Biomarkers of Neurodegeneration 2

Challenges in the practical implementation of blood biomarkers for Alzheimer's disease

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Blood biomarkers have emerged as accessible, cost-effective, and highly promising tools for advancing the diagnostics of Alzheimer's disease. However, transitioning from cerebrospinal fluid biomarkers to blood biomarkers—eg, to verify amyloid β pathology—requires careful consideration. This Series paper highlights the main challenges in the implementation of blood biomarkers for Alzheimer's disease in different possible contexts of use. Despite the robustness of measuring blood biomarker concentrations, the widespread adoption of blood biomarkers requires rigorous standardisation efforts to address inherent challenges in diverse contexts of use. The challenges include understanding the effect of pre-analytical and analytical conditions, potential confounding factors, and comorbidities that could influence outcomes of blood biomarkers and their use in diverse populations. Additionally, distinct scenarios present their own specific challenges. In memory clinics, the successful integration of blood biomarkers in diagnostic tests will require well-established diagnostic accuracy and comprehensive assessments of the effect of blood biomarkers on the diagnostic confidence and patient management of clinicians. In primary care settings, and even more when implemented in population-based screening programmes for which no experience with any biomarkers for Alzheimer's disease currently exists, the implementation of blood biomarkers will be challenged by the need for education of primary care clinical staff and clear guidelines. However, despite the challenges, blood biomarkers hold great promise for substantially enhancing the diagnostic accuracy and effectively streamlining referral processes, leading to earlier diagnosis and access to treatments. The ongoing efforts that are shaping the integration of blood biomarkers across diverse clinical settings pave the way towards precision medicine in Alzheimer's disease.

Introduction

Over the past decade, the diagnostics of Alzheimer's disease have moved towards a clinical–biological approach supported by robust cerebrospinal fluid (CSF) and PET biomarkers that provide in-vivo evidence for the main pathophysiological hallmarks of the disease: amyloid plaques and neurofibrillary tangles. These widely accepted biomarker modalities are now integral to clinical practice and are readily available in most specialised memory clinics. However, in many health-care systems worldwide, Alzheimer's disease is not often diagnosed at a specialised care level but rather at a primary care level, without access to established and validated biomarker modalities for Alzheimer's disease.[1]

Technological advancements have allowed for successful detection of the pathological hallmarks of Alzheimer's disease through blood biomarkers in extensively characterised research cohorts.[2–10] Blood biomarkers are generally more accessible, less invasive, and more scalable than CSF biomarkers and are also more cost-effective than PET, thus making blood biomarkers a promising option for simplifying the diagnostic process for patients with cognitive impairment.[11] If blood biomarkers are implemented, then the biomarkers could not only streamline diagnoses but also reduce costs. Of note, integration of blood biomarkers into primary care settings could facilitate early, reliable diagnosis and swift referrals to specialists.[12]

The evolution in diagnostic techniques aligns with the growing accessibility of disease-modifying treatments. Early diagnosis has become paramount, underscored by the ongoing revision of the clinical criteria for Alzheimer's disease put forth by the Alzheimer's Association, which now acknowledge the potential of plasma biomarkers for categorisation, disease diagnosis, and staging.[13] The updated guidelines place great emphasis on blood biomarkers, thereby highlighting their pivotal role in shaping the future of the diagnosis and treatment of Alzheimer's disease. This Series paper overviews the main benefits and challenges in the implementation of blood biomarkers for Alzheimer's disease in different possible contexts of use (figure 1).

Blood biomarkers for Alzheimer's disease: types and their targets

Blood biomarkers for Alzheimer's disease can be categorised into various classes (table, figure 2). The first class includes biomarkers associated with the presence of two classic pathological hallmarks of Alzheimer's disease: amyloid β peptides (Aβ42-to-Aβ40 ratio [Aβ42/40]) and phosphorylated tau (p-tau). Compared with the concentrations of blood amyloid β, the concentrations of p-tau in the blood show a stronger correlation with those in the CSF and a larger fold change in individuals with Alzheimer's disease pathology, thereby enabling the prediction of PET-based or CSF-based amyloid β biomarker positivity and differentiating individuals with Alzheimer's disease from those with other neurodegenerative diseases.[5,6] The second class of blood biomarkers for Alzheimer's disease is associated with neuronal loss, neurodegeneration, or synaptic degeneration. The third class includes processes related to neuroinflammation

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Key messages

- Blood biomarkers for Alzheimer's disease have great potential for implementation in both clinical and trial settings due to their high diagnostic performance, scalability, and cost-effectiveness, with plasma phosphorylated tau217 currently being the most established and accessible Alzheimer's disease biomarker.
- Implementing blood biomarkers in the diagnostics of Alzheimer's disease requires the resolution of technical challenges such as pre-analytical and analytical harmonisation and standardisation.
- Integration of blood biomarkers into various health-care settings requires interpreting results across analytical platforms and diverse, real-life patient populations with varying levels of comorbidities and confounding factors.
- Overcoming challenges such as pre-analytical and analytical harmonisation and standardisation is crucial to enable the transformative effect of blood biomarkers on enhancing and democratising the diagnosis and prognosis of Alzheimer's disease.
- Policy makers and health-care systems need to use concrete guidelines for regulating informed and meaningful access to blood biomarkers, particularly with the advent of novel treatment strategies.

mediated by glial cells. Although all these blood biomarkers hold great potential, the integration of the blood biomarkers into clinical practice requires further advancements in terms of quality, harmonisation, interpretation, and delineation of the respective contexts of use.

