Review

Blood-based high sensitivity measurements of beta-amyloid and phosphorylated tau as biomarkers of Alzheimer’s disease: a focused review on recent advances

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ABSTRACT

Discovery and development of clinically useful biomarkers for Alzheimer’s disease (AD) and related dementias have been the focus of recent research efforts. While cerebrospinal fluid and positron emission tomography or MRI-based neuroimaging markers have made the in vivo detection of AD pathology and its consequences possible, the high cost and invasiveness have limited their widespread use in the clinical setting. On the other hand, advances in potentially more accessible blood-based biomarkers had been impeded by lack of sensitivity in detecting changes in markers of the hallmarks of AD, including amyloid-β (Aβ) peptides and phosphorylated tau (P-tau). More recently, however, emerging technologies with superior sensitivity and specificity for measuring Aβ and P-tau have reported high concordances with AD severity. In this focused review, we describe several emerging technologies, including immunoprecipitation-mass spectrometry (IP-MS), single molecule array and Meso Scale Discovery immunoassay platforms, and appraise the current literature arising from their use to identify plaques, tangles and other AD-associated pathology. While there is potential clinical utility in adopting these technologies, we also highlight the further studies needed to establish Aβ and P-tau as blood-based biomarkers for AD, including validation with existing large sample sets, new independent cohorts from diverse backgrounds as well as population-based longitudinal studies. In conclusion, the availability of sensitive and reliable measurements of Aβ peptides and P-tau species in blood holds promise for the diagnosis, prognosis and outcome assessments in clinical trials for AD.

INTRODUCTION

There are over 50 million people worldwide suffering from dementia, a number which is projected to triple by 2050.1 The high prevalence of dementia is accompanied by a massive social and economic burden; the current annual cost of dementia is estimated at US$1 trillion and is set to double by 2030.1 The most common cause of dementia is Alzheimer’s disease (AD), which accounts for approximately 60%–80% of all dementia cases.2 AD is characterised by progressive memory deficits as well as non-amnestic cognitive symptoms, including impairments in language, visuospatial and executive function that severely impact activities of daily living. Neuropathologically, AD is characterised by progressive synaptic dysfunction and neuronal loss in brain regions that are essential for higher cognitive functions,3 culminating in brain atrophy and clinical symptoms in patients. The hallmarks of AD include cortical extracellular amyloid-β (Aβ) plaques composed of highly aggregated, fibrillar 40-amino-acid to 42-amino-acid Aβ peptides (Aβ40 and Aβ42), as well as intracellular neurofibrillary tangles (NFTs) and dystrophic neurites surrounding the plaques, both composed of paired helical filament-forming, abnormally hyperphosphorylated tau protein aggregates.2 As neurodegeneration is thought to be well underway by the time patients exhibit clinical symptoms,4 these core pathophysiological features have been targets for AD biomarker development—a key area in dementia research that holds promise for early detection of the disease which in turn helps improve the drug development process for AD (see later). While postmortem neuropathological evaluation remains the gold standard for confirmatory diagnosis of AD pathology, the core AD diagnostic biomarkers in living individuals with positron emission tomography (PET) assessment of in vivo Aβ and tau cortical burden) or cerebrospinal fluid (CSF measurements of Aβ42, tau and P-tau) analyses have gained acceptance5,6 but remain relatively inaccessible.

APPLICATIONS AND CHALLENGES OF BLOOD-BASED AD BIOMARKERS

Shift towards a biological definition of AD

Historically, AD was conceived of as a clinical-pathological construct, such that cognitive symptoms defined the presence of AD.5 However, approximately 30% of individuals clinically diagnosed as AD do not display significant AD neuropathological changes at autopsy or by in-vivo imaging.5 The National Institute on Aging and Alzheimer’s Association (NIA-AA) research framework has recently suggested that AD should be defined as a biological construct using biomarkers...
Neurodegeneration

that are characteristic of AD pathophysiology, such as Aβ and phosphorylated tau (P-tau). This biological definition of AD is potentially useful in both research and clinical care settings. In the former, it facilitates efforts to understand the sequence of events leading to cognitive impairment that is associated with AD neuropathology. In the latter, it allows clinicians to confirm the aetiological diagnosis with greater certainty and provide biomarker-guided targeted therapies when they are available. Furthermore, it improves early identification (screening) of older people at risk of developing AD, providing access to important patient cohorts crucial for the clinical evaluation of promising therapeutic strategies of those with significant burden of pathological proteins.

Recruitment of appropriate trial subjects for disease-modifying therapies

Given that current AD treatments provide only symptomatic relief, there has been a focus on clinical trials of disease-modifying therapies (DMT), targeting specific AD pathologies, in particular amyloidosis, but recently also tau pathology. In DMT trials, it is imperative to show that an intervention modifies the target pathophysiological process and improves clinical symptoms. Disappointingly, past DMT trials have largely reported no or minimal cognitive benefits. A possible explanation is the inclusion of participants lacking the target pathology since recruitment was based mainly on clinical assessment. Another possibility is the inclusion of individuals too advanced in the disease process, with a clinical course which may be difficult to modify regardless of the efficacy of the drug candidates. Thus, it is necessary to conduct biomarker-driven subject selection in DMT trials to facilitate target engagement and reliably assess the efficacy of interventions. An example of such a trial is a phase II clinical study of BAN2401 (also called lecanemab) in early AD subjects with positive biomarkers for brain amyloid pathology. BAN2401 is a humanised monoclonal antibody that selectively binds to, neutralises and eliminates soluble toxic Aβ aggregates. BAN2401 showed a significant, dose-dependent reduction in PET Aβ that such that approximately 80% of participants demonstrated reduced Aβ aggregate densities and were reclassified from Aβ positive to negative (based on amyloid PET visual read) at 18 months with the highest tested dose. Importantly, participants also showed slower cognitive decline.

