Degenerative Neurological and Neuromuscular Disease

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REVIEW

Advances in Blood Biomarkers for Alzheimer's Disease: Ultra-Sensitive Detection Technologies and Impact on Clinical Diagnosis

Yi Zhang[1,*], Kefan Bi[2,*], Linfu Zhou[3], Jie Wang[2], Lingtong Huang[4], Yan Sun[5], Guoping Peng[5], Wei Wu[2]

[1]Department of Laboratory Medicine, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China; [2]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, National Medical Center for Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China; [3]Department of Biochemistry, Zhejiang University School of Medicine, Hangzhou, People's Republic of China; [4]Department of Critical Care Units, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China; [5]Department of Neurology, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Wei Wu; Guoping Peng, Email zjuwuwei@zju.edu.cn; guopingpeng@zju.edu.cn

Abstract: Alzheimer's disease has escalated into a critical public health concern, marked by its neurodegenerative nature that progressively diminishes cognitive abilities. Recognized as a continuously advancing and presently incurable condition, AD underscores the necessity for early-stage diagnosis and interventions aimed at delaying the decline in mental function. Despite the proven efficacy of cerebrospinal fluid and positron emission tomography in diagnosing AD, their broader utility is constrained by significant costs and the invasive nature of these procedures. Consequently, the innovation of blood biomarkers such as Amyloid-beta, phosphorylated-tau, total-tau et al, distinguished by their high sensitivity, minimal invasiveness, accessibility, and cost-efficiency, emerges as a promising avenue for AD diagnosis. The advent of ultra-sensitive detection methodologies, including single-molecule enzyme-linked immunosorbent assay and immunoprecipitation-mass spectrometry, has revolutionized the detection of AD plasma biomarkers, supplanting previous low-sensitivity techniques. This rapid advancement in detection technology facilitates the more accurate quantification of pathological brain proteins and AD-associated biomarkers in the bloodstream. This manuscript meticulously reviews the landscape of current research on immunological markers for AD, anchored in the National Institute on Aging—Alzheimer's Association AT(N) research framework. It highlights a selection of forefront ultra-sensitive detection technologies now integral to assessing AD blood immunological markers. Additionally, this review examines the crucial pre-analytical processing steps for AD blood samples that significantly impact research outcomes and addresses the practical challenges faced during clinical testing. These discussions are crucial for enhancing our comprehension and refining the diagnostic precision of AD using blood-based biomarkers. The review aims to shed light on potential avenues for innovation and improvement in the techniques employed for detecting and investigating AD, thereby contributing to the broader field of neurodegenerative disease research.

Keywords: Alzheimer's disease, blood, biomarkers, detection technologies, pre-analytical

Introduction

Alzheimer's disease (AD), the most prevalent form of dementia, accounts for 60–70% of all dementia cases.[1] This age-related neurodegenerative disorder leads to irreversible neuronal loss, particularly in the cortex and hippocampal regions of the brain. The prevalence of AD among the elderly population is on a rising trend. In the United States, it is estimated that there are currently 6.7 million individuals aged 65 and older living with AD. Without medical breakthroughs in preventing, slowing, or treating AD, this figure is expected to grow to 13.8 million by 2060. Additionally, in 2020 and 2021, when COVID-19 entered the ranks of the top ten causes of death, Alzheimer's was the seventh-leading cause of

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death. Alzheimer's remains the fifth-leading cause of death among Americans age 65 and older.[2] The core manifestations of AD-impairment of cognitive functions, memory loss, and declines in intelligence, judgment, and language skills-reflect just a fraction of the challenges posed by this neurodegenerative disorder. AD Dementia stands as a predominant cause of disability among the elderly populace, concurrently ranking as the seventh most prevalent cause of mortality across all diseases.[3] Beyond the individual suffering and the immediate impact on families and caregivers, AD significantly affects socioeconomic development through various channels. From another perspective, recent analyses of cross-sectional and longitudinal data from large Alzheimer's disease cohorts reveal that the onset of AD and the pathological changes in brain regions begin approximately 20 years before clinical symptoms emerge. Early indications include the accumulation of amyloid-beta (A-beta) protein in the brain (approximately 15–20 years before clinical onset), followed by a reduction in cortical metabolism (approximately 10–15 years before clinical onset) and brain atrophy (approximately 5–10 years before clinical onset).[4,5] Therefore, early diagnosis, prevention, and intervention treatments have become key focal points of AD research, holding critical significance in reality.

