability has been demonstrated confirming the utility of F18-PI-2620 to evaluate longitudinal change of tau deposition during disease course [177]. Finally, three recently published studies have evaluated [18F]MK-6240 in vivo [178–180]. All these studies exhibited favorable kinetic and high binding levels of [18F]MK-6240 to brain regions typically affected from NFT deposition in AD subjects. [18F]MK-6240 PET images in another cohort of patients positive for cerebral amyloid-βbeta deposition (cognitive unimpaired/impaired elderly controls, MCI and AD patients) showed a pattern corresponding to the anatomic distribution of tau as expected in the Braak model [178].

8.3 Non-AD tauopathies

Tau imaging may also be relevant for other tauopathies, such as CBD and PSP. These atypical parkinsonian syndromes are characterized by abundant filamentous tau inclusions that are made of isoforms with four microtubule-binding repeats in tubular or straight filaments. In PSP and CBD abnormal aggregation of pathologically misfolded and hyperphosphorylated tau proteins mainly occur in the basal ganglia in the early stages, spreading later to multiple brain areas: brainstem, posterior frontal lobe, cerebellum and association cortices for PSP; primary motor cortex, frontal lobe, brainstem or cerebellum for CBD [181–184]. Nevertheless, a variety of overlapping syndromes are frequent in tauopathies.

To date few studies using low specific first-generation PET tracers have examined tau binding in parkinsonian disorders. They revealed distinct patterns of tracer retention in PSP and CBD, compared to controls, showing elevated PET signal in the basal ganglia and affected cortical regions, in agreement with postmortem data [185].

Elevated tau deposition in PSP cases was observed in the basal ganglia, thalamus, dentate nucleus of the cerebellum, and midbrain [186–192]. However, an extensive overlap and age-dependent increase in both the PSP and control groups has been reported [187]. Significant differences in 18F-AV-1451 distribution between patients with PD and PSP, with increased uptake in the globus pallidus, midbrain, and subthalamus in PSP cases, have been also described [193,194]. Interestingly, in some studies the radioligand accumulation correlated with the clinical disease severity [189,195,196], but the lack of correlations between tau binding and symptom severity was observed in the other cohorts [188,194]. A recent study by Whitwell and colleagues using flortaucipir reported that the clinical heterogeneity present in PSP is mirrored by anatomical and tau burden heterogeneity within the brain [197].

Studies analyzing the feasibility of tau PET in patients with CBS showed elevated asymmetrical tracer deposition in dentate nucleus of the cerebellum, midbrain, subthalamic nucleus, globus pallidus and putamen, precentral and postcentral cortex, superior frontal and parietal lobe, fitting with the regional distribution of tau pathology [198,199]. Furthermore, longitudinal investigations documented a correlation between tau accumulation over time and disease progression [166,200]. However, the degree of uptake in CBS using currently available tau ligands is variable, and some patients resulted negative [201,202].

Overall, studies using in vivo tau PET in PSP and CBD individuals that received a postmortem confirmation have shown conflicting results questioning the usefulness of tau imaging in early diagnosis of such conditions. Indeed, the available tracers are not specific for 4R tau pathology and show substantial off-target binding in the midbrain and basal ganglia to monoamine oxidase B ~~(MAO-B)~~ and to neuromelanin-containing cells [192,203].

Second generation of tracers with improved off-target binding are under evaluation also in non-AD ~~taoupathist~~tauopathies. Brendel and colleagues found a great potential to diagnose patients with suspected PSP using 18F-PI-2620 which proved high affinity to recombinant 4R tau fibrils and PSP brain homogenate [204,205].

9. Discussion and perspectives

Epidemiological projections indicate that AD evolving epidemic represents a global threat for healthcare systems [54]. Minimally invasive and globally accessible tests are needed to cope with this expected burdensome demand and to manage individuals in suspected preclinical/prodromal stages of NDDs [206,207]. Blood-based biomarkers represent cost-, resource- and time effective tools [208]. They hold the potential to enable large-scale biological screening of individuals who are very unlikely to have AD-related pathophysiology and would support the request of second-

level investigations (e.g. PET imaging or CSF assessment). Blood-based biomarkers not only open up the opportunity of a multi-step diagnostic work-up but can also facilitate the re-engineering of drug Research & Development (R&D) pipelines, from subjects' enrollment, target engagement, to treatment efficacy monitoring. In Oncology, serial liquid biopsies offer clues about the evolution of cancer in individual patients across disease stages, enabling individualized genetically and biologically guided therapies [207].

The development of novel ultrasensitive measurement techniques has enabled the detection of tau-related biomarkers, facilitating a liquid biopsy-driven paradigm shift in the field of NDDs, including ~~Alzheimer's disease~~AD [207]. Plasma t-tau seems to have a limited utility as diagnostic marker in NDDs when assessed alone [47]. On the other hand, plasma p-tau may find a context-of-use in the differential diagnosis between AD and non-AD dementias, including primary tauopathies and vascular dementia [25–29,75]. The combination of tau with other putative biomarkers (e.g. NFL) into panels or ratios seems to increase the diagnostic accuracy of tau biomarkers alone [22]. Sets of multiple biomarkers may accordingly find a context-of-use in ~~both~~ the clinical diagnostic workup of neurodegenerative dementias ~~and~~, in patients' selection and their follow-up for ~~targeted therapies~~disease modifying clinical trials.

Studies investigating p-tau isoforms other than p-tau181 (e.g. p-tau217) [30,33,209], and alternative proteolytic fragments like the N1-tau [25] fragment have shown encouraging results in differentiating AD and MCI from HC and in predicting the amyloid β- in preclinical AD. Several studies, performed in AD and NDDs, indicate that plasma p-tau217 is a reliable predictor of tau pathology (as assessed through tau-PET)[210], amyloid-β-mediated tau pathophysiology, longitudinal cortical/subcortical atrophy and AD-like cognitive decline. These findings coupled with the evidence that p-tau217 discriminates AD from non-AD conditions [30,31] explain why plasma p-tau217 will be used as exploratory marker in different COU, including patients' selection and follow-up in clinical trials. The evidence about plasma p-tau217 also support its integration in the ATN matrix for disease diagnosis, prognosis and progression monitoring in clinical practice [31].

~~not yet available~~ A better understanding of time-trajectories of these alternative phosphorylation sites of p-tau ~~may~~is mandatory to help understand the more adequate context-of-use of each isoform from preclinical to full-blown AD.

~~Tau protein misfolding and accumulation in toxic species and tangles is a critical pathophysiological process of AD and of primary tauopathies [1]; further, this protein contributes~~

~~to the pathophysiology of other brain proteinopathies (e.g. α-synuclein, TDP-43, FUS), leading to mixed or overlapping neuropathologies [203].~~

~~Biomarkers tracking *in vivo* tau pathology are embedded in the most recent 2011 NIA-AA and 2014 IWG-2 diagnostic criteria for AD, thus representing key elements for the diagnostic workup of AD [11,135]. Currently CSF total-tau, 181phosphorylated-tau, and tau-molecular imaging (PET), represent validated biomarkers for investigating tau-related pathology in AD [11,135].~~

~~However, CSF assessment through lumbar puncture and PET assessment through radiotracers and imaging device are invasive, time and resource demanding.~~

~~According to recent investigations plasma t-tau seems to have a limited utility as diagnostic marker in NDDs when assessed alone [46]. On the other hand, plasma p-tau help differentiate AD from non-AD dementias, including primary tauopathies and vascular dementia [24–28,71]. The combination of plasma p-tau and t-tau into ratios with other candidate biomarkers (e.g. NFL) seems anyway to increase the diagnostic power of these analytes [22]. Studies investigating p-tau isoforms other than p-tau181 (e.g. 217p-tau) [29,32,204], and alternative proteolytic fragments like the N1-tau [24] fragment have shown encouraging results in differentiating AD and MCI from HC but these data should be regarded with caution and may deserve a more extensive validation.~~

In primary tauopathies CSF p-tau and t-tau, as assessed by traditional techniques, are not reliable biomarkers for the diagnostic workup of 4R-tauopathies~~. In fact, both CSF t-tau and p-tau and their combination do not discriminate primary tauopathies from HC and the tau-related phenotypes within the pathologic tau-spectrum~~ [64]. These biomarkers are useful in both clinical and research settings for differentiating NDDs with an underlying AD pathology, which can mimic primary tauopathies [59,60]. The detection of different tau isoforms and fragments (e.g. 33 KDa vs 55 KDa isoforms) [72] and of specific tau strains (e.g. 3R and 4R tau) by means of PMCA and RT-QuIC [41] are promising ~~strategies~~ tools to stratify the pathologic tau-spectrum and ~~its clinical continuum.~~ to identify different phenotypes. The prognostic value of tau biomarkers ~~for~~ in~~the~~ 4R-tauopathies remains to be clarified~~, especially when measured in plasma with the use of novel ultrasensitive techniques, which allow minimally invasive and repeatable examinations~~.

~~As regards~~In the ALS-FTD spectrum, CSF t-tau and p-tau may contribute in discriminating AD from FTD and in identifying patients with FTD phenotypes ~~but~~ with an underlying AD-pathology [91]. The diagnostic value of p-tau and t-tau alone in differentiating the FTD-ALS continuum from ~~healthy controls~~HCs is controversial [89,91,98]. Nevertheless p-tau/t-tau ratio seems to be ~~lower in FTD and ALS compared to HC and may indirectly indicate~~useful in FTD for the identification of patients with a TDP-43 underlying pathology ~~related variants~~ [89] ~~.~~ and may be

useful in ALS to discriminated clinical endophenotypes (UMN-predominant *vs.* LMN-predominant variants) [99]. A possible explanation could be the lower cerebral tau burden in TDP-43 related FTD-ALS phenotypes. Baseline CSF t-tau and p-tau/t-tau ratio could represent a prognostic marker for both FTD and ALS [97] but further studies are needed to identify the proper context-of-use of these biomarkers.

In the reported studies, the high heterogeneity of the clinical diagnostic criteria used for patients' selection, the lack of post-mortem validation, and the unpredictable relationship between phenotypes and underlying pathology hamper the understanding of tau protein role as NDD biomarker.

In α-synucleinopathies the co-occurrence of underlying tauopathy is commonly reported and tau may have a permissive role on α-synuclein aggregation. Reduced levels of t-tau have been reported in PD compared to HC [108,109], while p-tau levels and p-/t-tau ratio seems to increase over time [109]. T-tau and p-tau concentration may vary according to PD phenotypes, thus suggesting a potential role of tau in differentiating clinical phenotypes (it is reported a reduction in CSF p-tau levels in patient with postural instability and gait disorder and an increase in patients with REM sleep behavior disorder) [112,113]. An potential association between tau burden and cognitive impairment in PDD and DLB has been postulated; nevertheless but data are conflicting and still inconclusive [117,118]. Similarly the diagnostic value of CSF p-tau and t-tau in atypical parkinsonism (e.g. MSA and DLB) is controversial [117,121,122,124,132,211][130,131].

Recent investigations on plasma reported an increase of p-tau concentration in PD discriminating PD from HC, and inversely correlated with MMSE scores. Further studies are needed to establish the diagnostic and prognostic value of both p-tau and t-tau in α-synucleinopathies and their role in differentiating PD phenotypes.

The development and validation ofAs regards tau-PET tracers, tau for the in vivo tracking of tau pathology has posed several challenging questions [138]. Tau-PET tracer uptake has been included in the current diagnostic criteria for AD as a surrogated marker of tau-accumulation [12]. Tau accumulation in the medial and basal temporal cortices was observed in MCI patients was demonstrated, thus suggesting a potential role of tau-PET tracers in the prodromal AD and a potential value of tau-PET uptake as a biomarker toin monitoring the efficacyect of disease-modifying therapies in clinical trials [165,167]. Further longitudinal observations are required to corroborate this hypothesis. A possible role of tau-PET imaging in the differential diagnosis between 4R tauopathies is suggested by some studies highlighting a different deposition pattern of tau in PSP compared to CBS. Off-target binding in the midbrain and basal ganglia is currently limiting the use of fist generation tracers but . This issue was partly resolved with the

development of second-generation tau tracers [145]. Further evidence on the physiological and pathologic variables influencing tau-PET tracers uptake, on the off-label binding and on the comparison of different tracers is warranted to understand the appropriate context-of-use of these biomarkers.

