

The Progression of Current Biomarkers for the Diagnosis of Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is a common degenerative disease with no cure. Atrophy of brain tissue, accumulation of amyloid deposition, and neuronal tangles are its main features and the most commonly used biomarkers for diagnosing AD. According to them, cerebrospinal fluid (CSF) measurement and imaging techniques are currently the mainstream methods for AD diagnosis. However, the high price and the need for technology cannot meet people's willingness to screen on a large scale, so people are forced to look for new biomarkers in cheaper and readily available blood. This brief commentary divides into three sections (CSF, imaging and blood) to describe the biomarkers found in AD. Among these biomarkers, the current development direction (machine automation) and joint diagnosis research are briefly described for biomarkers incorporated into clinical standards. The present research status and limitations of biomarkers that have received significant attention are discussed. Finally, the article summarizes the current status of biomarkers in diagnosing AD and looks forward to the feasibility of biomarkers in blood in the future.

Keywords: Alzheimer's disease (AD), cerebrospinal fluid (CSF), biomarkers, blood, magnetic resonance imaging (MRI), positron emission tomography (PET).

1. Introduction

As is common knowledge, Alzheimer's disease (AD) is a neurological illness that cannot be cured, and its main pathological features are neurofibrillary tangles and senile plaques, which are composed of hyperphosphorylated tau protein and amyloid beta ($A\beta$) peptide, respectively [1]. According to the latest data of the Alzheimer's Association Report, the number of American patients over 65 years old is expected to double to 13.8 million in 2060, and the number of reported deaths from AD has been increasing since 2000 [2].

According to the NIA-AA diagnostic criteria, there are three phases of Alzheimer's disease: preclinical AD, mild cognitive impairment (MCI) resulting from AD, and dementia resulting from AD [3]. Excessive brain deposit production can occur severe years before cognitive impairment in hereditary patients. Moreover, the possibility of acquiring AD in persons with MCI is more than six times higher than in people without cognitive impairment [4]. Both have indicated that early diagnosis time could obtain longer for prevention and treatment, and early prevention also has received significant attention in drug research and non-drug intervention.

In the early stage, due to the limitations of technology, the clinical diagnostic criteria for AD was the discovery of relevant pathological phenomena after death or significant dysfunction. It was not until 2007 that the research standard IWG began to support the evidence of biomarkers in AD [5]. So far, the commonly used diagnostic methods include cognitive testing, volumetric magnetic resonance imaging (MRI), positron emission tomography (PET), and cerebrospinal fluid (CSF) measurement. Besides, MRI is used to observe hippocampal atrophy in the brain, while PET can assess the situation of $A\beta$ deposition. However, they are still not standardized, a massive gap in biomarker research that needs filling. Apart from cognitive tests, the rest of the methods are generally costly and require a particular technology, could not be achieved at the general office, and it was stabbed in the spinal cord to obtain CSF is invasive and may cause particular damage to patients, and large-scale clinical implementation is also challenging. Making detection more convenient and more aware, the clinical urgently needs AD biomarkers [2]. Blood markers require venous puncture, meet all the above characteristics, convenient transportation, and have a standardized process. Therefore, it is considered

the most promising new diagnostic method for widespread clinical use, but considerable research and development challenges exist.

This review briefly summarizes the current status of biomarkers for diagnosing AD, analyzes the limitations of biomarkers that have been clinically used, and the shortcomings of markers that are being focused on. Meanwhile, the importance and clinical significance of developing blood and other non-invasive features are emphasized.