The Pathological Mechanisms of Alzheimer's Disease

The pathological mechanisms underlying AD remain elusive. Classic pathological theories include the formation of senile plaques comprised of A-beta deposition and neurofibrillary tangles (NFTs) induced by phosphorylated Tau. As research delves deeper into the pathogenesis of AD, an increasing number of pathological mechanisms and potential biomarkers have been discovered. However, regrettably, effective therapeutic interventions have yet to be identified.

The Amyloid Cascade Hypothesis

In 1985, researchers put forward a pivotal theory regarding the pathogenesis of AD, known as the "amyloid hypothesis". This hypothesis posited that a critical pathological hallmark of AD is the abnormal accumulation of A-beta in the brain, leading to neuronal death and cognitive dysfunction.[6,7] A-beta is generated from amyloid precursor protein (APP) through enzyme-mediated reactions involving α, β, and γ secretases. APP plays a crucial role in neural differentiation, cell adhesion, and signal transduction and is encoded on human chromosome 21; the processing pathways of APP can be bifurcated into two major routes: the non-amyloidogenic pathway and the amyloidogenic pathway.

In the non-amyloidogenic pathway, APP undergoes initial cleavage by α-secretase and subsequent processing by γ-secretase. This enzymatic cascade leads to the generation of soluble APPα (sAPPα) and the P3 fragment. Notably, the P3 fragment, characterized as a neurotoxic substance, is released into the extracellular milieu.[8] Conversely, in the amyloidogenic pathway, APP is first cleaved by β-secretase (BACE1) at specific sites, producing sAPP β and β-CTF (C-terminal fragment), followed by γ-secretase cleavage of β-CTF to generate A-beta peptides and APP intracellular domain(AICD).[9] A-beta peptides primarily exist in two forms, A-beta1-40 and A-beta1-42, naturally present in cerebrospinal fluid and brain tissue,[10] composed of 39 to 43 amino acids with a molecular weight of approximately 4kDa.[11] Under certain conditions, A-beta tends to aggregate into oligomers or fibrils, eventually forming extracellular amyloid plaques, which are a primary cause of cognitive decline and memory loss in AD patients.[12] As one of the main pathological features of AD, extracellular amyloid plaques are mainly composed of two C-terminal variants of A-beta: A-beta1-40 and A-beta1-42. In addition, A-beta1-42 has two more hydrophobic amino acids at the C-terminal than A-beta1-40, so A-beta1-42 has A higher incidence of fibrosis and greater toxicity. The measurement of these A-beta variants has been widely applied in the early diagnosis of AD.[13] Since its inception, the amyloid hypothesis has remained at the core of AD research. Despite controversies and challenges, it has substantially advanced the understanding of AD's pathological mechanisms and the development of therapeutic strategies.[14] The accumulation of A-beta protein in the brain is a primary driver of AD pathogenesis. Increased concentrations of A-beta42 are linked to the formation of insoluble amyloid fibrils in plaques.[15] Because mice do not spontaneously develop amyloid pathology, transgenic mice expressing human genes associated with familial AD mutations have become crucial tools for investigating the amyloid hypothesis in AD pathogenesis.[16] Early research has detailed that familial APP mutations V717F (Indiana) and APP K670N/M671L as the PDAPP and TG2576 models, represents a significant advancement in the field,[17,18] under the neuron-specific platelet-derived growth factor-β promoter, lead to amyloid deposition, Additionally, the I716V (Florida) and V717I (London) mutations result in elevated A-beta42 levels. Presenilin 1 (PS1) and Presenilin 2 (PS2), subunits of

