Blood phospho-tau in Alzheimer disease: analysis, interpretation, and clinical utility

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Abstract

Well-authenticated biomarkers can provide critical insights into the biological basis of Alzheimer disease (AD) to enable timely and accurate diagnosis, estimate future burden, and support therapeutic trials. Current cerebrospinal fluid and molecular neuroimaging biomarkers fulfill these criteria but lack the scalability and simplicity necessary or widespread application. Blood biomarkers of adequate effectiveness have the potential to act as first-line diagnostic and prognostic tools, and offer the possibility of extensive population screening and use that is not limited to specialized centres Accelerated progress in our understanding of the biochemistry of brain-derived tau protein and advances in ultrasensitive technologies have allowed for the development of AD-specific phosphorylated tau (p-tau) biomarkers in blood. In this Review we discuss how new information on the molecular processing of brain p-tau and secretion of specific fragments into biofluids is informing blood biomarker development, enabling the evaluation of preanalytical factors that affect quantification, and informing harmonized protocols for blood handling. We also review the performance of blood p-tau biomarkers in the context of AD and discuss their potential contexts of use for clinical and research purposes. Finally, we highlight outstanding ethical, clinical and analytical challenges, and outline the steps that need to be taken to standardize inter-laboratory and inter-assay measurements.

[H1] Introduction

In 1906, Alois Alzheimer described a form of early-onset dementia involving “a peculiar severe disease process of the cerebral cortex”, a disease that would later be named after him[1]. Over a century later, Alzheimer disease (AD) is the leading cause of late-onset dementia, directly affecting

over 50 million people and presenting with huge health, social and economic costs, which are estimated to increase exponentially in the coming decades[2–5]. AD is mostly diagnosed on the basis of syndromic changes and demographic features that seem to reflect AD-type dementia[6]. Despite vast clinical competencies, the slow disease course and overlap of symptoms with those of other dementias complicate accurate diagnosis of AD on the basis of clinical presentation alone[7,8]. In fact, up to a third of individuals diagnosed with AD exclusively on the basis of clinical phenotypes do not have AD neuropathological changes at post-mortem[7]. This suggests that selecting individuals for, and monitoring the outcomes of, therapeutic trials using clinical diagnosis alone will be less accurate than approaches that incorporate biological evidence, as recently proposed in the NIA-AA biological definition of AD[9]. This observation necessitated a search for biological markers of AD[10–12].

Definitive diagnosis of AD is established by autopsy confirmation of two major pathological hallmarks: extracellular amyloid plaques consisting of aggregated amyloid-β (Aβ) peptides, and intracellular neurofibrillary tangles containing abnormally phosphorylated tau[13] (Supplementary Table 1). Cerebrospinal fluid (CSF) and neuroimaging biomarkers that show associations with neuropathological evidence, and have the specificity and sensitivity to enable diagnosis during life, have been developed[14–19] (Supplementary Table 1). In living individuals, PET imaging of the intensity and distribution of Aβ plaques and tau neurofibrillary tangles and structural imaging of brain atrophy by magnetic resonance imaging (MRI) [14], and/or the evaluation of alterations in CSF levels of $A\beta_{1–42}$ (or $A\beta_{1–42}$:$A\beta_{1–40}$ ratio), phosphorylated tau (p-tau), and total-tau (or neurofilament light, NfL) can accurately detect AD-related pathophysiological changes[12,15,19]. These established biomarkers are in agreement with autopsy findings[16,20,21], and are thus included in research and clinical criteria for the definition and staging of AD[9,22,23]. According to one of the leading research frameworks — the AT(N*)* system — AD is biologically defined by biomarker evidence (either CSF or PET) of Aβ or tau pathophysiology, regardless of the accompanying clinical syndrome[9]. In this framework, developed by the National Insitute on Aging and the Alzheimer's Association (NIA-AA) in the USA, neurodegeneration (N) is defined by MRI-based identification of hippocampal atrophy or glucose

hypometabolism (fluorodeoxyglucose-PET), elevation of CSF total-tau or elevation of CSF NfL[9]. For a comparison of CSF and PET biomarker approaches, see Box 1.

Despite their proven diagnostic utility, the established AD biomarkers have several drawbacks that limit their widespread use[10,12,24]. For example, invasiveness and mild adverse reactions associated with lumbar puncture (CSF) and radiation exposure (PET) limit cohort enrolment and retention, especially in minority groups[25]. PET imaging also requires specialized facilities, necessitating substantial financial investment and limiting accessibility even in high-income countries[14]. At present, access to biomarker-based assessments is limited in non-specialist clinical settings such as primary care facilities, as well as in population and epidemiological studies. Lack of access to clinicobiological care is a major disadvantage for small communities in high-income countries and nearly all low-income and middle-income countries, where dementia incidence continues to increase rapidly[3,26,27]. With disease-modifying treatments becoming available[28], the need for more accessible and cost-effective biomarkers cannot be overstated.

The last decade has seen accelerated progress in the discovery and development of blood-based biomarkers for AD, with the aim of improving access to, and convenience and simplicity of biomarker-guided assessments[12,24,29]. The key benefits of using blood-based biomarkers of AD are discussed in Box 2. Initially, research into AD blood-based biomarkers focused mainly on markers of Aβ pathophysiology and neurodegeneration (Supplementary Table 1). However, a biomarker that can provide specific evidence of AD remained a high priority. In studies of CSF, increased tau phosphorylation was the most AD-specific AT(N) biomarker[30], inspiring attempts to develop blood-based p-tau biomarkers. However, the development of blood alternatives to CSF p-tau was originally impeded by analytical challenges[10–12]. More recently, an improved understanding of the biochemical processing of brain-derived tau, the availability of superior analytical technologies with improved sensitivity, and large well-defined cohorts with accurate molecular imaging of Aβ and tau have aided the discovery and development of p-tau biomarkers for clinical chemistry applications[29].

In this Review, we discuss the neurochemistry of tau forms in the brain and biofluids and how this informs biomarker development. Next, we critically review the pre-analytical factors that affect p-tau measurement in the blood as well as summarizing the diagnostic accuracy of different p-tau biomarkers in the AD continuum and the associations of these markers with in vivo measures of Aβ, tau and neurodegeneration. Furthermore, we discuss clinical and research contexts where blood p-tau could be used in place of CSF and PET alternatives. Finally, we examine outstanding, technical, clinical and ethical questions that should be answered to enable fuller clinical implementation.

[H1] Tau structure and phosphorylation

Tau protein is encoded by the *MAPT* gene and has six isoforms (ranging from 352–441 amino acids in length) in the adult human brain (a longer isoform is predominant in peripheral tissues)[31,32]. The tau protein sequence is divided into an N-terminal region, a mid-region, a microtubule-binding region, and a C-terminus (Fig. 1a). Owing to alternative splicing, the N-terminal region has zero, one or two inserts between amino acids 45 and 103 (generating 0N, 1N or 2N tau, respectively). The microtubule-binding region consists of four pseudo-repeat domains (R1–R4; Fig. 1). The mid-region has several threonine and serine residues, the phosphorylation of which by specific kinases has both physiological and pathophysiological implications. Although phosphorylated tau has known physiological functions, including maintenance of microtubule assembly and stability[33], phosphorylation beyond specific thresholds has pathophysiological consequences[34,35]. During AD, some fractions **[G]** of phosphorylated tau pool in the brain and progressively aggregate into insoluble filamentous deposits detectable in neuropathology and PET investigations[36,37]. Concurrently, some soluble p-tau fractions are increasingly secreted into CSF, where they can be detected and quantified to provide indirect evidence of disease state[19,37,38]. Pathological tau can be phosphorylated at multiple epitopes, including amino acids 181,199, 202, 205, 217, 231, 235, and 396[37,39–41]. In this Review, p-tauX refers to tau phosphorylated at amino acid X. Biomarker methods targeting these and other p-tau epitopes have been reported and are discussed in more detail below[37,42–49].

[H1] First-generation blood biomarkers

The use of blood p-tau as a biomarker was preceded by the development of blood-based markers of amyloidosis and neurodegeneration (thus addressing two of three AT(N) requirements). Some of these first-generation blood biomarkers are available for clinical use, for example, plasma NfL is approved for use in parts of Europe and immunoprecipitation-mass spectrometry (IP-MS) measurement of plasma Aβ is approved for in vitro diagnostic use in Europe and the US[10]. However, plasma Aβ and NfL have limitations that restrict their use, especially as standalone biomarkers for AD[12] (Boxes 3 and 4, and Supplementary Table 1).

[H2] Total-tau

CSF levels of total-tau reliably reflect neurodegeneration in AD; however, plasma levels of this marker have shown large inter-group overlaps that limit its diagnostic usefulness[50–54]. Nevertheless, evidence indicates that plasma total-tau has prognostic use: high baseline levels predict faster cognitive decline and neurodegeneration[51,52,55–57]. These associations are most obvious for participants with the highest plasma total-tau concentrations (for example, in the 90th percentile) [51,52,55–57]. Poor correlation between blood and CSF levels of total-tau suggests that the majority of total-tau in blood comes from peripheral sources and that the central nervous system (CNS)-derived fraction — an estimated 20% of plasma tau[58] — is too small to enable the detection of ongoing neurodegeneration[50,58,59]. Consequently, blood total-tau becomes diagnostically meaningful only in disorders with pronounced increases in CNS tau production and/or release into biofluids, for example, brain injury, traumatic brain injury, acute stroke, Creutzfeldt–Jakob disease, and some individuals with AD[52,60–64]. Alternative plasma total-tau assays have been developed, one detects the N-terminal-to-mid-region epitope 6–198 (NT1 tau)[65]. This assay is a good prognostic marker in cognitively unimpaired individuals and evidence indicates that it is more AD-specific than plasma NfL or a commercial total-tau method[66,67]. More recently, a new total-tau assay (referred to as NTA tau) targeting the N-terminus region of tau showed improved accuracy to detect Aβ abnormalities earlier in the AD continuum than established CSF total-tau assays, and demonstrated preliminary utility for use in plasma[68]. However, large-scale validation of its performance in blood is yet to be

reported[68]. We conclude that plasma total-tau assays are prognostic markers of incident AD but currently lack diagnostic robustness. The future development of total-tau assays that specifically target CNS-derived tau in blood might address these shortcomings. In this direction, unpublished results show that a novel assay targeting brain-derived tau (i.e, tau isoforms originating from the CNS) in blood provides a more accurate measure of neurodegeneration that is highly specific to autopsy-confirmed AD compared with non-AD tauopathies (Karikari, Ashton, Blennow, Zetterberg, personal communication).

