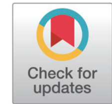
JCN Open Access REVIEW
pISSN 1738-6586 / eISSN 2005-5013 / J Clin Neurol 2022;18(4):401-409 / https://doi.org/10.3988/jcn.2022.18.4.401



Promising Blood Biomarkers for Clinical Use in Alzheimer's Disease: A Focused Update

Sun Ah Park[a,b,c]
Yu Jung Jang[a,c]
Min Kyoung Kim[a,c]
Sun Min Lee[b]
So Young Moon[b]

[a]Lab for Neurodegenerative Dementia, Department of Anatomy, Ajou University School of Medicine, Suwon, Korea
[b]Department of Neurology, Ajou University School of Medicine, Suwon, Korea
[c]Neuroscience Graduate Program, Department of Biomedical Sciences, Ajou University Graduate School of Medicine, Suwon, Korea

Alzheimer's disease (AD) is the most-common cause of neurodegenerative dementia, and it is characterized by abnormal amyloid and tau accumulation, which indicates neurodegeneration. AD has mostly been diagnosed clinically. However, ligand-specific positron emission tomography (PET) imaging, such as amyloid PET, and cerebrospinal fluid (CSF) biomarkers are needed to accurately diagnose AD, since they supplement the shortcomings of clinical diagnoses. Using biomarkers that represent the pathology of AD is essential (particularly when disease-modifying treatment is available) to identify the corresponding pathology of targeted therapy and for monitoring the treatment response. Although imaging and CSF biomarkers are useful, their widespread use is restricted by their high cost and the discomfort during the lumbar puncture, respectively. Recent advances in AD blood biomarkers shed light on their future use for clinical purposes. The amyloid β (Aβ)42/Aβ40 ratio and the concentrations of phosphorylated tau at threonine 181 and at threonine 217, and of neurofilament light in the blood were found to represent the pathology of Aβ, tau, and neurodegeneration in the brain when using automatic electrochemiluminescence technologies, single-molecule arrays, immunoprecipitation coupled with mass spectrometry, etc. These blood biomarkers are imminently expected to be incorporated into clinical practice to predict, diagnose, and determine the stage of AD. In this review we focus on advancements in the measurement technologies for blood biomarkers and the promising biomarkers that are approaching clinical application. We also discuss the current limitations, the needed further investigations, and the perspectives on their use.

Keywords Alzheimer's disease; biomarker; blood; diagnosis; precision.

Received December 30, 2021
Revised April 29, 2022
Accepted April 29, 2022

Correspondence
Sun Ah Park, MD, PhD
Lab for Neurodegenerative Dementia, Department of Anatomy and Department of Neurology, Ajou University School of Medicine; Neuroscience Graduate Program, Department of Biomedical Sciences, Ajou University Graduate School of Medicine, 164 Worldcup-ro, Yeongtong-gu, Suwon 16499, Korea
Tel +82-31-219-5030
Fax +82-31-219-5039
E-mail sap001@ajou.ac.kr

INTRODUCTION

The blood biomarkers that reliably represent Alzheimer's disease (AD)-specific and clinically relevant pathology would aid the care of the patients and the person at risk through easy access to the biomarkers. However, diagnosing and predicting AD using blood biomarkers has been challenging. The difficult journey of proteins, originating in the brain, in systemic circulation across the lymphatic system and blood-brain barrier means that protein levels are much lower in the blood than in the brain. This makes protein for a cerebrospinal fluid (CSF) biomarker difficult to detect using conventional tools in blood. Easy degradation by proteolytic enzymes and nonspecific binding to plasma proteins hampers accurate measurement.[1] The existence of a peripheral source of candidate biomarkers further complicates the matter.[2-4] These hurdles have been overcome with the development of technology that permits measuring proteins at very low concentrations. A series of well-designed studies using an ultrasensitive measurement system elucidated the value of selected blood biomarkers with reference to well-known CSF and neuroimaging biomarkers. The research framework of the ATN system, which sets AD stages based on amyloid β (Aβ)

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (https://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

(A)-, tau (T)- and neurodegeneration (N)-related biomarkers,[5] has been increasingly applied to the design and interpretation of drugs during their development. The system improves the reliability of a study and increases the chance of detecting a significant finding.[6] The recent accelerated approval of aducanumab is an example. The positive result on the surrogate endpoint, a reduction of amyloid plaque on amyloid positron emission tomography (PET), was the main basis for approval.[7,8] However, the accessibility of established biomarkers is restricted by the high cost of PET and the discomfort associated with lumbar puncture. Developing reliable blood-based biomarkers has therefore long been a major objective in AD research, and many efforts have been made. Some blood biomarkers have proven valuable, and these are expected to be added to the ATN framework[9,10] and clinical practice guidelines.

NEW TECHNOLOGIES FOR MEASURING BLOOD BIOMARKERS

Knowledge of the delicate measurement technologies is indispensable to grasp the recent advances in AD blood biomarkers. Protein biomarker measurements in biological fluids largely depend on immunoassay- and mass spectrometry (MS)-based techniques. Immunoassays require a specific antibody to detect the target protein, while MS measures the target protein by analyzing peptide fragments. The enzyme-linked immunosorbent assay (ELISA) is the most widely used immunoassay in biomarker studies.[11-13] However, its measurement range cannot fully cover the concentrations of blood proteins that are about 2%−10% of those in CSF, when we consider the Aβ and tau proteins as examples.[14] This may be why previous studies that used ELISA for blood-sample analysis have produced controversial results.[15] Recent progress in technologies has enormously contributed to the establishment of blood-based biomarkers for AD (Fig. 1). Automatic electrochemiluminescence (ECL) technology is increasingly replacing the conventional ELISA to improve test precision even for CSF biomarkers.[16,17] The Elecsys immunoassay (Roche Diagnostics, Penzberg, Germany),[18,19] EUROIMMUN ECL immunoassay (EUROIMMUN, Lüebeck, Germany),[20] and Mesoscale Discovery (MSD) platform (Meso Scale Diagnostics, Rockville, MD, USA)[21] have been increasingly used to assess blood biomarkers. These fully automatic ECL-based assays root in the sandwich principle that uses two specified antibodies for the capture of respective analytes (antigens) and detection of antigen-antibody complexes. The capture antibody attached to the surface of the working electrode or magnetic beads binds to the target protein after the incubation with the samples. The detector

