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CURRENT OPINION

Plasma biomarkers for neurodegenerative disorders: ready for prime time?

Wasiu G. Balogun[a], *Henrik Zetterberg*[b,c,d,e,f,g], *Kaj Blennow*[b,c] *and Thomas K. Karikari*[b,h]

Purpose of review
Several plasma biomarkers for Alzheimer's disease and related disorders (ADRD) have demonstrated clinical and technical robustness. However, are they ready for clinical implementation? This review critically appraises current evidence for and against the immediate use of plasma biomarkers in clinical care.

Recent findings
Plasma biomarkers have significantly improved our understanding of ADRD time-course, risk factors, diagnosis and prognosis. These advances are accelerating the development and in-human testing of therapeutic candidates, and the selection of individuals with subtle biological evidence of disease who fit the criteria for early therapeutic targeting. However, standardized tests and well validated cut-off values are lacking. Moreover, some assays (e.g., plasma Aβ methods) have poor robustness to withstand inevitable day-to-day technical variations. Additionally, recent reports suggest that common comorbidities of aging (e.g., kidney disease, diabetes, hypertension) can erroneously affect plasma biomarker levels, clinical utility and generalizability. Furthermore, it is unclear if health disparities can explain reported racial/ethnic differences in biomarker levels and functions. Finally, current clinically approved plasma methods are more expensive than CSF assays, questioning their cost effectiveness.

Summary
Plasma biomarkers have biological and clinical capacity to detect ADRD. However, their widespread use requires issues around thresholds, comorbidities and diverse populations to be addressed.

Keywords
Alzheimer's disease, Alzheimer's disease and related disorders, dementia, neurodegenerative disorder, plasma biomarker

INTRODUCTION

The development of blood-based biomarkers for Alzheimer's disease (AD) and related neurodegenerative disorders (ADRD) is ground-breaking, as they may help to improve biological understanding of these diseases and to accelerate screening (risk prediction) in clinical management and may also be useful for prognostication [1–4]. Blood biomarkers may also enable evaluation of the efficacy of candidate pharmacological and nonpharmacological agents, assessment of future disease risk in asymptomatic individuals, and longitudinal monitoring of people with symptoms [1–4].

Plasma biomarkers are anticipated to be simpler, more cost-effective, and easier-to-implement alternatives to cerebrospinal fluid (CSF) and neuroimaging biomarkers that are now the most established methods for clinical and research-based assessments of ADRD [1,3–5]. The core CSF biomarkers (β-amyloid $[A\beta]_{42}/A\beta_{40}$ ratio, phosphorylated tau 181 [p-tau],

[a]Department of Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA, [b]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, [c]Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden, [d]Department of Neurodegenerative Disease, UCL Institute of Neurology, [e]UK Dementia Research Institute at UCL, London, UK, [f]Hong Kong Center for Neurodegenerative Diseases, Hong Kong, China, [g]Wisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin and [h]Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Correspondence to Thomas K. Karikari, PhD, Assistant Professor, Clinical Neurochemistry Lab., Sahlgrenska University Hospital, House V3/SU, 43 180 Mölndal, Sweden. E-mail: Thomas.Karikari@gu.se and Department of Psychiatry, School of Medicine, University of Pittsburgh, 3811 O'Hara Street, 15213, Pittsburgh, PA, USA.
E-mail: Karikari@pitt.edu

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KEY POINTS

- Plasma biomarkers have shown great potential as surrogate indicators of brain pathology in Alzheimer's disease (AD) and Alzheimer's disease and related disorders.
- High-performing biomarkers include Aβ42/Aβ40, p-tau181, p-tau217, p-tau231, neurofilament light chain and glial fibrillary acidic protein, with other novel markers including brain-derived tau.
- These biomarkers have demonstrated prognostic and diagnostic utility to detect current and future AD and related disorders.
- Several plasma biomarkers additionally have robust analytical capacities including test- retest stability and day-to-day consistency in measurements, making them suitable for routine clinical use.
- Their generalizable clinical applications will require technical improvements in assay robustness (plasma Aβ42/Aβ40 ratio), large-scale validation in diverse populations, the establishment of cut-off values anchored to some kind of reference material, the existing high costs, and the effects of comorbidities to be addressed.

and total tau) jointly perform excellently to provide biological evidence of AD, in agreement with the principal pathological features of the disease – Aβ plaques, phosphorylated tau, and neurodegeneration respectively [5–7]. Neuroimaging alternatives to these CSF biomarkers respectively include positron emission tomography (PET) imaging of Aβ plaques and tau tangles, as well as magnetic resonance imaging (MRI) of hippocampal atrophy [5–7]. These markers are included in diagnostic and research guidelines and some are approved for clinical use by the US Food and Drugs Administration [5–8]. Yet, their invasiveness, high costs and difficulty to upscale hinder their widespread applications in primary care [1,2].