[H1] Phosphorylated tau as a biomarker

[H2] P-tau in the brain, CSF and blood

The brains of individuals with AD contain abnormally phosphorylated tau species spanning the complete protein sequence, including truncated forms[39,40] (Fig. 1a). Truncated tau is generated by proteolysis during pathophysiological processing[69–72], which enhances aggregation partly by liberating the aggregation-prone regions that polymerize into insoluble neurofibrillary tangles[71,72]. The abundance and distribution of neurofibrillary tangles can be characterized in vivo with tau PET and at post-mortem with immunohistochemistry[18,36]. Conversely, the brain-derived phosphorylated tau fragments that do not aggregate into fibrils make up the soluble fraction[73], portions of which are released into CSF and subsequently enter the blood[74,75]. This process seems to be specifically induced by Aβ pathology and thus absent in individuals with Aβ-negative non-AD tauopathies[75]. Therefore, CSF p-tau is an indirect marker of AD-type brain tau pathology that increases with disease progression and associates with incremental neurofibrillary tangle formation[17,75,76].

Until recently, CSF tau was thought to consist almost exclusively of mid-region forms, owing to pioneering work that showed the biomarker relevance of p-tau181 and total-tau using immunoassays that target defined mid-region epitopes[77]. These assays have now been translated into commercial products for clinical use[78–80]. Therefore, established CSF p-tau assays measure phosphorylated forms of CNS-derived tau released from the soluble, non-aggregated fraction and containing mid-region parts of the protein (Fig. 1a, b).

However, we now know that tau forms in CSF are not limited to mid-region entities, but contain measureable quantities of both N-terminal and mid-region tau (Fig. 1a,b)[41,47,48,65,68,75,81,82]. Tau fragments from the C-terminal end are less abundant in CSF, owing to their retention in the brain as integral components of neurofibrillary tangles[70,75,83] (Fig. 1a). Indeed, C-terminal fragments (for example, amino acids 306–378) make up fundamental prefibrillar structures that polymerize into neurofibrillary tangles in AD[84]. Furthermore, hexapeptide motifs in the microtubule-binding region are required for tau aggregation and trans-cellular transmission[85–89]. Major truncations of brain tau at amino acids 421[83], 391[83] and 368[71] should reduce amount of mid-region-bearing C-terminal tau fragments released into CSF. Indeed, only a few studies using highly sensitive and targeted approaches have quantified C-terminal tau in CSF[90,91]. CSF levels of a tau species ending at amino acid position 368 (tau368) in a ratio with total-tau (tau368/total-tau ratio) correlates tightly with tau PET measures of tau pathology[91].

Similar to the truncations that limit C-terminal tau release into CSF, truncation at amino acid 224[82] might further reduce the pool released into blood from CSF, resulting in predominantly N-terminal species in blood (Fig. 1). Indeed, compared with cognitively healthy controls, significantly lower amounts of the tryptic tau peptide containing the amino acids 226–230 are observed in the soluble brain fraction of individuals with AD, suggesting the potential involvement of this portion of tau in neurofibrillary tangle formation[90]. In one study, soluble tau aggregates were isolated from the brains of individuals with AD and injected into the brains of tau transgenic mice[92]. Tau phosphorylated at threonine-181, threonine-217 or threonine-231 had a lower capacity for initiating tau aggregation in the mice than aggregates containing more C-terminal p-tau forms (for example, p-tau262, 396 and 404). This observation suggests that tau forms phosphorylated on the N-terminal-to-mid-region epitopes are more abundant in the soluble, non-aggregating pool secreted into CSF and blood compared with C-terminal tau forms that tend to be more aggregation-prone. Consequently, successful blood p-tau assay development efforts have focused on N-terminal tau (Fig. 1b).

[H2] Blood p-tau biomarkers

Conventional immunoassays lack the sensitivity to detect the minute amounts of p-tau that are present in blood. The first blood p-tau method described was a p-tau231 assay for use in individuals with traumatic brain injury[63,93]. For AD, early methods used surface plasmon resonance[94] and immuno-magnetic reduction[95] to detect p-tau181. These assays exclusively target the threonine-181 phosphorylation site, in contrast to subsequent methods that followed traditional immunoassay principles and paired phosphorylation-specific antibodies with selected non-phosphorylation-specific antibodies (Fig. 1b). The next blood p-tau assay was developed by substituting the detection antibody in the Single molecule array (Simoa) **[G]** total-tau kit for a p-tau181-specific monoclonal antibody, resulting in an N-terminal-directed p-tau181 biomarker (Fig. 1b)[96]. Despite detecting higher p-tau levels in participants with AD and Down syndrome than control participants, this assay could not consistently measure p-tau in control samples owing to suboptimal analytical sensitivity[96].

Mielke *et al.*[97] and Karikari *et al.*[48] later reported novel plasma p-tau methods using Meso Scale Discovery (MSD; developed at Eli Lilly) and Simoa (developed at the University of Gothenburg, Sweden) technologies, respectively (Fig. 1b). The MSD assay uses antibody pairs directed at tau fragments phosphorylated at threonine-181 and concurrently bearing the N-terminal amino acids 111–130[38], whereas the Simoa approach specifically targets tau forms phosphorylated at threonine-181 and containing the N-terminal epitopes 6–18[48]. Both methods have been validated in subsequent studies[43,47,54,98–120] and the Gothenburg p-tau181 method has now been developed into a commercial Simoa product available from Quanterix (Fig. 1b). The N-terminus-directed approach was also used to develop assays for plasma and CSF p-tau217[42,49,121–123] and plasma p-tau231[45,47,124] (Fig. 1b).

Mass spectrometry enables the simulatenous detection of phosphorylation at multiple epitopes. Scientists at Washington University and the University of Gothenburg have developed multiplexed methods that simultaneously detect p-tau181, p-tau217 and p-tau202[58]. The Gothenburg method, which was presented at the Alzheimer's and Parkinson's Diseases Conference in 2021 but has not yet been formally published, also detects p-tau199, p-tau205 and p-tau231[125]. These methods use

IP-MS approaches to first enrich plasma tau by immunoprecipitating with antibodies directed at pre-defined epitopes. Each method then targets pre-specified peptides containing the given phosphorylation sites[58,125]. This successful development of plasma p-tau231 detection methods might suggest that cleavage at amino acid 224 is not an early event in AD pathogenesis, at least not in the preclinical phases where plasma p-tau231 performs best.

As p-tau biomarkers from multiple sources continue to become available, we recommend a common nomenclature of p-tauX followed by the innovating institution or company for example, p-tau231 Gothenburg, p-tau217 Lilly. When comparing biomarkers for the same p-tau epitope, descriptors like N-terminal and mid-region can be added[43].

[H2] Matrix type and pre-analytical effects

As with other blood-based biomarkers, repeatability of p-tau measurements is influenced by pre-analytical factors (Supplementary Table 2). Blood p-tau concentrations measured in ethylenediaminetetraacetic acid (EDTA)-plasma **[G]**, the default matrix, differ from other matrices for paired samples from the same blood draw. For example, compared with EDTA-plasma, absolute p-tau181 concentrations were reduced by half in serum and by a third in citrate-plasma **[G]**, but were increased by ~82% in heparin-plasma **[G]**[48,126]. Nevertheless, strong correlations in p-tau181 concentration **[Au:OK?]** were recorded between matrices[48,126–128]. Absolute p-tau181 concentrations decreased marginally with increasing freeze–thaw cycles in both EDTA-plasma and serum; this decrease was statistically singificant only for EDTA-plasma after freeze–thaw cycle four[126].

Despite the strong correlations in measured p-tau concentrations across matrices, the substantial variations in absolute concentrations necessitate the use of one specific matrix in a study or clinical routine, and matrix-specific cut-offs might become necessary[29,48,126,128]. Moreover, repeated freezeing and thawing of samples should be avoided or kept to a maximum of three cycles.

Recommended blood processing guidelines are summarized in Supplementary Table 3. For recommended interpretation of p-tau data, see Supplementary Box 1.

[H1] Clinical performance of blood p-tau

[H2] Preclinical AD

The preclinical phase of AD is referred to as an early stage of the disease in individuals without overt symptoms but who are positive for CSF or PET biomarkers; these individuals are often Aβ-positive but tau-negative[9,129]. Conversely, prodromal AD refers to those with MCI[9]. Among cognitively unimpaired older adults, plasma p-tau181 was marginally higher in Aβ-positive **[G]** individuals than in Aβ-negative **[G]** individuals — some studies reported significant differences between these two groups[43,48,112] but other studies did not[97]. Plasma p-tau181 accurately discriminated individuals with preclinical AD from cognitively healthy Aβ-negative older individuals [43,48,49,97,98,130] (area under the curve (AUC) was higher when classifying by Aβ-PET than by CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio[43]) and from cognitively healthy Aβ-negative young individuals[48]. These results were similar to those of predictive models that incorporate data on Aβ-PET, age, sex and *APOE* ε4 carriership[130] but were significantly better than prediction on the basis of age, *APOE* ε4 status, hippocampal volume, plasma total-tau, Simoa $A\beta_{1–42}$, $A\beta_{1–42}$:$A\beta_{1–40}$ ratio, total-tau:$A\beta_{1–42}$ ratio or NfL alone, or age and *APOE* ε4 status combined[48,97,101]. In the multicentre Alzheimer's Disease Neuroimaging (ADNI) cohort, plasma p-tau181 distinguished participants with preclinical AD from cognitively unimpaired Aβ-negative participants and all Aβ-negative participants (including those with non-AD dementia)[101]. The results of another study suggested that plasma p-tau181 is a less accurate marker of preclinical AD than plasma Aβ measured with IP-MS[130]. In the BioFINDER and TRIAD cohorts, plasma p-tau217 and p-tau231 each differentiated participants with preclinical AD from Aβ-negative cognitively healthy participants better than p-tau181[47,49], but a head-to-head study of all three p-tau forms reported equivalent performances[112]. Evidence indicates that plasma p-tau231 concentration begins to increase before the threshold for Aβ-PET positivity is reached, thus individuals classified as Aβ-negative by PET but with a reduced CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio[47] might already have increased plasma

p-tau231 concentration. Therefore, plasma p-tau231 might be most useful when used as a continuous biomarker instead of a dichotomous one, particularly in preclinical AD.