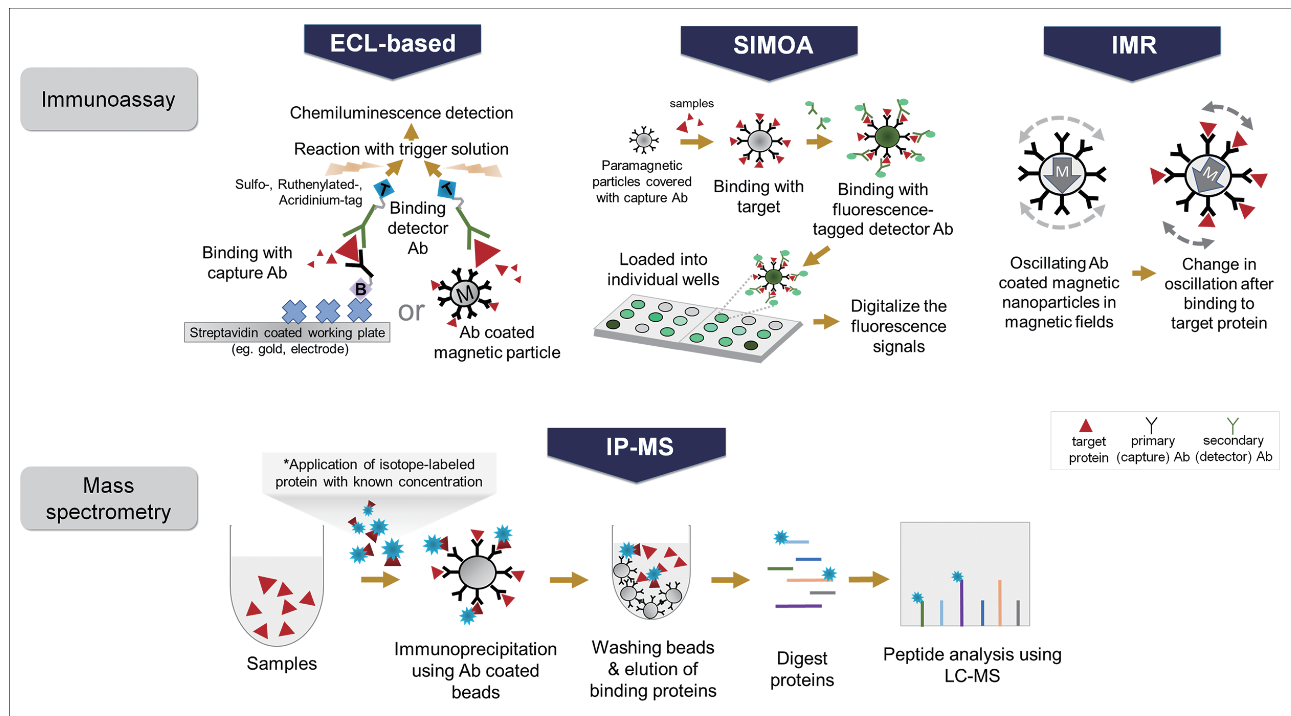


Fig. 1. Ultrasensitive techniques for measuring blood biomarkers. *Additional step for stable isotope spiking absolute quantitation[25] to improve amyloid β (Aβ) peptide quantification using isotope-labeled Aβ peptide at a specified concentration. Ab, antibody; B, biotin; ECL, electrochemiluminescence; IMR, immunomagnetic reduction; IP-MS, immunoprecipitation coupled with mass spectrometry; LC, liquid chromatography; M, magnetic; MS, mass spectrometry; SIMOA, single-molecule array; T, tag.

antibody is labeled with tags to magnify the signals (e.g., sulfo, ruthenylated, and acridinium tags), and additionally interacts with the target. While being exposed to electrical currents or magnetic fields, the sandwich complexes can remain on the working plate or magnetic beads while the unbounded ones are washed out. The complexes are then detected using chemiluminescent signals when the tags of the detector antibodies are exposed to the trigger solution. The strength of the emission is measured and then converted into the target protein concentration.

A single-molecule array (SIMOA; Quanterix, Billerica, MA, USA)[18] and immunomagnetic reduction (IMR; MagQu, Taiwan)[21] are other types of ultrasensitive immunoassays. The sensitivity of SIMOA is more than 100-fold higher than that of ELISA and more than 25-fold higher than that of the manually performed primitive method of ECL immunoassay[18] by detecting individual paramagnetic beads in a femtoliter-sized chamber, which generates a fluorescent product after binding to the target protein using a coated capture antibody and detector antibody. Since SIMOA digitizes individual fluorophores, the difference between a single immunocomplex can be counted on the beads.[18] The detection accuracy of ECL-based immunoassays is now comparable to or slightly higher than that of SIMOA after automated systems were developed and sensitive antibodies were used on a working electrode such as the MSD platform (https://www.mesoscale.com/), as shown in a direct comparative study that measured plasma Aβ42/Aβ40.[19]

IMR has the unique characteristic of using a single antibody and detecting the slowness of rotation of antibody-coated magnetic nanoparticles in a magnetic field after binding to the target antigen. IMR-based quantification of plasma Aβ40, Aβ42, and total tau (tTau) was recently approved in Taiwan to diagnose and evaluate the risk of AD.[22]

MS is a powerful and accurate method for measuring proteins at very low concentrations. Proteomic approaches, which are unbiased and targeted, have also been used to discover and validate AD biomarkers; however, they have been primarily used on CSF samples.[23] Highly accurate MS technology has been applied to detect target proteins at low blood concentrations, such as immunoprecipitation (IP) coupled with MS (IP-MS)[24] and IP-MS with Stable Isotope Spike Absolute Quantitation (SISAQ)[25] to improve Aβ peptide quantification while reducing the matrix effect of abundant plasma proteins by adding isotope-labeled Aβ peptides at a specified concentration. Measuring Aβ using IP-MS on the SISAQ platform has gained Clinical Laboratory Improvement Amendments and 'CE' approval in the USA and the EU, respectively, surpassing the quality standards for clinical in vitro diagnostic tests.[26]

Other than the ultrasensitive technique of the conventional ELISA that uses epitope-overlapping Aβ antibodies to capture and detect Aβ multimers after spiking the plasma samples, using synthetic Aβ42 to enhance the oligomerization of Aβ[27-29] was recently approved by the Korean National Evidence-based Healthcare Collaborating Agency as a supplementary diagnostic tool for AD (#HTA-2021-81, https://nhta.neca.re.kr/nhta/publication/nhtaU0601L.ecg).

