Biomarker-based staging of Alzheimer's disease: rationale and clinical applications

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Abstract (150 words)

Disease staging is at the core of the gold-standard neuropathological diagnosis of Alzheimer's disease (AD), where the spatial extent of brain pathology is used to estimate AD severity. While current *in vivo* diagnostic frameworks, in addition to imaging biomarkers, identify AD based on abnormal concentrations of amyloid-β and tau, breakthroughs in molecular imaging have enabled the staging of AD using *in vivo* biomarkers. Focusing on key principles of disease staging shared across several areas of medicine, this Perspective highlights the potential for *in vivo* staging of AD to transform the understanding of preclinical AD, improve enrollment criteria for disease-modifying clinical trials and impact clinical decision-making in the era of anti-amyloid therapeutics. We provide a state-of-the-art review of recent biomarker-based AD staging reports and highlight their contributions to the understanding of the natural history of AD. Furthermore, we outline hypothetical frameworks to stage AD severity using more accessible fluid biomarkers. Finally, by applying amyloid-PET staging to recently published anti-amyloid therapeutic trials, we highlight how biomarker-based disease staging frameworks can illuminate the numerous pathological changes that have already taken place in mildly symptomatic AD. We also discuss challenges related to validation and standardization of disease staging and provide a forward-looking perspective on potential clinical applications.

Main

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the primary cause of dementia worldwide, a primary contributor to global mortality and morbidity [1]. AD is defined by the presence of amyloid-β plaques and tau neurofibrillary tangle pathologies accompanied by neurodegeneration, which differentiate AD from other neurodegenerative diseases [2,3]. Notably, AD pathology starts to accumulate in the brain for approximately 10-20 years prior to the onset of symptoms, during the preclinical phase of disease. A clinical syndrome typically associated with AD pathology, characterized by insidious onset, slow progression, and early amnestic symptoms, is associated with a neuropathological diagnosis of AD [4]. However, 15-30% of individuals with a clinical diagnosis of probable AD dementia do not have evidence of amyloid-β plaques and tau neurofibrillary tangles at autopsy [5] or upon PET imaging [6]. These studies, in tandem with evidence from human genetics and observational cohorts [7–10] have transformed the definition of AD from being based on clinical symptoms to being based on biomarkers reflecting the underlying pathology [2]. In this framework, AD exists along a continuum beginning with early pathological changes, which are initially clinically silent, to initial symptoms to AD dementia.

In recent years, a large number of *in vivo* clinical trials have drawn attention to the need for AD staging rather than simply noting the presence or absence of AD pathological changes. In this review, we outline key concepts of disease staging in medicine and how they inform the rationale for biomarker-based staging of AD. We provide a state-of-the-art summary of disease staging systems used for AD, and consider future staging systems based on accessible biomarkers derived from blood plasma, and discuss the potential roles of disease staging in the design and interpretation of clinical trials. We conclude by outlining the potential clinical implementation of biomarker-based AD staging and discuss potential benefits and drawbacks of this approach.

Staging of disease:

Measurement of disease severity is crucial in many fields of medicine, allowing for assessing the accuracy of clinical diagnoses, directing patient care, determining when specific therapeutic interventions are effective, among many other uses [11]. Disease staging systems offer a structure for gauging the severity of a disease through the identification of key milestones in the progression

of the disease. In oncology, the Tumor / Node / Metastasis (TNM) staging system combines information about tumor (T) size, spread to nearby lymph nodes (N) and spreading to other organs beyond the primary site (M). For each letter, a number indicating the severity of the specific pathological feature is provided, which together combine to assign a specific stage to the cancer [12]. Despite the fact that all these pathological processes are naturally continuous, the standardization of TNM staging allows for the communication and study of cancer in a standardized manner than also helps determine appropriate treatment strategies for different disease stages [12].

While dichotomizing disease into presence / absence is helpful for many uses, most diseases occur on a continuum of severity. Disease staging systems are helpful for determining diagnostic accuracy, because individuals with more severe disease may be easier to diagnose than individuals with less severe disease. Many staging systems, including TNM staging and some AD staging systems, use the anatomical distribution of pathology as a marker of disease severity: diseases with greater spreading are considered more advanced. While it is expected that every individual will have their own disease evolution, general trends in the natural history of a disease allow for the recognition of key disease milestones. Furthermore, standardization of a disease staging systems allows a field of medicine to adopt a standardized language surrounding disease severity, which can help in the interpretation of observational and intervention studies which may use centre-specific methods to determine disease stage.

A disease staging system may be helpful for guiding management of patients with AD. In oncology, TNM staging helps to determine the treatment approach, such as surgery (for early localized lesions), local radiation therapy, or chemotherapy/immunotherapy. In AD, recently approved anti-amyloid therapies are likely to be most effective in early symptomatic disease and not in late symptomatic disease when severe tau pathology and neurodegeneration are present [13,14]. Even in individuals who are not eligible for disease-modifying interventions, clinical staging of dementia is helpful for counselling patients and their families about their prognosis to allow them prepare and get more help as needed [15]. While disease staging systems have tremendous utility in specific fields of medicine, biological AD staging systems currently are based on neuropathology,

which can only be applied after death. In contrast, in vivo staging of AD is currently based on disease symptoms. Below, we briefly review clinical of dementia.

Clinical staging of dementia

The severity of cognitive symptoms lies on a continuum. While not specific for AD, clinical staging of dementia symptoms allows for the delineation of important features that characterize the disease experience for persons living with dementia and caregivers [16]. Multiple methods to stage clinical dementia symptoms exist; here, we will briefly discuss the Clinical Dementia Rating (CDR) and the NIA-AA numeric clinical staging system (which corresponds closely to the Reisberg Global Deterioration Scale [17] stages) to outline how they are separate and complementary to biomarker-based staging. The CDR is a widely-used clinical tool to assess dementia severity by interviewing the patient and a knowledgeable informant (if available). A global CDR score is determined from 0-3, with 0 indicating no dementia and increasing numbers denoting increased dementia severity [18]. A score of 0.5 (very mild dementia) is closely associated with the syndrome of mild cognitive impairment [19], while CDR scores of 1, 2 and 3 denote mild dementia, moderate dementia, and severe dementia, respectively.