the γ-secretase complex, are also implicated in familial AD through mutations that promote A-beta42 accumulation.[19] Transgenic mice carrying combinations of these familial AD gene mutations exhibit common features such as A-beta plaque formation, gliosis, and synaptic degeneration. The TgCRND8 model, constructed using the PrP gene promoter to overexpress a combination of the Swedish mutation KM670/671NL and the Indiana mutation V717F, serves as a significant tool in AD research.[20] The J20 mouse model utilizes the PDGF-β promoter to overexpress two well-characterized mutations: the Swedish mutation (KM670/671NL) and the Indiana mutation (V717F). This model successfully replicates diffuse A-beta protein deposition in mice, serving as an invaluable tool for AD research.[21] The 5×FAD mouse model overexpresses human APP and PSEN1, incorporating the KM670/671NL (Swedish), V717I (London), and I716V (Florida) mutations, along with the PS1 gene mutations M146L and L286V, all under the control of a Thy1 promoter. Due to its stable and robust AD phenotype, this model is among the most extensively utilized in contemporary AD research.[22,23] This model is widely used to study the mechanisms associated with amyloid deposition in AD.

Recently, the amyloid hypothesis has been challenged. Due to the fail of clinical trials targeting A-beta and the withdrawal of a representative paper.[24] Although treatments targeting amyloid proteins for AD have consistently failed to yield satisfactory outcomes, amyloid biomarkers are increasingly validated as effective diagnostic tools.[25]

The Tau Hypothesis

A recent study has demonstrated that the presence of intracellular NFTs within the brain tissue of AD patients post-mortem constitutes the primary pathological hallmarks of the disease. This phenomenon is significantly correlated with the cognitive impairments observed during the progression of AD.[26] Tau protein, encoded by the MAPT gene, is a microtubule-associated protein with a molecular weight ranging between 50–75kDa. It plays a crucial physiological role by binding to and stabilizing the microtubules within neurons.[27] In the brains of AD patients, the tau protein is hyperphosphorylated at more than 20 residues, whereas in healthy individuals, phosphorylation is uneven and occurs at only 8–10 residues. These hyperphosphorylated tau proteins interact with each other within the neuronal cells, leading to the formation of NFTs.[28] NFTs, another major pathological characteristic of AD, are observed at the ultrastructural level as insoluble, twisted fibers with a periodicity of approximately 80nm, presenting as paired helical filaments or straight filaments.[29] Within this context, the tau protein consists of six isoforms composed of 352, 381, 382, 410, 412, and 441 amino acid residues, respectively.[30] Therefore, mainly the phosphorylated tau protein, in connection with the cellular NFTs observed in AD patients, is closely associated with the pathology of the disease. Phosphorylated tau protein, in particular, is considered an important biomarker for the early diagnosis of AD.

The Cholinergic Hypothesis

The cholinergic hypothesis represents one of the earliest propositions regarding the pathogenesis of AD. This hypothesis emerged from seminal neurochemical findings within the brains of AD patients, revealing disturbances in acetylcholine (ACh) metabolism, closely associating acetylcholinesterase (AChE) activity with neurotoxicity.[31,32] According to the classical cholinergic hypothesis, the disruption of cholinergic neurotransmission, attributed to a significant deficiency in acetylcholine, precipitates the cognitive impairments observed in AD patients. This notion suggests that a reduction in ACh metabolism could potentially ameliorate cognitive functions in AD. ACh is primarily hydrolyzed by AChE at the cholinergic synapses, Therefore, several AChE inhibitors have been approved by FDA for AD treatment. Furthermore, the deficiency in cholinergic function in AD is linked to the accumulation of A-beta and tau proteins. ACh undergoes hydrolysis catalyzed by AChE at cholinergic synapses, accelerating the accumulation of A-beta and tau proteins, and thus plays a crucial role in the pathogenesis of AD.[33] Studies indicate dynamic changes in AChE activity throughout the progression of AD, with decreased levels of brain AChE detected in the early stages of the disease, suggesting its potential as an early biomarker for AD pathogenesis.[34]

