Blood-based biomarkers for Alzheimer's disease: towards clinical implementation

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For many years, blood-based biomarkers for Alzheimer's disease seemed unattainable, but recent results have shown that they could become a reality. Convincing data generated with new high-sensitivity assays have emerged with remarkable consistency across different cohorts, but also independent of the precise analytical method used. Concentrations in blood of amyloid and phosphorylated tau proteins associate with the corresponding concentrations in CSF and with amyloid-PET or tau-PET scans. Moreover, other blood-based biomarkers of neurodegeneration, such as neurofilament light chain and glial fibrillary acidic protein, appear to provide information on disease progression and potential for monitoring treatment effects. Now the question emerges of when and how we can bring these biomarkers to clinical practice. This step would pave the way for blood-based biomarkers to support the diagnosis of, and development of treatments for, Alzheimer's disease and other dementias.

Introduction

Biomarkers that accurately reflect Alzheimer's disease pathology during life are an important part of inclusion criteria in clinical trials. They are also important for diagnosis in clinical practice, particularly now that disease-modifying therapies are becoming available. Classic pathophysiological hallmarks of Alzheimer's disease (amyloid β [Aβ], tau, and neurodegeneration) can currently be detected using either CSF or imaging techniques,[1] with amyloid-PET and tau-PET scans as the gold standards of amyloid and tau pathology in clinical trials.[2] However, these methods are either invasive or very expensive, or both. Thus, there is an important medical need to identify cost-effective biomarkers that can be more easily obtained in a less invasive manner, and that can be serially measured. It is likely that blood-based biomarkers will fulfill this need.

Blood-based biomarkers for Alzheimer's disease and other dementias are now becoming a reality. Results from well defined cohorts show high potential for implementation not only of the core pathological biomarkers (ie, Aβ and phosphorylated tau [pTau])[3] but also of blood markers of neurodegeneration (eg, neurofilament light chain [NfL]). These results show that, in people with Alzheimer's disease, these plasma biomarkers are abnormal in synchrony with CSF biomarker values, and thus could become powerful instruments for early and precise diagnosis, prognosis, or monitoring of disease progression and treatment effects in both clinical practice and trials. The identification of blood markers is a breakthrough in the field because it might provide the option to diagnose people with cognitive problems using a minimally invasive and cost-effective tool.

Herein, we describe the state of the art of the highly dynamic and accelerating field of blood-based biomarkers for Alzheimer's disease. We provide a comprehensive overview of recent progress (particularly since 2018) focusing on three biomarkers that are closest to clinical implementation—amyloid, pTau, and NfL. We also discuss the novel and emerging astrocyte biomarker glial fibrillary acidic protein (GFAP). Finally, we present a blood-based biomarker roadmap towards clinical implementation, addressing the specific activities needed to enable implementation in clinical care and in trials, highlighting the progress so far.

Blood-based biomarkers in sporadic Alzheimer's disease

$A\beta_{1-42}$ and $A\beta_{1-40}$

Aβ is the main pathological hallmark of Alzheimer's disease, and CSF and PET biomarkers for Aβ pathology become abnormal decades before dementia symptom onset.[1,4,5] A meta-analysis published in 2016 showed no differences in plasma concentrations of $A\beta_{1-42}$ and $A\beta_{1-40}$ in individuals with Alzheimer's disease compared with controls.[6] However, with the development of highly sensitive assays and technologies, recent results are much more promising.

Various reliable methods for precise and robust quantification of plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ are now available, each with their own advantages and disadvantages with regard to cost and practical aspects. Mass spectrometry assays and automated ultrasensitive immunoassays (eg, single molecule array; panel 1) can quantify either the specific full-length ($A\beta_{1-42}$ and $A\beta_{1-40}$) or N-terminally truncated forms ($A\beta_{x-42}$ and $A\beta_{x-40}$) of these peptides.[7–21] A plasma test based on the mass spectrometry analysis of Aβ has been approved according to the Clinical Laboratory Improvement Amendments (CLIA) to detect Aβ pathology.[22] Other methods can detect amyloid oligomerisation or Alzheimer's disease-specific structural changes of these plasma peptides.[23,24]

The clinical value of these assays for quantification of plasma Aβ has been validated in different cohorts covering the complete continuum of Alzheimer's disease. The assays have also been compared with established Alzheimer's disease biomarkers (CSF $A\beta_{1-42}$ and Aβ-PET) and in relation to cognitive performance and cognitive decline (both baseline and longitudinal changes).[6,8]

The ratio of plasma $A\beta_{1-42}$ to $A\beta_{1-40}$ (denoted as Aβ42/40) identified abnormal amyloid CSF or PET status in individuals across the clinical Alzheimer's disease continuum, with accuracies ranging between 82% and 97% for mass spectrometry assays[8–11,22] and between 62% and 79% for immunoassays.[11–21] Classification performance was better in those with dementia than in those with mild cognitive impairment, and improved after correction for carriership of the *APOE* ε4 allele.[11–22] Of note, a decrease in plasma Aβ42/40 of less than 20% was recorded in individuals with cerebral Aβ pathology compared with those without this pathology.[8] By contrast, about a 50% decrease in CSF Aβ42/40 was seen in individuals with pathological findings of Alzheimer's disease on amyloid PET scans compared with those without this pathology.[6,8,10] Multiple factors might account for this difference, including the production of plasma Aβ from peripheral sources, binding to peripheral blood proteins that are present at approximately 200-fold higher concentrations in plasma than in CSF, and liver metabolic rates. In line with CSF or PET amyloid data,[18] low plasma Aβ42/40 or presence of misfolded $A\beta_{1-42}$ are associated with cognitive decline and risk of progression to Alzheimer's disease among cognitively unimpaired individuals, people with subjective cognitive decline, or individuals with mild cognitive impairment.[13,18,23–29] The diagnostic value of plasma Aβ42/40 to differentiate Alzheimer's disease from non-Alzheimer's disease dementias has not yet been investigated.