Practical challenges in the implementation of plasma biomarkers

Availability and accessibility of analytical techniques

The availability of multiple tests from different vendors is the first step towards the democratisation of blood biomarker analyses; this is because the availability of multiple tests is likely to lead to reduced costs. The currently available methods include mass spectrometry approaches, which outperform immune-based methods of measuring plasma Aβ42/40 concentrations to detect brain amyloid β pathology.[14,57] Mass spectrometry is used in PrecivityAD (C2N Diagnostics),[58] a test that is approved by the US Food and Drug Administration (FDA) for clinical use. However, the global access to mass spectrometry-based assays is low because the assays require specific expertise and infrastructure, are time consuming, and yield lower throughput compared with immunoassays. Different immune-based methods—ie, electrochemiluminescence, chemiluminescence, and single molecule array (Simoa)—can be used to quantify different p-tau forms, glial fibrillary acidic protein (GFAP), or neurofilament light chain (NfL) in the plasma with high precision and robustness.[7,18,22,59–61] Despite the intrinsic disadvantages of immune-based methods (eg, antibody reproducibility, batch-to-batch variation, and potential cross-reactivity), some of these methods are fully automated and suitable for point-of-care testing and are already being widely used for CSF analysis (eg, Elecsys and Lumipulse). The availability of these methods in many routine diagnostic laboratories already will most likely facilitate the swift implementation of the corresponding plasma assays in these laboratories. Other automated platforms (eg, Meso Scale Discovery and Simoa) are widely available in many research laboratories and are expected to expand the capacity for measuring plasma biomarker concentrations to diagnose Alzheimer's disease once necessary regulatory approvals and corresponding national or local accreditations are in place.

Whether such platforms are also incorporated in routine diagnostic laboratories will most likely depend on hospital regulations, strategies, and budgets, as well as the clearance through regulatory approval of the different kits as in-vitro diagnostic tests. Importantly, improvements in blood collection methods such as dried blood spot could further allow access to plasma biomarker measurements to diagnose Alzheimer's disease as the collection methods would allow for the collection of samples in areas with inadequate laboratory infrastructure.[62,63] Technological and pre-analytical advancements are thus making blood biomarkers for Alzheimer's disease widely available. An analysis comparing the cost-effectiveness of blood biomarkers to that of traditional diagnostic tests showed that blood biomarkers could reduce costs by up to 40% depending on the amyloid PET positivity.[64,65] The use of blood biomarkers for Alzheimer's disease in combination with cognitive tests has further been shown to considerably reduce the estimated time required to complete a diagnostic test, as well as the associated costs,[66] thereby supporting the implementation of these biomarkers in clinical settings.

Standardisation of results for blood biomarker measurements

Standardisation of blood biomarker measurements across care settings and within clinical trials is imperative for the widespread adoption and implementation of blood biomarkers. When reproducible and consistent results are obtained across laboratories, platforms, and assay kit batches, the confidence of the end users and regulatory agencies in the use and interpretation of blood biomarkers increases. The experience with the implementation of CSF biomarkers indicates that high variability in the concentrations of measured biomarkers across and within laboratories severely delays clinical implementation,[67,68] and several pre-analytical and analytical challenges need to be addressed to standardise blood biomarker measurements worldwide. Based on learnings from the clinical adoption and implementation of CSF biomarkers for Alzheimer's disease, standardisation efforts for blood biomarkers for Alzheimer's disease were initiated soon after the successful development of the first highly sensitive