One study reported that, compared with non-carrier control participants, participants with asymptomatic familial AD have higher plasma p-tau181 concentrations starting ~16 years before symptom onset [100]. Similarly, a study in a Columbian autosomal-dominant AD kindred reported that, compared with non-carriers, plasma p-tau217 concentration increased in mutation carriers starting ~20 years before onset of mild cognitive impairment (MCI; excluding individuals with dementia)[49]

[H2] Prodromal AD

Plasma p-tau181 was higher in participants with Aβ-positive MCI than in participants with Aβ-negative MCI, outperforming plasma NfL and hippocampal volume for the differentiation between these two groups[48,101,112]. Plasma p-tau181 also differentiated participants with Aβ-positive MCI from each of Aβ-negative MCI, Aβ-negative cognitively unimpaired older participants, and Aβ-negative cognitively unimpaired young adults[48]. This finding suggests that plasma p-tau181 can distinguish Aβ-positive MCI from Aβ-negative individuals who are clinically at the MCI stage or have no evidence of cognitive impairment. Plasma p-tau231 and plasma p-tau217 distinguished Aβ-negative participants with MCI from Aβ-positive cognitively-unimpaired participants more accurately than p-tau181[47,49]. All three p-tau variants were higher in individuals with Aβ-positive MCI than in individuals with Aβ-negative MCI[112]. In individuals with Down syndrome, plasma p-tau181 concentration discriminated participants with prodromal AD from asymptomatic participants[116]

[H2] AD dementia

Evidence from multiple studies indicates that blood levels of p-tau biomarkers increase as individuals progress along the AD continuum, peaking at the dementia stage[48,49,54,98–101,103–107]. Plasma p-tau181 accurately differentiated participants with Aβ-positive AD dementia from Aβ-positive and Aβ-negative cognitively unimpaired participants, Aβ-negative participants with MCI, and

Aβ-positive participants with MCI[48,49,54,98–101,103–107]. Equivalent performances were recorded using serum p-tau181[48,103]. In the ADNI cohort, the diagnostic performance of plasma p-tau181 was similar to to CSF p-tau181 but better than plasma NfL[101]. In the BioFINDER-2 cohort (763 participants), plasma p-tau181 concentration separated Aβ-positive participants with AD dementia from Aβ-negative cognitively-unimpaired participants and Aβ-negative participants with MCI[48]. However, in a subsequent study in the same BioFINDER-2 cohort (699 participants), plasma p-tau217 outperformed p-tau181 for the differentation of Aβ-positive participants with AD from Aβ-negative cognitively unimpaired participants and Aβ-negative particiapants with MCI[49]. In a population-based multi-ethnic study, plasma p-tau217 and p-tau181 had equivalent capacities for the differentation of participants with AD dementia from control participants. Plasma p-tau231 distinguished individuals with Aβ-positive AD dementia from Aβ-negative cognitively unimpaired individuals with high accuracy[47].

Although the NIA-AA research framework requires Aβ-positivity for a diagnosis of AD, some individuals with a clinical diagnosis with AD dementia might lack Aβ pathology[9]. In the ADNI cohort, plasma p-tau181 concentration differentiated between Aβ-positive and Aβ-negative individuals diagnosed with AD[101]. However, classifying individuals as Aβ-positive or Aβ-negative remains complicated owing to discordance between CSF and PET measures of Aβ pathology; some CSF-Aβ-positive but Aβ-PET-negative individuals showed increased plasma p-tau181 compared with participants who were Aβ-negative in both CSF and PET, which suggests inherent biases in these methods of defining Aβ status[101].

Plasma p-tau181 concenrtation separated individuals with autopsy-verified AD dementia from Aβ-negative control participants with the same accuracy as CSF measures of p-tau181[131]. P-tau181 in antemortem plasma collected 8 years before death accurately separated participants with AD dementia from control participants[102]. Compared with plasma p-tau181, plasma p-tau217 associated better with post-mortem diagnosis[49], although this result was not replicated in another pathology-

verified cohort[106]. Plasma p-tau231 was as good as p-tau181 for the separation of individuals with autopsy-proven AD from control participants[47,117].

In familial AD, plasma p-tau181 and p-tau217 were higher in symptomatic mutation carriers than in cognitively healthy non-carriers[49,100]. Plasma p-tau181 accurately separated individuals with Down syndrome dementia from individuals with Down syndrome and no dementia[116] and age-matched control participants[96].

[H2] Differential diagnosis

A unifying characteristic of blood p-tau181, p-tau217 and p-tau231 measurements (except meaasurments taken using the immuno-magnetic reduction and surface plasmon resonance methods) is that each can be used to differentiate AD from non-AD tauopathies[47–49,98,99,102,103]. Antemortem plasma p-tau181 and p-tau231 concentration differentiated individuals with autopsy-verified AD from individuals without AD, and associated more strongly with postmortem diagnosis than clinical diagnosis[102,117]. Importantly, in individuals with AD, plasma p-tau181 and p-tau231 were increased to the same degree as it was in individuals with primary diagnosis of non-AD dementia but with mixed AD pathologies at autopsy (for example, accuracy for p-tau181 in mixed AD versus non-AD=90.1% and mixed AD versus controls=84.1%[102]), highlighting a specificity to AD pathophysiology[102,117]. Other studies have reported similarly accurate performances of plasma p-tau181 for the differential diagnosis of autopsy-confirmed AD versus related dementias not of the AD-type[98,99,106]. In addition, these studies further showed that the concentrations of plasma p-tau181 increase according to disease severity, and can distinguish between postmortem-verified AD from control individuals without evidence of neuropathology[98,99,102,106]. Plasma p-tau231 was highly accurate for the differentiation of individuals with AD from individuals with non-AD neurodegenerative disorders, but its performance was not statistically different from that of p-tau181[47]. Other direct comparisons of plasma p-tau217 and plasma p-tau181 have reported mixed results: p-tau217 performed better than p-tau181 in the BioFINDER-2 cohort[49] but subsequent independent studies reported no difference in the accuracies of the two markers[52,124,132].

[H2] Longitudinal progression

Plasma p-tau concentration increases with disease severity: baseline concentrations are higher in Aβ-positive individuals than Aβ-negative individuals at the same clinical stage, with concentrations further increasing at follow-up in Aβ-positive individuals [47–49,54,98,100–102,104,105,108,113,117,118,133–135]. In agreement with these observations, baseline and longitudinal measurements of plasma p-tau concentrations have shown associations with cognition, brain Aβ burden, brain tau burden and brain atrophy[47–49,54,98,100–102,104,105,108,113,118,133,134]. Furthermore, several studies have reported that individuals with high baseline concentrations of plasma p-tau have higher odds of cognitive deterioration and progression to AD dementia[48,54,98,101,113,118,133,134], and concomitantly abnormal plasma p-tau and plasma NfL levels confer poor prognostic outcomes[136].

The natural course of plasma p-tau181 and p-tau217 concentrations followed similar dynamics to CSF concentrations of p-tau. The earliest changes in plasma p-tau181 levels occurred before PET Aβ markers reached abnormal thresholds[114]. In healthy individuals, voxel-wise analysis of PET data found a weak but statistically significant association between the concentrations of plasma p-tau181 and p-tau217 versus Aβ-PET signal in regions known to accumulate amyloid early in the disease course [104,133]. The strongest associations between plasma p-tau concentration and Aβ-PET signal were observed in known 'late-accumulating' regions in individuals with MCI. [104,133]. Longitudinal changes in plasma p-tau181 concentration were quite small (and increased with diagnosis of AD) but matched longitudinal changes in CSF p-tau181 and showed statistically significant correlations with tau-PET uptake in temporoparietal regions assessed 6 years after blood samples were collected for p-tau measurements[101,104]. In another study, larger longitudinal increases were seen for p-tau217 at the group level compared with p-tau181 in previous studies[133]. Moreover, Aβ-PET-positive individuals showed more accelerated increases at follow-up than Aβ-PET-negative participants[133]. In both studies, baseline levels of plasma p-tau181 and p-tau217 were higher in participants with MCI who later progressed to AD dementia[104,133]. Baseline and longitudinal change of plasma p-tau181 were associated with grey matter volume loss in a cohort of individuals

diagnosed as cognitively unimpaired, MCI or AD dementia according to clinical presentation but without biomarker assessments[54].

In a neuropathology cohort, increases in plasma p-tau181 concentration were observed in individuals with AD compared with cognitively healthy individuals; these increases were most obvious 4–8 years before death and plateaued closer to post-mortem[102], a finding that has been corroborated by other studies[98,101,117]. Interestingly, in some non-AD pathologies, p-tau181 started to increase closer to death which might reflect late concomitant AD pathology[102]. In agreement with the observation that plasma p-tau levels decrease in advanced disease stages, the association between plasma p-tau181 concentration and tau-PET retention was stronger in individuals with MCI compared with those in the AD dementia stage, despite the latter group showing the highest level of tau-PET retention[48,97]. Additionally, individuals with pre-symptomatic and symptomatic familial AD had higher baseline levels of plasma p-tau181 than control participants; however, no statistically significant evidence of progressive increases in p-tau181 in the pre- symptomatic and symptomatic groups were found, suggesting stabilization in advanced disease[100]. This stabilization might be a result of to neuronal loss and/or damage.

Taken together, the findings discussed here suggest that blood p-tau biomarkers are a promising approach for the detection of AD, monitoring of progression and performing differential diagnosis, thus making them suitable for clinical diagnostic and prognostic use, and the evaluation of therapeutic candidates. Key publications are summarized in Supplementary Table 4.