Given these promising results, a global multi-centre phase III clinical study of BAN2401 (Clarity AD) has been initiated. Other examples include the phase III trials, ENGAGE and EMERGE, which aimed to assess the cognitive and functional impacts of the aggregated Aβ-binding monoclonal antibody drug aducanumab in individuals with mild cognitive impairment (MCI) due to early AD as determined by a positive PET Aβ scan. In a report at the end of 2019, it was noted that at the highest dose, participants in EMERGE had a significant reduction in the rate of cognitive decline using a standardised neuropsychological test (Clinical Dementia Rating-Sum of Boxes, CDR-SB). Similarly, in ENGAGE, a subgroup of participants who received the highest dose at least 10 times declined more slowly on the CDR-SB. In substudies, aducanumab which has been approved by the United States Food and Drug Administration as of June 2021, caused a dose-dependent reduction in brain amyloid burden. Overall, these clinical trials point to the importance of trial recruitment with supporting biomarker evidence to ensure that the appropriate participants are enrolled, and to provide evidence of the efficacy of the tested drugs in targeting AD pathology.

Current challenges in the use of AD biomarkers

While AD biomarkers measured by PET or CSF are highly indicative of AD pathophysiology, the challenges involved in their usage, including invasiveness of procedures, high cost, limited accessibility to scanners and cyclotrons, and limited utility as a screening tool, have impeded their widespread application in the clinical and research settings. In contrast, blood-based biomarkers would be a readily accepted and practical approach if they had sensitivity and specificity comparable to neuroimaging and CSF markers. However, most early studies on blood Aβ42, Aβ40 and Aβ42/Aβ40 ratio using conventional ELISA or multi-analyte Luminex immunoassays reported little or no difference between AD and control groups. Furthermore, the majority of these early studies were based on comparing blood Aβ in clinically diagnosed patients with AD with cognitively unimpaired controls which, given the uncertain value of clinical assessments in assessing pathological burden, might have underestimated and confounded the diagnostic value of plasma Aβ. The specific limitations of conventional blood Aβ measurements include the low abundance of Aβ in peripheral blood which may result from filtering effects of the blood–brain barrier, dilution in the large plasma volume, rapid metabolism and clearance, and adhesion to other plasma proteins leading to epitope masking and analytical interference. As a consequence, early measurements of Aβ concentrations in blood were much lower than those in CSF. This low abundance impeded reliable, quantitative measurements of peripheral Aβ in earlier studies using conventional immunoassays with their inherent limits in sensitivity.

Furthermore, measurement of soluble Aβ in the peripheral blood may not be reflective of brain amyloid plaque burden, as indicated by the poor correlation between blood and CSF Aβ levels. This limitation may have been compounded by early studies using assays which measured only soluble Aβ in the blood and could not recognise aggregated isoforms, leading to under-detection of Aβ oligomers (AβOs) that are widely postulated to be the major neurotoxic forms of Aβ. The ability to measure specific Aβ isoforms such as AβOs could therefore be more pathophysiologically relevant to brain amyloid burden.

Emerging technologies for blood-based amyloid measurements

In recent years, there has been a growing impetus for the development of new platforms and immunoassays for measuring peripheral Aβ. These developments helped address the major challenges listed earlier by having improved sensitivity compared with conventional assays, and/or the ability to measure Aβ variants that may be more relevant to AD pathology. The following section aims to summarise these emerging techniques or technologies, with brief descriptions of the assay principles followed by appraisals of their ability to identify individuals with significant brain Aβ burden (Aβ+) compared with those without (Aβ−, as confirmed by brain PET or CSF analyses). A summary of the results is provided in table 1 (with detailed cohort information given in online supplemental table S1). Group comparisons between Aβ+ and Aβ− are presented in table 1. To assess the diagnostic performance of blood Aβ in detecting elevated Aβ, area under the receiver operating characteristic curve (AUC) values were given where reported (table 1). For associations between blood Aβ and continuous variables such as the standardised uptake value ratio (SUVR) and CSF Aβ levels, correlation or regression results was considered (online supplemental table
Taken together, these data show blood Aβ biomarkers demonstrate improved discrimination performance when participants were classified into amyloid positive or negative based on PET or CSF amyloid measurements, compared with classifications based on clinical assessments. Nevertheless, blood Aβ typically showed much lower fold change (10%–15%) in plasma compared with CSF (40%–50%), likely due in part to a large proportion of plasma Aβ coming from peripheral sources of the peptide.12

