This is the accepted manuscript of Therriault J, Zimmer ER, Benedet AL, Pascoal TA, Gauthier S, Rosa-Neto P. Staging of Alzheimer's disease: past, present, and future perspectives. Trends Mol Med. 2022 Jun 15:S1471-4914(22)00137-X. doi: 10.1016/j.molmed.2022.05.008. Licensed CC-BY-NC-ND 4.-0.

Staging of Alzheimer's disease: past, present and future perspectives

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Abstract

For years, Alzheimer's disease (AD) was associated with the dementia stage of the disease, the tail end of a pathophysiological process that lasts approximately two decades. While early disease staging models focused on progressive deterioration of clinical functioning, PET and CSF biomarker studies highlighted the long preclinical phase of AD in which a cascade of detectable biological abnormalities precede cognitive decline. The recent proliferation of imaging and fluid biomarkers of AD pathophysiology provide the opportunity for the identification of several biological stages in the preclinical phase of AD. This review will discuss the use of clinical and biomarker information in the past, present and future staging of AD. We highlight potential applications of PET, CSF and plasma biomarkers for staging AD severity *in vivo*.

Keywords: Alzheimer's disease, staging, amyloid-β, tau, biomarkers

Staging of disease

A major challenge in many medical specialties is measuring disease severity to guide patient management and evaluate therapeutic efficacy. Disease staging systems rank a disease in relation to progressive levels of severity, where later stages are associated with a worse prognosis. Staging systems highlight specific milestones in the natural history of a disease that are detectable, reflect current or future symptomatic severity, and ideally provide clinical significance in selecting choice of therapeutic intervention [1]. Disease stages can be based on clinical history, etiology [2], anatomical distribution of pathology [3], or biological features [4], and can be identified based on physical examination, biomarker testing, or both.

Alzheimer's disease (AD) is a neurodegenerative disease, which results in progressing cognitive impairment and dementia. For decades, AD has been closely associated with the dementia stage of the disease. Evidence from autosomal dominant and sporadic forms of AD provide evidence that the defining features of AD, amyloid-β plaques and tau neurofibrillary tangles, accumulates over up to two decades before the onset of clinical dementia [5–7]. The recent proliferation of imaging and fluid biomarkers of AD pathophysiology provides the opportunity for staging of AD pathological changes in the preclinical phase of AD. This review will discuss the use of clinical and biomarker information in the past, present and future staging of AD. We focus on conceptual and methodological issues pertaining to disease classification and highlight novel promising biomarkers for the preclinical phase of AD.

Past: clinical staging of AD

Despite being defined in the early 1900s by Dr. Alois Alzheimer as being associated with plaques and tangles, the progressive functional decline of AD provided the basis for staging of AD severity for many decades. The diagnostic criteria for of AD relied on the nature of cognitive symptoms, progressive and insidious clinical progression in the absence of other causes [8]. To many clinicians, three overarching disease stages were apparent: mild forgetfulness, early and late dementia [9].

The Reisberg Global Deterioration Scale [9], developed in 1982, categorized the dementia process into seven stages based on cognitive and functional severity: no cognitive decline (stage 1), very mild cognitive decline, often accompanied by subjective memory concerns (stage 2), mild cognitive decline, where the earliest clinical deficits are observable in systematic observations (stage 3), moderate cognitive decline, where patients can no longer perform complex tasks efficiently (stage 4), moderately severe cognitive decline, where the individual begins to require assistance in activities of daily living (stage 5), severe cognitive decline, where patients depend entirely on their caregivers for survival (stage 6) and finally very severe cognitive decline, where verbal and psychomotor skills are lost (stage 7).

Therefore, the staging of cognitive decline ranged from no cognitive impairment to severe multidomain dementia. The focus of cognitive domains affected early in the disease process were largely centered around memory dysfunction, which became further emphasized in the 1984 diagnostic criteria for AD during the dementia stage [8]. With the diagnosis based on medical history and neurological examination (the abstract of the 1984 criteria reads "the diagnosis cannot be determined by laboratory tests"), clinical staging of AD severity provided a framework for the subsequent decades in routine clinical practice as well as in the description of cohorts in observational research. While clinically-determined stages lack specificity for AD, they nonetheless provide prognostically relevant information. Individuals without objective cognitive impairment who experience subjective cognitive decline (stage 2) display a nearly two-fold risk of developing Mild Cognitive Impairment (MCI) [10,11]. However, poor specificity of clinical symptoms to underlying AD neuropathology, especially at early clinical stages, created a need for other staging systems which incorporated biological and/or histopathological information.

Present: AD neuropathologic change & A/T/(N)

Neuropathological staging

Neuropathological staging of AD is based on the anatomical localization of neuropathology, with density of pathology assessed semi-quantitatively [12]. Current neuropathological staging models of AD involve an ABC scoring system, in which stages are assigned to amyloid-β plaques (A), Braak stage of tau neurofibrillary tangles (B) and CERAD score of neuritic plaques (C) [13,14].

Topography of amyloid-β plaques is staged according to Thal staging, a five-stage model in which amyloid-β first deposits across the whole neocortex (phase 1), followed by the isocortex (entorhinal and hippocampal cortices – phase 2), the striatum and diencephalon (phase 3), brainstem nuclei (phase 4) and finally the cerebellum (phase 5). Tau neurofibrillary tangles are staged according to the staging system devised by Braak & Braak [15–17], which begins with the transentorhinal cortex (stage I), entorhinal cortex and hippocampus (stage II), inferior temporal neocortex (stage III), association cortices (stages IV and V) and primary sensory cortices (stage VI). The CERAD (Consortium to Establish a Registry for Alzheimer's Disease) scoring system ranks the density of neuritic plaques in the neocortex (None, Sparse, Moderate, Frequent) [18]. Neuritic plaques are extracellular aggregates constituted of a central core of amyloid-β and a corona, which surrounds the central core, consisting of degenerating neurons containing tau [19]. Each staging system allows for the differentiation of several disease phases based on the anatomical distribution of neuropathologic change, whether into one of 5 Thal stages for amyloid-β, one of 6 Braak stages for tau neurofibrillary tangles, and one of 3 stages for neuritic plaques, with the additional possibility of scoring 0 if the specific pathology is absent. Stages are often collapsed to improve inter-rater reliability: for example, Braak stages I-II, III-IV and V-VI are frequently grouped together.

In summarizing an individual's neuropathological change score based on the staging systems, individuals are assigned an ABC score. Therefore, an example AD neuropathologic change classification could be anywhere from A0, B0, C0 to A3, B3, C3. In turn, ABC scores are converted into one of four levels of levels of AD neuropathologic change: none, low, intermediate, or high. Braak stage III/IV and above accompanied by significant amyloid-β plaques and neuritic plaques is considered to be a sufficient explanation for dementia [13,14].

Crucially, AD neuropathologic change staging systems are reported independently of clinical history (i.e. presence dementia or stage of cognitive impairment) [13,14]. Correspondingly, an important minority of individuals with histopathological evidence of AD at autopsy did not have cognitive impairment in their lifetime [20,21]. Furthermore, post-mortem AD staging systems revealed a proportion of individuals with histopathological evidence of AD who had subtle cognitive impairment that did not exhibit dementia [22–24]. While neuropathological staging of

AD had limited utility due to the fact that it could only be applied post-mortem, the cognition-independent feature of post-mortem histopathological staging systems provided a rationale for the similarly cognition-independent *in vivo* biomarker classification system of AD.

Identification of AD in vivo using A/T/(N)

The recently proposed unbiased biomarker classification system for AD provides three main classes of biomarkers: Aβ (A), Tau (T) and Neurodegeneration (N), denoted as A/T/(N) [25]. In this framework, abnormal levels of Aβ and phosphorylated tau are considered core features of AD, with neurodegeneration also being a feature of other neurodegenerative diseases, hence the appearance in parentheses [26]. Amyloid-β biomarkers include amyloid-PET and CSF concentrations of amyloid-β. Tau biomarkers include tau-PET and CSF concentrations of phosphorylated tau. The neurodegeneration category includes multiple biomarkers including FDG-PET, MRI atrophy (often of the hippocampus), CSF concentrations of total tau and of Neurofilament light chain. Similar to the neuropathological staging systems, then A/T/(N) system assigns a biomarker status to an individual independent of their cognitive status. This classification has numerous applications including enrichment of therapeutic trials [27] and prediction of cognitive decline [28]. With increasing clinical use of biomarkers for the differential diagnosis of individuals with cognitive impairment [29–31], it has often been speculated that this framework may also have practical applications in screening patients most likely to have AD and to help differentiate AD from non-AD dementia disorders.

While amyloid-β accumulation takes place on a continuum [32], amyloid-PET scans and CSF concentrations of amyloid-β are frequently dichotomized as pathological (positive) / normal (negative) in clinical and research settings [29,30,33]. Stratification of populations using amyloid-β levels is critical for diagnosing AD, assessing clinicopathological changes associated with amyloid-β, and for enriching populations for disease-modifying trials. Of note, the frequent observation of significant amyloid-β plaques in the brains of individuals without cognitive impairment corroborated previous findings in the neuropathology literature [20]. Despite being a core neuropathological feature of AD, cerebral amyloid-β aggregation measured with PET displayed poor correlations with brain glucose metabolism [34] or cognition [35,36]. This

supported the notion of an asymptomatic stage characterized by "silent" amyloid-β accumulation [36]. Studies of autosomal dominant AD further suggest that this silent phase begins approximately 20 years prior to the onset of symptoms [6].

