Chapter

Precision Medicine: Role of Biomarkers in Early Prediction and Diagnosis of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD), the most common form of dementia in the aged people, is a chronic and irreversible neurodegenerative disorder. Early prediction, intervention, and objective diagnosis are very critical in AD. In this chapter, we will introduce the current progress in the prediction and diagnosis of AD, including recent development in diagnostic criteria, genetic testing, neuroimaging techniques, and neurochemical assays. Focus will be on some new applied methods with more specific examples, that is, cerebrospinal fluid (CSF) and blood proteins and peptides, which might serve as biomarkers for the diagnosis of AD. We will also discuss biomarker-based diagnostic strategies and their practical application.

Keywords: Alzheimer's disease, mild cognitive impairment, diagnosis, prediction, biomarker

1. Introduction

Alzheimer's disease (AD), the most common type of dementia in aged people, is an untreatable neurodegenerative disorder characterized by abnormal accumulations of amyloid-β (Aβ) oligomers and intracellular neurofibrillary tangles (NFTs) in the brain attributable to hyperphosphorylated tau that results in progressive synaptic dysfunction and cognitive deficits [1, 2]. AD is the fifth leading cause of death for people aged 65 and over [3] and is officially listed as the sixth leading cause of death in the United States [4]. Presently, more than 47 million people are estimated to be living with dementia globally, and this number is projected to rapidly increase, reaching 75 million by 2030 and 135 million by 2050 [5]. In China, there were 135.2 million aged people in 2015, and 8.5 million of them were oldest-old (beyond 85 years and above). Based on age-specific prevalence of AD, China would have over 20 million AD patients in 2050 [6].

AD is a heterogeneous disease caused by a combination of environmental and genetic factors. Currently known risk factors for AD include age, sex, cardiovascular risk factors and metabolic risk factors, sleep apnea, family history, and certain genetic variants [7]. In an attempt to explain the complexity and multifactorial

nature of AD, various hypotheses are established. These include Aβ aggregation, tau aggregation, metal dyshomeostasis, oxidative stress, cholinergic dysfunction, inflammation, and downregulation of autophagy [8]. However, none of the hypotheses is capable of independently explaining the pathological conditions observed in AD. The amyloid cascade hypothesis is widely considered to be involved in the pathogenesis of AD [9]. The anatomic and temporal discordance between Aβ pathology, tau aggregation, and neurodegeneration has led to the postulation of Aβ being an initiator of a complex cascade that ends in tau-medicated neurodegeneration [7].

As the etiology and pathogenesis of AD have not been elucidated, none of the proposed pharmacologic treatments (medications) are authentic to slow or stop the neurodegeneration [10]. On the other hand, in clinical practice, a diagnosis of AD is primarily made on the base of clinical features, results of neurological and neuropsychological tests, and by exclusion of other causes of dementia, including vascular and frontotemporal dementia or other neurological diseases [11]. Although a variety of imaging techniques, and detection of levels of $A\beta_{40}$, $A\beta_{42}$, total tau protein (T-tau), and phosphorylated tau protein (P-tau) in CSF have been found to be able to support clinical diagnosis of mild cognitive impairment (MCI) and AD. However, CSF collection is invasive, and therefore, its sampling is quite difficult. Imaging techniques are expensive, which restrict their application either as routine screening tools or for repetition of tests to monitor the drug treatment or pathological progress [12].

The goal of precision medicine is to use biological knowledge and other related health information to predict individual disease risk, understand disease etiology, identify disease subcategories, improve diagnosis, and provide personalized treatment strategies [7]. To date, none of the effective intervention is available, which can cure or halt the progression of AD. However, studies have consistently shown that active management of Alzheimer's and other dementias can improve the quality of life of affected subjects and their caregivers [4]. The development of biomarkers for AD is making it possible to detect the disease and provide an accurate diagnosis earlier, which is beneficial for diagnosed individuals, their caregivers and loved ones, as well as society as a whole [13]. In particular, pathophysiological alterations associated with AD are thought to begin several decades before the onset of the disease [14]. Thus, early diagnosis would provide a crucial opportunity for intervention in AD progression. In addition, the use of biomarkers in all stages of Alzheimer's disease will facilitate to develop therapeutic strategy that targets the underlying brain changes at each stage. Moreover, the research of biomarkers discovery may contribute to enhance our understanding of the pathogenesis of the disease itself.

Therefore, the biomarker discovery is of utmost importance to improve diagnostics and prevention of disease and to monitor treatment effects. In this chapter, we introduced the update of AD diagnostic criteria, genetic research, and imaging and fluid (CSF and blood) biomarkers, highlighting the progress of biomarker research and advances in methodology.

2. Advance in diagnostic criteria

2.1 Development of AD biomarkers in early guidance

The first set of criteria proposed for diagnosis of AD was launched in 1984 by a workgroup from the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's disease and Related Disorders Association

(NINCDS–ADRDA) [15]. At that time, Alzheimer's pathological changes could not be measured in vivo. The criteria focused on clinical symptoms and required the presence of significant disability and impact on daily living. Thus, it allowed only a "probable" diagnosis of AD to be reached while the person was alive, and a definitive diagnosis could be made only if Alzheimer's pathology was found at autopsy [15]. In terms of distinguishing AD from other types of dementia, its specificity was low [16]. In 2007, the AD Research Diagnostic Criteria proposed by the International Working Group (IWG) first included true biomarkers in the criteria for active diagnosis of AD and considered AD to progress from preclinical, pre-dementia to dementia [17].

