



















Transitioning from cerebrospinal fluid to blood tests to facilitate diagnosis and disease monitoring in Alzheimer's disease

■ D. O. T. Alawode^{1,2} , A. J. Heslegrave^{1,2} , N. J. Ashton^{3,4,5,6} , T. K. Karikari³ , J. Simrén^{3,7} , L. Montoliu-Gaya³ , J. Pannee^{3,7} , A. O'Connor^{2,8} , P. S. J. Weston⁸ , J. Lantero-Rodriguez³ , A. Keshavan⁸ , A. Snellman^{3,9} , J. Gobom^{3,7} , R. W. Paterson⁸ , J. M. Schott⁸ , K. Blennow^{3,7} , N. C. Fox^{2,8}  & H. Zetterberg^{1,2,3,7} 

From the ¹Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology; ²UK Dementia Research Institute at UCL, London, UK; ³Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg; ⁴Wallenberg Centre for Molecular and Translational Medicine, Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden; ⁵Department of Old Age Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London; ⁶NIHR Biomedical Research Centre for Mental Health & Biomedical Research Unit for Dementia at South London & Maudsley NHS Foundation, London, UK; ⁷Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden; ⁸Dementia Research Centre, Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London, UK; and ⁹Turku PET Centre, University of Turku, Turku, Finland

Alawode DOT, Heslegrave AJ, Ashton NJ, Karikari TK, Simrén J, Montoliu-Gaya L, et al. Transitioning from cerebrospinal fluid to blood tests to facilitate diagnosis and disease monitoring in Alzheimer's disease. *J Intern Med* 2021; **290**: 583–601.

Abstract. Alzheimer's disease (AD) is increasingly prevalent worldwide, and disease-modifying treatments may soon be at hand; hence, now, more than ever, there is a need to develop techniques that allow earlier and more secure diagnosis. Current biomarker-based guidelines for AD diagnosis, which have replaced the historical symptom-based guidelines, rely heavily on neuroimaging and cerebrospinal fluid (CSF) sampling. While these have greatly improved the diagnostic accuracy of AD pathophysiology, they are less practical for application in primary care, population-based and epidemiological settings, or where resources are limited. In contrast, blood is a more accessible and cost-effective source of biomarkers in AD. In this review paper, using the recently proposed amyloid, tau and neurodegeneration [AT(N)] criteria as a framework towards a biological definition of AD, we discuss recent advances in biofluid-based biomarkers, with a particular emphasis on those with potential to be translated into blood-based biomarkers. We provide an overview of the research conducted both in CSF and in blood to draw conclusions on biomarkers that show promise. Given the evidence collated in this review, plasma neurofilament light

chain (N) and phosphorylated tau (p-tau; T) show particular potential for translation into clinical practice. However, p-tau requires more comparisons to be conducted between its various epitopes before conclusions can be made as to which one most robustly differentiates AD from non-AD dementias. Plasma amyloid beta (A) would prove invaluable as an early screening modality, but it requires very precise tests and robust pre-analytical protocols.

Keywords: Alzheimer's disease, Blood, Cerebrospinal fluid, Diagnosis, Disease monitoring, Fluid biomarkers.

Abbreviations: AD, Alzheimer's disease; A β , amyloid beta; APP, amyloid precursor protein; AT(N), amyloid, tau (neurodegeneration); BBB, blood–brain barrier; CJD, Creutzfeldt–Jakob disease; CSF, cerebrospinal fluid; CU, cognitively unimpaired; DS, Down syndrome; ECL, electrochemiluminescence; ELISA, enzyme-linked immunosorbent assay; FAD, familial Alzheimer's disease; FDG-PET, fluorodeoxyglucose positron emission tomography; MCI, mild cognitive impairment; MRI, magnetic resonance imaging; MS, mass spectrometry; NfL, neurofilament light chain; NFT, neurofibrillary tangle; NIA-AA, National Institute of Aging and Alzheimer's Association; PET, positron emission tomography; P-tau, phosphorylated tau; SCD, subjective cognitive decline; Simoa, single molecule array; TBI, traumatic brain injury; T-tau, total tau.

Introduction

AD, biomarkers and the AT(N) criteria

Alzheimer's disease (AD) is the most common form of dementia worldwide. It is characterized by (1) the presence of amyloid beta ($A\beta$) plaques in the brain parenchyma, which is often accompanied by $A\beta$ in cerebral blood vessels (amyloid angiopathy); (2) intraneuronal neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau; and (3) neurodegeneration [1-3]. According to the amyloid cascade hypothesis, accumulation of misfolded $A\beta$ years before clinical symptom onset is the initial trigger of AD pathogenesis [4]. This accumulation of $A\beta$, as well as the production of toxic oligomeric species, results in aberrant tau phosphorylation and misfolding, ultimately inducing neuronal loss and plaque-induced synaptic dysfunction [5]. This pathophysiological process is summarized in Fig. 1. Histopathological analysis of the brain at autopsy remains the gold standard for definitively diagnosing AD. However, molecular biomarkers have been developed to increase the accuracy of diagnosing AD clinically [6].

A biomarker is a naturally occurring, detectable indicator that can be measured to assess a

physiological or pathological state [7,8]. The importance of biomarkers is highlighted in the recent update of the National Institute of Aging and Alzheimer's Association (NIA-AA) research framework in 2018, in which a clinical diagnosis of AD is supported by biomarker evidence of a disease-specific pathophysiological signature, rather than by clinical symptoms alone [9]. A key reason for this is the inaccuracy of a diagnosis based solely on symptoms, with one multi-centre study observing the sensitivity and specificity of clinically probable AD to detect Braak stages V/VI to be 76.6% and 59.5%, respectively [10,11]. There are marked phenotypic differences within AD, especially in younger patients, and the symptoms overlap with other neurodegenerative disorders, including vascular dementia, and mood disturbances such as depression [12]. A secure diagnosis is important to ensure patients receive the correct management (of AD, or of alternative conditions), and to provide prognostic information, advice and support. Furthermore, it is now clear that histopathological changes predate symptom onset by several years in both familial and sporadic forms of AD [13-16]. While not currently clinically indicated, in the future it may become important to make a diagnosis of AD before symptom onset – if a

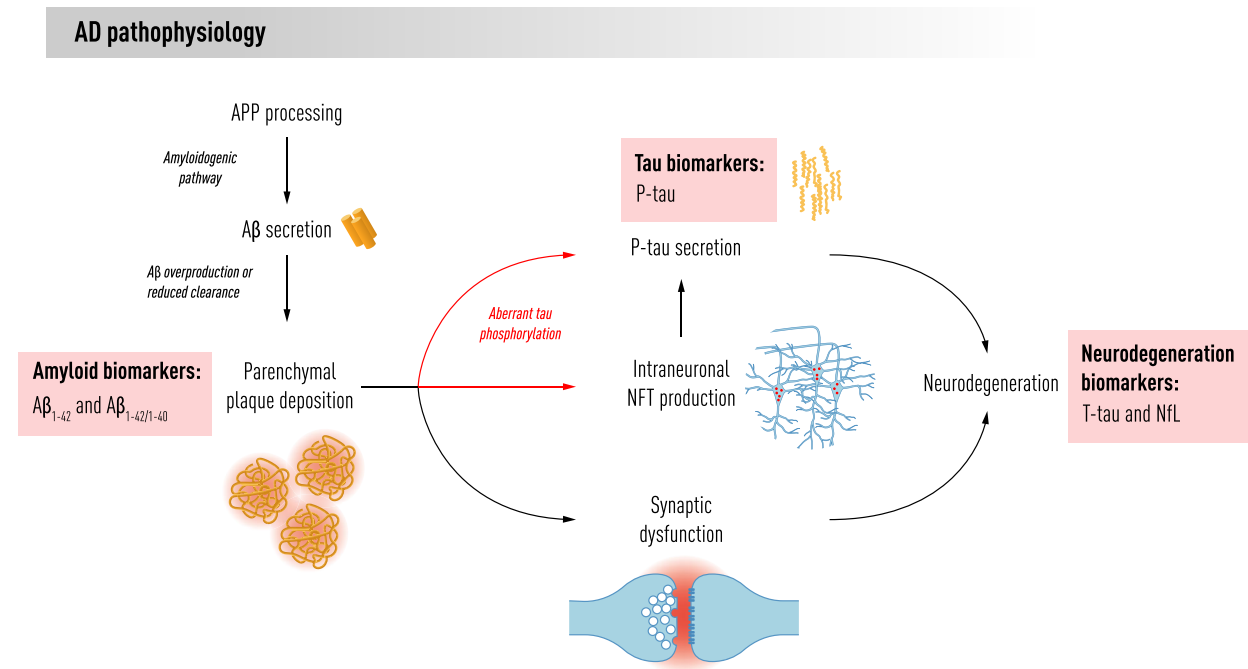


Fig. 1 AD pathophysiology and AT(N) criteria fluid biomarkers.

disease-modifying treatment is shown to be effective at this early stage.

Detection of AD pathology pre-symptomatically is already important for research and for clinical trials that seek to show disease modification at this stage. Clinical trials aiming to halt, or significantly slow, AD progression have thus far proven ineffective. This is possibly due to the inclusion of symptomatic patients who have progressed too far along the disease process, and in whom significant irreversible neuronal loss has already occurred [17]. Conversely, it may be due to some participants having a false AD diagnosis. This is particularly true of the solanezumab trial, where some recruited participants were later found to be amyloid PET-negative, hence were unlikely to have AD [18]. Furthermore, the lack of success in recent clinical trials may be due to too short trial duration and is further complicated by some participants displaying AD mixed with other disease pathologies, rather than being pure AD cases. Identifying individuals with AD pathology years prior to symptom onset will enable recruitment into clinical trials at a much earlier, and potentially more tractable, disease stage, and hence may prove more effective at identifying treatments to slow, or perhaps even halt, the disease process. Moreover, as participants in such trials would not be displaying cognitive symptoms, conventional cognitive/symptomatic endpoints are unlikely to be effective for identifying response to treatment, and so dynamic biomarkers which are sensitive to progression in pre-symptomatic disease will be important. Table 1 summarizes the use of available CSF and neuroimaging biomarkers in clinical trials, along with upcoming blood-based biomarkers.

There are two main types of biomarkers for molecular AD brain changes – neuroimaging biomarkers

(primarily positron emission tomography [PET] imaging) and fluid biomarkers (primarily cerebrospinal fluid [CSF]) [19]. The AT(N) criteria for AD diagnosis, which divide seven AD biomarkers into three groups based on the pathophysiological characteristic of AD they measure, include both of these classes of biomarkers [20] and are summarized in Table 2, where we also list a number of upcoming blood biomarkers. 'A' refers to A β pathology, as depicted by increased amyloid PET uptake, decreased CSF A β 1-42 (A β ₁₋₄₂) or decreased A β ₁₋₄₂/A β ₁₋₄₀ ratio (A β _{1-42/1-40}). 'T' refers to tau pathology, as depicted by positive tau PET tracer uptake or increased CSF phosphorylated tau (p-tau). Finally, '(N)' refers to neurodegeneration or neuronal injury, as depicted by decreased signal on [¹⁸F]-fluorodeoxyglucose (FDG)-PET, grey matter atrophy on structural magnetic resonance imaging (MRI), increased CSF total tau (t-tau) or increased CSF neurofilament light-chain (NfL) [20]. '(N)' is denoted in brackets to highlight that the biomarkers of neuronal injury are not specific to AD [9]. The fluid biomarkers in the AT(N) criteria can be seen alongside the pathophysiological process they reflect in Fig. 1.