2. Biomarkers in cerebrospinal fluid

Cerebrospinal fluid can directly reflect the biochemical changes of the brain because of its unmediated communication with the environment outside the brain, so it is the ideal biomarker for AD at present [6]. For CSF biomarkers, the primary physiological changes in the AD brain include three. According to the most widely accepted amyloid hypothesis, transmembrane amyloid precursor protein (APP) can be cleaved and metabolized by α -, β -, and γ -secretases to form two structures, A β 42 and A β 40, among which A β 42 is neurotoxic and deposits into extracellular insoluble senile plaques [7]. In addition, according to the neuronal fiber entanglement (NFT) hypothesis, the hyperphosphorylation of tau protein in the neuronal cytoplasm is caused by the imbalance of phosphatases and kinases, and abnormal hyperphosphorylation will lead to the formation of NFT in cells [7]. At the same time, hyperphosphorylated tau can accelerate neuronal damage. Therefore, A β 42, total tau (T-tau), and phosphorylated tau (P-tau) are the most commonly used biomarkers for AD in CSF.

2.1. A β

Of all the isomers of A β peptides, A β 42 is the most popular biomarker because it accumulates in large amounts in the brain, so if its content is massively reduced in CSF, it can be demonstrated that A patient is at risk for AD. Furthermore, it is the earliest known marker for diagnosing AD and the earliest to diagnose preclinical AD according to the number of biomarkers present in CSF. However, it can only predict the possibility that a low level of A β 42 will develop into AD dementia in the future. Still, there is no long-term study on the development tendency of AD in the healthy elderly, which is also a field that needs to be expanded [8].

In addition, A β 40 is also a critical biomarker; however, because it has no effect when used as a biomarker alone in CSF [9], some studies have shown that using the ratio A β 42/A β 40 can improve the diagnostic sensitivity and specificity than using A β 42 alone (89.4% vs. 74.9%, $p < 0.0001$) because the changes in A β 42 are also present in non-AD dementia patients [10]. The use of ratios can reduce range variation due to individual fluctuations.

2.2. The determination of the number of network layers

Tau protein also contributes significantly to the stabilization of tubulin. Although the increase in its total level is related to all aging and other neurodegenerative diseases, studies have shown that the rise of T-tau content in CSF will be 300% higher than the incidence of the control group [11]. However, the p-tau concentration is exclusively raised in Alzheimer's disease, and it has a high level of specificity for determining whether individuals with MCI would go on to acquire dementia caused by AD [12]. Currently, more and more studies have combined A β peptide and tau protein produced by two different pathological processes to turn the A β 42 and tau ratio into a new biomarker. According to Ritchie et al., the specificity of CSF p-tau /A β is between 22% and 86%, while the sensitivity is between 40% and 100%, which has a greater predictive power than T-tau or P-tau taken separately [11]. Because the measurement of different substances can help to reduce random errors in the measurement of a single substance, at the same time, it can compensate for the error caused by the natural variation of protein content in CSF.

Although these three biomarkers have been widely used, the critical values of biomarkers vary in different laboratories due to different test batches and reagent batches. Therefore, it is still a problem

to be solved as soon as possible to formulate unified and standardized critical values and general terms. Because of this problem, the Erlangen score is a standard that can be utilized to predict the progression of dementia caused by Alzheimer's disease in patients with MCI, which can help simplify and unify the judgment of AD biomarkers in CSF [13].

3. Imaging biomarkers

3.1. MRI

As a non-invasive method, MRI can be divided into structural imaging (sMRI) and functional imaging (fMRI) according to its ability to reflect brain tissue's structural and functional changes. Cerebral cortical atrophy is an essential marker of degenerative diseases, and the primary physiological phenomena are brain tissue damage and nerve deformation. It accumulates gradually over a long period and can begin up to 20 years before the emergence of MCI symptoms. In AD brains, the changed biomarkers were medial temporal lobe (MTL) atrophy, especially hippocampal atrophy, which was strongly associated with episodic memory impairment. SMRI can measure such brain structural changes, but only manual measurement can be used in the early stage. It is difficult to assess the subtle differences with the naked eye, which causes inconvenience for clinical use [14]. However, recent studies tend to automate and long-term continuous detection of sMRI, providing a new method for clinical diagnosis [14].

fMRI is an imaging method dependent on blood oxygen level dependence (BOLD) to observe neuronal activity, and it can also illustrate the shifts that take place in functional connections during the AD process. During attentive recollection, the default mode network (DMN) is active, but it is inhibited during external stimulus, and early amyloid deposition partially coincides with it. Therefore, using fMRI to detect the activation level of the biomarker DMN and the impairment status of connectivity function can track the pathogenesis of AD and predict cognitive decline in MCI patients [6]. In addition, studies have shown that AD patients can be accurately distinguished from ordinary people by combining machine learning methods.