~~10. Conclusions and perspectives~~

In conclusions, biomarkers development and related research have significantly contributed to the reengineering of AD as a clinical-biological framework, as reflected by the ATN system, where preclinical stages of the disease are identified and potentially treated for preventive strategies, including tau-targeting approaches. It is conceivable that the same paradigm~~ng~~ shift may take place also for NDD others than AD. Individuals with ~~no or subtle cognitive/motor/behavioral decline~~different clinical presentations but similar biomarkers may be grouped ~~i~~onto biological clusters facilitating a treatment ~~essentialy~~essentially based on pathophysiological mechanisms and not phenotypes ~~to treat with pathway-based, symptom (i.e., NDD clinical phenotype)-agnostic therapies~~[212,213], in line with the precision medicine paradigm [214].

~~Epidemiological projections indicate that AD evolving epidemic represents a global threat for healthcare systems, especially if a disease-modifying therapy becomes available [206]. Minimally invasive and globally accessible tests are needed to cope with this expected burdensome demand and to manage individuals in suspected preclinical/prodromal stages of NDDs, including Alzheimer's [207,208]. Blood-based biomarkers represent cost-, resource- and time-effective tools for critical clinical solutions [209]. They hold the potential to enable large-scale biological screening of individuals who are very unlikely to have AD-related pathophysiology and would support the request of second-level investigations (e.g. positron emission tomography [PET] imaging or cerebral-spinal fluid [CSF] assessment) that have reduced accessibility and are more invasive. Blood-based biomarkers not only open up the opportunity of a multi-step diagnostic work-up but can also facilitate the re-engineering of drug Research & Development (R&D) pipelines, from subjects' enrollment, target engagement, to treatment efficacy monitoring. In Oncology, serial liquid biopsies offer clues about the evolution of cancer in individual patients across disease stages, enabling individualized genetically and biologically guided therapies [208].~~

~~The development of novel ultrasensitive measurement techniques has enabled the detection of tau-related (and others) biomarkers, facilitating a liquid biopsy-driven paradigm shift in the field of neurodegenerative diseases, including Alzheimer's disease [208].~~

In conclusions, biomarkers development and related research have significantly contributed to the reengineering of AD as a clinical-biological framework, as reflected by the ATN system, where preclinical stages of the disease are identified and potentially treated for preventive strategies, including tau-targeting approaches.

It is conceivable that the same parading shift may take place also for NDD others than AD. Individuals with no or subtle cognitive/motor/behavioral decline may be grouped onto biological clusters to treat with pathway-based, symptom (i.e., NDD clinical phenotype)-agnostic therapies[210,211], in line with the precision medicine paradigm [212].

11. Expert opinion

Tau, as assessed in CSF in the form of t-tau and p-tau181, is an established diagnostic biomarker for AD.– The detection of tau in alternative easily accessible matrices like plasma currently represents one of the most exciting future directions for AD biomarker research. The further development of ultrasensitive measurement techniques and their more extensive validation represent accordingly a hot-topic in this field. The potential utility of alternative tau fragments and tau isoforms represents another challenge to improve AD diagnosis and stratification.

As regards primary tauopathies, alternative tau isoforms (e.g. 217p-taup-tau217) or proteolytic fragments (e.g. N-terminal fragments) quantified in CSF and in blood may represent candidate fluid diagnostic and prognostic markers overcoming the elassic limitations of current CSF t-tau and p-tau measurement in these conditionsNDDs.

In particular plasma p-tau217 could be used as exploratory marker in different drug development pipelines with distinct molecular target and for different COU, including patient selection, and theragnostic, and potently target engagement. The evidence about plasma p-tau217 also support its integration in the ATN matrix for disease diagnosis, prognosis and progression monitoring in clinical practice.

Nevertheless, the implementation of techniques enabling the identification of different tau strains (e.g. 3R- *vs.* 4R-tau), enabled by RT-QuIC and PMCA techniques, represents the most important frontier for fluid biomarker discovery in tauopathies.

The validation of biomarkers tracking tau pathology in the ALS-FTD spectrum is challenging. This could be also , due to the high clinical and phenotypical heterogeneity intrinsic for this pathologicof this spectrum. However, tau biomarkers may identify AD-related pathology, and, to a lesser extent, differentiate tau-related from TDP-43 related pathology.

Intriguingly, tau biomarkers may reveal co-pathologies in NDDs. Biomarkers tracking tau pathology in PD and atypical parkinsonism may in fact be potentially helpful for the segregation of clinical subtypes and to combine tailor disease-modifying treatments.

The development of tau-PET tracers and the overcome of first-generation tracers' limitations (especially the off-target binding and the necessity of a cyclotron) was a great step forward in the *in vivo* understanding of early pathophysiological changes in AD. Further studies are needed to define the context-of-use of tau-PET tracers in AD (especially in preclinical and prodromal AD) and their potential applications outside the AD-spectrum.

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Progress regarding the context-of-use of tau as biomarker of Alzheimer's disease and other neurodegenerative diseases

Abstract

Introduction Tau protein misfolding and accumulation in toxic species is a critical pathophysiological process of Alzheimer's Disease (AD) and other neurodegenerative disorders (NDDs). Tau biomarkers, namely cerebrospinal fluid (CSF) total-tau (t-tau), 181-phosphorylated tau (p-tau) and tau-PET tracers, have been recently embedded in the diagnostic criteria for AD. Nevertheless, the role of tau as a diagnostic and prognostic biomarker for other NDDs remains controversial.

Areas covered We performed a systematical PubMed-based review of the most recent advances in tau-related biomarkers for NDDs. We focused on papers published from 2015 to 2020 assessing the diagnostic or prognostic value of each biomarker.

Expert opinion The assessment of tau biomarkers in alternative easily accessible matrices, through the development of ultrasensitive techniques, represents the most significant perspective for AD-biomarker research. In NDDs, novel tau isoforms (e.g., p-tau217) or proteolytic fragments (e.g., N-terminal fragments) may represent candidate diagnostic and prognostic biomarkers and may help monitoring disease progression. Protein misfolding amplification assays, allowing the identification of different tau strains (e.g. 3R- *vs.* 4R-tau) in CSF, may constitute a breakthrough for the in vivo stratification of NDDs. Tau-PET may help tracking the spatial-temporal evolution of tau pathophysiology in AD but its application outside the AD-spectrum deserves further studies.

Keywords: Alzheimer's Disease, Fluid biomarkers, Neurodegenerative diseases, Tau, Tau-PET, Ultrasensitive techniques

Article highlights

- Tau protein misfolding and accumulation in toxic species and tangles is a critical pathophysiological process of Alzheimer's Disease (AD) and other neurodegenerative disorders (NDDs);
- Cerebrospinal fluid (CSF) total tau (t-tau) and phosphorylated tau (p-tau) are included in the current diagnostic criteria for AD; in primary tauopathies and in other NDDs (ALS-FTD spectrum and α-synucleinopathies) the role of tau as a diagnostic and prognostic biomarker is still controversial;
- The quantification of t-tau and p-tau and of emerging tau biomarkers in plasma by using ultrasensitive techniques are gaining momentum in the AD diagnostic workup;
- Novel tau fragments and tau isoforms detectable by means of ultrasensitive methods including Protein Misfolding Cyclic Amplification (PMCA) and Real-time quaking-induced conversion (RT-QuIC) may improve the in vivo stratification of NDDs;
- Tau-PET tracers are useful tools for the *in vivo* tracking of the spatial-temporal evolution of tau pathophysiology in AD but further studies are needed to define their proper context-of-use in other tauopathies.

1. Introduction

Tau is a natively unfolded microtubule-associated protein largely expressed in the central nervous system[1]. This protein plays an important role in microtubule polymerization, stabilization and remodeling, and is mainly expressed in the axons, where it also participates in regulating cellular transport processes[2,3]. Tau exists in 6 isoforms generated through the alternative splicing of the exons 2, 3 and 10 of the microtubule-associated protein tau (MAPT) gene[4]. The microtubule-binding domain (MBD) is encoded by the exon 10, whose alternative splicing produces 3 isoforms, each with 3 or 4 repeats, respectively called 3-repeat (3R) and 4-repeat (4R) tau[2]. Under pathological conditions, mutations in the MAPT gene along with a hyper-phosphorylation of tau may reduce the affinity of the MBD to the microtubules[5]. Accordingly, tau free-protein concentrations increase to a tipping point after which misfolded protein intermediates self-assembly into unwanted oligomers, fibrils, and neurofibrillary tangles (NFT)[5].

The neuronal and/or glial accumulation of aberrant tau aggregates represents the pathological hallmark of a broad spectrum of neurodegenerative disorders (NDDs) called tauopathies [6]. These include the so-called primary tauopathies, a group of NDDs, in which tau aggregates, including NFT, represent the main pathological hallmark. Primary tauopathies can be classified into 3R-tauopathies, 4R-tauopathies and mixed 3R/4R tauopathies. The major clinical phenotypes belong to the spectrum of Progressive Supranuclear Palsy (PSP), Corticobasal Degeneration (CBD) and Frontotemporal Lobar Degeneration with tau deposits (FTLD-Tau) [2,6]. Secondary tauopathies are conditions resulting from a complex interplay between tau and other misfolded proteins (e.g., amyloid-β - Aβ) or related to alternative causes (e.g., autoimmune processes). Alzheimer's Disease (AD) represents the best characterized and more intensively studied NDDs and is classified as secondary tauopathy[2,7]. More recently, other forms of secondary tauopathies have been described including chronic traumatic encephalopathy (CTE) and the autoimmune anti-IgLON5 encephalitis [2]. Tau cytoplasmic inclusions have also been reported in Frontotemporal Dementia (FTD); up to 30% of patients with the behavioral variant of frontotemporal dementia (bvFTD) and the majority (approximately 90%) of patients with the non-fluent variant of Primary progressive aphasia (nfvPPA) show tau pathologic depositions [8]. Also in α-synucleinopathies, where aberrant α-synuclein aggregates underpin the neurodegenerative mechanisms, NFT are likely to cause neurodegeneration and contribute to the disease burden [9,10].

Tau biomarkers are included in the diagnostic workup of AD according to the 2011 National Institute on Aging – Alzheimer's Association (NIA-AA), the 2014 International Working Group-2 (IWG-2) criteria and the AT(N) classification system [11,12]. In 2018 the AT(N) scheme embed biomarkers of amyloid-β-related pathology (A), tau-pathology (T) and other markers of

neurodegeneration (N) [13]. This classification includes cerebrospinal fluid (CSF) total-tau (t-tau), as biomarker of neurodegeneration (N) and 181-phosphorylated-tau (p-tau181 or simply p-tau), as biomarker of tau pathology [13]. These biomarkers are all increased in AD individuals. The AT(N) categorization for the first time added cerebral tau- positron emission tomography (PET) as core biomarker of AD. Indeed, the implementation of tau-PET tracers represents a recent step forward in the AD diagnostic workup [13].

Currently, the *ante-mortem* diagnosis of non-AD NDDs mainly relies on pure clinically based criteria. In this scenario, the diagnostic and prognostic value of tau protein in the spectrum of primary tauopathies remains unsatisfactory when measured with traditional approaches (e.g. ELISA) [14]. In fact, a biomarkers-based diagnosis is still an unmet need in NDDs and several methodological challenges should be addressed to overcome this limitation. In the last years, the use of ultrasensitive assays, the discovery of alternative tau byproducts in biofluids, and the development of innovative techniques, enabling the differentiation of tau strains, have represented potential breakthroughs for the *in vivo* dissection of tau pathology spectrum.

The validation of biomarkers tracking neurodegenerative processes and predicting pathophysiological progression at the earliest molecular/cellular changes is an unmet need for the early diagnosis, the prognostic evaluation and for patients selection and follow-up in targeted-therapy clinical trials [15].

We aim to review the current state of the art on the use of tau protein as a biomarker for NDDs. First, we investigated recent advances of this fluid biomarker in the diagnostic and prognostic workup of AD and other NDDs. Secondly, we evaluated the contribution of cerebral PET-imaging in tracking tau pathology across the NDDs spectrum.