While the AT(N) criteria highlight that both neuroimaging and fluid biomarkers can reliably confirm pathophysiological evidence of AD, fluid biomarkers offer the advantage of being able to detect the presence of multiple molecular pathologies in one bio-sample, as well as being of lower cost. However, a drawback of fluid biomarkers is the lack of anatomical information on the location and extent of pathologies, which can be gained from neuroimaging. Indeed, fluid biomarkers reflect a pathological process in the tissue, while neuroimaging, with a few exceptions, quantifies this pathology [21]. In this review, using the AT(N) criteria as a framework, we will address the

Table 1 Biomarker use in AD clinical trials

Intended use in trial	CSF biomarkers	Neuroimaging biomarkers	Blood biomarkers
Pre-screening			NfL, p-tau, A β ₁₋₄₂
Supporting diagnosis	T-tau, p-tau, A β ₁₋₄₂	Amyloid PET, Tau PET	
Drug effect monitoring	<i>Dependent on the mechanism of action of the drug</i>	<i>Dependent on the mechanism of action of the drug</i>	<i>Dependent on the mechanism of action of the drug</i>
Safety markers	Markers of inflammation and BBB integrity	MRI	NfL, markers of inflammation

Table 2 Summary of AT(N) criteria biomarkers

Criteria aspect	Pathology	Neuroimaging biomarkers	CSF biomarkers	Blood biomarkers
A	A β	Amyloid PET	A β ₁₋₄₂ or A β _{1-42/1-40}	A β _{1-42/1-40}
T	Tau	Tau PET	P-tau	P-tau
(N)	Neurodegeneration	MRI or FDG-PET	T-tau or NfL	NfL

evidence behind current CSF-based biomarkers for AD, with a particular focus on those that have potential for translation into blood-based biomarkers.

CSF and blood biomarkers for AD-related pathologies

Before delving into potential blood-based biomarkers for AD, it is important to consider some advantages and potential drawbacks common to all. Although CSF has the advantage of being in direct contact with the cerebral extracellular space, blood is less invasive to collect. Consequently, it is more suitable for obtaining repeated measurements from patients and is more easily accessible in low-resource and non-specialist settings worldwide [22-24]. While blood-based biomarkers have the potential to function as an initial diagnostic screening tool in a primary care setting, prior to more in-depth investigations in specialist centres [22,25], measuring biomarkers of brain diseases in the blood is not without its challenges, namely (1) analyte concentrations are 10- to 100-fold lower in the blood compared with CSF as a direct consequence of the blood-brain barrier (BBB) [26]; (2) some AD biomarkers are expressed by extra-cerebral tissues; (3) proteases in the blood may break down analytes of interest prior to their measurement [27]. This puts extra demand on the pre-analytical and analytical processes of relevance to blood biomarker measurements for CNS diseases.

Amyloid beta

A β ₁₋₄₀, A β ₁₋₄₂ and A β _{1-42/1-40} as amyloid biomarkers in CSF

A β in CSF is already well established as a biomarker for AD. A β is produced when amyloid precursor protein (APP) is processed along its plaque-forming (amyloidogenic) pathway. In this pathway, APP undergoes cleavage, first by β -secretase followed by γ -secretase, to produce an

A β peptide [28]. The length of the A β peptide is dependent on the site (or extent) of γ -secretase cleavage [29]. While A β peptides of varying amino acid lengths can be produced, the most abundant isoforms in CSF are A β ₁₋₃₈, A β ₁₋₄₀ and A β ₁₋₄₂ [30], with A β ₁₋₄₀ and A β ₁₋₄₂ being the most widely studied isoforms. All A β peptides differ in amino acid sequence mainly at the C terminus [31].

Initial studies looking at total CSF A β in AD compared with controls had mixed results. While some showed a slight decrease in AD [32-35], others found no change in total CSF A β concentration in AD compared with controls [36-38]. A major shift occurred following the discovery of A β ₁₋₄₀ and A β ₁₋₄₂ and the development of assays that are specific to these peptides. Investigations into the key differences between them revealed that A β ₁₋₄₂ is more hydrophobic and hence is more prone to aggregation than A β ₁₋₄₀ [31]. Furthermore, CSF concentrations of A β ₁₋₄₀ remain unchanged in AD, whereas CSF concentrations of A β ₁₋₄₂ decrease [39-41], suggesting that of the two, A β ₁₋₄₂ provides a better biomarker for AD.

While CSF A β ₁₋₄₂ concentrations have proven invaluable in diagnosing patients with probable AD dementia, A β ₁₋₄₂ concentrations are to some extent dependent on the total A β concentrations of each patient [42]. Although it is necessary to have a threshold concentration of CSF A β ₁₋₄₂ concentrations, below which an AD diagnosis is likely, inter-individual differences make these thresholds somewhat arbitrary. Looking at CSF A β ₁₋₄₂ concentrations alone may result in some patients being misdiagnosed as 'normal' when in fact concentrations may be abnormally low, if the CSF results had been related to their overall A β production and vice versa [43]. Harnessing the fact that CSF A β ₁₋₄₀ concentration is not altered in AD, but instead may provide a useful index of an individual's rate of A β production more generally,

using CSF $A\beta_{1-42/1-40}$, may improve the reliability of results compared to using CSF $A\beta_{1-42}$ alone. Lewczuk *et al.* [40] found measuring CSF $A\beta_{1-42/1-40}$ alongside $A\beta_{1-42}$ to improve diagnostic accuracy when comparing patients with AD to either controls or those with non-AD dementias. Although the differences in diagnostic accuracy between $A\beta_{1-42/1-40}$ and $A\beta_{1-42}$ were not statistically significant, likely due to low patient numbers. Additionally, Slaets *et al.* [41] reported that the addition of CSF $A\beta_{1-42/1-40}$ to a biomarker panel for AD diagnosis consisting of $A\beta_{1-42}$, $A\beta_{1-40}$ and tau phosphorylated at threonine 181 (p-tau₁₈₁) significantly improved diagnostic accuracy compared with the same panel without $A\beta_{1-40}$ and $A\beta_{1-42/1-40}$. However, it is worth noting that they observed no statistically significant difference in the area under the receiver operating characteristic curves between $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$. Furthermore, Struyfs *et al.* [30] and Bousiges *et al.* [44] both found that the addition of $A\beta_{1-42/1-40}$ improved the ability to differentiate AD from non-AD dementias, particularly frontotemporal lobe dementia and dementia with Lewy bodies. In non-shunted normal pressure hydrocephalus, all $A\beta$ peptides are reduced in CSF and measuring CSF $A\beta_{1-42}$ alone would result in a false positive, while the $A\beta_{1-42/1-40}$ corrects for this [45]. Finally, the concordance of CSF $A\beta_{1-42/1-40}$ with amyloid PET is higher than for CSF $A\beta_{1-42}$ alone [46], and the use of $A\beta_{1-42/1-40}$ mitigates against adsorption effects that could lead to falsely low $A\beta_{1-42}$ [47,48]. These studies clearly highlight the important role CSF $A\beta_{1-42/1-40}$ plays in detecting $A\beta$ pathology in AD.

$A\beta_{1-40}$, $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$ as amyloid biomarkers in blood

Building on the success of CSF $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$ in diagnosing AD, $A\beta$ is an attractive blood-based biomarker of AD because it easily crosses the BBB [49]. However, early investigations into the use of plasma $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$ as predictors of future AD development showed inconsistent results, with some reporting that high plasma $A\beta_{1-42}$ concentrations or a high $A\beta_{1-42/1-40}$ are risk factors for AD development, while others reported the opposite, and still others reported no significant differences in plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ between AD cases and controls [50-54]. The potential reasons for this include the following: the limited analytical sensitivity of the enzyme-linked immunosorbent assay (ELISA)-based techniques in use at the time; sub-optimal or variable sample handling protocols; and, in many cases, the use of

clinical criteria for diagnosis rather than evidence for $A\beta$ pathology.

Recent advances in immunoassay technology to detect and quantify single protein measurements have increased their analytical sensitivity and have made it possible to quantify protein biomarkers at subfemtomolar concentration levels. There have been three main developments that have allowed for this. One has been to replace the enzyme label of the detection antibody with a molecule that emits light upon an electrochemical reaction, so-called electrochemiluminescence (ECL) [55]. The second is a refinement of the basic ELISA technology, so-called single molecule array (Simoa), compartmentalizing the detection reaction within femtolitre-sized wells using magnetic beads onto which the immunocomplexes are captured, and digitalizing protein detection [56-58]. The final advancement has been the development of sensitive mass spectrometry (MS)-based assays to quantify plasma $A\beta$ peptides [59]. These technological advances have led to breakthroughs in efforts to detect and quantify $A\beta$ present in peripheral blood.

A study by Janelidze *et al.* [25], which used ultrasensitive Simoa immunoassay technology to measure plasma $A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations, found slight but significant correlations between plasma and CSF measurements of these analytes, but not of $A\beta_{1-42/1-40}$. Furthermore, plasma $A\beta_{1-40}$, $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$ were all significantly decreased in AD patients compared with controls and patients with either mild cognitive impairment (MCI) or subjective cognitive decline (SCD). This was also observed in CSF, but the differences in CSF were much more pronounced. Additionally, plasma $A\beta_{1-42/1-40}$ was lower in patients with MCI compared with both SCD and controls. The results from this study are in line with those seen in Rembach *et al.* [60], Jessen *et al.* [61] and Pesaresi *et al.* [62] and have been replicated by Vergallo *et al.* [63]. In addition to observing similar results to those above, Palmqvist *et al.* [64] showed that plasma $A\beta_{1-40}$, $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$ can accurately predict cerebral $A\beta$ deposition. Of particular importance is a cross-sectional study conducted by Palmqvist *et al.* [65], which highlights that plasma $A\beta_{1-40}$, $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$ reflect the changes seen in CSF, albeit not as dynamically, and that CSF and plasma $A\beta$ alterations precede positive amyloid PET findings. While Chatterjee *et al.* [66] did not observe a significant difference in plasma

$A\beta_{1-40}$ and $A\beta_{1-42}$ concentrations between the $A\beta$ -positive ($A\beta^+$) and $A\beta$ -negative ($A\beta^-$) groups, perhaps due to their small sample size, they did observe a significantly lower plasma $A\beta_{1-42/1-40}$ in the $A\beta^+$ group compared to the $A\beta^-$. Finally, in a study which observed the utility of blood biomarkers without classification of CSF and PET, Simrén *et al.* [67] demonstrated significantly lower $A\beta_{1-42/1-40}$ in AD patients compared with MCI and controls, however no change between MCI and controls. Interestingly, $A\beta_{1-42/1-40}$ was associated with longitudinal change in grey matter volume, which is more strongly seen in cognitively unimpaired (CU) individuals than impaired patients.

Similar success in blood $A\beta$ measurements has been observed using MS, which, due to detecting analyte ions (or gas-phase-produced fragments thereof) at their specific mass-to-charge ratio with high accuracy, has a greater analytical specificity and selectivity compared with immunoassays. An important difference compared with immunoassays is that while MS methods for plasma $A\beta$ rely on antibodies for enrichment of the low abundance of $A\beta$ peptides, quantification in MS is antibody-independent, as the stable isotope-labelled synthetic $A\beta$ peptide analogues, that are used as internal standards, are co-enriched with the endogenous peptides [68]. Furthermore, because samples analysed by MS are typically handled under denaturing conditions, in aqueous-organic solvents, results are less influenced by matrix effects [69-71]. Of note, Ovod *et al.* [72] highlighted that the half-life of $A\beta$ in plasma is one third that of CSF $A\beta$. Additionally, they observed lower absolute concentrations of plasma $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$ in the blood of $A\beta^+$ individuals, suggesting that plasma $A\beta$ concentrations correlate positively with CSF $A\beta$. Furthermore, Nakamura *et al.* [59] showed that plasma $A\beta_{1-42}$ and $A\beta_{1-42/1-40}$ accurately predicted amyloid PET positivity and negativity in two separate data sets, highlighting that plasma $A\beta$ is inversely proportional to brain $A\beta$ burden. Schindler *et al.* [73] observed similar results; however, they only saw a 10-15% change in plasma $A\beta_{1-42/1-40}$ between amyloid PET-positive and PET-negative individuals, whereas in CSF, this change is 50%. Interestingly, direct (same-sample) comparison of Simoa and MS-based quantification of $A\beta_{1-40}$ and $A\beta_{1-42}$ in a preclinical cohort suggests that the correlation with brain amyloid pathology is higher with MS than with Simoa, at least at this stage of disease [74].