Technical developments have led to the development of plasma biomarkers that require minimal expertise in sample collection. Plasma biomarkers have additional potential advantages including suitability for prescreening and lower costs that are important for large-scale clinical diagnostic, prognostic, interventional and observational applications [1–4,9▪▪].

We provide a short up-to-date review on recent findings in favor of plasma biomarkers as the next generation of accurate diagnostic and prognostic biomarkers to detect AD neuropathologic change. We additionally discuss the counterargument that despite their demonstrably high performances, widespread clinical use would require that specific issues that are critical to these endeavors are first and foremost addressed.

HIGH-PERFORMING BLOOD BIOMARKERS FOR NEURODEGENERATIVE DISORDERS: AN UPDATE

Several blood biomarkers have shown utility for AD and ADRD. In this review, we focus on biomarkers that have shown repeated utility across multiple independent studies. Plasma neurofilament light chain (NfL), an indicator of axonal injury/neurodegeneration, is probably the most widely used blood biomarker. Being a universal biomarker of a disease feature common to multiple neurodegenerative pathologies, plasma NfL levels are higher not only in AD but also in several other neurodegenerative diseases including amylotrophic lateral sclerosis, frontotemporal lobal degeneration and primary tauopathies such as progressive supranuclear palsy and corticobasal syndrome [10▪▪,11]. Other biomarkers include plasma Aβ42/Aβ40 and p-tau that have shown potential for AD detection. Plasma Aβ42/Aβ40 associates with brain Aβ pathology [12,13▪▪,14], whereas p-tau biomarkers (including p-tau181, p-tau217 and p-tau231) are known to increase according to Aβ and tau pathophysiologies [9▪▪,15,16▪▪,17▪▪]. In addition, plasma p-tau may predict future cognitive impairment [9▪▪,18▪▪,19▪▪, 20▪▪]. It is highly concordant with AD diagnosis at autopsy [21,22▪▪]. The presence of astroglia activation in AD [23▪▪,24▪▪,25▪▪], as well as in non-AD disorders, such as multiple sclerosis and frontotemporal lobar degeneration, support the use of plasma glial fibrillary acidic protein (GFAP) as a blood biomarker [26,27▪].

DIAGNOSTIC AND PROGNOSTIC PERFORMANCES SUGGESTING THAT BLOOD BIOMARKERS ARE READY FOR PRIME TIME

NfL: In most neurodegenerative disorders, plasma NfL levels are elevated because axonal degeneration is a prominent feature [28,29]. Individuals with AD across the disease spectrum – asymptomatic AD, prodromal AD, and familial AD – show elevated blood levels of NfL [10▪▪,30–32]. The concentration of NfL in blood inversely associates with cognitive function, and positively with CSF biomarkers, postmortem pathology, and imaging findings [10▪▪]. Furthermore, plasma NfL predicts longitudinal disease outcome including progression from asymptomatic to symptomatic phase [33–36]. Plasma NfL associates with MRI signatures of

neurodegeneration [34,37], as well as the severity of neurodegeneration at autopsy [29]. Additionally, NfL is analytically robust, with highly reproducible day-to-day values when measured in either plasma or serum [38▪,39▪].

Aβ42/Aβ40: One head-to-head comparison study suggested that plasma Aβ42/Aβ40 measured by immunoprecipitation-mass spectrometry (IP-MS) technology was the best-performing to detect brain Aβ compared with immuno-assay methods [40▪▪], although immunoassay methods including the ones from Quanterix and Roche recently showed considerably good performance for early detection of Aβ pathology [20▪▪,41▪▪]. Plasma Aβ42/Aβ40 associates well with, and predicts longitudinal changes in brain Aβ PET and CSF Aβ42/Aβ40 [12,13▪▪,14,42▪]. Moreover, plasma Aβ42/Aβ40 is one of the biomarkers that starts to change in the early preclinical phase of AD [12,13▪▪,14]. This biomarker also has only a small fold change between Aβ-positive and Aβ-negative individuals when compared with CSF Aβ42/Aβ40 [1,12], making the biomarker not robust in everyday laboratory practice [43▪▪]. Plasma Aβ42/Aβ40 performs well when samples are analyzed batch-wise with single lots of assay reagents.