2. Literature search methods

The aim of the present work is to review the most recent advances on tau as a biomarker for neurodegenerative diseases. A systematic PubMed-based literature search was performed to select relevant papers assessing the diagnostic and/or prognostic value of plasma tau (both t-tau, p-tau, and other tau fragments) in AD and other NDDs, as evaluated through novel ultrasensitive techniques and of CSF tau in other neurodegenerative diseases. We screened the papers using the following combination of keywords:

- for plasma tau in AD and other NDDs: (("plasma" OR "blood" OR "serum") AND ("tau") AND ("Alzheimer" OR "Corticobasal degeneration" OR "Progressive Supranuclear Palsy" OR "Amyotrophic Lateral Sclerosis" OR "Frontotemporal Dementia" OR "Parkinson" OR "Multiple System Atrophy" OR "Lewy Body"));

- for CSF tau in NDDs (AD excluded): (("CSF") AND ("tau") AND ("Corticobasal Degeneration" OR "Progressive Supranuclear Palsy" OR "Amyotrophic Lateral Sclerosis" OR "Frontotemporal Dementia" OR "Parkinson's Disease" OR "Multiple System Atrophy" Or "Dementia With Lewy Bodies")).

Only papers written in English, assessing the prognostic and/or diagnostic value of tau and published between 2015 and 2020, were included in the final analysis. A total number of 1480 and 297 articles for plasma tau and CSF tau respectively were initially screened. Fourteen papers on plasma tau and 14 on CSF tau were retained in the final analysis (Fig.2).

For each study, we analyzed the study population, the technique used for biomarkers detection, the diagnostic and/or prognostic performance of the investigated biomarker. Given the high heterogeneity of the papers included, we used the AuROC values as a concise measure of diagnostic accuracy to compare different studies. The diagnostic value of each marker to properly allocate patients to different diagnostic groups was identified when available and classified as follows: 'excellent' (area under ROC curve [AuROC] 0.90–1.00), 'good' (AuROC: 0.80–0.89), 'fair' (AuROC: 0.70–0.79), 'poor' (AuROC: 0.60–0.69), or 'fail' (i.e., no discriminatory capacity) (AuROC: 0.50–0.59) [16]. We used hazard ratios (HR) to compare the prognostic values. Possible comparisons across studies are only qualitative, and not based on statistical analysis.

3. Fluid biomarkers tracking tau-pathology: from conventional to ultrasensitive techniques

Fluid biomarkers represent promising tools for the in vivo tracking of early pathophysiological changes in NDDs. Their detection and quantification in different matrices and related technical issues are regarded as hot topics in biomarkers research[14].

Tau quantification in CSF represents the first and most widely validated approach for the in vivo detection of tau protein. Tau, quantified in the CSF both as t-tau and p-tau181 currently belongs to the core AD biomarkers, along with amyloid-β_{42} ($A\beta_{42}$), and its assessment is based on traditional Enzyme-linked immunoabsorbent assay (ELISA) techniques [11–13]. Accordingly, several commercial kits are to date available for both clinical and research purposes [17]. Even if broadly used and largely validated, standard tau quantification techniques present three main limitations: 1) they are unable to effectively quantify tau in "peripheral" matrices (e.g., plasma), where its concentration is significantly low [18], 2) they fail in detecting alternative tau species (e.g., p-tau-217) 3) are ineffective in differentiating tau isoforms (e.g., 3R and 4R tau), seeds (e.g., tau fibrils), based on their tridimensional conformation [19].

Traditional methods show unsatisfactory and suboptimal power in differentiating patients with NDDs from healthy controls (HC) when applied to biological matrices other than CSF (e.g., blood),

where these molecules are expressed at femtomolar ranges instead of picomoles [20]. The quantification of tau in non-CSF substrates is challenging because the concentration of the analyte may not reflect primary neurodegenerative changes. Variations in tau concentrations could be due to peripheral proteolytic processes, metabolic clearance, the interaction with other proteins and modifications of the of brain blood barrier permeability[21].

Novel techniques, developed and validated in the last five years, show better sensitivity in measuring tau levels than traditional methods by detecting up to subpicomolar and even subfemtomolar concentrations. These techniques mostly address the N-terminal rather than mid-region of tau. Single Molecule Array (Simoa), a fully-automated ELISA relying on antibody-coated magnetic beads, has been used to assess t-tau, p-tau [22–24] and N-terminal tau fragments [25] in blood samples of AD patients (Table 1). On the other hand, Electrochemiluminescence immunoassays (ECLIA) provide a quantification of t-tau [26], p-tau181 [27–29] and p-tau217 [30,31] in patients with AD, with high accuracy even in low sample volumes (Table 1). An upgrade of standard mass spectrometry (MS), combining immunoprecipitation reactions with MS [32,33], tracks different phosphorylated tau isoforms like p-tau217 and p-tau181 in plasma [33](Table 1). Also immunomagnetic reaction (IMR) – an antibody-mediated reaction generating changes in the magnetic fields that enable the quantification of the analyte [34,35] – detects t-tau [36–39] and p-tau181 [37,39] concentrations with a greater sensitivity compared to traditional techniques, but with suboptimal specificity (Table 1).

However, these ultrasensitive techniques fail in discriminating different tridimensional conformations of tau (e.g., 3R vs 4R tau). Nevertheless, the selective identification of different tau seeds represents a critical issue to understand the pathophysiology of the primary tauopathies. Based also on the hypothesis of a prion-like transcellular propagation of tau, real-time quaking-induced conversion (RT-QuIC) and protein misfolded cycle amplification (PMCA) have been successfully applied in NDDs [40]. These techniques were initially developed for the detection of prion proteins in Creutzfeldt-Jakob disease. RT-QuIC uses recombinant protein substrates undergoing shaking and incubation cycles to quantify seeding activity of prions (e.g. PrP) and prion-like proteins, such as α-synuclein (α-syn) and tau strains [41]. Similarly, in PMCA seeding activity of substrates derived from brain homogenates or recombinant proteins is amplified by cycles of sonification, enabling a sensitive detection and a selective amplification of specific protein seeds [40].

More extensive research is necessary for the analytical and clinical validation of these approaches by clarifying advantages and drawbacks. Finally, an accurate qualification process, mostly based on

clinical performance, will be required to define context(s)-of-use for pharmacological trials and clinical practice.

4. Advances in tau as a biomarker for Alzheimer's Disease: assessing novel matrices, techniques and targets

T-tau and p-tau proteins measured in CSF are core biomarkers of neurodegeneration in AD [11,12]. The finding of alternative tau isoforms specific for the different clinical and pathological AD subtypes may represent a significant advancement, in parallel with the recent development of ultrasensitive techniques that allow the measurement of tau proteins in blood.

To overcome several technical issues including the higher dilution of tau biomarkers in blood *vs.* CSF [42], several antibody-driven assays have been combined with different detection systems reaching subpicomolar and even subfemtomolar limits of detection (LOD) [43]. A metanalysis indicated that plasma t-tau is increased in AD [44]. A study performed with IMR techniques showed that plasma t-tau levels discriminated AD from controls with optimal accuracy (Table 2) [36]. By contrast, other studies conducted with Simoa and IMR reported higher plasma t-tau concentrations in AD or Mild Cognitive Impairment (MCI) patients compared to HC, but with consistent overlapping results [27,36,39,45,46]. Furthermore, plasma t-tau is not or only weakly correlated with CSF t-tau, p-tau in different studies [45,47–49]. An inverse association between t-tau and cortical thickness in AD-related areas has been reported [38,46]. Plasma t-tau was associated in AD, MCI and HC with a longitudinal decline of cognitive scores and cerebral 18FDG-uptake at PET imaging, increased ventricular volume, and a decrease of hippocampal volume [47,50]. Combining plasma t-tau into ratios (e.g., with plasma amyloid-β_{1-42}) increases the predictive value of cerebral tau accumulation, as assessed by PET, compared to t-tau alone [22]. On the other hand, plasma t-tau did not predict the amyloid-β-status as assessed by cerebral amyloid-PET [27] or CSF [26] in AD, MCI, and HC. Intriguingly baseline plasma t-tau levels seem to be associated with atrophy of the basal forebrain cholinergic system (BFCS) in subjective memory complainers, regardless of their amyloid-β-status [51], although no differences in plasma t-tau levels emerged between SCD and HC in a further study [48]. Finally, plasma t-tau levels were found to be associated with MAPT H1c haplotype in AD, MCI and HC, according to a genome-wide association study (GWAS) [52].

Full-length (FL) tau may account only for a small part of plasma tau proteins. Different proteolytic fragments of tau have been explored in plasma with ultrasensitive techniques so far. The levels of tau N1 fragments, measured with Simoa, are higher in AD and AD-MCI subjects than HC,

showing good to optimal accuracy in discriminating HC from AD and MCI subjects, respectively [25] (Table 2). The actual eligibility of these fragments is still a matter of debate [53].

Conversely, plasma p-tau181 seems to be a more specific AD biomarker. Plasma p-tau181 concentrations assessed by Simoa, IMR and ECLIA-based techniques are higher in AD and MCI-AD compared to HCs [23,27–29,39]. Plasma p-tau181 distinguishes AD patients from non-AD dementias taken together [28] and from vascular dementia (VaD) with optimal accuracy [23], from FTD with good to optimal accuracy [23,29] and from PSP, CBD and Parkinson's disease (PD) or Multiple System Atrophy (MSA) with good accuracy [23] (Table 2). Furthermore, plasma p-tau concentration is consistent with CSF p-tau levels in Aβ-PET positive individuals [28,29]. Plasma p-tau181 predicts the Aβ-PET status with good accuracy and is associated with both Aβ and tau PET positivity in MCI, AD, and HC [27] (Table 2). Moreover, in a cohort of AD, MCI and HC, plasma p-tau181 correlated with tau deposition within the brain [22].

Tau isoforms other than p-tau181 have been recently studied and showed a potential role in the differential diagnosis of AD from MCI and HC. Plasma p-tau217 was able to differentiate AD (clinically assessed) from non-AD patients and autopsy-confirmed AD from non-AD patients with slightly suboptimal accuracy [30] (Table 2). Plasma p-tau-217 levels predict the amyloid-β-status as assessed by amyloid-PET or CSF Aβ42/Aβ40 ratio with optimal accuracy across preclinical-AD, AD-MCI, moderate AD, non-AD-MCI and HC [33] (Table 2). Furthermore p-tau217 could discriminate amyloid-β positive and tau-PET negative participants from HC and has a potential role as an early, preclinical biomarker, being able to assess tau increase before detectable tau aggregation. Both plasma p-tau217 and p-tau181 correlated with their CSF counterparts and were shown to be released from CNS rather than from peripheral sources, thus probably representing more reliable AD biomarkers than plasma t-tau and p-tau202 [33]. Moreover, the cerebral Aβ plaques and tau tangles accumulation is independently associated with *ante-mortem* concentrations of plasma p-tau217 in an autopsy-confirmed cohort [54]

Recently, dynamic trajectories of subsequent site-specific phosphorylation of tau have been reported from preclinical to full-blown AD stages in CSF. While p-tau217 and p-tau181 increase as early as two decades before and tend to decline close to the symptomatic stage, the levels of t-tau and p-tau205 increase later (between 17-13 estimated years to symptom onset) and constantly increase along disease progression [55] Also p-tau231 assessed in CSF by using a mid-region target antibody increases in preclinical AD, largely before p-tau181 and p-tau217, when only subtle Aβ pathology is detectable [56]. These preliminary data suggest the potential role of alternative site-specific phosphorylated isoforms of CSF p-tau for the identification of preclinical AD and for the subsequent risk of phenoconversion. Dynamic changes of tau phosphorylation profile may also represent a potential useful biomarker to assess the efficacy of disease modifying therapies in clinical trials but a better understanding of these changes and a more extensive validation of these biomarkers and their potential context-of-use is needed. Furthermore, whether these changes are reflected in plasma and their timing is an issue that should be assessed.

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5. The diagnostic and prognostic role of tau as a biomarker in 4R-tauopathies

Tauopathies are pathologically characterized by a predominant intracellular accumulation of hyperphosphorylated tau fibrils [1,2] Primary tauopathies can be divided into 3R-tauopathies, including Pick's Disease (PiD), and 4R-tauopathies like PSP, CBD, Argyrophilic Grain Disease (AGD), Globular Glial Tauopathy (GGT). Mixed 3R- and 4R-tauopathies include Primary Age-Related Tauopathy (PART) and neurofibrillary tangles dementia[2]. The genetic FTLD due to MAPT mutations may be related either to 3R-, 4R- or 3/4 R-tau pathological patterns [3](Figure 1). The clinical phenotype of these forms is highly heterogeneous and may not reflect the underlying pathology [14,57].