The approval of more blood-based diagnostic platforms for clinical use is expected to continue in the AD field. However, it should be noted that a biomarker being accepted does not mean that it performs perfectly as an AD biomarker. A large cohort study has started to directly compare the various ultrasensitive techniques to quantify target biomarkers (Table 1)[19,30] A head-to-head comparison was conducted on the efficacy of eight promising measurement tools (two ECL-based immunoassays, two SIMOA methods, one antibody-free liquid chromatography-MS, and three IP-MS techniques) regarding the degree to which the plasma Aβ42/Aβ40 ratio reflects the CSF Aβ42/Aβ40 ratio and positive results in amyloid PET.[19] IP-MS with SISAQ developed at Washington University (St Louis, MO, USA) had the greatest accuracy, with an area under the receiver operating characteristic curve (AUC) of up to 0.872, with low intra- and intertest variabilities.[19] The Elecsys immunoassay from Roche Diagnostics had the best AUC (0.795) and lowest intertest variability among the immunoassays.[19] IP-MS had an AUC (0.817) superior to that of SIMOA (0.620) for both measuring the Aβ42/Aβ40 ratio and identifying positive amyloid PET findings in subjects without dementia.[30] Accuracy, convenience, and cost will determine which technique and target biomarkers will become the most widely used in clinical applications.

Aβ BIOMARKERS

Aβ pathology is important for the confirmatory diagnosis of AD.[31] It is indicated by reduced Aβ42 levels in the CSF[11,32] and increased cortical uptake in amyloid PET.[33] Since these Aβ biomarkers have been demonstrated to be useful in detecting AD from its early stage, measuring Aβ in blood has received considerable attention with the aim of establishing AD blood biomarkers. The possible usefulness of the plasma Aβ42/Aβ40 ratio in predicting and/or diagnosing AD has been suggested through using conventional immunoassay, ELISA, and multiplex techniques, but the results have been inconsistent.[34] One of the reasons for this was that a clinical diagnosis that did not consider AD pathology was used for defining the subjects in many studies, although there were several exceptions.[35-37] This approach does not differentiate subclinical AD from a normal control,[38] or non-AD demen-

Table 1. Head-to-head comparison of technologies in measuring the plasma amyloid β (Aβ)42/Aβ40 ratio

	Elecsys	EUROIMMUN ECL-I	SIMOA N4PE kit	SIMOA Neuro 3 kit	LC-MS-Ar[77]	IP-MS-WU[25,40]	IP-MS-GU[19]	IP-MS-Sh[24]
BioFINDER cohort [*n*=286: $A\beta^+/A\beta^-$=118/168 in CSF, $A\beta^+/A\beta^-$=110/176 in amyloid PET][19]								
CSF*	0.778 (0.725–0.832)	0.697 (0.635–0.758)	0.687 (0.626–0.748)	-	0.776 (0.721–0.830)	0.855 (0.810–0.899)‡	-	-
PET†	0.727 (0.669–0.784)	0.672 (0.609–0.735)	0.655 (0.591–0.719)	-	0.753 (0.696–0.811)	0.833 (0.787–0.879)‡	-	-
BioFINDER subcohort [*n*=200: $A\beta^+/A\beta^-$=86/114 in CSF and amyloid PET][19]								
CSF*	0.773 (0.709–0.837)	0.704 (0.631–0.777)	0,679 (0.605–0.753)	-	0.775 (0.711–0.839)	0.872 (0.824–0.920)‡	-	0.825 (0.767–0.882)
PET†	0.773 (0.709–0.837)	0.704 (0.631–0.777)	0.679 (0.605–0.753)	-	0.775 (0.711–0.839)	0.872 (0.824–0.920)‡	-	0.825 (0.767–0.882)
BioFINDER subcohort [*n*=227: $A\beta^+/A\beta^-$=91/136 in CSF, $A\beta^+/A\beta^-$=86/141 in amyloid PET][19]								
CSF*	0.795 (0.738–0.853)	0.697 (0.628–0.767)	0.706 (0.639–0.773)	0.636 (0.563–0.709)	0.763 (0.700-0.827)	0.838 (0.785-0.891)‡	0.678 (0.605–0.750)	-
PET†	0.728 (0.663–0.793)	0.667 (0.596–0.738)	0.649 (0.577–0.721)	0.600 (0.525–0.675)	0.742 (0.676–0.809)	0.814 (0.760–0.868)‡	0.632 (0.557–0.707)	-
ADNI cohort [*n*=122: $A\beta^+/A\beta^-$=59/63 in amyloid PET][19]								
PET†	0.740 (0.651–0.829)	-	0.685 (0.590–0.781)	0.634 (0.534–0.734	-	0.845 (0.772–0.917)‡	0.662 (0.565–0.758)	0.821 (0.747–0.895)

	SIMOA, Aβ40, Aβ42 kits	IP-MS[30]
British birth cohort [*n*=441: $A\beta^+/A\beta^-$=82/359 in amyloid PET][30]		
PET†	0.620 (0.548–0.691)	0.817 (0.770–0.864)‡

Data are the areas under the receiver operating characteristic curves for detecting Aβ pathology, with the 95% confidence interval in parentheses.
*To detect CSF Aβ pathology; †To detect amyloid PET positivity; ‡Indicates the highest values.
ADNI, Alzheimer Disease Neuroimaging Initiative; CSF, cerebrospinal fluid; ECL-I, electrochemiluminescence immunoassay; IP-MS, immunoprecipitation coupled with mass spectrometry; IP-MS-GU, IP-MS by the University of Gothenburg; IP-MS-Sh, IP-MS by Shimadzu; IP-MS-WU, IP-MS with SISAQ (Stable Isotope Spike Absolute Quantitation) by Washington University; LC-MS-Ar, liquid chromatography coupled with MS by Araclon; SIMOA, single-molecule array; PET, positron-emission tomography.

tia from AD.[39] Another explanation for the discrepancies is the insufficiencies of conventional immunoassays in measuring low blood concentrations of Aβ42 and Aβ40.