In 2011, the National Institute on Aging and the Alzheimer's Association (NIA-AA) proposed revised guidelines for diagnosing AD [18–21]. This updated diagnostic criteria and guidelines incorporated biomarker tests in addition to clinical symptoms, and provided the researchers tools for diagnosing AD. It identified AD as a continuum with three distinct stages: preclinical stage, mild cognitive impairment (MCI), and dementia. There are two types of biomarker, the former including CSF $A\beta_{42}$ or amyloid positron emission tomography (PET) and the latter including CSF tau/P-tau, MRI hippocampus or medial axillary atrophy, and low glucose metabolism on PET or SPECT [18]. In 2012, the NIA-AA also developed new guidelines to help pathologists to describe and categorize the brain changes associated with Alzheimer's and other dementias on autopsy [22]. Parallel to the hypothetical pathophysiological sequence of AD, a biomarker model was proposed by Jack et al. [23, 24]. It revealed that the biomarker abnormality occurs first in Aβ levels, which can either be in the form of an upregulation in plasma or down-regulation in CSF in usually cognitively normal individuals and can be detected by biochemical analysis [6, 23, 24]. The next stage of biomarker abnormality was usually amyloid deposition in the brain detected by abeta PET. Subsequently, the changes of biomarkers include neuronal injury, indicated by increased levels of CSF total phosphorylated tau proteins, and cerebral atrophy revealed by structural MRI, as well as neurodegeneration and synaptic dysfunction detected by reduced fluorodeoxyglucose (FDG) uptake through PET. These results of biomarker studies showed that they are correlated with different disease stages, which thus correlate with and support the changes in the "abeta hypothesis" [6]. Furthermore, research criteria for diagnosing preclinical states of AD developed by the International Working Group (the IWG-2 criteria) require the individual to be asymptomatic and have a marker of AD pathology or an AD autosomal dominant mutation on chromosome 1, 14, or 21 [16]. This is describing an at-risk state where progression of AD is not inevitable.

2.2 NIA-AA 2018 update guidelines

Recently, NIA-AA guidelines for AD have been updated [25]. In these latest guidelines, Alzheimer's biomarkers are divided into three categories (the A/T/N system). The classification uses three types of biomarkers as shown in **Table 1**. "A" refers to amyloid β (Aβ) as measured either by amyloid PET imaging of amyloid plaques or in the cerebrospinal fluid (CSF) as $A\beta_{42}$ or the $A\beta_{42}$ to $A\beta_{40}$ ratio. "T" refers to tau pathology as measured by CSF phosphorylated tau or tau PET imaging of parenchymal neurofibrillary tangles. "N" refers to neurodegeneration or neuronal injury and dysfunction, as measured by elevated levels of CSF total tau, decreased glucose metabolism shown on FDG-PET imaging, and brain atrophy shown with structural MRI. While "A" and "T" are considered to have diagnostic specificity for AD, "N" is not specific for AD diagnoses because it can reflect any number of etiologies in addition to AD. The A/T/N biomarkers may reflect the

Biomarker class	CSF marker	Imaging marker
Amyloid (A)	CSF $A\beta_{42,}$or $A\beta_{42}/A\beta_{40}$ ratio	Amyloid PET
Tau (T)	CSF phospho-tau	Tau PET
Neurodegeneration (N)	CSF total tau	Anatomic MRI; FDG PET

Table 1.
AT(N) biomarker grouping of the NIA-AA Framework.

presence (state) or progression (stage) of a disease. An individual with biomarker evidence of Aβ deposition alone (abnormal amyloid PET scan or low CSF $A\beta_{42}$ or $A\beta_{42}/A\beta_{40}$ ratio) with a normal pathologic tau biomarker would be assigned the label "Alzheimer's pathologic change" [25]. The term "Alzheimer's disease" would be applied if biomarker evidence of both Aβ and pathologic tau is present. Alzheimer's pathologic change and AD are not regarded as separate entities but earlier and later phases of the "Alzheimer's continuum" (an umbrella term that includes both). These definitions are applied independently from clinical symptoms [25].

In addition, together with cognitive symptoms (C), AT (N)(C) measures have different roles for definition and staging, A and T indicate specific neuropathologic changes that define Alzheimer's disease, that is, Aβ biomarkers determine whether or not an individual is in the Alzheimer's continuum. Pathologic tau biomarkers determine if someone who is in the Alzheimer's continuum has Alzheimer's disease. (N) and (C) are not specific to AD and are therefore placed in parentheses. They indicate staging severity [25].

NIA-AA 2018 guidelines are still research framework and cannot be considered as routine clinical care [25]. However, clearly, with the update of these guidelines, the definition of AD shifts from symptom-based definition to biology-based definition. This is leading to a better understanding of the underlying mechanisms of the disease and aiding in the development of new interventions to delay or prevent disease progression and biomarker research [25].