Currently, the diagnosis of 4R-tauopathies relies on the post-mortem detection of disease-specific patterns of abnormal tau aggregates [3]. Nevertheless, the in vivo diagnosis of these disorders is based on purely clinical-based criteria without reliable biomarkers to track the different tau pathologies [3,14,58].

The potential role of CSF tau concentration as a diagnostic and prognostic biomarker in 4R-tauopathies (especially PSP and corticobasal syndrome - CBS) has been investigated by several studies since 1997, with conflicting results. The measurement of CSF t-tau and p-tau concentrations is helpful in the diagnostic workup of AD. Indeed the characteristic increase of tau species (t-tau or p-tau) in the CSF is specific to AD pathology and lacks in non-AD dementias (including PSP and CBS) [59,60]. Further, CSF t-tau and p-tau do not distinguish PD from atypical parkinsonism with enough accuracy (in particular from PSP) [61]. Nevertheless these biomarkers when combined with others in CSF − α-synuclein, Aβ42, neurofilament (NFL) [62], soluble amyloid precursor protein α (sAPPα), soluble amyloid precursor protein β (sAPPβ), monocyte chemoattractant protein-1 (MCP-1), YKL-40 [63] − may discriminate PSP from PD individuals, as well as CBS from PD and AD/FTD though with conflicting results (Table 3). Similarly, p-tau and t-tau CSF concentrations do not differentiate idiopathic normal pressure hydrocephalus (iNPH), a well-known parkinsonian syndrome-mimic, from PSP [64] (Table 3). Nonetheless, the combination of t-tau, Aβ40 and MCP-1 differentiate iNPH from other neurodegenerative movement disorders taken as a whole (e.g. PD, MSA, PSP and CBD irrespective of their underlying pathology) with good accuracy (AuROC: 0.80) [65].

Within the tau-spectrum, a trend towards higher CSF tau (both t-tau and p-tau) levels has been reported in CBS compared to PSP and HCs [66–68]. Accordingly, Aerts and colleagues showed that t-tau and p-tau differentiated CBD from PSP with fair diagnostic accuracy (AuROC: 0.77 and 0.76, respectively) and that increased tau levels were associated with lower Mini Mental State Examination (MMSE) scores [69]. These data suggest a role of tau biomarkers in the

differential diagnosis of tauopathies and in the stratification of different phenotypes (e.g. motor-dominant *vs.* cognitive-dominant). However, these results should be taken with caution since AD-pathology underpins CBS in about 20% of cases, thus representing a potential bias for the correct interpretation of CSF tau levels. Additionally, these data come from small samples, and patients' selection are based on obsolete diagnostic criteria [58,70].

Tau isoforms may help in differentiating tauopathies from other neuropathologies and to stratify the tau-spectrum. The quantification of alternative tau fragments compared to the classic ones detected by standard ELISA methods is promising [71]. Borroni and colleagues, showed that 33kDa-tau/55kDa-tau ratio discriminate PSP from HCs (AuROC: 0.90) and other NDDs (e.g. AD, CBD, FTD etc.) with good to excellent diagnostic accuracy (AuROC: 0.93 for AD, AuROC: 0.87 for CBD, AuROC: 0.86 for FTD) [68,72]. These authors reported a lower 33kDa-tau/55kDa-tau ratio, as assessed by semiquantitative immunoprecipitation, in patients with PSP compared to other tauopathies and α-synucleinopathies. They also showed a correlation of this ratio with brainstem atrophy and motor impairment in PSP [68,72]. Other groups did not replicate these results and further investigations are needed [73].

Available commercial kits currently quantify tau concentration targeting its central core region. More recently, alternative tau fragments detected using antibodies recognizing the N-terminal fragment τ12-BT2 has been assessed, showing an excellent diagnostic accuracy in differentiating PSP from AD [74](Table 3). Furthermore, a mass spectrometry-based study (see table 1), identified 18 different CSF tau fragments with divergent patterns of expression in PSP compared to AD and to HCs [71].

In last year the application of RT-QuIC and PCMA technologies may isolate and quantify different tau strains (e.g. 4R and 3R tau) in CSF, thus representing an ideal tool to stratify *in vivo* 3R, 4R and mixed 3R/4R tauopathies [41].

Stepping aside from CSF, a single study explored the potential discriminating value of plasma p-tau181 in tauopathies, using ultrasensitive detection techniques (Simoa). Plasma p-tau was able to differentiate PSP and CBS from AD with good diagnostic accuracy (Table 2), being associated with Aβ42 pathology assessed by cerebral amyloid-PET examinations [75]. The validation of tau-biomarkers in plasma in large longitudinal cohorts using ultrasensitive techniques is warranted.

Tau biomarkers may have a prognostic context-of-use in 4R-tauopathies. Constantinescu and colleagues showed that t-tau CSF levels may predict mortality in a cohort of PSP patients (see table 3 for the Hazard Ratio) [76]. Increased CSF t-tau levels at baseline are associated with a faster decline as measured by clinical progression scores like the Schwab and England ADL (SEADL);

decreased baseline p-tau predicts low SEADL scores and rapid decrease in PSP Rating scale (PSPRS). The prognostic value of tau is increased when combining biomarkers into ratios (e.g., baseline p-tau, t-tau and NFL concentrations) [77]. Currently, the role of tau as prognostic biomarker in 4R-tauopathies is limited and should be clarified in longitudinal studies.

6. The diagnostic and prognostic role of tau in ALS-FTD spectrum

Frontotemporal dementia (FTD) and Amyotrophic lateral sclerosis (ALS) are NDDs with significantly clinical and pathological overlaps.

FTD is a heterogeneous cognitive disorder, recognizing multiple clinical and pathological phenotypes [78,79], related to the degeneration of frontal and temporal lobes [78]. Three main clinical variants of FTD have been described: the behavioral variant of FTD (bvFTD) [80], primary progressive aphasia (PPA) and the motor FTD syndromes (CBS and PSP) [81]. PPA can be further divided into a semantic variant (svPPA) and in a non-fluent variant (nfvPPA) [82]; the logopenic variant of PPA (lvPPA) is conversely considered an atypical AD variant rather than a proper FTD-syndrome [78]. On the other hand, three main pathological FTD phenotypes have been described, namely FTD with TDP-43 aggregates (FTD-TDP), FTD with tau depositions (FTD-Tau) and FTD with FUS aggregates (FTD-FUS) [8]. It should be noticed that the correlation between the clinical and the pathological phenotype is not always predictable. While nfvPPA is associated in most cases with FTD-tau (85% of all cases) and svPPA with FTD-TDP43 pathology (90% of cases), bvFTD can be associated with both FTD-tau and FTD-TDP43 histological patterns [8].

ALS is progressive NDD characterized by motor symptoms related to the progressive degeneration of the upper (UMN) and lower motoneuron (LMN) [83]. ALS is strictly associated to TDP-43 pathology in about 95% of patients, alternative pathological inclusions (e.g. SOD-1 inclusions) may be reported in specific variants in a minority of cases [83].

The clinical and pathological boundaries between FTD and ALS are blurry and the current diagnostic criteria consider ALS and FTD as a complex clinical *continuum* and pathologic *spectrum* as well [84,85]. In fact, ALS patients show cognitive impairment in about 50% of cases, meeting current criteria for diagnosing dementia in 10-15% of cases [86], and behavioral impairment in up to 45-50% of patients baseline [87]. Conversely, FTD patients may present motor symptoms in approximately 30% of cases showing motor neuron dysfunction (12.5% of bvFTD patients) [88].
The assessment of Tau as a biomarker in the ALS-FTD spectrum is controversial and recent studies on CSF tau showed conflicting results.

Some authors observed a higher concentration of CSF t-tau in FTD patients compared to HC, distinguishing them with a good diagnostic accuracy [89–91], (see Table 3 for AUROC

values). On the other hand, other investigations failed in detecting any significant difference [92–95]. These conflicting results may be due to the fact that FTD is a highly heterogeneous diagnostic frame, including different clinical and pathological phenotypes. Inconsistent results have been reported in ALS studies [91,93,96–100].

Regarding the differential diagnosis between FTD and AD, p/t-Tau, t-tau and its ratios with Aβ42 may be of clinical interest; FTD patients show lower levels of t-tau [91,94,95,101] and higher of p/t-Tau [94] when compared to AD patients. Moreover, the ratios between t-tau and Aβ42 are able to distinguish between FTD and AD patients [91,95,101].

The CSF p-Tau and t-Tau ratio (p/t-Tau) is more interesting for the clinical workup. p/t-Tau was observed to be significantly lower in ALS patients [89,96–99] and in FTD patients [89,90,92,102], when compared to HC. Not surprisingly, ALS showed lower levels of CSF p/t-Tau than FTD phenotypes with a likely related tauopathy. This ratio was significantly lower in FTD variants with suspected TDP-related pathology (both patients with autopsy-proven TDP-related pathology and TDP-related mutation carriers) compared to FTD phenotypes with a likely underlying tauopathy [89,90,92,94,102,103] (Table 3). Low p/t-Tau may therefore represent a marker of an underpinning TDP-43 pathology. From a pathophysiological point of view this could be due to a lower tau burden in TDP-related compared to tau-related FTLD variants. On the other hand, the different proportion of patients with concomitant MND and amyloid-β co-pathology in the two groups may contribute to these observed differences [102]. Furthermore p/t-Tau ratio seems to correlate with motor cortex thickness and seems to be associated with UMN involvement in ALS [99]. Accordingly p-tau, t-tau or their ratio may find a context-of-use in the differentiation of clinical endophenotypes within the ALS spectrum (e.g. preponderant UMN *vs.* LMN involvement) but further investigations are needed.

Different concentrations of CSF tau fragments other than classic tau analytes have been reported by some authors in TDP-related *vs.* tau-related FTLD [90]. This suggests that differential proteolytic processes may occur in different FTLD variants but this hypotesis and its implications for novel biomarkers research deserves further studies and a more extensive assessment [90].

As regards the role of CSF tau ad a prognostic biomarker, one study showed a potential prognostic value of baseline CSF t-tau in ALS, being a reduction of overall survival observed in individuals with higher CSF t-tau values (Table 3) [97]. Moreover p/t-tau ratio has been associated with survival in FTD [102] and disease progression in ALS [100].

Moving to surrogated progression biomarkers, a negative correlation between baseline CSF t-tau and the longitudinal increase of ALS Functional Rating Scale Revised (ALSFRS-R) score was described [100].

7. The diagnostic and prognostic role of tau in α-synucleinopathies

Parkinson's disease (PD), Dementia with Lewy Bodies (DLB) and Multiple system atrophy (MSA) belong to a group of NDDs collectively called α-synucleinopathies. They are pathologically characterized by abnormal deposition of α-synuclein such as the (α-syn)-rich neuron intracytoplasmic inclusions (Lewy bodies, Lewy neuritis and glial cytoplasmic inclusions) or the oligodendrocytes cytoplasmic inclusions [104]. Nevertheless, other misfolded proteins have been recognized in degenerated neurons, including tau proteins [105] highlighting the necessity of a more complex pathophysiological model.

In contrast to tau, which seems to require cofactors, in vitro and in vivo studies demonstrated how α-syn is able to self-polymerize [106] [107]. Several pathophysiological mechanisms have been proposed to explain the interaction between α-syn and tau. According to different models α-syn can act as the "amyloidogenic seed" promoting tau aggregation with subsequent interaction between the two proteins enhancing each other's fibrillization [108] or as the pathological chaperone for tau fibrillization [109]. Whatever the mechanism, oligomeric forms of α-syn and tau co-exist in α-synucleinopathies and influence each other and perpetuate mutual aggregation, leading to hybrid oligomers' formation [9,110,111].