In particular, studies have revealed a connection between iron metabolism and accumulation in senile plaques and neurons, and iron may be associated with A β and tau protein involved in the pathogenesis of AD because it can be redox active forms of ferrous, neuronal oxidative damage. Thus, the iron deposit is a new AD biomarker. Quantitative susceptibility mapping (QSM) technology belongs to MRI, which can quantify magnetic tissue sensitivity and detect nuclei with iron deposition [15]. Iron deposits exist in the deep gray matter of the brain. Early AD can be judged by detecting QSM values in different brain regions, and AD can also be distinguished from other dementias [15]. At present, this new detection method still has many limitations. QSM cannot distinguish the magnetic sensitivity of different types of iron, and there is no clear detection standard and enough research data [15].

3.2. PET

PET (positron emission tomography) is an imaging method suitable for detecting tumors and neurological diseases. It can detect pathological changes in the brain before cognitive impairment occurs in AD. 18F-fluorodeoxyglucose (FDG-PET) is a widely used radioactive tracer that can see changes in glucose metabolism rate in the brain, thereby judging changes in neuronal activity. A decline in glucose metabolism has been seen in individuals with Alzheimer's disease in the parietal-temporal region, the posterior cingulate gyrus, and the frontal cortex of the brain. Moreover, FDG-PET can effectively distinguish healthy older adults from patients with other types of dementia, especially in determining dementia with DLB and AD with 90% sensitivity and 80% specificity [16].

In addition, using amyloid tracers with an affinity for A β plaques allows visualization of amyloid deposits in the brain. The first to be used is ^{11}C -labeled Pittsburgh compound B (^{11}C -PiB), a radioactive analogue of thioflavin-T, which can bind well to A β 42 and A β 40 and can be rapidly cleared in vivo [16]. However, the short half-life of ^{11}C (about 20 minutes) requires immediate

detection, which may cause inconvenience for clinical diagnosis. Flutemetamol (18F), which has been approved for use by the FDA, is a PiB derivative labeled with 18F, and is anticipated to have a half-life of 110 minutes, making imaging far more durable [16]. Hampel et al. believed that the deposition of β -amyloid in the cortex is not directly responsible for cognitive decline, but an early event that is already stable before the patient develops MCI [6]. Moreover, the presence of brain deposits in MCI patients cannot directly diagnose AD, so the clinical role of amyloid-PET is more to exclude other types of dementia. Still, it cannot distinguish AD from DLB [16]. In particular, when MRI, FDG-PET, and BiP-PET were combined, the MRI and BiP-PET combination had the highest accuracy and the best predictive value for the temporal cortex [17].

The imaging technology applied to the diagnosis of AD is the most common now, which has well applicability for the purpose of diagnosing and forecasting the development of MCI in the future, and discrimination of other degenerative neurological diseases. It is mainly limited by the high price of the instrument and the need for professional and technical personnel to participate in the detection, an inevitable problem for the widespread implementation of local clinics.