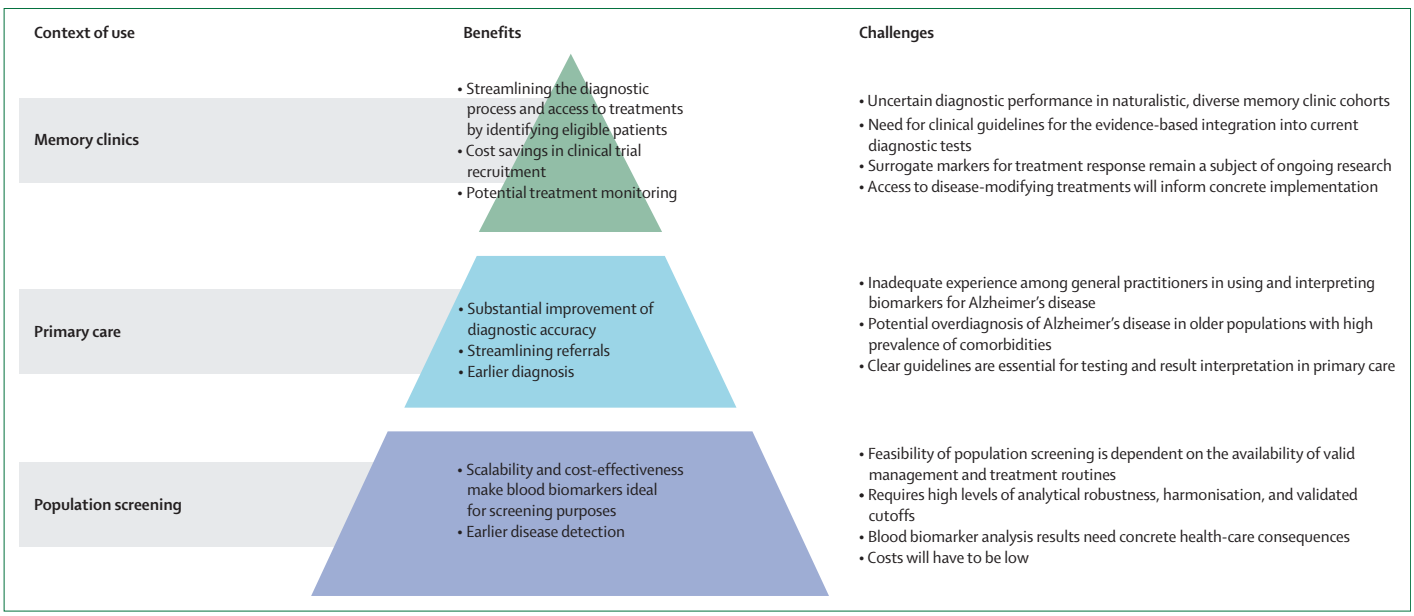

Figure 1: **Potential benefits and challenges associated with the use of blood biomarkers for Alzheimer's disease in different possible contexts of use**

blood tests for Alzheimer's disease, guided by the Global Biomarker Standardization Consortium of the Alzheimer's Association.

In the case of CSF biomarkers, a major source of variability was attributed to pre-analytical sample handling, which was mitigated through the development of empirical evidence-based standardised operating procedures (SOPs) for sample handling.[69] Likewise, the initial SOPs for plasma sample handling were published[70] to mitigate any effects of plasma sample handling on the accuracy of blood biomarker measurements. SOPs are continuously being reviewed and updated by interpreting additional empirical data currently being obtained—eg, by testing the effect of pre-analytical sample handling on biomarkers other than those investigated in the first version of the SOP, the effect of pre-analytical variables that have not yet been tested, such as fasting versus non-fasting and exercise before blood withdrawal on the concentrations of blood biomarkers for Alzheimer's disease,[71,72] or the effect of sample thawing before blood biomarker measurements.[73]

Analytical variability in CSF was also largely mitigated through efforts to standardise batch-to-batch variation by immunoassay manufacturers by using fully automated platforms over manual, labour-intensive laboratory procedures such as ELISA[74] and the development of certified reference materials that can be used to calibrate commercial assays for value alignment.[75] Especially for amyloid β and p-tau, several head-to-head assay and technology comparison studies have already been performed or are ongoing to identify the assays that are diagnostically the most sensitive and analytically the most robust.[14,18,22,76] Together with aspects such as wide-scale assay availability, cost-effectiveness of a measurement with a particular platform, turnaround times and logistics, and scalability, head-to-head assay comparisons will help to identify assays to be implemented in daily routines. Efforts to establish certified reference materials for blood biomarkers are in early stages but are ongoing. The initial feasibility to develop reference material for blood-based NfL has already been shown.[77] Until certified reference materials are available for all blood biomarkers for Alzheimer's disease, in-house normal reference ranges and cutoff concentrations, as well as careful in-house quality control of day-to-day and batch-to-batch variations, are essential to ensure the diagnostic accuracy of assays.

Overall, standardisation challenges will vary greatly depending on the blood biomarker and analytical approaches used for its measurement. Compared with immunoassays, mass spectroscopy-based detection methods provide a more reliable quantification of low-abundance proteins in protein-rich matrices such as the blood, as evidenced by the results observed for plasma amyloid β.[9,57] Differences in the specificity of antibodies used in immunoassays from different vendors can also affect analytical variability. Currently, robust and reliable analysis of Aβ42/40 using immune-based methods is the greatest challenge. Pre-analytical studies show that the concentrations of Aβ42/40 decrease with suboptimal sample handling.[70,78,79] In addition, the fold change difference in Aβ42/40 concentration between individuals with Alzheimer's disease and those without is small, highlighting the importance of accuracy in biomarker measurement. Analysis of p-tau and NfL is substantially more robust, with greater pre-analytical stability under sample handling variations,[70] especially for p-tau, which shows greater fold change differences between healthy individuals and individuals with disease.[80]

Finally, true standardisation will require the derival and validation of cutoffs that are translatable between methodological approaches for respective blood biomarkers.