[H1] Association with Aβ, tau & degeneration

[H2] P-tau and Aβ pathophysiology

In multiple cohorts, the association between plasma p-tau concentration and CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio or Aβ-PET retention was greater in Aβ-positive participants than Aβ-negative participants at the same clinical stage [48,49,54,98–101,103–107,110,114,134]. Plasma p-tau181 and p-tau231 concentrations

correlated with the degree of Aβ-PET signal uptake in the cortex, with the strongest associations observed in the precuneus, striatum and frontal cortex[47,48]. For p-tau181, baseline associations with Aβ-PET retention were stronger in individuals with MCI and AD dementia (widespread in cortical and sub-cortical regions) than in Aβ-negative control participants (limited to the precuneus, temporal and superior frontal areas but without subcortical involvement)[48,101,104]. It is important to note that Aβ-negative individuals can have sub-threshold levels of amyloid deposition in their brains[137]. Plasma p-tau231 concentration correlated with Aβ-PET uptake in cognitively unimpaired Aβ-positive individuals but, unlike with other p-tau epitopes, a correlation was also observed in cognitively unimpaired Aβ-negative individuals who had incipient Aβ-PET abnormalities[47]. This observation indicates that p-tau231 concentration is sensitive to subtle amyloid accumulation and begins to increase before the threshold of Aβ positivity is reached; this conclusion is also supported by the results of CSF studies[43,45]. Conversely, another study reported that correlations between plasma p-tau217 concentration and Aβ-PET uptake were only statistically significant in Aβ-positive individuals with AD[49], and in a further study, plasma p-tau217 enabled the discrimination of Aβ-positive, tau-negative cognitively unimpaired participants from Aβ-negative, tau-negative cognitively unimpaired participants[138]. In multiple studies, plasma p-tau181, p-tau181:$Aβ_{1–42}$ ratio **[Au:OK?]** and p-tau217 concentrations accurately predicted abnormal Aβ-PET scans[48,49,101,110,134]. The first studies to compare all three p-tau variants showed no statistically significant difference in their ability to predict Aβ-PET abnormality[112] and to detect biomarker-positive AD[124].

Plasma $Aβ_{1–42}$:$Aβ_{1–40}$ ratio measured with IP-MS is another high-performing blood biomarker for Aβ pathology[139,140]. However, in re-analysis of previously-published datasets this marker showed small changes (~10%) between PET Aβ-positive individuals and Aβ-negative individuals (compared with 37.7% for CSF $Aβ_{1–42}$:$Aβ_{1–40}$ ratio in the same set of participants who had paired CSF and plasma samples available) as also demonstrated before in several independent studies[139–142]. This small change in plasma $Aβ_{1–42}$:$Aβ_{1–40}$ ratios between Aβ-PET-positive and Aβ-PET-negative individuals (representing approximately a quarter of the fold change seen for CSF) leads to large overlaps that are susceptible to minor analytical variations, as demonstrated recently[141]. Conversely, the fold changes of CSF and plasma p-tau231 between PET Aβ-positive and Aβ-negative individuals were

more comparable (~166% for CSF p-tau231 versus ~85.6% for plasma p-tau231, meaning that the fold change in CSF is only reduced by half in plasma). This limits the susceptibility of plasma p-tau231 to small technical variations[141]. Box 4 illustrates robustness of $A\beta_{1–42}$:$A\beta_{1–40}$ ratio and p-tau measured in CSF versus plasma to predict Aβ-PET positivity.

[H2] P-tau and tangle pathology

In multiple studies, plasma p-tau concentration correlated with tau-PET burden across the AD continuum[47–49,98,99,104]. Yet, plasma p-tau181 and p-tau217 levels were higher in Aβ-positive, tau-negative individuals compared with Aβ-negative, tau-negative participants, suggesting that plasma p-tau changes ahead of tau-PET[48,138] and corroborating longitudinal evidence[104]. Compared with plasma p-tau181, plasma p-tau231 showed more consistent step-wise associations with tau-PET burden from Braak stages I–II through III–IV to V–VI[47]. The correlation between p-tau concentration and NFT burden (in vivo and neuropathological) tends to be less strong in AD dementia and Braak stage V–VI than at earlier stages of the disease[47,48,97,101,102]. Plasma p-tau181, p-tau217 and p-tau231 concentrations were associated with the extent of both amyloid and tau pathologies measured either by PET or at neuropathology[102,117,143], and was statistically found to mediate 75% of the relationship of Aβ with tau aggregates[138]. Associations between plasma levels of p-tau231, p-tau217 and p-tau181 tended to decline in late Braak stages[47–49,97,98,102]. Head-to-head comparisons found no differences among plasma p-tau231, p-tau217 and p-tau181 for prediction of tau-PET positivity[112]. Future studies of plasma p-tau202 and p-tau205 (key epitopes of interest in neuropathological diagnosis) will be important for tau pathology staging. Fig. 2 provides an example of plasma p-tau associations with Aβ-PET and tau-PET burden.

[H2] P-tau and neurodegeneration

Longitudinal change in plasma p-tau181 concentration was associated with progressive glucose hypometabolism and grey matter loss in characteristic AD-affected temporal regions[54,105]. These associations were observed only in Aβ-positive participants, whereas plasma NfL concentration was associated with these signs of neurodegeneration independent of Aβ status[105]. Plasma p-tau181 concentration negatively correlated with grey matter volume in cognitively unimpaired participants at baseline and 36 months later[109]. In cognitively impaired participants, plasma p-tau181 cocnentration negatively correlated with grey and white matter volume at baseline and at follow-up 12–48 months later[109]. Longitudinal change in plasma p-tau181 concentration (but not plasma levels of NfL, glial fibrillary acidic protein, total-tau, $A\beta_{1–42}$ or $A\beta_{1–42}$:$A\beta_{1–40}$ ratio) was associated with change in grey matter volume both in people with normal and impaired cognition including AD dementia[54]. Plasma p-tau231 concentration, p-tau181 concentration, p-tau181:total-tau ratio and p-tau181:$A\beta_{1–42}$ ratio were associated with baseline and 1-year change in hippocampal atrophy but not with cerebrovascular disease[47,48,110]. Longitudinal change in plasma p-tau217 correlated with progressive atrophy of the hippocampus and temporal cortex in cognitively healthy controls, individuals with preclinical AD and individuals with MCI (but not in a group of only Aβ-positive individuals with MCI)[133].

Taken together, these findings indicate that, despite not being able to provide structural information, blood p-tau levels associate well with and predict brain Aβ, tau and neurodegenerative profiles. Therefore, these accessible biomarkers seem to reflect AD pathological changes in the brain.

[H1] Head-to-head comparison of different p-tau forms

In several studies, the new CSF p-tau biomarkers — p-tau217, p-tau231 and N-terminal-directed p-tau181 — became abnormal earlier in the AD continuum than the established mid-region-targeting p-tau diagnostics[37,42,43,45,144]. These observations suggest that N-terminal fragments of cleaved p-tau forms are released into biofluids presumably as an early response to emerging Aβ abnormalities.

As the disease progresses, the other p-tau forms become available in CSF, leading to identical accuracies as the N-terminal p-tau forms to differentiate between AD and non-AD dementias[42,45]. CSF p-tau217 concentration correlated better with Braak-staged tau-PET burden than did CSF p-tau181 concentration (both Eli Lilly assays), although it is unclear how these markers compare with the mid-region tau-targeting p-tau181 assays currently used clinically as AD diagnostic tests [121]. Conversely, antemortem CSF p-tau231 concentration was a better predictor of mixed AD pathology in definite Creutzfeldt–Jakob disease than CSF p-tau217 or p-tau181[145]. Similar results have been reported in blood — p-tau231 and, to a lesser extent, p-tau217 seem to be more accurate markers of preclinical AD than p-tau181 but perform similarly to p-tau181 in the detection of MCI and AD dementia, and for the differentiation of AD from non-AD neurodegenerative disease[47,49], although cross-cohort replication of these findings is needed. Indeed, the results of studies published in the last 5 years show that blood p-tau181, p-tau217 and p-tau231 perform equally well in the detection detect of Aβ and tau pathology as assessed by PET[52], and that p-tau181 and p-tau217 both have a high accuracy for AD dementia prediction[146] and differential diagnosis of tauopathies[132].

Taken together, the findings discussed here suggest that plasma p-tau231, p-tau217 and p-tau181 could be used interchangeably for clinical purposes; a conclusion that is supported by multi-marker prediction models developed in the Swedish BioFINDER cohort[146]. Indeed, head-to-head comparison showed no difference between the different p-tau forms in their ability to predict Aβ-PET and tau-PET outcome[112]. As expected, a high degree of correlation was observed between levels of the p-tau variants in CSF and in plasma[42,43,47,121]. Biochemically, as tau is phosphorylated, often unselectively, at threonine-231, threonine-217 and threonine-181 by the same kinases[147,148], we find it unlikely that the differences in p-tau biomarker performances could be influenced by the kind of kinases that phosphorylate tau at the indicated sites. Perhaps targeting the N-terminal sites and/or fragments with different assays, instead of targeting the phosphorylation epitopes alone, would provide deeper insights into p-tau time course in AD.

In CSF, it seems that N-terminal-directed p-tau biomarkers might be more suitable for detection of pre-dementia AD than current markers that target mid-region tau. In blood, plasma p-tau231, p-tau217 and p-tau181 – each measured on N-terminal tau forms – had similar diagnostic performances and capacities to predict brain Aβ and tau, suggest interchangeability for clinical purposes. An apparent exception is for plasma p-tau231, for which the Gothenburg method of detection seems superior to a new assay from ADx Neurosciences/Amsterdam University[124].

[H1] Future prospects

[H2] Primary care screening and population studies

Dementia rates continue to increase worldwide, and primary care centres remain the first point of call for many patients[2]. The importance of early diagnosis of AD, before the dementia stage is reached, is increasingly being recognised, especially considering the recent FDA approval of the amyloid-targeting drug aducanumab. Therefore, primary care physicians have an important role in the efficient identification of individuals at high risk of AD. In individuals not evaluated for CSF or PET biomarkers, plasma p-tau181 and p-tau231 concentrations were higher in those with a preliminary diagnosis of MCI or AD than in young and older control participants over 60 years old[47,48]. In cohorts classified exclusively on the basis of clinical diagnosis, plasma p-tau181 and p-tau217 concentrations detected current and future AD dementia[54,106], with performances similar to CSF or PET findings. Moreover, plasma p-tau181 concentration was able to distinguish between clinically-defined AD dementia with and without Aβ pathology[101].