4. Biomarkers in the blood

4.1. A β and tau

Compared with obtaining cerebrospinal fluid, venous puncture to obtain blood is safer, cheaper, and can be used on a large scale, so blood sampling is more suitable for clinical practice. A barrier (blood-brain barrier, abbreviated BBB) separates the brain from the rest of, and limits the movement of protein, and peptides in the blood is only a small amount of exchange. Still, in the BBB in patients with AD, there may be some damage to other molecules that are allowed to enter the blood circulation, which is the reason about using the blood as a test liquid, is also the biggest obstacle to limiting the development of blood biomarkers [18]. A β is the most important biomarker of AD pathology, and detecting A β in plasma is also of great significance. Plasma A β 42 and A β 42/A β 40 can also be used to diagnose AD, but the results of earlier studies were contradictory, and there was no correlation between A β in cerebrospinal fluid and plasma [19]. This phenomenon may be due to the fact that A β in plasma comes from other parts of the human body, so the concentration of A β detected is not only produced by brain tissue [6]. In addition, the A β peptide has strong hydrophobicity, which can bind to some test tube walls and some plasma proteins, so the detection results of enzyme-linked immunosorbent assay (ELISA) were significantly affected in the early stage [19]. Therefore, there is a considerable obstacle to using A β in blood for diagnosing AD. In addition, studies of the equally essential biomarkers T-tau and p-tau are limited by their rapid clearance from the blood and limited detection levels in early studies.

As is known to all, from brain tissue protein detection in blood, there are many problems: one is the brain protein metabolism in the blood and clearing, it is blood protein of highly complex, increased the analysis of factors to consider, three is many interference factors, not only is affected by other diseases, and diet, diurnal variation, could also have an impact on it [18]. At present, in order to avoid this interference and improve the detection sensitivity and specificity, ultra-sensitive methods and technologies have been introduced, such as mass spectrometry (MS), immunomagnetic reduction (IMR), electrochemiluminescence (ECL) and single molecule array (Simoa) [19]. With the help of these technologies, the detection of A β and tau protein in plasma has made significant progress in diagnosing AD. Among them, the detection of p-tau using ECL can find that the concentration of p-tau in the plasma of AD patients is higher than that of controls, while the detection of t-tau using Simoa can predict the future development of AD without cognitive impairment and MCI [20]. In addition, the A β 42/40 ratio measured by immunoprecipitation-mass spectrometry (IP-MS) can predict that amyloid PET is positive in patients at all stages of AD [20]. However, these research results need to be further repeated experiments, and it is far from being used for AD diagnosis.

4.2. NFL

In addition, although neurofibrillary tangles (NFL) are a marker of axonal injury and a biomarker of most neurodegenerative diseases, they are highly correlated in plasma and CSF. Moreover, with the emergence of ultrasensitive technology (Simoa method), its detection sensitivity is much higher than that of previous technology, and it is able to pick up on a considerable rise in plasma NFL content in patients with AD before the onset of symptoms [21]. Although NFL does not distinguish between AD and other neurodegenerative diseases, it is still a good choice for primary screening.

4.3. Inflammation

The pathogenesis of AD is greatly influenced by inflammation as well. Jong-Chan et al. supposed that inflammation is an early event, which may occur before the appearance of A β and tau, which provides a new way to detect preclinical AD. Inflammatory factors are released by activated microglia and astrocytes, which can promote the expression and deposition of β -amyloid [22]. Moreover, it has been proved by experimental data that inflammatory response occurs in AD and MCI, and it exists in the blood [23]. In addition, inflammatory mediators include pro-inflammatory, inflammatory, and anti-inflammatory mediators. They are present in every neurodegenerative disease, which complicates the search for specific and accurate AD biomarkers. There is no recognized biomarker for AD, but a number of inflammatory agents have been linked to AD. Among them, interleukin-1 (IL-1), a pro-inflammatory factor, can affect hippocampal neurons and is related to cholinergic dysfunction in AD, indicating that it is intimately associated with the pathogenesis of AD [22]. Diffuse plaques are connected with interleukin-6 (IL-6) in the earliest stages of AD. In particular, according to research conducted by Anuradha et al., the levels of inflammatory factors such as IL-1, IL-6, and transforming growth factor beta 1 (TGF- β 1) were found to be elevated in AD patients, these factors are now thought to be potential biomarkers for the disease.[24]. Although there is no definite study to find the explicit use of inflammatory factors in the pathogenesis of AD, this does not affect its potential as a biomarker for AD.