	Biomarker	Significance
Pathological hallmarks of Alzheimer's disease	Aβ42/40	Decrease in blood correlates with cerebral amyloid β pathology (PET or CSF), especially in the early stages of Alzheimer's disease.[14–17] Better differentiation of individuals with Alzheimer's disease from those with other neurodegenerative diseases. Low fold change between amyloid β-positive and amyloid β-negative individuals, based on measurements of PET or CSF amyloid β.
	Phosphorylated tau (p-tau)	Different isoforms (p-tau181, p-tau231, p-tau217, etc) show strong performance in detecting Alzheimer's disease pathology.[4,18–20] p-tau217: best-performing blood biomarker due to greatest dynamic range[21] and sensitivity. Plasma p-tau217 performs equivalent to CSF testing for identifying amyloid β-positivity.[5,6,22–24] p-tau231: potential for early detection of Alzheimer's disease pathology.[25] p-tau205: potential for improved identification of advanced Alzheimer's disease pathology,[26] in correspondence with tau PET accumulation.[27,28] Differences in concentrations of p-tau biomarkers might be greater depending on different assays or analysis platforms, or both, than differences between isoforms.[18,22]
Neuronal and synaptic degeneration	Neurofilament light chain	Cross-disease or disease-unspecific biomarker of neurodegeneration[29] and increases in Alzheimer's disease and other neurodegenerative diseases.[30,31] High baseline concentrations[32] and longitudinal changes[33] associated with higher risk of dementia.
	Brain-derived total tau protein	Has been proposed to assess Alzheimer's disease-specific neurodegeneration.[34]
	β-synuclein	Pre-synaptic protein tracking synaptic degeneration. Elevated in Alzheimer's disease and Down syndrome.[35–38] Diagnostic and prognostic value in preclinical Alzheimer's disease, shows an increase in concentration earlier than p-tau181.[35] Correlates with cognitive measures and cerebral atrophy in Alzheimer's disease.[37,38]
Neuroinflammation mediated by glial cells	Glial fibrillary acidic protein	Astrocytic marker strongly associated with early cerebral amyloidopathy,[39,40] early stages of Alzheimer's disease,[7] and tau aggregation mediated by amyloid β.[41–44] Scarce evidence of direct association with astrogliosis.[45] Elevated in traumatic brain injury,[46] spinal cord injury,[47] CNS inflammatory diseases (eg, multiple sclerosis, neuromyelitis optica spectrum disorder),[48,49] and several non-Alzheimer's disease neurodegenerative diseases, including frontotemporal lobar degeneration spectrum,[50–52] Parkinson's disease,[53] and amyotrophic lateral sclerosis.[54] High accuracy for discriminating individuals with Alzheimer's disease from the controls,[7,41,55] but its low specificity restricts its use in diagnosing the cause of cognitive impairment.
	Other inflammation-related molecules	CX3CL1, metalloproteinases, and TREM2 have been studied as biomarkers for Alzheimer's disease.

Aβ42/40=Aβ42-to-Aβ40 ratio. CSF=cerebrospinal fluid. CX3CL1=C-X3-C motif chemokine ligand 1. p-tau=phosphorylated tau. TREM2=triggering receptor expressed on myeloid cells 2.

Table: **Types of blood biomarkers for Alzheimer's disease, their targets, and main characteristics**

Traditionally, CSF biomarker cutoffs have not been standardised, but rather laboratory-specific cutoffs have been applied.

Research challenges in the implementation of plasma biomarkers

Effects of confounding factors and comorbidities in real-world populations

Most studies on blood biomarkers for Alzheimer's disease published to date have been conducted in select populations with generally low rates of medical comorbidities. However, of late, attention is being paid to how medical comorbidities can confound the results obtained for measuring blood biomarkers for Alzheimer's disease. In a community-based study, the plasma concentrations of p-tau217 and p-tau181 were higher in individuals with chronic kidney disease (CKD), myocardial infarction, stroke, and hypertension than in individuals without these conditions, and plasma p-tau concentrations were marginally lower in individuals with elevated BMI (30–34 kg/m^2) and a history of smoking than in individuals without these factors.[81]

The association between CKD and plasma p-tau concentrations has received the most attention because the magnitude of difference in plasma p-tau concentrations between individuals with and without CKD was similar to that between individuals with and without amyloid PET positivity.[81] Of note, the study by Mielke and colleagues[81] primarily included participants who were not cognitively impaired, potentially leading to smaller differences in the expected p-tau concentrations, as compared with what would possibly have been observed in case of participants with cognitive impairment. Additionally, the role of CKD as a confounder in cognitively impaired individuals, in whom any Alzheimer's disease pathology is most likely more advanced, remains uncertain.

In a community-based cohort of individuals aged between 70 years and 71 years, CKD was associated with higher concentrations of plasma NfL despite having similar MRI and CSF biomarkers.[82] Of note, participants with dementia had similar concentrations of plasma NfL as individuals with CKD.[82] Another study reported that serum creatinine concentrations and BMI were associated with plasma NfL, GFAP, and p-tau concentrations, with p-tau concentrations being least affected, and medical comorbidities only had a minor effect on the performance of clinical prediction models that used plasma biomarkers.[83]

The use of ratios of plasma proteins has been suggested to attenuate associations between CKD and plasma biomarker concentrations, assuming that the proteins in the numerator and denominator are similarly affected. In fact, a recent study using mass spectrometry reported that when compared with p-tau alone, the ratios of p-tau to non-phosphorylated tau were more reliable indicators of the pathology of Alzheimer's disease at different clinical stages,[84] and this trend is observed