Despite this array of positive results, the contradictory results observed by other studies investigating plasma $A\beta$ cannot be ignored. Consistent with early investigations into plasma $A\beta$, Giedraitis *et al.* [75] and Tamaoka *et al.* [76] reported no association between plasma $A\beta_{1-40}$ or $A\beta_{1-42}$ concentrations and AD pathology. Both Hansson *et al.* [77] and Lövheim *et al.* [78] agree with this finding, with Hansson *et al.* [77] also finding no correlation between plasma and CSF $A\beta$. One possible explanation for the contradictory results is the inter-study variation in pre-analytical practices [66], which has been addressed by the development of a standardized guideline for pre-analytical variables in AD blood-based biomarker research in 2015 [79]. Importantly, discrepancies between blood and CSF biomarkers may reflect sampling issues in both. A systematic review conducted by Hansson *et al.* [80] in 2018, looking at the variation in pre-analytical methods for handling CSF samples prior to AD biomarker measurement, revealed a broad range of protocols was used in the 49 studies investigated. Out of the 15 variables assessed, the only two variables that remained consistent were the storage conditions (-80°C) and the lumbar puncture sampling location (L3-5). In some cases, these variations have a significant effect on the biomarkers of interest and hence on results obtained from the study. For example, CSF $A\beta_{1-42}$ is significantly affected by storage tube type [81-83], and some studies have found that centrifuging CSF samples prior to analysis may cause significant reductions in CSF $A\beta_{1-42}$, likely due to the high propensity of $A\beta_{1-42}$ to aggregate [84,85]. While Hansson *et al.* [80] focussed their review towards CSF samples and have recently published an updated standardized pre-analytical protocol for measuring AD biomarkers in CSF [86], the results obtained in their 2018 review highlight the need for universal pre-analytical protocols, not only for CSF, but also for blood sample handling.

Alternatively, these contradictory results may be due to the variation in patient cohort characteristics between studies. $A\beta$ concentrations vary depending on the patient's stage of disease, which reflects the increasing plaque burden as the disease progresses. This, combined with the fact that $A\beta$ is ubiquitously expressed in extra-cerebral tissues, may explain the variations in results obtained when investigating plasma $A\beta$ concentrations. Indeed, a large proportion of plasma $A\beta$ is not brain-derived, resulting in a much lower (10-15%) reduction in plasma $A\beta_{1-42/1-40}$ compared with CSF

A $\beta_{1-42/1-40}$ (50%) [73]. A final possible explanation for the variation in results may be matrix effects caused by plasma proteins in the blood [87]. These matrix effects can be limited by the dilution of the plasma sample prior to analysis [25]. In fact, several studies have now shown that A $\beta_{1-42/1-40}$ reflects cerebral A β pathology, provided it is determined using methods which minimize matrix effects, such as MS [59,72,88]. However, variations in analytical protocols and instruments used mean that this is not always done, resulting in greater interference caused by other molecules present in the plasma, and hence greater variation in results obtained across different studies.

Finally, some of the improvements in diagnostic performance recorded for plasma A β tests during recent years may be due to improved diagnostic work-up of the study participants so that most of them have been classified as A β^+ or A β^- based on CSF or PET biomarkers. This has made it less likely that the control group contains individuals with preclinical amyloid pathology and that the AD group contains individuals with cognitive deterioration, having already ruled out non-AD neurodegenerative diseases. Studies in memory clinic or population-based cohorts without prior stratification by CSF or PET biomarkers should ascertain the true diagnostic potential of plasma A β , giving insight into its real-world use.

A β_{1-43} as a potential amyloid biomarker

While A β_{1-40} and A β_{1-42} remain the two most widely studied isoforms of A β , longer A β peptides, including A β_{1-43} , have been observed within the brains of AD patients. Early investigations into A β revealed that although A β_{1-42} is the most abundant A β peptide in plaques, A β_{1-43} comprises a minor component, with A β_{1-40} predominantly being present in cerebral microvessels rather than in parenchymal plaques [89,90]. However, recent studies have shown that A β_{1-43} may play a greater role in AD than previously thought. Parvathy *et al.* [91] found that both A β_{1-42} and A β_{1-43} are associated with early disease progression, with deposition of both peptides being observed prior to AD diagnosis. Additionally, in mouse models of familial AD (FAD), Saito *et al.* [92] showed that not only does A β_{1-43} have a greater propensity to aggregate and is more neurotoxic than A β_{1-42} , but it also accumulates in AD brains more frequently than A β_{1-40} , observations which are supported by the findings of Welander *et al.* [93] and Keller *et al.* [94]. Furthermore, Jäkel *et al.* [95] observed a

positive correlation between A β peptide length and plaque load (A β_{1-43} > A β_{1-42} > A β_{1-40}). These results deviate somewhat from the observations of Iizuka *et al.* [89], who found A β_{1-42} to be the major component of plaques, with A β_{1-43} being a minor component, and A β_{1-40} only being present in cerebrovascular amyloid. These differences in results are possibly due to the very small cohort size used by Iizuka and colleagues. Similarly, Perrone *et al.* [29] found CSF A β_{1-43} to have a positive correlation with A β_{1-42} concentrations, with CSF A β_{1-43} concentrations being significantly reduced in FAD mutation carriers. These studies highlight that A β_{1-43} plays a role in AD, albeit less well investigated.

Despite the above evidence, there remains very little published literature on attempts to produce a functioning biomarker assay for A β_{1-43} in AD. One reason for this is that A β_{1-43} has a very similar diagnostic accuracy to CSF A β_{1-42} ; hence, it is unlikely to provide additional diagnostic value over existing biomarkers [96,97]. However, A β_{1-43} may prove useful in differentiating between different groups of AD patients. One study observed a significantly greater reduction in CSF A β_{1-43} , but not A β_{1-42} , in early-onset AD compared with late-onset AD [97], while another study showed that A β_{1-43} , but not A β_{1-42} , could identify amnesic MCI patients who progressed to AD [98]. In addition, Lauridsen *et al.* [98] observed a significant decrease in CSF A β_{1-43} over the 2-year follow-up period, with no significant difference seen in CSF A β_{1-42} concentrations. It is clear that A β_{1-43} plays a role in AD; hence, there is a need to investigate this peptide further, particularly in blood.

Phosphorylated tau

Tau is a microtubule-associated protein that is a natural component of healthy, mature neurones [99]. A very small percentage of tau may be phosphorylated in healthy individuals. However, in AD, tau is 3-4 times more phosphorylated and aggregates intraneuronally into NFTs composed predominantly of p-tau [99-101]. Tau was first identified as a CSF biomarker for AD in 1993 using ELISA [102]. Since 1993, ELISA methods for measuring t-tau that detect all tau isoforms, irrespective of their phosphorylation, have been developed. Along with the 6 different isoforms of tau in the CNS, produced by alternate splicing, there are up to 85 possible tau phosphorylation sites [103]. Studies have revealed that the concentration of

p-tau in CSF accurately depicts the extent of p-tau deposition within the AD brain [104], and in contrast to t-tau, there is essentially no change in concentrations of certain p-tau species in other neurological conditions like acute stroke [105] or Creutzfeldt–Jakob disease (CJD) [106], nor in other tauopathies and neurodegenerative diseases [107–111]. This suggests that several p-tau species are specific to AD when measured in biofluids, and can be used to distinguish AD from other neurodegenerative disorders. It is thought that both p-tau and t-tau increase in CSF as a direct response to A β pathology, as opposed to being markers of neuronal loss, as previously assumed [88,112]. Rather, it may be the resultant tau pathology caused by A β -induced tau secretion that causes neurodegeneration in AD, since neurodegeneration and cognitive loss do not occur in the absence of tau [113]. This is consistent with earlier studies in mouse models, which show increases in CSF endogenous murine tau concentration without evidence of neuronal loss in APP transgenic mice [114]. In addition to phosphorylation, increasing evidence indicates that both N-glycosylation and O-glycosylation are implicated in AD, emphasized by the fact that tau carries potential N-glycosylation and O-glycosylation sites [115]. However, no established biomarkers to study the pathophysiological relevance of this in humans exist yet. In this section, we will discuss tau phosphorylated at three sites – threonine 181 (p-tau₁₈₁), threonine 217 (p-tau₂₁₇) and threonine 231 (p-tau₂₃₁).

p-tau181, 217 and 231 as tau biomarkers in CSF

Early studies looking at CSF p-tau concentrations in AD using ELISA revealed that irrespective of which p-tau epitope was measured, p-tau is significantly elevated in AD compared with age-matched CU controls, as well as patients with non-AD dementias [109,110,116,117,118]. Further investigations into the efficacy of combining p-tau measurements with CSF A β ₁₋₄₂ and/or A β _{1-42/1-40}, and CSF t-tau have led to CSF p-tau, particularly p-tau₁₈₁, being included in the AT(N) criteria for AD diagnosis and the NIA-AA research framework for defining AD [9,20]. However, more recently, there has been question as to whether certain p-tau epitopes function better than others as AD biomarkers.

Of all the p-tau epitopes, immunoassays detecting CSF p-tau₁₈₁ are by far the most widely studied. Unless otherwise specified, 'p-tau' is almost always assumed to refer to mid-region p-tau₁₈₁ [119,120].

However, CSF is known to predominantly contain a mixture of both N-terminal and mid-region tau fragments, with C-terminal fragments being relatively scarce [121–123]. CSF p-tau₁₈₁ has proven useful in differentiating AD from controls and other tauopathies and neurodegenerative diseases, while also predicting cognitive decline in preclinical cases of AD [124–126]. However, in 2020, two separate studies – one using ELISA [127] and the other using MS [128] – observed that CSF p-tau₂₁₇ displayed a larger-fold change with AD pathology than p-tau₁₈₁. A third study concluded that CSF p-tau₂₁₇ serves as a better marker of cognitive decline than CSF p-tau₁₈₁ [129], and a fourth study, using a novel ultrasensitive immunoassay on the Simoa platform, observed much less overlap between diagnostic groups (AD vs controls and amyloid PET-positive vs amyloid PET-negative) with p-tau₂₁₇ than with p-tau₁₈₁ [130]. In summary, these studies argue that p-tau₂₁₇ is the superior tau pathology biomarker; therefore, it should be used more widely in clinical practice. Both Janelidze *et al.* [127] and Barthelemy *et al.* [128] observed that while CSF p-tau₁₈₁ clearly distinguished AD from the non-AD groups studied, CSF p-tau₂₁₇ more markedly distinguished between the groups, and it showed a stronger correlation with tau PET and amyloid PET in AD patients.

To investigate these results further, Karikari *et al.* [131] conducted a head-to-head comparison of novel CSF p-tau₂₁₇ and p-tau₁₈₁ biomarkers, containing the N-terminal amino acid 6–18 epitope (N-p-tau₂₁₇ and N-p-tau₁₈₁, respectively), with the performance of already established p-tau₁₈₁ biomarkers, which target the mid-region epitopes (mid-p-tau₁₈₁), in AD and MCI patients in three cohorts. In their two validation cohorts, N-p-tau₂₁₇ and N-p-tau₁₈₁ increased in MCI-AD patients, whereas mid-p-tau₁₈₁ remained within normal range. Additionally, N-p-tau₂₁₇ and N-p-tau₁₈₁ both equally identified increased A β pathology and differentiated MCI-AD from non-AD MCI and A β ⁻ CU individuals significantly better than mid-p-tau₁₈₁. The performance of N-p-tau₂₁₇ and N-p-tau₁₈₁ was virtually indistinguishable from one another, suggesting that CSF p-tau₂₁₇ may not be a more accurate biomarker for AD pathology, but rather it functions better than the p-tau₁₈₁ biomarkers to which it was compared to – mid-p-tau₁₈₁. Furthermore, N-p-tau₂₁₇ and N-p-tau₁₈₁ both increase in synchrony with A β pathology changes, whereas mid-p-tau₁₈₁ increases at a later

disease stage [120,131,132]. Interestingly, Emeršič *et al.* [133] found CSF p-tau₂₁₇ to also be elevated in both AD and CJD, suggesting that p-tau₁₈₁ is more specific to AD, and may serve to better confirm AD diagnosis.

Studies looking at CSF p-tau₂₃₁ have shown huge promise, with early investigations finding CSF p-tau₂₃₁ to identify AD with 85% sensitivity and 97% specificity [118], and more recent studies observing a more prominent increase in CSF mid-p-tau₂₃₁ in AD compared with a gold standard mid-p-tau₁₈₁ immunoassay [120]. Of particular importance is a study conducted by Ashton *et al.* [134], which observed that compared with CSF p-tau₁₈₁ and p-tau₂₁₇, CSF p-tau₂₃₁ was more sensitive to the earliest changes in parenchymal A β pathology before amyloid PET positivity had occurred.