**Next generation ELISAs**

As mentioned, early generation ELISAs were largely unable to accurately detect and measure AD-associated changes in blood
Aβ. However, significant improvements have been made in newer ELISAs to enhance their performance. For instance, the EUROMMUN ELISA study employed C-terminal and N-terminal antibodies, while most previous ELISAs did not, and consequently detected different Aβ fragments. Furthermore, improvements in assay design and conjugation method may also have resulted in increased sensitivities, thus enabling the detection of more subtle variations in biomarker profiles than attainable with early generation ELISAs. These improvements likely led to similar performance between the EUROMMUN ELISA and the ultrasensitive single molecule array (Simoa) assay (see later) in a head-to-head comparison, where the accuracy of detecting PET Aβ+ in non-demented participants through measurements of plasma Aβ42/Aβ40 ratio was similar, and plasma Aβ42/Aβ40 as well as Aβ42/t-tau ratios measured by both methods correlated to the same extent with amyloid PET and CSF Aβ. Nevertheless, more head-to-head comparisons among the different available ELISAs are needed.

Simoa immunoassay

The Simoa immunoassay is a digital ELISA, allowing concentrations to be determined digitally rather than by measurement of the total analogue signal (Quanterix, USA). Briefly, after formation of the immunocomplex on paramagnetic beads, the beads are transferred to thousands of femtoliter-sized wells, each sized to hold only one bead. By confining the fluorophores generated by individual enzymes to extremely small volumes (approximately 40 fL), a high local concentration of fluorescent products is achieved. Therefore, a readable signal is detected even if only a single sandwich complex is present on the bead. The analyser counts the number of wells containing an enzyme-labelled bead and wells containing a bead. The ratio between the counts provides the average enzyme per bead number, and the concentration of the target analyte in the sample is derived from a standard curve.

The first version of the Simoa assay for blood Aβ was published in 2011. Using this assay, two large, independent studies, Janelidze et al and Verberk et al, reported no or weak correlations between the plasma Aβ biomarkers and established measurements such as SUVR, CSF Aβ42 or CSF Aβ42/Aβ40 ratio \((r=0.16\) to 0.38). In distinguishing between PET Aβ+ and Aβ− subjects, Janelidze et al reported a modest AUC of 0.60–0.62 for the plasma Aβ biomarkers, while Verberk et al reported numerically higher AUCs of 0.66–0.79. Likewise, in differentiating between CSF Aβ+ and Aβ− subjects, Janelidze et al reported AUCs of 0.66–0.68 for the plasma Aβ biomarkers, while Verberk et al reported AUCs of 0.66–0.83. The varying reported ranges may be due to differences in the cohorts used, adjustments of AUCs (for age, gender and/or apolipoprotein E genotype) or the combination of detection antibody pairs used. Janelidze et al used the first-generation singleplex from Quanterix which employed N-terminal antibody 6E10, which binds to the RHD sequence located at residues 5–7 of the Aβ peptide, as the capture antibody and isoform-specific C-terminal antibodies, namely H31L21 and 2G3 for Aβ42 and Aβ40, respectively, as the detector antibodies. However, such antibody pair combination may result in lower assay specificity. In contrast, Verberk et al used the Neurology 3-Plex which swapped the antibodies that are used to capture and detect plasma Aβ. Matching the antibody orientation of Verberk et al, a small-scale study by Thijssen et al selected the C-terminal antibodies 21F12 and 2G3 as capture antibodies for Aβ42 and Aβ40, respectively, and N-terminal antibody 3D6 that binds to the first five residues of the Aβ peptide, as the detector antibody. When compared with the commercially available assay—which used an N-terminal antibody to capture both peptides, followed by 21F12 or 2G3 for detection of Aβ42 and Aβ40, respectively—the prototype assay performed better in discriminating between Aβ+ and Aβ− subjects (AUC 0.95 vs 0.85) and produced a stronger correlation between plasma Aβ42/Aβ40 ratio and CSF Aβ42 (\(r=0.71\) vs \(r=0.53\)) (online supplemental table S1). Overall, these studies indicated that while Simoa provides superior sensitivity, the combination of detection/capture antibody pairs should be carefully considered to maximise assay performance.

A major advantage of the Simoa immunoassay is its increased sensitivity when compared with conventional immunoassays, measuring proteins at femtomolar concentration and allowing detection of low abundance targets such as peripheral Aβ. Moreover, the assay may be performed in a fully automated setup, increasing efficiency and output, and minimising variability of results common in a manual setup. The scalability of this platform implies that it is highly adaptable to clinical care and clinical trial settings. However, as discussed earlier, further optimisation of the assays to validate its sensitivity, specificity and consistency is warranted. Besides the diagnostic utility, Simrén et al showed that longitudinal change in Simoa Aβ42/Aβ40 measures were sensitive to grey matter loss in cognitively unimpaired individuals, suggesting the potential prognostic utility of Simoa measured Aβ.