3. Genetic susceptibility

AD can be divided into early-onset familial AD (EOAD) and late-onset AD (LOAD). Early-onset AD accounts for less than 1–5% and is caused by highly penetrable variants, the majority of which are attributable to mutations in one of the three genes, amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2) [26, 27]. Individuals with Alzheimer's mutations in any of these three genes tend to develop symptoms before age 65, and the average age is 50 years [28]. More than 95% of AD cases are LOAD, which are "sporadic" with no apparent familial recurrence of the disease and are caused by a more complex underlying genetic architecture, and typically appear in older individuals (age 65 years and over) [29].

E4 allele of apolipoprotein E (APOE4) is the only verified genetic risk factor for late-onset AD. It is present in approximately 15% of the normal population; however, it occurs in 50% of those developing AD. APOE encodes a lipid carrier apolipoprotein E (ApoE) that is found both in the periphery and the central nervous system [7]. APOE4 shows the complex interplay of mechanisms contributing to sporadic AD, including reduced cholesterol transport, less efficient Aβ clearance and more aggregation, triggering neurotoxicity through tau phosphorylation, increased brain neuronal activity and atrophy, reduced synaptic plasticity, and greater neuroinflammation [7].

In addition to APOE4, genome-wide association study (GWAS) has identified more than 30 genomic loci that are associated with AD risk [7]. Ten susceptible loci for LOAD with the most consistent results include APOE, CLU, PICALM, CR1, BIN1, EPHA1, MS4A, ABCA7, CD33, and CD2AP. These AD risk loci are associated with different biological processes, including immune system, endocytosis, lipid homeostasis, and Aβ metabolism, highlighting the complexity of AD and point toward potentially novel directions for drug discovery and treatment [30]. Besides, rare variants (allele frequency < 1%) that influence the risk for LOAD have also been identified in several genes, including TREM2, PLD3, UNC5C, AKAP9, ADAM10, and ABI3 [7].

Genetics can provide a valuable starting point for advancement. To date, the vast majority of genetic work in AD has been the search for individual genes or combinations of genes associated with a dichotomous outcome of an AD diagnosis. For example, a study used survival analysis modeling to integrate AD risk variants and develop a polygenic hazard score for age of onset, which show a strong genetic component in AD that can be useful in predicting risk. Thus, genetic knowledge may also facilitate precision medicine. This approach has recently been proposed for dementia [7].

4. Biomarkers of AD

4.1 Imaging

As mentioned above, three Alzheimer's neuroimaging biomarkers are currently used for research and, in some cases, are used to aid in clinical diagnosis. Elevated cortical tau shown with PET imaging is a biomarker for neurofibrillary tangles; decreased glucose metabolism shown by FDG-PET imaging and atrophy shown by structural MRI are biomarkers for neurodegeneration or neuronal injury [31]. Deposition of Aβ can be detected by amyloid-specific imaging agents for positron emission tomography-computed tomography (PET/CT) as early as 15 years before the onset of AD symptoms, whereas the next most sensitive metric, cerebral hypometabolism (FDG-PET/CT) is detectable only 10 years prior to symptom onset. Aβ PET/CT is thought to precede by 10 years the declines in even the most sensitive cognitive metrics including episodic memory [32].

4.2 Fluid biomarkers for AD

In parallel to imaging biomarkers, additional types of biomarkers currently being studied in AD and used mainly for research purposes are found in CSF and blood.

4.2.1 CSF biomarker

The most validated CSF biomarkers for AD are $A\beta_{42}$, total tau (T-tau), and tau phosphorylated at threonine 181 (P-tau181) [33]. These biomarkers have consistently shown a marked change in AD dementia and also in the early prodromal phase of the disease. In CSF of AD patients, a decreased level of $A\beta_{42}$ has consistently been found, whereas the concentrations of tau and P-tau are increased [33]. The levels of CSF tau and P-tau have been found to correlate with brain atrophy in AD, while a reduction of $A\beta_{42}$ in CSF is shown to correlate with brain atrophy in non-demented subjects indicating a potential preclinical stage [34]. In addition, high CSF T-tau and P-tau predict the progression of cognitive symptoms better

than $A\beta_{42}$ during a clinically relevant time period (1–2 years) [35]. Based on their high diagnostic performance, as state above, these core AD CSF biomarkers have been included in the diagnostic criteria for AD [18, 25]. However, CSF biomarkers show 20–30% interlaboratory and interassay variability [36]. In order to reduce this variability, standardization efforts include the creation of a mass spectrometry (MS)-based reference measurement procedures (RMP) for CSF $A\beta_{42}$ [37] and certified reference materials (CRM) for the main AD CSF biomarkers [38]. Precise measurements have also been achieved by novel assays developed on fully automated laboratory equipment [39]. Moreover, other Aβ protein levels and ratios (tau/$A\beta_{42}$, $A\beta_{42}/A\beta_{40}$, $A\beta_{42}/A\beta_{38}$) also become abnormal with the signature of AD [40]. For example, reduced $A\beta_{42}/A\beta_{40}$ ratio is characteristic of AD dementia and prodromal AD [41].