Given these results, we suggest that individuals presenting to primary care physicians with cognitive concerns should be first examined according to standard clinical procedures, starting with a comprehensive evaluation of the patient's demographics, medical history, present comorbidities, duration of cognitive symptoms, and basic neurological examination. If the suspicion of a neurodegenerative disease persists, cognitive testing can be performed. The clinician can subsequently make a request for blood biomarker testing (Fig. 3). Elevated levels of plasma p-tau

would suggest that AD pathology is responsible for the observered cognitive impairment, whereas normal plasma p-tau levels would indicate non-AD causes. If p-tau is normal, increased blood NfL concentration would suggest the presence of non-AD neurodegeneration. However, normal blood concentration of NfL would indicate cognitive impairment owing to non-neurodegenerative causes. Algorithms or models incorporating these markers could be applied, where individuals determined as low-risk (clearly normal biomarker concentrations) are considered not to have suspected AD, but medium-risk (gray zone of positivity) and high-risk (clearly increased) individuals are referred to specialist care (Fig. 3). This specialist care might involve more advanced examinations such as CSF and/or PET analyses. We must stress that blood biomarkers might help the clinician in decision-making but should in no case substitute a proper neurological assessment.

Although a straightforward approach to blood biomarker use in primary care is to suggest potential causes of suspected cognitive decline, screening as part of routine clinical assessment of older adults (as is performed for diabetes and common cancers) would enable early identification and management of individuals who are asymptomatic but have hallmarks of preclinical disease. This early identification could be used to identify individuals for inclusion in population studies and therapeutic trials, with the aim of estimating disease prevalence and better-understanding longitudinal trajectories[24].

[H2] Confirmatory diagnosis in specialist care

Whether referred from primary-care or directly seeking specialist care, patients are expected to be more receptive to blood collection than to lumbar puncture or PET imaging. Together with standard (as described above) and neurology-focused (for example, detailed cognitive testing) clinical assessments, the assessment of blood p-tau biomarkers might help confirm the presence or absence of AD pathology. As discussed above, blood concentration of p-tau is highly increased in individuals with AD dementia compared with Aβ-negative control participants; this increase is of the same magnitude as the increases observed in CSF p-tau concentration[49,101]. Blood p-tau and CSF p-tau concentrations show equivalent accuracies for the differential diagnosis of AD and for the

prediction of longitudinal progression[101,146]. Therefore, blood p-tau could replace CSF p-tau for as a biomarker for definitive and differential diagnosis of AD. We expect that first-line application of blood-biomarkers would help in the diagnosis of a substantial number of individuals. However, real-world challenges might arise when dealing with individuals defined as having a medium or high risk of AD on the basis of blood p-tau results, but whose plasma $A\beta_{1–42}$:$A\beta_{1–40}$ ratio (if measured) are at sub-threshold or borderline levels. This situation might be resolved by then triaging patients with CSF or PET analysis to confirm AT(N) status (Fig. 3). Moreover, in situations where individuals have biomarker evidence of AD but also show clinical signs of other neurodegenerative pathologies, MRI might also be useful, in addition to CSF or PET biomarker identification of AD, for the verification of neurodegeneration and the identification of conditions such as vascular disease and normal pressure hydrocephalus. In the future, blood biomarker panels that integrate p-tau with markers for the full spectrum of AD and non-AD conditions will be important for differential diagnosis and identification of concomitant pathologies.

[H2] Clinical trial recruitment and outcome evaluation

The ability of plasma p-tau measurements to identify Aβ pathophysiology in individuals with symptomatic AD demonstrates the potential importance of this marker in identifying and recruiting Aβ-positive symptomatic participants for clinical trials. However, we expect that blood p-tau will also be important for the recruitment of asymptomatic Aβ-positive cohorts, including participants with presymptomatic familial AD[48,49,98,100,101]. The results of simulation studies using data from the ADNI cohort indicate that pre-screening of asymptomatic participants to select plasma p-tau181-positive individauls prior to screening with Aβ-PET results in a cost-saving of approximately 60% compared with Aβ-PET-only screening, in addition to savings in time, cost and logistics[101] (Fig. 4). Promising markers of preclinical AD (for example, plasma p-tau231 and p-tau217) are likely to be most useful for this purpose. Equivalent approaches with any of the p-tau markers (plasma p-tau181, p-tau217 or p-tau231) could be applied to recruit symptomatic individuals. A recent publication showed that plasma p-tau217 has a high positive predictive value but a low negative predictive value for Aβ-PET and tau-PET positivity owing to its poor sensitivity in people with low concentrations[149]. For this

reason, it was recommended that a good strategy to use plasma p-tau217 as a screening test for Aβ is as a rule-in biomarker for cognitive impairment and as a rule-out biomarker for those with normal cognition[149].

For outcome measures in therapy trials, the high longitudinal stability of plasma p-tau181[101] and high intra-individual increases in p-tau217[133] could be used to evaluate effects of therapeutic intervention: significant decreases in plasma concentration of p-tau181 or p-tau217, or a reduction in the rate of increase over time, could indicate beneficial effects of anti-Aβ or anti-tau treatment. In the first example of plasma p-tau being used as a marker in a therapeutic trial, Eli Lilly reported at the 2021 Alzheimer's Association International Conference that decreases in plasma p-tau217 concentration accompanied reductions in Aβ-PET and tau-PET signal following donanemab treatment compared with both placebo and pre-treatment levels in the TRAILBLAZER-ALZ study[150].Furthermore, recent studies showed that plasma p-tau181 or p-tau217 predicted longitudinal changes in tau-PET accumulation, including in individuals who showed normal tau-PET uptake at baseline[119,151]. Moreover, modelling studies indicated that for trials using tau-PET as the readout, prescreening using plasma p-tau would reduce the required sample size by 43%-68%[119,151].

[H2] Epidemiological and genetic studies

To date, most published epidemiological and genetic studies of AD did not include biomarkers. Therefore, the relationship between AD risk factors and the AT(N) biomarkers remains unclear[152]. Blood p-tau measurments, together with amyloid, neurodegeneration and other markers, could be encoporated into large-scale epidemiological and genetic studies with the aim of identifying resilience and risk factors for AD. For example, a study published in 2021 examined the link between plasma concentrations of p-tau181 and AD polygenic risk[107], and we are likely to see more of these kinds of studies in the future. We expect that incoporation of these biomarkers will be particularly useful in multi-ethnic and community-based cohorts, including those with high genetic risks and

cardiovascular burden, and will employ similar approaches to those described above for primary-care and population cohorts.

[H1] Outstanding challenges

The discussions above point to a revolutionary future, in which widespread and routine analyses of blood p-tau, likely combined with $A\beta_{1–42}$:$A\beta_{1–40}$ ratio, NfL and glial fibrillary acidic protein, become routine practice in clinical assessments and research studies. However, several outstanding challenges must be addressed to accelerate this anticipated progress.

[H2] Analytical standardization

Currently, blood p-tau measurements rely on research-grade assays developed in independent laboratories using specific methods and targeting distinct epitopes (Fig. 1). Despite excellent biomarker capacity, validation efforts have been limited to independent cohorts, resulting in missed opportunities to directly compare the performance of different assays and understand how each biomarker changes at the different stages of the disease process. Standardization efforts, including round-robin studies **[G]** and development of reference materials and methods, are needed to harmonize readings and enable direct comparisons. Head-to-head comparisons published in 2021 and 2022 reported high correlations between the Gothenburg, Eli Lilly and Quanterix p-tau methods [112,124]. More comparison studies are warranted, and lessons learned from the CSF biomarker standardization efforts of the International Federation of Clinical Chemistry and Laboratory Medicine, the Alzheimer's Association's Global Biomarker Standardization Consortium and the Alzheimer's Biomarkers Standardization Initiative might help accelerate this process.

Transfer of methods into commercial products has already started (for example, the Gothenburg plasma p-tau181 method is now commercialised by Quanterix for use on the Simoa platform) brings p-tau analyses to anyone with instrument access. However, this raises urgent needs to standardize measurements through stringent pre-analytical and analytical protocols. Reference quality-control

samples with expected results should be included in every commercial kit and measured at the start and the end of each analytical run[153] to assess inter-user variability, minimize heterogeneity and generate universal cut-offs. Additionally, the assembly of an expert working group to survey and develop practical operational guidelines, reference materials, methods and laboratory certification programs for each p-tau form would ensure standard practices. The International Federation of Clinical Chemistry and Laboratory Medicine's Working Group on CSF proteins is already fulfilling this role for CSF p-tau.

[H2] Diversity in study cohorts

Recruiting a diverse range of participants for studies of AD biomarkers, including blood p-tau[29], has been a challenge[154,155]. With just two exceptions[106,110] the dozens of p-tau biomarker studies published to date involved exclusively white participants. Significant variations in CSF p-tau and total-tau concentrations between white individuals and individuals of other ethnicities have been reported[26,156]. Therefore, establishing whether the (patho)physiological regulation of blood p-tau levels differs between populations and investigating the biochemical factors mediating such variations is essential. This knowledge would help determine if generalized use of biomarker cut-offs, which are often generated in selected white American and European populations, is feasible. To this end, a recent study that evaluated 76 pairs of non-Hispanic white American and African American individuals of equivalent age, sex, cognition and *APOE* ε4 genotype reported that plasma p-tau231 and p-tau181 were less accurate to detect abnormalities in Aβ-PET and the CSF $A\beta_{1-42}$:$A\beta_{1-40}$ ratio in the African American group[157].

[H2] Clinical application

Real-world clinical data on the performance of p-tau biomarkeres are lacking; published findings are mostly from well-characterized cohorts classified by PET or CSF markers. We do not yet know how observations from such cohorts will translate to the setting of routine memory clinics, which see patients with greater heterogeneity in demographics, disease presentations and biomarker-based assessments. Therefore, whether blood p-tau can be used as a single marker or to replace CSF

biomarkers that have been tested in larger varieties of disease conditions remains unclear. Realistically, we might need to exercise caution in projecting immediate diagnostic use of blood p-tau as a CSF substitute until large-scale clinical characterization studies are performed.