pTau forms

Tangles containing hyperphosphorylated tau in full-length or truncated forms are another major pathological hallmark of Alzheimer's disease. So far, studies in which plasma total tau was measured found too much overlap between clinical groups and low diagnostic value, even when using ultrasensitive technologies.[30–34] This finding contrasts starkly with results obtained when analysing post-translational modified forms of tau. Tau has more than 70 post-translational modification sites, including more than 40 phosphorylation sites and several truncated forms.[35] Different pTau forms are measurable in both CSF and plasma. Similar to Aβ, various methods are available, such as mass spectrometry and immune-based approaches, to detect amyloid or tau pathology with high accuracy (80–98%).[7,36–44]

The concentrations of plasma tau phosphorylated at different sites (pTau181, pTau217, or pTau231) are strongly increased (more than two-fold) in individuals with clinically diagnosed Alzheimer's disease compared with both cognitively unimpaired controls[41–43,45] and people with non-Alzheimer's disease dementias.[37–47] Plasma pTau181 has gained momentum as a biomarker since an initial report showing that plasma pTau181 concentrations in patients with Alzheimer's disease correlated with tau-PET.[37] Findings of subsequent independent studies strongly suggest that plasma pTau181 reflects Alzheimer's disease-specific neuropathology[38,39,48] because pTau181 is also elevated in people with Alzheimer's disease compared with individuals with non-Alzheimer's disease dementias, including other tauopathies.[38–40,46,48] In a prospective cohort study of both cognitively impaired and unimpaired individuals, both baseline and longitudinal changes in plasma pTau181 were associated with widespread tau aggregation 6 years later.[49] Moreover, pTau181 differentiated participants with amyloid pathology across different clinical stages and correlated with increased tau-PET, particularly in brain areas affected by

Panel 1: Technologies commonly used for blood-based biomarker measurements

ELISA
Protein concentration is measured by antibody pairs (capture and detection) able to specifically capture the analyte of interest into the wells of a plate and generate sandwich immunocomplexes (capture antibody–analyte–detection antibody). The detection antibody is labelled with an enzyme that catalyses the conversion of a substrate to a product, which generates fluorescence or a colour change proportional to the amount of analyte within the sample (usually within the nmol/L or pmol/L range).

Electrochemiluminescence immunoassays
An antibody-based approach similar to ELISA; the detection antibody is labelled with an electrochemically active molecule that generates an electrochemiluminescence signal that is proportional to the amount of analyte within the sample. This technology is in principle more sensitive than ELISA and entails fewer washing steps, which often result in some loss of reporter signal.

Single molecule array
An antibody-based approach similar to ELISA; sandwich immunocomplexes are coupled to magnetic beads rather than to a solid plate. Each single bead is loaded into its own single well with the corresponding substrate, and a fluorescence signal is then generated. The very low volume of the wells (around 40 fL) ensures a high local concentration of fluorescent signal, allowing the detection of single molecules. This technique can, thus, measure proteins at very low concentrations (fmol/L range), providing 100–1000 times higher sensitivity than ELISA.

Immunoprecipitation mass spectrometry
Antibodies coupled to beads are used to first isolate the analyte of interest from the samples. The analyte is then eluted and quantified by mass spectrometry using an isotope-labelled form of the target as an internal standard.

Immuno-infrared sensor
This technology can detect structural protein changes (eg, protein misfolding) and has been used specifically to detect changes in the secondary structure of amyloid β peptides.

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Alzheimer's disease pathology.[38–40] In addition, increased pTau181 concentrations in plasma were associated with grey matter atrophy.[38,50] These associations were observed only in individuals with Aβ pathology,[51] which underpins pTau181's specificity for Alzheimer's disease-associated neuropathological changes. pTau181 also differentiated patients with mild cognitive impairment who progressed to Alzheimer's disease from those who did not progress[39,44,52] and had better performance as a plasma biomarker than did Aβ42/40.[44] Similar results have been observed in individuals without dementia, with high pTau181 associated with higher risk of progressing to Alzheimer's disease.[39,40 11]

Additional promising pTau markers are emerging. Compared with pTau181, CSF pTau217 showed greater differences between Alzheimer's disease and controls when using mass spectrometry approaches.[42,53] Findings of a large, multicentre cohort study showed that plasma pTau217 can differentiate Alzheimer's disease from non-Alzheimer's disease dementias with high accuracy (96%), similar to the performance of established CSF or tau-PET biomarkers.[41] Plasma pTau217 also identified tau-PET positive cases with high accuracy. Using neuropathological ratings of cerebral tau-tangle pathology, plasma pTau217 concentrations correlate with the density of cortical tau pathology in Alzheimer's disease, but not in other tauopathies such as FTD-tau, emphasising the specificity of plasma pTau for Alzheimer's disease tau pathology.[41] The study also showed that plasma pTau217 starts to increase about 20 years before the onset of mild cognitive impairment in autosomal dominant Alzheimer's disease, which is congruent with results showing that plasma pTau217 becomes abnormal before tau-PET.[54] This finding suggests that the origin of plasma pTau changes might not be the same as the biological processes causing tau-PET signal. Direct comparison studies using both mass spectrometry and immunochemical platforms showed that pTau217 and pTau181 perform well in discriminating different modalities (autopsy-confirmed Alzheimer's disease *vs* frontotemporal lobar degeneration, tau-PET negativity, and Aβ-PET negativity).[42,45,53,55,56] However, pTau217 was better able to detect Aβ-PET positivity, particularly when using mass spectrometry approaches.[42,53] pTau217 also showed slightly better accuracy when discriminating Alzheimer's disease from other dementias and a stronger association with tau-PET.[56]