Interestingly, levels of CSF α-syn and p-tau are directly correlated in α-synucleinopathies. This could be because the accumulation of α-syn in PD brain could inhibit the release of p-tau in CSF through unknown mechanisms, supporting the relationship between α-syn and tau in α-synucleinopathies [112]**.** With regard to CSF, reduced levels of α-syn, t-tau, p-tau and amyloid-β at disease onset have been found in PD compared to HC [112][113]. Furthermore, p-tau levels and p-/t-tau ratio increased over time after PD onset despite stable levels of t-tau/α-syn ratio. Patients exhibiting postural instability and gait impairment predominant phenotype had lower CSF p-tau and Aβ42 concentrations than those with tremor-dominant phenotype [112]. Male sex and SNCA polymorphism rs316181 were linked to increased levels of p-tau, while an increased t-tau/Aβ42 ratio was associated with REM behavior disorder [113]**.**

The diagnostic value of CSF tau biomarkers is higher when they are combined into ratios [112][114][115][113][115]**.** The p-tau/α-syn ratio (which is increased in PD compared to HC), showed the highest accuracy for PD diagnosis [115] (Table 3)**.** Roughly 75% of PD patients with a disease course longer than 10 years evolve to PD with dementia (PDD) [116]. CSF t-tau has been reported to be increased in PDD compared to HC, while p-tau did not show differences in the two groups [117]. Longitudinal studies in PD patients found neither a predictive value for the subsequent development of dementia for CSF t-tau and p-tau levels measured at PD onset [118],

nor an association with dementia severity in PDD [117]. In contrast, plasma t-tau and p-tau181 levels measured through immunomagnetic reduction (IMR) were increased in DLB compared to HC, and reduced compared to PDD [37] (Table 2).

DLB is the second most common cause of dementia after Alzheimer's disease (AD). DLB belongs to the spectrum of α-synucleinopathies, nevertheless, a mixed neuropathology with amyloid-β plaques and tau pathology is present in nearly 40% of patients (α-syn+AD) and are associated with an increased rate of dementia and a decreased survival time [119]. Although previous studies reported increased levels of t-tau in DLB compared to HC [120], these findings were not replicated in more recent studies [121]. The use of different diagnostic criteria and populations may in part explain these inconsistent results. In general, CSF t-tau and p-tau levels are lower in DLB than AD [120], suggesting that levels of p-tau in CSF could represent a valuable marker for the differential diagnosis between these two NDDs [123]. On the other hand, t-tau levels are higher in DLB in comparison to PD and PDD, supporting the hypothesis that limbic and cortical Lewy body pathology is the main and specific pathologic correlate of dementia in PD [124]. In a neuropathological study, the regional distribution of tau and amyloid-β is different in α-syn+AD patients and AD, with a greater proportion of tau in the temporal neocortex for DLB patients and in frontal neocortex in AD. Moreover, the severity of tau burden was correlated with worse *ante-mortem* cognitive performances in DLB individuals [125]. Tau pathology has also been correlated to the pattern of brain atrophy in MRI. Indeed, increased CSF t-tau levels were associated with high global atrophy scores [126] and elevated CSF p-tau with a more selective posterior atrophy [127]. CSF ratios t-tau/Aβ42 and p-tau/Aβ42 recently proved to predict the presence of AD co-pathology in DLB, with higher levels of both ratios in α-syn+AD brains when compared to α-syn-AD brains, while no correlation was found analyzing single biomarkers. Besides, higher CSF t-tau/Aβ42 and lower CSF Aβ42 levels were associated with a higher burden of neocortical α-syn [121].

MSA is a NDD characterized by autonomic failure associated with a poorly levodopa-responsive parkinsonian syndrome or cerebellar ataxia, thus defining two main phenotypes respectively identified as MSA-P and MSA-C [128,129]. Differential diagnosis between PD and MSA could be challenging because of the clinical overlap in the early stages and a reliable biomarker for the differential diagnosis of MSA from PD yet represents an unmet need. Data on CSF biomarkers in MSA are conflicting. In some studies, CSF t-tau concentration was higher in MSA than PD patients or HC [130][131], whereas others reported a reduction of its level [132]. By contrast, p-tau concentration was similar in MSA and HC [130][132]. Conflicting data have been published on the p-tau/t-tau ratio, reported either higher [130][132] or lower [133][134] in MSA compared to PD. The combination of p-tau181, t-tau and DJ1 (a protein related to PD pathogenesis

whose function is still unknown in MSA) proved high sensitivity and specificity (respectively 82% and 81%) to discriminate MSA from PD [131], as well as the p-tau/Aβ42 ratio (specificity 71%, sensibility 93%), which is significantly increased in MSA [134] (Table 3). Similarly, the AD index (CSF Aβ40/42 ratio × t-tau) and the ratio t-tau/α-syn were higher in MSA than HC, while no significant differences were found comparing PD and HC [130]. Finally, no CSF biomarkers proved to be reliable for the differential diagnosis between MSA-C and MSA-P [114].

Fluid biomarkers for α-synucleinopathies were recently investigated also in blood. Red blood cells (RBC) seem to be a preferential niche for misfolded proteins [135] and oligomeric α-syn was described in RBC of patients with PD [136]. Analysis of RBC in PD patients showed increased levels of phospho-tau (p-tau) in PD compared to HC [110]. Interestingly, RBC t-tau concentration was inversely correlated to MMSE scores both in the general PD cohort and in PD drug naïve patients, suggesting a central role of tau pathology in promoting cognitive decline in PD [110]. Plasma t-tau and p-tau were higher in MSA compared to HC when measured through IMR [137][37], while levels of p-tau measured through Simoa did not differ between MSA and HC [24].

8. Tau PET in Tauopathies

In the field of neurodegenerative dementias, in vivo imaging with radioligands designed to detect each underlying proteinopathy aims to find a gold standard biological marker. Amyloid-β imaging has been incorporated into AD diagnostic criteria since 2007 [138]. However, cerebral amyloid-PET uptake did not correlate with cognitive performance nor neurodegeneration in AD. Biomarkers of tau pathology may be closely related to neuronal injury and changes in cognition. Tau-PET will support the investigation of the spatial-temporal evolution of tau pathophysiology in AD and other NDD as well as the clinical validation of fluid biomarkers. Therefore, research effort is focusing on the development of tau-PET radiotracers in order to reveal underlying tau deposits in a broad range of tauopathies, such as AD, FTD, PSP.

A significant number of tau PET tracers have been synthesized so far, but many of those lack sufficient specificity and selectivity [139,140]. The main difficulties to develop these tracers are related to [141]: 1) lipophilicity, which is necessary to cross the blood-brain barrier and the cell membrane (tau is located both intra and extracellularly); by contrast, excessive lipophilicity could lead to unspecific binding; 2) affinity for tau, relevant to obtain high selectivity for tau molecule and overcoming the high Aβ concentrations interfering with tau-ligand binding, but requiring prolonged scanning time to reach the steady-state; 3) different conformations of tau aggregates and different tau isoforms [142], making it challenging to develop a unique tau radiotracer for all types of tauopathies. Based on both in vitro and in vivo results, three families of radiotracers were

initially synthesized: 1) the THK family (THK117, its (S)-enantiomer THK5317, and THK5351; 2) 18F-AV1451, also known as T807 or Flortaucipir; 3) PBB3. The so-called first-generation tau tracers finally showed a lack of specificity, with relevant off-target binding to monoamine oxidase B (MAO-B) for the HK family and Flortaucipir [143], neuromelanin for Flortaucipir [144], and Aβ-plaques and α-syn for PBB3 [145,146]. Hence, the second generation of tau PET tracers has been developed: RO948, PI2620, JNJ311, MK6240, PM-PBB3 and AM-PBB3 [147]. However, the most widely studied agent is [18F]AV-1451 (Flortaucipir). It was the first approved tau PET tracer for estimation of the density and distribution of aggregated tau neurofibrillary tangles in adults with cognitive impairment and suspected AD [148].

8.1 Aging

First-generation tracers demonstrated retention of uptake confined to the medial temporal lobe in normal cognitively elderly, in accordance with pathological data [149], likely reflecting an age-related process of tau-deposition [150]. Accumulation of tau in the temporal cortex, without an associated significant Aβ burden, may suggest a promoting effect of tau in mild amnestic deficits; hippocampal atrophy or a PART related pathology in such subjects.

8.2 Alzheimer's Disease

There is now convincing evidence that cortical tau binding at PET imaging reflects tau accumulation reported from histopathological studies in AD brains [149]. 18F-AV1451 demonstrated high affinity in vitro for paired helical filaments (PHF) of 3R/4R tau isoforms reported in AD brains [151,152]. A recent study in 82 individuals with or without dementia showed a high concordance between the visual reading of 18F-AV1451 PET scans and staging of cortical neurofibrillary tangles, reinforcing the concept that Flortaucipir effectively reflects pathological changes of AD [153]. Similar results were replicated in vivo, with 18F-AV1451 demonstrating to accurately differentiate clinically diagnosed AD from HC and other NDDs [154]. Higher levels of tau tracer retention in the inferior lateral temporal region, but also in the posterior cingulate and lateral parietal regions, provided the best discrimination areas between AD patients and HC [155–158]. Other studies comparing cognitively normal subjects with MCI patients showed differences in binding restricted to medial temporal regions (parahippocampal and entorhinal cortex) [159,160], as well as lateral temporal and parietal areas when examining only amyloid-β-positive MCI individuals [158]. Furthermore, some studies reported greater retention of 18F-AV1451 in the cortex of younger compared to older AD patients, with a cut off of 75 years-old [161], and in early-onset compared to late-onset AD patients with a cut off of 65

years-old [160], as similarly reported in previous post-mortem studies [162]. Moreover, neocortical Flortaucipir retention was found in preclinical AD cases and rarely in amyloid-β negative cases [161,163].

The extent of tau accumulation intimately correlates with the severity of MCI due to AD and AD dementia, providing an objective index for disease severity. The few available longitudinal tau PET studies in AD demonstrated a tracer retention related to disease duration [164–167]. However, results from the largest studies, which compared cognitively unimpaired Aβ–negative and Aβ–positive individuals, MCI and AD dementia patients, are conflicting. Cho and colleagues studied 107 participants (45 Aβ-negative cognitively unimpaired, 7 Aβ-positive cognitively unimpaired, 31 MCI, and 24 AD dementia) who completed both 18F-flortaucipir and 18F-florbetaben at baseline and who were followed up for 2 years. The authors found a predominant tau accumulation in the medial and basal temporal cortices in MCI and in the lateral temporal cortices in AD dementia [167], thus supporting a progressive tau accumulation pattern in line with the Braak model [149]. Conversely, Jack and colleagues evaluated the longitudinal change in tau PET signal during a one-year follow-up in a group of 126 individuals (59 cognitively unimpaired Aβ-negative, 37 cognitively unimpaired Aβ-positive, and 30 cognitively impaired Aβ-positive). They demonstrated a tau accumulation in the Aβ-positive clinically unimpaired group in the medial temporal lobe and in the medial parietal areas, including posterior cingulate cortex. This suggests that initial accumulation of tau aggregates in AD may not be restricted to the medial temporal lobes as implied by Braak staging [165]. Overall these evidence proved that longitudinal tau PET may be an useful biomarker in clinical trials to monitor the effect of disease-modifying therapies tailored to reduce tau as well as cerebral amyloid-β plaque burden [168]. In AD increased 18F-AV1451 uptake is strongly co-localized with hypometabolic regions and has been associated with worse performance on various cognitive domains in regionally specific patterns [169]. Accordingly, 18F-AV1451 distribution may predict the clinical variants of AD suggesting on-site neurotoxicity provoked by tau aggregates in posterior cortical atrophy, logopenic variant of primary progressive aphasia, or behavioral/dysexecutive variant of AD [169–172]. The main challenge for tau tracers remains the capability to detect preclinical / prodromal / early AD stages. Importantly, the sensitivity for an early diagnosis is limited by the fact that in the early AD stages (e.g. MCI) neurofibrillary tangles are only present in deep brain regions. In more advanced AD stages 18F-AV1451 discriminate mild-to-moderate AD patients from HC [154].

Currently, there are not conclusive studies on second-generation tau tracers in AD patients and we do not have any head-to-head comparison between them. Available studies suggest that they may

be more useful in identifying earlier Braak stages. They also show substantially less non-specific binding or higher affinities to primary tauopathies [173]. These agents include [18F]MK-6240 (Merck & Co), PM-PBB3 (APRINOIA Therapeutics), F18-PI-2620 (Life Sciences) (50) and RO-948 (Roche). RO-948 tracer showed similar patterns of cerebral uptake then 18F-AV1451 [174]. Moreover, RO-948 showed a greater stability, a higher retention in the medial temporal lobe and lower intracerebral "off-target" binding than Flortaucipir [175,176]. In AD subjects, PET images of F18-PI-2620 showed a significantly higher uptake than control subjects in temporal lobe, parietal and cingulate cortex. Importantly, in non-demented control subjects it showed robust initial brain uptake and fast washout from the brain, as well as no age dependency. This last finding suggests that F18-PI-2620 could improve the discrimination between non-demented control and AD subjects in elderly. Moreover, excellent test-retest variability has been demonstrated confirming the utility of F18-PI-2620 to evaluate longitudinal change of tau deposition during disease course [177]. Finally, three recently published studies have evaluated [18F]MK-6240 in vivo [178–180]. All these studies exhibited favorable kinetic and high binding levels of [18F]MK-6240 to brain regions typically affected from NFT deposition in AD subjects. [18F]MK-6240 PET images in another cohort of patients positive for cerebral amyloid-β deposition (cognitive unimpaired/impaired elderly controls, MCI and AD patients) showed a pattern corresponding to the anatomic distribution of tau as expected in the Braak model [178].