P-tau181, 217 and 231 as tau biomarkers in blood

The challenges of measuring biomarkers of brain diseases in the blood have already been mentioned above. Previously, the low concentrations of tau in blood made it difficult to measure. However, the development of ultrasensitive immunoassay technologies has mitigated these difficulties [17]. Nonetheless, there remains one specific challenge which appears to be particularly problematic for tau. Tau is extremely stable in CSF, whereas in blood, it has a very short half-life (~10h) [88]. This could be due to proteases causing an increased rate of tau degradation [27,88]. Indeed, several studies investigating plasma tau clearance following hypoxic brain injury have highlighted the efficient clearance mechanisms of tau in blood [135,136]. However, it is possible to minimize tau degradation by adopting fast and efficient pre-analytical sample processing measures.

In one of the first studies of its kind, Shekhar *et al.* [137] attempted to quantify serum p-tau₁₈₁ in a small pilot study, consisting of AD dementia, MCI and control groups. They observed an elevated concentration of p-tau₁₈₁ in both the AD and MCI groups compared to controls, as well as in AD compared to MCI. Shortly after, in another pilot study, Tatebe *et al.* [138] attempted to quantify plasma p-tau₁₈₁ in AD dementia, Down syndrome (DS) and control groups, using a novel p-tau₁₈₁ Simoa assay which detects N-p-tau₁₈₁. They observed a significantly higher concentration of p-tau₁₈₁ in both the AD and DS groups compared to their respective age-matched controls, as well as a strong correlation between plasma and CSF p-

tau₁₈₁ concentrations. These findings have been further corroborated by other studies in CU individuals and those with AD dementia, MCI and non-AD dementias [139-143]. In a much larger-scale study, Mielke *et al.* [139] found that plasma p-tau₁₈₁ was more strongly associated with A β and tau PET imaging than plasma t-tau, and more sensitively and specifically predicted increased brain A β concentrations. This was further corroborated in a recent multi-centre study conducted by Karikari *et al.* [143], which showed that not only can p-tau₁₈₁ identify AD with high diagnostic accuracy, but it also increases minimally in individuals diagnosed with AD but who are amyloid PET-negative, and increases more prominently in individuals with decreased CSF A β prior to amyloid PET positivity. Moreover, Janelidze *et al.* [140] showed that plasma p-tau₁₈₁ can accurately predict future progression to AD dementia in individuals who were initially CU. In a longitudinal study, Lantero-Rodriguez *et al.* [144] observed that plasma p-tau₁₈₁ accurately predicts AD pathology and discriminates between AD and non-AD pathology, at least 8 years prior to death and subsequent neuropathological diagnosis. Similarly, O'Connor *et al.* [145] observed, in their longitudinal study of FAD, that plasma p-tau₁₈₁ concentrations were higher in mutation carriers than non-carriers from 16 years prior to estimated symptom onset. Furthermore, Moscoso *et al.* [146] have recently shown that longitudinal changes in plasma p-tau₁₈₁ are associated with longitudinal neurodegeneration in AD-specific brain regions, as measured by FDG-PET and grey matter volume. Together, this evidence suggests plasma p-tau₁₈₁ poses a promising blood-based biomarker for both AD diagnosis and for patient recruitment into clinical trials. Furthermore, it may provide longitudinal information relating to AD-specific neurodegeneration that could be employed as a treatment response measure in therapeutic clinical trials.

Studies into the utility of plasma p-tau₂₁₇ in AD diagnosis began relatively recently but have had promising results. An investigation into core CSF and blood AD biomarkers in relation to amyloid PET revealed that plasma and CSF p-tau₂₁₇ concentrations change simultaneously [65]. Following on from this, one cohort study found plasma p-tau₂₁₇ to be increased in CU individuals with abnormal (i.e. positive) amyloid PET but normal tau PET, suggesting changes in plasma p-tau₂₁₇ precede the detectability of insoluble tau aggregates by tau PET [147]. Before conclusions can be

made as to whether plasma p-tau₂₁₇ will function as a useful biomarker for early AD pathology, investigations must first be conducted to compare plasma p-tau₂₁₇ in AD with other neurodegenerative diseases, particularly CJD, since CSF p-tau₂₁₇ was found to be increased in this condition [133].

A recent study also demonstrates the high diagnostic performance of p-tau₂₃₁ in blood [148]. While at the cognitive impairment stage p-tau₁₈₁ and p-tau₂₃₁ are seemingly similar in diagnostic accuracy, the p-tau₂₃₁ epitope begins to increase early in the preclinical stage of the disease, similar to the findings in CSF [148]. The early increase is suggested to be a response to accumulating amyloid pathology under a threshold of amyloid PET positivity.

Neurodegeneration

T-tau as a neurodegeneration biomarker in CSF

CSF t-tau in AD has been proposed to reflect the severity of A β -induced neurodegeneration and neuronal or axonal injury [49,140]. As with p-tau, high concentrations of t-tau have been observed consistently in AD patients [119]. Changes in CSF t-tau are not specific to AD, as t-tau is also increased in other cases of neuronal injury, including stroke, traumatic brain injury (TBI) and CJD [49]. However, recent studies have suggested that the t-tau being measured in AD biofluids is secreted alongside p-tau, and reflects A β -induced tau secretion from living neurones [112]. While these neurones will eventually degenerate and die, the t-tau being measured in AD is not thought to be a direct marker of this [149]. In contrast, the high CSF t-tau with normal CSF p-tau, measured in conditions like stroke, TBI and CJD, is a direct result of massive neuronal death, and in these cases, t-tau is a marker of neuronal injury [149]. Therefore, in combination with raised p-tau, increased CSF t-tau does reflect AD pathology, rather than simply being a non-specific effect of neuronal damage.

T-tau as a neurodegeneration biomarker in blood

One of the earliest studies investigating plasma t-tau in AD yielded discouraging results, reporting no significant increase in plasma t-tau being seen in AD compared to non-AD dementias [150]. However, this study was most likely limited by the low sensitivity of the ELISA technology used. Since the development of more sensitive ELISA

technology, particularly through the use of Simoa, numerous studies have reported increased plasma t-tau concentrations in AD [17,136,151,152], with some observing a strong correlation between plasma and CSF t-tau [151], and others observing a weak [152] or absent correlation [136]. Furthermore, one study reported reduced plasma t-tau concentrations in AD [153]. While the general consensus is that plasma t-tau concentrations increase in AD, Zetterberg *et al.* [136], Dage *et al.* [17] and Mattsson *et al.* [152] all observed significant overlap in plasma t-tau ranges between their AD and non-AD groups, including age-matched CU controls. An additional study found an association between elevated plasma t-tau concentrations and cognitive decline; however, this was independent of elevated brain A β [154]. It is possible that the inconsistent results thus far in measuring plasma t-tau may be due to the currently available assays measuring a form of tau that is particularly susceptible to protease degradation [140]. Interestingly, Pase *et al.* [155] showed in a multi-centre study that plasma t-tau can act as a risk-stratifier for progression to AD dementia. One strength of this study was *post-mortem* correlation with tau pathology observed in a subset of the cohorts investigated. Nonetheless, the current evidence suggests plasma t-tau may not be a useful diagnostic blood biomarker for AD, but high concentrations may provide prognostic evidence of incident neurodegeneration, similar to the performance of a t-tau assay using N-terminal anti-tau antibodies which were recently described [156,157].

NfL as a neurodegeneration biomarker in CSF

Neurofilaments are an important structural component of the neuronal cytoskeleton [158], and one specific subunit of neurofilaments, NfL, is primarily expressed in large-calibre myelinated axons [159]. Increased CSF NfL concentrations have been associated with white matter lesions and subcortical brain damage in AD [160], as well as other neurodegenerative and non-neurodegenerative diseases [161]. Hence, NfL is not specific to AD, but it functions as an excellent biomarker for neuronal death and axonal loss. Furthermore, CSF NfL concentrations are significantly increased in AD compared to CU controls, serving as an accurate marker of progression from MCI to AD and reflecting neurodegeneration independent of A β pathology [119,161,162,163,164].

NfL as a neurodegeneration biomarker in blood

Interest in NfL as a blood biomarker came about in relation to longitudinal studies, due to blood being easier to sample serially than CSF. Following the development and validation of the first assay to reliably measure serum NfL concentrations in 2013 using ECL [165], more sensitive assays have been developed using Simoa technology [166]. Indeed, in a comparison between three analytical platforms – ECL, standard ELISA and Simoa – Simoa was found to be the most sensitive at quantifying serum NfL concentrations [167]. Using this ultrasensitive Simoa assay, Mattsson *et al.* [166] showed for the first time that plasma NfL correlates with CSF NfL, but also with other hallmarks of AD. Furthermore, blood NfL has high diagnostic accuracy for AD, and it is increased prior to symptom onset, making it a promising biomarker for neuronal injury in this disease. These results have since been corroborated by the vast majority of studies across both sporadic and familial disease [168–173], with Schultz *et al.* [172] observing that similar to CSF NfL, plasma NfL concentrations correlate with white matter damage in the brain, and Ashton *et al.* [173] demonstrating that plasma NfL correlates strongly with the severity of NFT pathology in AD seen in *post-mortem* analysis. Due to the lack of specificity of NfL for AD, its value is unlikely to be in differentiating AD from other neurodegenerative diseases, but rather to distinguish neurodegeneration (including AD) from non-degenerative causes of cognitive impairment (e.g. primary psychiatric causes) [174,175]. Additionally, it can be used as a non-invasive screening tool to identify patients at risk of cognitive decline, as well as a dynamic biomarker to monitor treatment efficacy and to track disease progression.

T-tau vs. NfL as neurodegeneration biomarkers in AD

Both t-tau and NfL are useful markers of neurodegeneration in AD. CSF t-tau has the added advantage of correlating with A β pathology changes [88,112], which is not the case for CSF NfL [176]. However, the evidence presented suggests that NfL translates better into a blood biomarker for AD neurodegeneration than t-tau. Indeed, plasma NfL is robust to even a 48-h delay in centrifugation of whole blood, in contrast to the known issues with plasma tau being susceptible to degradation by proteases [177]. Therefore, it is possible that plasma NfL may replace t-tau in an initial blood-based diagnostic work-up for AD to confirm the presence of neurodegeneration, followed by CSF t-

tau being used in tertiary centres to aid the confirmation of A β -induced neurodegeneration.

An integrated hypothesis for AD pathogenesis

AD is an extremely complex disease. To date, research has shown that microglia are the primary mediators of neuroinflammation in AD brains. However, the role of neuroinflammation in AD pathogenesis remains highly debated. Some papers argue that neuroinflammation is neuroprotective, designed to clear A β plaques, while others argue that it is neurotoxic by promoting AD progression through cytokine release, phagocytosis of synapses and consequent neurodegeneration [178–182]. Furthermore, one review argues that microglia play both a neuroprotective and a neurodegenerative role, depending on the stage of AD [183].

In their recent review, Edwards [113] proposed a unifying hypothesis for AD pathogenesis, whereby they suggest the primary driver for AD progression following amyloid plaque deposition and A β -induced synaptic damage is an inadequate microglial response. The authors introduce the idea that the magnitude with which microglia respond increases with disease progression, proposing that microglia are responsible for removing damaged synapses and hence play a neuroprotective role in AD. Consequently, this protective role of microglia prevents damage from propagating down the axon, thus breaking the cycle of A β -induced synaptic dystrophy. This provides an alternative explanation for why some elderly individuals without dementia are found to have a similar burden of plaques and tangles to that seen in patients with clinically advanced AD at *post-mortem* [184]. In essence, the plaque load an individual can tolerate prior to neurodegeneration occurring may be dependent on the genetic characteristics of their microglia, which determines the rate at which damaged synapses are phagocytosed [113].

A number of pathological mechanisms are addressed by Edwards [113], each of which present proteins which could function as fluid biomarkers for AD. In addition to A β , tau and NfL, these mechanisms and corresponding biomarkers include (1) markers of low-level A β release (glutamate); (2) markers of dystrophic synapses (neurogranin, SNAP-25, synaptotagmin); (3) markers of microglial activation (TREM-2, YKL-40); and (4) complement-mediated synapse loss (complement

proteins, *e.g.* C3). Tests for some of these proteins have shown promising results in CSF studies [185–188], but translating them into blood tests will be difficult. Investigations have revealed that neurogranin [189] and soluble TREM-2 [190] do not function well as blood biomarkers for AD. Additionally, YKL-40 was found to be significantly increased in the AD and MCI groups compared to controls [191]. However, there was a significant overlap between the groups, and it did not correlate with CSF A β_{1-42} or CSF p-tau $_{181}$. The proteins discussed by Edwards [113] are highly expressed in extra-cerebral tissues. Consequently, any brain-derived signal in blood is likely to be overwhelmed by release of proteins from other tissues.