The recent use of fully automated and/or ultrasensitive methods, such as ECL-based assays, SIMOA, and IP-MS technology, with reference to CSF and imaging biomarkers in well-defined subjects has clearly revealed that a decrease in the plasma Aβ42/Aβ40 ratio is an indicator of brain amyloidosis.[19,24,25,40,41] A strong correlation of it with amyloid PET,[24,40] and with the CSF Aβ42/Aβ40 ratio[19,24,25,40] is observed. In contrast, the plasma Aβ42/Aβ40 ratio in nanoparticle-based IMR assays has been found by higher in AD than in controls.[42,43] This contradiction of IMR was not clearly explained, but it was speculated that IMR increased Aβ42 levels by diminishing the binding of Aβ42 to plasma proteins in the assay.[44] An association of increased Aβ level with an AD diagnosis and amyloid PET positivity was also found when using an ELISA-based multimer detection system (MDS) that measures oligomerized Aβ.[27-29] This MDS utilizes the aggregation tendency of blood Aβ to measure multimeric Aβ levels. The distinctive assay principle of MDS is thought to produce the opposite result.[27]

The plasma Aβ42/Aβ40 ratio has been shown to be valuable in the early detection of amyloid pathology during the early preclinical stage of AD. IP-MS technology seems to be the most-accurate analytical tool for determining that ratio at current. The requirements of a specialized technique and a long processing time, however, is a hurdle for the widespread use of IP-MS.

TAU BIOMARKERS

In addition to Aβ tau deposits forming neurofibrillary tangles in neurons define AD from other neurodegenerative disorders.[31] CSF tTau levels are considered to constitute a neurodegenerative marker, while phosphorylated tau is thought to represent the AD-specific pathology of neurofibrillary tangles.[43] Trials to develop CSF-matching blood biomarkers for tau pathology have been performed based on advanced ultrasensitive assays. A series of reports suggested that plasma

phosphorylated tau at threonine 181 (pTau181) and threonine 217 (pTau217) are useful AD biomarkers. An increase in plasma pTau181 levels based on the ECL-based technique using the MSD[45] and SIMOA[46] platforms predicts AD development at the preclinical and prodromal stages. An increase in pTau181 level can distinguish AD from non-AD neurodegenerative disorders.[45-47] pTau217 is another protein shown to be useful for early detection[19,48] and for differentiating AD from non-AD dementia.[47,49] The quantities of pTau181 and pTau217 in plasma are correlated with their corresponding levels in the CSF[4] and with accumulations of the amyloid and tau proteins in the brain on PET imaging.[49] These findings collectively suggest that the phosphorylation of these epitopes is AD-specific.[4] The pTau217 concentration in plasma was found to increase earlier than that of pTau181 according to the amyloid pathology when the two were measured using IP-MS.[4] The increase in pTau217 preceded the apparent tau accumulation on tau PET,[50] which suggests that it is potentially useful as the earliest pathology biomarker for tau. The superiority of pTau217 over pTau181 was also identified in the stronger correlation with tau burdens on tau PET,[47] and the better performance in discriminating AD from non-AD neurodegenerative disorders,[47,49] monitoring clinical progress,[48] and predicting dementia onset at the preclinical and prodromal stages.[51]

Another plasma form of pTau, which is phosphorylated at threonine 231, was recently measured using the SIMOA platform and was found to be highly accurate in differentiating amyloid PET positivity from negativity and AD from non-AD.[52] The change in the level of this pTau protein was evident from a very early stage, even before the standard thresholds of amyloid PET positivity and rise in plasma pTau181 and CSF pTau217 levels.[52] However, a recent direct comparisons of SIMOA-measured pTau181 and pTau231, and between MSD-measured pTau181 and pTau217 indicated that MSD-measured pTau217 followed by MSD-measured pTau181 performed better in predicting abnormal tau accumulation on tau PET at the asymptomatic stage of AD; however, pTau231 revealed a lower performance.[53] The contradictory results regarding pTau231[52,53] increase the need for more investigations into its value as an AD biomarker. Plasma pTau181 and pTau217 also need further validation, particularly considering racial differences. The accuracy of pTau in diagnosing autopsy-confirmed AD varies according to the racial background when assessed using the MSD assay platform. Accuracy was high in non-Hispanic blacks (AUC=0.94 and 0.96 for pTau181 and pTau217, respectively) but low in non-Hispanic whites (AUC=0.65 and 0.75, respectively).[54] A direct comparison between Aβ and tau biomarkers in early AD diagnosis has just started. The accuracy of plasma Aβ42/Aβ40 in IP-MS (AUC=0.817) was higher than that of the pTau181 level (AUC=0.707) on the SIMOA platform in identifying amyloid PET positivity among elderly subjects without dementia.[30] However, its accuracy was higher than that of the Aβ42/Aβ40 ratio (AUC=0.620) when these were both computed using SIMOA. The relative benefits and accuracies of Aβ and tau biomarkers require further investigation, particularly with the inclusion of plasma pTau217 and pTau231.

NEURODEGENERATIVE BIOMARKERS

In addition to Aβ and pTau accumulation, which form amyloid plaque and neurofibrillary tangles, neuritic plaque is the most clinically relevant AD pathology.[31] The product of the dystrophic neural process caused by neuronal injury is the main component of neuritic plaque. The cytoskeletal components of neuronal axons are released into the CSF and then into the systemic circulation when axons are damaged.[55] Specifically, neurofilament light (NFL), the most abundant of the neurofilament proteins, is detectable in blood using ultrasensitive methods. CSF and plasma NFL levels are correlated,[56] with both increasing in many neurological disorders such as acute brain injury, stroke, demyelinating disorders, and neurodegenerative dementia.[55,57,58] Blood NFL levels increase with deterioration of the clinical status and accurately reflect clinical profiles.[15,59] Tracing the change in serum NFL concentrations has been shown to allow clinicians to predict the onset of dementia 16.2[60] and 15 years[61] in advance for early-onset AD with a causative mutation. However, the cross-sectional level of serum NFL is less powerful for the early detection of mutation carriers, as this becomes clear only 6.8 years before the expected clinical onset.[60] The increase in plasma NFL in sporadic AD cases is very delayed, as it is only evident at the prodromal stage (mild cognitive impairment)[59,62] or after dementia onset.[57] This is explained by the finding that NFL levels increase with age,[57,63] and most sporadic AD cases develop at an older age. These results collectively make it more difficult to detect the increase in NFL in response to AD in the elderly population until the symptomatic stage.[57] The increase in NFL does not persist until an advanced stage of AD. No additional increase in the NFL level or a decrease in the Aβ42/Aβ40 ratio was observed at a Clinical Dementia Rating (CDR)>1 when the Aβ42/Aβ40 ratio, pTau181, and NFL in plasma were compared using SIMOA. pTau181 continually increases in AD until CDR=3,[64] and so clinicians should therefore remain cautious when interpreting NFL levels in subjects at advanced stages of dementia. Blood NFL levels are dynamic, representing the speed of neurodegeneration. They change most dramatically during the rapidly progressive stage[59] and around the onset of

dementia.[60]