In contrast to amyloid-PET, which shows little association with cognitive symptoms [35], tau-PET load shows associations with disease severity (Figure 1). Specifically, the topographical distribution of tau-PET displays strong correlations with metabolic dysfunction [37], atrophy [38], and domain-specific cognitive dysfunction [39,40]. Moreover, the magnitude of tau-PET SUVRs reflects the degree of cognitive impairment and dementia [39,41,42]. Furthermore, tau-PET uptake in the temporal neocortex also displays high diagnostic accuracy for AD vs other causes of cognitive impairment in multicentre studies [43]. At the cross-sectional level, tau-PET abnormality is almost exclusively observed in the presence of amyloid-PET abnormality [44,45]. Taken together, these studies suggested the elevated tau (generally operationalized as tau-PET in the neocortex) is closely associated with AD's clinical impairment stage (MCI and Dementia). In contrast, amyloid-PET abnormality seems to be required for tau-PET abnormality, and predate it by several years, though the precise temporal lag between these two biomarkers is unknown.

The A/T/(N) biomarker framework is not a staging system *sensu stricto*. Disease staging systems imply a sequence of pathological events, which is not presupposed in a biomarker classification system such as the A/T/(N) system [25,26]. Instead, the A/T/(N) system is operationalized for the identification (not staging) of biological AD in living individuals. Therefore, the application of A/T/(N) biomarker profiles allows for tracking AD through its preclinical, mild cognitive impairment and dementia stages. In this framework, staging of AD remains guided by clinical symptoms, where increasing symptomatic severity connotes more advanced disease stage (Box 1).

There remains an unmet need to stage AD *in vivo* using biomarkers during the preclinical stage. The reasons for this are threefold: (i) the accumulation of protein aggregates in the absence of symptoms lasts longer than AD's symptomatic phase, therefore adding granularity to presymptomatic biological changes will permit the identification of points in the disease course where individuals may respond optimally to treatment (ii) disease-modifying therapeutic interventions in the dementia stage of AD have been unsuccessful (iii) prevalence estimates of

asymptomatic biological AD exceed those of symptomatic AD at all age groups [46], indicating that the number of individuals who may benefit from disease-modifying interventions is greater than the number of people living with clinically symptomatic AD.

Future: Multidimensional biomarker-based staging of AD

Advances in *in vivo* biomarkers provide a novel framework for staging of pathological changes characteristic of early AD in asymptomatic individuals, as well as tracking the severity of pathological changes into the symptomatic phases. We highlight three promising aspects of *in vivo* AD staging: (i) leveraging the topographical information from PET imaging to optimize sensitivity to detect stage-specific AD pathological changes (ii) adding granularity to the spectrum of tau pathology from emerging pTau biomarkers (iii) the opportunity to monitor multidimensional aspects of AD pathophysiology simultaneously with CSF or plasma samples.

Biological Staging AD using the topography of PET imaging

Advances in molecular imaging are expected to refine existing AD diagnostic classification systems [47]. While most research has focused on dichotomous classification of amyloid-PET and tau-PET imaging into positive and negative groups, the spatial resolution of PET provides the opportunity for staging based on the anatomical distribution of pathology. Staging systems may provide additional information by leveraging the topographical distribution of PET uptake, which may aid in the patient monitoring during the course of AD.

Detection of elevated concentrations of neuropathologies in specific brain regions before global abnormality presents as a unique feature of PET imaging. Multiple studies have proposed data-driven staging systems of regional amyloid-β concentrations. While the precise order of regional accumulation varies between studies, the general pattern of results supports earliest accumulation in medial neocortical structures (medial prefrontal cortex, posterior cingulate, precuneus), which are followed by the striatum, and eventually, medial temporal regions [48–50]. Region-specific approaches have increased sensitivity for predicting cognitive changes compared to global measures [51], though replicability across radioligands, cohorts and analytic methods can be challenging [50]. However, in a recent multicentre study of over 3000 individuals, staging

amyloid-β pathology according to regional abnormality was able to classify subjects scanned with different radioligands and was associated with distinct risk profiles of cognitive decline [52]. These studies suggest that while the topography of amyloid-β is not closely associated with contemporaneous cognitive impairment, staging systems of regional pathology convey important prognostic information about future cognitive decline.

PET imaging of protein aggregates in AD benefits from standing on the shoulders of giants. The decades of neuropathological work which has examined the regional distribution of pathological aggregates, as well as synapse loss, have led to the creation of staging systems used in large-scale observational research studies as well as in routine neuropathological examinations [12]. The validation of tau-PET radioligands have been particularly informed by the canonical distributions of pathology documented in Braak stages. Early tau-PET studies provided evidence that the distribution of tau-PET uptake conformed to Braak stages (most studies collapsed Braak stages into I-II, III-IV and V-VI) [53–55]. Tau-PET signal in early stages is observed in individuals without cognitive impairment, with and without significant amyloid-β load. Elevated medial temporal tau-PET in the absence of amyloid-β pathology may reflect primary age-related tauopathy (PART) [56]. Significantly elevated tau-PET in regions comprising Braak stages III-IV (temporal neocortex and association cortices) is almost exclusively observed in individuals with abnormal amyloid-β biomarkers, and is generally accompanied by at least mild cognitive decline [57]. Finally, tau-PET in brain regions comprising later Braak stages (association cortices and primary sensory cortices) is almost exclusively observed in individuals with cognitive impairment [58]. Therefore the topographical information provided by PET imaging can be used to optimize the stage-specific detection of pathology that characterizes AD (Figure 2).

A critical advantage of PET imaging over neuropathological assessments is the ability to track the evolution of pathology in the same individual over time. Longitudinal tau-PET studies have provided strong evidence of a diversity of tau accumulation patterns, frequently associated with brain connectivity [59–62]. A recent study of over 2300 individuals identified four patterns of tau-PET accumulation, with each pattern characterized by specific clinically-relevant phenotypic and prognostic features [63]. Furthermore, a recent multicentre study identified distinct patterns of neocortical tau-PET uptake in individuals with preclinical AD, characterized by either

asymmetrical tau-PET distribution in the temporal lobe, or high uptake in the precuneus [64]. While many studies have highlighted substantial variability in tau accumulation, the general pattern of early accumulation in the medial temporal lobe, followed by aggregation in the temporal neocortex, association cortex and finally primary sensory cortices seems to be a consistent pattern and may serve as a basis for AD staging using tau-PET. While understanding the heterogeneity of AD will be helpful for personalized interventions [65], staging systems such as Braak staging are nonetheless useful for estimating AD severity, despite not perfectly capturing the variability in tau accumulation described in large PET studies.

Expanding the continuum of the tau biomarker category

Tau's complex biology and pathophysiology necessitate the distinction of different kinds of tau biomarkers. While the tau biomarker category is useful, recent PET and fluid tau biomarker studies suggests that different tau biomarkers measure different aspects of the AD process [66] (Box 2).

In particular, several recent studies of biomarkers for pathological tau phosphorylated at specific phosphorylation sites have led to the emerging viewpoint that specific tau phosphorylation signatures are associated with specific disease stages. In a recent study of autosomal dominant AD, abnormality in CSF concentrations of pTau217 rose before concentrations of pTau181, both of which preceded concentrations of pTau205 [67]. In particular, CSF pTau217 and pTau181 were closely associated with the initiation of amyloid-β plaque aggregation. Another study highlighted that early changes in pTau231 were more closely associated with levels of amyloid-β pathology in amyloid-negative individuals than pTau217 and pTau181, suggesting that pTau231 is associated with very early AD pathological change [68,69]. The early increase of CSF concentrations of pTau231 is in agreement with neuropathological studies reporting that pTau231 is observed prior to the formation of tau filaments in pre-neurofibrillary tangles [70]. A recent study investigating pTau235 suggests that elevated concentrations of pTau235 are preceded by pTau231 and pTau217 [71]. Another observational study suggested that CSF concentrations of pTau231, pTau217 and pTau181 all began increasing in relation to subthreshold amyloid-β concentrations [69]. Recent data suggest that pTau217 is more closely associated with both cerebral amyloid-β and tau than is pTau181 [72], and that pTau217 mediates the relationship between cerebral amyloid-β and tau

[73]. Taken together, these studies suggest a close association between pTau metabolism and exposure to cerebral amyloid-β dysmetabolism.

