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Guidelines and Consensus

Blood-based biomarkers of Alzheimer's disease—A guideline for clinical use

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ABSTRACT

As the population ages, cognitive disorder has emerged as a prevalent condition among the elderly, with Alzheimer's disease (AD) being the most common type. This presents a significant social and economic challenge to the global public health system. While specific diagnostic methods for AD, such as cerebrospinal fluid testing or positron emission tomography, are available, their limited use in clinical practice is often attributed to their invasiveness or high cost. Recently, there has been growing interest in the potential of blood-based biomarkers for early screening, clinical diagnosis, and monitoring of treatment for AD. These biomarkers offer simplicity, low cost, and high patient compliance, and have shown promise in reflecting pathological changes in the brain. To standardize and guide the clinical application of blood-based biomarkers for AD, we have compiled a summary of recent advancements in clinical research and developed ten recommendations using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) methodology. These recommendations focus on the use of blood-based biomarkers for clinical diagnosis, early screening for AD, and predicting disease progression, thereby promoting their application in clinical settings.

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1. Introduction

Alzheimer's disease (AD) is the most common disease-causing cognitive impairment in the elderly. However, even in specialized memory clinics, it is difficult for clinicians to differentiate AD patients from other types of dementia, such as frontotemporal dementia, Parkinson's disease (PD)-related dementia, and vascular dementia, leading to a misdiagnosis rate of over 30% for AD patients.[1] The neuropathological diagnosis of AD depends on the deposition of β-amyloid protein (Aβ) in the neurons and the intracellular aggregation of hyperphosphorylated tau protein (p-tau).[2] Therefore, the development of diagnostic tools that are easy to obtain, reliable, and cost-effective is crucial for the diagnosis of AD, especially for patients at the early stage. Since the clinical symptoms lag behind the neuropathology for one or two decades, a large accumulation of Aβ deposits may already exist in the brain before cognitive impairment becomes apparent.[3] In addition, various antibody therapies targeting Aβ pathology have emerged, which can clear amyloid plaques in the brain and thereby slow down cognitive decline. The early detection of neuropathological changes in AD is essential for these disease-modifying therapies (DMT) to achieve effective clinical outcomes.[4–6] Therefore, identifying early-stage AD patients is crucial for the emerging DMT in clinical practice, allowing more patients to achieve optimal therapeutic effects. The detection of AD-specific pathologies and the achievement of personalized and accurate diagnosis are crucial in evaluating disease stages, progression, and treatment efficacy. Recent studies have found that diagnostic markers for AD, such as Aβ and p-tau established by the National Institute on Aging-Alzheimer's Association (NIA-AA), can be detected in the blood and exhibit consistent changes with brain pathology, suggesting the feasibility of blood biomarkers in the clinical diagnosis of AD.[7,8] Experts from two committees of the Chinese Medical Association, the Dementia and Cognitive Disorder Committee and the Clinical Laboratory Diagnostics Committee gathered to discuss the recent clinical research progress and international consensus to propose this guideline for the clinical application of blood-based biomarkers for AD in China.

2. Clinical diagnosis of AD based on cerebrospinal fluid and imaging biomarkers

Both Aβ and p-tau can be detected in cerebrospinal fluid (CSF).[2] The ratio of Aβ42 to Aβ40 in the CSF shows a significant decrease in AD.[9] Combined with amyloid-targeting positron emission tomography (PET), there is a strong correlation between changes in Aβ in the CSF and amyloid deposition in the brain, and Aβ42 or Aβ42/Aβ40 in CSF shows high accuracy in predicting brain Aβ deposition.[10] Post-translationally modified tau proteins, such as several phosphorylated tau proteins, only show significant changes in the CSF of AD patients, including p-tau181, p-tau205, p-tau217, and p-tau231.[11] P-tau217 can better distinguish AD dementia from other types of dementia compared to p-tau181,[12] while the levels of microtubule binding region tau (MTBR tau) have the strongest correlation with tau-PET results and cognitive function.[13] Several Aβ PET tracers have been approved abroad, such as flutemetamol. Recently, florbetaben has also been approved for clinical testing by the China National Medical Products Administration (NMPA). The tau PET tracer flortaucipir was also approved by the US Food and Drug Administration (FDA).[14] In summary, the current established methods based on CSF and PET testing have good diagnostic capabilities for AD pathology. However, these testing methods are limited in their clinical application due to factors such as invasiveness (CSF collection via lumbar puncture) and the radioactivity of PET imaging probes.