Immunoprecipitation-mass spectrometry

This technique involves the isolation and enrichment of Aβ peptides from plasma by immunoprecipitation (IP) using a specific antibody, followed by identification and relative quantification of the individual Aβ isoforms including Aβ42, Aβ40, Aβ38 or APP669-711 with highly sensitive mass spectrometry (MS). While the principle underlying this approach is similar for the studies, there are key differences in methodologies. Kaneko et al and Nakamura et al used matrix-assisted laser desorption/ionisation-time-of-flight MS (MALDI-TOF/MS), whereas Ovod et al and Keshavan et al used liquid chromatography tandem mass spectrometry MS (LC-MS/MS). Pannee et al used MALDI-TOF/MS profiling followed by LC-MS/MS to confirm the identities of the Aβ peptides. In comparison with LC-MS/MS which generates multiple mass spectra for each fragmented peptide and compares these spectra to a database for protein identification, in MALDI-TOF/MS only one MS spectrum is generated, compiling the masses of all peptides. As such, MALDI-TOF/MS provides a quicker analysis and higher throughput, making it more feasible for large-scale studies. However, MALDI-TOF/MS is less accurate and reliable in protein identification due to the lack of true sequence dependence of data since only a mass spectrum is generated for all peptides.

Next, the studies have selected different antibodies and number of rounds of IP, which may affect the overall specificity and sensitivity of the assay. In addition to Aβ42 and Aβ40, Kaneko et al and Nakamura et al measured APP669-711 (Aβ3–40) while Pannee et al and Ovod et al measured Aβ38 (APP672-709). The purpose of these measurements was to correct for inter-individual variability in the overall metabolic production of Aβ. Similar to Aβ40, both APP669-711 and Aβ38 are thought to increase due to higher overall Aβ production, but are unaffected by AD pathology. Thus, the ratio of each individual isoforms and Aβ42 may serve as a better predictor of...
brain amyloid burden than Aβ42 alone. For instance, CSF Aβ42/Aβ38 ratio was reported to be better than CSF Aβ42 alone at predicting PET Aβ positivity, with a performance that is comparable to CSF Aβ42/Aβ40 ratio.\(^{34}\)

The published AUC results suggested that plasma APP669-711/Aβ42 ratio (AUC ≥ 0.86), Aβ42/Aβ40 ratio (AUC ≥ 0.80) or Aβ40/Aβ42 ratio (AUC ≥ 0.87) was able to discriminate between Aβ+ and Aβ− subjects. In their study, Nakamura et al. evaluated the classification ability (Aβ+ vs Aβ−) of a composite biomarker—an average of the normalised values of APP669-711/Aβ42 ratio and Aβ40/Aβ42 ratio. The composite biomarker showed the highest classification ability (AUC ≥ 0.91) among the Aβ biomarkers. A later study by Keshavan et al. measured and compared the ability of three blood-based candidates, namely LC-MS measures of plasma Aβ and Simoa-measures of plasma Aβ and tau phosphorylated at threonine 181 (P-tau181), to detect Aβ PET status in a population-based cohort of non-dementia individuals.\(^{35}\)

In this study, the LC-MS plasma composite biomarker (AUC = 0.82) showed the highest AUC among other plasma biomarkers, including LC-MS Aβ42 (AUC = 0.74), Simoa Aβ42 (AUC = 0.59) and Aβ42/Aβ40 ratio (AUC = 0.62). The plasma LC-MS Aβ42/Aβ40 ratio showed an AUC of 0.82. Although the composite biomarker showed the highest classification ability among the Aβ biomarkers, there was reservation regarding the interpretation of the data. A composite biomarker should be derived from two independent markers but APP669-711/Aβ42 ratio and Aβ40/Aβ42 ratio are not independent—Aβ42 was accounted for two times in the formula and being the most impactful on the analyses, it could potentially skew the overall results and conclusion.

A major advantage of IP-MS is the consistency of the Receiver Operating Characteristic (ROC) results across different studies, despite differences in methodology. All studies reported high AUCs for the combined ratios of Aβ42 with Aβ40 or APP669-711, indicating the superior ability of these ratios to detect elevated brain Aβ. Furthermore, this approach allows for the detection of multiple Aβ-related peptides in human plasma such as APP669-711, for which there are currently no commercially available antibody-based assay kits. Nonetheless, there are limitations to IP-MS which may reduce feasibility for widespread clinical use, such as complexity of the procedures, long processing times and inconsistency in analytical procedures across different diagnostic labs. Development of an automated assay system to standardise the analytic factors and increase throughput is needed to support its usage in a larger setting.

**Elecys immunoassays**

The Elecsys immunoassay is an electrochemiluminescence (ECL) method using a sandwich principle performed in a fully automated setup (Roche Diagnostics). First, samples are mixed with biotinylated antibodies specific to the target Aβ isoforms and ruthenium-labelled detection antibodies, resulting in the formation of a sandwich complex. Next, streptavidin-coated magnetic beads are added which bind to the biotinylated antibodies. For measurement, the reaction mixture is aspirated into a measuring cell where the beads are magnetically captured onto the surface of an electrode. To start the reaction, voltage is applied to the electrode, leading to emission of photons from the ruthenium complex, which is measured by a photomultiplier. The analyte concentration is determined via a calibration curve.