8.3 Non-AD tauopathies

Tau imaging may also be relevant for other tauopathies, such as CBD and PSP. These atypical parkinsonian syndromes are characterized by abundant filamentous tau inclusions that are made of isoforms with four microtubule-binding repeats in tubular or straight filaments. In PSP and CBD abnormal aggregation of pathologically misfolded and hyperphosphorylated tau proteins mainly occur in the basal ganglia in the early stages, spreading later to multiple brain areas: brainstem, posterior frontal lobe, cerebellum and association cortices for PSP; primary motor cortex, frontal lobe, brainstem or cerebellum for CBD [181–184]. Nevertheless, a variety of overlapping syndromes are frequent in tauopathies.

To date few studies using low specific first-generation PET tracers have examined tau binding in parkinsonian disorders. They revealed distinct patterns of tracer retention in PSP and CBD, compared to controls, showing elevated PET signal in the basal ganglia and affected cortical regions, in agreement with postmortem data [185].

Elevated tau deposition in PSP cases was observed in the basal ganglia, thalamus, dentate nucleus of the cerebellum, and midbrain [186–192]. However, an extensive overlap and age-dependent increase in both the PSP and control groups has been reported [187]. Significant differences in 18F-AV-1451 distribution between patients with PD and PSP, with increased uptake in the globus pallidus, midbrain, and subthalamus in PSP cases, have been also described [193,194]. Interestingly, in some studies the radioligand accumulation correlated with the clinical disease severity [189,195,196], but the lack of correlations between tau binding and symptom severity was observed in the other cohorts [188,194]. A recent study by Whitwell and colleagues using flortaucipir reported that the clinical heterogeneity present in PSP is mirrored by anatomical and tau burden heterogeneity within the brain [197].

Studies analyzing the feasibility of tau PET in patients with CBS showed elevated asymmetrical tracer deposition in dentate nucleus of the cerebellum, midbrain, subthalamic nucleus, globus pallidus and putamen, precentral and postcentral cortex, superior frontal and parietal lobe, fitting with the regional distribution of tau pathology [198,199]. Furthermore, longitudinal investigations documented a correlation between tau accumulation over time and disease progression [166,200]. However, the degree of uptake in CBS using currently available tau ligands is variable, and some patients resulted negative [201,202].

Overall, studies using in vivo tau PET in PSP and CBD individuals that received a postmortem confirmation have shown conflicting results questioning the usefulness of tau imaging in early diagnosis of such conditions. Indeed, the available tracers are not specific for 4R tau pathology and show substantial off-target binding in the midbrain and basal ganglia to monoamine oxidase B and to neuromelanin-containing cells [192,203].

Second generation of tracers with improved off-target binding are under evaluation also in non-AD tauopathies. Brendel and colleagues found a great potential to diagnose patients with suspected PSP using 18F-PI-2620 which proved high affinity to recombinant 4R tau fibrils and PSP brain homogenate [204,205].

9. Discussion and perspectives

Epidemiological projections indicate that AD evolving epidemic represents a global threat for healthcare systems [54]. Minimally invasive and globally accessible tests are needed to cope with this expected burdensome demand and to manage individuals in suspected preclinical/prodromal stages of NDDs [206,207]. Blood-based biomarkers represent cost-, resource- and time effective tools [208]. They hold the potential to enable large-scale biological screening of individuals who are very unlikely to have AD-related pathophysiology and would support the request of second-

level investigations (e.g. PET imaging or CSF assessment). Blood-based biomarkers not only open up the opportunity of a multi-step diagnostic workup but can also facilitate the re-engineering of drug Research & Development (R&D) pipelines, from subjects' enrollment, target engagement, to treatment efficacy monitoring. In Oncology, serial liquid biopsies offer clues about the evolution of cancer in individual patients across disease stages, enabling individualized genetically and biologically guided therapies [207].

The development of novel ultrasensitive measurement techniques has enabled the detection of tau-related biomarkers, facilitating a liquid biopsy-driven paradigm shift in the field of NDDs, including AD [207]. Plasma t-tau seems to have a limited utility as diagnostic marker in NDDs when assessed alone [47]. On the other hand, plasma p-tau may find a context-of-use in the differential diagnosis between AD and non-AD dementias, including primary tauopathies and vascular dementia [25–29,75]. The combination of tau with other putative biomarkers (e.g. NFL) into panels or ratios seems to increase the diagnostic accuracy of tau biomarkers alone [22]. Sets of multiple biomarkers may accordingly find a context-of-use in the clinical diagnostic workup of neurodegenerative dementias , in patients' selection and their follow-up for disease modifying clinical trials.

Studies investigating p-tau isoforms other than p-tau181 (e.g. p-tau217) [30,33,209], and alternative proteolytic fragments like the N1-tau [25] fragment have shown encouraging results in differentiating AD and MCI from HC and in predicting the amyloid β- in preclinical AD. Several studies, performed in AD and NDDs, indicate that plasma p-tau217 is a reliable predictor of tau pathology (as assessed through tau-PET)[210], amyloid-β-mediated tau pathophysiology, longitudinal cortical/subcortical atrophy and AD-like cognitive decline. These findings coupled with the evidence that p-tau217 discriminates AD from non-AD conditions [30,31] explain why plasma p-tau217 will be used as exploratory marker in different COU, including patients' selection and follow-up in clinical trials. The evidence about plasma p-tau217 also support its integration in the ATN matrix for disease diagnosis, prognosis and progression monitoring in clinical practice [31].

A better understanding of time-trajectories of these alternative phosphorylation sites of p-tau is mandatory to help understand the more adequate context-of-use of each isoform from preclinical to full-blown AD.

In primary tauopathies CSF p-tau and t-tau, as assessed by traditional techniques, are not reliable biomarkers for the diagnostic workup of 4R-tauopathies [64]. These biomarkers are useful in both clinical and research settings for differentiating NDDs with an underlying AD pathology, which can mimic primary tauopathies [59,60]. The detection of different tau isoforms and

fragments (e.g. 33 KDa vs 55 KDa isoforms) [72] and of specific tau strains (e.g. 3R and 4R tau) by means of PMCA and RT-QuIC [41] are promising tools to stratify the pathologic tau-spectrum and to identify different phenotypes. The prognostic value of tau biomarkers in 4R-tauopathies remains to be clarified.

In the ALS-FTD spectrum, CSF t-tau and p-tau may contribute in discriminating AD from FTD and in identifying patients with FTD phenotypes with an underlying AD-pathology [91]. The diagnostic value of p-tau and t-tau alone in differentiating the FTD-ALS continuum from HCs is controversial [89,91,98]. Nevertheless p-tau/t-tau ratio seems to be useful in FTD for the identification of patients with a TDP-43 underlying pathology [89] and may be useful in ALS to discriminate clinical endophenotypes (UMN-predominant *vs*. LMN-predominant variants) [99]. Baseline CSF t-tau and p-tau/t-tau ratio could represent a prognostic marker for both FTD and ALS [97] but further studies are needed to identify the proper context-of-use of these biomarkers.

In α-synucleinopathies the co-occurrence of underlying tauopathy is commonly reported. T-tau and p-tau concentration may vary according to PD phenotypes, thus suggesting a potential role of tau in differentiating clinical phenotypes [112,113]. An association between tau burden and cognitive impairment in PDD and DLB has been postulated but data are conflicting and inconclusive [117,118]. Similarly the diagnostic value of CSF p-tau and t-tau in atypical parkinsonism (e.g. MSA and DLB) is controversial [117,121,122,124,132,211][130,131].

As regards tau-PET tracers, tau accumulation in the medial and basal temporal cortices was observed in MCI patients, thus suggesting a potential role of tau-PET tracers in the prodromal AD and a potential value of tau-PET uptake in monitoring the efficacy of disease-modifying therapies in clinical trials [165,167]. A possible role of tau-PET imaging in the differential diagnosis between 4R tauopathies is suggested by some studies highlighting a different deposition pattern of tau in PSP compared to CBS. Off-target binding in the midbrain and basal ganglia is currently limiting the use of fist generation tracers but was partly solved with the development of second-generation tau tracers [145]. Further evidence on the physiological and pathologic variables influencing tau-PET tracers uptake, on the off-label binding and on the comparison of different tracers is warranted to understand the appropriate context-of-use of these biomarkers.

In conclusions, biomarkers development and related research have significantly contributed to the reengineering of AD as a clinical-biological framework, as reflected by the ATN system, where preclinical stages of the disease are identified and potentially treated for preventive strategies, including tau-targeting approaches. It is conceivable that the same paradigm shift may take place also for NDD others than AD. Individuals with different clinical

presentations but similar biomarkers may be grouped into biological clusters facilitating a treatment essentially based on pathophysiological mechanisms and not phenotypes [212,213], in line with the precision medicine paradigm [214].

11. Expert opinion

Tau, as assessed in CSF in the form of t-tau and p-tau181, is an established diagnostic biomarker for AD. The detection of tau in alternative easily accessible matrices like plasma currently represents one of the most exciting future directions for AD biomarker research.

Alternative tau isoforms (e.g. p-tau217) or proteolytic fragments (e.g. N-terminal fragments) quantified in CSF and in blood may represent candidate fluid diagnostic and prognostic markers overcoming the limitations of current CSF t-tau and p-tau measurement in NDDs. In particular plasma p-tau217 could be used as exploratory marker in different drug development pipelines with distinct molecular target and for different COU, including patient selection, and theragnostic. The evidence about plasma p-tau217 also support its integration in the ATN matrix for disease diagnosis, prognosis and progression monitoring in clinical practice.

Nevertheless, the implementation of techniques enabling the identification of different tau strains (e.g. 3R- *vs.* 4R-tau), enabled by RT-QuIC and PMCA, represents the most important frontier for fluid biomarker discovery in tauopathies.

The validation of biomarkers tracking tau pathology in the ALS-FTD spectrum is challenging, due to the high clinical and phenotypical heterogeneity of this spectrum. However, tau biomarkers may identify AD-related pathology, and, to a lesser extent, differentiate tau-related from TDP-43 related pathology.

Intriguingly, tau biomarkers may reveal co-pathologies in NDDs. Biomarkers tracking tau pathology in PD and atypical parkinsonism may in fact be potentially helpful for the segregation of clinical subtypes and to combine tailor disease-modifying treatments.

The development of tau-PET tracers and the overcome of first-generation tracers' limitations (especially the off-target binding and the necessity of a cyclotron) was a great step forward in the *in vivo* understanding of early pathophysiological changes in AD. Further studies are needed to define the context-of-use of tau-PET tracers in AD (especially in preclinical and prodromal AD) and their potential applications outside the AD-spectrum.

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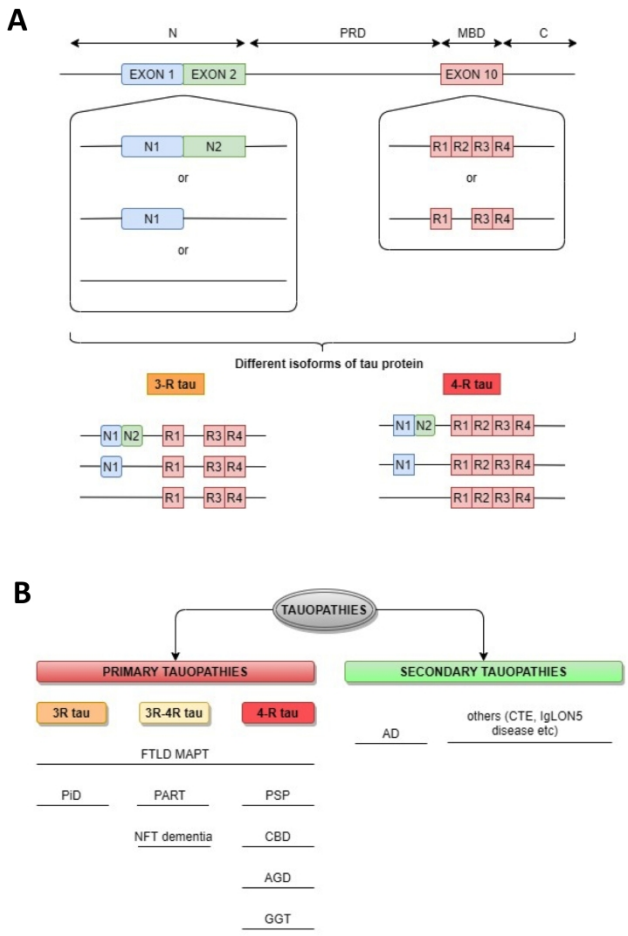

Figure 1. Tau isoforms and the pathological classification of tauopathies.Six tau isoforms are generated through the alternative splicing of exons 2,3 and 10 of the Microtubule-associated protein tau (MAPT) gene. The microtubule-binding domain (MBD) is encoded by exon 10, whose alternative splicing generates three tau isoforms with 3 or 4 repeats called 3R and 4R tau respectively (panel A). Tauopathies are classified accordingly into primary tauopathies, which are further divided into 3R, 4R and mixed 3R/4R tauopathies, and secondary tauopathies (panel B).