If tau phosphorylation at different sites indeed reflects different points in the AD pathophysiological process, it is conceivable to stage individuals according to their abnormality in multiple pTau biomarkers. Correspondingly, panels of phosphorylated tau pathology may give additional information over a single phosphorylated tau epitope (Box 3). A single plasma or CSF sample may therefore provide information about the extent of tau phosphorylation, with the understanding that phosphorylation at specific sites may be more closely associated with the aggregation of amyloid-β plaques, tau neurofibrillary tangles, both, or even other pathophysiological events. However, the strength of these relationships is currently unclear, and studies are restricted to highly selected cohorts. Moreover, due to the closely correlated nature of pTau epitopes in most individuals [69,71,74,75], it is unknown how many individuals will be positive in one, but negative in another, and to what extent this information is prognostically meaningful at the individual level. Another concern in relation to the individual-level predictive value of pTau panels is the high coefficients of variation of fluid biomarkers; issues relating to false positives and false negatives may be present. Head-to-head studies of fluid biomarkers of pTau epitopes are needed to determine the site-specific associations between different pTau concentrations in CSF or plasma with neurofibrillary tangles changes in the brain. Future studies can be guided by hypotheses from neuropathological studies reporting that some phosphorylation sites may preferentially reflect tau aggregation in early Braak stages, while others sites may be associated with tau aggregation in later Braak stages [70].

Plasma

Considered a pipe dream only years ago, the development of ultrasensitive assays for amyloid-β and phosphorylated tau in plasma has provided evidence for the feasibility AD biomarker assessments using minimally invasive, scalable and accessible methods. Assays based on immunoprecipitation coupled with mass-spectrometry can predict amyloid-PET status based on concentrations of amyloid-β in plasma [76]. Similarly, concentrations of phosphorylated tau in plasma show high sensitivity and specificity for AD pathological change, correlate with CSF and PET measures of tau pathology *in vivo*, correlate with neurofibrillary tangle burden at autopsy,

and differentiate AD from other dementia syndromes [77–81]. Because clinical criteria for AD have limited sensitivity and specificity for AD pathology [82], the addition of plasma-based biomarkers of phosphorylated tau to clinical workups may provide information to physicians evaluating individuals with age-related cognitive impairment [83], or for predicting future cognitive decline [84]. Plasma assessments of AD pathophysiology may represent clinical tool with the potential to improve dementia-related care globally.

Similar to CSF samples, single blood sample provides the ability to assess multiple aspects of the AD pathological processes. This constitutes a promising advantage in contrast to neuroimaging, for which three separate scans would be required to determine a patient's A/T/(N) imaging biomarker status [28], in turn related to substantially increased cost, patient burden and decreased accessibility.

Current evidence suggests that plasma biomarkers should be considered as screening biomarkers and not as diagnostic biomarkers [85,86]. If evidence of efficacy of anti-amyloid or anti-tau therapies for AD exist, there will be a need to confirm that specific individuals who will begin treatment (whether at preclinical, MCI or dementia stages) are amyloid-β positive, or amyloid-β and tau positive. Presence of AD biomarker abnormalities will need to be confirmed before treatment is initiated, and may preclude the need for more costly / invasive CSF or PET assessments. Alternatively, plasma assessments could be used as routine pre-screening, with CSF examination or PET imaging used to confirm the presence of pathology before initiating treatment.

Despite the promise of plasma measures of AD pathology, more research is needed on the associations between plasma biomarkers and more established AD biomarkers. Early evidence suggests that plasma pTau181 becomes abnormal approximately 6 years after an individual reaches abnormal levels of amyloid-β [87]; the time frame to abnormality in other pTau phosphorylation sites such as pTau231, pTau217 and pTau235 are currently unclear but expected to be slightly shorter [69,81]. In familial AD, plasma pTau181 concentration starts to increase around 15 years prior to expected clinical disease onset [88]. Recent studies have also associated pTau181 with cortical thinning as well as cerebral glucose hypometabolism [89], indicating that plasma pTau181 may help track neurodegenerative processes in AD. However, due to the novelty

of plasma-based pTau measurements, several questions remain [83]. Studies assessing the positive predictive value of abnormal pTau181 levels in relation to more established tau biomarkers such as tau-PET [41,90] will be required to increase confidence in the significance of plasma pTau181 concentrations at the individual level. It is unlikely that the dynamic range of individual phosphorylated tau species are sufficient to stage disease. While recent phase III data from the aducanumab trials provide evidence for lowering of plasma pTau181 concentrations after treatment [91], it is also unknown how plasma pTau biomarkers fare in terms of measuring of the slowing (or removal) of tau aggregates in the brain. Hence, in situations where high accuracy is required such as in clinical trials, quantification using PET imaging to measure changes in regional tau aggregation may be preferable.

Emerging biomarkers

While amyloid-β plaques and tau neurofibrillary tangles are the defining features of AD, numerous biological processes become abnormal in AD and may indicate specific disease stages. The 2018 NIA-AA biological research framework for AD proposed the possibility of adding biomarkers to the AT(N) system, denoted as A/T/X(N), where the "X" represents novel candidate biomarkers of additional pathophysiological mechanisms [26]. Although there are many potential candidates for the X [92], including cerebrovascular biomarkers, the most abundant cell types in the human cortex, the so-called glial cells – microglia, astrocytes, and oligodendrocytes – are the subject of substantial research interest [93].

Microglia cells are the brain's immune cells and, when activated, change their morphology, assume phagocytic properties, and release inflammatory mediators [94]. In AD, it is thought that microglial activation happens in waves, with an early and a late peak. These results come from PET evidence with TSPO tracers ([^{11}C]PK11195 and others) [95]. It is suggested that TSPO is overexpressed in the outer layer of mitochondria in activated microglial cells [96]. Additionally, soluble triggering receptor expressed on myeloid cells 2 (sTREM2) is considered a promising fluid biomarker of microglial activation that changes at different stages of AD [97]. Although findings are still conflicting, several studies found increased sTREM2 levels in the CSF of MCI and AD patients [98–102], and TREM2 is considered to have protective functions [103]. Recently,

426 microglial activation has been identified as a key player in amyloid-β and tau propagation from
427 affected to unaffected regions [104,105].
428
429 Astrocytes are physically intercalated with neurons, and a single astrocyte simultaneously
430 exchanges information with multiple neurons. They are the homeostatic cells of the brain, playing
431 critical roles in modulating neurotransmission, regulating brain energy metabolism, and
432 maintaining CNS ionic and fluid balance [106]. In AD pathology, astrocytes undergo molecular,
433 morphological, and functional changes, assuming a state collectively termed as reactive
434 astrogliosis (or reactive astrocytes) [106] . It is currently a consensus that reactive astrocytes co-
435 exist in multiple states [107,108]. Thus, it is unlikely that a single astrocyte biomarker will capture
436 astrocyte heterogeneity. Reactive astrogliosis was first seen in living AD individuals with
437 topographical information from [^{11}C]DED PET, a tracer that binds to monoamine oxidase B
438 (MAO-B). [^{11}C]DED PET binding presents elevated signal in the brain of amyloid-β+ prodromal
439 AD individuals [109]. They also demonstrated that [^{11}C]DED PET binding associates with cortical
440 atrophy and [^{18}F]FDG hypometabolism [110,111]. Recently, novel astrocyte PET tracers have
441 been developed such as the [^{18}F]SMBT-1 [112], also a MAO-B tracer derivate from the THK
442 family, and the [^{11}C]BU99008 [113], a tracer that binds to the imidazoline 2 binding sites (IS2B).
443 Early findings indicates that [^{11}C]BU99008 captures an specific type of reactive astrocytes (IS2B+
444 astrocytes) in AD [114,115]. A recent meta-analysis evaluated 3,204 CU and AD individuals
445 identified that [^{11}C]DED PET binding is also increased at later stages of AD (but the peak increase
446 remains in the early prodromal phase) [116]. This meta-analysis also confirmed that Glial
447 Fibrillary Acidic Protein (GFAP) and YKL-40 levels in the CSF, and S100B in the blood are
448 consistently altered in AD. More recent evidence indicates that GFAP is increased in the CSF and
449 plasma of CU amyloid-β+ individuals [117,118]. Thus, it seems that GFAP is being released (or
450 leaking) in response to emerging amyloid-β pathology, which indicates that they may react to early
451 soluble species of amyloid-β [119]. Oligomeric forms of amyloid-β are the core pathology of the
452 recently proposed pre-amyloid-β plaque phase of AD [120].
453
454 Oligodendrocytes are CNS myelinating cells, which wrap axons to provide insulation and a
455 protective layer. Recent evidence indicates that disruption of oligodendrocyte progenitor cells may
456 cause myelin abnormalities in AD [121]. In fact, micro- and macrostrutral changes in white matter

have been widely described in AD, including degeneration and demyelination. Since the generation of myelin is the main function of adult oligodendrocyte progenitor cells, it is possible that these cells are behind, at least in part, the white matter abnormalities seen in AD. Myelin quantification with MRI [122] may be an indirect marker of oligodendrocyte dysfunction in AD. Specific markers for tracking oligodendrocyte dysfunction remain to be developed.

Disease staging and comorbidities

The notion of "pure" AD as a pathological entity (i.e. abnormal amyloid-β and tau in the absence of pathologies that characterize other neurodegenerative diseases) has been slowly replaced by the recognition that multiple neuropathological comorbidities are common in most dementia cases [123,124]. Therefore, adding biomarkers of other neuropathologies may contribute to a superior understanding of clinical presentations associated with AD, and those associated with other pathologies, as well as their potential interactions.