Conclusion

In conclusion, we have considered biomarkers which have the potential to be translated into blood biomarkers for AD. In particular, plasma p-tau $_{181}$ and NFL show huge promise, with both having significant evidence highlighting that assays for these markers work in both research laboratories and in specialist settings. Plasma NFL could potentially screen for a range of pathologies, not just AD, and act as a therapy response marker. As plasma p-tau $_{181}$ reflects both amyloid and tau pathology, it would be applicable in differential diagnoses compared to other dementias, as well as potentially functioning as a therapy response marker, given the changes seen in longitudinal studies. However, prior to clinical implementation, plasma p-tau $_{181}$ requires further analysis comparing assays targeting N-terminal and mid-region p-tau $_{181}$.

Plasma A β would have value in early, or even pre-symptomatic, screening and recruitment to clinical trials. However, it would need cautious interpretation due to the prevalence of amyloid positivity increasing with age in individuals who will not develop AD in their lifetime. Nonetheless, the inter-laboratory variation in pre-analytical protocols has led to inconsistent plasma A β results. Therefore, a new standardized guideline for pre-analytical variables in AD blood-based biomarker research must be established for worldwide use, with implications for protocols which deviate from the proposed guideline.

Finally, plasma p-tau $_{217}$ and p-tau $_{231}$ studies look promising. However, more head-to-head comparisons of assays measuring different phospho-forms

of tau, using identical methods, are needed to reach a conclusion on which of these biomarkers most robustly separate AD from non-AD neurodegenerative dementias.

Given the rapidly changing field, it is unclear which of these biomarkers will ultimately prove most useful to answer different clinical and research questions. As is often the case with technical advances, there are associated ethical issues, including the fact that the ease of testing with blood-based measures may lead to inappropriate use, such as direct-to-consumer predictive testing without counselling or support being available. However, what is clear is that blood-based biomarkers are set to transform both clinical and research practice – and will have wide, even global, applicability.

Conflict of interest

DOTA has no conflicts of interest. JMS has received research funding and PET tracer from AVID Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly); has served as a consultant at advisory boards, or at data monitoring committees consulted for Roche, Eli Lilly, Biogen, Merck, GE and Axon Neuroscience SE; and is Chief Medical Officer for Alzheimer's Research UK. KB has served as a consultant at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, 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. NCF has served as a consultant at advisory boards, or at a data monitoring committee for Roche, Biogen and Ionis. HZ has served at scientific advisory boards for Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx; has given lectures in symposia sponsored by Celectricon, Fujirebio, Alzecure and Biogen; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

Funding

DOTA is supported by the International Journal of Experimental Pathology and the UK Dementia Research Institute at UCL. AOC acknowledges support from an Alzheimer's Society Clinical

Research Training Fellowship (AS-CTF-18-001) and previous support from the Rosetrees Trust. JMS acknowledges the support of the National Institute for Health Research, University College London Hospitals Biomedical Research Centre, the Medical Research Council and the Alzheimer's Association. KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer's 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), the European Union Joint Programme for Neurodegenerative Disorders (JPND2019-466-236) and the National Institute of Health (NIH), USA (#1R01AG068398-01). HZ 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), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement no 860197 (MIRIADE) and the UK Dementia Research Institute at UCL.

References

- DeKosky ST. Epidemiology and pathophysiology of Alzheimer's disease. *Clin Cornerstone*. 2001;**3**:15–26.
- Winner B, Kohl Z, Gage FH. Neurodegenerative disease and adult neurogenesis. *Eur J Neurosci*. 2011;**33**:1139–51.
- Stelzmann RA, Norman Schnitzlein H, Reed MF. An English translation of Alzheimer's 1907 paper, "über eine eigenartige erkrankung der hirnrinde". *Clin Anat*. 1995;**8**:429–31.
- Hardy J, Higgins G. Alzheimer's disease: the amyloid cascade hypothesis. *Science*. 1992;**256**:184–5.
- Selkoe DJ. Alzheimer's disease is a synaptic failure. *Science*. 2002;**298**:789–91.
- Coart E, Barrado LG, Duits FH, Scheltens P, van der Flier WM, Teunissen CE, et al. Correcting for the absence of a gold standard improves diagnostic accuracy of biomarkers in Alzheimer's disease. *J Alzheimers Dis*. 2015;**46**:889–99.
- World Health O. *International Programme on Chemical S. Biomarkers in Risk Assessment : Validity and Validation*. Geneva: World Health Organization; 2001.
- Atkinson AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;**69**:89–95.
- Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimer's Dement*. 2018;**14**:535–62.
- Beach TG, Monsell SE, Phillips LE, Kukull W. Accuracy of the clinical diagnosis of Alzheimer disease at national institute on aging Alzheimer disease centers, 2005–2010. *J Neuropathol Exp Neurol*. 2012;**71**:266–73.
- Sabbagh MN, Lue L-F, Fayard D, Shi J. Increasing precision of clinical diagnosis of Alzheimer's disease using a combined algorithm incorporating clinical and novel biomarker data. *Neurol Ther*. 2017;**6**:83–95.
- Eikelboom WS, van Rooij JGJ, van den Berg E, Coesmans M, Jiskoot LC, Singleton E, et al. Neuropsychiatric symptoms complicating the diagnosis of Alzheimer's disease: a case report. *J Alzheimers Dis*. 2018;**66**:1363–9.
- Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med*. 2012;**367**:795–804.
- Reiman EM, Quiroz YT, Fleisher AS, Chen K, Velez-Pardo C, Jimenez-Del-Rio M, et al. Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. *Lancet Neurol*. 2012;**11**:1048–56.
- Fagan AM, Shaw LM, Xiong C, Vanderstichele H, Mintun MA, Trojanowski JQ, et al. Comparison of analytical platforms for cerebrospinal fluid measures of β -Amyloid 1–42, total tau, and P-tau181 for identifying Alzheimer disease amyloid plaque pathology. *Arch Neurol*. 2011;**68**:1137–44.
- Villemagne VL, Burnham S, Bourgeat P, Brown B, Ellis KA, Salvado O, et al. Amyloid β deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet Neurol*. 2013;**12**:357–67.
- Dage JL, Wennberg AMV, Airey DC, Hagen CE, Knopman DS, Machulda MM, et al. Levels of tau protein in plasma are associated with neurodegeneration and cognitive function in a population-based elderly cohort. *Alzheimers Dement*. 2016;**12**:1226–34.
- Honig LS, Vellas B, Woodward M, Boada M, Bullock R, Borrie M, et al. Trial of Solanezumab for mild dementia due to Alzheimer's disease. *N Engl J Med*. 2018;**378**:321–30.
- Zetterberg H, Bähr M. Disease signatures: biomarkers/indicators of neurodegeneration. *Mol Cell Neurosci*. 2019;**97**:1–2.
- Jack CR, Bennett DA, Blennow K, Carrillo MC, Feldman HH, Frisoni GB, et al. A/T/N: an unbiased descriptive classification scheme for Alzheimer disease biomarkers. *Neurology*. 2016;**87**:539–47.
- Hampel H, Lista S, Teipel SJ, Garaci F, Nisticò R, Blennow K, et al. Perspective on future role of biological markers in clinical therapy trials of Alzheimer's disease: a long-range point of view beyond 2020. *Biochem Pharmacol*. 2014;**88**:426–49.
- Molinuevo JL, Ayton S, Batrla R, Bednar MM, Bittner T, Cummings J, et al. Current state of Alzheimer's fluid biomarkers. *Acta Neuropathol*. 2018;**136**:821–53.

- 23 Albani D, Marizzoni M, Ferrari C, Fusco F, Boeri L, Raimondi I, et al. Plasma A beta(42) as a biomarker of prodromal Alzheimer's disease progression in patients with amnesic mild cognitive impairment: evidence from the PharmaCog/E-ADNI Study. *J Alzheimers Dis.* 2019;**69**:37–48.
- 24 Shi L, Baird AL, Westwood S, Hye A, Dobson R, Thambisetty M, et al. A decade of blood biomarkers for Alzheimer's disease research: an evolving field, improving study designs, and the challenge of replication. *J Alzheimer's Dis.* 2018;**62**:1181–98.
- 25 Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, Vanwesten D, Jeromin A, et al. Plasma beta-amyloid in Alzheimer's disease and vascular disease. *Sci Rep.* 2016;**6**:26801.
- 26 Blennow K, Zetterberg H. Understanding Biomarkers of Neurodegeneration: Ultrasensitive detection techniques pave the way for mechanistic understanding. *Nat Med.* 2015;**21**:217–9.
- 27 Zetterberg H, Burnham SC. Blood-based molecular biomarkers for Alzheimer's disease. *Mol Brain.* 2019;**12**:26.
- 28 Cacace R, Sleegers K, Van Broeckhoven C. Molecular genetics of early-onset Alzheimer's disease revisited. *Alzheimer's Dement.* 2016;**12**:733–48.
- 29 Perrone F, Bjerke M, Hens E, Sieben A, Timmers M, De Roeck A, et al. Amyloid- β 1–43 cerebrospinal fluid levels and the interpretation of APP, PSEN1 and PSEN2 mutations. *Alzheimer's Res Therapy.* 2020;**12**:1–43.
- 30 Struyfs H, Van Broeck B, Timmers M, Fransens E, Sleegers K, Van Broeckhoven C, et al. Diagnostic accuracy of cerebrospinal fluid amyloid-beta isoforms for early and differential dementia diagnosis. *J Alzheimers Dis.* 2015;**45**:813–22.
- 31 Jarrett JT, Berger EP, Lansbury PT. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: Implications for the pathogenesis of Alzheimer's disease. *Biochemistry.* 1993;**32**:4693–7.
- 32 Farlow M. Low cerebrospinal-fluid concentrations of soluble amyloid β -protein precursor in hereditary Alzheimer's disease. *Lancet.* 1992;**340**:453–4.
- 33 Pirttilä T, Koivisto K, Mehta PD, Reinikainen K, Kim KS, Kilku O, et al. Longitudinal study of cerebrospinal fluid amyloid proteins and apolipoprotein E in patients with probable Alzheimer's disease. *Neurosci Lett.* 1998;**249**:21–4.
- 34 Tabaton M, Nunzi MG, Xue R, Usiak M, Auttilogambetti L, Gambetti P. Soluble amyloid β -protein is a marker of Alzheimer amyloid in brain but not in cerebrospinal fluid. *Biochem Biophys Res Commun.* 1994;**200**:1598–603.
- 35 Van Nostrand WE, Wagner SL, Shankle WR, Farrow JS, Dick M, Rozemuller JM, et al. Decreased levels of soluble amyloid beta-protein precursor in cerebrospinal fluid of live Alzheimer disease patients. *Proc Natl Acad Sci - PNAS.* 1992;**89**:2551–5.
- 36 Motter R, Vigo-Pelfrey C, Kholodenko D, Fransens E, Sleegers K, Van Broeckhoven C, et al. Reduction of β -amyloid peptide 42 in the cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol.* 1995;**38**:643–8.
- 37 Southwick PC, Yamagata SK, Echols CL, Higson GJ, Neynaber SA, Parson RE, et al. Assessment of amyloid β protein in cerebrospinal fluid as an aid in the diagnosis of Alzheimer's disease. *J Neurochem.* 2002;**66**:259–65.
- 38 van Gool WA, Kuiper MA, Walstra GJ, Wolters EC, Bolhuis PA. Concentrations of amyloid beta protein in cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol.* 1995;**37**:277–9.
- 39 Shoji M, Matsubara E, Kanai M, Watanabe M, Nakamura T, Tomidokoro Y, et al. Combination assay of CSF tau, A beta 1–40 and A beta 1–42(43) as a biochemical marker of Alzheimer's disease. *J Neurol Sci.* 1998;**158**:134–40.
- 40 Lewczuk P, Esselmann H, Otto M, Maler JM, Henkel AW, Henkel MK, et al. Neurochemical diagnosis of Alzheimer's dementia by CSF A beta42, A beta42/A beta40 ratio and total tau. *Neurobiol Aging.* 2004;**25**:273–81.
- 41 Slaets S, Le Bastard N, Martin J-J, Sleegers K, Van Broeckhoven C, De Deyn PP, et al. Cerebrospinal fluid A β 1–40 improves differential dementia diagnosis in patients with intermediate P-tau181P levels. *J Alzheimers Dis.* 2013;**36**:759–67.
- 42 Wiltfang J, Esselmann H, Bibl M, Hüll M, Hampel H, Kessler H, et al. Amyloid beta peptide ratio 42/40 but not A beta 42 correlates with phospho-Tau in patients with low- and high-CSF A beta 40 load. *J Neurochem.* 2007;**101**:1053–9.
- 43 Hansson O, Lehmann S, Otto M, Zetterberg H, Lewczuk P. Advantages and disadvantages of the use of the CSF Amyloid β (A β) 42/40 ratio in the diagnosis of Alzheimer's Disease. *Alzheimer's Res Therapy.* 2019;**11**:34.
- 44 Bousiges O, Cretin B, Lavaux T, Philippi N, Jung B, Hezard S, et al. Diagnostic value of cerebrospinal fluid biomarkers (phospho-Tau181, total-Tau, A β 42, and A β 40) in prodromal stage of Alzheimer's disease and dementia with lewy bodies. *J Alzheimers Dis.* 2016;**51**:1069–83.
- 45 Jeppsson A, Zetterberg H, Blennow K, Wikkelsø C. Idiopathic normal-pressure hydrocephalus Pathophysiology and diagnosis by CSF biomarkers. *Neurology.* 2013;**80**:1385–92.
- 46 Janelidze S, Pannee J, Mikulskis A, Chiao P, Zetterberg H, Blennow K, et al. Concordance between different amyloid immunoassays and visual amyloid positron emission tomographic assessment. *JAMA Neurol.* 2017;**74**:1492–501.
- 47 Willemse E, van Uffelen K, Brix B, Engelborghs S, Vanderstichele H, Teunissen C. How to handle adsorption of cerebrospinal fluid amyloid- β (1–42) in laboratory practice? Identifying problematic handlings and resolving the issue by use of the A β 42 /A β 40 ratio. *Alzheimer's Dement.* 2017;**13**:885–92.
- 48 Toombs J, Foiani MS, Wellington H, Paterson RW, Arber C, Heslegrave A, et al. Amyloid β peptides are differentially vulnerable to preanalytical surface exposure, an effect incompletely mitigated by the use of ratios. *Alzheimer's Diagnosis Assessment Dis Monitoring.* 2018;**10**:311–21.
- 49 Lee JC, Kim SJ, Hong S, Kim Y. Diagnosis of Alzheimer's disease utilizing amyloid and tau as fluid biomarkers. *Exp Mol Med.* 2019;**51**:1–10.
- 50 Mayeux R, Honig LS, Tang MX, Manly J, Stern Y, Schupf N, et al. Plasma A β 40 and A β 42 and Alzheimer's disease: relation to age, mortality, and risk. *Neurology.* 2003;**61**:1185–90.
- 51 Pomara N, Willoughby LM, Sidtis JJ, Mehta PD. Selective reductions in plasma A β 1–42 in healthy elderly subjects during longitudinal follow-up: a preliminary report. *Am J Geriatric Psychiatry.* 2005;**13**:914–7.
- 52 van Oijen M, Hofman A, Soares HD, Koudstaal PJ, Breteler MMB. Plasma A β 1–40 and A β 1–42 and the risk of dementia: a prospective case-cohort study. *Lancet Neurol.* 2006;**5**:655–60.