Using CSF Aβ42/Aβ40 ratio as a marker of Aβ status against which plasma Aβ was compared, Palmqvist et al. reported a decrease in Elecsys-measured plasma Aβ42/Aβ40 ratio in Aβ+ subjects,\(^{36}\) and there was also a marked decrease in plasma Aβ42 and Aβ40, especially in AD dementia subjects.\(^{35}\) The plasma Aβ42, Aβ40 and Aβ42/Aβ40 ratio predicted Aβ positivity with an AUC of 0.71, 0.54 and 0.77, respectively. When using plasma Aβ42 and Aβ40 as separate predictors in a logistic regression, the AUC improved slightly to 0.80, and further increased to 0.85 after adding APOE genotype into the model. As APOE4 allele carriers have significantly lower CSF Aβ42 levels, the combination of APOE genotype and blood-based biomarkers could further support the diagnosis of AD.\(^{37}\) In the study, a moderate positive correlation (r = 0.48) was reported between CSF and plasma Aβ42/Aβ40 ratios. The inclusion of APOE genotype in a combined model (Aβ42 and Aβ40) significantly improved the AUC, suggesting that future biomarkers studies may consider the inclusion of AD risk factors (eg, age, gender, APOE) to improve prediction.\(^{35}\) The current results are promising and warrant further validation in independent cohorts to determine the utility of Elecsys-measured plasma Aβ for the detection of elevated brain Aβ.

**Meso Scale Discovery platform**

Similar to the Elecsys immunoassay, the Meso Scale Discovery (MSD platform) is an ECL-based immunoassay (Meso Scale Diagnostics) which allows multiplexing. The MSD plate has a working electrode surface where specific capture antibodies are immobilised on independent and well-defined spots. Depending on the capture antibody immobilised on each spot, multiple target analytes may be captured from the sample. Furthermore, both techniques use different ECL labels and antibody pairs, which may impact on their sensitivity and specificity. Plates containing samples, capture antibody and detection antibody conjugated with MSD SULFO-TAG labels are loaded into the MSD instrument where a voltage is applied to the plate electrodes, causing the captured labels to emit light. Multiple excitation cycles amplify signals to increase the sensitivity of the assay and analyte concentrations are determined via a calibration curve.

Using PET for Aβ status, Vogelsang et al. reported no significant difference in plasma Aβ42 and Aβ40/Aβ42 ratio between Aβ+ and Aβ− groups.\(^ {37}\) The study also showed a moderate but positive correlation between CSF and plasma Aβ42/Aβ40 ratios (r = 0.43), comparable to results reported for Elecsys (see earlier). This is unsurprising given the similar underlying assay principles. Currently, there is little evidence supporting the use of MSD platform in the measurement of plasma Aβ as a blood-based biomarker. Since the study cohort was small (n=41), future studies using a larger cohort may provide more indication on the potential use of this platform.

**Immunomagnetic reduction**

Immunomagnetic reduction (IMR) quantifies the concentrations of target proteins/peptides in a sample by measuring the percentage reduction in the alternating current magnetic susceptibility of the IMR reagent caused by the binding of the antibody-coated magnetic nanobeads with the targets (MagQua, Taiwan ROC). When the IMR reagent which contains the antibody-coated magnetic beads is mixed with the sample, the target analytes bind with the antibodies and part of the magnetic beads become enlarged, leading to a reduction in the oscillation speed and magnetic signal of the beads. The reduction percentage is referred to as the IMR signal. Sample analyte concentration is then calculated based on the established relationship between protein standard concentration and IMR signal.
In contrast to other immunoassays, IMR-measured plasma Aβ42 was increased in AD dementia.15 A possible explanation is that other immunoassays are mostly based on sandwich ELISA which relies on the binding of both capture and detection antibodies to measure plasma Aβ. Since Aβ is frequently bound to plasma proteins, this may induce a potential stereoscopic obstacle for two antibodies to associate with one Aβ molecule simultaneously, leading to partial loss of signal. In comparison, the IMR method uses a single antibody to capture Aβ molecule in the plasma. Therefore, the IMR method has a higher possibility of capturing and detecting the target Aβ molecules in various conformations, such as isolated, complex or oligomeric forms.

Two IMR-based studies have reported on the association of plasma Aβ with brain Aβ burden, with Tzen et al18 showing a significant association between IMR-measured plasma Aβ42/Aβ40 ratio and PET SUVR (β=0.65) while Teunissen et al18 reported a negative correlation between plasma Aβ42 and CSF Aβ42 (r=−0.35). Future studies may need to compare AUC performances between PET and CSF markers, as well as their associations with IMR within the same cohort. Based on the current results, there is a lack of consistent findings to support diagnostic utility for this approach.

**Amplified plasmonic exosome platform**

While the above-mentioned techniques and platforms mainly addressed Aβ sensitivity issues, amplified plasmonic exosome (APEX) and the subsequently described methods have the potential to specifically measure different Aβ aggregation species. APEX is based on the finding that exosomes can bind to extracellular Aβ42 proteins via glycoproteins or glycolipids on the exosomal plasma membrane. In their study, Lim et al established that exosomes have a higher binding affinity to larger Aβ42 aggregates which also have a stronger propensity to form aggregated, fibrillar structures.40 Briefly, after binding the target to the APEX nanosensor via the capture antibody, insoluble optical deposits are formed on the sensor through in-situ enzymatic amplification. This deposition enhances the surface plasmon resonance signal, as represented by a greater spectral shift. Through modifications in the sensor’s design and fabrication (eg, patterned silicon nitride membrane, double-layered nanostructure, back illumination), APEX is designed to achieve nanoscale detection. To measure exosome-bound Aβ42 from plasma, Aβ42 is directly enriched from plasma via the Aβ42 capture antibody on the APEX nanosensor, and the relative amount of CD63, an established exosomal marker associated with the captured Aβ42, is measured via the CD63 detection antibody. Measurements are made relative to a sample-matched negative control, where the same sample was incubated over a control sensor functionalised with IgG isotype control antibody.