While biomarkers for amyloid-β and tau pathologies are becoming well established, the development of biomarkers for other protein aggregates are still in their infancy. There is tremendous interest in developing a biomarker for α-synuclein, the main component of Lewy bodies. Two meta-analyses demonstrated that α-synuclein levels in the CSF are consistently reduced in Parkinson's disease [125,126]. However, there are important variabilities in the measured levels a-synuclein across studies [127]. A recent developed method, the real-time quaking-induced conversion (RT-QuIC), that estimates a-synuclein aggregation, using CSF as the biological matrix, demonstrated high sensitivity (90%) and specificity (~99%) to discriminate PD and controls [128]. By contrast, less advancements have been made in the development of a-synuclein PET tracers. Remarkably, it is not uncommon to find deposits of αSyn in autopsied AD brains [129]. Indeed, CSF α-synuclein levels were significantly higher in the MCI and AD patients [130]. Interestingly, distinct patterns of association between CSF α-synuclein and pTau seem to offer promise to differentiate clinical progression due to AD or in association with Lewy body pathology [131]. In blood, no differences have been found in plasma α-synuclein between AD and controls [132,133]. These early results should be carefully evaluated since 99% of the α-synuclein resides in the red blood cells, the other 1% is in total plasma, platelets, and peripheral blood mononuclear cells [134].

The transactive response DNA-binding protein of 43 kDa (TDP-43) is a nuclear RNA/DNA binding protein involved in the regulation of RNA processing. Accumulation of hyperphosphorylated and ubiquitinated TDP-43 aggregates is commonly found in cases of frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-TDP or FTLD-U) and amyotrophic lateral sclerosis [135,136]. Post-mortem data suggest that TPD-43 inclusions are a co-pathology usually found in the medial temporal lobe of many AD patients [137]. The concomitant AD and TDP-43 pathology are associated to faster cognitive changes and brain atrophy [138–140]. This high prevalence of TDP-43 pathology gave rise to a new pathological entity termed called 'limbic-predominant age-related TDP-43 encephalopathy' (LATE), in which deposits of TDP-43 are widespread distributed in the amygdala, middle frontal gyrus and hippocampus [141]. LATE is characterized by an amnestic presentation similar to amnestic AD. Early findings suggest that TDP-43 can be measured in plasma [142,143]. A recent systematic review described multiple antibodies that can may become reproductive immunoassays for measuring TDP-43, but this is still a challenging task [144]. Biomarkers of other protein aggregates are a priority in AD research but in terms of validation they are still far behind amyloid-β and tau biomarkers. However, while biomarkers for neuropathological comorbidities will help explain a patient's clinical symptoms, it is important to emphasize that their usefulness for staging AD will be limited, as these other neuropathological comorbidities do not define AD, but rather define other neurodegenerative diseases.

Role of Biological AD Staging for Clinical Trials

Recent clinical trial data support the use of *in vivo* disease staging in selecting patients most likely to benefit from a specific therapeutic intervention. A phase 2 randomized controlled trial of the monoclonal antibody donanemab, which pre-screened participants using both amyloid- and tau-PET met primary endpoints, defined as a 25-30% slower decline in the Integrated Alzheimer's Disease Rating Scale, a composite measure of cognition and activities of daily living [145] (ClinicalTrials.gov number: NCT03367403). Potential study participants were pre-screened based on clinical criteria in addition to biomarker criteria, resulting in the inclusion of just over 10% of the population screened included in the study. Participants were only included if they had evidence

of tau-PET abnormality and if tau-PET levels were below a predefined upper threshold. It was hypothesized that subjects who do not yet display advanced tau aggregation may respond better to anti-amyloid therapy.

Future disease-modifying trials in AD may benefit from using the topographical information from tau-PET in determining inclusion criteria. Because tau aggregation in medial temporal regions often takes place in the absence of symptoms, asymptomatic amyloid-β positive individuals constitute a highly heterogenous group with respect to future cognitive decline. Therefore, restricting enrollment criteria to specific disease stages may inform the time frame in which future cognitive symptoms can be anticipated in individuals without cognitive symptoms at baseline.

Another potential use of disease staging in clinical trials is using biomarker-based staging as an outcome measure. The transition from amyloid-positive to amyloid-negative has already been demonstrated in many monoclonal antibody anti-amyloid therapies [145–150]. Future clinical trials may use tau-PET biomarkers to look for the expansion of tau to subsequent Braak stages. Stability (or even reduction) of PET-based Braak stage at follow-up could be used as evidence of biomarker efficacy. If disease-modifying treatments are successful, there will be a greater need for in vivo staging of AD using biomarkers [151].

Concluding remarks

Staging of AD has evolved over the past four decades from clinically-defined stages based on symptomatic severity to a more complex clinical-pathological model integrating information from multiple biomarker modalities. This review highlighted pTau panels, in which a single collection of plasma or CSF could be used to provide information on multiple tau phosphorylation sites, as a method of staging AD *in vivo*. We also highlighted novel potential "X" biomarkers, which promise to refine our understanding of AD progression. Furthermore, using the topography of PET abnormalities presents to opportunity to stage AD *in vivo* while simultaneously allowing for comparison with established post-mortem frameworks for staging AD. Finally, biomarker-based AD staging systems have the advantage of superior sensitivity and specificity for AD as compared to clinical staging (see Clinician's corner). However, several important challenges lie ahead (see outstanding questions section). Many AD biomarker studies are conducted in highly selected

cohorts made up of volunteers who are motivated to participate in biomedical research on AD. Correspondingly, they are unlikely to represent the general population. In this connection, recent studies have identified lower concentrations of pTau181 and total tau in the CSF of African American individuals after correcting for age, sex, APOE4 and cognitive impairment [152]. While the reasons underlying these differences are unclear, these results highlight that great care must be taken when applying biomarker thresholds derived from observational studies whose demographics are not reflective of the general population. Similarly, great care is needed to ensure that the biological milestones used to stage AD are relevant to all populations to which they will be applied.

Unbiased staging of disease lies at heart of personalized clinical management. When successful disease-modifying therapies exist, staging of AD using biomarkers will be critical for selecting patients who will respond to a specific therapy. Until then, disease staging systems provide a window into the natural history of AD and provide a framework for the development of new biomarkers and therapeutic targets.

Acknowledgements and funding:

JT is funded by the Canadian Institutes of Health Research Doctoral Award.

References

1 Gonnella, J.S. *et al.* (1984) Staging of Disease. *JAMA - J. Am. Med. Assoc.* 251, 637
2 Markson, L.E. *et al.* (1991) Clinical outcomes management and disease staging. *Eval. Health Prof.* 14, 201–227
3 Amin, M.B. *et al.* (2017) The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA. Cancer J. Clin.* DOI: 10.3322/caac.21388
4 Insel, R.A. *et al.* (2015) Staging presymptomatic type 1 diabetes: A scientific statement of jdrf, the endocrine society, and the American diabetes association. *Diabetes Care* DOI: 10.2337/dc15-1419
5 Jack, C.R. *et al.* (2013) Tracking pathophysiological processes in Alzheimer's disease: An updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 12, 207–216
6 Bateman, R.J. *et al.* (2012) Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *N. Engl. J. Med.* 367, 795–804
7 Villemagne, V.L. *et al.* (2013) Amyloid-β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: A prospective cohort study. *Lancet Neurol.* 12,

357–367
8 McKhann, G. *et al.* (1984) Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* 34, 939–939
9 Reisberg, B. *et al.* (1982) The Global Deterioration Scale for Assessment of Primary Degenerative Dementia. *Am. J. Psychiatry* 139, 1136–1139
10 van Harten, A.C. *et al.* (2018) Subjective cognitive decline and risk of MCI: The Mayo Clinic Study of Aging. *Neurology* DOI: 10.1212/WNL.0000000000005863
11 Kryscio, R.J. *et al.* (2014) Self-reported memory complaints: Implications from a longitudinal cohort with autopsies. *Neurology* DOI: 10.1212/WNL.0000000000000856
12 Del Tredici, K. and Braak, H. (2020) To stage, or not to stage. *Curr. Opin. Neurobiol.* 61, 10–22
13 Hyman, B.T. *et al.* (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimer's Dement.* DOI: 10.1016/j.jalz.2011.10.007
14 Montine, T.J. *et al.* (2012) National institute on aging-Alzheimer's association guidelines for the neuropathologic assessment of Alzheimer's disease: A practical approach. *Acta Neuropathol.* 123, 1–11
15 Braak, H. and Braak, E. (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* 82, 239–259
16 Braak, H. *et al.* (2006) Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 112, 389–404
17 Braak, H. and Braak, E. (1995) Staging of alzheimer's disease-related neurofibrillary changes. *Neurobiol. Aging* 16, 271–278
18 Mirra, S.S. *et al.* (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). - Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 41, 479–486
19 Crain, B.J. (2014) Escourolle and Poirier's Manual of Basic Neuropathology, Fifth Edition. *J. Neuropathol. Exp. Neurol.* DOI: 10.1097/nen.0000000000000120
20 Knopman, D.S. *et al.* (2003) Neuropathology of Cognitively Normal Elderly. *J. Neuropathol. Exp. Neurol.* 62, 1087–1095
21 Schmitt, F.A. *et al.* (2000) "Preclinical" AD revisited: Neuropathology of cognitively normal older adults. *Neurology* 55, 370–376
22 Abner, E.L. *et al.* (2017) Outcomes after diagnosis of mild cognitive impairment in a large autopsy series. *Ann. Neurol.* DOI: 10.1002/ana.24903
23 Petersen, R.C. *et al.* (2006) Neuropathologic Features of Amnestic Mild Cognitive Impairment. *Arch. Neurol.* DOI: 10.1001/archneur.63.5.665
24 Schneider, J.A. *et al.* (2009) The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann. Neurol.* DOI: 10.1002/ana.21706
25 Jack, C.R. *et al.* (2016) A/T/N: An unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology* 87, 539–547
26 Jack, C.R. *et al.* (2018) NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's Dement.* 14, 535–562
27 Sperling, R.A. *et al.* (2014) The A4 Study: Stopping AD Before Symptoms Begin? *Sci. Transl. Med.* 6, 228fs13-228fs13
28 Jack, C.R. *et al.* (2019) Associations of Amyloid, Tau, and Neurodegeneration Biomarker