The NIA-AA clinical staging system also applies a numeric staging system, yet is intended to apply only to individuals on the Alzheimer's continuum (i.e. with biomarker evidence of Alzheimer's pathological change) [2,20]. In this clinical staging system, stage 1 indicates normal cognitive function, with stage 2 also corresponding to cognitive function within normal ranges but featuring subjective decline as reported by the participant, or a history of decline from previous level of functioning. Stage 3 denotes cognitive performance below a specific range of functioning without any loss of ability to perform activities of daily living. Stages 4, 5, and 6 correspond to mild, moderate and severe dementia, respectively.

While clinical stages provide important and accessible information to the patient and caregiver, clinical dementia severity is typically result of multiple neuropathological processes, especially with more advanced age [21]. Neuropathological assessments, in contrast, can identify the severity of AD as well as a number of other neurodegenerative diseases. Below, we briefly review the

neuropathological staging of AD and how these studies have informed clinico-pathological relationships.

Neuropathological staging of Alzheimer's disease

The gold standard for the identification of AD remains a neuropathological evaluation. In post-mortem evaluations, staging of AD is based on a semi-quantitative assessment of the anatomical distribution of specific pathological changes. The current staging models involve an ABC scoring system, which assigns stages to amyloid-β plaques (A), tau neurofibrillary tangles (B) according to the Braak stage, and neuritic plaques (C) based on the CERAD score [3,22].

For amyloid-β plaques, the Thal staging system classifies their topography into five stages. Amyloid-β first appears in the 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) [23]. Tau neurofibrillary tangles are staged using the Braak and Braak system, which starts with the transentorhinal cortex (stage I) and progresses to the entorhinal cortex, hippocampus (stage II), inferior temporal neocortex (stage III), association cortices (stages IV and V), and primary sensory cortices (stage VI) [24,25]. Neuritic plaques, consisting of amyloid-β core surrounded by aggregated tau in degenerating neurons, are ranked by density in the neocortex (none, sparse, moderate, frequent) using the CERAD scoring system [26]. These staging systems allow differentiation of disease phases based on the anatomical distribution of neuropathological changes.

To summarize an individual's neuropathological change, an ABC score is assigned. For example, an AD neuropathologic change classification could range from A0, B0, C0 to A3, B3, C3. These ABC scores are then converted into four levels of AD neuropathologic change: none, low, intermediate, or high. Braak stages III/IV and above, accompanied by significant amyloid-β and neuritic plaques, are considered sufficient to explain dementia [3,22]. Therefore, AD staging systems are used to determine not only whether AD was present / absent (diagnosis) but also the severity of the disease and its potential relationship to symptoms. Despite the limitations of post-mortem assessments, disease staging systems in AD have allowed for the standardization of AD diagnosis

[27]. Early clinico-pathological studies also allowed for the determination of the relationships between various brain pathologies and clinical symptom severity [28,29].

Importantly, AD neuropathologic change staging is reported independently of clinical history, such as the presence or stage of cognitive impairment. Because AD pathology accumulates for many years prior to the onset of AD symptoms, many individuals with AD pathology are cognitively unimpaired [30]. Although neuropathological staging of AD is limited to post-mortem application, the cognition-independent nature of these staging systems supports the development of in vivo biomarker classification systems that are similarly independent of cognitive status.

The need to stage AD based on biomarkers reflecting pathophysiology

For decades, AD was nearly synonymous with the clinical symptoms of progressive amnestic dementia [31]. However, several neuropathological examinations observed that relatively milder AD pathology was frequently observed in the brains of individuals without cognitive symptoms [32,33], or of individuals with mild cognitive symptoms who did not meet criteria for dementia [34,35]. With these studies came an understanding that the clinical manifestations associated with probable AD dementia are often the result of advanced pathological changes. Subsequent in vivo studies also reported frequent elevated amyloid-PET uptake in cognitively unimpaired individuals [30,36–38], confirming that very early AD-associated changes are often asymptomatic.

Well-established biomarker models suggest that the dementia stage of AD is the tail end of a pathological process that has been underway for approximately two decades [7,9,10,39]. While the survival rate of individuals diagnosed with dementia varies according to a number of factors including sex, age and medical comorbidities, several estimates suggest that individuals live for approximately 6-8 years after a diagnosis of AD dementia [40]. Correspondingly, it can be estimated that the dementia phase of AD accounts for just 25-30% of the natural history of AD. The fact that the majority of the AD natural history is clinically silent highlights the need to stage AD (in particular the preclinical phase) using biomarkers that reflect AD neuropathology.

The value of staging AD biologically is further underscored by the higher prevalence of AD pathology compared to individuals with probable AD dementia [37]. Therefore, the number of individuals who might benefit from AD-modifying therapies exceeds the number of individuals with symptomatic AD, provided there is evidence of efficacy from ongoing randomized controlled trials in preclinical populations such as the AHEAD-3, AHEAD-45 and DIAN-TU studies (do we need REFS here?). Overall, the long preclinical phase of AD can be framed as a window in which multiple different therapeutic strategies can be employed. Staging systems using AD biomarkers will be important tools in determine which individuals may respond optimally to specific therapeutic strategies.