[H2] Therapeutic trials

Although we expect blood p-tau measurements to have a crucial role in future clinical trials, we must be aware of its prospects and potential limitations. Plasma p-tau associates with both amyloid and tau pathologies whether measured at autopsy or by in vivo biomarkers[47–49,98,143]. These observations mean that despite being highly specific to AD pathophysiology, it may be challenging to determine if increases in plasma p-tau concentration are primarily driven by Aβ plaque accumulation or by non-Aβ-dependent tau build-up or both, particularly in humans where these processes cannot be decoupled. This could affect the specificity of plasma p-tau as an outcome measure in trials of anti-Aβ or anti-tau therapies. Even in terms of amyloid, it is unclear if p-tau levels increase specifically in relation to Aβ plaques or this is also observed in plaques composed of other amyloid proteins. For example, a recent study showed that CSF p-tau181 and p-tau217 levels were increased in mouse models overexpressing either Aβ or the familial Danish dementia type of amyloid[158]. Moreover, the increases in CSF were observed in the absence of tangle pathology[158]. For therapeutic trails in humans, a treatment-associated reduction in plasma p-tau217 was observed in the TRAILBLAZER-ALZ study of the Aβ-targeting drug donanemab[150], although one could argue that whether this was a direct response to brain Aβ clearance or to the associated decrease in tau pathology (as reflected by a reduction in tau-PET signal) remains unclear. This challenge might be addressed by developing tau markers that are specific for Aβ-induced tau phosphorylation. Such a marker would be useful as a surrogate for estimating the efficacy of anti-Aβ therapies.

[H2] (Patho)physiological confounders

Blood p-tau concentrations represent a balance between p-tau production and clearance. Therefore, conditions that affect this balance by enhancing or diminishing blood tau production or clearance (for example, kidney disease[159] and liver malfunction[160]) could compromise the diagnostic accuracy

of blood p-tau measurments and their utility in therapy evaluation. In a similar way, a trial of the anti-Aβ drug solanezumab reported an increase in blood Aβ levels, which indicated the removal of Aβ from the brain; however, other evidence suggested that the increase was instead a result of solanezumab binding to Aβ and blocking its clearance from the blood[161]. Supporting evidence from a preclinical model showed that solanezumab did not alter the amounts of Aβ species in mouse brain, but rather formed complexes with these Aβ forms[162].

Some non-AD tauopathies seem to induce tau phosphorylation at AD-typical epitopes. For example, in Aβ-negative individuals with some *MAPT* pathogenic mutations (for example, R406W[163]), p-tau217 concentrations are increased to levels similar to those found in indiviudals with AD, whereas normal serum p-tau181 levels were found in individuals carrying other such mutations (for example, P301L)[103]. Conversely, individuals with concomitant frontotemporal lobar degeneration (FTLD)-TDP43 pathology and *GRN* mutations had lower serum p-tau181 concentrations than individuals with the same pathology but without a pathogenic *GRN* mutation[103]. Selective increases in blood concentrations of p-tau217, but not p-tau231, have been recorded in individuals with autopsy-verified Aβ-negative Creutzfeldt–Jakob disease; p-tau217 level correlated with extent of neurodegeneration[145]. Futhermore, recent data show increases in plasma p-tau concentration following acute neurological injury, for example in cardiac surgery[164].

We need to identify and account for such confounders to minimize the risk of misinterpreting p-tau results.

[H2] Ethical implications

In the future, it might become possible to provide medical advice by comparing an individual's p-tau level to published cut-offs, perhaps via direct-to-consumer tests similar to exisiting methods of genetic and blood glucose testing[165]. However, such unrestricted accress to blood p-tau analyses presents ethical challenges. We anticipate that p-tau analyses will increase the ease and accuracy of diagnosis, but will not eliminate the need for cognitive testing and other clinical evaluations.

Therefore, diagnostic or prognostic advice based solely on p-tau (and other blood biomarkers) would be problematic, if not unethical. However, the possibility of combining direct-to-consumer p-tau tests with family history, genetic, cardiovascular and cognitive assessments, which are each now accessible to the consumer via predictive-testing channels would further complicate the ethical challenges.

Even when provided by qualified clinicians, the disclosure of diagnostic and prognostic information based on blood biomarker data might cause distress to patients (especially in the present day when access to disease-modifying therapies remains limited), as has been reported for other neurodegenerative diseases[166,167]. We must recognize these concerns and put measures in place to investigate and understand how best to navigate such sensitive topics.

[H1] Conclusion

The integration of rapid developments in ultrasensitive analytical technologies and our increased understanding of the biochemical processing of tau have enabled blood biomarker development to probe tau phosphorylation in AD. These advances are based on the discovery that blood tau forms are mostly N-terminal fragments that somewhat differ from the epitopes targeted by established CSF p-tau biomarkers. Blood p-tau concentration increases with disease severity specifically in AD and is associated with key disease hallmarks, providing insights into disease staging and progression, and enabling differential diagnosis. Importantly, baseline and longitudinal increases in blood p-tau are more pronounced in (and sometimes exclusive to) Aβ-positive individuals. Antemortem p-tau is associated more accurately with pathological diagnosis than clinical diagnosis, suggesting that integrating blood analyses into routine clinical evaluation could improve accuracy. This viewpoint is made more realistic by the recent development of commercial kits that guarantee unrestricted access to routine and widespread blood p-tau evaluation. In the near future, regular blood p-tau screening in primary care (as done for cholesterol, diabetes and some cancers) could help identify emerging AD pathophysiology and streamline referrals for specialist care. In secondary care, blood p-tau analyses could resolve low-risk cases, with medium-risk and high-risk cases requiring triaging

with CSF, PET, MRI and other established procedures. Furthermore, blood biomarkers could be used as a pre-screening tool in clinical trials, and in large-scale population and epidemiological studies. Nonetheless, analytical challenges, such as the need for method harmonization, and ethical challenges, such as those involved in disclosing disease risk to patients and caregivers, need to be addressed. Additionally, real-world routine clinical data are needed to establish for which purpose(s) blood p-tau could replace CSF or PET markers in clinical evaluation. Finally, there is an urgent need for studies in diverse populations; these include people of racial and ethnic backgrounds different from those of European ancestry that are well studied, as well as individuals whose socioeconomic statuses differ from those included in recent studies. In conclusion, we consider blood p-tau to be an excellent biomarker of brain Aβ and tau pathologies with potential uses in routine clinical assessments, therapeutic trials, and research cohort studies, making effective yet accessible and cost-effective biomarker testing for AD a reality.

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This study presented pre-analytical factors that affect blood p-tau measurement and quantification in different matrices and conditions.

127. Verberk, I. M. W. *et al.* Characterization of pre-analytical sample handling effects on a panel of Alzheimer's disease–related blood-based biomarkers: Results from the Standardization of Alzheimer's Blood Biomarkers (SABB) working group. *Alzheimer's & Dementia* **n/a**, (2021).

This study investigated preanalytical factors for blood sample handling in biomarker assessments, and presented recommended guidelines for other investigators.

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129. Sperling, R. A. *et al.* 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. *Alzheimers Dement* **7**, 280–292 (2011).

130. Keshavan, A. *et al.* Population-based blood screening for preclinical Alzheimer's disease in a British birth cohort at age 70. *Brain* **144**, 434–449 (2021).

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131. Grothe, M. J. *et al.* Associations of Fully Automated CSF and Novel Plasma Biomarkers With Alzheimer Disease Neuropathology at Autopsy. *Neurology* (2021) doi:10.1212/WNL.0000000000012513.

Grothe et al., demonstrated that CSF and plasma p-tau181 levels reflect AD neuropathological changes in autopsied brain tissues.

132. Thijssen, E. H. *et al.* Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study. *The Lancet Neurology* **20**, 739–752 (2021).

This study showed that plasma p-tau217 and p-tau181 are interchangeable for the differential diagnosis of autopsy-verified AD versus other tauopathies.

133. Mattsson-Carlgren, N. *et al.* Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain* **143**, 3234–3241 (2020).

This study reported the longitudinal profiles of plasma p-tau217 and their association with brain amyloid, tau and neurodegeneration in the BioFINDER study.

134. Shen, X.-N. *et al.* Plasma amyloid, tau, and neurodegeneration biomarker profiles predict Alzheimer's disease pathology and clinical progression in older adults without dementia. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* **12**, e12104 (2020).

135. Simrén, J., Ashton, N. J., Blennow, K. & Zetterberg, H. Blood neurofilament light in remote settings: Alternative protocols to support sample collection in challenging pre-analytical

conditions. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* **13**, e12145 (2021).

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This study showed that integrating plasma p-tau and neurodegeneration abnormality data can provide individualized longitudinal prognostic trajectories.

137. Chételat, G. *et al.* Amyloid imaging in cognitively normal individuals, at-risk populations and preclinical Alzheimer's disease. *NeuroImage: Clinical* **2**, 356–365 (2013).

138. Janelidze, S. *et al.* Associations of Plasma Phospho-Tau217 Levels With Tau Positron Emission Tomography in Early Alzheimer Disease. *JAMA Neurol* **78**, 149–156 (2021).

This study pointed to the likelihood the plasma p-tau levels become abnormal ahead of tau PET in AD.

139. Nakamura, A. *et al.* High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* **554**, 249–254 (2018).

140. Schindler, S. E. *et al.* High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology* **93**, e1647–e1659 (2019).

141. Benedet, A. L. *et al.* The accuracy and robustness of plasma biomarker models for amyloid PET positivity. *Alzheimer's Research & Therapy* **14**, 26 (2022).

142. Janelidze, S. *et al.* Head-to-Head Comparison of 8 Plasma Amyloid-β 42/40 Assays in Alzheimer Disease. *JAMA Neurology* **78**, 1375–1382 (2021).

143. Mattsson-Carlgren, N. *et al.* Soluble P-tau217 reflects amyloid and tau pathology and mediates the association of amyloid with tau. *EMBO Molecular Medicine* **13**, e14022 (2021).

This study reported that plasma p-tau217 is associated with amyloid pathology in early AD and tau pathology in advanced stages, and modulates the relationship between amyloid and tau in vivo.