A recent study analysed plasma pTau231 in several cohorts, including a neuropathological cohort of individuals with Alzheimer's disease and non-Alzheimer's disease dementias, and compared plasma pTau231 with plasma pTau181.[43] pTau231 differentiated Alzheimer's disease from non-Alzheimer's disease dementias with a performance similar to pTau181. However, based on both Aβ-PET and tau Braak stages, pTau231 started to change in earlier stages of Alzheimer's disease pathology,[43] suggesting that pTau231 might be especially valuable as an early pathological marker of Alzheimer's disease even before the onset of symptoms. However, another study of a community-based population did not replicate the superiority of pTau231 over pTau181 or pTau217 for predicting amyloid or tau-PET.[57] Future studies should address the value of different pTau forms—ie, whether the differences are truly biological or depend on the technology used.

pTau forms have been shown to detect Alzheimer's disease pathology across the clinical Alzheimer's disease continuum, and could thus be used as Alzheimer's disease-specific diagnostic markers. Evaluation in the earliest possible stages of Alzheimer's disease, and in cognitively unimpaired individuals, is a crucial next step. A recent study in a cohort of individuals with subjective cognitive decline or mild cognitive impairment found that the best performing model for predicting Alzheimer's disease was a combination of plasma pTau217 or pTau181, *APOE* genotype, and three brief cognitive tests of memory and executive function.[52] The combination of these tests and biomarkers could facilitate recruitment for Alzheimer's disease trials, due to the ease of performing blood tests compared with CSF analysis or PET scans. The plasma NfL concentration or plasma Aβ42/40 measured with immunoassays did not contribute to this model.[52] However, another study showed that plasma Aβ42/40 measured by mass spectrometry outperformed both plasma pTau181 concentration and plasma Aβ42/40 measured by single molecule array for detecting Aβ-PET positivity among participants without dementia.[11] Additional studies are needed to determine the specificities of the tests and their most suitable context of use. In addition, unlike plasma Aβ42/40, plasma pTau concentration gradually increases during disease progression,[58] thereby suggesting that pTau could be potentially useful to monitor disease stage. This feature might be useful in clinical trials of drug candidates targeted at slowing disease progression. The main limitation of the studies to date are that they have been primarily done by a few groups using retrospective cohorts in specialised centres. Prospective validation and inclusion of more heterogeneous populations are some of the necessary next steps for research.

Besides phosphorylated sites, the differential truncation patterns observed in circulating tau fragments could themselves capture important aspects of Alzheimer's disease-related neurodegeneration and cognitive decline. The N-terminal fragment of tau (NT1) in plasma is increased in individuals with Alzheimer's disease compared with cognitively unimpaired controls and predicts cognitive decline and neurodegeneration among cognitively unimpaired elderly individuals.[59,60]

Neurofilament light chain

NfL is an axonal scaffolding protein and one of two core neurofilament proteins in the CNS (the other being α-internexin). Neurofilaments are essential for both the

growth and stability of axons and also in synaptic organisation and function in the CNS.[61] NfL has emerged as a strong cross-disease biomarker candidate for neurodegeneration.[62,63] This biomarker can be measured in CSF and blood and was the first neurospecific biomarker for which clinical value was proven in a multitude of publications after development of an ultrasensitive assay.[64] Among neuroinflammatory and neurodegenerative diseases, the correlation between CSF and blood concentrations of NfL is good to excellent (*r* values of 0·70–0·97).[65] The highest NfL concentrations are seen in frontotemporal, vascular, and HIV-associated dementias, as well as in amyotrophic lateral sclerosis and atypical parkinsonian disorders.[66] In sporadic Alzheimer's disease, CSF NfL shows the second highest fold-change among Alzheimer's disease-associated fluid biomarkers (after CSF total tau [tTau]).[6] Increased concentrations of NfL in CSF and plasma are associated with amyloid-PET and tau-PET positivity, as well as with longitudinal neurodegeneration, as determined by MRI, but with a larger overlap across groups than in familial Alzheimer's disease.[67,68] Concentrations of NfL increase with age, which complicates interpretation of results. In clinical research and practice, NfL is used as a general biomarker of neuroaxonal injury or degeneration, irrespective of the underlying cause. It can be used to indicate a neurodegenerative process among patients with psychiatric symptoms.[69] The biomarker could thus be used as an initial diagnostic test that, if positive, could lead to additional examinations with more specific biomarkers to better understand the underlying cause of the neurodegeneration. NfL could also serve as a biomarker of disease severity in clinical trials of disease-modifying treatments, and to optimise and monitor treatment effects in clinical practice, similar to its uses in multiple sclerosis.[70]