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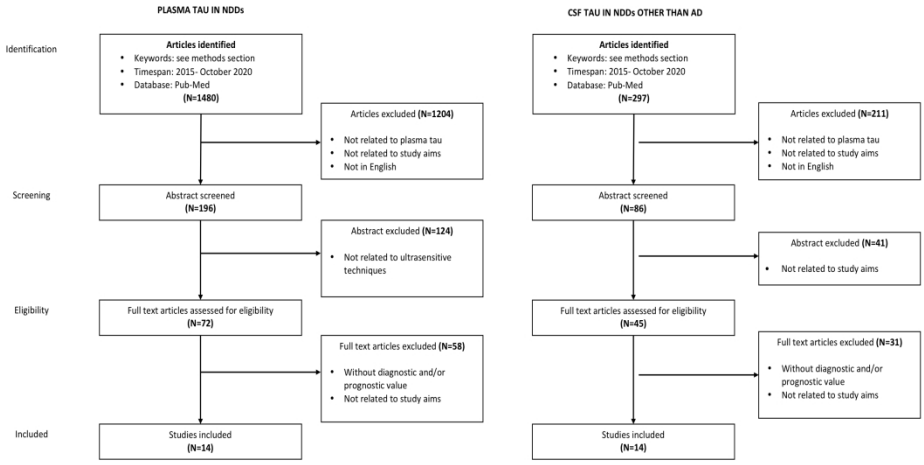

Figure 2. Paper selection flow-chartThe paper selection algorithm for plasma tau in NDDs and CSF tau in NDDs other than AD is summarized in the figure. Abbreviations: AD: Alzheimer's Disease, CSF: cerebrospinal fluid, NDDs: neurodegenerative diseases.

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Technology	Key characteristics	Tau species detected	Comments
Simoa	Fully-automated digitalized standard-ELISA. *How it works* 1. Specific antibody-coated paramagnetic microbeads and fluorescence-emitting detection antibodies are embedded with the analytes. 2. The sample containing immunocomplexes (bead, bound protein and detection antibody) is loaded into arrays of subfemtomolar-sized reaction chambers, large enough to hold one bead. 3. The emitted signal reflects the presence of single enzyme-associated immunocomplexes on single beads. ~~(https://www.quanterix.com/simoa-bead-technology)~~	t-tau p-tau181 N-terminal tau fragment	*Advantages* - greatest sensitivities; - multiplexing (short turn-around times and high throughput); - flexibility and cost-saving; - most established ultrasensitive technology for blood-biomarkers in AD. *Limitations* - standardization of the protocol and further cross-assay evaluations are needed.
ECLIA	Electrochemiluminescent labels generate light when activated by the interaction with ligands. The magnitude of the signal reflects the concentration of the analyte. *How it works* 1. Labels interact with ligands; 2. The interaction generates a luminescent signal, whose magnitude is proportional to the analytes concentration. The reaction relies on a complex of ruthenium and TPA and can be incorporated into different immunoassays. ~~(https://www.creative-biolabs.com/drug-discovery/diagnostics/eclia-based-kits-development.htm)~~	t-tau p-tau181 p-tau217	*Advantages* - rapid measurement, wide measuring range, high sensitivity (up to femtomolar range); - low sample volume; - MSD platform is a multiplex technology enabling parallel measurements of different biomarkers and matrices (e.g., plasma, CSF); - optimal accuracy for different tau-species. *Limitations* - further head-to-head comparisons are required to assess sensitivity and specificity.

IP-LC/MS	Combines immunoprecipitation and liquid chromatography (LC) /mass spectrometry (MS) *How it works* 1. peptides are isolated with specific antibodies [30]; 2. Peptides are physically separated based on their mass and biochemical properties by means of LC/MS.	p-tau181 p-tau217	*Limitations* - a multiple-step extraction phase to purify and concentrate the analytes is required to increase sensitivity and reduce the interference of contaminants; - labor-intensive, low-throughput and time-consuming method.
IMR	An antibody-mediated reduction in the magnetic field generates a signal for analyte concentration quantification. *How it works* 1. Magnetic antibody-coated nanoparticles oscillate under multiple AC magnetic fields; 2. Binding of target molecules enlarges or clusters magnetic nanoparticles, leading to a reduction in their magnetic susceptibility, which is proportional to the concentration of the analytes.	t-tau p-tau181	*Advantages* - does not require beads to purify the antigens, relying on a single-antibody detection strategy - great sensitivity (up to the femtomolar range) *Limitations* - suboptimal specificity.

Table 1. Overview of ultrasensitive measurement techniques for the detection of tau species in plasma

Abbreviations: AC: alternating current; AD: Alzheimer's disease; ECL: electrochemiluminescence; ECLIA: electrochemiluminescence immunoassay; ELISA: enzyme-linked immunosorbent assay; IMR: immunomagnetic reduction; IP/LC-MS: immunoprecipitation coupled with liquid chromatography-mass spectrometry; LoD: limit of detection; MSD: mesoscale discovery; p-tau181: phosphorylated-tau at threonine 181; p-tau217: phosphorylated-tau at threonine 217; Simoa: single molecule array; TPA: tripropylamine; t-tau: total tau.

Reference	Population	Study design	Technique	Tau species	Diagnostic value	Prognostic value
Lue LF. et al., 2017	n=129 - U.S. cohort: n=32 CU (n=16); AD dementia (n=16) - Taiwan cohort: n=97 CU (n=66); AD dementia (n=31)	Cross-sectional	IMR	t-tau	CU vs. probable AD: - AuROC=0.81 (U.S. cohort) - AuROC=0.96 (Taiwan cohort) - AuROC=0.92(combined cohort) (clinical diagnosis as reference)	NA
Mielke MM. et al., 2017	n=458 CU (n=335); MCI (n=123)	Longitudinal	Simoa ~~HD-1 Analyzer~~	t-tau	NA	Risk of evolving to MCI in CU: - middle tertile (HR=2.43) - highest tertile (HR=2.02)
Mielke MM et al., 2018	n=269 CU (n=172); MCI (n=57); AD (n=40)	Cross-sectional	Simoa ~~HD-1 Analyzer~~	t-tau	Amyloid-negative vs. amyloid-positive: AuROC=0.60 (amyloid-PET as reference)	NA
			MSD	p-tau181	Amyloid-negative vs. amyloid-positive: AuROC=0.80 (amyloid-PET as reference)	NA
Yang C. et al., 2018	n=73 CU (n=23); AD-MCI (n=29); AD (n=21)	Cross-sectional	IMR	p-tau181	CU vs. AD-MCI: AuROC=0.85; AD-MCI vs. AD: AuROC=0.78 (clinical diagnosis as reference)	NA
Lin CH. et al., 2018	n=205 CU (n=35); PD (n=102); DLB (n=6); MSA (n=22); PSP (n=6); CBD (n=3); FTD-P (n=6); FTD without P (n=25)	Cross-sectional	IMR	p-tau181	FTD vs. parkinsonian syndromes (PD, DLB, PSP and CBD) $A\beta_{1-42}$ x p-tau181 at a cut-off value of 92.66 $(pg/ml)^2$ AuROC=0.93 (clinical diagnosis as reference)	NA
Chen Z. et al., 2019	n=151 - Discovery cohort: n=65 CU (n=19); AD-MCI (n=21); AD (n=25) - Validation cohort n=86	Cross-sectional	Simoa ~~HD-1 Analyzer~~	N-terminal detected tau (including tau fragments)	CU vs.AD-MCI: AuROC=0.88 CU vs AD: AuROC=0.96 (clinical diagnosis as reference standard) (discovery cohort)	NA

	CU (n=41); AD-MCI (n=22); AD (n=23)					
Park JC. et al., 2019	n=76 CU (n=52); MCI (n=9); AD dementia (n=15)	Cross-sectional and longitudinal	Simoa ~~HD-1 Analyzer~~ (t-tau) xMAP ($A\beta_{1-42}$)	t-tau	tau-positive vs. tau-negative: -t-tau: AuROC=0.80 -t-tau/$A\beta_{1-42}$: AuROC=0.89 (amyloid-PET as reference)	NA
Pase MP. et al., 2019	n=1820 - Cohort 1: n=1453* (CU > 65 years) - Cohort 2: n=367˚ (MCI, SMC) *at FU: - dementia onset n=134 AD dementia (n=105); non-AD dementia (n=29) ˚at FU: - AD dementia onset (n=19)	Prospective	Simoa ~~HD-1 Analyzer~~	t-tau	NA	Cohort 1: - each SD unit increase in the log levels is associated with a 35% increased risk of AD (HR=1.35) - after adjustment for APOE ε4 allele and vascular risk factors: • all dementia risk: HR=1.31 • AD risk: HR=1.38 Cohort 2: each SD unit increase in the log levels is associated with a 54% increased risk of AD (HR=2.33) after adjusting for age and sex
Janelidze S. et al., 2020	n=589 - Cohort 1: n=182 CU (n=64); MCI (n=28); AD (n=38); non-AD dementia (n=52) - Cohort 2: n=344 CU (n=219); MCI (n=125) - Cohort 3 (neuropathology cohort): n=63 AD (n=16); non-AD	Both cross-sectional and longitudinal designs	MSD	p-tau181	Cohort 1: - tau-positive vs. negative participants AuROC=0.87 with tau-PET as reference; - AD vs. non-AD dementia: AuROC=0.94 (clinical diagnosis as reference) Cohort 1 and cohort 2 combined: - Aβ-+ vs. Aβ - participants AuROC=0.80 (Aβ PET as reference)	Cohort 2: higher p-tau levels are associated with progression to AD for both CU (HR=2.48) and MCI (HR=3.07)

	dementia (n=47)				Cohort 3: AD vs. non-AD dementia: AuROC=0.85 (neuropathology as reference)	
Thijssen E. et al., 2020	n=404 Cohort 1: n=362 CU (n=69); MCI (n=47); AD (n=56); CBS (n=39); PSP (n=48); bvFTD (n=50); nfvPPA (n=27); svPPA (n=26) Cohort 2: n=42 (MCI, AD)	Cross-sectional (retrospective) and longitudinal	MSD/~~Lilly~~ immunoassays	p-tau181	Cohort 1: - AD vs. FTLD (n=190): AuROC=0.89 (clinical diagnosis as reference); - Aβ-PET positive participants vs. negative: AuROC = 0.86 with amyloid-PET as reference; - authopsy-confirmed AD (n =15) vs. FTLD-tau (n=52) AuROC=0.86 (neuropathology as reference)	NA
Karikari T. et al., 2020	n=1331 - Discovery cohort: n=37 CU (n=18); AD (n=19) - Validation cohort 1: n=763 CU (n=140); MCI (n=45); AD (n=33); FTD (n=8) - Validation cohort 2: n=763 CU (n=337); MCI (n=191); AD (n=126); bvFTD/PPA (n=18); PD/MSA (n=36); VD (n=12); PSP/CBS (n=21) - Primary care cohort: N=105 CU (n=83); MCI (n=12); AD (n=10)	Longitudinal	Simoa	p-tau181	Across cohorts - AD vs. CU: AuROC=0.90-0.98 Validation cohort 1 - AD vs. FTD: AuROC=0.76-0.82 (clinical diagnosis as reference) - tau-PET + vs. tau-PET -: AuROC=0.83-0.93 across cohorts (tau-PET as reference) - AD vs. amyloid-β -: AuROC=0.99 (amyloid-PET as reference) Validation cohort 2 - AD vs VaD: AuROC=0.92 - AD vs PSP/CBS: AuROC=0.89 - AD vs PD/MSA: AuROC=0.82 (clinical diagnosis as reference) Primary care cohort - AD vs CU young adults: AuROC = 1.0 - AD vs CU older adults: AuROC=0.84 (clinical diagnosis as reference)	NA
Palmqvist S. et al., 2020	n=1402 - Cohort 1 (neuropathology cohort): n=81	Cross-sectional	MSD/~~Lilly~~ immunoassays	p-tau 217	Cohort 1: AD vs. non-AD: AuROC=0.89 (neuropathology as reference) Cohort 2:	NA