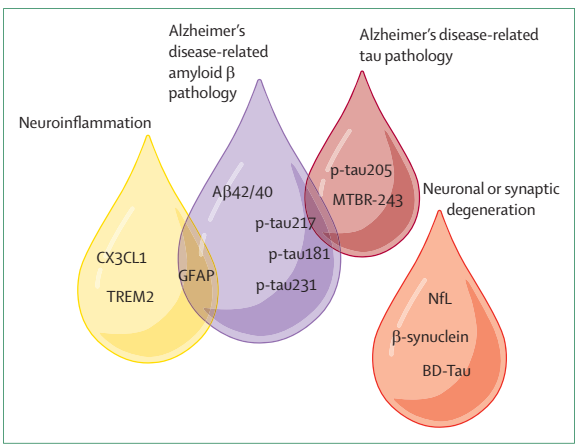

Figure 2: **Currently researched classes of blood biomarkers for Alzheimer's disease**
Aβ42/40=Aβ42-to-Aβ40 ratio. CX3CL1=C-X3-C motif chemokine ligand 1. TREM2=triggering receptor expressed on myeloid cells 2. GFAP=glial fibrillary acidic protein. Aβ=amyloid β. p-tau=phosphorylated tau. MTBR-243=microtubule binding region of tau containing the residue 243. NfL=neurofilament light chain. BD-Tau=brain-derived tau.

for ratios of other proteins related to Alzheimer's disease such as Aβ42/40.[83,85] Although more research in larger and more demographically representative samples of individuals with CKD is needed, plasma protein ratios seem to be promising indices to attenuate the association of CKD with blood biomarkers for Alzheimer's disease.

Evidence also suggests alterations in blood biomarkers for Alzheimer's disease in individuals with hypoxic–ischaemic injury after an out-of-hospital cardiac arrest.[86] Furthermore, after traumatic brain injury, the concentrations of blood p-tau biomarkers increased,[87] possibly mediated by changes in the permeability of the blood–brain barrier. In contrast to CKD, the alterations in blood biomarkers for Alzheimer's disease might not be problematic for the diagnosis of Alzheimer's disease because patients with acute hypoxic–ischaemic injury or recent traumatic brain injury will not be investigated for a screening or diagnostic biomarker for Alzheimer's disease.

Some blood biomarkers for Alzheimer's disease are also affected by treatments for medical conditions. For example, the angiotensin receptor blocker and neprilysin inhibitor sacubitril–valsartan, used for treating heart failure, increased plasma Aβ42 concentrations and the Aβ42/40 ratio by approximately 30%.[88] Of note, sacubitril-valsartan did not affect the plasma concentrations of p-tau181, p-tau217, GFAP, or NfL.

Overall, although tremendous progress has been made in research on blood biomarkers for Alzheimer's disease, prospective, longitudinal data from real-world studies that are demographically representative of the populations at risk for Alzheimer's disease are scarce. The performance of blood biomarkers in prospective cohorts with prespecified cutoffs and analytical plans will be important to assess the performance of a new test, including how plasma tests are affected by comorbidities and other factors such as diet and lifestyle. Despite the limitations, medical comorbidities do not correspond to large differences observed in the fold change for some blood biomarkers, such as that for p-tau217 (300–700%), between cognitively impaired individuals with Alzheimer's disease and cognitively unimpaired individuals or individuals with other neurodegenerative diseases, which partly mitigates concerns regarding the influence of comorbidities on the interpretation of results of high-performance plasma biomarkers. Furthermore, many blood tests used in routine clinical practice are affected by medical comorbidities; careful interpretation of results in the context of these comorbidities has been possible in other areas of medicine and should also be possible in the case of blood biomarkers for Alzheimer's disease.

In summary, strategies to reduce the influence of medical comorbidities include the use of plasma biomarker ratios, different thresholds for diagnosis in individuals with relevant comorbid conditions, referral for PET or CSF biomarker analyses when blood biomarkers are not expected to provide meaningful information, or a combination of these strategies.

Differences in the measurement of blood biomarkers across diverse populations

In clinical practice, blood biomarker measurements are often interpreted by comparing the findings with predefined reference intervals or cutoffs. The unavailability of reliable reference intervals for highly diverse populations could lead to the misinterpretation of laboratory test results and contribute to misdiagnosis and inappropriate clinical intervention in a diverse real-world setting with greater heterogeneity among individuals. In real-world settings, demographic factors (eg, race or ethnicity, age, sex), medical comorbidities, and social determinants of health (eg, economic status, education, and quality of and access to health care) are expected to have varying profiles across different populations.