Preclinical AD (amyloid-β and tau abnormality in the absence of symptoms), as well as preclinical Alzheimer's pathological change (amyloid-β abnormality without tau abnormality) represent highly heterogeneous categories in terms of disease severity. In fact, in individuals with preclinical AD, there is a highly heterogeneous risk of clinical progression to MCI and/or all-cause dementia. Recent multicenter longitudinal studies provided evidence of high risk of cognitive decline for individuals with preclinical AD [41,42]. Crucially, however, the degree of risk of cognitive decline and clinical progression varied according the topography of tau-PET abnormality. In individuals with elevated neocortical tau-PET, the risk of clinical progression to all-cause dementia was higher than in those with elevated tau-PET signal restricted to the medial temporal lobe. Rates of cognitive decline were also higher for individuals with elevated neocortical tau compared to other groups. Asymptomatic individuals with Alzheimer's pathological change, in contrast, had relatively lower rates of cognitive decline and clinical progression [41]. Taken together, these results suggest that staging biological AD in the preclinical phase has important implications for understanding risk of cognitive decline.

In addition to greater sensitivity (the ability to detect pre-clinical changes), biomarker-based disease staging also has greater specificity: by understanding the severity of AD specifically, it may be possible to understand the proportion of cognitive impairment attributable to AD and not to other causes. Because dementia is frequently associated with multiple neuropathological conditions [21], understanding when AD is the likely cause of symptoms will be crucial given that amyloid-β positivity may be incidental in some individuals with clinical dementia [43]. The recent

development of biofluid assays for alpha-synuclein, a non-AD pathology, has highlighted how non-AD co-pathologies, such as Lewy body disease, contribute to cognitive decline in individuals with abnormal amyloid-β and tau [44,45]. As biomarkers for other neurodegenerative diseases continue to develop [46], biomarker-based disease staging stands to contribute to personalized medicine approaches for AD and other neurodegenerative diseases.

PET-based disease staging

Advancements in molecular imaging are anticipated to refine current diagnostic classification systems for AD [47]. While most research has concentrated on categorizing amyloid-PET and tau-PET imaging as either positive or negative, the spatial resolution of PET allows for staging based on the anatomical distribution of pathology. Staging systems have the potential to provide additional information by utilizing the topographical distribution of PET uptake, which can aid in monitoring patients during the progression of AD. Because amyloid-β and tau are the hallmark pathologies of AD, staging AD according to these pathologies may give more precise estimates of AD severity specifically, in contrast to other PET biomarkers of neurodegeneration or neuroinflammation that may be abnormal in several other neurodegenerative diseases.

Amyloid-PET

Several studies have proposed data-driven staging systems based on regional burden of amyloid-β plaques imaged by PET. Although the exact sequence of regional accumulation may vary slightly among studies and populations (i.e., sporadic vs autosomal dominant AD), the general pattern indicates initial accumulation in medial neocortical structures (such as the medial prefrontal cortex, posterior cingulate, and precuneus), followed by the striatum, and ultimately the medial temporal regions [48–50]. However, achieving replicability across different radioligands, cohorts, and analytic methods can be challenging [50]. Nevertheless, one study of over 650 participants from the Alzheimer's Disease Neuroimaging Initiative identified 4 stages of amyloid-PET uptake, with later stages being more represented in individuals with AD dementia [51]. Subsequent studies based on this staging model identified longitudinal trajectories of amyloid-PET deposition [52] and specific patterns of future cognitive decline in relation to a participant's baseline amyloid-PET stage [53]. Furthermore, a multicenter study involving over 3000 individuals found that staging amyloid-β

pathology based on regional abnormalities was able to classify subjects scanned with different radioligands and was associated with distinct risk profiles of cognitive decline [54]. A recent study staging the spatial extent of amyloid-PET also reported important associations between amyloid-PET stage and CSF p-tau, tau-PET binding and future cognitive decline [55]. Moreover, a study also suggested that amyloid-PET visual reads, taking into account the number of "positive" brain regions, can reliably identify and differentiate between early and established amyloid-PET uptake [56]. These studies suggest that staging systems based on regional amyloid-β pathology provide important information about biomarker changes in early disease as well as prognostic information for future cognitive decline.

An alternative to topographical staging of amyloid-PET is the use of global amyloid-PET burden, facilitated by the Centiloid scale. The Centiloid scale is a framework to standardize amyloid-PET SUVRs measured from different amyloid-PET imaging agents [57]. A Centiloid value of 0 represents the mean of a group of amyloid-negative young adults (younger than 45 years), while a Centiloid value of 100 represents the mean of a group of individuals with mild-to-moderate AD dementia [57]. Centiloid values can exceed 100 but rarely go considerably below 0. While different cut-points for amyloid-PET positivity on the Centiloid scale have been proposed, most cut-points converge around 20-25 CL [58]. This suggests that the majority of the Centiloid scale exists within the "positive" range (i.e., 25 Centiloid and above), which may provide additional information beyond dichotomization about the natural history of AD. Case-to-autopsy studies have suggested that a Centiloid value above 20 is strongly associated with at least moderate density of amyloid-β plaques, while Centiloid values above 50 are strongly associated with a neuropathological diagnosis of AD (i.e. including significant tau tangles and neuritic plaques) [59]. Furthermore, a Centiloid score of 19 was identified as the point at which amyloid-PET SUVRs reliably began to increase over time [60], and a smaller study identified Centiloid 24 as a point where amyloid-PET SUVRs were more likely to increase [61]. Therefore, while not based on the regional distribution of amyloid-PET (though some degree of correlation is expected), staging of AD based on the magnitude of amyloid-β pathology can provide insights into severity of AD-related biological changes and risk of cognitive decline.