144. Barthélemy, N. R. *et al.* Cerebrospinal fluid phospho-tau T217 outperforms T181 as a biomarker for the differential diagnosis of Alzheimer's disease and PET amyloid-positive patient identification. *Alzheimer's Research & Therapy* **12**, 26 (2020).

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Acknowledgements

T. K. K. was funded by the Swedish Research Council (Vetenskapsrådet #2021-03244**),** the Alzheimer's Association Research Fellowship (#AARF-21-850325), the BrightFocus Foundation (#A2020812F), the International Society for Neurochemistry's Career Development Grant, the Swedish Alzheimer Foundation (Alzheimerfonden; #AF-930627), the Swedish Brain Foundation (Hjärnfonden; #FO2020-0240), the Swedish Dementia Foundation (Demensförbundet), the Swedish Parkinson Foundation (Parkinsonfonden), Gamla Tjänarinnor Foundation, the Aina (Ann) Wallströms and Mary-Ann Sjöbloms Foundation, the Agneta Prytz-Folkes & Gösta Folkes Foundation (#2020-00124), the Gun and Bertil Stohnes Foundation, and the Anna Lisa and Brother Björnsson's Foundation. N. J. A. was supported by the Swedish Alzheimer Foundation (Alzheimerfonden; #AF-931009), Hjärnfonden and the Swedish Dementia Foundation (Demensförbundet). M. S. C. receives funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (Grant agreement No.

948677); the Instituto de Salud Carlos III (PI19/00155); and the Spanish Ministry of Science, Innovation and Universities (Juan de la Cierva Programme grant IJC2018-037478-I). H. Z. is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532); the European Research Council (#681712); Swedish State Support for Clinical Research (#ALFGBG-720931); the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862); and the UK Dementia Research Institute at Universitly College London. K. B. is supported by the Swedish Research Council (#2017-00915); the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615); the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243); the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986); and European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236). The funders had no role in data collection, analysis or decision to publish.

Competing interests

[Au: I haved edited this section to list companies in alphabetical order according to journal style.]

H. Z. has served at scientific advisory boards for CogRx, Denali, Pinteon Therapeutics, Roche Diagnostics, Samumed, Siemens Healthineers and Wave, and has given lectures in symposia sponsored by Alzecure, Bioeng and Fujirebio. H. Z. is also a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). K. B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). M. S. C. has served as a consultant and at advisory boards for Roche Diagnostics International Ltd and has given lectures in symposia sponsored by Roche Diagnostics, S.L.U and Roche Farma, S.A. The other authors report no competing interests.

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Table 1. Key publications on blood p-tau as a biomarker for AD. [Au: This table is too long for our format. Tables must fit two A4 portrait pages (Times New Roman 8pt.). I suggest you move this table to supplementary information.]

Table 2. Analytical guidelines for the measurement of blood p-tau biomarker results. [Au: the information in this table is a little too technical for one of our Review articles. I suggest you move the table to the supplementary information.]

Figure 1 | Molecular processing of tau in the brain and biofluids informs the development of blood p-tau biomarkers. [Au: I have made quite a few edits to reduce the length of this figure legned, which should be maximum ~200 words.]

a | The top image shows the complete tau protein, which is present in the brain[39,40,75] **[Au: simplification to reduce length OK?]**. **[Au: deleted to reduce length of legend, this is clear enough from the figure.]** Mass spectrometric data show that tau is post-translationally modified at several positions, including truncations at amino acids 368[71], 391[83] and 421[83] that enhance fibrillar aggregation[18,36] **[Au: Deletion to reduce the length of the figure legend OK? implications of fibrillary aggregation will be clear to the majority of our readers.]**. Truncated C-terminal fragments are retained in brain tangles, whereas N-terminal and mid-region forms make up the majority of the soluble pool[73] **[Au: Edits for clarity and to reduce length OK?]**, fractions of which are released into CSF and blood[74,75]. The middle image shows CSF tau, which lacks the extreme C-terminal part of the protein but contains mid-region epitopes **[Au: deleted to reduce length of figure OK? This is clear enough from part b.]**. The bottom image shows blood tau, which extends from the N-terminal to the start of the microtubule binding region (around amino acid 254). **[Au: Deletion to reduce length of legend OK? this is clear enough from part b]** Levels of p-tau in blood are a small fraction (up to 5%) of the CSF levels in the same individual[48], suggesting that brain-derived p-tau is released into blood via the CSF. **b |** The top part of image shows the epitopes of the established CSF p-tau and total-tau assays that are currently being used in the clinic. **[Au: Deletions to reduce the length of the figure legend OK? This seems clear enough from the figure.]** The bottom part of the image shows blood p-tau biomarkers, including those measured using immunoassay and immunoprecipitation-mass spectrometry technologies. **[Au: deteled to reduce the length of the figure legend]** Although the p-tau epitopes of interest are in the proline-rich region (yellow), measuring these on N-terminal-directed fragments provides more reliable biomarkers[48].

Figure 2 | Association of plasma p-tau181 with Aβ-PET and tau-PET load

Brain images showing voxel-wise correlations of plasma p-tau181 concentration with in vivo Aβ-PET (^{18}F-AZD4694) and tau-PET (^{18}F-MK-6240) load, overlaid on structural MRI templates (models were adjusted for age and sex, as well as diagnosis when all subjects were included). Each panel shows correlations for the entire cohort as well as the cognitively unimpaired and cognitively impaired sub-groups. The colour scales reflect the strength of the correlation in different brain areas; the areas with strongest associations are in red. Axial (top), sagittal (middle), and coronal (bottom) views of the brain are shown. This figure demonstrates that the plasma p-tau181 concentrations associate with amyloid and tau pathologies in the brain of the same individuals, with the degree of association being stronger in people with cognitive symptoms compared with those without. **[Au: I suggest you add a sentence to explain the key reason for including this example. What key finding are you trying to highlight?]** Data used to prepare this figure were from the TRIAD cohort, McGill University. **[Au: have these images been previously published elsewhere?]**

Figure 3 | Potential applications of blood p-tau biomarkers in primary and specialist care. **[Au: Added numbers to help the reader navigate the figure]**

In the suggested pathway, an individual with cognitive complaints (1) would undergo a clinical interview to establish whether or not a neurological aetiology is suspected (2) **[Au: suggest this sentence to ensure that you explan the flowchart from the start.]**. Still in a primary care setting, blood p-tau screening would be used to categorize individuals into low (normal blood p-tau concentration), medium (borderline concentrations), and high (clearly abnormal blood p-tau concentration) AD risk groups (3) **[Au: Edits to this setnence OK? From the diagram it seems that the risk groups are defined purely on the basis of blood p-tau level. This was not clear from the original wording.]**. The low-risk group could be further evaluated with plasma NfL to identify the presence of non-AD neurodegeneration, whereas individuals at medium and high risk would be referred for specialist care (4). Neurological examination and neuropsychological screening are important, as part of a routine diagnostic workflow, for clinicians to identify patients whose clinical presentations point to suspected AD or other neurodegenerative diseases, and to request for biomarker analysis accordingly **[Au: suggest you add a few words to explain the role**

of neurological exam and neuropsychological screening in the process.]. In the specialist care setting, further investigation with CSF or neuroimaging measures of amyloid, tau and neurodegeneration might be required, for example, individuals with elevated blood p-tau and normal cognition might be prognosed as having preclinical AD but the sensitive nature of this category demands confirmation with CSF or PET. Individuals with evident dementia and clearly abnormal blood p-tau measures might not need to be assessed with other biomarker modalities. Following diagnosis, these carefully classified patients would proceed to treatment, clinical management and enrolment in clinical trials. The disease risk stratification approach described here will require prior defintion and verification of p-tau biomarker cut-offs in mutiple indendent cohorts **[Au: Original legend was too long for our format, so I have removed some of the more detailed information and added this summary in yellow, what do you think?].** For individuals who present directly for specialist care, thus bypassing primary care assessment, detailed neurological and cognitive assessments and blood p-tau measures could be used for first-line evaluations, with blood NfL added when necessary **[Au: Meaning of this sentence was not clear to me, i have made some assumptions so please check my edits carefully.]**.

[Au: removed mention of BioRender here. I have flagged your use of BioRender to our Editorial Assisstant and we will include the relevant copyright line in the published mansucript, if necessary.]

Figure 4 | Advantages of blood p-tau pre-screening to recruit asymptomatic individuals for anti-AD clinical trials.

a | Based on the assumption that 1,000 participants with preclinical AD are required for a study, and that the population from which participants are being recruited has a 20% rate of Aβ positivity by PET (for example, see ref[101]), two options arise. **b |** The traditional approach would be to perform Aβ-PET scans on 5,000 individuals. Assuming a modest cost of US$3,000 per scan, a total investment of $15 million will be needed. The difficulties involved in finding a sufficient number of older adults willing to undergo PET scanning should also be considered. **c |** We propose a new pre-screening approach supported by evidence from our recent study of the multicentric Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort[101]. Assuming 40% of the

target preclinical cohort have increased plasma p-tau — estimated from the ADNI data using a cut-off point found to predict Aβ-PET abnormalities[101] — a test population of 5,000 individuals would yield ~2,000 individuals with elevarted plasma p-tau **[Au: Edits to this sentence for clarity and to reduce length of legend OK? I assumed that the 40% figure came from the ADNI data, is this correct?]**. These 2,000 individuals would then undergo PET screening, identifying the required 1,000 Aβ-positive individuals for recruitment **[Au: simplification of this sentence for clarity OK?]**. If blood p-tau prescreening costs $50/ per individual, a total of $250,000 would be spent at this stage. Aβ-PET scanning costs in the refined sub-cohort of 2,000 individuals would require only $6 million. Together, $6.25 million would be spent, leading to a 58% cost-saving compared with the traditional method, alongside substantial savings in recruitment efforts and volunteer time and convenience.

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Box 1 | CSF versus imaging biomarkers [Au: suggested title for box OK?]

[Au: OK to move this paragraph to a box from the introdcution? The original introduction section was longer than I would recommend and this information is not essential for the reader to understand the rest of the review.]