P-tau: Several plasma p-tau species have been developed and validated, with p-tau181, p-tau217 and p-tau231 being the most well studied [1,4,44]. There is strong evidence that plasma p-tau is a reliable biomarker for AD, with demonstrated utility in multiple clinical contexts; these include AD time course [45], definitive diagnosis and differential diagnosis versus other causes of cognitive impairment [15,16▪▪,17▪▪,21,22▪▪,46▪], disease prognosis in primary care [15,17▪▪], and in participant selection and efficacy monitoring in therapeutic trials [18▪▪,20▪▪]. These performances have been independently authenticated in dozens of research cohorts, with some studies further showing that plasma p-tau measures correlate well with CSF p-tau in paired samples [1,4,44]. Moreover, plasma p-tau often performs equivalently to CSF p-tau to differentiate Aβ-positive AD dementia individuals from Aβ-negative non-AD dementia and control participants [16▪▪,47▪▪]. The specificity of plasma p-tau to AD pathophysiology (compared with other biomarkers like NfL and GFAP) makes it a potential first line of action in the diagnostic workup [1,2]. Moreover, plasma p- tau shows larger fold change between symptomatic AD patients and controls compared with plasma Aβ42/Aβ40, which leads to high test-retest reproducibility and robustness, supporting utility in clinical laboratory practice [38▪,48▪▪].

GFAP: GFAP is an intermediate filament protein highly expressed in astrocytes whose main physiological function is to provide network support and structure to cells [49]. Plasma GFAP associates with *in vivo* Aβ pathology across the AD continuum [23▪▪,24▪▪,50]. Plasma GFAP associates better with Aβ-PET than CSF GFAP [24▪▪]. The higher preanalytical stability of plasma versus CSF GFAP partly but not fully explains this observation [51▪], with the further speculation that plasma GFAP levels may be affected by blood-brain barrier dysfunction. Beyond AD, a rise in GFAP levels in frontotemporal dementia may indicate the late presymptomatic stage, as well as the severity of the disease [26,27▪]. Plasma GFAP is also increased in neuroinflammatory conditions, including multiple sclerosis, and is a top biomarker candidate for the progressive form of the disease [52,53].

Novel plasma total-tau (t-tau) biomarkers: Recently reported plasma biomarkers of clinical value include those that have sought to develop improved t-tau assays in blood. Similar to plasma p-tau methods that target N-terminal tau protein fragments that seem to be more abundant in blood compared with mid-region and C-terminal forms [1,54], the development of t-tau assays have capitalized on the same approach. The NT1 assay [55] targets tau molecular forms containing amino acids (aa) 6–198 by pairing the antibodies Tau12 (epitope: 6–18) with BT2 (aa 194–198).

Another assay, tau NTA, targets an even shorter N-terminal-bearing fragment, and tends to be increased earlier in the disease process [56▪▪]. Both of these outperform the existing plasma t-tau assay from Quanterix [55,56▪▪]. More recently, a new plasma t-tau assay that is specific to tau of brain-origin was described [57▪▪]. This assay – referred to as brain-derived tau (BD-tau) – avoids tau of peripheral origin, with levels in plasma and CSF correlating strongly; a strong correlation is also seen between plasma BD-tau and CSF t-tau [57▪▪]. Plasma BD-tau associates well with Aβ and tau pathology *in vivo* and at autopsy, and also differentiates AD from non-AD neurodegenerative diseases [57▪▪].

FINDINGS SUGGESTING THAT BLOOD BIOMARKERS ARE NOT READY FOR PRIME TIME

Blood biomarkers represent a paradigm shift and game changer in the AD field. However, there are still some issues to address to enable their widespread use and acceptance. These include the following points:

Analytical sensitivity

Despite their proven capacity to measure pico- to femto-molar quantities of brain proteinopathies in remote blood, some of the existing biomarker

methods have limitations for the accurate detection of very low levels of their target analytes [1]. Since AD develops slowly over several years to a decade or possibly beyond, disease prevention and treatment strategies will benefit greatly from plasma biomarkers that can accurately identify disease risk several decades before older adulthood. One of such markers with sensitivity limitation is p-tau217 which is otherwise highly effective at detecting AD pathology [1].

Lack of assay standardization

Certified reference methods and materials for assay standardization are lacking for all of the biomarkers reviewed in this paper. This puts high demands on assay manufacturers to produce kits with low lot-to-lot variation. Laboratories using these assays must also implement control programs through which longitudinal stability of the measurements in relation to the studies in which reference limits and cut-offs were established is monitored and maintained.

Lack of validated abnormality thresholds

Generalized, multicenter application will require that plasma biomarkers have been vigorously validated to generate cut-off points (traceable to some type of reference material) that work well across populations, similar to what is currently available for neuroimaging and CSF biomarkers.