Glial fibrillary acidic protein

GFAP is a major cytoskeletal constituent of astrocytes. In Alzheimer's disease, reactive astrocytosis has been implicated as a potential driver or effect of Alzheimer's disease pathological changes.[71] In a neuropathology study of a population-based cohort, both GFAP expression and protein concentrations were higher in areas surrounding dense Aβ plaques and increased with tau accumulation.[72] Despite GFAP being less thoroughly investigated than Aβ, tau, and NfL, promising results for its usefulness as a fluid biomarker have been obtained using ultrasensitive immunoassays. Plasma or serum GFAP concentrations are elevated in individuals within the clinical Alzheimer's disease continuum.[18,19,44,73,74] GFAP concentrations can differentiate abnormal Aβ-PET status with about 81% accuracy when considering the complete clinical spectrum from cognitively unimpaired up to Alzheimer's disease,[18] and between 76% and 80% accuracy when considering individuals without dementia.[18,19,75] Plasma GFAP concentrations increased linearly with increasing Aβ-PET burden, but the linear association disappeared with a high Aβ-PET burden.[76] Moreover, increases in plasma or serum GFAP were also related to clinical disease severity, as shown by associations with syndrome diagnosis, neuropsychological test performance, and MRI atrophy measures.[18,19,73,74] In patients with mild cognitive impairment, plasma GFAP predicted subsequent development of Alzheimer's disease.[75] Furthermore, in cognitively unimpaired individuals, higher concentrations of GFAP in serum were associated with steeper rates of cognitive decline[44,77,78] and higher risk of dementia.[77,78] Of note, the predictive value of plasma or serum GFAP is independent of plasma Aβ42/40.[18,77] Since astrocyte activation is not specific to Alzheimer's disease pathophysiology, GFAP might be a potential biomarker for other types of dementia as well. Indeed, some studies found that serum or plasma GFAP concentrations were increased in subtypes of frontotemporal dementia.[74,79,80] Concentrations were also elevated in Lewy body dementia compared with both controls and people with Parkinson's disease.[74] Plasma or serum GFAP could thus be used both for diagnosis (in diagnostic panels) and for prognosis. However, further studies are needed to define these potential clinical applications across different types of dementia and neurodegenerative diseases. In view of the early and gradual increases in GFAP co-occurring with amyloid pathology, and given its prognostic value, plasma GFAP could also play a part in trials—eg, to support inclusion of individuals with early stages of Alzheimer's disease, for enrichment, and to monitor treatment responses.

Blood-based biomarkers in genetically determined Alzheimer's disease

Alzheimer's disease has a monogenetic determined cause in rare cases, with a mutation in either the *PSEN1*, *PSEN2*, or *APP* gene, or a multiplication of the *APP* gene, which also occurs in Down syndrome due to the chromosome 21 trisomy.[5,81] The advantage of studying genetically determined Alzheimer's disease is that the young age of onset (on average 40–60 years) reduces the interference of general ageing on biomarker concentrations. In addition, the age of onset is predictable per mutation. This situation allows biomarkers to be studied over the disease course and has contributed tremendously to the conceptual understanding of the sequence of changes in Alzheimer's disease.[5,81] Since the introduction of high-sensitivity assays, several cohort studies—both large and small—have analysed plasma Aβ, pTau, and NfL (but not yet GFAP) in genetically determined Alzheimer's disease.[41,47,81–86]

Increased plasma $Aβ_{1-42}$

Contrary to sporadic Alzheimer's disease, higher plasma $Aβ_{1–42}$ concentration and Aβ42/40 have been detected in both carriers of autosomal dominant Alzheimer's disease mutations and in individuals with Down syndrome,

including children, and the $A\beta_{1-42}$ concentrations and Aβ42/40 did not correlate with their CSF counterparts.[81,85] Elevated concentrations of $A\beta_{1-42}$ in blood in people with genetic Alzheimer's disease probably reflect overproduction of $A\beta_{1-42}$ caused by mutations affecting APP processing.[87] However, the dynamics of plasma $A\beta_{1-42}$ concentrations in genetic Alzheimer's disease are not yet fully understood.[81,87,88] A mass spectrometry analysis reported a relative reduction of plasma Aβ42/40 towards dementia onset and increased Aβ42/40 in symptomatic individuals, which depended on mutation type.[87] In people with Down syndrome, the initially increased concentrations in blood of $A\beta_{1-42}$ and Aβ42/40 also decreased nearer to the time of symptom onset, followed by increased $A\beta_{1-42}$ thereafter.[82,85] One should be cautious applying these results to older individuals (eg, 75–80 years) with sporadic Alzheimer's disease. Overall, these results suggest that in genetic Alzheimer's disease, plasma Aβ concentrations are increased and fluctuate over the lifetime depending on both genetic variation and the patient's disease stage; the mechanisms underlying these relationships are not yet fully understood.

Increased plasma pTau and NfL

Biomarker dynamics of pTau and NfL in individuals with genetically determined Alzheimer's disease are consistent with findings in people with sporadic Alzheimer's disease and provide insights into the timing of changes in these biomarkers. Regarding pTau forms, plasma concentrations of pTau181 and pTau217 begin to increase in autosomal dominant Alzheimer's disease 16–24 years before symptom onset, which aligns with the start of amyloid accumulation in the brain as measured with Aβ-PET and CSF Aβ.[41,47,89,90] Similar to the CSF biomarkers of Alzheimer's disease, the concentrations of plasma pTau appear to stabilise and can even decrease slightly after symptom onset.[91] Blood NfL concentrations also increase early, about 16 years before symptom onset in the Dominantly Inherited Alzheimer Network cohort study and 22 years before symptom onset in the Colombian kindred study.[83,84] However, plasma NfL could distinguish between mutation carriers and unaffected family controls only 3 years before symptom onset.[83] Similar to sporadic Alzheimer's disease, a continued increase in NfL concentrations was observed in symptomatic individuals.[83] Among individuals with Down syndrome, plasma NfL also began to increase more than 20 years before dementia onset.[81,82] Furthermore, baseline and longitudinal NfL concentrations correlated well with other signs of neurodegeneration and clinical diagnosis, and increases in NfL predicted future cognitive decline.[82,84–86,92,93] These findings in genetically determined Alzheimer's disease support the potential use of plasma pTau181, pTau217, and NfL for diagnostic, prognostic, and disease monitoring purposes very early in the disease course.