- 53 Graff-Radford NR, Crook JE, Lucas J, Boeve BF, Knopman DS, Ivnik RJ, *et al.* Association of low plasma A β 42/A β 40 ratios with increased imminent risk for mild cognitive impairment and Alzheimer disease. *Arch Neurol.* 2007;**64**:354–62.
- 54 Irizarry MC. Biomarkers of Alzheimer disease in plasma. *Neurotherapeutics.* 2004;**1**:226–34.
- 55 Li L, Chen Y, Zhu J-J. Recent advances in electrochemiluminescence analysis. *Anal Chem.* 2017;**89**:358–71.
- 56 Cohen L, Walt DR. Highly sensitive and multiplexed protein measurements. *Chem Rev.* 2019;**119**:293–321.
- 57 Kan CW, Tobos CI, Rissin DM, Wiener AD, Meyer RE, Svancara DM, *et al.* Digital enzyme-linked immunosorbent assays with sub-attomolar detection limits based on low numbers of capture beads combined with high efficiency bead analysis. *Lab Chip.* 2020;**20**:2122–35.
- 58 Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, *et al.* Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. *Nat Biotechnol.* 2010;**28**:595–9.
- 59 Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V, *et al.* High performance plasma amyloid- β biomarkers for Alzheimer's disease. *Nature.* 2018;**554**:249–54.
- 60 Rembach A, Faux NG, Watt AD, Pertile KK, Rumble RL, Trounson BO, *et al.* Changes in plasma amyloid beta in a longitudinal study of aging and Alzheimer's disease. *Alzheimer's Dement.* 2014;**10**:53–61.
- 61 Jessen F, Amariglio RE, van Boxtel M, Breteler M, Ceccaldi M, Chételat G, *et al.* A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. *Alzheimer's Dement.* 2014;**10**:844–52.
- 62 Pesaresi M, Lovati C, Bertora P, Mailland E, Galimberti D, Scarpini E, *et al.* Plasma levels of beta-amyloid (1–42) in Alzheimer's disease and mild cognitive impairment. *Neurobiol Aging.* 2006;**27**:904–5.
- 63 Vergallo A, Megret L, Lista S, Cavedo E, Zetterberg H, Blennow K, *et al.* Plasma amyloid beta 40/42 ratio predicts cerebral amyloidosis in cognitively normal individuals at risk for Alzheimer's disease. *Alzheimer's Dement.* 2019;**15**:764–75.
- 64 Palmqvist S, Janelidze S, Stomrud E, Zetterberg H, Karl J, Zink K, *et al.* Performance of fully automated plasma assays as screening tests for Alzheimer disease-related beta-amyloid status. *Jama Neurol.* 2019;**76**:1060–9.
- 65 Palmqvist S, Insel PS, Stomrud E, Janelidze S, Zetterberg H, Brix B, *et al.* Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease. *Embo Mol Med.* 2019;**11**(12):e.11170.
- 66 Chatterjee P, Elmi M, Goozee K, Shah T, Sohrabi HR, Dias CB, *et al.* Ultrasensitive detection of plasma amyloid-beta as a biomarker for cognitively normal elderly individuals at risk of Alzheimer's disease. *J Alzheimer's Dis.* 2019;**71**:775–83.
- 67 Simrén J, Leuzy A, Karikari TK, Hye A, Benedet AL, Lantero-Rodriguez J, *et al.* The diagnostic and prognostic capabilities of plasma biomarkers in Alzheimer's disease. *Alzheimer's Dement.* 2021;1–12.
- 68 Brinkmalm A, Portelius E, Öhrfelt A, Brinkmalm G, Andreasson U, Gobom J, *et al.* Explorative and targeted neuroproteomics in Alzheimer's disease. *Biochim Biophys Acta.* 2015;**1854**:769–78.
- 69 Crutchfield CA, Thomas SN, Sokoll LJ, Chan DW. Advances in mass spectrometry-based clinical biomarker discovery. *Clin Proteomics.* 2016;**13**:1.
- 70 Oeckl P, Otto M. A review on MS-based blood biomarkers for Alzheimer's disease. *Neurol Ther.* 2019;**8**:113–27.
- 71 Pannee J, Törnqvist U, Westerlund A, Ingelsson M, Lannfelt L, Brinkmalm G, *et al.* The amyloid- β degradation pattern in plasma—a possible tool for clinical trials in Alzheimer's disease. *Neurosci Lett.* 2014;**573**:7–12.
- 72 Ovod V, Ramsey KN, Mawuenyega KG, Bollinger JG, Hicks T, Schneider T, *et al.* Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimer's Dement.* 2017;**13**:841–9.
- 73 Schindler SE, Bollinger JG, Ovod V, Mawuenyega KG, Li Y, Gordon BA, *et al.* High-precision plasma β -amyloid 42/40 predicts current and future brain amyloidosis. *Neurology.* 2019;**93**:e1647–e1659.
- 74 Keshavan A, Pannee J, Karikari TK, Rodriguez JL, Ashton NJ, Nicholas JM, *et al.* Population-based blood screening for preclinical Alzheimer's disease in a British birth cohort at age 70. *Brain.* 2021;**144**(2):434–49.
- 75 Giedraitis V, Sundelöf J, Irizarry MC, Gårevik N, Hyman BT, Wahlund L-O, *et al.* The normal equilibrium between CSF and plasma amyloid beta levels is disrupted in Alzheimer's disease. *Neurosci Lett.* 2007;**427**:127–31.
- 76 Tamaoka A, Fukushima T, Sawamura N, Ky I, Oguni E, Komatsuzaki Y, *et al.* Amyloid β protein in plasma from patients with sporadic Alzheimer's disease. *J Neurol Sci.* 1996;**141**:65–8.
- 77 Hansson O, Zetterberg H, Vanmechelen E, Vanderstichele H, Andreasson U, Londos E, *et al.* Evaluation of plasma Abeta (40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. *Neurobiol Aging.* 2010;**31**:357–67.
- 78 Lövheim H, Elgh F, Johansson A, Zetterberg H, Blennow K, Hallmans G, *et al.* Plasma concentrations of free amyloid β cannot predict the development of Alzheimer's disease. *Alzheimer's Dement.* 2017;**13**:778–82.
- 79 O'Bryant SE, Gupta V, Henriksen K, Edwards M, Jeromin A, Lista S, *et al.* Guidelines for the standardization of preanalytic variables for blood-based biomarker studies in Alzheimer's disease research. *Alzheimer's Dement.* 2015;**11**:549–60.
- 80 Hansson O, Mikulskis A, Fagan AM, Teunissen C, Zetterberg H, Vanderstichele H, *et al.* The impact of preanalytical variables on measuring cerebrospinal fluid biomarkers for Alzheimer's disease diagnosis: a review. *Alzheimer's Dement.* 2018;**14**:1313–33.
- 81 Cullen VC, Fredenburg RA, Evans C, Conliffe PR, Solomon ME. Development and advanced validation of an optimized method for the quantitation of A β 42 in human cerebrospinal fluid. *AAPS J.* 2012;**14**:510–8.
- 82 Perret-Liaudet A, Pelpel M, Tholance Y, Dumont B, Vanderstichele H, Zorzi W, *et al.* Risk of Alzheimer's disease biological misdiagnosis linked to cerebrospinal collection tubes. *J Alzheimer's Dis.* 2012;**31**:13–20.
- 83 Vanderstichele HMJ, Janelidze S, Demeyer L, Coart E, Stoops E, Herbst V, *et al.* Optimized standard operating procedures for the analysis of cerebrospinal fluid A β 42 and the ratios of A β isoforms using low protein binding tubes. *J Alzheimer's Dis.* 2016;**53**:1121–32.