3. Methodology

We conducted a search on PubMed, Web of Science, Chinese Medical Association Journal Database, and China National Knowledge Infrastructure from January 1, 2010, to December 31, 2023, using the following search terms in the title/abstract: (serum OR plasma OR blood) AND (MCI OR mild cognitive impairment OR Alzheimer's OR Alzheimer) AND (Aβ OR amyloid OR amyloid beta OR tau OR p-tau OR t-tau OR NfL OR neurofilament OR GFAP OR glial fibrillary acidic protein OR TRPC6) AND (sensitivity OR specificity OR diagnosis OR diagnostic). This search returned a total of 838 publications. After reviewing and screening all titles and abstracts, we excluded review articles, preprints, and case reports, resulting in the inclusion of 293 peer-reviewed research articles. Members from two Committees of the Chinese Medical Association, the Dementia and Cognitive Disorder Committee and the Clinical Laboratory Diagnostics Committee have discussed the guidelines in a face-to-face meeting and approved the draft. The criteria for selecting papers included: (1) using CSF Aβ42/Aβ40, Aβ PET or Tau PET as reference standards for diagnosing blood-based biomarkers; (2) excluding the use of homebrew reagent kits for single cohort validation; (3) validating blood biomarkers in two or more independent cohorts based on the "A/T/N" system; and (4) validating candidate blood biomarkers in Chinese cohorts based on the "A/T/N" system. Over 80 studies met these criteria and were included in the paper. Following initial screening and analysis of the literature, members of the Chinese Society of Dementia and Cognitive Impairment and the Chinese Society of Laboratory Medicine came together to conduct a

thorough review of each study, evaluating the quality and relevance of the evidence. After extensive discussions and assessments, the authors reached a consensus on the recommendations. They meticulously synthesized the evidence to formulate and summarize the 10 recommendations, ensuring that they were rooted in robust and pertinent findings. The recommended evidence levels are graded using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) method, which can be divided into high (A), moderate (B), low (C), and very low (D) levels of evidence.

4. Research progress of blood-based biomarkers for AD

4.1. Detection of Aβ in the blood

Aβ in the brain can be released into the blood through the blood-brain barrier. Currently, various detection methods, including ultra-sensitive electrochemiluminescence technology (Meso Scale Discovery, MSD), single-molecule array technology (SIMOA), and immunoprecipitation mass spectrometry (IP-MS), can detect Aβ in the blood. The MSD and SIMOA technologies are upgrades based on the traditional sandwich assay method. The MSD technology captures antibodies on a microplate, while the SIMOA technology labels the detection antibody on magnetic beads and enriches the magnetic beads in the microplate. In contrast to MSD and SIMOA technologies, IP-MS enriches the target protein in the sample through an immunoprecipitation method and detects the sample concentration using traditional protein mass spectrometry techniques. Due to differences in antibodies or methods used, these assays are different in the ability to detect amyloid levels. In a head-to-head comparison study of eight methods for detecting Aβ in the plasma, it was found that the method based on IP-MS performed the best in predicting abnormal changes in CSF Aβ42/Aβ40, with an AUC of 0.86.[15]

Although the detection of Aβ42/Aβ40 in the blood can to some extent predict changes in brain amyloid pathology, the magnitude of Aβ42/Aβ40 changes in the blood is much lower compared to the CSF. In the China Aging and Neurodegenerative Initiative (CANDI) clinical cohort study, it was found that the Aβ42/Aβ40 in the CSF of AD patients was 0.05, while it was 0.09 in cognitively normal individuals, representing a change of over 40%. However, in the blood, the Aβ42/Aβ40 in AD patients and normal individuals was 0.05 and 0.06, respectively, with only around 10% difference in magnitude.[16, 17] This makes it difficult to use blood Aβ42/Aβ40 as a biomarker for diagnosing AD. The deposition of amyloid proteins in the brain occurs many years before the onset of cognitive impairment, leading to changes in blood Aβ42/Aβ40 even before there are noticeable changes in cognition.[18] This can help identify individuals in cognitively normal elderly populations with amyloid protein pathology in the brain but normal cognition, who may be at risk of developing AD in the future. Therefore, establishing a standardized and stable blood-based Aβ detection system is still of great significance for the diagnosis of AD.