To this end, studies have examined the effect of race or ethnicity in European,[89,90] American,[91–93] and Asian cohorts;[94–96] demographic factors;[97–99] and comorbidities on the measurement of blood biomarkers for Alzheimer's disease and their diagnostic and prognostic performances. Although some demographic and comorbidity factors (as previously discussed) showed substantial associations with blood biomarkers for Alzheimer's disease, whether their potential confounding effects are clinically relevant needs to be analysed.[83] For instance, studies could consider whether accounting or further adjusting for these factors, which are influencing the results of the blood biomarkers, leads to a substantial and major improvement in the performance of blood biomarkers.[83]

Future studies can also assess whether specific cutoffs in relation to demographic factors (eg, age-specific or sex-specific cutoffs) will enhance the performance of blood biomarkers.[98] Another consideration is the differential prevalence of demographic and comorbidity factors across geographical regions,[100–103] which would in turn influence

whether the clinical performance and applicability of blood biomarkers are adversely affected in specific populations. Next, socioeconomic diversity (particularly economic inequality) could have resulted in low active inclusion of low-income and middle-income countries in previous studies and potentially in future clinical validation studies (due to poorly resourced health-care systems and inadequate financial affordability or access to advanced research facilities in these countries).[104] More studies need to examine the use of blood biomarkers in under-represented countries, particularly given the differences in social and health exposures (eg, education, medical comorbidities, and genetic risk variants) between some low-income countries and some high-income countries where association with changes in biomarkers for Alzheimer's disease have been studied.[104] Carefully designed studies will be needed to avoid inaccurate conclusions derived from the complexities of overlap in the effects of these factors.

Challenges in the implementation of plasma biomarkers in the clinical context

Regulatory challenges for the implementation of blood biomarkers

The Geneva roadmap depicts five phases to systematically address the requirements for the implementation of novel fluid biomarkers into clinical practice.[80,105] Independent groups examining well-defined cohorts in research settings have reported the analytical and clinical validity for most key plasma biomarkers for Alzheimer's disease based on retrospective and longitudinal studies (ie, phases 1–3).[106–110] Studies evaluating phases 4 and 5 are now being initiated—ie, evaluation of the utility of plasma biomarkers from a technical and clinical perspective in a real-world scenario.

From a technical point of view, in-vitro diagnostic assays approved by certified bodies are to be used to support clinical decision making; these assays undergo extensive analytical and clinical validation to guarantee robust, reproducible, and reliable results across different batches.[2,111] Aβ42 and Aβ40 assays from Sysmex Corporation have been granted in-vitro diagnostic approval in Japan, and the FDA has granted Breakthrough Device Designation to the PrecivityAD Test, the Elecsys Amyloid Plasma Panel, and the Simoa p-Tau217 and p-Tau181 tests to facilitate the in-vitro diagnostic process.[2] Other assays are expected to follow a similar path.

The development of reference materials will allow for tracking assay performance across different laboratories and assays. Of note, the Alzheimer's Association has compiled the first recommendations for the appropriate use of blood biomarkers for Alzheimer's disease in clinical settings and trials, which also summarise the research priorities to move the field forward.[112] Ultimately, the successful implementation of blood biomarkers will require the development of cost-effective assessments and appropriate use criteria, which in turn would depend on the context of use and regional particularities of each health-care unit and system.

Diagnostics

Before the widespread implementation of blood biomarkers for Alzheimer's disease in routine diagnostic tests in memory clinics, clear guidelines on their usage and interpretation should be established. First, a potential challenge would be the evidence-based appropriate integration of blood biomarkers in the current diagnostic test, such as examining effective ways of combining the biomarkers with existing clinical measures that are non-invasive and widely available. For instance, whether the combination of blood biomarkers with other accessible measures, such as parameters evaluated by clinicians (often based on medical history, clinical symptoms, and available imaging measures such as brain CT or MRI, or both), apolipoprotein E genotypes, and cognitive assessments, improves the diagnostic[113] and prognostic[8,114] performance of blood biomarkers needs to be considered. Similarly, whether the addition of blood biomarkers improves the performance of clinical measures substantially needs to be ascertained. Preliminary promising data presented at the Alzheimer's Association International Conference 2023 and obtained as part of the Swedish BioFINDER Primary Care study[115] showed that the use of plasma biomarkers for Alzheimer's disease (based on a predefined algorithm using plasma p-tau217 and Aβ42/40) increased the accuracy of detecting the pathology of Alzheimer's disease by primary care physicians from 55% to more than 85% in a cohort of 265 participants.

Validation in independent cohorts could enhance the relevance of such findings as this information could be key to assigning appropriate care in the early phases of treating individuals with Alzheimer's disease (eg, treatment opportunities, counselling). Second, recommendations should be provided on appropriate clinical interventions to be undertaken after receiving results of blood biomarker tests. For example, assuming two cutoffs based on appropriate thresholds for sensitivity and specificity, older individuals could be stratified into three groups on the basis of their probabilities of amyloid PET positivity—namely, high, intermediate, and low risk,[12] with the clinical decisions for each risk group being comprehensibly different. Participants in the high-risk group might receive pharmaceutical intervention that is approved for treating Alzheimer's disease (symptomatic treatments or anti-amyloid therapies, or both), as well as referral for disease-modifying clinical trials. Participants in the low-risk group might require other clinical evaluations to identify the non-Alzheimer's disease causes of cognitive symptoms. In contrast, participants in the intermediate-risk group might be referred for further confirmatory testing using CSF or PET biomarkers for Alzheimer's disease.