4.4. miRNAs

MicroRNAs, also known as miRNAs, are non-coding RNAs that are made up of around 22 nucleotides, which are the key factors regulating critical physiological processes of the human body and are also potential biomarkers of AD [25]. Exosomes are small extracellular vesicles that can communicate between cells and widely exist in biological fluids, including blood. Because the concentration of miRNA in exosomes is higher than in fluids, miRNAs contained in exosomes are widely used in research [26]. Many miRNAs have been found to be associated with AD, but the current research is not mature and the diagnostic effect of a single miRNA has not been reached. At the same time, there are many problems in this field, such as the isolation of exosomes from blood, and the limited understanding of exosomes themselves [25].

5. Conclusions

AD is currently the most typical degenerative neurological disease, and its mortality rate is increasing worldwide, especially in developing countries [2]. Currently, there are limited methods for clinical diagnosis of AD, and CSF detection and imaging techniques are the most commonly used methods. Among them, biomarkers that have been approved include A β , tau protein, brain atrophy, etc., and their accuracy and specificity are constantly improving with research, and automated diagnostic techniques are continually improving. Of course, the most important diagnostic criteria are gradually being developed.

However, in order to identify prospective AD patients as soon as feasible, the price and technical requirements of existing diagnostic methods are obstacles to large-scale screening. Therefore, blood has received research attention as the most readily available sample. The field of blood biomarkers is growing, and A β and tau protein detection progress is very promising. However, there are also some

difficulties in the study of blood, such as the complexity of components and the interference of other diseases [18]. Still, these do not affect blood to become the most potential diagnostic samples, and more relevant research should be invested in the future. In addition, detecting a single biomarker cannot achieve 100% detection, and considering the joint diagnosis between biomarkers is also the research direction [11]. At the same time, it is also crucial to predict the future development direction of MCI patients.