When used together with clinical evaluation, the AT(N) system (recently revised to ATX(N) to accommodate emerging biomarkers[168]) enables patient classification according to biological and clinical severity[17,169,170]. Nevertheless, the International Working Group's 2021 update to the AT(N) framework recommends prioritization of CSF over PET[23] **[Au: addition to this sentence for clarity OK?]**. Analytically, a single lumbar puncture provides enough CSF for multiple **[Au:OK? "repeated" implied to me a longitudinal analysis, which I don't think was your meaning.]** AT(N) biomarker measurements and biobanking for future analyses. Conversely, Aβ-PET, tau-PET and MRI (or fluorodeoxyglucose-PET) evaluations each require a separate assessment, with no possibility for retrospective analyses. Regarding pathophysiology, soluble Aβ[171–174] and tau[37,75,76]

abnormalities tend to be reflected slightly earlier in CSF than in the insoluble brain aggregates targeted by PET, suggesting the former might be more suitable for preclinical evaluations. Moreover, the availability of completely automated technologies that are validated and standardized for use in multiple laboratories has simplified CSF analyses and improved transferability[79,80,175]. Economically, CSF examination costs a fraction of the price of a single PET visit[101,130,176] **[Au: Edit to avoid repetition OK?]**. However, neuroimaging provides brain-wide information on anatomical distribution and intensity of pathological protein aggregates, which is not possible with CSF biomarkers **[Au: addition for clarity OK?]** [177,178]. Furthermore, tau-PET tends to be more reliable for longitudinal monitoring in the late stages of dementia, when neurodegeneration is extensive but insoluble protein aggregates are mature and accessible to ligands[76]. Nevertheless, some commonly used tau-PET tracers are suboptimal for detecting early stages of tau accumulation and differentiating AD from non-AD tauopathies[177,178].

Box 2 | Benefits of blood-based biomarkers of AD **[Au: OK to move this section here from the introduction? The introduction was too long and this information is fairly general.]**

Compared with CSF or PET approaches **[Au: Addition for clairty OK?]**, blood sampling is minimally invasive, more flexible (collection is feasible at home or in the community) and allows for time and cost savings. Moreover, retrospective analyses can be performed on frozen blood samples **[Au: edits to this sentence for clarity OK?]** . Additionally, the scalability and accessibility of blood sampling is ideal for large-scale clinical use, as well as for observational and intervention studies[24]. Blood biomarker use is also likely to increase enrolment and retention in population-based and clinic-based studies, and expand participant diversity[12,24]. Such studies would be expected to provide new information on the biological basis of dementia and associated risk factors, with clinical and public health implications[29]. In clinical trials, blood biomarkers could be used for 'pre-screening', to select initial cohorts for further assessment with CSF or PET biomarkers[12,24] **[Au: Edits to this sentence for clarity OK?]**. Emerging centrifugation-free and freeze-free approaches to blood collection would facilitate the use of blood-based biomarkers in challenging environments, for example, during pandemics and in remote communities[135,179]. From a clinical perspective, although blood biomarkers are expected to be useful in the specialized clinics that currently use CSF or PET, the real

transformative potential is most likely to be realized in environments that don't currently have access to biomarker-supported decision-making[4,27]. Additionally, blood biomarkers could **[Au:OK? To indicate that this is not yet the case]** be employed in primary care clinics, in combination with cognitive testing and other clinical algorithms, to streamline referrals to appropriate secondary care[15,24].

Box 3 | Robustness defines a clinically useful biomarker

[Au: The text in the original box figure did not fit our format. Figures in boxes need to be as small and simple as possible, and we generally avoid including large portions of text in figures anyway. I have moved the text into its own box. What do you think?]

Clinical performance of a biomarker

Diagnostic accuracy validated in independent research studies:

- A clinically useful biomarker needs to have high sensitivity (proportion of positive results among those with the disease) and high specificity (proportion of negative results among those without the disease) validated in several independent research studies.
- Biological factors that might negatively affect diagnostic accuracy must be characterized. These factors include patient characteristics (for example, age, comorbidities, medication use) and within-individual biological factors (for example, genetics, circadian variations, stress).

Analytical performance of a biomarker

The following factors govern the total measurement error:

- Pre-analytical factors, for example, differences in sampling technique, time to centrifugation or shipment, storage.
- Analytical variability, that is, inevitable differences in measured levels inherent to any measurement technique.
- Bias, that is, differences in levels between rounds of measurements, instruments or batches of reagent.

Robustness of a biomarker

For a biomarker to be robust and give conistent and clincially useful classification of patients, the percent total analytical error must be substantially lower than the percent fold change (that is, mean magnitude of change in levels between individuals with the disease of interest and controls or individuals with other diseases). See box 4 for an example **[Au: Ok to direct the reader to Box 2 here?]**.

Box 4 | Robustness of plasma $A\beta_{1–42}$:$A\beta_{1–40}$ ratio and p-tau for predicting Aβ-PET positivity

[Au: Edits to title to better reflect the content of the box now that the section of text has been removed from the figure. OK?]

Reliability of blood biomarkers depends on clinical and analytical performances (box 3). An ideal marker should have high sensitivity, high specificity and large fold changes that allow it to withstand unavoidable variations in analytical errors including longitudinal bias across measurements. For a robust biomarker, the analytical bias is substantially lower than the fold change such that small variations do not significantly impact assay performance.

To demonstrate how analytical bias affects the robustness of a biomarker, we performed a synthesis of publicly-available data to compare how well plasma and CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio or p-tau predict Aβ-PET outcome (figure; details and R code can be found in Supplementary Information). The yellow traces show the distribution of biomarker values in individuals who are Aβ-negative according to PET, whereas the red traces show the distribution among individuals who are Aβ-positive according to PET **[Au: OK to add this to help our non-expert readers navigate the figure?]**. Biomarker distribution densities are shown on a $\log_{10}$ scale and boxplots using original scale are included; cut-offs for the separation of Aβ-positive and Aβ-negative individuals are indicated by dashed lines **[Au: Edits to this sentence for clarity OK?]** .

CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio (part a; AUC= 85.9%,) was superior to plasma IP-MS $A\beta_{1–42}$:$A\beta_{1–40}$ ratio (part c; AUC=75.8%, p=0.006) for separation of Aβ-positive from Aβ-negative individuals. Notably, CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio had a 37.5%-fold change, whereas plasma $A\beta_{1–42}$:$A\beta_{1–40}$ ratio had only a 8.6%-fold change, similar to published data[130,139,140,142]. Conversely, CSF and plasma p-tau231 (parts b

and d) in Aβ-positive individuals were increased by 166.0% and 85.%, respectively, compared with Aβ-negative individuals **[Au: additions to this sentence for clarity OK?]**.

Therefore, CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio can be considered a robust biomarker with a clear biomodal distribution, whereas plasma $A\beta_{1–42}$:$A\beta_{1–40}$ ratio might have robustness issues owing to the low fold change observed between the two groups. Plasma p-tau has a larger fold change, and hence a robustness advantage, over plasma $A\beta_{1–42}$:$A\beta_{1–40}$ ratio. This means that an identical analytical bias would affect plasma Aβ42/Aβ40 more acutely than plasma p-tau. Plasma $A\beta_{1–42}$:$A\beta_{1–40}$ ratio might therefore be problematic to implement in clinical routine, as several factors can affect analytical performance (see box 3 and Supplementary Table 2).

[Box 4 fig]

Key points

- Blood p-tau181, p-tau217 and p-tau231 biomarkers that reflect brain tau and Aβ pathophysiology have been developed and validated. **[Au: Deletion OK? Key points must be one sentence only]**
- The levels of p-tau species in blood increase with increasing Aβ accumulation and clinical severity in individuals with AD; these changes are absent in individuals with cognitive impairment not due to AD.
- Blood concentration of p-tau is associated with, and predicts changes in, CSF and PET measures of Aβ, tau and neurodegeneration; antemortem blood p-tau concentration predicts definite neuropathological diagnosis several years later, outperforming clinical diagnosis during life.
- Blood p-tau has potential uses for definitive and differential diagnosis in specialized care, for pre-screening in primary care and therapeutic trials, as well as for population-based and epidemiological studies.

- Future studies in real-world settings (for example, heterogeneous and diverse memory clinic cohorts) will show if blood p-tau can serve as a stand-alone confirmatory biomarker or replace CSF or PET biomarkers in specific scenarios.
- Outstanding challenges such as the need for analytical guidelines, inter-laboratory method comparison and standardization, cut-off value generation and validation, appropriate use criteria for clinical implementation, and consideration of the ethics of direct-to-consumer tests should be addressed to enable accelerated progress.

Glossary

[Au: I have highlighted suggestions for glossary terms throughout your manuscript with a [G]. Please provide succinct, one-sentence definitions for these specialist terms.]

Fractions: this represents part of a whole; for example, the soluble and insoluble parts of tau togther form the pool of tau in the brain.

Single molecule array (Simoa): An ultrasensitive immunoassay technology platform that allows small quantities of target analytes to be detected in biological fluids (e.g., blood) that are remote from the brain.

Ethylenediaminetetraacetic acid (EDTA)-plasma: A clear component of blood obtained by collecting whole blood into a tube containing known concentration of the cheating agent and anticoagulant EDTA for a defined amount of time and centrifuging the mixture to separate the upper layer of plasma from the heavier cellular components.

Citrate-plasma: Blood matrix prepared by adding a clotting-preventing citrate compound to whole blood for a fixed amount of time, and centrifuging to separate the clear liquid layer from the cellular material.

Heparin-plasma: A clear-liquid component of blood obtained by adding heparin salt anti-coagulant to whole blood to induce the separation of the upper layer of interest from the more-dense cellular components.

Aβ-positive: abnormal levels of amyloid plaques in their brain, as determined at autopsy or measured in vivo using Aβ-PET or the CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio.

Aβ-negative: normal amounts of amyloid plaques in the brain, not found to be associated with amyloid pathology; this is determined either at postmortem or analysed using Aβ-PET or the CSF $A\beta_{1–42}$:$A\beta_{1–40}$ ratio according to pre-defined thresholds.

Round-robin studies: this refers to interlaboratory studies where the same tests are independently performed at multiple centres or laboraties on identical samples and the results compared to assess variability of the assay.