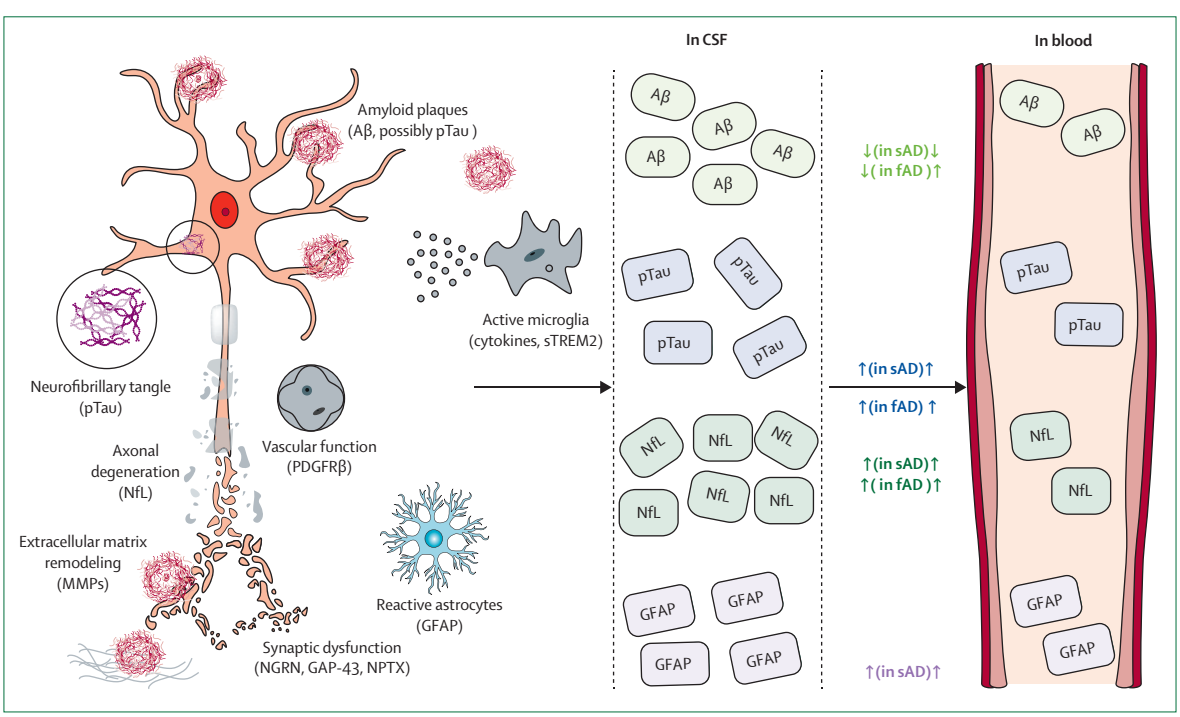

Figure 1: **Pathological mechanisms involved in Alzheimer's disease and their associated biofluid-based biomarkers**
Alzheimer's disease has a complex pathophysiology. Biofluid-based biomarkers that can be reliably measured in both blood and CSF are: Aβ, pTau, NfL, and GFAP. Biomarkers with strong potential in CSF only include: cytokines, sTREM2, PDGFRβ, MMPs, NGRN, GAP-43, and NPTX. Aβ=amyloid β. NfL=neurofilament light chain. pTau=phosphorylated tau. GFAP=glial fibrillary acidic protein. MMP=matrix metalloproteinase. sAD=sporadic Alzheimer's disease. fAD=familial Alzheimer's disease.

Factors associated with heterogeneity

As blood-based biomarkers of amyloid and tau pathology and neurodegeneration approach clinical use, it is essential to understand what factors affect the concentrations of these markers to best interpret the results (figure 1).[94] This information is especially important for the development of reference ranges. Because initial blood biomarker studies are done in well characterised populations, the examination of the blood markers and the factors that affect them in diverse population-based and community-based cohorts is crucial. This is especially important from a primary care standpoint, for which blood-based biomarkers are better suited compared with invasive and costly CSF and neuroimaging markers.[95] However, patients in primary care have maximum heterogeneity in terms of neurodegenerative diseases and comorbidities.

Factors such as age, sex, comorbidities, medication, lifestyle factors, and genetic variation can affect the clinical interpretation of blood biomarkers. Most study findings suggest that concentrations of NfL, GFAP, pTau181, pTau217, and total tau in blood increase with age, whereas Aβ42/40 decreases with age.[12,58,96,97] This finding could be accounted for by increased prevalence of neuropathology at older ages and changes in turnover of the biomarkers. Therefore, when assessing age-related associations of Alzheimer's disease-specific blood biomarkers (Aβ, pTau), it is important to stratify by in-vivo assessments of amyloid and tau pathology and to examine such associations among cognitively unimpaired individuals without pathology. The age-related aspects of non-specific markers of neurodegeneration (NfL, GFAP) are more difficult to study. Reports of sex differences in biomarker concentrations