Despite the promising CSF core biomarkers for the identification of presymptomatic AD and discriminate AD cases well from healthy subjects, the inherent heterogeneity in the progression of mounting plaque and tangle load over time between patients, as well as the presence of mixed pathologies and different comorbidities, are considered [42]. For example, elevated amyloid deposition is frequently found in cognitively normal subjects, and CSF levels of Aβ and Aβ imaging with PIB-PET do not correlate with cognitive decline [43]. Thus, it is needed to augment the CSF core biomarkers with novel proteins to improve diagnostic accuracy in longitudinal studies [44]. Recently, new biomarkers reflecting other aspects of pathophysiology have been reported, for example, CSF neurofilament light chain (NFL), neurogranin, and YKL-40 proteins have reached at an advanced clinical validation stage [45]. A recent meta-analysis showed that the core CSF biomarkers of neurodegeneration (T-tau, P-tau, and $A\beta_{42}$) and CSF NFL were strongly associated with AD, and NSE, VLP-1, HFABP, and YKL-40 were moderately associated with AD [33]. Among these, NFL, NSE, VLP-1, and HFABP are related to neurodegeneration, and YKL-40 is associated with glial activation [33]. Of note, another protein, neurogranin, involved in synaptic dysfunction and degeneration, is found with higher CSF levels in patients with AD. It is seemingly specific for AD and does not change in the majority of other neurodegenerative disorders [35]. Taken together, the integration of complementary pathophysiological biomarker candidates covering additional key AD mechanisms will likely result in an incremental performance optimization for the detection, diagnosis, and differential diagnosis of primary neurodegenerative diseases and dementia disorders [45].

4.2.2 Blood-based biomarker

Blood collection is routinely performed, minimally invasive and cheap and suitable for recurrent measures. Blood-based biomarkers may allow for efficient monitoring of disease processes in AD and could be used as a screening tool in primary care [46]. Amyloid β (Aβ) is a widely researched plasma biomarker for AD. Evidence supporting the transport of Aβ across the blood-brain barrier and through CSF suggests that 30–50% of plasma Aβ originates from the CNS [47]. However, diagnostic relevance of plasma Aβ for AD process yields conflicting results [33, 48, 49]. In terms of this, a meta-analysis found that lower $A\beta_{42}$:$A\beta_{40}$ ratios are significantly associated with the development of AD and dementia [49]. Another meta-analysis showed that plasma or serum concentration of $A\beta_{40}$ did not differ significantly between patients with AD subjects and controls [33]. Recently, using the INNO-BIA kit based on a multiplex xMAP technique, Hanon et al. found that plasma $A\beta_{42}$ and $A\beta_{40}$ are lower in AD than in amnestic MCI and non-amnestic MCI, respectively. Plasma $A\beta_{42}$ correlated with age,

Mini–Mental State Examination, and APOE ε4 allele [48]. Another AD pathology, that is, tau, a meta-analysis suggested that plasma T-tau are strongly associated with AD [33].

Indeed, CNS-specific proteins with very low concentrations in the blood are difficult to quantify using standard immunochemical technologies, such as ELISA (enzyme-linked immunosorbent assay), which is a major challenge in developing blood biomarkers [20]. This might be one of the reasons for the inconsistency of the analysis results in the previous studies. Recent technical breakthroughs in the field of ultrasensitive assays have started to improve it [50]. These technologies include single-molecule array (Simoa) technology and immunomagnetic reduction (IMR) [50]. Simoa technology can detect single protein molecules in blood, which captured target proteins on microscopic beads decorated with specific antibodies and then labeled the immunocomplexes (one- or zero-labeled target protein molecules per bead) with an enzymatic reporter capable of generating a fluorescent product. After isolating the beads in 50-fl reaction chambers designed to hold only a single bead, fluorescence imaging is detected [51]. The average sensitivity improvement of the Simoa immunoassays versus conventional ELISA was >1200-fold, with coefficients of variation of <10% [52]. By using this technique, Mattsson et al. found associations between elevated plasma tau and AD hallmarks, but these were mild and differed between cohorts, and high plasma tau is associated with rapid progression in later disease stages [53]. More recently, by using this platform, Tatebe et al. reported the quantitative data on the plasma levels of P-tau181 in controls and patients with AD and Down syndrome (DS). These data suggest that the plasma P-tau181 is a promising blood biomarker for brain AD pathology [54]. Mielke et al. reported that plasma total tau and P-tau181 levels are higher in AD dementia patients than those in cognitively unimpaired and total tau and P-tau181 levels are higher in AD dementia patients than those in cognitively unimpaired, and plasma P-tau181 are more strongly associated with both Aβ and tau PET [55]. Interestingly, the neuronal injury marker NFL mentioned above was also found to be increased in plasma of the patients with MCI and patients with AD dementia with Aβ pathologic features by using Simoa technology [46].

Another ultrahigh-sensitive technology is referred to as a superconducting quantum interference device (SQUID) immunomagnetic reduction (IMR) assay. Magnetic nanoparticles are coated with an antibody, and on binding of the analyte, the oscillation of the particles in an alternating magnetic field is decreased in a concentration-dependent manner [56]. Using the SQUID-based IMR, the low detection limit for amyloids and tau protein is found to be 1–10 pg/mL [57, 58]. Thus, it makes possible the measurement of plasma biomarkers for the diagnosis of AD [58–61]. For example, by IMR technology, the previous studies suggested that the plasma $A\beta_{42}$ is a useful biomarker for AD. The $A\beta_{42}/A\beta_{40}$ ratio improves the diagnostic power of the plasma Aβ biomarkers [58], and plasma $A\beta_{42}$ correlates with CSF $A\beta_{42}$ in AD [59]. Additional researches indicated that plasma $A\beta_{42}$ and tau can be used to assist in the clinical diagnosis of AD [60], and the concentration of P-tau181 in plasma can be used to differentiate memory disorder/cognitive decline in early-stage AD patients [61]. Clearly, these ultrahigh-sensitive assay technologies provide novel methods to measure low-level proteins especially in blood. These AD-specific proteins such as Aβ, and tau-related proteins or the protein biomarkers at low concentrations in the bloodstream for AD and may serve as clinical tools for the diagnosis of AD.