Tau-PET

The validation of tau-PET radioligands has significantly benefited from the knowledge of the canonical distributions of tau tangle pathology described from autopsy studies. Early tau-PET studies provided evidence that the distribution of tau-PET uptake aligns with Braak stages (with most studies combining stages I–II, III–IV, and V–VI) [62–65]. Tau-PET signal in early stages can be observed in individuals without cognitive impairment, both with and without significant amyloid-β presence [64,66,67]. Elevated tau-PET in the medial temporal regions without amyloid-β pathology may indicate primary age-related tauopathy (PART) [68,69]. Markedly increased tau-PET in regions corresponding to Braak stages III–IV (temporal neocortex and association cortices) is predominantly observed in individuals with abnormal amyloid-β biomarkers and is typically accompanied by at least mild cognitive symptoms [66,67]. Lastly, tau-PET in brain regions corresponding to advanced Braak stages (association cortices and primary sensory cortices) is predominantly observed in individuals with cognitive impairment [66,67,70]. These tau-PET results extend observations from autopsy studies suggesting that advanced Braak stages are nearly incompatible with normal cognitive function [29,71]. Taken together, these results suggest that clinical manifestations of AD represent advanced AD neuropathological changes and highlight the utility of tau-PET for staging AD severity.

Similar to postmortem observations [72], tau-PET stage also provides important prognostic information. A recent multicenter longitudinal study of over 1300 individuals provided evidence of high risk of cognitive decline for individuals with preclinical AD [41]. Crucially, however, the degree of risk of cognitive decline and clinical progression varied according the topography of tau-PET abnormality. In individuals with elevated neocortical tau-PET, the risk of clinical progression to all-cause dementia was higher than in those with elevated tau-PET signal restricted to the medial temporal lobe. Rates of cognitive decline were also higher for individuals with elevated neocortical tau compared to other groups. Asymptomatic individuals with Alzheimer's pathological change, in contrast, had relatively lower rates of cognitive decline and clinical progression [41]. Taken together, these results suggest that staging biological AD in the preclinical phase has important implications for understanding risk of cognitive decline.

Because disease staging systems provide an opportunity to model the natural history of a disease, disease staging systems using tau-PET have provided evidence of different changes in other

biomarkers in relation to tau-PET progression. For example, a study staging tau-PET with [^{18}F]MK6240 observed that both amyloid-PET SUVR and different p-tau biomarkers from cerebrospinal fluid (CSF) plateaued at Braak stage IV [66]. Furthermore, subtle memory decline could be observed at the group-level starting at PET-based Braak stage II, whereas advanced PET-based Braak stages were incompatible with normal cognition. Knowledge of biomarker-based disease stage would also be useful for future studies aiming to validate novel biomarkers in order to understand their association with AD severity [73].

Similar to amyloid-PET, it is important to consider that topographical distribution is not the only method of staging tau-PET. Alternative methods include assigning stages based on quantitative values within specific meta-regions, for example as was performed in the phase II and phase III donanemab randomized trials [74,75]. It is anticipated that reasonable correspondence would be observed between quantitative information and spatial distribution, though this may not always be the case. Nevertheless, a proof-of-concept of the utility of disease staging based on biomarkers has recently been published in the donanemab randomized controlled trials: in both the phase II [74] and the phase III [75] trials, individuals with intermediate tau-PET uptake had better responses to donanemab therapy than did individuals with more advanced disease. These results lend support to the notion that anti-amyloid therapy is likely to be more beneficial early in the disease course, before substantial tau accumulation and neurodegeneration have taken place.

The finding that biomarker-based disease staging can be used to determine who may respond optimally to specific treatment interventions highlights the need for more accessible biomarkers that can inform treatment eligibility. The need for amyloid-PET to confirm the presence of amyloid-β plaque pathology, tau-PET to stage disease and multiple MRIs to monitor for adverse events would place a very large burden on patients as well as the healthcare system and heavily limit the ability of patients in non-urban areas to access treatment. Crucially, fluid, and especially blood-based biomarkers, which can measure multiple different pathologies at once from a single sample, may circumvent the need for multiple PET scans, at least for the majority of patients.

Fluid biomarker-based disease staging

An alternative to imaging techniques for staging AD *in vivo* is the use of fluid (CSF and blood) biomarkers. Multiple fluid biomarkers have been developed that demonstrate strong associations with AD neuropathology, amyloid-β and tau burden by PET, as well as clinical diagnosis and prognosis (see [46] for a review). Notably, fluid biomarkers reflect the balance between production, secretion/release, and clearance of the analytes of interest at the time of fluid collection, which is correlated with, but different from, imaging measures which visualize the lifetime accumulation of pathology [76]. In some studies, fluid measures have been demonstrated to detect amyloid-β pathology prior to amyloid-PET [77,78]. Therefore, individuals with abnormal fluid biomarkers but normal amyloid-PET may have very early AD pathology, and fluid biomarker may thus enable staging starting earlier than imaging measures. However, the main advantages of fluid biomarkers are logistical: compared to imaging methods, they need far less complex infrastructure, are less expensive, and multiple biomarkers can be measured using a single biofluid sample. Fluid biomarker panels, which include multiplexed immunoassays or mass spectrometry-based measurements, could be useful in AD staging.

Studies in both autosomal dominant [79] and sporadic AD [66,73,80] have demonstrated that different p-tau species may become abnormal in different stages of the disease [81]. Plasma and CSF p-tau231 are thought to become abnormal very early in the disease progression and are associated with amyloid-β pathology [80,82–84]. Plasma and CSF p-tau217 also detect amyloid-β pathology, even in cognitively normal individuals with preclinical AD [85–87]; in addition p-tau217 is highly correlated with tau neurofibrillary tangle pathology [82,85,88–90]. Further, plasma p-tau217 increases longitudinally, making the fold change in the symptomatic stage of AD higher than that for p-tau181 and p-tau231 (PMID: 36456833). CSF (REFs 79, 91, + PMID: 36510321) and plasma (REF #73, Montoliu-Gaya) levels of p-tau205 [73,79,91] are altered in later stages of the disease and are more strongly associated with tau-PET than p-tau231 or p-tau217. The same is true for the microtubule-binding region (MTBR) tau variants tau368 (PMID: 31834365, PMID: 3654422) and MTBR-tau243 [92,93]. Other fluid tau biomarkers including p-tau235 [94], NTA [95,96], or NT1 [97,98] have also shown alterations within the AD continuum. Overall, more research is needed to further validate the recently-developed “late” fluid biomarkers that are more closely associated with tau tangle pathology. Previous studies have proposed that tau undergoes a sequential phosphorylation process in AD, in which phosphorylation of specific sites primes phosphorylation at other sites [99].