Mitochondrial and Metabolic Dysfunctions in AD

AD is potentially driven by oxidative imbalance within the central nervous system, where reactive oxygen species (ROS) disrupt metabolic processes.[35] Mitochondrial dysfunction is a hallmark of AD, with oxidative stress in the cortex and hippocampus being

major features of the disease progression.[36] Elevated oxidative stress in hippocampal tissues leads to cognitive decline, as ROS can damage DNA, oxidize proteins, and peroxidize lipids, impairing mitochondrial function and causing neuronal degeneration. A-beta deposition disrupts mitochondrial homeostasis and enzyme activity, damaging mitochondrial membrane potential. The disruption of the electron transport chain, coupled with ROS accumulation and reduced ATP production, exacerbates oxidative damage.[37] In preclinical AD models and AD patients, elevated levels of oxidative stress biomarkers such as 4-hydroxy-2-nonenal (HNE), F2-isoprostanes, and F4-isoprostanes have been observed. Additionally, 8-hydroxy-2'-deoxyguanosine (8-OHdG) serves as a prominent biomarker indicating significant DNA oxidative damage.[38–40] The lipid peroxidation markers, including 8,12-iso-iPF2a-VI, are also significantly elevated.[41] These findings highlight the critical role of reactive oxygen species (ROS) and oxidative stress in the pathogenesis of AD. The mitochondrial dysfunction hypothesis posits that mitochondrial defects are primary triggers for A-beta accumulation and neurofibrillary tangle (NFT) formation, resulting in synaptic degradation and neuronal death. Energy metabolism disturbances and impaired glucose metabolism are also significant features in the pathogenesis of AD.[42] Factors such as A-beta plaque formation, mitochondrial damage, and insulin resistance contribute to the metabolic dysregulation observed in AD.[43] Glucose is a critical energy source for neurons, and studies have shown that brain glucose utilization decreases by nearly 45% during the progression of AD.[43,44] In AD mouse models, GLUT1 deficiency accelerates A-beta deposition, leading to neurodegeneration.[45] The accumulation of A-beta may impair glucose metabolism, causing oxidative damage to synapses, synaptic dysfunction, and cognitive decline. Additionally, A-beta accumulation promotes the aggregation of the translocase of the outer mitochondrial membrane (TOM) complex, damaging the activity of respiratory chain enzymes, particularly complexes III and IV.[46] These disruptions highlight the significant role of impaired glucose metabolism in the pathogenesis of AD. Glucose metabolism defects in tauopathy mouse models are associated with memory impairment, long-term synaptic depression, and increased tau phosphorylation mediated by p38 mitogen-activated protein kinase (MAPK).[47] Insulin resistance, a key feature of both AD and type 2 diabetes, is marked by a reduction in insulin levels by approximately 80% in AD patients compared to healthy individuals. Impaired insulin signaling affects the processing of tau and A-beta, both of which are crucial in AD pathogenesis. Elevated levels of ApoE4 contribute to reduced glucose utilization in the AD brain. ApoE plays a vital role in regulating cholesterol transport and lipid metabolism. Gene expression profiling in transgenic mice carrying human ApoE alleles indicates that those with the ApoE4 allele exhibit defective expression of insulin signaling-related genes (IRS, GLUT4, PPARG, and IDE) compared to mice with the protective ApoE2 allele.[48,49] These findings underscore the significant role of disrupted glucose metabolism and insulin signaling in the development and progression of AD.

Evolving Trends in Blood Immunomarker Testing: From ATN to ATN-IVS, Single to Multiparameter Assays