Besides, the studies of blood-based biomarkers also cover the following aspects: searching for other disease pathology related to proteins in blood; blood-based biomarker panels; and markers of inflammation, oxidative stress, mitochondrial dysfunction, and neuronal and microvascular injury [62].

5. Mass spectrometry (MS)-based methods and approaches

Numerous reports have demonstrated that MS-based methods can be robust, and accurate MS has been playing an important role in studying peptide and protein identities, structures, modifications, and interactions that collectively drive their biological functions. MS-based technology has been used to study the pathogenesis of AD and biomarkers in body fluids, such CSF, plasma, urine, and saliva.

5.1 Proteomics

MS-based proteomics technology is well suited for the biomarker discovery for diseases such as AD [63–65]. During the last 10 years, apart from the gel-based techniques (e.g., 2D-PAGE and 2D-DIGE), gel-free techniques (e.g., stable isotope labeling or using label-free methods) have been dominating the field of MS-based quantitation in proteomics [66]. Including our previous study [67], the method of iTRAQ with multidimensional liquid chromatography and tandem mass spectrometry has been used to reveal many candidate proteins as potential biomarkers of MCI or AD [67–69]. One of our quantitative proteomics-based studies revealed the differentially expressed proteins in AD subjects [67]. These proteins were found involved in various biological processes and pathways, such as Aβ metabolism, inflammatory and immune response, and oxidative stress, which have previously been reported to be linked with AD, supporting the existing theories of AD pathophysiology. Furthermore, some new technologies such as SWATH-MS will also be applied to further enhance probability of AD biomarkers. SWATH-MS is a specific further variant of data-independent acquisition (DIA) methods and is emerging as a technology that combines deep proteome coverage capabilities with quantitative consistency and accuracy [70].

Apart from quantitative proteomics, the development of assays to quantify particular post-translational modification of proteins is also being considered [65, 71, 72]. For example, the carbonylation of proteins associated with oxidative stress has been studied in AD [72]. Using Western blotting with two-dimensional gel electrophoresis (2D-Oxyblot), we investigated the specifically carbonylated proteins in the hippocampi [73] and serum [74] of triple transgenic mouse model of AD (3 × Tg-AD) at the early age of month, some carbonylated proteins were identified as significantly oxidized proteins compared with the control in both of the samples. This suggests that oxidative stress is an early event in AD progression, and these oxidized proteins in the serums may provide potential biomarkers of AD at the early stage. This is similar to two previous studies [75, 76]; where the authors observed serum protein carbonylation in MCI and found increased levels of carbonylation at this stage of cognitive decline.

Together, the proteomic approach is comparatively new and more advanced for biomarker analysis of proteins and provides a complementary way to obtain such a comprehensive data.

5.2 Targeted proteomic approaches

There are generally three different stages in the development of new biomarkers: the discovery phase (i.e., screening), the verification phase, and the validation phase. Multiple reaction monitoring (MRM), also known as selected reaction monitoring, is a targeted mass spectrometry approach to protein quantitation and is emerging to bridge the gap between biomarker discovery and clinical validation [77, 78]. Highly multiplexed MRM assays are readily configured and enable simultaneous verification of large numbers of candidates facilitating the development

of biomarker panels which can increase specificity [77, 78]. MRM can enhance the lower detection limit for peptides due to its ability to rapidly and continuously monitor exclusively for the specific ions of interest. MRM analysis combine with stable isotope also offers multiplexing capability and increases the reliability of quantification [77, 78]. As AD is a multifactorial disease, a panel of proteins is more suitable as biomarker for AD. Thus, MRM is a valuable tool to verify biomarker candidates for AD and possible future practical applications. Several studies have emerged using MRM to identify CSF-based protein biomarkers of AD [79–81]. In addition to MRM, parallel reaction monitoring (PRM) technique has also been used to evaluate biomarker candidates for AD [82, 83]. PRM is related to the SRM approach but has the advantage of acquiring full fragment spectra instead of a choice of preselected fragments; interfering signals are avoided, whereas quantitation and high sensitivity are conserved [64]. In this way, other biochemical pathways and proteins which are not directly correlated to Aβ accumulation could be monitored, such as synaptic function, secretory vesicle function, and in the innate immune system.