4.2. Detection of phosphorylated tau in the blood

Relative to the levels of Aβ in the plasma, the levels of phosphorylated tau protein in the plasma are lower, with some sites of phosphorylated tau protein in AD patient plasma being less than 1 pg/mL. In recent years, new technologies and suitable antibody pairs have emerged, allowing various phosphorylated tau proteins in the plasma, such as p-tau181, p-tau217, and p-tau231 to be detected using SIMOA, MSD, and IP-MS methods.[15, 19–24] These phosphorylated tau proteins in the plasma are closely related to abnormal changes in the Aβ42/Aβ40 ratio in CSF, brain amyloid protein deposition, and tau protein neurofibrillary tangles, which are characteristic pathological features of AD. By contrast, in other neurodegenerative diseases related to tau proteins, such as progressive supranuclear palsy and frontotemporal dementia, there is no significant change in the levels of phosphorylated tau protein in the plasma. Therefore, the presence of p-tau proteins in the plasma can indicate whether a patient has AD pathology.

Unlike the small changes in the Aβ42/Aβ40 ratio in the plasma, the levels of phosphorylated tau protein in AD patient plasma increase by 2–4 times. In the CANDI study, the levels of plasma p-tau181 in AD patients were twice those of cognitively normal individuals.[16, 17] More importantly, phosphorylated tau proteins show a certain predictive ability at different stages of cognitive impairment. P-tau217 can differentiate between Aβ-positive and Aβ-negative populations in the early stage of cognitive impairment. Different forms of phosphorylated tau protein have different magnitudes of change at different disease stages and therefore have different predictive values for pathological changes in the brain. When using the IP-MS method to simultaneously detect changes in the plasma phosphorylated tau proteins, it was found that p-tau217, p-tau231, and p-tau205 have more accurate predictive abilities for brain pathology than other forms of phosphorylated tau proteins.[25] For example, p-tau231 appears at the stage when amyloid proteins begin to accumulate, but the changes become less significant after the amyloid accumulation. This may be due to the different time points at which the phosphorylation sites appear.[26, 27] Therefore, further evaluations

are warranted when using different forms of phosphorylated tau proteins for early screening or assessing disease pathology in clinical applications.

4.3. Glial fibrillary acidic protein (GFAP) in the blood

Patients with neurodegenerative diseases, including AD, usually have a pathological state of abnormally activated astrocytes in the brain, as indicated by a significant increase in GFAP-positive astrocytes.[28] These activated astrocytes are involved in the process of neuronal injury and pathology related to neurodegenerative diseases, representing a form of neuroinflammation. Although GFAP, as a characteristic marker of astrocytes, shows changes in its levels in the CSF of many neurodegenerative disease patients, its changes in the blood are closely related to Aβ pathology.[29, 30] The mechanism for the characteristic changes of plasma GFAP in populations with Aβ pathology is currently unclear. GFAP has the ability to predict amyloid deposition in the brain, similar to p-tau181 in the plasma.[17] In longitudinal studies, GFAP has been able to predict disease progression in cognitively normal individuals or those with mild cognitive impairment.[31, 32] Furthermore, it has been found that in Aβ-positive cognitively normal individuals, those with high levels of GFAP in the blood showed a more rapid accumulation of tau pathology in the brain, indicating the potential of the blood GFAP to predict tau pathology in cognitively normal individuals.[33] In contrast to GFAP, chitinase-3-like protein 1 (YKL-40) does not show specific changes in AD patients and is not a specific marker for astrocyte activation in AD. Importantly, the concentration of plasma GFAP protein is high, exceeding 100 pg/mL in AD patients, making it easy to develop a blood-based GFAP diagnostic system.