Profiles with Rates of Memory Decline among Individuals Without Dementia. *JAMA - J. Am. Med. Assoc.* 321, 2316–2325

29 Johnson, K.A. *et al.* (2013) Appropriate use criteria for amyloid PET: a report of the Amyloid Imaging Task Force, the Society of Nuclear Medicine and Molecular Imaging, and the Alzheimer's Association. *Alzheimers. Dement.* 54, 476–490

30 Rabinovici, G.D. *et al.* (2019) Association of Amyloid Positron Emission Tomography with Subsequent Change in Clinical Management among Medicare Beneficiaries with Mild Cognitive Impairment or Dementia. *JAMA* 321, 1286–1294

31 FDA (2020) , FDA approves first drug to image tau pathology in patients being evaluated for Alzheimer's disease. .

32 Jack, C.R. *et al.* (2017) Defining imaging biomarker cut points for brain aging and Alzheimer's disease. *Alzheimer's Dement.* 13, 205–216

33 Therriault, J. *et al.* (2021) Determining amyloid-β positivity using 18F-AZD4694 PET imaging. *J. Nucl. Med.* 62, 247–252

34 Altmann, A. *et al.* (2015) Regional brain hypometabolism is unrelated to regional amyloid plaque burden. *Brain* DOI: 10.1093/brain/awv278

35 Sperling, R.A. *et al.* (2020) Association of Factors With Elevated Amyloid Burden in Clinically Normal Older Individuals. *JAMA Neurol.* (in press), 1–11

36 Jansen, W.J. *et al.* (2015) Prevalence of cerebral amyloid pathology in persons without dementia: A meta-analysis. *JAMA - J. Am. Med. Assoc.* 313, 1924–1938

37 Chiotis, K. *et al.* (2017) Longitudinal changes of tau PET imaging in relation to hypometabolism in prodromal and Alzheimer ' s disease dementia. *Mol. Psychiatry* DOI: 10.1038/mp.2017.108

38 Xia, C. *et al.* (2017) Association of in vivo [18 F]AV-1451 tau PET imaging results with cortical atrophy and symptoms in typical and atypical Alzheimer disease. *JAMA Neurol.* 74, 427–436

39 Ossenkoppele, R. *et al.* (2016) Tau PET patterns mirror clinical and neuroanatomical variability in Alzheimer's disease. *Brain* 139, 1551–1567

40 Therriault, J. *et al.* (2021) Amyloid-dependent and amyloid-independent effects of Tau in individuals without dementia. *Ann. Clin. Transl. Neurol.* 8, 2083–2092

41 Pascoal, T.A. *et al.* (2020) 18F-MK-6240 PET for early and late detection of neurofibrillary tangles. *Brain* 143, 2818–2830

42 Therriault, J. *et al.* (2020) Topographical distribution of amyloid-β, tau and atrophy in behavioral / dysexecutive AD patients. *Neurology* DOI: 10.1212/WNL.0000000000011081

43 Leuzy, A. *et al.* (2020) Diagnostic performance of RO948 F 18 tau positron emission tomography in the differentiation of alzheimer disease from other neurodegenerative disorders. *JAMA Neurol.* 77, 955–965

44 Therriault, J. *et al.* (2021) Frequency of biologically-defined AD in relation to age, sex, APOEε4 and cognitive impairment. *Neurology*

45 Jack, C.R. *et al.* (2019) The bivariate distribution of amyloid-β and tau: relationship with established neurocognitive clinical syndromes. *Brain* 142, 3230–3242

46 Jack, C.R. *et al.* (2019) Prevalence of Biologically vs Clinically Defined Alzheimer Spectrum Entities Using the National Institute on Aging-Alzheimer's Association Research Framework. *JAMA Neurol.* 76, 1174–1183

47 Scheltens, P. *et al.* (2021) Alzheimer's disease. *Lancet* 397, 1577–1590

48 Mattsson, N. *et al.* (2019) Staging β-Amyloid Pathology with Amyloid Positron Emission Tomography. *JAMA Neurol.* 76, 1319–1329
49 Palmqvist, S. *et al.* (2017) Earliest accumulation of β-amyloid occurs within the default-mode network and concurrently affects brain connectivity. *Nat. Commun.* 8,
50 Fantoni, E. *et al.* (2020) The Spatial-Temporal Ordering of Amyloid Pathology and Opportunities for PET Imaging. *J. Nucl. Med.* 61, 166–171
51 Pascoal, T.A. *et al.* (2020) Topographical distribution of Aβ predicts progression to dementia in Aβ positive mild cognitive impairment. *Alzheimer's Dement. Diagnosis, Assess. Dis. Monit.* 12,
52 Collij, L.E. *et al.* (2020) Multitracer model for staging cortical amyloid deposition using PET imaging. *Neurology* 95, e1538–e1553
53 Schöll, M. *et al.* (2016) PET Imaging of Tau Deposition in the Aging Human Brain. *Neuron* 89, 971–982
54 Johnson, K.A. *et al.* (2016) Tau positron emission tomographic imaging in aging and early Alzheimer disease. *Ann. Neurol.* 79, 110–119
55 Lowe, V.J. *et al.* (2018) Widespread brain tau and its association with ageing, Braak stage and Alzheimer's dementia. *Brain* DOI: 10.1093/brain/awx320
56 Crary, J.F. *et al.* (2014) Primary age-related tauopathy (PART): a common pathology associated with human aging. *Acta Neuropathol.* 128, 755–766
57 Therriault, J. *et al.* (2022) Biomarker modeling of Alzheimer's disease using PET-based Braak staging. *Nat. Aging* DOI: 10.1038/s43587-022-00204-0
58 Jagust, W. (2018) Imaging the evolution and pathophysiology of Alzheimer disease. *Nat. Rev. Neurosci.* DOI: 10.1038/s41583-018-0067-3
59 Franzmeier, N. *et al.* (2020) Patient-centered connectivity-based prediction of tau pathology spread in Alzheimer's disease. *Sci. Adv.* 6,
60 Jacobs, H.I.L. *et al.* (2018) Structural tract alterations predict downstream tau accumulation in amyloid-positive older individuals. *Nat. Neurosci.* 21, 424–431
61 Pascoal, T.A. *et al.* (2021) Longitudinal 18F-MK-6240 tau tangles accumulation follows Braak stages. *Brain* 144, 3517–3528
62 Vogel, J.W. *et al.* (2020) Spread of pathological tau proteins through communicating neurons in human Alzheimer's disease. *Nat. Commun.* 11, 2612
63 Vogel, J.W. *et al.* (2021) Four distinct trajectories of tau deposition identified in Alzheimer's disease. *Nat. Med.* 27, 871–881
64 Young, C.B. *et al.* (2022) Divergent Cortical Tau Positron Emission Tomography Patterns Among Patients With Preclinical Alzheimer Disease. *JAMA Neurol.* DOI: 10.1001/jamaneurol.2022.0676
65 Vogel, J.W. and Hansson, O. (2022) Subtypes of Alzheimer's disease: questions, controversy, and meaning. *Trends Neurosci.* 45, 342–345
66 Ossenkoppele, R. *et al.* (2021) Tau PET correlates with different Alzheimer's disease-related features compared to CSF and plasma p-tau biomarkers. *EMBO Mol. Med.* 13, 1–15
67 Barthélemy, N.R. *et al.* (2020) A soluble phosphorylated tau signature links tau, amyloid and the evolution of stages of dominantly inherited Alzheimer's disease. *Nat. Med.* 26, 398–407
68 Ashton, N.J. *et al.* Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. *Pre-print*