5.3 Immunoprecipitation (IP) methods coupled with mass spectrometry

Due to wide dynamic range and low abundance of Aβ peptides, the most common experimental procedure to quantitate Aβ peptides in CSF or blood requires a sample preparation step before MS analysis. Many methods are currently available to purify/concentrate the Aβ peptides, such as solid-phase extraction (SPE), immunoprecipitation (IP), size exclusion, ultrafiltration and liquid-liquid extraction, immunodepletion, etc. [64]. Among these, IP is a common method. By using IP coupled with SRM-MS method, a recent publication reported that plasma $Aβ_{42}$ concentration correlated with the CSF $Aβ_{42}/Aβ_{40}$ ratio and had good accuracy for predicting the sensitivity and specificity of elevated brain Aβ [25]. Similarly, Nakamura et al. recently proposed a set of plasma biomarkers, the amyloid-β precursor protein (APP) 669–711/$Aβ_{42}$ and $Aβ_{40}/_{42}$ ratios and their composites, for AD diagnosis with high sensitivity and specificity. Their composites displayed an accuracy of 90% in predicting Aβ brain burden at an individual level, as confirmed with PET imaging [84]. Of note, as reviewed by Brinkmalm et al. [85], in normal APP and Aβ metabolism, Aβ is most likely regulated by amyloid-degrading enzymes [86]. Different lengths of Aβ peptides exist in vivo, depending on different degradation pathways of APP [87]. To date, more than 40 different endogenous APP and Aβ peptides, including modifications, have been identified in the CSF [88]. Thus, these approaches can not only give a more accurate quantification of Aβ peptides in blood or CSF but also can be used to detect various Aβ species, which are beneficial to screen candidate biomarker for AD. For example, using the high selectivity of anti-Aβ antibodies in combination with mass spectrometry to determine the molecular mass with high accuracy, Vigo-Pelfrey et al. demonstrated the complex nature of Aβ peptides in the CSF and reported several different N- and C-terminal variants of Aβ [89]. In addition, IP-MS method has also been used to measure the protein levels in the CSF; using this method, a marked increase in the CSF levels of both synaptosomal-associated protein 25 (SNAP-25) and synaptotagmin-1 (SYT1) was found in AD dementia and prodromal AD cases [90, 91]. Interestingly, the levels of both SNAP-25 and SYT1 are reduced in cortical areas in the AD brain [90], thus suggesting that a set of synaptic proteins covering different components of the synaptic unit may be valuable tools in clinical studies on the relevance of synaptic dysfunction and degeneration in AD pathogenesis. This may also be used in the clinical evaluation of patients. The results indicate that this strategy is advantageous for detecting low abundance proteins, especially from CNS, or various Aβ peptides as a biomarker of AD.

6. Other technologies and methods

6.1 Metabolomics

Metabolomics is the newest omics platform that offers great potential for the diagnosis and prognosis of neurodegenerative diseases. This reflects alterations in genetics, transcription, and protein profiles and influences from the environment. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are two analytical platforms regularly used for detection. NMR is a particularly powerful tool for metabolite structural test. An MS-based approach is a sensitive one to identify and quantify in complex biological systems [65]. Metabolomics encompasses several techniques including untargeted metabolomics, targeted metabolomics, lipidomics, and fluxomics [92–94]. Untargeted metabolomics measures hundreds of metabolites in order to identify metabolic signatures related to a particular disease state or phenotype. This approach provides relative changes in metabolites and is useful for discovery projects where affected metabolic pathways are unknown. Targeted metabolomics provides quantitative measurements of a defined set of metabolites in a pathway of interest (e.g., glycolysis or TCA cycle). Lipidomics estimates changes in lipid profiles and requires specialized protocols for the detection and analysis of water-insoluble metabolites. Fluxomics incorporates stable isotope tracers to provide a dynamic, as opposed to static, assessment of metabolic changes and is performed in cells or in vivo [95]. Metabolomics has been widely used in the study of mechanisms and biomarkers of Alzheimer's disease. Metabolomics analysis conducted with biological samples of patients with MCI and AD identified metabolic changes associated with preclinical and clinical AD, such as plasma, CSF, and saliva (**Table 2**) [95–104]. These findings suggest that metabolomics-based biomarkers could be used to improve disease diagnosis, which will allow target pathways altered earlier in AD.

6.2 MicroRNA (miRNA)

miRNAs are a class of small non-coding RNAs of 20–22 nucleotides in length, which regulate more than 50% of protein-coding genes [105], and are associated with many neurodegenerative diseases, such as AD [106]. Fransquet and Ryan comprehensively reviewed the methods and findings from 26 studies comparing the measurement of miRNA in blood between AD cases and controls [107]. Of 8098 individually measured miRNAs, 23 that were differentially expressed miRNAs were found to be significant in two or more studies. Only six miRNAs (miR-107, miR-125b, miR-146a, miR-181c, miR-29b, and miR-342) were consistent in their direction of expression between these studies [107]. Interestingly, miR-107 has been found to be associated with the dysregulation of proteins involved in aspects of AD pathology, as well as being consistently downregulated in AD brains [107].Thus, the differentially expressed miRNAs and the corresponding targets will be potential biomarkers and provide evidence for new strategies for design of drugs for AD treatment.