References

- [1] Walsh DM, Selkoe DJ. A β Oligomers - a decade of discovery. *Journal of neurochemistry*. 2007; 101:1172-1184.
- [2] 2022 Alzheimer's disease facts and figures. *Alzheimer's & dementia*. 2022; 18:700-789.
- [3] Jack CR, Albert MS, Knopman DS, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia*. 2011; 7:257-262.
- [4] BOYLE PA, WILSON RS, AGGARWAL NT, TANG Y, BENNETT DA. Mild cognitive impairment: Risk of Alzheimer's disease and rate of cognitive decline. *Neurology*. 2006; 67:441-445.
- [5] Dubois B, Prof, Feldman HH, Prof, Jacova C, PhD, et al. Advancing research diagnostic criteria for Alzheimer's disease: the IWG-2 criteria. *Lancet neurology*. 2014; 13:614-629.
- [6] Hampel H, Lista S, Khachaturian ZS. Development of biomarkers to chart all Alzheimer's disease stages: The royal road to cutting the therapeutic Gordian Knot. *Alzheimer's & dementia*. 2012; 8:312-336.
- [7] Niemantsverdriet E, Valckx S, Bjerke M, Engelborghs S. Alzheimer's disease CSF biomarkers: clinical indications and rational use. *Acta neurologica Belgica*. 2017; 117:591-602.
- [8] Rosén C, Hansson O, Blennow K, et al. Fluid biomarkers in Alzheimer's disease - current concepts. *Molecular neurodegeneration*. 2013; 8:20-20.
- [9] Mehta PD, Pirttilä T, Mehta SP, Sersen EA, Aisen PS, Wisniewski HM. Plasma and Cerebrospinal Fluid Levels of Amyloid β Proteins 1-40 and 1-42 in Alzheimer's Disease. *Archives of neurology (Chicago)*. 2000; 57:100-105.
- [10] Lewczuk P, Matzen A, Blennow K, et al. Cerebrospinal Fluid A β 42/40 Corresponds Better than A β 42 to Amyloid PET in Alzheimer's Disease. *J Alzheimers Dis*. 2017;55(2):813-822.
- [11] Ritchie C, Smailagic N, Noel-Storr AH, et al. CSF tau and the CSF tau/ABeta ratio for the diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). *Cochrane database of systematic reviews*. 2017;2017:CD010803-CD010803.
- [12] Hawksworth J, Fernández E, Gevaert K. A new generation of AD biomarkers: 2019 to 2021. *Ageing research reviews*. 2022; 79:101654-101654.
- [13] Baldeiras I, Santana I, Leitão MJ, et al. Erlangen Score as a tool to predict progression from mild cognitive impairment to dementia in Alzheimer's disease. *Alzheimers Res Ther*. 2019;11(1):2.
- [14] Matsuda H. MRI morphometry in Alzheimer's disease. *Ageing research reviews*. 2016; 30:17-24.
- [15] Nikparast F, Ganji Z, Zare H. Early differentiation of neurodegenerative diseases using the novel QSM technique: what is the biomarker of each disorder? *BMC neuroscience*. 2022; 23:1-23.
- [16] Rice L, Bisdas S. The diagnostic value of FDG and amyloid PET in Alzheimer's disease—A systematic review. *European journal of radiology*. 2017; 94:16-24.
- [17] Trzepacz P, MD, Yu P, PhD, Sun J, MS, et al. Comparison of Neuroimaging Modalities for the Prediction of Conversion from Mild Cognitive Impairment to Alzheimer's Dementia. *The American journal of geriatric psychiatry*. 2013;21: S137-S137.
- [18] Henriksen K, O'Bryant SE, Hampel H, et al. The future of blood-based biomarkers for Alzheimer's disease. *Alzheimer's & dementia*. 2014;2013; 10:115-131.
- [19] Hu S, Yang C, Luo H. Current trends in blood biomarker detection and imaging for Alzheimer's disease. *Biosensors & bioelectronics*. 2022; 210:114278-114278.

- [20] Zetterberg H, Sahlgrenska akademien, Institutionen för neurovetenskap och fysiologi, sektionen för psykiatri och neurokemi, et al. Blood-based biomarkers for Alzheimer's disease—An update. *Journal of neuroscience methods*. 2019; 319:2-6.
- [21] Bălașa AF, Chircov C, Grumezescu AM. Body Fluid Biomarkers for Alzheimer's Disease-An Up-To-Date Overview. *Biomedicines*. 2020;8(10):421.
- [22] Schuitmaker A, Dik MG, Veerhuis R, et al. Inflammatory markers in AD and MCI patients with different biomarker profiles. *Neurobiology of aging*. 2008;2009; 30:1885-1889.
- [23] Xue-Ning S, Li-Dong N, Yan-Jiang W, et al. Inflammatory markers in Alzheimer's disease and mild cognitive impairment: a meta-analysis and systematic review of 170 studies. *Journal of Neurology, Neurosurgery and Psychiatry*. 2019;90(5):590.
- [24] Anuradha U, Kumar A, Singh RK. The clinical correlation of proinflammatory and anti-inflammatory biomarkers with Alzheimer disease: a meta-analysis. *Neurological sciences*. 2021;2022; 43:285-298.
- [25] Backes C, Meese E, Keller A. Specific miRNA Disease Biomarkers in Blood, Serum and Plasma: Challenges and Prospects. *Molecular diagnosis & therapy*. 2016; 20:509-518.
- [26] Dong Z, Gu H, Guo Q, et al. Profiling of Serum Exosome MiRNA Reveals the Potential of a MiRNA Panel as Diagnostic Biomarker for Alzheimer's Disease. *Molecular neurobiology*. 2021; 58:3084-3094.