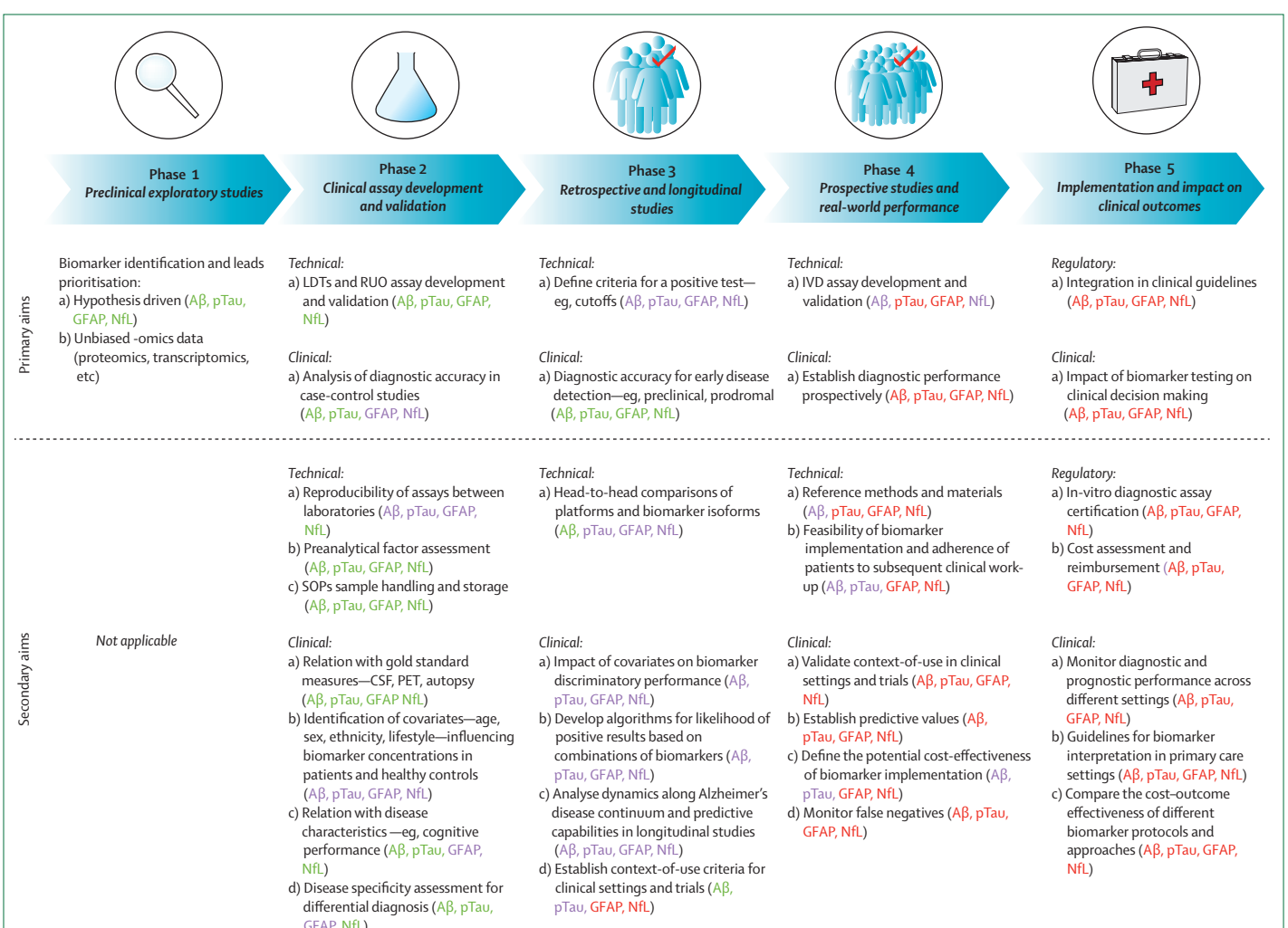

Figure 2: **Roadmap for the five phases in the development of blood-based biomarkers for the diagnosis of Alzheimer´s disease**
Colour codes indicate the levels of evidence obtained for each marker as estimated by the authors—green: accomplished to a large extent for this marker; purple: accomplished to some extent for this marker; red: no results are available yet that address this aspect for this marker. Aβ=amyloid β. NfL=neurofilament light chain. pTau=phosphorylated tau. GFAP=glial fibrillary acidic protein. LDT=laboratory-developed test. RUO=research use only. SOP=standard operating procedure. IVD=in-vitro diagnostic.

have varied. One study reported higher total tau concentrations for women,[98] but other studies have not observed a sex difference.[32,33,99] NfL concentration, measured in serum or plasma, and Aβ42/40 have not been found to differ by sex.[11,15,96,99]

Individuals with a higher body-mass index (BMI), particularly people in the overweight or obese categories, have lower concentrations of plasma NfL.[100] In an ethnically diverse population study, increased BMI was associated with lower concentrations of plasma pTau181, pTau217, and NfL, but was not related to Aβ42/Aβ40 or tTau.[45] Although obesity is associated with brain atrophy,[101] this observation is primarily accounted for by the higher blood volume that corresponds to increasing weight. Additional studies examining the effects of BMI on other blood-based biomarkers of Alzheimer's disease are needed before clinical implementation.

Cardiovascular comorbidities have also been shown to affect blood biomarker concentrations. One study reported that plasma Aβ concentrations were lower in individuals with hypertension, ischaemic heart disease, and diabetes.[12] However, replication in larger cohorts and with the examination of other biomarkers is needed. Renal disease can also affect blood biomarker concentrations because of reduced clearance.

The examination of blood-based biomarkers in diverse communities is needed to understand racial, ethnic, and geographical differences, which have been shown to affect Alzheimer's disease CSF biomarker and Aβ-PET values.[102,103] Blood biomarker studies in diverse populations showed that biomarker concentrations were not affected by race or ethnicity, or by sex.[78] However, in an autopsy study, plasma pTau181 and pTau217 were better able to distinguish the presence of Alzheimer's disease neuropathology changes in non-Hispanic Black individuals (area under the curve: 0·94–0·96) than in non-Hispanic White individuals (0·65–0·75).[45] Of note, it is essential to consider differences in blood biomarker concentrations in the context of other factors and not to overinterpret the results. As mentioned, BMI and renal disease can affect concentrations of Alzheimer's disease-related blood-based biomarkers. The age-adjusted prevalence of obesity is higher among Black and Hispanic women and men compared with White people, and it is higher in rural than in urban areas.[104] The same is true for chronic kidney disease.[105] Therefore, it is important to consider racial and ethnic differences in the frequency of comorbidities and other factors, and not simply state that there are racial or ethnic differences in the biomarker itself without any other context.

The road towards clinical implementation

Implementation of novel fluid biomarkers into clinical practice will require several aspects to be systematically addressed. The Geneva roadmap[106] describes a five-phase framework for biomarker development. After initial exploratory studies (phase 1), clinical assay development and validation (phase 2) of biomarkers needs to be done. In phase 3, use of biomarkers should be studied in retrospective and longitudinal cohorts, followed by evaluation of biomarkers in prospective validation studies in real-world settings (phase 4). The last phase (phase 5) is focused on clinical implementation.[106] We have adapted this roadmap by adding technical analytical aspects for blood-based biomarkers (figure 2). Phases 1 and 2 of the implementation roadmap have been

Panel 2: Glossary for in-vitro biomarker assays

Laboratory-developed test or so-called in-house test
Usually designed, developed, and used within a single laboratory. They are not legally marketed for either research or clinical use.