Technical robustness

Plasma p-tau, NfL, and GFAP have wide analytical ranges, large fold changes between diagnostic groups, are not significantly affected by preanalytical handling factors, and thus demonstrate strong technical robustness that can withstand small day-to-day measurement biases [38▪,39▪,48▪▪,58]. However, plasma Aβ42/Aβ40 – whether measured by IP-MS or by immunoassay methods – has small fold changes and narrow analytical range that are susceptible to preanalytical variations [1,38▪,43▪▪,48▪▪].

Cost

Recent simulation analyses estimated the cost of a single plasma biomarker testing to be as low as $50 to drive significant cost-savings compared with CSF and neuroimaging [9▪▪,13▪▪]. However, the cost of approved tests or diagnostic use in the United States is currently much higher than this value. An example is the PrecivityAD test from C2N Diagnostics, which combines plasma Aβ42/Aβ40 ratio with age and *APOE* ε4 genotype information to predict brain Aβ load. This test costs $1250 per analysis, which is almost half the average cost of Aβ PET imaging [59]. Another plasma Aβ42/Aβ40 ratio test from Quest Diagnostics, which is based on a CSF assay [60] with not much having been published on the plasma version, is believed to cost about $500. Importantly, both methods are more expensive than the FDA-approved CSF Aβ42/Aβ40 ratio test available from Lumipulse, questioning the cost advantage argument often put forward in favor of plasma biomarkers.

Research cohort composition not reflecting the wider population: The demographics of participants in research cohorts among whom biomarker testing is performed are uneven, with the majority of cohort studies in the United States focusing on middle-class non-Hispanic whites. To this end, individuals of other demographics – including self-identified racial/ethnic groups, other socioeconomic statuses, and those living in disadvantaged areas – need to be actively included to ensure that the results obtained are generalizable to the larger population [61].

Differences in biomarker levels and performances between populations

A few reports have suggested that plasma biomarker levels and performances tend to differ between participants of different ethnoracial backgrounds [62▪▪,63▪▪] whereas another study did not report any differences [64▪▪]. Importantly, other studies have pointed to a likelihood that the intensity of brain pathological changes appear to be less pronounced in participants of non-European ancestry who also tend to be less affected by the presence of the major genetic risk *APOE* ε4 [65▪▪,66]. These results need to be actively investigated to, among other things, identify potential disease resilience/resistance factors.

Effects of comorbidities

A diagnosis of common comorbidities of aging – particularly those that affect organs where tau protein is highly expressed (e.g., kidney disease, hypertension, diabetes) – can erroneously affect plasma biomarker levels and clinical performances [67▪▪,68▪▪]. On the other hand, autopsy-verified mixed dementias are more common in Black populations versus non-Hispanic White individuals [69]. However, it is unknown if and how these multimorbidities affect biomarker accuracies. It is imperative to comprehensively evaluate effects of these and a wider spectrum of comorbidities in diverse populations round the world [61].

CONCLUSION

Much effort has been made to develop and clinically validate several plasma biomarkers, including plasma Aβ42/Aβ40, p-tau181, p-tau217, p-tau231, NfL, and GFAP. These markers have shown immense diagnostic and prognostic utility to detect AD and ADRD in multiple independent cohorts. Given their ability to identify pathophysiological disease changes including when compared with autopsy diagnosis, and that most have high preanalytical stability, these markers are appropriate for clinical and prognostic applications. Nonetheless, issues such as assay standardization, the establishment of cut-off values, technical robustness (particularly for Aβ42/Aβ40 ratio), high costs, large-scale validation in diverse populations, and the effects of comorbidities need to be addressed to enable fuller understanding and generalizability of findings.

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Conflicts of interest

W.G.B. and T.K.K. have no conflicts of interest. HZ has served at scientific, advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Karikari TK, Ashton NJ, Brinkmalm G, *et al.* Blood phospho-tau in Alzheimer's disease: analysis, interpretation, and clinical utility. Nat Rev Neurol 2022; 18:400–418.
2. Teunissen CE, Verberk IMW, Thijssen EH, *et al.* Blood-based biomarkers for Alzheimer's disease: towards clinical implementation. Lancet Neurol 2021; 21:66–77.
3. Ashton NJ, Leuzy A, Karikari TK, *et al.* The validation status of blood biomarkers of amyloid and phospho-tau assessed with the 5-phase development framework for AD biomarkers. Eur J Nucl Med Mol Imaging 2021; 48:2140–2156.
4. Alawode DOT, Heslegrave AJ, Ashton NJ, *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.
5. Hampel H, Cummings J, Blennow K, *et al.* Developing the ATX (N) classification for use across the Alzheimer disease continuum. Nat Rev Neurol 2021; 17:580–589.
6. Dubois B, Villain N, Frisoni GB, *et al.* Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. Lancet Neurol 2021; 20:484–496.
7. Jack CR, Bennett DA, Blennow K, *et al.* NIA-AA Research Framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement 2018; 14:535–562.
8. Sabbagh MN, Hendrix S, Harrison JE:. FDA position statement ''Early Alzheimer's disease: developing drugs for treatment, guidance for industry''. Alzheimers Dement Transl Res Clin Interv 2019; 5:13–19.