69 Suárez-Calvet, M. *et al.* (2020) Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in Aβ pathology are detected. *EMBO Mol. Med.* 12, 1–19
70 Augustinack, J.C. *et al.* (2002) Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol.* 103, 26–35
71 Lantero-Rodriguez, J. *et al.* (2021) P-tau235: a novel biomarker for staging preclinical Alzheimer's disease. *EMBO Mol. Med.* DOI: 10.15252/emmm.202115098
72 Janelidze, S. *et al.* (2020) Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nat. Commun.* 11, 1–12
73 Mattsson-Carlgren, N. *et al.* (2021) Soluble P-tau217 reflects amyloid and tau pathology and mediates the association of amyloid with tau. *EMBO Mol. Med.* 13, 1–11
74 Leuzy, A. *et al.* (2021) Comparing the Clinical Utility and Diagnostic Performance of CSF P-Tau181, P-Tau217, and P-Tau231 Assays. *Neurology* 97, e1681–e1694
75 Ashton, N.J. *et al.* (2021) Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol.* 141, 709–724
76 Nakamura, A. *et al.* (2018) High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* DOI: 10.1038/nature25456
77 Karikari, T.K. *et al.* (2020) Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol.* 19, 422–433
78 Janelidze, S. *et al.* (2020) Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat. Med.* 26, 379–386
79 Thijssen, E.H. *et al.* (2020) Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat. Med.* 26, 387–397
80 Palmqvist, S. *et al.* (2020) Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA* DOI: 10.1001/jama.2020.12134
81 Ashton, N.J. *et al.* (2021) Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol.* DOI: 10.1007/s00401-021-02275-6
82 Beach, T.G. *et al.* (2012) Accuracy of the clinical diagnosis of Alzheimer disease at National Institute on Aging Alzheimer Disease Centers, 2005-2010. *J. Neuropathol. Exp. Neurol.* DOI: 10.1097/NEN.0b013e31824b211b
83 Gauthier, S. *et al.* (2020) Impact of p-tau181 and p-tau217 levels on enrolment for randomized clinical trials and future use of anti-amyloid and anti-tau drugs. *Expert Rev. Neurother.* 0,
84 Cullen, N.C. *et al.* (2021) Individualized prognosis of cognitive decline and dementia in mild cognitive impairment based on plasma biomarker combinations. *Nat. Aging* 1, 114–123
85 Ashton, N.J. *et al.* (2020) An update on blood-based biomarkers for non-Alzheimer neurodegenerative disorders. *Nat. Rev. Neurol.* 16, 265–284
86 Petersen, R.C. (2022) Are plasma markers for Alzheimer's disease ready for clinical use? *Nat. Aging* DOI: https://doi.org/10.1038/s43587-022-00176-1
87 Moscoso, A. *et al.* (2020) Time course of phosphorylated-tau181 in blood across the Alzheimer's disease spectrum. *Brain* DOI: 10.1093/brain/awaa399
88 O'Connor, A. *et al.* (2020) Plasma phospho-tau181 in presymptomatic and symptomatic

familial Alzheimer's disease: a longitudinal cohort study. *Mol. Psychiatry* 181,
89 Hansson, O. *et al.* (2020) Plasma phosphorylated tau181 and neurodegeneration in Alzheimer's disease. *Ann. Clin. Transl. Neurol.* DOI: 10.1002/acn3.51253
90 Ossenkoppele, R. *et al.* (2018) Discriminative accuracy of [18F]flortaucipir positron emission tomography for Alzheimer disease vs other neurodegenerative disorders. *JAMA - J. Am. Med. Assoc.* 320, 1151–1162
91 Haeberlein, S.B. *et al.* (2022) Two Randomized Phase 3 Studies of Aducanumab in Early Alzheimer's Disease. *J. Prev. Alzheimer's Dis.*
92 Hampel, H. *et al.* (2021) Developing the ATX(N) classification for use across the Alzheimer disease continuum. *Nat. Rev. Neurol.* DOI: 10.1038/s41582-021-00520-w
93 Carter, S.F. *et al.* (2019) Astrocyte Biomarkers in Alzheimer's Disease. *Trends Mol. Med.* 25, 77–95
94 Prinz, M. *et al.* (2019) Microglia Biology: One Century of Evolving Concepts. *Cell* 179, 292–311
95 Fan, Z. *et al.* (2017) An early and late peak in microglial activation in Alzheimer's disease trajectory. *Brain* DOI: 10.1093/brain/aww349
96 Zimmer, E.R. *et al.* Tracking neuroinflammation in Alzheimer's disease: The role of positron emission tomography imaging. , *Journal of Neuroinflammation*. (2014)
97 Suárez-Calvet, M. *et al.* (2019) Early increase of CSF sTREM2 in Alzheimer's disease is associated with tau related-neurodegeneration but not with amyloid-β pathology. *Mol. Neurodegener.* DOI: 10.1186/s13024-018-0301-5
98 Kleinberger, G. *et al.* (2014) TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci. Transl. Med.* DOI: 10.1126/scitranslmed.3009093
99 Suárez-Calvet, M. *et al.* (2016) sTREM 2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers . *EMBO Mol. Med.* DOI: 10.15252/emmm.201506123
100 Heslegrave, A. *et al.* (2016) Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. *Mol. Neurodegener.* DOI: 10.1186/s13024-016-0071-x
101 Brosseron, F. *et al.* (2018) Characterization and clinical use of inflammatory cerebrospinal fluid protein markers in Alzheimer's disease. *Alzheimer's Res. Ther.* DOI: 10.1186/s13195-018-0353-3
102 Piccio, L. *et al.* (2016) Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. *Acta Neuropathol.* DOI: 10.1007/s00401-016-1533-5
103 Lewcock, J.W. *et al.* (2020) Emerging Microglia Biology Defines Novel Therapeutic Approaches for Alzheimer's Disease. *Neuron* 108, 801–821
104 d'Errico, P. *et al.* (2021) Microglia contribute to the propagation of Aβ into unaffected brain tissue. *Nat. Neurosci.* DOI: 10.1038/s41593-021-00951-0
105 Pascoal, T.A. *et al.* (2021) Microglial activation and tau propagate jointly across Braak stages. *Nat. Med.* DOI: 10.1038/s41591-021-01456-w
106 Souza, D.G. *et al.* The astrocyte biochemistry. , *Seminars in Cell and Developmental Biology*. (2019)
107 Liddelow, S.A. *et al.* (2017) Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* DOI: 10.1038/nature21029
108 Escartin, C. *et al.* (2021) Reactive astrocyte nomenclature, definitions, and future directions. *Nat. Neurosci.* 24, 312–325

109 Carter, S.F. *et al.* (2012) Evidence for Astrocytosis in Prodromal Alzheimer Disease Provided by 11C-Deuterium-L-Deprenyl: A Multitracer PET Paradigm Combining 11C-Pittsburgh Compound B and 18F-FDG. *J. Nucl. Med.* 53, 37–46
110 Carter, S.F. *et al.* (2019) Longitudinal association between astrocyte function and glucose metabolism in autosomal dominant Alzheimer's disease. *Eur. J. Nucl. Med. Mol. Imaging* DOI: 10.1007/s00259-018-4217-7
111 Vilaplana, E. *et al.* (2020) Cortical microstructural correlates of astrocytosis in autosomal-dominant Alzheimer disease. *Neurology* DOI: 10.1212/WNL.0000000000009405
112 Harada, R. *et al.* (2021) 18F-SMBT-1: A selective and reversible PET tracer for monoamine oxidase-b imaging. *J. Nucl. Med.* DOI: 10.2967/jnumed.120.244400
113 Tyacke, R.J. *et al.* (2018) Evaluation of ^{11}C-BU99008, a positron emission tomography ligand for the Imidazoline $_2$ binding site in human brain. *J. Nucl. Med.*
114 Calsolaro, V. *et al.* (2021) Astrocyte reactivity with late-onset cognitive impairment assessed in vivo using 11C-BU99008 PET and its relationship with amyloid load. *Mol. Psychiatry* DOI: 10.1038/s41380-021-01193-z
115 Kumar, A. *et al.* (2021) Astroglial tracer BU99008 detects multiple binding sites in Alzheimer's disease brain. *Mol. Psychiatry* DOI: 10.1038/s41380-021-01101-5
116 Bellaver, B. *et al.* (2021) Astrocyte Biomarkers in Alzheimer Disease: A Systematic Review and Meta-analysis. *Neurology* DOI: 10.1212/WNL.0000000000012109
117 Pereira, J.B. *et al.* (2021) Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. *Brain* DOI: 10.1093/brain/awab223
118 Benedet, A.L. *et al.* (2021) Differences between Plasma and Cerebrospinal Fluid Glial Fibrillary Acidic Protein Levels across the Alzheimer Disease Continuum. *JAMA Neurol.* DOI: 10.1001/jamaneurol.2021.3671
119 Fontana, I.C. *et al.* Amyloid-β oligomers in cellular models of Alzheimer's disease. , *Journal of Neurochemistry*. (2020)
120 Uhlmann, R.E. *et al.* (2020) Acute targeting of pre-amyloid seeds in transgenic mice reduces Alzheimer-like pathology later in life. *Nat. Neurosci.* DOI: 10.1038/s41593-020-00737-w
121 Vanzulli, I. *et al.* (2020) Disruption of oligodendrocyte progenitor cells is an early sign of pathology in the triple transgenic mouse model of Alzheimer's disease. *Neurobiol. Aging* DOI: 10.1016/j.neurobiolaging.2020.05.016
122 van der Weijden, C.W.J. *et al.* (2021) Myelin quantification with MRI: A systematic review of accuracy and reproducibility. *Neuroimage* DOI: 10.1016/j.neuroimage.2020.117561
123 Schneider, J.A. *et al.* (2007) Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology* 69, 2197–2204
124 Power, M.C. *et al.* (2018) Combined neuropathological pathways account for age-related risk of dementia. *Ann. Neurol.* DOI: 10.1002/ana.25246
125 Eusebi, P. *et al.* (2017) Diagnostic utility of cerebrospinal fluid α-synuclein in Parkinson's disease: A systematic review and meta-analysis. *Mov. Disord.* DOI: 10.1002/mds.27110
126 Zhou, B. *et al.* The diagnostic and differential diagnosis utility of cerebrospinal fluid α - synuclein levels in Parkinson's disease: A meta-analysis. , *Parkinson's Disease*. (2015)
127 Fayyad, M. *et al.* Parkinson's disease biomarkers based on α-synuclein. , *Journal of Neurochemistry*. (2019)
128 Fairfoul, G. *et al.* (2016) Alpha-synuclein RT-QuIC in the CSF of patients with alpha-