In their small cohort of 72 subjects, Lim et al reported a strong positive correlation between the level of plasma exosome-bound Aβ42 and SUVR (r=0.95). Using the same cohort, Tanaka et al41 performed the first head-to-head comparison study between APEX and Simoa and showed that plasma exosome-bound Aβ42 (AUC=0.99) outperformed Simoa-measured Aβ42/Aβ40 ratio (AUC=0.82) and Aβ42 alone (AUC=0.78) in distinguishing between PET Aβ+ and Aβ− subjects. The superior performance of the plasma exosome-bound Aβ42 necessitates further validation in larger independent cohorts to establish APEX as a biomarker platform.

**Multimer detection system**

Multimer detection system (MDS) is a sandwich ELISA that preferentially detected oligomers over monomers.12 A unique epitope exists in the Aβ monomer, with multiple copies of this epitope found in the multimer. Thus, if antibodies targeting the unique/overlapping epitope were used for both capturing and detecting antibodies, the monomer would only be occupied by one of the antibodies and no signal would be produced. In contrast, multiple copies of the unique epitope in a multimer would allow binding of both the capturing and detecting antibodies to produce detectable signals.

In an earlier study, An et al42 reported that MDS was unable to discriminate crude AβO levels in the plasma of patients with AD from controls due to low concentrations. However, spiking the plasma samples with synthetic Aβ42 resulted in significant increases in AβO levels in the patients with AD, but not in controls.43 A possible explanation is that oligomerisation of Aβ is influenced by potential factors in plasma of patients with AD, which may be absent or in lower concentrations in controls. Adopting a similar concept to measure plasma AβOs by MDS, Wang et al45 spiked synthetic Aβ42 peptide and used epitope-overlapping Aβ antibodies at the N-terminus 3-4 of Aβ.45 They reported a moderate correlation between plasma AβO levels and PIB PET SUVR (r=0.43) or CSF Aβ42 levels (r=−0.44). The present study samples were small (n=50). Furthermore, the current long incubation time (144 hours) needs to be reduced to be feasible for clinical use.

**Interdigitated microelectrode sensor system**

Kim et al introduced the use of an interdigitated microelectrode (IME) sensor system, together with 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS)—a molecule which aids in converting aggregated Aβ into its monomeric form; for the measurement of plasma Aβ in the heterogenous and monomeric states.44 Briefly, the chip’s sensing zone was coated with anti-Aβ antibody to capture the Aβ in plasma. Two microchannels were incorporated on top of the chip to simultaneously load two different plasma samples—the EPPS treated and non-treated samples. The interaction between plasma Aβ and anti-Aβ antibodies was measured using an impedance measurement system, which was constructed to cancel noise signals from parasitic capacitance of IME and subsequently amplify the low-level signals. The impedance change increases as the concentration of Aβ increases. To mitigate interindividual and intraindividual variations in Aβ levels, a self-standard ratio was calculated by dividing the concentration of homogenous Aβ monomers (EPPS treated) by that of heterogenous Aβ (non-treated).

Using blood collected from two clinical institutes, Kim et al reported a positive correlation between the self-standard ratio and PET SUVR in both cohorts (r=0.551 and 0.414, respectively). Similar to the majority of the other studies, more results, including AUC, are needed to establish the utility of this method.

**Blood-derived P-tau: a promising marker of AD pathophysiology**

Besides Aβ, there have been concerted efforts to accurately measure another established CSF biomarker, namely P-tau, in blood. These attempts are driven by the development of ultra-sensitive technologies enhanced by automation and improved throughput, such as the Simoa platform (see earlier). Following initial analytical challenges that hampered successful application of blood P-tau assays, recent advances in the understanding of tau biochemical processing in the brain as well as the subsequent
release of soluble P-tau into biofluids have enabled unprecedented rapid development of P-tau biomarkers in blood. First, a blood-based alternative of the established CSF marker of tau pathology, P-tau181, was developed by focusing on fragments containing the N-terminal portion of the protein that appears to be released into blood more abundantly than the mid-region forms that conventional CSF assays detect. Blood P-tau181 assessment methods from different sources, mainly the University of Gothenburg and Eli Lilly (using Simoa and MSD technologies, respectively), have shown excellent analytical and diagnostic performances in several landmark publications (tables 2 and 3, with detailed cohort information given in online supplemental table S2). Next, to be developed were P-tau biomarkers focusing on other phosphorylated sites, for example, P-tau21745 and P-tau23146 which have shown comparable diagnostic utility and, in some cases, superior predictive abilities particularly in the preclinical stages of AD (tables 2 and 3). Furthermore, IP-MS technology-based multiplexed assays have been developed that can measure multiple P-tau biomarkers in the same plasma samples simultaneously, including various tau species phosphorylated at residues apart from threonine-181.47