9. Karikari TK, Benedet AL, Ashton NJ, *et al.* Diagnostic performance and ■■ prediction of clinical progression of plasma phospho-tau181 in the Alzheimer's Disease Neuroimaging Initiative. Mol Psychiatry 2021; 26:429–442.
This publication was the first to demonstrate the diagnostic accuracies and longitudinal performance of plasma p-tau181.

10. Ashton NJ, Janelidze S, Al Khleifat A, *et al.* A multicentre validation study of the ■■ diagnostic value of plasma neurofilament light. Nat Commun 2021; 12:3400.
This large-scale study across two independent centers showed that plasma NfL levels are higher in a range of neurological and psychiatric disorders.

11. Ashton NJ, Hye A, Rajkumar AP, *et al.* An update on blood-based biomarkers for non-Alzheimer neurodegenerative disorders. Nat Rev Neurol 2020; 16:265–284.

12. Schindler SE, Bollinger JG, Ovod V, *et al.* High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. Neurology 2019; 93: e1647–e1659.

13. Keshavan A, Pannee J, Karikari TK, *et al.* Population-based blood screening for ■■ preclinical Alzheimer's disease in a British birth cohort at age 70. Brain 2021; 144:434–449.
This paper reported the value and cost savings of plasma Aβ42/Aβ40 and p-tau181 to detect brain amyloidosis in a cognitively normal cohort of individuals born in the same year in Britain.

14. Nakamura A, Kaneko N, Villemagne VL, *et al.* High performance plasma amyloid-β biomarkers for Alzheimer's disease. Nature 2018; 554:249–254.

15. Karikari TK, Pascoal TA, Ashton NJ, *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; 19:422–433.

16. Palmqvist S, Janelidze S, Quiroz YT, *et al.* Discriminative accuracy of plasma ■■ phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. JAMA 2020; 324:772–781.
The first study to report the biomarker potential of plasma p-tau217 and to compare its performances to that plasma p-tau181.

17. Ashton NJ, Pascoal TA, Karikari TK, *et al.* Plasma p-tau231: a new biomarker ■■ for incipient Alzheimer's disease pathology. Acta Neuropathol 2021; 141:709–724.
This publication showed that plasma p-tau231 is a marker of emerging Alzheimer's disease that tends to be increased in a stepwise manner before and after the thresholds of Aβ PET are reached.

18. Ashton NJ, Janelidze S, Mattsson-Carlgren N, *et al.* Differential roles of Aβ42/ ■■ 40, p-tau231 and p-tau217 for Alzheimer's trial selection and disease monitoring. Nat Med 2022; https://doi.org/10.1038/s41591-022-02074-w.
This study reported that plasma Aβ42/Aβ40 and p-tau231 are the markers that become first increased in the asymptomatic phase of AD, with other markers such as plasma p-tau181 and GFAP following suit later. In longitudinal analysis, plasma p-tau217 was the only marker that showed significant increases at follow-up. The results suggest different roles for these markers in therapeutic trial selection and during-trial monitoring.

19. Palmqvist S, Tideman P, Cullen N, *et al.* Prediction of future Alzheimer's ■■ disease dementia using plasma phospho-tau combined with other accessible measures. Nat Med 2021; 27:1034–1042.
The reports indicated that plasma p-tau217 or p-tau181, when combined with APOE e4 genotype and cognitive performance can be effective for prognostication of future AD.

20. Milà-Alomà M, Ashton NJ, Shekari M, *et al.* Plasma p-tau231 and p-tau217 as ■■ state markers of amyloid-β pathology in preclinical Alzheimer's disease. Nat Med 2022; 28:1797–1801.
In this cohort of cognitively normal older adults, plasma p-tau231 and p-tau217 performed well to identify those with pathological amounts of brain Aβ.

21. Lantero Rodriguez J, Karikari TK, Suárez-Calvet M, *et al.* Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to postmortem and improves the clinical characterisation of cognitive decline. Acta Neuropathol 2020; 140:267–278.