synucleinopathies. *Ann. Clin. Transl. Neurol.* DOI: 10.1002/acn3.338
129 Twohig, D. and Nielsen, H.M. α-synuclein in the pathophysiology of Alzheimer's disease. , *Molecular Neurodegeneration*. (2019)
130 Korff, A. *et al.* (2013) α-synuclein in cerebrospinal fluid of Alzheimer's disease and mild cognitive impairment. *J. Alzheimer's Dis.* DOI: 10.3233/JAD-130458
131 Wang, H. *et al.* (2018) A longitudinal study of total and phosphorylated α-synuclein with other biomarkers in cerebrospinal fluid of Alzheimer's disease and mild cognitive impairment. *J. Alzheimer's Dis.* DOI: 10.3233/JAD-171013
132 Shi, M. *et al.* (2010) Significance and confounders of peripheral DJ-1 and alpha-synuclein in Parkinson's disease. *Neurosci. Lett.* DOI: 10.1016/j.neulet.2010.06.009
133 Chang, K.A. *et al.* (2016) Plasma soluble neuregulin-1 as a diagnostic biomarker for Alzheimer's disease. *Neurochem. Int.* DOI: 10.1016/j.neuint.2016.04.012
134 Barbour, R. *et al.* (2008) Red blood cells are the major source of alpha-synuclein in blood. *Neurodegener. Dis.* DOI: 10.1159/000112832
135 Neumann, M. *et al.* (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science (80-.).* DOI: 10.1126/science.1134108
136 Cairns, N.J. *et al.* (2007) TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am. J. Pathol.* DOI: 10.2353/ajpath.2007.070182
137 Chen-Plotkin, A.S. *et al.* TAR DNA-binding protein 43 in neurodegenerative disease. , *Nature Reviews Neurology*. (2010)
138 Josephs, K.A. *et al.* (2008) Abnormal TDP-43 immunoreactivity in AD modifies clinicopathologic and radiologic phenotype. *Neurology* DOI: 10.1212/01.wnl.0000304041.09418.b1
139 Josephs, K.A. *et al.* (2014) TDP-43 is a key player in the clinical features associated with Alzheimer's disease. *Acta Neuropathol.* DOI: 10.1007/s00401-014-1269-z
140 Wilson, R.S. *et al.* (2013) TDP-43 pathology, cognitive decline, and dementia in old age. *JAMA Neurol.* DOI: 10.1001/jamaneurol.2013.3961
141 Nelson, P.T. *et al.* (2019) Limbic-predominant age-related TDP-43 encephalopathy (LATE): Consensus working group report. *Brain* 142, 1503–1527
142 Foulds, P. *et al.* (2008) TDP-43 protein in plasma may index TDP-43 brain pathology in Alzheimer's disease and frontotemporal lobar degeneration. *Acta Neuropathol.* DOI: 10.1007/s00401-008-0389-8
143 Williams, S.M. *et al.* (2017) Blood-Based Oligomeric and Other Protein Variant Biomarkers to Facilitate Pre-Symptomatic Diagnosis and Staging of Alzheimer's Disease. *J. Alzheimer's Dis.* DOI: 10.3233/JAD-161116
144 Goossens, J. *et al.* TDP-43 as a possible biomarker for frontotemporal lobar degeneration: a systematic review of existing antibodies. , *Acta neuropathologica communications*. (2015)
145 Mintun, M.A. *et al.* (2021) Donanemab in Early Alzheimer's Disease. *N. Engl. J. Med.* 384, 1691–1704
146 Honig, L.S. *et al.* (2018) Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease. *N. Engl. J. Med.* 378, 321–330
147 Doody, R.S. *et al.* (2014) Phase 3 Trials of Solanezumab for Mild-to-Moderate Alzheimer's Disease. *N. Engl. J. Med.* DOI: 10.1056/nejmoa1312889
148 Sevigny, J. *et al.* (2016) The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature* 537, 50–56

149 Salloway, S. *et al.* (2021) A trial of gantenerumab or solanezumab in dominantly inherited Alzheimer's disease. *Nat. Med.* 27, 1187–1196
150 Tian Hui Kwan, A. *et al.* (2020) Lessons Learnt from the Second Generation of Anti-Amyloid Monoclonal Antibodies Clinical Trials. *Dement. Geriatr. Cogn. Disord.* 3, 334–348
151 Levey, A.I. (2021) Progress with Treatments for Alzheimer's Disease. *N. Engl. J. Med.* 384, 1762–1763
152 Morris, J.C. *et al.* (2019) Assessment of Racial Disparities in Biomarkers for Alzheimer Disease. *JAMA Neurol.* 76, 264–273
153 Sperling, R.A. *et al.* (2011) Toward defining the preclinical stages of Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement.* DOI: 10.1016/j.jalz.2011.03.003
154 Dubois, B. *et al.* (2016) *Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria*, 12
155 Gauthier, S. *et al.* (2006) Mild cognitive impairment. *Lancet*
156 Albert, M.S. *et al.* (2011) The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement.* DOI: 10.1016/j.jalz.2011.03.008
157 Liang, C.S. *et al.* (2021) Mortality rates in Alzheimer's disease and non-Alzheimer's dementias: a systematic review and meta-analysis. *Lancet Heal. Longev.* DOI: 10.1016/S2666-7568(21)00140-9
158 Morris, J.C. (1993) The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 43, 2412–2414
159 McKhann, G. *et al.* (2011) The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging- Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 263–269

Glossary:

Alzheimer's disease: progressive neurodegenerative disease defined biologically by the accumulation of cerebral amyloid-β plaques and tau neurofibrillary tangles.

Alzheimer's clinical syndrome: clinical syndrome associated with AD pathophysiology. Typically conceptualized as amnestic predominant multidomain cognitive impairment resulting in dementia.

Amyloid-β: peptide produce by proteolytic processing of amyloid precursor protein (APP) by beta- and gamma-secretases. Amyloid-β peptides are the main component of extracellular plaques. Amyloid-β accumulation is considered to be an early central event in the pathogenic cascade of AD.

Braak staging: six-stage hierarchical AD staging system based on the anatomical distribution of tau neurofibrillary tangles. Early stages document abnormalities in medial temporal regions, while later stages include abnormalities extending to primary sensory cortices.

CSF: Cerebrospinal fluid. CSF assays permit the investigation of protein production and clearance in the brain.

Dementia: clinical syndrome characterized by major deficits in two or more cognitive domains, resulting in major inference with ability to carry out activities of daily living.

Disease staging: framework for ranking progressive levels of disease severity based on the reliable identification of specific points in the disease course. Later stages are associated with worse prognosis.

Mild cognitive impairment: clinical syndrome characterized by cognitive decline greater than expected for an individual's age and education level, but that does not cause interference in activities of daily living.

Neurofibrillary tangles: abnormal aggregates of intraneuronal hyperphosphorylated tau. One of the defining features of Alzheimer's disease.

Neurodegeneration: process of neuronal injury or neuronal death. Biomarkers include FDG-PET, MRI and plasma NfL.

Tau: microtubule-associated protein (MAP) involved in neuronal microtubule stabilization and intraneuronal transport. Becomes misfolded in Alzheimer's disease.

pTau: phosphorylated tau. Tau may become phosphorylated at one of several sites.

PET: Positron Emission Tomography: molecular imaging technique to quantify physiological functions using imaging agents radiolabeled with positron-emitting isotopes.

Preclinical AD: the observation of abnormal concentrations of both amyloid-β and tau biomarkers in individuals without objective cognitive impairment.

Prodromal AD: MCI with the presence of Alzheimer's disease (abnormal amyloid-β and tau), considered to precede AD dementia.

Text Boxes:

Box1: AD biomarker-assisted clinical staging

Currently, biomarkers are used to support the diagnosis of AD, while clinical presentation itself is used to stage AD severity. Positive amyloid and tau biomarkers can be observed in individuals without cognitive impairment (preclinical AD), in those with Mild Cognitive Impairment, and in those with dementia.