	AD (n=34); non-AD (n=47) - Cohort 2: n=699 CU (n=301); MCI (n=178); AD dementia (n=121); non-AD dementia: PD/PDD/MSA, PSP/CBS, bvFTD/PPA, VD (n=99) - Cohort 3: n= 622 PSEN1 mutation carriers n=365; age- and sex-matched noncarriers: n=257				- AD vs. non-AD dementia: AuROC=0.96 (clinical diagnosis as reference) - tau + vs.tau -: AuROC=0.93 (tau-PET as reference) - amyloid + vs. amyloid -: AuROC=0.87 (amyloid-PET as reference)	
Barthélemy NR. et al., 2020	n=128 - Discovery cohort: n=36 CU (n=17); non-AD MCI (n=2); preclinical AD (n=5); AD-MCI (n=8); moderate AD (n=2) - Validation cohort: n=92 CU (n=31); non-AD MCI (n=11); preclinical AD (n=20); AD-MCI (n=24); moderate AD (n=6)	Cross-sectional	IP-LC/MS	p-tau181	Amyloid + vs. -: - discovery cohort: AuROC=0.98 - validation cohort: AuROC=0.75 (amyloid-PET or CSF $A\beta_{1-42}/A\beta_{1-40}$ as reference)	NA
				p-tau217	Amyloid positive vs. amyloid-negative subjects: - discovery cohort: AuROC=0.99 - validation cohort: AuROC=0.92 (amyloid-PET or CSF $A\beta_{1-42}/A\beta_{1-40}$ as reference)	NA
Janelidze S. et al., 2020	n=490 CU (n=225); SCI (n=89); MCI (n=176)	Cross-sectional and longitudinal	MSD/~~Lilly~~ immunoassays	p-tau217	$A\beta\text{-PET}^{+}$ /tau-PET^{-} CU vs. $A\beta\text{-PET}^{-}$ /tau-PET^{-} :AuROC=0.832 (amyloid-PET as reference)	NA

Table 2. Diagnostic and prognostic value of plasma tau species in NDDs assessed with ultrasensitive measurement techniques

Abbreviations: AD: Alzheimer's Disease; AuROC: area under the receiver operating curve; Aβ: amyloid β; Aβ1–40: amyloid β-peptide 1–40; Aβ1–42: amyloid β-peptide 1–42; bvFTD: behavioral variant Frontotemporal Dementia; CBS: Corticobasal Syndrome; CU: Cognitively Unimpaired; FTD: Frontotemporal Dementia; FTLD: Frontotemporal Lobar Degeneration; FTD-P: Frontotemporal Dementia with parkinsonism; FU: follow-up; HR: hazard ratio; IMR: immunomagnetic reduction; IP LC/MS: immunoprecipitation coupled to liquid chromatography/mass spectrometry; MCI: mild cognitive impairment; MSD: meso scale discovery; MSA: Multiple System Atrophy; NA: not assessed; nfvPPA: non-fluent variant primary progressive aphasia; PD: Parkinson's disease; PDD: Parkinson's disease dementia; PPA: Primary Progressive Aphasia; PSP: Progressive Supranuclear Palsy; p-tau181: phospho-tau181; p-tau217: phospho-tau217; SD: standard deviation; Simoa: single molecule array; SCI: subjective cognitive impairment; -SMC: subjective memory complainer; svPPA: semantic variant primary progressive aphasia; t-tau: total-tau; VaD: vascular dementia; xMAP: multi-analyte profiling.

Reference	Population	Study design	Technique	Tau species	Diagnostic value	Prognostic value
Magdalinou NK. et al., 2015	n=190 CU (n=30); PD (n=31); PSP (n=33); CBS (n=14); MSA (n=31); AD (n=26); FTD (n=16); "unclassified" parkinsonism (n=9)	Prospective	ELISA ~~INNOTEST~~ (t-tau; p-tau; $A\beta_{1-42}$)	t-tau, p-tau	t-tau, p-tau combined with $A\beta_{1-42}$, NFL, α-syn, sAPPα, sAPPβ, MCP-1, YKL-40): - PD vs. APSs (PSP, CBS, MSA): AuROC=0.95 - PD vs. PSP: AuROC=0.95 - PD vs. CBS: AuROC=0.98 - CBS vs. AD/FTD: AuROC=0.93 (clinical diagnosis as reference)	NA
Wagshal D. et al., 2015	n=87 CU (n=26); AD (n=37); PSP (n=24)	Cross-sectional	~~INNO-BIA AlzBio3 with~~ xMAP platform (t-tau; p-tau181)/ ELISA ~~s~~ (alternative N-terminal and mid-tau fragments)	t-tau, p-tau181, tau fragments	t-tau, p-tau181 and tau fragments: - AD vs. PSP: AuROC=0.79-0.95; - AD vs. CU: AuROC=0.65-0.73 N-terminal fragment tau12-BT2 shows the best accuracy: - AD vs PSP: AuROC=0.95 - AD vs CU: AuROC=0.73	NA
Wilke C. et al., 2015	n=180 CU (n=20); ALS (n=60)	Cross-sectional	ELISA ~~INNOTEST~~	t-tau, p-tau181	CU vs. ALS: - t-tau: AuROC=0.68 - p-tau/t-tau: AuROC=0.75 (clinical diagnosis as reference)	NA
Llorens F. et al., 2016	n=140 CU (n=55); ET (n=8); PD (n=40); PDD (n=10); DLB (n=18); MSA (n=11)	Cross-sectional	ELISA (t-tau)/MSD (α-syn)	t-tau	CU vs. DLB: - t-tau/α-syn: AuROC=0.88 - t-tau: AuROC=0.77 (clinical diagnosis as reference)	NA
Bourbouli M. et al., 2017	n=100 CU (n=17); ALS (n=32); FTD (n=51)	Cross-sectional	~~Sandwich~~ ELISA	t-tau, p-tau181	t-tau: - CU vs. ALS: AuROC=0.80 - CU vs. FTD: AuROC=0.77; TDP-43 x t-tau/p-tau181 in: - CU vs. ALS: AuROC=0.97 - CU vs. FTD: AuROC=0.90 (clinical diagnosis as reference)	NA
Constantinides VC. et al., 2017	n=86 CU (n=18); PSP (n=19); MSA (n=15); CBD (n=17); PD (n=17)	Cross-sectional	ELISA ~~INNOTEST~~	t-tau	t-tau/$A\beta_{1-42}$ in: - MSA vs. PD: AuROC=0.80 - cut-off point 0.344: 71.4 % sensitivity, 93.3 % specificity - cut-off point 0.305: 78.6 % sensitivity, 80 % sensitivity (clinical diagnosis as reference)	NA

Delgado-Alvarado M. et al., 2017	n=80 CU (n=40); PD (n=40)	Cross-sectional	ELISA ~~INNOTEST~~	t-tau, p-tau181	CU vs. PD: - t-tau/α-syn: AuROC=0.79 - p-tau181/α-syn: AuROC=0.86 - p-tau181/α-syn and TNF-α: AuROC=0. 91 (clinical diagnosis as reference)	NA
Irwin DJ. et al., 2018	n=83 CU (n=36); autopsy-confirmed AD (n=23); autopsy-confirmed DLB (n=24)	Cross-sectional	xMAP platform	t-tau, p-tau181	Predicting α-syn + AD pathology: - t-tau: AuROC=0.80 - p-tau/$A\beta_{1-42}$: AuROC=0.80 - t-tau/$A\beta_{1-42}$: AuROC=0.92; Predicting of neocortical Lewy Bodies pathology t-tau/$A\beta_{1-42}$: AuROC=0.76 (neuropathology as reference)	NA
Scarafino A. et al., 2018	n=166 ALS (n=85); AM (n=30); non-NDDs (n=51)	Longitudinal	ELISA	t-tau, p-tau181	t-tau in: - ALS vs. AM: AuROC = 0.70 - ALS vs. non-NDDs: AuROC=0.74 (clinical diagnosis as reference)	Higher baseline t-tau levels are associated with decreased overall survival (HR=2.08)
Schirinzi T. et al., 2018	n=128 CU (n=58); PSP (n=39); PD (n=31)	Cross-sectional	ELISA ~~INNOTEST~~	t-tau, p-tau181	PSP vs. CU: - p-tau (cut-off 206 pg/ml): AuROC=0.67 - t-tau (cut-off 34.5 pg/ml): AuROC=0.69; PSP vs. PD: - p-tau/t-tau (cut-off 0.185 pg/ml): AuROC=0.70 (clinical diagnosis as reference)	NA
Constantinescu R. et al., 2019	n=151 PD (n=68); APS (n=83) including MSA (n=34), PSP (n=34), CBS (n=15)	Prospective	ELISA ~~INNOTEST~~	t-tau, p-tau181	NA	In PSP t-tau is associated with increased mortality (HR=9.59)
Abu-Rumeileh S. et al., 2020	n=169 CU (n=43); ALS (n=80); AM (n=46)	Longitudinal	ELISA ~~INNOTEST~~	t-tau, p-tau181	ALS vs. CU: - t-tau: AuROC=0.74 - p-tau/t-tau:AuROC=0.81; ALS vs. AM: - p-tau/t-tau: AuROC=0.75 (clinical diagnosis as reference)	NA
Bousiges O. et al., 2020	n=166 CU (n=21); DLB	Cross-sectional	ELISA ~~INNOTEST~~	t-tau; p-tau181	t-tau: - DLB vs. AD: AuROC=0.92	NA

	(n=67); AD (n = 65);				- DLB vs. AD: AuROC=0.94; p-tau181: - DLB vs. AD: AuROC = 0.93 (clinical diagnosis as reference)	
Ye LQ. et al., 2020	n=240 CU (n=24); AD (n=82); FTD (n=20); HD (n=27); MSA (n=24); SCA3 (n=27); ALS (n=36)	Cross-sectional	ELISA	t-tau; p-tau181	t-tau: - CU vs. FTD: AuROC=0.76 - ALS vs. AD AuROC=0.87 t-tau/$A\beta_{1-42}$: - CU vs. FTD: AuROC=0.80 - AD vs. FTD: AuROC=0.95 (clinical diagnosis as reference)	NA

Table 3. Diagnostic and prognostic value of CSF tau species in non-AD NDDs

Abbreviations: α-syn: α-synuclein; AD: Alzheimer's Disease; ALS: Amyotrophic Lateral Sclerosis; AM: Amyotrophic Lateral Sclerosis mimics; APS: Atypical Parkinsonian Syndrome; AuROC: area under the receiver operating curve; $A\beta_{1-42}$: amyloid β-peptide 1–42; CBS: Corticobasal Syndrome; CU: Cognitively Unimpaired; DLB: Dementia with Lewy Bodies; ELISA: enzyme-linked immunosorbent assay; ET: Essential Tremor; FTD: Frontotemporal Dementia; HD: Huntington's Disease; HR: hazard ratio; MCP-1: monocyte chemoattractant protein-1; MSD: mesoscale discovery; MSA: multiple system atrophy; NA: not assessed; NDD: neurodegenerative disease; NFL: neurofilament light chain; PD: Parkinson's Disease; PDD: Parkinson's Disease Dementia; PSP: Progressive Supranuclear Palsy; p-tau: phopsho-tau; p-tau181: phospho-tau181; sAPPα: soluble amyloid precursor protein α; sAPPβ: soluble amyloid precursor protein β; SCA3: spinocerebellar ataxia-type 3; TDP-43: TAR DNA-binding protein 43; t-tau: total-tau; TNF-α: tumor necrosis factor α; xMAP: multi-analyte profiling; YKL-40: chitinase-3-like protein 1.