A neuropathological study demonstrated that the pattern of tau hyperphosphorylation is associated with different stages of tau tangle maturity [100]. Altogether, these results suggest that the combination of different tau biomarkers may be used for staging AD.

In a recent study, CSF Aβ42/40, p-tau217, p-tau205, MTBR-tau243 and non-phosphorylated tau were used to create a staging model for the whole AD *continuum* in two independent cohorts [101]. The CSF biomarkers became abnormal in a similar order for all participants (*i.e.*, first CSF Aβ42/40, then p-tau217, p-tau205, microtubule binding region tau at 243 [MTBR-tau243] and finally non-phosphorylated tau). The panel of CSF biomarkers were used to stage AD, and more severe stages were associated with a higher rate of amyloid- and tau-PET positivity and cognitive impairment. Finally, the CSF stages predicted longitudinal trajectories of other biomarkers and clinical progression. Translation of this approach to blood-based biomarkers could enable AD staging based on a single blood sample, as depicted in Figure 2.

Although fluid biomarkers are highly promising, there are important limitations that must be addressed. Assays designed to measure the same analyte may have different levels of performance [88,102–105], so a staging model would likely be assay-specific. Some comorbidities, such as chronic kidney disease, may have an effect on fluid biomarker levels [106–108] that could affect a staging system, although this may be mitigated via the use of biomarker ratios [109,110]. Pre-analytical conditions may affect biomarker values, and best practices should be followed to minimize these concerns [111,112]. Furthermore, in general, the mean fold-change between healthy controls and individuals with AD is lower in plasma than in CSF [105,113,114], especially for plasma Aβ42/40 [114]. Factors such as *APOE* genotype, sex, or glial activation [115] could also potentially affect fluid biomarker levels and the relationship of stages to other outcomes, such as clinical stage.

Fluid biomarkers may enable more rapid and less expensive AD staging compared to imaging measures, which have logistical drawbacks. Because some individuals are reluctant to undergo lumbar puncture (LP), and personnel to perform LP may be limited, accurate blood biomarkers would be particularly helpful in enabling greater accessibility of AD staging.

Subtypes vs staging: space and time

The shift towards a biological definition of AD [2] has allowed for the recognition of a diversity of clinical syndromes secondary to amyloid-β and tau pathologies. Whereas previously AD was considered to cause amnestic-predominant dementia [116], retrospective studies identified AD as the cause of non-amnestic focal cortical syndromes including posterior cortical atrophy, primary progressive aphasia and behavioural variant frontotemporal dementia [117]. With the advent of tau-PET, seminal studies suggested that the topographical distribution of tau tangles were closely associated with different clinical presentations of AD [118,119], which has now been confirmed for a diversity of clinical syndromes using tau-PET imaging [120–125] and neuropathological assessments [126]. These studies highlighted substantial individual-level variability in the topographical distribution of tau pathology, which diverge from the group-level averages described in the Braak staging system.

Recent multicenter tau-PET studies have provided strong evidence for the existence of disease subtypes, classified according to the distribution of tau-PET signal. A recent study involving over 2300 individuals identified four distinct patterns of tau-PET accumulation, each associated with specific clinically relevant characteristics and prognostic features [127]. Additionally, a multicenter study revealed unique patterns of neocortical tau-PET uptake in individuals with preclinical AD, characterized by either asymmetrical distribution in the temporal lobe or high uptake in the precuneus [128]. Based on these findings, it has been suggested that multiple AD subtypes exist (at least as characterized by tau tangle distribution patterns), and importantly, that they are more prevalent than previously estimated [128].

While several studies have highlighted considerable variability in tau accumulation, there appears to be a consistent general pattern of initial accumulation in the medial temporal lobe, followed by aggregation in the temporal neocortex, association cortex, and finally primary sensory cortices. This pattern may serve as a foundation for staging AD based on tau-PET imaging, despite the acknowledged heterogeneity of the disease. Alternatively, in vivo staging systems could be developed for different sub-types of AD if the existing medial temporal / widespread temporal / primary sensory staging system is not useful.

The existence of disease subtypes (discussed above as defined by tau-PET patterns, though other subtyping systems exist [129]) is not mutually exclusive with the utility of disease staging systems. Subtyping and staging have different goals in the study of AD. An illustrative example of the different information provided by staging and subtyping is of individuals with different clinical variants of AD. Multiple individuals may have similar degrees of global cognitive impairment (for example, a Clinical Dementia Rating of 0.5) that differ dramatically in terms of the focus (such as amnestic-predominant or visuospatial predominant variants). Furthermore, such individuals may be at a similar tau stage (for example, stage V) with different relative distribution of pathology within brain regions that encompass stage V. Here, subtype evidently provides information that is not captured by staging. Nevertheless, disease staging systems are well-suited for understanding the natural history of AD and to identify important points in the disease course that may respond to specific therapeutic interventions.