Additionally, interpretation of positive biomarker test results for Alzheimer's disease in older individuals who are cognitively unimpaired on objective assessment remains debatable. Although evidence suggests that the presence of positive biomarkers for Alzheimer's disease (mostly defined by amyloid and tau PET scans) in cognitively unimpaired older participants is, as a group, associated with accelerated

cognitive decline,[116,117] the interindividual trajectories are quite variable, with a proportion remaining cognitively stable over time.[117,118] The clinical trajectory in this case has been hypothesised to be influenced by factors beyond Alzheimer's disease pathology, such as resilience against the pathological processes of Alzheimer's disease (which could be attributed to healthy lifestyle, education, or protective genetic makeup), as well as severity of pathophysiological processes (eg, neuroinflammation, oxidative stress, synaptic loss, and dysfunction) and non-Alzheimer's disease copathologies, which could exacerbate cognitive symptoms.[117–119] Moreover, whether the addition of blood biomarkers would have a substantial effect in the diagnostic confidence and patient management of clinicians needs to be considered. The long-term effects on health and economic outcomes also remain to be evaluated.

Treatment

A major breakthrough in the field of Alzheimer's disease is the introduction of disease-modifying treatments, specifically anti-amyloid β immunotherapy, which has been approved in the USA, with approval pending in Europe and elsewhere.[120,121] The introduction of this new drug class presents many biomarker-related challenges, mainly because of the high costs and restricted accessibility of CSF and PET biomarkers for Alzheimer's disease pathology (biomarkers required to initiate treatment). In this context, blood biomarkers have the potential to streamline the process for approval of anti-amyloid β immunotherapy, by identifying individuals eligible for treatment; stratifying individuals on the basis of their pathophysiological stage; and monitoring and managing the treatment. Monitoring and managing the treatment can be especially relevant, considering that accelerated approval from the FDA might be granted when the drug is shown to affect a biological mechanism in a manner considered reasonably likely to help patients.[122,123]

With respect to recruitment of individuals in clinical trials for Alzheimer's disease, research suggests that including blood biomarkers for Alzheimer's disease at different trial enrolment stages could reduce the number of necessary lumbar punctures and PET scans, resulting in time savings of up to 50%, a reduction in screening failures of about 50%, and cost savings of up to 75%.[124–126] Thus, the approach of using blood biomarkers for Alzheimer's disease at different trial enrolment stages is now being implemented in the recruitment for ongoing trials (eg, NCT04468659). Plasma p-tau217 has also been used as a stand-alone biomarker to identify the presence of Alzheimer's disease pathology in individuals for inclusion in a trial (NCT05026866). However, in current clinical practice, anti-amyloid β treatment is initiated only after verifying the presence of amyloid β pathology using CSF or PET biomarkers. Considering that the accuracy for amyloid β pathology is similar to that for CSF assays and the best-performing plasma assays,[6,23,24] the challenges in transitioning from CSF to blood most probably include the insufficient clinical experience with using blood biomarkers, current low clinical accessibility to blood biomarkers, and regulatory guidelines for initiating treatment.

Trials on anti-amyloid β have reported greater benefits for individuals with Alzheimer's disease who have low tau burden, suggesting a need for stratification.[121] Although tau PET has traditionally helped in stratification,[121,127] emerging fluid biomarkers such as p-tau205 and microtubule binding region of tau containing the residue 243 could be considered accessible tools for effective stratification in the future.[128] In clinical trials, patients receiving anti-amyloid β treatment are followed up using amyloid β PET (to evaluate the clearance of amyloid β) and MRI (for safety reasons, specifically with respect to the development of amyloid β-related imaging abnormalities). In clinical practice, few memory clinics will have the capacity to assess the reduction in amyloid β concentration using PET (which could prompt cessation of treatment, as done in phase 2 and 3 trials for donanemab),[121,127] thus indicating a potential need for surrogate markers. Unfortunately, no blood markers in clinical trials have so far been able to mimic the pronounced removal of amyloid β observed using PET,[120,121,129] and thus, further research is necessary before blood biomarkers can be used to track target engagement and treatment response in anti-amyloid β treatments. Frequent MRI follow-ups required to identify the development of amyloid β-related imaging abnormalities[130] could burden health-care systems, and hence, blood biomarkers could be pivotal in prescreening for the requirement of MRI. However, concrete data are insufficient and future studies should investigate potential markers such as NfL and GFAP.

Primary care

Implementation of blood biomarkers in primary care poses more challenges than that in memory clinic settings. In contrast to most memory clinics, physicians do not have previous experience with using biomarkers for Alzheimer's disease and interpreting results, which can be particularly challenging in older populations examined in primary care settings who have a high prevalence of both Alzheimer's disease and non-Alzheimer's disease pathologies. Using blood biomarkers as a diagnostic test for Alzheimer's disease in older populations could result in overdiagnosis as the blood biomarkers might be interpreted as the primary cause of cognitive impairment, when in fact, these biomarkers could be only an asymptomatic or mild copathology to—eg, vascular pathology, other neurodegenerative diseases, or other non-degenerative causes of cognitive disturbance. Another challenge related to the primary care population is the high prevalence of comorbidities, which can affect plasma concentrations of blood biomarkers.