4.4. Neurodegenerative markers in the blood

Although AD patients all have amyloid and neurofibrillary tangle pathology, the degree of neurological injury or cognitive impairment varies, thus necessitating markers that accurately reflect neurodegenerative changes in the brain. Unlike the many markers closely associated with neurological damage in CSF, there is currently a lack of suitable neurological injury markers in the blood, especially for AD-specific neurological injury markers. Neurofilament light chain (NfL) protein has significant changes in the blood in various neurodegenerative diseases such as amyotrophic lateral sclerosis, multiple sclerosis, and in patients with human immunodeficiency virus infection.[34] Compared to healthy individuals, there is an increase in NfL in the blood of AD patients, especially in the late stages of AD. In a cohort study of familial AD, it was found that these AD patients showed a gradual increase in the blood NfL levels approximately ten years before clinical disease onset.[35,36] In patients with amyloid cerebrovascular pathology, the change in plasma NfL is most pronounced.[17] Additionally, studies have found that plasma NfL levels are closely related to age and cerebrovascular diseases, among other age-related co-morbidities. Therefore, when using plasma NfL to assess the degree of neurological injury in AD patients or to evaluate clinical drug trials and treatment effects, more factors need to be taken into account.[37]

Central nervous system-derived tau protein accounts for only about 20% of the protein in the blood, resulting in a weak correlation between plasma T-tau levels and CSF T-tau levels.[38] Recent findings have shown a high correlation between serum brain-derived tau protein and CSF brain-derived tau protein, while there is no correlation between serum T-tau protein and CSF T-tau protein.[39] This suggests that brain-derived tau protein may be a potential blood-based biomarker for predicting AD neurodegenerative changes.

Transient receptor potential canonical 6 (TRPC6) is a non-selective cation channel protein that plays an important role in the survival of neurons, the development of dendrites, and the establishment of neural networks. After binding with amyloid precursor protein (APP), TRPC6 blocks the recognition of the cleavage site of APP by γ-secretase, thereby specifically reducing the production of Aβ.[40] Clinical studies have found that TRPC6 is significantly reduced in AD patients' brains and blood, with no significant changes in PD patients.[41] In a recent multicenter clinical trial (NMPA, 20200005) in China, involving 734 normal controls and 366 AD patients verified by Aβ-PET, it was found that the blood TRPC6 mRNA levels in AD patients were significantly reduced compared to the control group. The decrease in TRPC6 is associated with the deterioration of cognitive function, Aβ deposition in the brain, and brain atrophy in AD patients. Therefore, TRPC6 mRNA levels are expected to serve as blood-based biomarkers for early AD, providing a basis for early drug therapy.

4.5. Application of Aβ and tau in exosomes in the AD

Exosomes are small vesicles with a diameter of 30–150 nm that are actively secreted by cells into the extracellular space after the fusion of multivesicular bodies with the cell membrane.[42] In the central nervous system, both neurons

and glial cells can secrete exosomes, and they have cell specificity.[43] Exosomes play an important role in the progression and spread of neurodegenerative diseases. Studies have found that Aβ and tau proteins can be secreted in the form of exosomes and that exosome markers such as Alix have been found in amyloid plaques, suggesting that exosomes may be related to the occurrence of AD.[44,45] As a new type of biomarker carrier system, exosomes provide new ideas and methods for the diagnosis and treatment of neurodegenerative diseases. Although there are still many challenges, with the continuous development of technology and research, it is hoped that exosomes can be better utilized in the prevention and treatment of these diseases in the future.

5. Application of blood-based biomarkers in clinical diagnosis of AD

Currently, the clinical usage standards for AD biomarkers, including Aβ PET in 2013[46] and the usage standards based on CSF AD biomarkers in 2018,[47] indicate that these biomarkers can be used to differentiate AD dementia from non-AD dementia. However, there is still controversy regarding whether these biomarkers can be used for detecting AD risk in cognitively normal individuals or in patients with subjective cognitive impairment.[48] Blood-based biomarkers for AD, due to their convenience, can help doctors screen and assess the likelihood of AD in patients when combined with clinical and other test results, thus optimizing the clinical diagnostic process for AD. Therefore, they will be an important supplement to the clinical diagnosis of AD, even though they currently cannot replace CSF or PET imaging tests.