- 84 Bjerke M, Portelius E, Minthon L, Wallin A, Anckarsäter H, Anckarsäter R, et al. Confounding factors influencing amyloid beta concentration in cerebrospinal fluid. *Int J Alzheimer's Dis.* 2010;**2010**:1–11.
- 85 Hu WT, Watts KD, Shaw LM, Howell JC, Trojanowski JQ, Basra S, et al. CSF beta-amyloid 1–42 - what are we measuring in Alzheimer's disease? *Ann Clin Transl Neur.* 2015;**2**:131–9.
- 86 Hansson O, Rutz S, Zetterberg H, Bauer E, Hähl T, Manuilova E, et al. Pre-analytical protocol for measuring Alzheimer's disease biomarkers in fresh CSF. *Alzheimer's Dement.* 2020;**12**(1):e12137.
- 87 Zetterberg H, Blennow K. From cerebrospinal fluid to blood: the third wave of fluid biomarkers for Alzheimer's disease. *J Alzheimers Dis.* 2018;**64**:S271–S279.
- 88 Zetterberg H. Blood-based biomarkers for Alzheimer's disease-An update. *J Neurosci Meth.* 2019;**319**:2–6.
- 89 Iizuka T, Shoji M, Harigaya Y, Kawarabayashi T, Watanabe M, Kanai M, et al. Amyloid β -protein ending at Thr43 is a minor component of some diffuse plaques in the Alzheimer's disease brain, but is not found in cerebrovascular amyloid. *Brain Res.* 1995;**702**:275–8.
- 90 Bourassa P, Tremblay C, Schneider JA, Bennett DA, Calon F. Beta-amyloid pathology in human brain microvessel extracts from the parietal cortex: relation with cerebral amyloid angiopathy and Alzheimer's disease. *Acta Neuropathol.* 2019;**137**:801–23.
- 91 Parvathy S, Davies P, Haroutunian V, Purohit DP, Davis KL, Mohs RC, et al. Correlation between $A\beta$ x-40-, $A\beta$ x-42-, and $A\beta$ x-43-containing amyloid plaques and cognitive decline. *Archives Neurol.* 2001;**58**:2025–31.
- 92 Saito T, Suemoto T, Brouwers N, Slegers K, Funamoto S, Mihira N, et al. Potent amyloidogenicity and pathogenicity of $A\beta$ 43. *Nat Neurosci.* 2011;**14**:1023–32.
- 93 Welander H, Frånberg J, Graff C, Sundström E, Winblad B, Tjernberg LO. $A\beta$ 43 is more frequent than $A\beta$ 40 in amyloid plaque cores from Alzheimer disease brains. *J Neurochem.* 2009;**110**:697–706.
- 94 Keller L, Welander H, Chiang H-H, Tjernberg LO, Nennesmo I, Wallin ÅK, et al. The PSEN1 I143T mutation in a Swedish family with Alzheimer's disease: clinical report and quantification of AB in different brain regions. *Eur J Human Genetics: EJHG.* 2010;**18**:1202–8.
- 95 Jäkel L, Boche D, Nicoll JAR, Verbeek MM. $A\beta$ 43 in human Alzheimer's disease: effects of active $A\beta$ 42 immunization. *Acta Neuropathol Commun.* 2019;**7**:141.
- 96 Bruggink KA, Bea Kuiperij H, Claassen JAHR, Verbeek MM. The diagnostic value of CSF amyloid- β 43 in differentiation of dementia syndromes. *Curr Alzheimer Res.* 2013;**10**:1034–40.
- 97 Lauridsen C, Sando SB, Møller I, Berge G, Pomary PK, Grøntvedt GR, et al. Cerebrospinal fluid $A\beta$ 43 is reduced in early-onset compared to late-onset Alzheimer's disease, but has similar diagnostic accuracy to $A\beta$ 42. *Front Aging Neurosci.* 2017;**9**:210.
- 98 Lauridsen C, Sando SB, Shabnam A, Møller I, Berge G, Grøntvedt GR, et al. Cerebrospinal fluid levels of amyloid beta 1–43 in patients with amnesic mild cognitive impairment or early Alzheimer's disease: a 2-year follow-up study. *Front Aging Neurosci.* 2016;**8**:1–43.
- 99 Iqbal K, Liu F, Gong CX, Grundke-Iqbal I. Tau in Alzheimer disease and related Tauopathies. *Curr Alzheimer Res.* 2010;**7**(8):656–64.
- 100 Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci - PNAS.* 1986;**83**:4913–7.
- 101 Gong CX, Iqbal K. Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Curr Med Chem.* 2008;**15**:2321–8.
- 102 Vandermeeren M, Vandermeeren M, Vandermeeren M, Six J, Van de Voorde A, Martin JJ, et al. Detection of τ proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme-linked immunosorbent assay. *J Neurochem.* 1993;**61**:1828–34.
- 103 Martin L, Latypova X, Terro F. Post-translational modifications of tau protein: Implications for Alzheimer's disease. *Neurochem Int.* 2011;**58**:458–71.
- 104 Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. *Lancet Neurol.* 2003;**2**:605–13.
- 105 Hesse C, Rosengren L, Andreasen N, Davidsson P, Vanderstichele H, Vanmechelen E, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. *Neurosci Lett.* 2001;**297**:187–90.
- 106 Riemenschneider M, Wagenpfeil S, Vanderstichele H, Otto M, Wiltfang J, Kretschmar H, et al. Phospho-tau total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. *Mol Psychiatr.* 2003;**8**:343–7.
- 107 Blennow K, Wallin A, Ågren H, Spenger C, Siegfried J, Vanmechelen E. tau protein in cerebrospinal fluid - a biochemical marker for axonal degeneration in Alzheimer disease? *Mol Chem Neuropathol.* 1995;**26**:231–45.
- 108 Sjögren M, Davidsson P, Wallin A, Granérus A-K, Grundström E, Askmark H, et al. Decreased CSF- β -amyloid 42 in Alzheimer's disease and amyotrophic lateral sclerosis may reflect mistreatment of β -amyloid induced by disparate mechanisms. *Dement Geriatr Cogn.* 2002;**13**:112–8.
- 109 Vanmechelen E, Vanderstichele H, Davidsson P, Van Kerschaver E, Van Der Perre B, Sjögren M, et al. Quantification of tau phosphorylated at threonine 181 in human cerebrospinal fluid: a sandwich ELISA with a synthetic phosphopeptide for standardization. *Neurosci Lett.* 2000;**285**:49–52.
- 110 Buerger K, Zinkowski R, Teipel SJ, Tapiola T, Arai H, Blennow K, et al. Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of Tau protein phosphorylated at threonine 231. (Archives of Neurology). *JAMA, J Am Med Assoc.* 2002;**288**:2241.
- 111 Parnetti L, Lanari A, Amici S, Gallai V, Vanmechelen E, Hulstaert F. CSF phosphorylated tau is a possible marker for discriminating Alzheimer's disease from dementia with Lewy bodies. *Neurol Sci.* 2001;**22**:77–8.
- 112 Sato C, Barthélemy NR, Mawuenyega KG, Patterson BW, Gordon BA, Jockel-Balsarotti J, et al. Tau kinetics in neurons and the human central nervous system. *Neuron.* 2018;**97**:1284–98.e7.
- 113 Edwards FA. A unifying hypothesis for Alzheimer's disease: from plaques to neurodegeneration. *Trends Neurosci.* 2019;**42**:310–22.
- 114 Maia LF, Kaeser SA, Reichwald J, Hruscha M, Martus P, Staufenbiel M, et al. Changes in amyloid- β and tau in the cerebrospinal fluid of transgenic mice overexpressing amyloid precursor protein. *Sci Transl Med.* 2013;**5**:194re2-re2.
- 115 Haukedal H, Freude K. Implications of glycosylation in Alzheimer's disease. *Front Neurosci-Switz.* 2021;**14**.

- 116 Verbeek MM, Kremer BP, Jansen RW, de Jong D. Tau protein phosphorylated at threonine 181 in cerebrospinal fluid as a possible biomarker for Alzheimer's disease. *Neurobiol Aging*. 2004;**25**:S364-S.
- 117 Welge V, Fiege O, Lewczuk P, Mollenhauer B, Esselmann H, Klafki HW, et al. Combined CSF tau, p-tau181 and amyloid-beta 38/40/42 for diagnosing Alzheimer's disease. *J Neural Transm*. 2009;**116**:203-12.
- 118 Kohnken R, Buerger K, Zinkowski R, Miller C, Kerkman D, DeBernardis J, et al. Detection of tau phosphorylated at threonine 231 in cerebrospinal fluid of Alzheimer's disease patients. *Neurosci Lett*. 2000;**287**:187-90.
- 119 Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol*. 2016;**15**:673-84.
- 120 Suárez-Calvet M, Karikari TK, Ashton NJ, Lantero Rodriguez J, Milà-Alomà M, Gispert JD, et al. Novel tau biomarkers phosphorylated at T181, T217 or T231 rise in the initial stages of the preclinical Alzheimer's continuum when only subtle changes in A β pathology are detected. *Embo Mol Med*. 2020;**12**:e12921-n/a.
- 121 Meredith JE, Sankaranarayanan S, Guss V, Lanzetti AJ, Berisha F, Neely RJ, et al. Characterization of novel CSF tau and ptau biomarkers for Alzheimer's disease. *PLoS One*. 2013;**8**:e76523.
- 122 Barthélemy NR, Fenaille F, Hirtz C, Sergeant N, Schraen-Maschke S, Vialaret J, et al. Tau protein quantification in human cerebrospinal fluid by targeted mass spectrometry at high sequence coverage provides insights into its primary structure heterogeneity. *J Proteome Res*. 2016;**15**:667-76.
- 123 Chen Z, Mengel D, Keshavan A, Rissman RA, Billinton A, Perkinson M, et al. Learnings about the complexity of extracellular tau aid development of a blood-based screen for Alzheimer's disease. *Alzheimer's Dement*. 2019;**15**:487-96.
- 124 Vos SJB, Xiong C, Visser PJ, Jasielc MS, Hassenstab J, Grant EA, et al. Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study. *Lancet Neurol*. 2013;**12**:957-65.
- 125 Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol*. 2010;**6**:131-44.
- 126 Schoonenboom NSM, Reesink FE, Verwey NA, Kester MI, Teunissen CE, van de Ven PM, et al. Cerebrospinal fluid markers for differential dementia diagnosis in a large memory clinic cohort. *Neurology*. 2012;**78**:47-54.
- 127 Janelidze S, Stomrud E, Smith R, Palmqvist S, Mattsson N, Airey DC, et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. *Nat Commun*. 2020;**11**(1):1683.
- 128 Barthélemy NR, Bateman RJ, Hirtz C, Marin P, Becher F, Sato C, 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. *Alzheimers Res Ther*. 2020;**12**(1):26.
- 129 Mielke MM, Aakre JA, Algeciras-Schimnic A, Proctor N, Machulda MM, Knopman DS, et al. Comparison of cerebrospinal fluid phosphorylated tau 181 and 217 for cognitive progression. *Alzheimer's Dement*. 2020;**16**:e040503.
- 130 Kvartsberg H, Hanes J, Benedet AL, Ashton NJ, Pascoal TA, Rosa-Neto P, et al. Quantification of tau phosphorylated at threonine 217 using a novel ultrasensitive immunoassay distinguishes Alzheimer's disease from healthy controls. *Alzheimer's Dement*. 2020;**16**:e043467.
- 131 Karikari TK, Emersič A, Vrillon A, Lantero-Rodriguez J, Ashton NJ, Kramberger MG, et al. Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis. *Alzheimer's Dement*. 2020;**17**:755-67.
- 132 Buchhave P, Minthon L, Zetterberg H, Wallin ÅK, Blennow K, Hansson O. Cerebrospinal fluid levels of β -amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Arch Gen Psychiatr*. 2012;**69**:98-106.
- 133 Emersič A, Karikari TK, Rodríguez-Lantero J, Ashton NJ, Rot U, Kramberger MG, et al. CSF phosphorylated tau-217 is increased in Alzheimer's and Creutzfeldt-Jakob diseases and correlates with amyloid pathology. *Alzheimer's Dement*. 2020;**16**:e045296.
- 134 Ashton NJ, Benedet AL, Pascoal TA, Karikari TK, Lantero-Rodriguez J, Mathotaarachchi S, et al. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. *Research Square*. 2021;1-21.
- 135 Randall J, Mörtberg E, Provuncher GK, Fournier DR, Duffy DC, Rubertsson S, et al. Tau proteins in serum predict neurological outcome after hypoxic brain injury from cardiac arrest: Results of a pilot study. *Resuscitation*. 2012;**84**:351-6.
- 136 Zetterberg H, Wilson D, Andreasson U, Minthon L, Blennow K, Randall J, et al. Plasma tau levels in Alzheimer's disease. *Alzheimer's Res Therapy*. 2013;**5**:9.
- 137 Shekhar S, Kumar R, Rai N, Kumar V, Singh K, Upadhyay AD, et al. Estimation of Tau and phosphorylated Tau181 in serum of Alzheimer's disease and mild cognitive impairment patients. *PLoS One*. 2016;**11**:e0159099.
- 138 Tatebe H, Kasai T, Ohmichi T, Kishi Y, Takeya T, Waragai M, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. *Mol Neurodegener*. 2017;**12**:63.
- 139 Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement*. 2018;**14**:989-97.
- 140 Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med*. 2020;**26**:379.
- 141 Karikari TK, Pascoal TA, Ashton NJ, Janelidze S, Benedet AL, Rodriguez JL, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol*. 2020;**19**:422-33.
- 142 Thijssen EH, La Joie R, Wolf A, Strom A, Wang P, Iaccarino L, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med*. 2020;**26**:387.
- 143 Karikari TK, Benedet AL, Ashton NJ, Lantero Rodriguez J, Snellman A, Suárez-Calvet M, et al. Diagnostic performance and prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's Disease Neuroimaging Initiative. *Mol Psychiatr*. 2020;**26**:429-42.