Strict guidelines regarding who should be tested will need to be adapted to the current indications for anti-amyloid treatment and symptomatic treatment programmes as well as national or regional dementia care programmes specifying the responsibilities for primary and secondary care. In the absence of effective disease-modifying drugs for

preclinical stages, freely testing individuals who wish to be tested could cause more harm than benefit due to the long period of biomarker positivity without any cognitive symptoms,[118,131,132] regardless of how local guidelines are structured otherwise. However, these results could provide opportunities for older populations to engage in dementia prevention trials and revise their plans (eg, retirement, financial and estate planning).[132,133] In areas for which anti-amyloid treatment is approved, a recommendation to test patients with mild cognitive impairment or mild dementia and an underlying suspicion of Alzheimer's disease (usually a progressive, amnestic syndrome) could accelerate the start of treatment by identifying eligible patients early and referring them to secondary care. The positive predictive value is higher in such a group of eligible individuals with increased pre-test probability, making a blood test for Alzheimer's disease pathology potentially suitable for aiding Alzheimer's disease diagnostics and also facilitating the initiation of cholinesterase inhibitor treatment in areas in which this responsibility lies in primary care. Conversely, in the case of patients for whom the clinician has a low suspicion of Alzheimer's disease, blood biomarkers could instead aid in ruling out Alzheimer's disease since the negative predictive value is higher in such a population.[12]

Population-level screening

As scalability and cost-effectiveness are among the most important advantages of blood biomarkers compared with other disease-specific biomarker modalities, the use of blood biomarkers for screening a general (undiagnosed) population to detect Alzheimer's disease pathology represents the pinnacle of their practical implementation. Currently, no health-care system is prepared for screening of Alzheimer's disease in the relevant age (eg, >50 years) and at-risk population groups, as has already been realised for other disease conditions such as various forms of cancer. However, a recent trial suggests that many of the currently explored treatment options for Alzheimer's disease yield their highest effects when initiated early, ideally preclinically or presymptomatically.[121] Population screening could, thus, enable early intervention and possibly even prevention strategies and improved diagnostics and care for underdiagnosed patient groups, leading to overall better public health.[134]

However, as expectations of individuals might vary with respect to the diagnosis, prognosis, and prevention of Alzheimer's disease, clinicians and patients should have access to understandable information and platforms for clear communication and shared decision making, considering the differences in the needs and preferences of each individual.[132]

Population screening will ultimately be highly dependent on the availability of valid treatment options. However, until blood biomarkers are ready to be used as screening tools for Alzheimer's disease, the general challenges discussed in this Series paper have to be resolved to reach sufficient—ie, near perfect (negative predictive value of close to 100) diagnostic accuracy—to avoid false-negative (which prevent individuals from receiving clinical follow-up and treatment) or false-positive (which lead to unnecessary stress or serious health consequences) test results.[2]

Finally, the costs of any given blood biomarker test for population screening will need to be low, and health-care decisions about their implementation will be informed by the concrete consequences of a respective test result. Targeted efforts and studies are needed to create a knowledge base about the feasibility of future screening for Alzheimer's disease in the relevant population.[135]

The analysis of dried blood after a simple fingerprick is one technological development that will further increase the potential for blood biomarkers to be used in screening in underserved areas and settings where blood sample processing or cooling is not possible. Promising results have been presented for the measurement of NfL and p-tau concentrations using dried blood samples.[136]

Conclusions

The implementation of blood biomarkers for the diagnosis of Alzheimer's disease presents considerable challenges, ranging from pre-analytical and analytical standardisation to interpreting the results in real-world populations with diverse demographics, confounding factors, and comorbidities. Although the scalability and cost-effectiveness of blood biomarkers are evident, resolution of these challenges is essential for ensuring the integration of blood biomarkers into different health-care settings, including memory clinics, primary care, and population screening programmes. The successful resolution of these challenges will define the transformative effect of blood biomarkers on facilitating and enhancing the diagnosis and treatment of Alzheimer's disease. The next challenge is for policy makers and health-care systems worldwide to develop adequate guidelines which, once approved, would render these biomarkers and disease-modifying therapies a reality for everyone, everywhere.

Contributors

All authors contributed equally to the conceptualisation, data curation, writing, and reviewing of the manuscript. MS and DA supervised and coordinated the overall process.

Declaration of interests

MS has served on the advisory boards for Roche and Novo Nordisk; received speaker honoraria from Bioarctic, Novo Nordisk, and Roche; and receives research support (to the institution) from Alzpath, Bioarctic, Novo Nordisk, and Roche (outside the scope of the submitted work). MS is a co-founder of Centile Bioscience, which develops imaging-based diagnostic support solutions and serves as an Associate Editor of *Alzheimer's Research & Therapy*. IMWV declares honoraria from Quanterix. MdC declares contracts or grants from the Ministerio Español de Ciencia e innovación (PROYECTOS I+D+I – 2020 – Retos de investigación), Europe Research 2020 dynamisation actions:payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Novo Nordisk and Springer Healthcare; participation as an associate editor at *Alzheimer's Research & Therapy*; on the review board of Galen and Hilary Weston Foundation, and the review board of Alzheimer's Research UK; and travel support from the Alzheimer's Association. JT declares consulting fees from the Neurotorium Educational Platform. SP declares support from Avid, ki:elements, and Alzheimer's Drug Discovery Foundation; and payment

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