Summary of the recent blood P-tau181 findings

Most current studies employed Simoa or MSD for the measurement of blood P-tau181, with results broadly similar across the two platforms. Several studies showed that blood P-tau181 concentrations were increased in MCI due to AD and AD dementia, but not in non-AD dementias including frontotemporal dementia (FTD) and vascular dementia (VaD).48–54 Specifically, blood P-tau181 was consistently elevated in the Aβ+ subjects (eg, Aβ+ MC1 and Aβ+ AD).48 50 51 52 54–56 In a recent study, it was reported that the earliest increases in plasma P-tau181 occurred shortly before PET and CSF Aβ markers reached abnormal levels, with changes accelerated as the severity of Aβ pathology increased.57 Moreover, plasma P-tau181 reached abnormal levels only after PET and CSF Aβ biomarkers reached relatively advanced abnormality levels—it was estimated that plasma P-tau181 reached abnormal levels approximately 6.5 and 5.7 years after CSF and PET Aβ measures.57

Next, plasma P-tau181 was also shown to be different from non-AD neurodegenerative diseases (AUCs=0.82–0.94).48 51–53 Plasma P-tau181 detected cross-sectional elevated brain Aβ (Aβ+) in combined CU, MCI and dementia subjects (all subjects; AUCs=0.72–0.95),48 47 48 51–54 as well as in subgroups of non-demented (CU+MCI; AUCs=0.71–0.81),30 51 52 54 CU (AUCs=0.67–0.86)48 46 48 53–55 and MCI subjects (AUCs=0.68–0.94).46 48 51 52 53–55 The range of AUCs observed may be due to differences in the definitions of Aβ positivity (which could be related to the different PET Aβ tracers or CSF cut-offs used in the different studies) and the cohorts in terms of prevalence of tau pathology, which is also highly associated with plasma P-tau181. Some of the above-mentioned studies further demonstrated P-tau181 associations, cross-sectionally and longitudinally, with NFT burden,46 48 51 53–55 cortical atrophy brain,56 hippocampal atrophy,56 59 and cognitive impairment.27 54 59 As illustrated in the majority of these studies, besides performing the association analyses in the whole cohort, it may be important to stratify the subjects by their cognitive (or diagnostic) and Aβ status as the observed associations could be driven by a particular subgroup, such as the Aβ+ subjects. This may not be surprising given that blood P-tau181 likely reflects the pathophysiological processes specifically associated with AD pathology.

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<th>Table 2 Comparisons of blood phosphorylated tau (P-tau) among diagnostic groups stratified by Aβ status</th>
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<td>↑ Blood P-tau in Aβ+ AD vs Aβ− MCI</td>
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<td>↑ Blood P-tau in Aβ+ AD vs Aβ− MCI</td>
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Continued
Table 2 Continued

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<th>Result</th>
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<td>P-tau181</td>
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<td>Simoa</td>
<td>P-tau231</td>
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Aβ, amyloid-β; AD, Alzheimer’s disease; CU, Cognitively unimpaired; IP-MS, immunoprecipitation-mass spectrometry; MCI, mild cognitive impairment; MSD, Meso Scale Discovery; Simoa, single molecule array; UCSF, University of California at San Francisco.

Table 3 AUCs of blood phosphorylated tau (P-tau) in distinguishing between Aβ+ and Aβ− participants

<table>
<thead>
<tr>
<th>AUC</th>
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<td>P-tau181</td>
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<td>0.76</td>
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<td>0.80</td>
<td>CU+MCI+AD</td>
<td>MSD</td>
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</tr>
<tr>
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<td>52</td>
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<tr>
<td>0.91</td>
<td>CU+MCI+AD+FTLD</td>
<td>MSD</td>
<td>53</td>
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<td>Simoa</td>
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<td>0.68</td>
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<td>CU+MCI (CIND)</td>
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<td>0.71</td>
<td>CU+MCI+prior neurological conditions</td>
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<td>0.75</td>
<td>CU+MCI</td>
<td>MSD</td>
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<td>CU+MCI</td>
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<td>P-tau217</td>
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<td>0.93</td>
<td>MCI (validation cohort)</td>
<td>Simoa</td>
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Comparison of biomarker performance between P-tau species

A few papers have evaluated P-tau181 and P-tau217 in the very early phases of MCI/dementia. A study examining familial AD found significantly higher P-tau217 concentrations 20 years before the expected year of onset (EYO) of symptoms in individuals diagnosed with MCI, while another reported significant increases in P-tau181, 16 years before EYO in symptomatic Familial AD mutation carriers (both MCI and AD). More recently, head-to-head comparisons of N-terminal-directed P-tau181 and P-tau217 biomarkers in CSF showed similar performances; however, both were superior to conventional P-tau181 measured on mid-region fragments. These findings support the idea that both the measured plasma P-tau biomarkers are increased in the preclinical phase of FAD. Further, studies evaluating these P-tau biomarkers in later stages of disease showed tight associations between biomarker levels in plasma samples taken during life and AD neuropathology assessed post-mortem, with AUC values for differentiating AD from non-AD neurodegenerative disorders at 0.89 for P-tau217, 0.97 for P-tau181 and 0.997 for P-tau231 (online supplemental table S2). These findings further support the hypothesis that increased P-tau specifically reflect AD pathology. However, further studies directly comparing these tau biomarkers in plasma using the same assay technology in the same cohort are needed to ascertain whether P-tau181, P-tau217 and P-tau231 substantially differ in diagnostic utility in different phases of the AD continuum. In asymptomatic AD, P-tau231 shows increases in individuals with subthreshold levels of PET-measured Aβ burden in the first quartile, while CSF P-tau217 and plasma P-tau181 were increased in the third or fourth quartile. Finally, a few studies have demonstrated that longitudinal increases of blood P-tau levels were associated with longitudinal brain atrophy and cognitive decline, particularly in the patients with AD, and may differentiate MCI converters from non-converters, suggesting potential utility in longer-term monitoring in therapeutic trials.