As mentioned earlier, the decrease in plasma Aβ42/Aβ40 is very small (around 10%),[16] so its clinical application in diagnosis is very limited. The stability of detecting Aβ using techniques such as IP-MS or SIMOA needs to be further improved, which also limits its clinical application. In cognitively normal individuals with amyloid pathology in the brain, plasma Aβ42/Aβ40 decreases[18]; and in predicting the progression of mild cognitive impairment, plasma Aβ42/Aβ40 can improve the predictive ability of phosphorylated tau protein.[49] Therefore, establishing a sensitive and stable plasma Aβ42/Aβ40 detection method can facilitate clinical diagnosis. However, various factors make it difficult to use the current Aβ detection methods in clinical practice, such as the lack of standardized sample collection for Aβ42 and Aβ40, the impact of lipid content in blood samples, and the lack of specific effects of various comorbidities in the elderly on Aβ levels in the blood. In the future, there is a need to develop standardized Aβ reference materials, establish a stable Aβ detection system, and evaluate the factors affecting Aβ levels in the blood in large multicenter clinical cohorts and community populations.

Multiple phosphorylated tau protein sites show a significant increase in the plasma of AD-related MCI patients or AD patients, so they can effectively predict the characteristic pathology of AD in the brain, thereby assisting in distinguishing AD dementia from other neurodegenerative diseases. Recent studies have shown that the use of more stable and sensitive techniques such as IP-MS can improve the predictive ability of p-tau protein in the blood, reaching a level similar to that of CSF biomarkers.[50] Although compared to p-tau217, the change in plasma p-tau181 in AD patients is less, and its appearance is later, due to the content of p-tau181 being more than 10 times that of p-tau217, its detection is easier.[51]

Recommendations:

(1) The predictive value of blood-based Aβ42/Aβ40 assays in diagnosing AD dementia is moderate and can be used as an early screening tool (Class IIa recommendation, Level A evidence).
(2) Blood-based p-tau181 and p-tau217 are highly correlated with AD pathology in AD-related MCI patients or AD patients (Class I recommendation, Level A evidence).
(3) Blood-based GFAP helps to distinguish AD dementia from non-AD dementia (Class I recommendation, Level A evidence).
(4) Although not specific enough for AD diagnosis, blood-based NfL can be used to assess neurodegeneration and to help monitor the progression of AD pathology (Class IIb recommendation, Level A evidence).

6. Potential application of blood-based AD biomarkers in early screening

Early intervention for AD is the key to effective DMT, so accurately and economically screening individuals for AD pathology as early as possible is crucial. Establishing a blood-based biomarker detection system for AD can significantly reduce the cost of early screening. By conducting blood-based biomarker testing, it is possible to determine the low, medium, and high risk of AD, and decide which individuals need further examination, such as CSF or PET scans. Screening blood-based biomarkers can reduce unnecessary CSF or PET scans and ensure timely diagnosis and treatment for patients who truly need it, which is great important for clinical practice.

If there is a need for further intervention in the treatment window of AD, to treat before the onset of the disease, it is necessary to screen out the high-risk population with existing amyloid pathology in the brain and at risk of developing

AD in cognitively normal individuals. In this case, the advantages of the blood-based biomarker system for AD are prominent. In the population aged 60 or older with cognitive impairment, less than 30% of them have amyloid pathology in the brain. Screening asymptomatic AD patients using PET or CSF biomarkers would require a lot of manpower and resources. The A4 study is the first phase III clinical trial for preclinical AD,[52] which took three and a half years and over 4000 Aβ PET scans to enroll 1169 participants who met the study criteria. Therefore, in ongoing clinical trials of anti-Aβ immunotherapy for preclinical AD, blood-based biomarkers are already being used for screening.[53–55]

The advantage of blood-based biomarkers is in screening among cognitively normal and mild cognitive impairment populations. If blood-based biomarkers are used alone, their ability to predict brain pathology needs to exceed 90% in order to be clinically feasible. Currently, blood-based biomarkers cannot accurately predict the future development of the disease, so there is a need to establish a comprehensive blood-based biomarker screening system. In addition, elderly people with amyloid pathology in the brain do not further deteriorate clinically for 5–10 years or even a lifetime.[56,57] Therefore, screening of the population at risk of AD is needed to reduce unnecessary treatment for the non-AD risk population. In addition, for interventional clinical trials with low side effects, such as clinical trials of lifestyle intervention for AD, blood-based biomarkers can be used for clinical screening.