Clinical trial applications

From a biomarker-based staging perspective, many recent disease-modifying trials have targeted individuals with mild clinical symptoms but severe AD pathology [74,130–132]. As reviewed in previous sections, by the time an individual is symptomatic, it is likely that advanced AD pathological changes are present, including late-stage neocortical tau pathology [7,8]. Using results from published clinical trials, it can also be determined that a large number of other biological changes have already taken place by the time symptomatic subjects are enrolled (Figure 3). To illustrate this concept, we assigned a Centiloid value to subjects from the TRIAD cohort based on their global amyloid-PET SUVR, and plotted abnormality in other disease features including CSF Aβ42/40, CSF p-tau217, medial temporal tau-PET, neocortical tau-PET, neurodegeneration and cognitive decline in relation to Centiloid values. It can be observed that by the time subjects were enrolled in published anti-amyloid monoclonal antibody trials, significant changes in p-tau217, medial temporal tau, neocortical tau, as well as neurodegeneration had already taken place at the group-level. This illustrates how randomized trials targeting early symptomatic AD have individuals with more severe AD pathological changes. Given the large number of biological changes that have already taken place, it can be hypothesized that disease-modifying interventions are timed sub-optimally in existing studies. In this connection, a report of a post-mortem examination of a patient treated with aducanumab reported sparse amyloid-β plaques and advanced

neurofibrillary tangle pathology (Braak stage V) [133]. While the patient had relatively lower density of neurofibrillary changes as compared to a reference group of individuals at Braak V-VI [133], amyloid-β plaque reduction in this advanced disease stage may have only mild effects on tau tangle aggregation. However, more case-to-autopsy studies of individuals treated with anti-amyloid therapies will refine the understanding of anti-amyloid treatment with downstream tau accumulation, where mixed results have been reported with tau-PET to date [74,75,130].

Figure 3 also provides examples of ongoing anti-amyloid trials in the asymptomatic phase of the disease which target much earlier AD pathological change such as the AHEAD 3 and AHEAD 4-5 studies [134]. Notably, another study testing lecanemab and the anti-tau agent E2814 in asymptomatic individuals with dominantly inherited AD (DIAN-TU) is ongoing. While results of these trials will not be known for several years, it is anticipated that targeting earlier disease stages will result in better outcomes as less disease irreversible processes (i.e., neurodegeneration) will have unfolded at earlier stages. Despite the recent negative results for primary outcomes in the recent A4 study [135], which also targeted preclinical AD, the agents used in the above-mentioned ongoing trials have already demonstrated evidence of substantial amyloid-β plaque reduction and clinical benefit.

In addition to informing AD trial interpretation, biomarker-based staging may furthermore provide a useful framework for determining eligibility for future disease-modifying trials. While many trials currently require evidence of amyloid-β pathology for eligibility, novel biomarker approaches have begun to further refine eligibility criteria. For example, the AHEAD 3 and AHEAD 4-5 randomized trials include participants with “intermediate” and “elevated” amyloid-PET uptake, respectively [134]. Because amyloid-PET positive asymptomatic individuals are a highly heterogeneous group in terms of their risk of cognitive decline [41], tau biomarkers may also be used to help refined enrolment criteria. Disease staging using either tau-PET [41,136,137] or p-tau biomarkers [138–141] can help inform risk of future decline and stratify prespecified analyses accordingly, in turn helping refine power calculations for primary trial outcomes [142].

Finally, biomarker-based staging may prove useful in designing key secondary outcomes providing evidence of disease modification. For example, different rates of tau-PET stage

progression in placebo vs active groups is a promising strategy given the strong association of elevated brain amyloid-β with tau-PET spreading [143–146] and stage progression [66,147]. In this connection, the lecanemab phase III results observed slowing of [^{18}F]MK6240 tau-PET accumulation in early temporal regions but not in large composite ROIs [130], suggesting that biomarker outcomes can be optimized to reflect stage-specific changes [81].

Taken together, biomarker-based disease staging can help determine eligibility and help interpret potential disease-modifying signals from randomized controlled trials, especially in relation to disease severity of the participants at baseline. Future biomarker-based staging studies can also investigate time needed on different disease-modifying therapies based on the severity of disease at baseline.

Limitations of disease staging

It is important to also consider the limitations of disease staging. Staging systems are by design simplifications; they are models based on key disease features which at the group-level tend to happen in sequence. In this connection, the existence of tau-PET subtypes and atypical clinical presentations of AD are reminders that disease staging systems cannot explain all important features of AD. Furthermore, most diseases are naturally continuous processes and the selection of specific milestones may thus be somewhat artificial. A similar concern is often raised with biomarker dichotomization; disease staging at least partially attenuates this concern through the use of multiple stages.

Reproducibility is another concern. For example, while a greater number of stages helps provide more granularity into the natural history of a disease, there is a corresponding risk of lower reproducibility (in terms of inter-rater or inter-method agreement). This has been noted for Braak staging at autopsy [148], hence the collapsing of stages I-II, III-IV and V-VI to increase agreement between pathologists and academic centres [24]. Similarly, the simultaneous use of multiple in vivo biomarker categories opens the door to lower reproducibility purely from measurement error.

Another limitation of disease staging, specifically with respect to AD, is the relatively low amount of longitudinal biomarker staging data (with the exception of amyloid-PET which has existed for

much longer). The understanding of relative and absolute risks of biological AD stage progression with respect to age, *APOE* ε4 carrier status and other clinical and demographic factors will likely be refined in the coming years. Correspondingly, the association of biological AD stage with relative and absolute risk of dementia, hospitalization and mortality will be necessary to inform clinical practice and counsel patients. Finally, biomarker-based staging should complement but not replace clinical staging of dementia. It is also expected that brain and/or cognitive resilience will complicate associations between biological AD severity and severity of cognitive impairment; it is conceivable that individuals with exceptional cognitive reserve (related to education or other social determinants of health) may display less severe cognitive symptoms with respect to their biological stage.