6.3 Exosomes

Exosomes contain proteins, messenger RNAs (mRNAs), and microRNAs (miRNAs) that reflect their cellular origin, and they play a prominent role in cellular signaling, expulsion of toxic proteins, and transfer of cellular pathogens to other cells. CNS-derived exosomes (NEDs) are present in biological fluids (blood, CSF, and urine) and circulate in the interstitial space, both in the brain and in the periphery [108]. It may serve as markers of underlying CNS changes that occur in

Category	Samples	Methods	Candidate metabolites[a]	References
Untargeted metabolomic	Plasma	UPLC/HILIC -QTOF-MS	All groups: 4-aminobutanal ↓, spermine ↑ L-arginine ↑ L-ornithine ↑	[96]
Lipidomic	Plasma	UPLC-MS	Ceramides ↑: Cer16:0 Cer18:0, Cer24:1 Phosphatidylcholines ↓: PC36:5, PC38:6	[97]
Lipidomics	Plasma	UPLC-QTOF-MS	Phosphatidylcholine 40:4 ↑ Triglyceride 57:1 ↓ ChoE/triglyceride ↓	[98]
Targeted metabolomics	Serum	UPLC-TQ-S-MS/MS	Aβ pathology ↑: PCs and SMs Tau pathology ↑: long-chain acylcarnitines, PC ae C36:2, and SM.C20:2	[99]
Untargeted metabolomics	Serum	FIA-MS/MS UPLC-MS/MS	Glycerophospholipids ↓ Sphingolipids ↑	[100]
Untargeted metabolomic	Saliva	FUPLC-Q-TOF/MS	Phenyllactic acid ↑ hypoxanthine ↓ Sphinganine-1-phosphate ↑ Ornithine ↑, inosine ↓ 3-Dehydrocarnitine ↓	[101]
Targeted metabolomic	Saliva	^{1}H NMR	Propionates ↑	[102]
Untargeted metabolomics	CSF	UPLC-MS/MS	S-adenosylhomocysteine ↓, glycine ↓, S-adenosylmethionine ↑	[103]
Targeted metabolomics	PCSF	FMOC-derivatized UHPLC-MS/MS	Methionine sulfoxide ↑, guanine ↑, Anthranilate ↓, diacetylspermine ↓, 3-Methoxy-anthranilate ↑, Cadaverine ↑, histamine ↑, 3-HydroxyKynurenine ↓	[104]

Abbreviations: Cer: Ceramides; ChoE/TG: indicates co-elution of ChoE and TG molecules; FIA-MS/MS: flow injection analysis-MS/MS; FMOC: 9-fluorenylmethyl chloroformate; FUPLC: faster ultra-performance liquid chromatography; HILIC: hydrophilic interaction liquid chromatography; MS: mass spectrometry; NMR: nuclear magnetic resonance; PC: Phosphatidylcholines; PCSF: Postmortem cerebrospinal fluid; SM: sphingomyelin; TOF: Time of flight; UPLC: high-performance liquid chromatography.
[a]Compared with the control: ↑: upregulated; ↓: downregulated.

Table 2.
AD-related metabolomics studies reported in the literatures.

advance of changes in circulating proteins. Importantly, CNS-derived exosomes have unique surface markers that reflect their origin. By using the corresponding antibodies, targeted examinations of neuron-, astrocyte-, or endothelial cells can be performed (**Table 3**) [109–113]. Several proteins in neural-derived plasma exosomes have been identified to associate with preclinical AD [112], and cargo proteins of plasma astrocyte-derived exosomes in AD have also been detected [110]. Interestingly, alterations in plasma NDE levels of P-tau, $A\beta_{42}$, neurogranin, and repressor element 1-silencing transcription factor were found among AD and MCI cases that converted to AD within 36 months compared with stable MCI cases and normal control subjects [113]. In addition, miRNAs released from exosomes appear to be associated with multiple neurodegenerative conditions linking to AD, which is marked by hyperphosphorylated tau proteins and accumulation of Aβ plaques

Samples	Materials	Exosomes-proteins/ miRNAs[a]	Methods	References
AD = 57 Control = 57	Plasma	Total tau ↑ P-T181-tau ↑ P-S396-tau ↑ Aβ1–42 ↑	ELISA	[109]
AD = 12 Control = 10	Plasma	GDNF ↓ P-T181-tau ↑ BACE-1 ↑ sAPPβ ↑ P-S396-tau ↑	ELISA	[110]
AD = 26 Control = 26	Plasma	P-serine-312-IRS-1 ↑	ELISA	[111]
AD = 46 Control = 46	Plasma	LAMP-1 ↑, Ubiquitin ↑, HSP70 ↓.	ELISA	[112]
AD = 10 MCI = 20 Control = 10	Plasma	P-T181-tau ↑ P-S396-tau ↑ Aβ1-42 ↑ NRGN ↓ REST ↓	ELISA	[113]
MCI = 43 DAT = 51	Plasma, CSF	mir-193b ↓	qPCR, WB	[115]
AD = 50 Control = 50	Plasma	mir-342-3p ↓ mir-342-5p ↓ mir-23b-3p ↓ mir-24-3p ↓ mir-338-3p ↓ mir-3065-5p ↓	MicroRNA sequencing	[116]
AD = 28 Control = 27	CSF	mir-29c ↓ mir-136-3p ↓ mir-16-2 ↓ mir-331-5p ↓ mir-485-5p ↑	miRNA assay qPCR	[117]
Health Control = 23/36 MCI = 3/8 AD = 23/16	Serum	Fold change > 1.5 Adjust p-value < 0.05 (HC vs AD): hsa-miR-20a-5p ↑ hsa-miR-3065-5pb ↑ hsa-miR-582-5p ↑ Fold change < 0.83 Adjust p-value < 0.05 (HC vs AD): hsa-miR-342-3p ↓ hsa-miR-1306-5p ↓	RT-qPCR Deep sequencing	[118]

Abbreviations: BACE-1: β-site amyloid precursor protein-cleaving enzyme 1; DAT: dementia of Alzheimer type; GDNF: glial-derived neurotrophic factor; HSP70: heat-shock protein 70; IRS: insulin receptor substrate; LAMP-1: lysosome-associated membrane protein 1; NRGN: neurogranin; REST: repressor element 1-silencing transcription factor; WB: Western blot analysis; RT-qPCR: Reverse transcription-quantitative real-time PCR.
[a]Expression changes in AD, compared with the control: ↑: upregulated; ↓: downregulated.