22. Smirnov DS, Ashton NJ, Blennow K, *et al.* Plasma biomarkers for Alzheimer's ■■ disease in relation to neuropathology and cognitive change. Acta Neuropathol 2022; 143:487–503.
in this blood-to-autopsy study, plasma p-tau biomarkers were accurate to identify brain Alzheimer's disease neuropathologic change.

23. Chatterjee P, Pedrini S, Stoops E, *et al.* Plasma glial fibrillary acidic protein is ■■ elevated in cognitively normal older adults at risk of Alzheimer's disease. Transl Psychiatry 2021; 11:1–10.
This was one of the pioneering studies to show that plasma GFAP is higher in cognitively normal adults with Aβ pathology compared with those without.

24. Benedet AL, Milà-Alomà M, Vrillon A, *et al.* Differences between plasma and ■■ cerebrospinal fluid glial fibrillary acidic protein levels across the alzheimer disease continuum. JAMA Neurol 2021; 78:1471–1483.
This study was the first to demonstrate that plasma GFAP performs better to predict brain Aβ pathology than CSF GFAP measured in the same set of individuals.

25. Ferrari-Souza JP, Ferreira PCL, Bellaver B, *et al.* Astrocyte biomarker signa- ■■ tures of amyloid-β and tau pathologies in Alzheimer's disease. Mol Psychiatry 2022; 27:4781–4789.
This report demonstrated that in AD, CSF GFAP associates well with brain Aβ pathology while CSF YKL-40 is preferentially associated with tau pathology, suggesting divergence in the association of these astrocytic biomarkers with principal components of Alzheimer pathology.

26. Benussi A, Ashton NJ, Karikari TK, *et al.* Serum glial fibrillary acidic protein (GFAP) is a marker of disease severity in frontotemporal lobar degeneration. J Alzheimers Dis 2020; 77:1129–1149.

27. Katisko K, Cajanus A, Huber N, *et al.* GFAP as a biomarker in frontotemporal ■ dementia and primary psychiatric disorders: diagnostic and prognostic performance. J Neurol Neurosurg Psychiatry 2021; 92:1305–1312.
A first demonstration that GFAP in blood is higher not only in AD but also in non-AD neurodegenerative diseases like frontotemporal dementia and also in primary psychiatric disorders.

28. Gaetani L, Blennow K, Calabresi P, *et al.* Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry 2019; 90:870–881.

29. Ashton NJ, Leuzy A, Lim YM, *et al.* Increased plasma neurofilament light chain concentration correlates with severity of postmortem neurofibrillary tangle pathology and neurodegeneration. Acta Neuropathol Commun 2019; 7:5.

30. Bäckström D, Linder J, Mo SJ, *et al.* NfL as a biomarker for neurodegeneration and survival in Parkinson disease. Neurology 2020; 95:e827–e838.

31. Hansson O, Janelidze S, Hall S, *et al.* A biomarker for differential diagnosis of parkinsonian disorder. Neurology 2017; 88:930–937.

32. Lin C-H, Li C-H, Yang K-C, *et al.* A biomarker for disease severity and progression in Parkinson disease. Neurology 2019; 93:e1104–e1111.

33. Mattsson N, Cullen NC, Andreasson U, *et al.* Association between longitudinal plasma neurofilament light and neurodegeneration in patients with Alzheimer disease. JAMA Neurol 2019; 76:791–799.

34. Mattsson N, Andreasson U, Zetterberg H, Blennow K. Association of plasma neurofilament light with neurodegeneration in patients with Alzheimer disease. JAMA Neurol 2017; 74:557–566.

35. Forgrave LM, Ma M, Best JR, DeMarco ML. The diagnostic performance of neurofilament light chain in CSF and blood for Alzheimer's disease, frontotemporal dementia, and amyotrophic lateral sclerosis: a systematic review and meta-analysis. Alzheimers Dement (Amst) 2019; 11:730–743.

36. Kapoor R, Smith KE, Allegretta M, *et al.* Serum neurofilament light as a biomarker in progressive multiple sclerosis. Neurology 2020; 95:436–444.

37. Mielke MM, Syrjanen JA, Blennow K, *et al.* Plasma and CSF neurofilament light: Relation to longitudinal neuroimaging and cognitive measures. Neurology 2019; 93:e252–e260.

38. Ashton NJ, Suárez-Calvet M, Karikari TK, *et al.* Effects of preanalytical ■ procedures on blood biomarkers for Alzheimer pathophysiology, glial activation and neurodegeneration. Alzheimers Dement Diagn Assessm Dis Monit 2021; 13:e12168.
This study described how preanalytical handling factors affect plasma and serum biomarker levels.