Conclusions

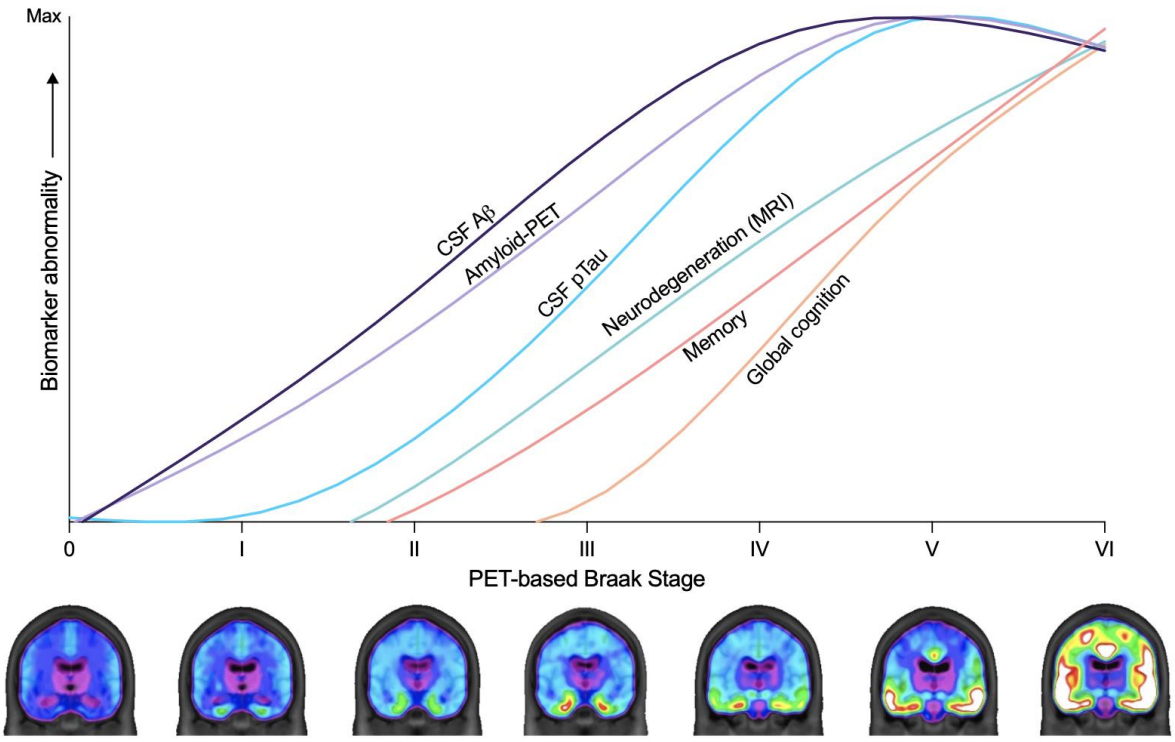
Recent advances in the *in vivo* staging of AD have increased the understanding of the natural history of AD and aided in determining eligibility for disease-modifying trials. The application of disease staging systems for AD will also allow for increased sensitivity to detect early disease (i.e., by looking at early tau-PET regions or by assessing fluid biomarkers that are elevated early in disease such as p-tau231 and p-tau217). In turn, other biomarkers which become elevated late in the natural history of AD such as neocortical tau-PET, p-tau205 in CSF (#91 + PMID: 36510321) and plasma [73] and CSF levels of the MTBR-fragments tau368 (PMID: 3654422) and MTBR-243 [93] can help increase specificity for AD and indicate the presence of advanced disease. Translation and reproducibility of these biomarker signatures into blood will substantially increase the ability of AD staging systems to inform clinical practice. Caution and care must be applied when translating novel biomarkers to unselected populations, particularly if they consist of groups that have been traditionally under-represented in medical research [108,149,150].

List of display items:

Box: Principles of disease staging.

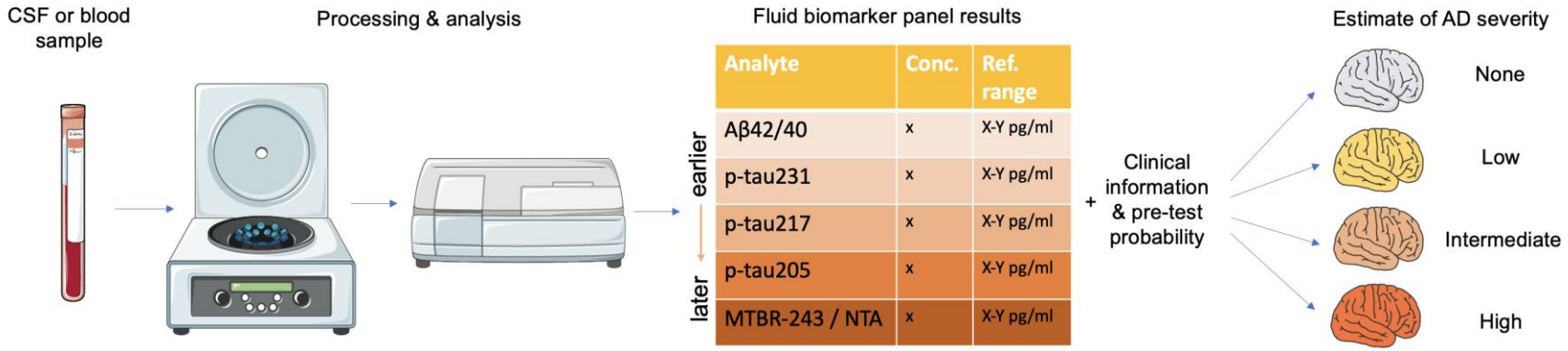
One of the primary difficulties in various medical fields is determining the severity of a disease to guide patient care and assess the effectiveness of treatment. Disease staging methods categorize a disease based on its increasing levels of severity, where the later stages indicate a worse outcome. These staging techniques identify specific milestones in the progression of a disease that are detectable, reflect current or future symptomatic severity, and ideally inform the selection of therapeutic interventions. The stages of a disease can be based on its clinical history, origin, anatomical distribution of pathology, or biological characteristics, and can be determined through physical examination, biomarker testing, or a combination of both. At present, biomarkers are employed to aid in the identification of Alzheimer's disease (AD), while the disease's severity is determined based on its clinical manifestation. People who do not have cognitive impairments (preclinical AD), as well as those who have mild cognitive impairment (MCI) or dementia, may exhibit positive amyloid-β and tau biomarkers.