Currently, the primary adjunct diagnostic approaches for AD primarily involve imaging techniques, such as Positron Emission Computed Tomography (PET) and Magnetic Resonance Imaging (MRI), alongside the analysis of cerebrospinal fluid (CSF) biomarkers. Imaging methodologies, however, only offer high reliability in the intermediate to late stages of AD, presenting limited efficacy for early-stage diagnosis and being cost-prohibitive, thus hindering their widespread application.[50] CSF analysis targets AD-related biomarkers, including A-betaproteins, Tau proteins, beta-secretase, and apolipoprotein, as a crucial early diagnosis method. Nonetheless, obtaining CSF samples through lumbar puncture is an invasive procedure that carries a risk of complications, posing certain risks to patients. Furthermore, the invasiveness required to collect CSF samples adversely affects the potential for early diagnosis, disease monitoring, and the assessment of therapeutic efficacy.[51] In contrast, blood-based biomarker detection, especially immunological indicators, garners increased attention as a more accessible, lower-risk, and cost-effective diagnostic option for AD. In 2018, the National Institute on Aging—Alzheimer's Association (NIA-AA) introduced a research framework that centers on the CSF testing-based AT(N) schema as the principal diagnostic indicator.[52] This framework delineates a novel classification system for AD that incorporates fluid or imaging biomarkers indicative of A-beta pathology (A), tau pathology (T), and neurodegeneration (N)—collectively termed the "ATN" classification. This system aims to categorize individuals with AD pathology into subgroups according to the presence or absence of biomarker evidence for each pathogen. Building upon the foundational 2018 framework, moreover, in 2023, the NIA-AA further refined its approach by organizing biomarkers into three broad categories: core AD biomarkers, non-specific biomarkers pivotal in AD

pathogenesis yet also present in other brain disorders, and biomarkers identifying common non-AD co-pathologies. Moreover, the updated framework introduced three additional biomarker categories: "I" for inflammatory/immune mechanisms and two categories for prevalent non-AD co-pathologies, "V" for vascular brain injury and "S" for synucleinopathy (Table 1). This enhancement of the classification system underscores a comprehensive strategy to elucidate the complex pathology of AD, facilitating more precise diagnosis and enabling targeted therapeutic interventions.

Amyloid β

A-beta peptides, particularly A-beta1-38, A-beta1-40, and A-beta1-42, constitute one of the primary pathological hallmarks of AD, produced through the action of gamma-secretase on various cleavage sites of the precursor protein. Among these, A-beta1-40 and A-beta1-42 garner significant attention due to their propensity to aggregate into neurotoxic plaques in the brain, damaging neurons, microglia, and other neural cells tasked with clearing them.[53] Over the past two decades, A-beta1-42 has emerged as a critical biomarker in the pathology of AD, with the ratio of A-beta1-42 to A-beta40 in the CSF demonstrating a strong correlation with the diagnosis and accuracy of AD.[54,55] Clinical cohort studies have noted a significant decrease in the ratio of A-beta1-42 to A-beta40 in the plasma of AD patients, a trend highly consistent with biomarker results in the CSF.[56] Immunoprecipitation-mass spectrometry (IP-MS) techniques have observed a declining trend in the levels of A-beta1-42 and A-beta1-40 in plasma, achieving over 90% sensitivity and specificity in distinguishing between high (A-beta+) and low (A-beta-) levels of A-beta, hence facilitating early warning and prediction of AD.[57] Similarly, measurements using Single Molecule Array (Simoa) technology have indicated a downward trend in the concentrations of A-beta1-42 and A-beta1-40 in plasma, consistent with the trends observed in CSF levels. This decline is also evident in patients with mild cognitive impairment (MCI).[58] However, results obtained through Magnetic Immunoassay (MIR) technology indicate an increase in A-beta1-42 levels and a decrease in A-beta40 levels in AD patients, diverging from the findings in control groups and MCI patients, likely due to lower A-beta concentrations in plasma and the insufficient sensitivity of MIR technology for A-beta detection. Moreover, traditional Enzyme-Linked Immunosorbent Assay (ELISA) techniques have shown inconsistencies in replicating the observed decrease in A-beta1-42 in CSF. Early work using Luminex xMAP technology in plasma also failed to reproduce the observed reduction of

Table 1 The National Institute on Aging-Alzheimer's Association Delineates Changes in Fluid Biomarkers 2018vs2023