Other than NFL, several axon- and synapse-related proteins in blood have been examined as potential biomarkers that typify neurodegeneration,[65] but none of them have approached clinical usefulness. The tTau level in CSF is useful as a neurodegeneration biomarker for AD to be incorporated into ATN staging.[31] However, its plasma levels had a weak correlation with matching CSF tTau levels when measured using SIMOA.[66] Elevated plasma tTau was suggested to predict the development of all-cause dementia including AD through a meta-analysis[67] and large cohort study;[66] however, unlike for NFL, its predictive value was not confirmed in recent studies.[68,69]

Direct comparisons further indicate the superiority of NFL over tTau regarding the discrimination of AD from healthy controls (AUC=0.94 vs. 0.56;[68] AUC=0.83 vs. 0.80[69]) and AD from MCI (AUC=0.78 vs. 0.72[69]) on the SIMOA platform. Increased NFL is not an AD-specific event but rather a universal marker of neurodegeneration, independent of causing it. NFL levels are therefore suitable as a screening tool to determine whether a neurological symptom originated from neuronal damage, and for monitoring the progress and speed of neurodegeneration. Knowledge of the longitudinal change in the level of NFL in the blood instead of its value at a single time point, as well as consideration of AD-specific biomarkers such as Aβ42/Aβ40 and pTau, would simultaneously increase its usefulness as a biomarker.

CURRENT LIMITATIONS AND PERSPECTIVES

Accumulating evidence of the diagnostic and disease-tracing value of blood biomarkers in AD indicates that they will soon be suitable for use. However, several remaining hurdles must be overcome (Fig. 2). First, a consensus on which technique is suitable for the standardized measurements of blood biomarkers in clinical practice is required. Second, reliable cutoff values for individual biomarkers must be determined. Third, a large biomarker gray zone in which differentiation is unclear is problematic when interpreting the available data.[19,54,70] Fourth, a method for weighting the impact of confounding factors such as aging, comorbidities, and race dif-

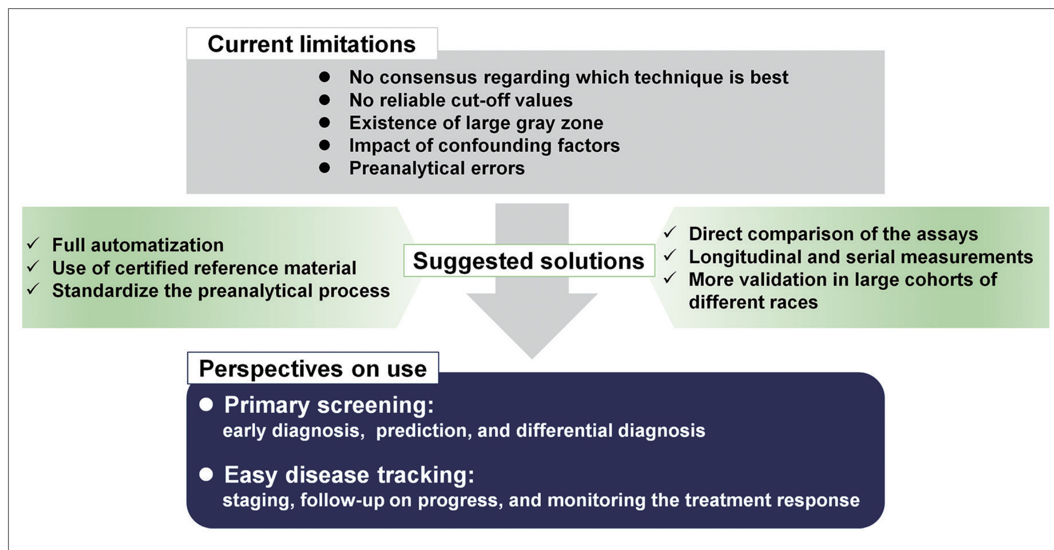


Fig. 2. Current limitations, suggested solutions, and possible clinical applications of blood biomarkers.

Table 2. Blood biomarkers with solid evidence of utility in AD

Biomarker	Early detection	Specific to AD	Correlation with clinical data	Correlation with CSF*	Correlation with PET
Aβ42/Aβ40	Yes, preclinical	Yes	Yes	Yes	Amyloid
pTau181	Yes, preclinical	Yes	Yes	Yes	Amyloid & tau
pTau217	Yes, preclinical	Yes	Yes	Yes	Amyloid & tau
NFL	Yes, prodromal	No	Yes	Yes	Amyloid (asym), tau (sym)†

*Correlation with the corresponding level in CSF; †Stage-dependent correlation: preclinical asym correlation with amyloid PET, and clinical sym correlation with tau PET.[78]
AD, Alzheimer's disease; asym, asymptomatic stage; CSF, cerebrospinal fluid; NFL, neurofilament light; PET, positron emission tomography; pTau181, phosphorylated tau at threonine 181; pTau217, phosphorylated tau at threonine 217; sym, symptomatic.

ferences on biomarker levels needs to be developed.[54,71-73] Fifth, the preanalytical errors that profoundly affect assay results must be addressed.[61]

The assays should be automated to secure the validity of the biomarkers and corresponding assay tools. The adoption of certified reference materials is necessary to control the assay quality and permit data comparisons between different measurement techniques. Blood-sample handling and the measurement process must be standardized to improve precision. Direct comparing various tools in the same cohort and performing longitudinal tracking instead of single measurements for validation would overcome some of the issues that need to be addressed. Further studies in large community-based cohorts with different racial backgrounds are necessary to establish the utility of individual biomarkers for use on specified technology. The development of certified reference materials for CSF Aβ42 measurements according to the Global Biomarker Standardization Consortium[74] is noteworthy progress in terms of a worldwide trial to monitor assay quality and to harmonize different techniques, with the aim of obtaining reliable cutoff values for fluid biomarkers. Cooperative investigations are expected to continue to improve the precision and utility of biomarkers.[74]