Research-use-only assay
Assays that are commercially available and approved by the regulatory authorities (eg, European Commission [CE-marked] or US Food and Drug Administration [FDA]) but do not have an intended clinical decision purpose. They are not legally marketed for clinical use.

In-vitro diagnostic assay
Assays that are intended for clinical decision making. These assays are approved by the authorisation bodies (eg, CE-marked or FDA-approved) and generally commercially available.

	Description	Consequences and impact	Expected timeframe
Memory clinic	Biomarkers are added to the repertoire of diagnostic tests in memory clinics. Performed in addition to medical and neurological examination, neuropsychological investigation, and imaging. In the future, might replace CSF or PET for Alzheimer's disease confirmation in some cases.	Diagnosis will be available in all memory clinics, not merely tertiary or academic centres. Broadens biomarker testing for Alzheimer's disease.	Short term (hopefully 3–5 years)
Primary care	Biomarkers to be used as a screening test, together with a brief cognitive test (eg, MMSE or MoCA). Results used to reassure patients or refer them for further testing to memory clinic. Confirmation of Alzheimer's disease pathology by CSF or PET in memory clinic.[52]	Will entail a stepwise diagnostic algorithm that is feasible even when accuracy is suboptimal. Use of biomarkers by the primary care provider would increase the number of individuals detected at the preclinical or prodromal stage, who would be followed up and better informed along the course of their disease, directly improving patient care (eg, expectation management).	Intermediate term (hopefully 5–10 years)
Population screening	Three prerequisites for screening are: (1) near 100% accuracy of screening test; (2) low cost of screening test; and (3) availability of treatment. Even when accuracy is achieved, high costs and low availability mean that a population-wide screening programme for Alzheimer's disease is not yet on the horizon.	Although population screening is unlikely for the foreseeable future, it is important to start thinking about strategies to communicate and deal with presymptomatic Alzheimer's disease in the community.	Long term (unlikely within the next 10 years)

MMSE=mini mental state examination. MoCA=Montreal Cognitive Assessment.

Table 1: **Different scenarios of diagnostic use of blood-based biomarkers**

addressed to a large extent for Aβ, pTau,[107] and NfL. Work on phases 1 and 2 for GFAP, and phase 3 for all biomarkers, is ongoing. Phases 4 and 5 still need to be addressed for all biomarkers.

In terms of assay requirements, phases 1–3 of the roadmap can use thoroughly validated, laboratory-developed tests or research-use-only assays (panel 2). For diagnostic assays to be used in clinical care (phases 4 and 5 of the roadmap), robust and scalable in-vitro diagnostic assays approved by the certifying bodies are needed. Another relevant prerequisite for implementation of biomarkers into trials or clinical practice is development of standardised operating procedures for processing and storage, preferably procedures that can accommodate analysis of multiple biomarkers. The effects of different preanalytical factors[108] are being carefully mapped as part of the Global Biomarker Standardization Consortium.[109] Studies by the Consortium have shown that a delay in the processing of blood directly after collection or centrifugation negatively affects Aβ concentrations when kept at room temperature, irrespective of the technology used.[110] However, this reduction can be mitigated by keeping the samples cold. No such effect was observed for pTau, NfL, or GFAP, and no effects were observed for repeated freezing and thawing. The results of this study led to a consensus standard operating procedure for blood-based biomarker collection.[110]

With respect to combined analyses for multiple biomarkers, validation of biomarkers in retrospective samples (phase 3 of the roadmap) also includes development of diagnostic decision tools, such as algorithms that incorporate information gathered from the biomarkers when they have been assessed in diagnostic panels. Since blood-based biomarkers allow for serially repeated measurements, it is important to understand whether within-individual rates of change provide more information than do absolute values. For example, the rate of change in serum NfL concentration can distinguish mutation carriers (ie, individuals with presymptomatic familial AD) from non-mutation carriers earlier than absolute values, whereas absolute values might be more useful in the symptomatic phase.[84]

Criteria on appropriate use of biofluid-based biomarkers for individual patients should be defined in phase 4 (eg, when and how these biomarkers should be used). An especially challenging consideration is the application of biomarkers at presymptomatic and prodromal stages of disease. Personalised prediction of dementia risk based on MRI and CSF biomarkers has been described for mild cognitive impairment.[111] Considering that the predictive value of plasma pTau181 is similar to that of CSF pTau181,[36] such personalised models could also be developed for blood-based biomarkers.

Communication of biomarker results to potential users (ie, clinicians) and patients will be key during phase 5 of the implementation roadmap. Even without curative treatments, people value information highly, for example, to understand the origin of their complaints, to better deal with signs and symptoms, for advanced care planning, and to be able to make informed choices for the future.[112,113]

	Markers	Alzheimer's disease stage	Consequence
Prescreening in at-risk populations	Aβ, pTau	Predementia	Cost-effective and practical early Alzheimer's disease detection
Inclusion criterion	Aβ and pTau to prescreen for Alzheimer's disease, eventually combined with NfL and GFAP and cognitive measures in an algorithm, yielding cutoffs for an inclusion *vs* exclusion decision[114]	Predementia and dementia	Cost-effective and practical early Alzheimer's disease detection
Enrichment and stratification during inclusion	pTau, GFAP, and NfL concentrations, eventually split into different progression cutpoints to use their prognostic value	Predementia and dementia	Improves the power of trials
Target engagement	Drug-specific targets—eg, Aβ markers to show targeted Aβ-interfering effects	Predementia and dementia	Detects a biological effect
Outcome measures	NfL, Aβ, and pTau; NfL has more widespread use than Aβ or pTau;[115] surrogacy to be proven for all	Predementia and dementia	Treatment efficacy and effects; understanding the biological effects of drugs

Aβ=amyloid β. pTau=phosphorylated tau. NfL=neurofilament light chain. GFAP=glial fibrillary acidic protein.