39. Altmann P, Ponleitner M, Rommer PS, *et al.* Seven day preanalytical stability of ■ serum and plasma neurofilament light chain. Sci Rep 2021; 11:11034.
This paper showed that NfL is highly robust and stable over a seven-day period when measured in either plasma or serum.

40. Janelidze S, Teunissen CE, Zetterberg H, *et al.* Head-to-head comparison of 8 ■■ plasma amyloid-β 42/40 assays in alzheimer disease. JAMA Neurol 2021; 78:1375–1382.
This publication compared performances of different plasma Aβ assays in a head-to-head comparison.

41. Palmqvist S, Stomrud E, Cullen N, *et al.* An accurate fully automated panel of ■■ plasma biomarkers for Alzheimer's disease. Alzheimers Dement 2022.
This publication described the diagnostic utility of a panel of plasma biomarkers available on the Roche Elecsys platform.

42. Alcolea D, Delaby C, Muñoz L, *et al.* Use of plasma biomarkers for AT(N) ■ classification of neurodegenerative dementias. J Neurol Neurosurg Psychiatry 2021; 92:1206–1214.
This study investigated the potential of combining plasma Aβ42/40, p-tau181 and NfL to generate a blood-based AT(N) classification scheme for AD.

43. Benedet AL, Brum WS, Hansson O, *et al.* Alzheimer's Disease Neuroimaging ■■ Initiative: the accuracy and robustness of plasma biomarker models for amyloid PET positivity. Alzheimers Res Ther 2022; 14:26.
This study described how hypothetical changes in assay repeatability affected plasma biomarker diagnostic performances.

44. Zetterberg H, Blennow K. Moving fluid biomarkers for Alzheimer's disease from research tools to routine clinical diagnostics. Mol Neurodegener 2021; 16:10.

45. Moscoso A, Grothe MJ, Ashton NJ, *et al.* Time course of phosphorylated-tau181 in blood across the Alzheimer's disease spectrum. Brain 2020; 144:325–339.

46. Grothe MJ, Moscoso A, Ashton NJ, *et al.* Initiative for the ADN: ■ associations of fully automated CSF and novel plasma biomarkers with Alzheimer disease neuropathology at autopsy. Neurology 2021; 97: e1229–e1242.
This study showed that plasma and CSF biomarkers including p-tau181 are highly predictive of autopsy-verified diagnosis and pathological features of AD.

47. Ashton NJ, Puig-Pijoan A, Milà-Alomà M, *et al.* Plasma and CSF biomarkers in ■■ a memory clinic: head-to-head comparison of phosphorylated tau immunoassays. Alzheimers Dement 2022. doi: 10.1002/alz.12841.
This is the largest head-to-head comparison of different plasma p- tau biomarkers from multiple academic and industrial laboratories.

48. Verberk IMW, Misdorp EO, Koelewijn J, *et al.* Characterization of preanalytical
■■ sample handling effects on a panel of Alzheimer's disease–related blood-based biomarkers: results from the Standardization of Alzheimer's Blood Biomarkers (SABB) working group. Alzheimers Dement 2022; 18:1484–1497.

This study documented preanalytical handling procedures from different laboratories and how these factors affect biomarker levels.

49. Yang Z, Wang KKW. Glial Fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci 2015; 38:364–374.

50. Pereira JB, Janelidze S, Smith R, *et al.* Plasma GFAP is an early marker of amyloid-β but not tau pathology in Alzheimer's disease. Brain 2021; 144:3505–3516.

51. Simrén J, Weninger H, Brum WS, *et al.* Differences between blood and
■ cerebrospinal fluid glial fibrillary acidic protein levels: the effect of sample stability. Alzheimers Dement 2022; 18:1988–1992.

This report indicated that plasma GFAP had higher technical robustness that CSF GFAP.

52. Abdelhak A, Huss A, Kassubek J, *et al.* Serum GFAP as a biomarker for disease severity in multiple sclerosis. Sci Rep 2018; 8:14798.

53. Barro C, Healy BC, Liu Y, *et al.* Serum GFAP and NfL levels differentiate subsequent progression and disease activity in patients with progressive multiple sclerosis. Neurol Neuroimmunol Neuroinflamm 2023; 10:e200052.

54. Karikari TK, Emersic A, Vrillon A, *et al.* Head-to-head comparison of clinical performance of CSF phospho-tau T181 and T217 biomarkers for Alzheimer's disease diagnosis. Alzheimers Dement 2021; 17:755–767.