CONCLUSIONS

The accumulating evidence supports the utility of blood AD biomarkers for predicting, diagnosing, and staging AD (Table 2). Identification of AD biomarkers has been achievable thanks to improvements in measurement tools and validation of the results by referring to established CSF and neuroimaging biomarkers. Continuous endeavors to improve and standardize the methods are underway to overcome the current limitations. Blood biomarkers are expected to be used in clinical practice soon. The Aβ42/Aβ40 ratio, the pTau181 and pTau217, and NFL levels in blood correspond to the pathology of Aβ, neurofibrillary tangles, and neurodegeneration, respectively. Simultaneously considering these blood AD biomarkers may be helpful in estimating the stage and in differentiating the diagnosis of AD from other brain diseases.[5] Combining biomarkers with other available data would improve the accuracy of AD diagnoses, as for plasma pTau217 when a brief cognitive test and apolipoprotein E (APOE) genotyping were combined.[51] The most likely scenario is that blood biomarkers will be used for initial screening before more-confirmatory tests, such as CSF biomarkers and PET imaging using a specific ligand, and for monitoring the disease onset, progress, and response to therapy.[75,76] Adopting blood biomarkers in clinical practice will reduce the economic burden of AD and increase the early and accurate detection of subjects who will potentially benefit from preventive and disease-modifying treatments when these become available. A focused review of blood biomarkers with solid evidence is timely, and it may help clinicians to prepare for using these biomarkers in medical practice.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

ORCID iDs

Sun Ah Park https://orcid.org/0000-0002-3027-3259
Yu Jung Jang https://orcid.org/0000-0002-0188-5352
Min Kyoung Kim https://orcid.org/0000-0002-1407-1067
Sun Min Lee https://orcid.org/0000-0001-5917-015X
So Young Moon https://orcid.org/0000-0002-1025-1968

Author Contributions

Conceptualization: Sun Ah Park, Sun Min Lee, So Young Moon. Data collection: all authors. Funding acquisition: Sun Ah Park. Supervision: Sun Ah Park. Visualization: Yu Jung Jang, Min Kyoung Kim. Writing—draft: Sun Ah Park. Writing—review & editing: all authors.

Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Funding Statement

This work was supported by Basic Science Research Program (NRF-2019R1A5A2026045) and the Original Technology Research Program for Brain Science (NRF-2018M3C7A1056293) of the National Research Foundation of Korea (NRF) funded by the Korean government, MSIT.

REFERENCES

1. Wilson MR, Yerbury JJ, Poon S. Potential roles of abundant extracellular chaperones in the control of amyloid formation and toxicity. *Mol Biosyst* 2008;4:42-52.
2. Chen M, Inestrosa NC, Ross GS, Fernandez HL. Platelets are the primary source of amyloid beta-peptide in human blood. *Biochem Biophys Res Commun* 1995;213:96-103.
3. Kent SA, Spires-Jones TL, Durrant CS. The physiological roles of tau and Aβ: implications for Alzheimer's disease pathology and therapeutics. *Acta Neuropathol* 2020;140:417-447.
4. Barthélemy NR, Horie K, Sato C, Bateman RJ. Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. *J Exp Med* 2020;217:e20200861.
5. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 2018;14:535-562.
6. Cummings J, Feldman HH, Scheltens P. The "rights" of precision drug development for Alzheimer's disease. *Alzheimers Res Ther* 2019;11:76.
7. Sevigny J, Chiao P, Bussière T, Weinreb PH, Williams L, Maier M, et al. The antibody aducanumab reduces Aβ plaques in Alzheimer's disease. *Nature* 2016;537:50-56.
8. US Food and Drug Administration. Aducanumab (marketed as Aduhelm) information [Internet]. Silver Springs, NY: US Food and Drug Administration; 2021 [cited 2021 Jul 8]. Available from: https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/aducanumab-marketed-aduhelm-information.
9. Hampel H, Cummings J, Blennow K, Gao P, Jack CR Jr, Vergallo A. Developing the ATX(N) classification for use across the Alzheimer disease continuum. *Nat Rev Neurol* 2021;17:580-589.