Multi-marker approach towards AD biomarkers

Since AD is pathologically characterised by amyloid plaques, NFTs as well as neurodegeneration, a comprehensive ATN (amyloid, tau, neurodegeneration) classification system has been advanced to better account for the complex pathophysiological processes central to AD pathogenesis. To the extent that blood-based biomarkers accurately reflects brain changes, a corresponding combination of biomarkers may yield superior utility as well. For example, using data from an Asian cohort of patients with AD with concomitant cerebrovascular diseases (CVDs), it has been reported that combining Simoa P-tau181 with Aβ42 measures yielded better AUCs for amyloid positivity and hippocampal atrophy than any single
marker evaluated (P-tau181, total tau, Aβ40 and Aβ42). An earlier study reported similar improved performance of combining IMR measurements of Aβ and tau in identifying AD in both prodromal and dementia phases. However, as various P-tau species seem to be associated with both amyloid and tau pathologies, they could potentially be used together as biomarkers to detect A and T pathophysiology, especially in settings where IP-M5 plasma Aβ measurements are not feasible, or when plasma Aβ measures cannot distinguish A+ and A− cases. Nonetheless, the fact that different P-tau markers appear to be altered at different stages of AD pathology could be a source of complications.

Finally, amyloid and tau pathologies are also associated with various pathophysiological conditions, including synaptic dysfunction, neuroaxonal damage and injury, neuroinflammation and oxidative stress; as well as with concomitant CVsDs which may be relatively frequent in Asian and other less-studied populations and interact additively or synergistically with AD in worsening cognitive functions. It would therefore be of interest to investigate if combining Aβ and tau markers with those for synaptic, neuroaxonal injury, inflammatory, oxidative stress and endothelial/vascular injury in a multi-marker panel may yield improved clinical utility and further insights into disease mechanisms. A potential blood biomarker candidate is neurofilament-L (NfL), which shows good correlation with CSF NfL and reflects severity of axonal degeneration and injury. NfL is a disease-unspecific marker, as elevations are observed in several neurodegenerative disorders including AD, thus limiting its differential diagnostic potential. Nonetheless, studies have reported on the utility of blood NfL for predicting and monitoring longitudinal changes in cognition, brain atrophy and hypometabolism. Unlike P-tau181, which was associated with cognitive decline and prospective neurodegeneration that are characteristic for AD, the associations of blood NfL were not AD specific. Furthermore, both blood biomarkers were independently associated with longitudinal changes in cognition and AD-typical neurodegeneration. Taken together, the combination of blood NfL to the multi-marker panel may aid as a screening tool to identify individuals with ongoing neurodegeneration and improve prediction of disease progression in AD, as well as monitor treatment response in disease-modifying trials (eg, downstream effects of the tested drugs on neurodegeneration).

BLOOD BIOMARKERS

ETHICAL AND CONSUMER ISSUES ASSOCIATED WITH BLOOD BIOMARKERS

In the near future, the increasing ability to receive accurate diagnostic and prognostic information with the ease of a blood test will revolutionise AD research and care. Given the concerns surrounding the risk of developing AD and related dementias, such testing may also be the basis of profitable direct-to-consumer (DTC) tests which may come with significant ethical and social issues, as highlighted in. Briefly, DTC blood testing may raise concerns about confidentiality and discrimination, as when a DTC testing company sells consumers’ data to third parties without informed consent. In regions where laws offer limited protections against biomarker-based discrimination, insurers could use the positive blood test result to refuse an individual of disability or long-term care insurance for prodromal individuals, and companies may deny opportunities for employment or promotion. Next, in societies that lack proper awareness of AD, DTC blood testing may amplify AD-associated stigma and discrimination even in the absence of cognitive symptoms. Therefore, it is necessary to communicate the facts and educate the public to dispel misconceptions about the disease. Furthermore, policies to protect individuals with positive DTC test results from discrimination and stigmatisation should be implemented. Before these issues are addressed, it is advisable that blood testing for dementia is restricted by regulation to the clinical setting.

Concluding remarks

Recent advances in the development of ultrasensitive, high throughput analytical technologies and platforms have enabled the discovery and potential clinical application of promising biomarkers that reflect AD brain pathology in blood samples. These biomarkers will be critical in both diagnostic and prognostic assessments for AD as well as in longitudinal monitoring and therapeutic trials. However, there is a need for further research to (1) directly compare the performance of different biomarker platforms; (2) validate initial findings in larger cohorts from diverse backgrounds and (3) evaluate the utility of multi-marker panels, in order to fully realise the potential of blood-based biomarkers for AD and associated conditions.

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Competing interests HZ has served at scientific advisory boards for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteer Therapeutics, Nervgen, AlzTherapies and CosyRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Programme. KB has served as a consultant, at advisory boards, of Brsponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Programme. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/ Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Programme.

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