Table 2: **Different applications of blood-based biomarkers in clinical trial design**

The major clinical contexts for use of blood-based diagnostic biomarkers are outlined in table 1. Use of blood-based biomarkers in specialist memory clinics could be implemented in the next 3–5 years. Initiation of the diagnostic process in primary care settings could be expected within the next 5–10 years. Population screening (often done in the primary care setting) is a long-term vision.

Currently, Alzheimer's disease biomarker testing is limited because of the high cost and low availability of amyloid PET and CSF biomarkers. Blood-based biomarkers could, therefore, enable widespread testing for Alzheimer's disease. However, implementation will not only depend on the outcomes of future blood-based biomarker studies but also could differ depending on the local organisation of health-care systems and available treatment options. For instance, even though the US Food and Drug Administration approved the anti-amyloid drug aducanumab on June 7, 2021, approval in other parts of the world is still awaited. Also, CSF and PET are currently the only approaches recommended for measurement of amyloid status. However, some specialised care providers might already be using blood-based biomarkers to select appropriate patients. Anticipated approval of aducanumab in other parts of the world—and expected approval of other similar drug candidates that are at advanced stages of testing—will probably accelerate the introduction of blood-based biomarkers to clinics.

Search strategy and selection criteria

We searched PubMed using the search terms "Alzheimer's disease" AND ("amyloid" OR "Tau" OR "pTau" OR "NfL" OR "GFAP") AND ("plasma" OR "serum" OR "blood"). For specific sections, additional search terms included "familial Alzheimer's disease", "Down's syndrome", "genetically determined Alzheimer's disease", "population-based cohorts", "BMI", "sex", "disease prediction", "implementation", "in vitro diagnostic assays", "drug development", or "clinical trials". We also searched the references within the selected papers for relevant articles. We reviewed only papers in English. We did not apply date restrictions to the search. The last search was done on Aug 25, 2021. Results from 2016 and older papers were included only if deemed necessary to understand the subject under discussion. We included studies that aimed to establish the use of the aforementioned proteins as body fluid biomarkers in the context of Alzheimer's disease. Only studies done in humans were included. The final reference list was generated on the basis of relevance to the topics covered in this Review.

Implementation in clinical trials

The introduction of novel blood-based biomarkers for Alzheimer's disease will most likely improve the design and conduct of clinical trials evaluating disease-modifying therapies. Because of the long preclinical stage of Alzheimer's disease,[1] inclusion of participants who are at early preclinical stages in clinical trials will be dependent on biological markers. Blood-based biomarkers could potentially be used as inclusion criteria (including enrichment and stratification) or to evaluate target engagement and treatment efficacy (table 2).[116]

Use of blood-based biomarkers to detect the presence of pathological features of Alzheimer's disease (eg, amyloid plaques and tau tangles) is important for selection of individuals eligible for treatments such as the anti-amyloid drug aducanumab. For example, concentrations in plasma of pTau are an inclusion measure in novel trial designs for drugs targeting Alzheimer's disease (Teunissen CE, unpublished observation). Moreover, biomarkers reflecting the presence of other mechanisms (eg, neuroinflammation) will be important to determine if other treatments (or combinations of treatments) should be prescribed. Blood-based biomarkers might become useful for monitoring biological efficacy (eg, plasma Aβ and pTau to analyse clearance of amyloid and tau aggregates), treatment effects, side-effects (eg, amyloid-related imaging abnormalities), and safety of extending the dosing interval.[117] Blood-based biomarkers are advantageous in this context, compared with CSF and imaging biomarkers, due to their non-invasiveness, reduced costs, and lower burden to the patient and health-care systems (eg, with respect to tracer cost and scanning time). However, advantages of imaging include the spatial resolution and visibility of accrued damage.

Conclusions and future directions

Use of blood-based biomarkers for diagnosis and prognosis of Alzheimer's disease is nearing clinical use, both in specialist clinics and in the primary care setting, largely due to availability of ultrasensitive detection methods. A crucial next step is to define the use of these biomarkers at the individual patient level. In a few years, we expect that blood-based biomarkers of Alzheimer's disease will be ready for clinical implementation—perhaps even earlier in clinical trials. These advances also hold promise for the development of novel neurospecific protein biomarkers. There is a relative paucity of blood-based biomarkers to reflect the full complexity of Alzheimer's disease pathology (figure 1), such as biomarkers of microglia activation or synaptic dysfunction. The difficulty with microglia biomarkers in blood is the interference of inflammation in other organs; no specific brain microglia biomarkers are known. For synaptic dysfunction, CSF neurogranin concentrations have shown strong promise, but contrasting results have been obtained in blood so far.[118] Nevertheless, with the emergence of feasible blood-based arrays and high-throughput proteomics technologies, novel diagnostic and prognostic biomarkers could be identified. Validation and implementation of blood-based biomarkers will facilitate the development of precision medicine. Importantly, the knowledge acquired from the Alzheimer's disease biomarker field will pave the way to address the next important, unmet clinical need: identification of specific biomarkers to support the diagnosis of, and development of treatments for, other types of dementia.

Contributors

CET was responsible for the literature search, figures, writing, interpretation, and overall coordination of the study. IMWV, EHT, LV, OH, HZ, WMvdF, MMM, and MdC were responsible for the literature search, writing, interpretation, and critical review. MdC was also involved in designing the figures.

Declarations of interests

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