Figure 1: *In vivo* clinical and biomarker changes in relation to AD pathological stage severity.



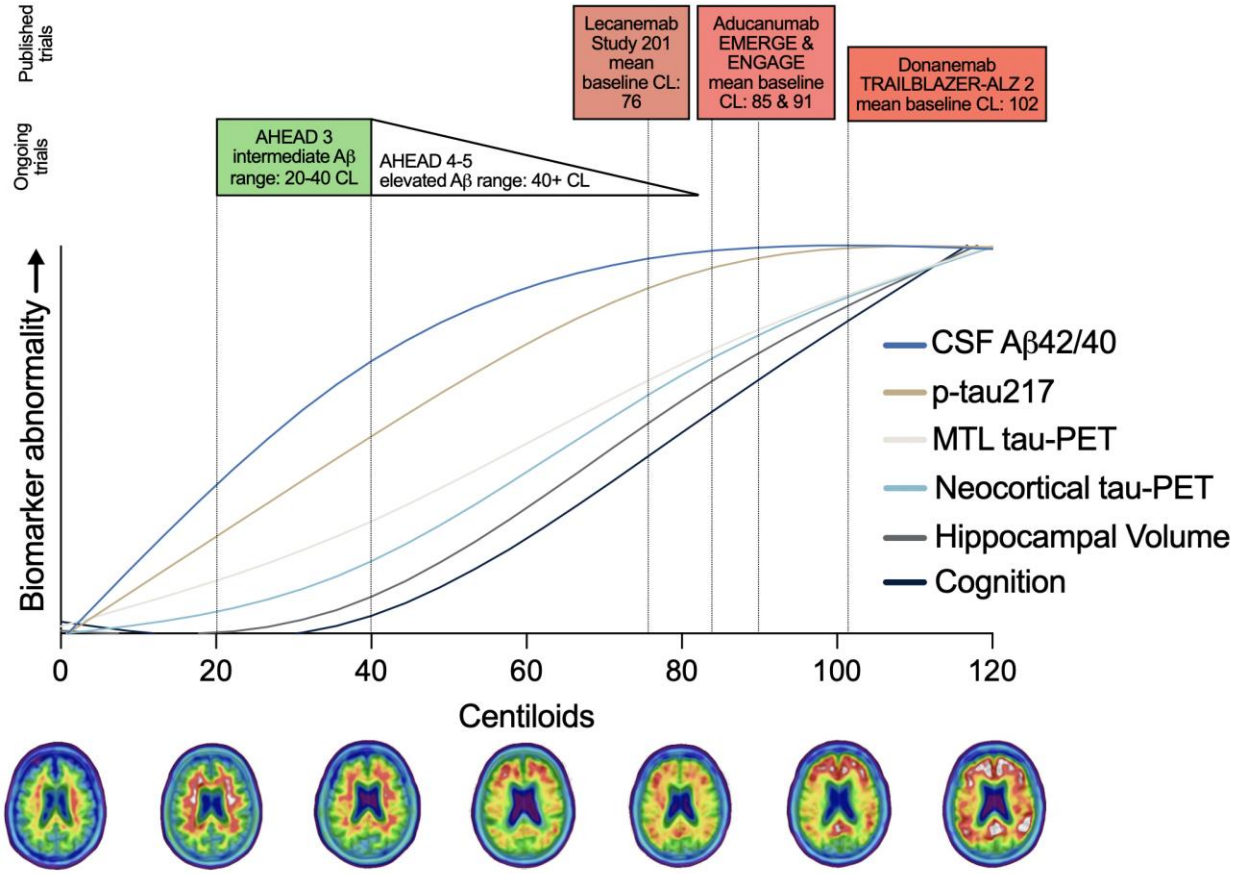
In vivo staging of disease provides a framework to model the natural history of AD. Using *in vivo* disease staging, the evolution of several biomarkers can be compared in relation to established neuropathological staging systems used in the diagnoses of AD at autopsy. Furthermore, as an alternative to biomarker dichotomization, disease staging provides the opportunity to understand the relationship between clinical changes and core pathological markers. Figure adapted from Therriault et al. Nature Aging, 2022.

Figure 2: Hypothetical fluid-based AD staging procedure.



In this hypothetical framework, several fluid biomarkers, or plasma biomarker "panels", could be used to stage the severity of AD pathological changes. It is hypothesized that different plasma biomarkers may provide complementary information about AD pathological changes, for example, biomarkers such as Aβ42/40 and p-tau231 can provide reliable information about amyloid-PET positivity, while novel biomarkers such as p-tau205, microtubule binding region tau 243 (MTBR-243) or N-Terminal Tau A (NTA) are more indicative of tau tangle pathology. Other high-performance biomarkers such as p-tau217 display very strong correlations with amyloid-β in early disease, as well as strong correlation with both amyloid-β and tau pathologies in symptomatic disease. Mass-spectrometry methods, which are able to quantify multiple analytes in a single run, may be better suited for this purpose. Alternative strategies include taking into account the magnitude of fluid biomarker abnormality to stage disease. Crucially, many of the analytes described here are also available in blood, suggesting the potential for plasma-based disease staging.

Figure 3: Biomarker-based staging informs the design and expectations of disease modification for clinical trials



Clinical and biomarker changes in relation to amyloid-PET abnormality. Using PET-based disease staging, it is possible to make inferences regarding the magnitude of pathological changes that have already taken place in individuals with asymptomatic symptomatic AD (biomarker curves are taken from the TRIAD cohort). For example, the mean Centiloid (CL) value of published anti-amyloid trials suggests that substantial tau accumulation and neurodegeneration have already taken place by the time of enrollment. Hence, their potential to modify the course of disease may be modest at these advanced stages. Newer trials targeting earlier AD in asymptomatic individuals may be more successful in this regard.

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