10. Koychev I, Jansen K, Dette A, Shi L, Holling H. Blood-based ATN biomarkers of Alzheimer's disease: a meta-analysis. *J Alzheimers Dis* 2021; 79:177-195.
11. Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* 2009;302:385-393.
12. Mulder C, Verwey NA, van der Flier WM, Bouwman FH, Kok A, van Elk EJ, et al. Amyloid-beta(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. *Clin Chem* 2010;56:248-253.
13. Park SA, Chae WS, Kim HJ, Shin HS, Kim S, Im JY, et al. Cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease in South Korea. *Alzheimer Dis Assoc Disord* 2017;31:13-18.
14. Palmqvist S, Insel PS, Stomrud E, Janelidze S, Zetterberg H, Brix B, et al. Cerebrospinal fluid and plasma biomarker trajectories with increasing amyloid deposition in Alzheimer's disease. *EMBO Mol Med* 2019;11:e11170.
15. Olsson B, Lautner R, Andreasson U, Öhrfelt A, Portelius E, Bjerke M, et al. CSF and blood biomarkers for the diagnosis of Alzheimer's disease: a systematic review and meta-analysis. *Lancet Neurol* 2016;15: 673-684.
16. Hansson O, Seibyl J, Stomrud E, Zetterberg H, Trojanowski JQ, Bittner T, et al. CSF biomarkers of Alzheimer's disease concord with amyloid-β PET and predict clinical progression: a study of fully automated immunoassays in BioFINDER and ADNI cohorts. *Alzheimers Dement* 2018;14:1470-1481.
17. Kaplow J, Vandijck M, Gray J, Kanekiyo M, Huyck E, Traynham CJ, et al. Concordance of Lumipulse cerebrospinal fluid t-tau/Aβ42 ratio with amyloid PET status. *Alzheimers Dement* 2020;16:144-152.
18. Kuhle J, Barro C, Andreasson U, Derfuss T, Lindberg R, Sandelius Å, et al. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clin Chem Lab Med* 2016; 54:1655-1661.
19. Janelidze S, Teunissen CE, Zetterberg H, Allué JA, Sarasa L, Eichenlaub U, et al. Head-to-head comparison of 8 plasma amyloid-β 42/40 assays in Alzheimer disease. *JAMA Neurol* 2021;78:1375-1382.
20. Mielke MM, Hagen CE, Xu J, Chai X, Vemuri P, Lowe VJ, et al. Plasma phospho-tau181 increases with Alzheimer's disease clinical severity and is associated with tau- and amyloid-positron emission tomography. *Alzheimers Dement* 2018;14:989-997.
21. Lue LF, Kuo YM, Sabbagh M. Advance in plasma AD core biomarker development: current findings from immunomagnetic reduction-based SQUID technology. *Neurol Ther* 2019;8(Suppl 2):95-111.
22. AlzForum. Taiwan FDA approves MagQu plasma Aβ and tau tests [Internet]. Cambridge, MA: AlzForum; 2021 [cited 2021 Feb 5]. Available from: https://www.alzforum.org/news/research-news/taiwan-fda-approves-magqu-plasma-av-and-tau-tests.
23. Portelius E, Brinkmalm G, Pannee J, Zetterberg H, Blennow K, Dahlén R, et al. Proteomic studies of cerebrospinal fluid biomarkers of Alzheimer's disease: an update. *Expert Rev Proteomics* 2017;14:1007-1020.
24. Nakamura A, Kaneko N, Villemagne VL, Kato T, Doecke J, Doré V, et al. High performance plasma amyloid-β biomarkers for Alzheimer's disease. *Nature* 2018;554:249-254.
25. Ovod V, Ramsey KN, Mawuenyega KG, Bollinger JG, Hicks T, Schneider T, et al. Amyloid β concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimers Dement* 2017;13:841-849.
26. AlzForum. Plasma Aβ test wins approval—Are p-tau tests far behind? [Internet]. Cambridge, MA: AlzForum; 2020 [cited 2020 Nov 24]. Available from: https://www.alzforum.org/news/research-news/plasma-av-test-wins-approval-are-p-tau-tests-far-behind.
27. An SSA, Lee BS, Yu JS, Lim K, Kim GJ, Lee R, et al. Dynamic changes of oligomeric amyloid β levels in plasma induced by spiked synthetic Aβ42. *Alzheimers Res Ther* 2017;9:86.
28. Wang MJ, Yi S, Han JY, Park SY, Jang JW, Chun IK, et al. Oligomeric forms of amyloid-β protein in plasma as a potential blood-based biomarker for Alzheimer's disease. *Alzheimers Res Ther* 2017;9:98.
29. Babapour Mofrad R, Scheltens P, Kim S, Kang S, Youn YC, An SSA, et al. Plasma amyloid-β oligomerization assay as a pre-screening test for amyloid status. *Alzheimers Res Ther* 2021;13:133.
30. Keshavan A, Pannee J, Karikari TK, Rodriguez JL, Ashton NJ, Nicholas JM, et al. Population-based blood screening for preclinical Alzheimer's disease in a British birth cohort at age 70. *Brain* 2021;144: 434-449.
31. Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement* 2012;8:1-13.
32. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol* 2007;64:343-349.
33. Rowe CC, Villemagne VL. Brain amyloid imaging. *J Nucl Med* 2011; 52:1733-1740.
34. Chouraki V, Beiser A, Younkin L, Preis SR, Weinstein G, Hansson O, et al. Plasma amyloid-β and risk of Alzheimer's disease in the Framingham Heart Study. *Alzheimers Dement* 2015;11:249-257.
35. Hansson O, Zetterberg H, Vanmechelen E, Vanderstichele H, Andreasson U, Londos E, et al. Evaluation of plasma Abeta(40) and Abeta(42) as predictors of conversion to Alzheimer's disease in patients with mild cognitive impairment. *Neurobiol Aging* 2010;31:357-367.
36. Toledo JB, Vanderstichele H, Figurski M, Aisen PS, Petersen RC, Weiner MW, et al. Factors affecting Aβ plasma levels and their utility as biomarkers in ADNI. *Acta Neuropathol* 2011;122:401-413.
37. Kim HJ, Park KW, Kim TE, Im JY, Shin HS, Kim S, et al. Elevation of the plasma Aβ40/Aβ42 ratio as a diagnostic marker of sporadic early-onset Alzheimer's disease. *J Alzheimers Dis* 2015;48:1043-1050.
38. Jansen WJ, Ossenkoppele R, Knol DL, Tijms BM, Scheltens P, Verhey FR, et al. Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. *JAMA* 2015;313:1924-1938.
39. Serrano-Pozo A, Qian J, Monsell SE, Blacker D, Gómez-Isla T, Betensky RA, et al. Mild to moderate Alzheimer dementia with insufficient neuropathological changes. *Ann Neurol* 2014;75:597-601.
40. Schindler SE, Bollinger JG, Ovod V, Mawuenyega KG, Li Y, Gordon BA, et al. High-precision plasma β-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology* 2019;93:e1647-e1659.
41. Janelidze S, Stomrud E, Palmqvist S, Zetterberg H, van Westen D, Jeromin A, et al. Plasma β-amyloid in Alzheimer's disease and vascular disease. *Sci Rep* 2016;6:26801.
42. Chiu MJ, Chen TF, Hu CJ, Yan SH, Sun Y, Liu BH, et al. Nanoparticle-based immunomagnetic assay of plasma biomarkers for differentiating dementia and prodromal states of Alzheimer's disease - A cross-validation study. *Nanomedicine* 2020;28:102182.
43. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. *Nat Rev Neurol* 2010;6: 131-144.
44. Teunissen CE, Chiu MJ, Yang CC, Yang SY, Scheltens P, Zetterberg H, et al. Plasma amyloid-β (Aβ 42) correlates with cerebrospinal fluid Aβ 42 in Alzheimer's disease. *J Alzheimers Dis* 2018;62:1857-1863.
45. Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med* 2020;26:379-386.
46. Lantero Rodriguez J, Karikari TK, Suárez-Calvet M, Troakes C, King A, Emersic A, et al. Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathol* 2020;140:267-278.