Recommendations:

(5) Blood-based Aβ42/Aβ40 can be used for early screening of high-risk individuals with AD (Class IIa recommendation, Level A evidence).
(6) Blood-based p-tau181 and p-tau217 can be used for early screening of high-risk individuals with AD (Class I recommendation, Level A evidence).
(7) After pre-screening for AD pathology, high-risk individuals need to confirm for AD-specific pathology through CSF Aβ42/40, p-tau181 or p-tau217 detection, or Aβ PET (Class I recommendation, Level A evidence).

7. The application of blood-based biomarkers in predicting disease progression in AD

In longitudinal studies, many AD-related biomarkers such as Aβ42/Aβ40, p-tau181, p-tau217, p-tau231, GFAP, and NfL are closely associated with disease progression. In familial AD patients and carriers of familial AD mutations, plasma NfL begins to show significant changes 10 years before cognitive impairment, and increases gradually with the pathological development.[58,59] This may be related to NfL's ability to reflect the process of neurodegenerative disease. Similarly, plasma p-tau[60–62] and GFAP[32] increase significantly with disease progression, but unlike NfL, they are closely related to disease progression only in AD patients. Additionally, clinical trials using Aβ antibodies have shown significant decreases in blood p-tau and GFAP proteins while improving brain amyloid pathology.[53] This further suggests that blood-based biomarkers for AD can be used to predict disease progression or observe the therapeutic effects of drugs.

However, in the clinical application of using blood-based biomarkers to predict disease progression, it may be necessary to select different biomarkers according to different scenarios. For example, plasma NfL shows significant early changes in familial AD patients,[35,36] but only increases significantly in the dementia stage in sporadic AD patients.[60] Additionally, in clinical study of anti-Aβ treatments, changes in NfL in the CSF have been observed, but not in the plasma.[53] This may be because plasma NfL is influenced by multiple factors.[17] Furthermore, the pattern of changes varies in different phosphorylated tau proteins at different stages of AD, such as p-tau217 showing the highest degree of change with disease progression, and having the strongest correlation with brain atrophy and cognitive decline.[60]

Recommendation:

(8) Blood-based p-tau181, p-tau217, GFAP, and NfL can be used for the evaluation of AD disease progression (Class IIa recommendation, Level A evidence).

8. The combined use of blood-based biomarkers with other biological markers

As mentioned above, different blood-based biomarkers for AD may have differences in various scenarios, and the complexity of AD pathology makes it difficult to accurately assess brain pathology and predict disease progression using a single blood-based biomarker alone. Therefore, it may be necessary to improve accuracy by combining multiple factors. The CANDI cohort study found that whether Aβ42/Aβ40 or p-tau181, when combined with apolipoprotein E (*APOE*) ε4 gene or APOE ε4 protein detection, significantly improves their ability to predict brain amyloid pathology, especially in the mild cognitive impairment stage or preclinical stage.[16] In individuals with amnestic MCI, the combination of blood-based biomarkers can also accurately predict brain amyloid deposition.[37] In the dementia stage, plasma p-tau181 and p-tau217 can predict brain amyloid pathology well, possibly because AD dementia patients

have accumulated enough p-tau protein in the brain. So, it is difficult to further improve their predictive ability even in combination with genotype or Aβ42/Aβ40. If the level of p-tau is normal in dementia patients, NfL testing can help diagnose non-AD dementias such as frontotemporal dementia.

Although the combination of p-tau181 or p-tau217 with NfL or plasma Aβ42/Aβ40 has limited improvement in predictive ability in individuals with mild cognitive impairment or dementia, the combination with structural imaging, such as characteristic hippocampal atrophy in AD patients, can further improve the diagnostic ability for brain amyloid pathology.[16] A study found that combining p-tau217 and three simple cognitive tests has greater accuracy in predicting future AD than clinical doctors, reaching the level of predicting disease progression with CSF biomarkers.[63] The use of multiple blood-based biomarkers, or the combination of blood-based biomarkers with other tests for the diagnosis or prediction of AD, may require further research to establish a standard protocol for clinical use.