- 144 Lantero Rodriguez J, Karikari TK, Suárez-Calvet M, Troakes C, King A, Emersic A, et al. Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathol.* 2020;**140**:267–78.
- 145 O'Connor A, Karikari TK, Poole T, Ashton NJ, Lantero Rodriguez J, Khatun A, et al. Plasma phospho-tau181 in presymptomatic and symptomatic familial Alzheimer's disease: a longitudinal cohort study. *Mol Psychiatry.* 2020.
- 146 Moscoso A, Grothe MJ, Ashton NJ, Karikari TK, Rodríguez JL, Snellman A, et al. Longitudinal associations of blood phosphorylated tau181 and neurofilament light chain with neurodegeneration in Alzheimer disease. *JAMA Neurol.* 2021;**78**(4):396–406.
- 147 Janelidze S, Berron D, Smith R, Strandberg O, Proctor NK, Dage JL, et al. Associations of plasma phospho-Tau217 levels with tau positron emission tomography in early Alzheimer disease. *JAMA Neurol.* 2021;**78**:149.
- 148 Ashton NJ, Pascoal TA, Karikari TK, Benedet AL, Lantero-Rodriguez J, Brinkmalm G, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol.* 2021;**141**:709–24.
- 149 Zetterberg H. Tauomics and kinetics in human neurons and biological fluids. *Neuron.* 2018;**97**:1202–5.
- 150 Ingelsson M, Blomberg M, Benedikz E, Wahlund L-O, Karlsson E, Vanmechelen E, et al. Tau immunoreactivity detected in human plasma, but no obvious increase in dementia. *Dement Geriatr Cogn.* 1999;**10**:442–5.
- 151 Chiu M-J, Chen Y-F, Chen T-F, Yang S-Y, Yang F-P, Tseng T-W, et al. Plasma tau as a window to the brain—negative associations with brain volume and memory function in mild cognitive impairment and early Alzheimer's disease. *Hum Brain Mapp.* 2014;**35**:3132–42.
- 152 Mattsson N, Zetterberg H, Janelidze S, Insel PS, Andreasson U, Stomrud E, et al. Plasma tau in Alzheimer disease. *Neurology.* 2016;**87**:1827–35.
- 153 Larry Sparks D, Kryscio RJ, Sabbagh MN, Ziolkowski C, Lin Y, Sparks LM, et al. Tau is reduced in AD plasma and validation of employed ELISA methods. *Am J Neurodegenerative Dis.* 2012;**1**:99–106.
- 154 Mielke MM, Hagen CE, Wennberg AMV, Airey DC, Savica R, Knopman DS, et al. Association of plasma total tau level with cognitive decline and risk of mild cognitive impairment or dementia in the mayo clinic study on aging. *JAMA Neurol.* 2017;**74**:1073–80.
- 155 Pase MP, Beiser AS, Himali JJ, Satizabal CL, Aparicio HJ, DeCarli C, et al. Assessment of Plasma Total Tau Level as a Predictive Biomarker for Dementia and Related Endophenotypes. *JAMA neurology.* 2019;**76**:598–606.
- 156 Chen Z, Mengel D, Keshavan A, Rissman RA, Billinton A, Perkinson M, et al. Learnings about the complexity of extracellular tau aid development of a blood-based screen for Alzheimer's disease. *Alzheimer's Dement.* 2019;**15**:487–96.
- 157 Chhatwal JP, Schultz AP, Dang Y, Ostaszewski B, Liu L, Yang H-S, et al. Plasma N-terminal tau fragment levels predict future cognitive decline and neurodegeneration in healthy elderly individuals. *Nat Commun.* 2020;**11**:6024.
- 158 Lista S, Toschi N, Baldacci F, Zetterberg H, Blennow K, Kilimann I, et al. Diagnostic accuracy of CSF neurofilament light chain protein in the biomarker-guided classification system for Alzheimer's disease. *Neurochem Int.* 2017;**108**:355–60.
- 159 Schlaepfer WW, Lynch RG. Immunofluorescence studies of neurofilaments in the rat and human peripheral and central nervous system. *J Cell Biol.* 1977;**74**:241–50.
- 160 Sjögren M, Blomberg M, Jonsson M, Wahlund LO, Edman Å, Lind K, et al. Neurofilament protein in cerebrospinal fluid: a marker of white matter changes. *J Neurosci Res.* 2001;**66**:510–6.
- 161 Zetterberg H, Skillbäck T, Mattsson N, Trojanowski JQ, Portelius E, Shaw LM, et al. Association of cerebrospinal fluid neurofilament light concentration with Alzheimer disease progression. *JAMA Neurol.* 2016;**73**:60–7.
- 162 Mattsson N, Insel PS, Palmqvist S, Portelius E, Zetterberg H, Weiner M, et al. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. *EMBO Mol Med.* 2016;**8**:1184–96.
- 163 Hu Y-Y, He S-S, Wang X-C, Duan Q-H, Khatoon S, Iqbal K, et al. Elevated levels of phosphorylated neurofilament proteins in cerebrospinal fluid of Alzheimer disease patients. *Neurosci Lett.* 2002;**320**:156–60.
- 164 Petzold A, Keir G, Warren J, Fox N, Rossor MN. A systematic review and meta-analysis of CSF neurofilament protein levels as biomarkers in dementia. *Neurodegener Dis.* 2007;**4**:185–94.
- 165 Gaiottino J, Norgren N, Dobson R, Topping J, Nissim A, Malaspina A, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. *PLoS One.* 2013;**8**:e75091.
- 166 Mattsson N, Andreasson U, Zetterberg H, Blennow K, Neuroimaging AsD. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. *Jama Neurol.* 2017;**74**:557–66.
- 167 Kuhle J, Barro C, Andreasson U, Derfuss T, Lindberg R, Sandelius Å, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med.* 2016;**54**:1655–61.
- 168 Weston PSJ, Poole T, Ryan NS, Nair A, Liang Y, Macpherson K, et al. Serum neurofilament light in familial Alzheimer disease A marker of early neurodegeneration. *Neurology.* 2017;**89**:2167–75.
- 169 Lewczuk P, Ermann N, Andreasson U, Schultheis C, Podhorna J, Spitzer P, et al. Plasma neurofilament light as a potential biomarker of neurodegeneration in Alzheimer's disease. *Alzheimers Res Ther.* 2018;**10**:71.
- 170 Lin YS, Lee WJ, Wang SJ, Fuh JL. Levels of plasma neurofilament light chain and cognitive function in patients with Alzheimer or Parkinson disease. *Sci Rep.* 2018;**8**(1):17368.
- 171 Sánchez-Valle R, Heslegrave A, Foiani MS, Bosch B, Antonell A, Balasa M, et al. Serum neurofilament light levels correlate with severity measures and neurodegeneration markers in autosomal dominant Alzheimer's disease. *Alzheimers Res Ther.* 2018;**10**(1):113.
- 172 Schultz SA, Strain JF, Adedokun A, Wang Q, Preische O, Kuhle J, et al. Serum neurofilament light chain levels are associated with white matter integrity in autosomal dominant Alzheimer's disease. *Neurobiol Dis.* 2020;**142**:104960.
- 173 Ashton NJ, Leuzy A, Lim YM, Troakes C, Hortobágyi T, Höglund K, et al. Increased plasma neurofilament light chain concentration correlates with severity of post-mortem

- neurofibrillary tangle pathology and neurodegeneration. *Acta Neuropathol Commun.* 2019;**7**(1):5.
- 174 Ashton N, Janelidze S, Al Khleifat A, Leuzy A, van der Ende E, Karikari T, et al. Diagnostic value of plasma neurofilament light: a multicentre validation study. *Nature Portfolio.* 2021;1–14.
- 175 Hansson O, Janelidze S, Hall S, Magdalinou N, Lees AJ, Andreasson U, et al. Blood-based NFL: a biomarker for differential diagnosis of parkinsonian disorder. *Neurology.* 2017;**88**:930–7.
- 176 Mattsson N, Insel PS, Palmqvist S, Portelius E, Zetterberg H, Weiner M, et al. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer's disease. *Embo Mol Med.* 2016;**8**:1184–96.
- 177 Simrén J, Ashton NJ, Blennow K, Zetterberg H. Blood neurofilament light in remote settings: alternative protocols to support sample collection in challenging pre-analytical conditions. *Alzheimer's Dement.* 2021;**13**(1):e12145.
- 178 Wyss-Coray T, Mucke L. Inflammation in neurodegenerative disease—a double-edged sword. *Neuron.* 2002;**35**:419–32.
- 179 Hanisch U-K, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci.* 2007;**10**:1387–94.
- 180 Rogers J, Mastroeni D, Leonard B, Joyce J, Grover A. Neuroinflammation in Alzheimer's disease and Parkinson's disease: are microglia pathogenic in either disorder? *Int Rev Neurobiol.* 2007;**82**:235–46.
- 181 Ransohoff RM. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci.* 2016;**19**:987–91.
- 182 Ransohoff RM. How neuroinflammation contributes to neurodegeneration. *Science.* 2016;**353**:777–83.
- 183 Hansen DV, Hanson JE, Sheng M. Microglia in Alzheimer's disease. *J Cell Biol.* 2018;**217**:459–72.
- 184 Katzman R, Terry R, DeTeresa R, Brown T, Davies P, Fuld P, et al. Clinical, pathological, and neurochemical changes in dementia: a subgroup with preserved mental status and numerous neocortical plaques. *Ann Neurol.* 1988;**23**:138–44.
- 185 Kvartsberg H, Duits FH, Ingelsson M, Andreasen N, Öhrfelt A, Andersson K, et al. Cerebrospinal fluid levels of the synaptic protein neurogranin correlates with cognitive decline in prodromal Alzheimer's disease. *Alzheimer's Dement.* 2015;**11**:1180–90.
- 186 Suárez-Calvet M, Kleinberger G, Araque Caballero MÁ, Brendel M, Rominger A, Alcolea D, et al. sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *Embo Mol Med.* 2016;**8**:466–76.
- 187 Heslegrave A, Heywood W, Paterson R, Magdalinou N, Svensson J, Johansson P, et al. Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. *Mol Neurodegener.* 2016;**11**:3.
- 188 Wennström M, Surova Y, Hall S, Nilsson C, Minthon L, Hansson O, et al. The inflammatory marker YKL-40 is elevated in cerebrospinal fluid from patients with Alzheimer's but not Parkinson's disease or dementia with Lewy bodies. *PLoS One.* 2015;**10**:e0135458.
- 189 Kvartsberg H, Portelius E, Andreasson U, Brinkmalm G, Hellwig K, Lelental N, et al. Characterization of the postsynaptic protein neurogranin in paired cerebrospinal fluid and plasma samples from Alzheimer's disease patients and healthy controls. *Alzheimer's Res Ther.* 2015;**7**:40.
- 190 Ashton NJ, Suarez-Calvet M, Heslegrave A, Hye A, Razquin C, Pastor P, et al. Plasma levels of soluble TREM2 and neurofilament light chain in TREM2 rare variant carriers. *Alzheimer's Res Ther.* 2019;**11**:94.
- 191 Craig-Schapiro R, Perrin RJ, Roe CM, Xiong C, Carter D, Cairns NJ, et al. YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiat.* 1969;**2010**:903–12.

Correspondence: D. O. T. Alawode and H. Zetterberg, Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, London, UK.
(e-mail: d.alawode.16@ucl.ac.uk (D.O.T.A.); henrik.zetterberg@clinchem.gu.se (H.Z.)). ■