47. Thijssen EH, La Joie R, Strom A, Fonseca C, Iaccarino L, Wolf A, et al. Plasma phosphorylated tau 217 and phosphorylated tau 181 as biomarkers in Alzheimer's disease and frontotemporal lobar degeneration: a retrospective diagnostic performance study. *Lancet Neurol* 2021;20:739-752.
48. Mattsson-Carlgren N, Janelidze S, Palmqvist S, Cullen N, Svenningsson AL, Strandberg O, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain* 2020;143:3234-3241.
49. Palmqvist S, Janelidze S, Quiroz YT, Zetterberg H, Lopera F, Stomrud E, et al. Discriminative accuracy of plasma phospho-tau217 for Alzheimer disease vs other neurodegenerative disorders. *JAMA* 2020;324:772-781.
50. Janelidze S, Berron D, Smith R, Strandberg O, Proctor NK, Dage JL, et al. Associations of plasma phospho-Tau217 levels with tau positron emission tomography in early Alzheimer disease. *JAMA Neurol* 2021;78:149-156.
51. Palmqvist S, Tideman P, Cullen N, Zetterberg H, Blennow K, Dage JL, et al. Prediction of future Alzheimer's disease dementia using plasma phospho-tau combined with other accessible measures. *Nat Med* 2021;27:1034-1042.
52. Ashton NJ, Pascoal TA, Karikari TK, Benedet AL, Lantero-Rodriguez J, Brinkmalm G, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol* 2021;141:709-724.
53. Mielke MM, Frank RD, Dage JL, Jeromin A, Ashton NJ, Blennow K, et al. Comparison of plasma phosphorylated tau species with amyloid and tau positron emission tomography, neurodegeneration, vascular pathology, and cognitive outcomes. *JAMA Neurol* 2021;78:1108-1117.
54. Brickman AM, Manly JJ, Honig LS, Sanchez D, Reyes-Dumeyer D, Lantigua RA, et al. Plasma p-tau181, p-tau217, and other blood-based Alzheimer's disease biomarkers in a multi-ethnic, community study. *Alzheimers Dement* 2021;17:1353-1364.
55. Yuan A, Nixon RA. Neurofilament proteins as biomarkers to monitor neurological diseases and the efficacy of therapies. *Front Neurosci* 2021;15:689938.
56. Alagaratnam J, von Widekind S, De Francesco D, Underwood J, Edison P, Winston A, et al. Correlation between CSF and blood neurofilament light chain protein: a systematic review and meta-analysis. *BMJ Neurol Open* 2021;3:e000143.
57. Ashton NJ, Janelidze S, Al Khleifat A, Leuzy A, van der Ende EL, Karikari TK, et al. A multicentre validation study of the diagnostic value of plasma neurofilament light. Nat Commun 2021;12:3400.
58. Barro C, Zetterberg H. Neurological symptoms and blood neurofilament light levels. *Acta Neurol Scand* 2021;144:13-20.
59. Mattsson N, Cullen NC, Andreasson U, Zetterberg H, Blennow K. Association between longitudinal plasma neurofilament light and neurodegeneration in patients with Alzheimer disease. *JAMA Neurol* 2019;76:791-799.
60. Preische O, Schultz SA, Apel A, Kuhle J, Kaeser SA, Barro C, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med* 2019;25:277-283.
61. Weston PS, Poole T, O'Connor A, Heslegrave A, Ryan NS, Liang Y, et al. Longitudinal measurement of serum neurofilament light in presymptomatic familial Alzheimer's disease. *Alzheimers Res Ther* 2019;11:19.
62. Pereira JB, Westman E, Hansson O; Alzheimer's Disease Neuroimaging Initiative. Association between cerebrospinal fluid and plasma neurodegeneration biomarkers with brain atrophy in Alzheimer's disease. *Neurobiol Aging* 2017;58:14-29.
63. Quiroz YT, Zetterberg H, Reiman EM, Chen Y, Su Y, Fox-Fuller JT, et al. Plasma neurofilament light chain in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional and longitudinal cohort study. *Lancet Neurol* 2020;19:513-521.
64. Xiao Z, Wu X, Wu W, Yi J, Liang X, Ding S, et al. Plasma biomarker profiles and the correlation with cognitive function across the clinical spectrum of Alzheimer's disease. *Alzheimers Res Ther* 2021;13:123.
65. Park SA, Han SM, Kim CE. New fluid biomarkers tracking non-amyloid-β and non-tau pathology in Alzheimer's disease. *Exp Mol Med* 2020;52:556-568.
66. Pase MP, Beiser AS, Himali JJ, Satizabal CL, Aparicio HJ, DeCarli C, et al. Assessment of plasma total tau level as a predictive biomarker for dementia and related endophenotypes. *JAMA Neurol* 2019;76:598-606.
67. Ding X, Zhang S, Jiang L, Wang L, Li T, Lei P. Ultrasensitive assays for detection of plasma tau and phosphorylated tau 181 in Alzheimer's disease: a systematic review and meta-analysis. *Transl Neurodegener* 2021;10:10.
68. Illán-Gala I, Lleo A, Karydas A, Staffaroni AM, Zetterberg H, Sivasankaran R, et al. Plasma tau and neurofilament light in frontotemporal lobar degeneration and Alzheimer disease. *Neurology* 2021;96:e671-e683.
69. Sugarman MA, Zetterberg H, Blennow K, Tripodis Y, McKee AC, Stein TD, et al. A longitudinal examination of plasma neurofilament light and total tau for the clinical detection and monitoring of Alzheimer's disease. *Neurobiol Aging* 2020;94:60-70.
70. Cullen NC, Leuzy A, Janelidze S, Palmqvist S, Svenningsson AL, Stomrud E, et al. Plasma biomarkers of Alzheimer's disease improve prediction of cognitive decline in cognitively unimpaired elderly populations. *Nat Commun* 2021;12:3555.
71. Korley FK, Goldstick J, Mastali M, Van Eyk JE, Barsan W, Meurer WJ, et al. Serum NfL (neurofilament light chain) levels and incident stroke in adults with diabetes mellitus. *Stroke* 2019;50:1669-1675.
72. Akamine S, Marutani N, Kanayama D, Gotoh S, Maruyama R, Yanagida K, et al. Renal function is associated with blood neurofilament light chain level in older adults. *Sci Rep* 2020;10:20350.
73. Khalil M, Pirpamer L, Hofer E, Voortman MM, Barro C, Leppert D, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. *Nat Commun* 2020;11:812.
74. Boulo S, Kuhlmann J, Andreasson U, Brix B, Venkataraman I, Herbst V, et al. First amyloid β1-42 certified reference material for re-calibrating commercial immunoassays. *Alzheimers Dement* 2020;16:1493-1503.
75. Hansson O. Biomarkers for neurodegenerative diseases. *Nat Med* 2021;27:954-963.
76. Zetterberg H, Bendlin BB. Biomarkers for Alzheimer's disease-preparing for a new era of disease-modifying therapies. *Mol Psychiatry* 2021;26:296-308.
77. Janelidze S, Palmqvist S, Leuzy A, Stomrud E, Verberk IMW, Zetterberg H, et al. Detecting amyloid positivity in early Alzheimer's disease using combinations of plasma Aβ42/Aβ40 and p-tau. *Alzheimers Dement* 2022;18:283-293.
78. Benedet AL, Leuzy A, Pascoal TA, Ashton NJ, Mathotaarachchi S, Savard M, et al. Stage-specific links between plasma neurofilament light and imaging biomarkers of Alzheimer's disease. *Brain* 2020;143:3793-3804.