Recommendations:

(9) APOE genotype testing combined with blood-based AD biomarkers can be used for the diagnosis of AD-related MCI or preclinical AD populations (Class IIa recommendation, Level A evidence).
(10) Structural imaging combined with blood-based AD biomarkers can be used for the diagnosis of AD-related MCI or preclinical AD populations (Class IIb recommendation, Level B evidence).

9. Application criteria for blood-based biomarkers of AD in clinical practice

To verify the accuracy and reliability of blood-based biomarkers for AD in clinical diagnosis, approved examination methods such as CSF Aβ42/Aβ40 or Aβ PET should be used as reference standards. Additionally, since tau PET can also reflect the pathology of tau protein fiber entanglement in the brain, tau PET tracers are also approved for clinical use and can serve as reference standards for the diagnosis of blood-based biomarkers.[7]

In addition to establishing reference standards for blood-based biomarkers, standardized procedures for blood sample collection are important. Both Aβ and tau proteins are prone to aggregation and adhesion to the walls of collecting tubes, so the sample collection is crucial for accurately detecting blood-based biomarkers for AD. Establishing a unified peripheral blood collection and processing scheme and promoting its use is the most important preparatory work for standardized testing.[64] Currently, research has found that different types of blood sample collection tubes (the plasma should be collected in ethylenediaminetetraacetic acid [EDTA] anticoagulant tubes), the time interval between sample collection and centrifugation, parameters of the centrifuge, type of sample transfer tubes (low-adhesion polypropylene tubes are recommended), the volume of each sample transfer tube, and the number of freeze-thaw cycles (recommended to be controlled within three times) may all affect the test results.[65,66] Various detection systems for AD blood-based biomarkers have been developed. These systems should undergo strict performance validation before starting clinical use to scientifically and rigorously evaluate their clinical value. Additionally, different detection systems have different reference ranges and cutoff values,[15] so each hospital should establish its own reference range and validate the cutoff value provided by the reagent kit; different laboratories should actively explore ways to promote result inter-recognition.

Furthermore, research has found differences in the levels of biomarkers between different racial groups. In comparison with Caucasians, the levels of Aβ and p-tau in the plasma of Latin American or Black individuals differ in normal populations. Currently, the specific differences between Eastern and Western populations are not clear, so it is necessary to design uniform inclusion and sample processing standards, and carry out head-to-head research to determine the specific differences in AD blood-based biomarkers between these populations. Variations in lifestyle, environmental factors, and comorbidities may also lead to changes in the levels of blood-based biomarkers. Low body mass index (BMI), cardiovascular diseases, and chronic kidney diseases can increase the levels of p-tau217 in the plasma.[67] Additionally, NfL is closely related to age, but the levels of NfL in the plasma of AD patients may also be affected by other disease factors, such as cerebrovascular diseases and peripheral nervous system disorders.[68–70] Therefore, large-scale clinical trials with diverse populations are needed to clarify the impact of these biological variations on the levels of blood-based biomarkers, in order to interpret the results scientifically and reliably, and enhance the diagnostic value of the biomarkers.

10. Conclusions and prospects

Blood-based biomarkers have already played a role in the design of clinical trials for AD, accelerating the progress of clinical trials and reducing economic costs. In the future, greater efforts should be undertaken to promote the application of blood-based biomarkers, to determine the diagnostic thresholds of blood-based biomarkers, and to further clarify their clinical significance. To enhance the diagnostic value of blood-based biomarkers, efforts should be made to establish a stable detection system, control pre-analytical variables, and perform quality control. Moreover, in

diagnosing or predicting AD, whether using combinations of multiple blood-based biomarkers, or blood-based biomarkers combined with other risk factors for AD, a convenient and efficient prediction model needs to be established to help doctors make clinical decisions quickly upon receiving test results. Additionally, the current work on blood-based biomarkers for AD mainly focuses on biomarkers closely related to AD pathology such as Aβ and p-tau, and lacks biomarkers that are truly associated with neuronal and synaptic damage. Therefore, there are still many issues to be resolved in the clinical practice of blood-based biomarkers for AD, and it is hoped that with the development of more high-quality clinical research in the future, the clinical application of blood-based biomarkers for AD will be improved. This guideline does not have legal effects and is only for reference by physicians.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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