Table 3.
AD-related CNS-derived exosomes (proteins/miRNAs) reported in the literatures.

[114]. Specific profiles of exosomal miRNAs from human biological fluids, such as plasma and CSF, have prompted the potential application of miRNAs as diagnostic biomarkers (**Table 3**) [115–118]. These findings further support the search of exosome-based biomarkers for AD and other neurodegenerative diseases.

7. Conclusions

AD is the most common type of dementia and is becoming a major challenge for global health and social care. The last 20 years have seen an enormous expansion in research on biomarkers for AD. The use of biomarkers, such as T-tau, P-tau, and $A\beta_{42}$ (and $A\beta_{42}/A\beta_{40}$ ratio), together with brain imaging now provides the ability to detect evidence of the AD pathophysiological process in vivo. However, CSF biomarker and brain imaging are not used as screening tools. Research efforts have focused on the development and validation of non-invasive blood-based biomarkers. Recent advances in technical developments of novel ultrasensitive immunoassay, mass spectrometry methods, metabolomics, and exosomes show promise for blood biomarkers with potential applications as screening tools for AD (**Figure 1**). These opened a window for the study of AD biomarkers.

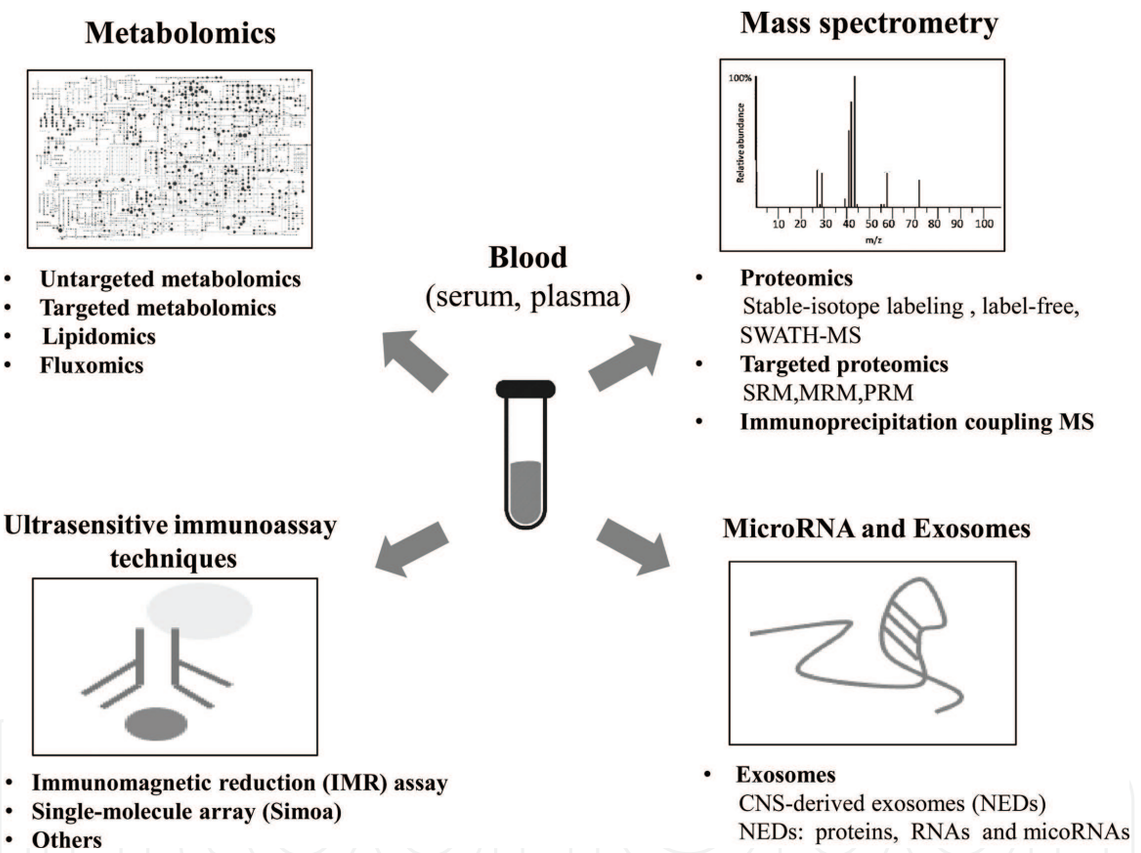

Figure 1.
Overview of the feasible and commonly used methods for AD blood biomarker discovery.

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Conflict of interest

The authors declare that they have no competing interests.

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