Biomarker Category	2018	2023
Body Fluid Samples	CSF	CSF or Plasma
A	Aggregated Aβ or associated pathologic state: Aβ 42, or Aβ 42/Aβ 40 ratio	Aβ42 (Core 1)
T	Aggregated tau (neurofibrillary tangles) or associated pathologic state: phosphorylated tau	T1(phosphorylated and secreted AD tau): p-tau 217, p-tau 181, ptau 231(Core 1)
		T2(AD tau proteinopathy): pT205, MTBR-243, non-phosphorylated tau fragments (Core 2)
N	Neurodegeneration or neuronal injury: total tau	Injury, dysfunction, or degeneration of neuropil: NFL
I	/	(inflammation) Astrocytic Activation: GFAP
V	/	/
S	/	α-synuclein: αSyn-SAA

Abbreviations: Aβ, β amyloid; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein.

A-beta1-42 seen in CSF.[59] Recent studies utilizing ELISA kits with enhanced sensitivity (EUROIMMUN) have more accurately quantified the plasma A-beta1-42/A-beta40 ratio, with results aligning with PET findings.[60] In individuals carrying autosomal dominant AD mutations and patients with Down syndrome (including children), the A-beta1–42 concentrations and A-beta1-42/40 did not correlate with their CSF counterpart.[61] An increase in plasma A-beta1-42 concentration and the A-beta1-42/40 ratio has been detected, possibly reflecting the overproduction of A-beta1-42 due to mutations affecting the APP.[9] However, the diagnostic significance and value of changes in plasma A-beta1-42 concentration in hereditary AD patients remain unclear, necessitating further research.

Tau Protein

Tau protein, consisting of a sequence of 441 amino acids, is a neural microtubule-associated protein.[62] When Tau is excessively phosphorylated, it experiences a decreased affinity for neuronal microtubules. This phosphorylated Tau (p-Tau) tends to aggregate into tangles and form amyloid deposits, causing neuron damage.[63,64] p-Tau and total Tau (t-Tau) are two critical biomarkers for detection. Studies have shown that t-Tau levels in the CSF of AD patients are significantly elevated, with concentrations three times that of normal levels, demonstrating strong disease relevance, and Tau protein levels in the blood can diagnose and differentiate between normal individuals and AD patients.[65] A study in plasma revealed that t-Tau levels were significantly higher in AD patients compared to the normal control group, offering a means to diagnose and distinguish between these two populations.[66] Following adjustments for age and sex, an increase of one standard deviation (SD) in the logarithmic scale of plasma total tau levels was found to be associated with a 35% increase in the risk of AD dementia. Incorporating plasma t-Tau levels into a model that already accounts for age and sex significantly enhanced the stratification accuracy for the risk of AD dementia among participants. This refinement in risk assessment underscores the utility of plasma t-Tau as a potent biomarker for early detection and risk stratification in AD dementia. These findings suggest that plasma t-Tau levels may enhance the prediction of future dementia, are associated with dementia endophenotypes, and could serve as a biomarker for risk stratification in dementia prevention trials.[67] This may relate to its inclusion as a marker of neurodegenerative change/neuronal injury (N) within the AT(N) biomarker framework. Phosphorylation of tau at Thr181 was the first phospho-epitope identified in biofluids and has historically been considered the gold standard for p-Tau measurements. According to the AT(N) classification, p-Tau is included as a marker for Tau pathology (T). Elevated p-tau levels at threonine 181 (T181) serve as significant indicators or predictors of neurofibrillary tangle pathology. The correlation between p-tau levels at T181 and the presence of neurofibrillary tangles underscores its utility as a biomarker for detecting and forecasting the progression of neurodegenerative conditions associated with AD. Recent research has expanded upon this foundation, revealing that phosphorylation at threonine positions 181 (pThr181), 217 (pThr217), and 231 (pThr231) of the tau protein can precisely distinguish individuals with PET findings from those with negative results. This advancement enhances the potential for p-Tau variants as diagnostic tools in the clinical assessment of AD, providing a more nuanced approach to understanding its pathophysiology.[68]