55. Chen Z, Mengel D, Keshavan A, *et al.* Learnings about the complexity of extracellular tau aid development of a blood-based screen for Alzheimer's disease. Alzheimers Dement 2018; 15:487–496.

56. Snellman A, Lantero-Rodriguez J, Emeršič A, *et al.* N-terminal and mid-region
■■ tau fragments as fluid biomarkers in neurological diseases. Brain 2022; 145:2834–2848.

This publication demonstrated that N-terminal nonphosphorylated tau forms associate better with early Aβ pathological changes than mid-region-tau forms. The study was also the first to describe the plasma NTA assay.

57. Gonzalez-Ortiz F, Turton M, Kac PR, *et al.* Brain-derived tau: a novel blood-
■■ based biomarker for Alzheimer's disease-type neurodegeneration. Brain 2022; https://doi.org/10.1093/brain/awac407.

This study showed the characterization and clinical performance of a CNS-tau specific antibody and a blood-based assay that is selective for brain-derived tau.

58. Lierop ZYGJ van, Verberk IMW, *et al.* Preanalytical stability of serum biomarkers for neurological disease: neurofilament-light, glial fibrillary acidic protein and contactin. *Clin Chem Lab Med* 2022;60:842–850.

59. Alzforum. Plasma Aβ test wins approval—are p-tau tests far behind? 2020.

60. Weber DM, Tran D, Goldman SM, *et al.* High-throughput mass spectrometry assay for quantifying β-amyloid 40 and 42 in cerebrospinal fluid. Clin Chem 2019; 65:1572–1580.

61. Karikari TK. Blood tests for Alzheimer's disease: increasing efforts to expand and diversify research participation is critical for widespread validation and acceptance. J Alzheimers Dis 2022; 90:967–974.

62. Schindler SE, Karikari TK, Ashton NJ, *et al.* Effect of race on prediction of brain
■■ amyloidosis by plasma aβ42/aβ40, phosphorylated tau, and neurofilament light. Neurology 2022; 99:e245–e257.

This paper reported significant differences in plasma p-tau and NfL between self-identified non-Hispanic White and African-American participants of the same age, sex, cognitive performance and education.

63. O'Bryant SE, Zhang F, Petersen M, *et al.* Neurodegeneration from the AT(N)
■■ framework is different among Mexican Americans compared to non-Hispanic Whites: A Health & Aging Brain among Latino Elders (HABLE) study. Alzheimers Demen Diagn Assessm Dis Monitor 2022; 14:e12267.

This study showed than neurodegeneration biomarker levels are different in older adults who either self-identified as Hispanic or non-Hispanic White.

64. Windon C, Iaccarino L, Mundada N, *et al.* Adni: comparison of plasma and
■■ CSF biomarkers across ethnoracial groups in the ADNI. Alzheimers Demen Diagn Assessm Dis Monitor 2022; 14:e12315.

This publication pointed to a lack of racial/ethnic difference in plasma and CSF AD biomarkers, including when demographic covariates are accounted for.

65. Naslavsky MS, Suemoto CK, Brito LA, *et al.* Global and local ancestry
■■ modulate APOE association with Alzheimer's neuropathology and cognitive outcomes in an admixed sample. Mol Psychiatry 2022; 27:4800–4808.

The results of this study indicated differences in cognitive predictors and APOE e4 as a risk factor among people of high European and African ancestry.

66. Schlesinger D, Grinberg LT, Alba JG, *et al.* African ancestry protects against Alzheimer's disease-related neuropathology. Mol Psychiatry 2013; 18:79–85.

67. Syrjanen JA, Campbell MR, Algeciras-Schimnich A, *et al.* Associations of
■■ amyloid and neurodegeneration plasma biomarkers with comorbidities. Alzheimers Dement 2022; 18:1128–1140.

Syrjanen *et al.* demonstrated associations between plasma Aβ42, Aβ40, Aβ42/Aβ40 ratio and NfL with common comorbidities of aging in older adults.

68. Mielke MM, Dage JL, Frank RD, *et al.* Performance of plasma phosphorylated
■■ tau 181 and 217 in the community. Nat Med 2022; 28:1398–1405.

This publication showed that plasma p-tau181 and p-tau217 associate with common comorbidities of aging in older adults, with effect sizes between those with and without kidney disease paralleling those in PET Aβ-positive versus Aβ-negative individuals.

69. Barnes LL, Leurgans S, Aggarwal NT, *et al.* Mixed pathology is more likely in black than white decedents with Alzheimer dementia. Neurology 2015; 85:528–534.