Stages of preclinical AD were first defined in 2011: (1) No cognitive impairment with the presence of amyloid-β pathology; (2) no cognitive impairment with the presence of amyloid-β pathology and neurodegeneration and finally (3) amyloid-β pathology, neurodegeneration and subtle cognitive decline [153]. A revision in 2016 described preclinical AD as requiring both amyloid-β and tau pathologies, without the need for neurodegeneration [154].

Mild Cognitive Impairment is a term used to describe noticeable cognitive decline relative to age norms that does not significantly interfere with activities of daily living [155]. MCI is a heterogeneous clinical entity that does not require the presence of AD pathology; however, AD as a biological process can result in MCI. Correspondingly, AD research criteria have incorporated the use of imaging and cerebrospinal fluid biomarkers to identify AD in individuals with MCI [156].

The dementia stage is the stage most commonly associated with AD. It is characterized by substantial cognitive impairment affecting more than one cognitive domain. Impact of cognitive decline on activities of daily living is substantial, and neuropsychiatric symptoms are common. Despite the fact that dementia constitutes the tail end of a pathophysiological process that takes approximately two decades (for AD at least), individuals can live with dementia for several years [157]. During this time, cognitive symptoms continue to progressively worsen. Correspondingly, dementia stage can be subdivided into mild, moderate and severe, which commonly correspond to a Clinical Dementia Rating score of 1, 2 or 3, respectively [158]. The Reisberg Global Deterioration Scale provides increased granularity for staging dementia symptoms during the

symptomatic phase of the disease. Although the biological approach is the cornerstone of current trials of disease-modifying interventions in Alzheimer's disease, clinical diagnosis still rests on the criteria set by the National Institute on Aging and Alzheimer's Association in 2011 [159].

Box2: PET and CSF biomarkers of AD pathophysiology are complementary

Positron Emission Tomography (PET) is a non-invasive molecular imaging modality which permits the assessment of regional tissue function *in vivo*. PET provides a highly sensitive (picomolar to nanomolar range) technique for assessing a diversity of specific physiological processes including regional blood flow (perfusion), synaptic density, metabolic activity, drug delivery, as well as quantification of neuropathology.

It is important to draw a conceptual distinction between CSF concentrations of amyloid-β and pTau with PET measures of insoluble amyloid-β and neurofibrillary tangle aggregates. While they both reflect different aspects of the same pathological process, concentrations of AD pathology measured with CSF and PET are not interchangeable. CSF biomarkers reflect the concentration of abnormal proteins which have leaked from brain tissue into the CSF, measured using immunochemical or mass-spectrometry techniques. The availability of these proteins is influenced by rates of production and clearance, and reflect a specific point in time in the AD pathological cascade. PET biomarkers, in contrast, bind with high sensitivity and selectivity for insoluble forms of AD pathophysiology, namely amyloid-β plaques or tau neurofibrillary tangles. Therefore, CSF biomarkers are often conceptualized as measures of a pathological process that are associated with the presence of specific neuropathological abnormalities in the brain. Correspondingly, PET biomarkers are considered to reflect the accumulation of pathology over time. Because CSF biomarkers reflect concentrations of soluble proteins at a given period of time and PET biomarkers reflect the aggregation of these proteins, a temporal offset between CSF and PET biomarkers is expected. In fact, several studies support the notion that at the group level, CSF measures of AD pathology begin to change before PET measurements.

The topographical information conferred from PET imaging permits the staging of pathology according to anatomical localization of pathology (in line with neuropathological staging models). For these reasons, it can be argued that CSF biomarkers are indicators of disease *state* (i.e. they

indicate the presence or absence of AD pathophysiology), while PET biomarkers can provide information about disease *state* and disease *stage*.

Box3: pTau panels

The ability to measure multiple aspects of disease pathophysiology with a single sample is an important advantage of fluid biomarkers over PET biomarkers. The advantage may be leveraged in relation to the recent explosion of biomarkers for tau phosphorylated at different sites. Evidence from autosomal dominant and sporadic AD suggest that different tau phosphorylation sites become elevated at different points in the disease course. Therefore, pTau panels, in which multiple pTau epitopes are evaluated concurrently, may provide AD staging information beyond the information available from a single pTau epitope.

Elevations in specific pTau phosphorylation sites could be used to stage AD *in vivo*. For example, a subject with only abnormality in pTau231 and/or pTau217, but who does not have abnormal concentrations of pTau 235 or pTau181 may be at an earlier stage of AD as compared to an individual with abnormal concentrations of each of these sites. Moreover, a better understanding of the temporal order of abnormality of these phosphorylation sites, provided they are replicable, will inform pTau panel staging models. Longitudinal studies comparing the predictive power of individual pTau phosphorylation sites are also needed to determine stage-specific associations between pTau phosphorylation sites and cognitive decline.

More research is needed to determine the extent to which different pTau phosphorylation sites are preferentially associated with either cerebral amyloid-β plaques or neurofibrillary tangles. However, the high specificity of pTau for AD may indicate that pTau at certain epitopes are closely associated with the presence of amyloid-β in the brain. This may be an advantage when evaluating AD pathology in plasma, where peripheral expression of amyloid-β in peripheral tissues confounds the estimation of cerebral amyloid-β associated with AD. Therefore, elevated pTau at sites closely associated with cerebral amyloid-β may be used as surrogate markers of amyloid-β.

Clinician's corner

Accepted models of AD pathophysiology provide evidence that accumulation of amyloid-β and tau pathologies take place over up to two decades before the onset of clinical symptoms. Therefore, staging of AD using biomarkers has some advantages over clinically-derived stages. Because AD is characterized by a long preclinical period in which neuropathologies accumulate in the absence of symptoms, biomarker-based staging of AD will have superior sensitivity for detecting changes in the asymptomatic stage of the disease. The measurement of cognitive decline in AD is characterized by floor effects and practice effects on cognitive testing. Moreover, cognitive reserve complicates the relationship between severity of neuropathological changes and severity of cognitive decline.

Furthermore, because multiple comorbidities are associated with cognitive decline, cognitively-derived stages also lack specificity for AD. Biomarker-based disease staging provides the potential for monitoring AD pathophysiology in the asymptomatic phase of the disease. Tracking levels of amyloid-β, abnormal tau phosphorylation, and the topography of neurofibrillary tangles provides the opportunity to stage AD specifically, complementing information from cognitive testing.

Currently, AD biomarkers are used in specialized centres to rule in / rule out the presence of AD. It is conceivable that in the future, in vivo biomarkers can provide information about AD severity in addition to presence / absence. For example, tracking the extent of tau abnormality using PET may permit for *in vivo* identification of Braak neurofibrillary tangle stage.

More accessible measures of AD pathophysiology are soon coming to the clinic. Measurements of phosphorylated tau in plasma have high specificity for AD and may aid in the differential diagnosis of cognitive impairment. Furthermore, multiple measurements of phosphorylated tau at specific phosphorylation sites may be able to identify different disease stages without the need to highly specialized equipment or perceived invasive procedures (PET and CSF).

Figure legends

Figure 1. Biological interpretation and staging AD pathophysiology using Positron Emission Tomography. Amyloid, Tau and Neurodegeneration PET biomarkers in representative individuals across that AD continuum. **A:** Amyloid-PET (such as [^{18}F]AZD4694) tracers bind to mature amyloid-β plaques; Tau-PET tracers (such as the [^{18}F]MK4620) tracers bind to neurofibrillary tangles; FDG-PET is used as an index of tripartite synaptic activity (coupling between energy usage by neurons and astrocytes). **A:** Cognitively unimpaired individuals with no AD pathology are negative for amyloid and tau. **B:** Amyloid-β accumulation is observed in the absence of cognitive impairment. **C**: Subtle tau accumulation restricted to the medial temporal lobes also occurs in the absence of overt cognitive impairment. Note this individual is still considered as tau-negative using summary measures of tau positivity. **D:** Cognitively impaired individuals are both A+ and T+ positive, indicating the presence of preclinical AD. **E:** and A+T+ in mild cognitive impairment. to mild dementia. **F:** A+T+ biomarker profile in mild dementia. **G:** A+T+ in the moderate dementia stage. As disease progresses vulnerable regions become hypometabolic as depicted in [^{18}F]FDG PET images. In this framework, dichotomized amyloid-β and tau biomarkers are used to identify the presence of AD, while clinical stages describe AD severity.

Figure 2: Stage-specific optimization of cerebral tau pathology detection

Tau neurofibrillary tangle aggregation in AD is characterized by substantial variability in magnitude and topography. Because this variability in tau is associated with disease symptoms as well as neurodegeneration, how to optimally detect and report tau abnormality remains an important question in AD. Therefore it may be beneficial to use different regions of interest (ROIs) when assessing tau pathology at different stages of AD. Quantification of tau pathology in asymptomatic elderly adults may be facilitated by specifically investigating brain regions characterized by very early tau accumulation such as the transentorhinal cortex, entorhinal cortex, and hippocampus. Using larger ROIs in asymptomatic elderly individuals may dilute the isolated medial temporal signal by concurrently sampling brain regions in which tau is not elevated, and may therefore miss detectable tau pathology. In contrast, the diagnosis of dementia due to AD may be aided by investigating tau uptake outside the medial temporal lobe, which indicates a more

advanced pathological state. Trade-offs between sensitivity and specificity should be considered with respect to study design and population.