Neurofilament Light

Neurofilament light chain (NfL) is a cytoskeletal protein encoded by the NEFL gene, predominantly located within the intermediate filaments of neural axons and serving as a crucial component of giant myelinated axons.[69] It plays a vital role in the stability and growth of axons. Upon axonal damage, NfL is released into the CSF and subsequently enters the bloodstream, which can be detected through ultra-sensitive immunoassays. As a result, NfL is categorized within the AT(N) biomarker framework as an indicator of neurodegenerative changes (N) in both CSF and blood. The levels of NfL in serum or plasma are consistent with those in CSF and are elevated in both familial and sporadic forms of AD. Notably, in familial AD, an increase in NfL concentrations can be detected up to 10 years before the onset of significant clinical symptoms.[70] A recent histopathological study has revealed a negative correlation between pre-mortem plasma concentrations of NfL and post-mortem NfL staining density in the medial temporal lobe tissue samples. Specifically, individuals with higher plasma NfL concentrations exhibited lower NfL staining densities within their brain tissues. Moreover, the study demonstrated that the levels of NfL are significantly associated with cortical atrophy, correlating with the stage of disease and variations in the burden of A-beta.[71] Consequently, an increase in plasma NfL

concentrations could indicate the severity of brain atrophy, metabolic decline, and white matter integrity reduction, as markers for neurodegenerative changes occurring in various brain regions. Although not specific to AD diagnosis, NfL still has the potential to serve as a blood biomarker for AD and other neurodegenerative diseases.[72] Utilizing ultra-sensitive detection technologies like Simoa, low concentrations of plasma NfL can be identified, making it a valuable peripheral biomarker for assessing cognitive decline and identifying individuals at risk of neurodegeneration and brain atrophy. Recent research indicates that blood levels of NfL can act as a biomarker for monitoring pre-symptomatic neurodegenerative changes and disease progression in AD, including sporadic AD, early-stage dementia, and the prodromal phase of autosomal dominant AD.[73]

Glial Fibrillary Acidic Protein

Glial fibrillary acidic protein (GFAP) is a crucial astrocyte cytoskeleton component and a biomarker for astrocyte activation.[73] Studies have shown that, compared to cognitively unimpaired (CU) individuals, patients with AD and other related neurodegenerative diseases exhibit significantly higher levels of GFAP in the CSF.[74] Furthermore, CU elderly individuals at risk for AD, as indicated by positive amyloid PET results, also show elevated levels of GFAP in their plasma.[75] Given that GFAP levels rise with the accumulation of A-beta and tau proteins, GFAP is more likely to be considered a marker for amyloid aggregation rather than an indicator of neuroinflammation.[76] An earlier study demonstrated a significant increase in GFAP levels among A-beta+ MCI patients and those MCI patients who progressed to dementia, compared to A-beta− MCI and stable MCI patients.[77] Recent research by Pratishtha Chatterjee et al found that GFAP levels in MCI and AD patients increase over time compared to controls.[61] While the longitudinal increase of NfL in the MCI stage was insignificant, a notable increase was observed in AD patients compared to controls. Recently, a study revealed that the GFAP level in the blood was higher in the A-beta-positive group than in the negative groups, and in individuals with AD or mild cognitive impairment (MCI) compared to the healthy controls.[78] These findings reveal a sequential change in biomarkers during the disease progression, reflecting the underlying pathological process.[77]

Exosomes

Exosomes are a class of extracellular vesicles with diameters ranging from 40–160 μm (average 100 μm) containing various proteins, nucleic acids, metabolites and lipids, which play a crucial role in intercellular communication.[79] While performing their physiological functions, exosomes also accelerate pathological processes of several neurological diseases, such as prion disease,[80] Parkinson's disease,[81] and amyotrophic lateral sclerosis.[82] As for AD, exosomes carrying cargoes including A-beta oligomers, tau proteins, and inflammatory factors lead to the progression of AD pathology through multiple pathways.[83–85]

Exosomes can be produced by all cells, and thus can be detected in almost all body fluids. By isolating neuronal-derived exosomes from blood samples, the levels of specific proteins and/or nucleic acids they carry can assist in the diagnosis and prediction of AD. Jia et al, showed that the concentrations of exosomal A-beta1-42, t-Tau, and p-Tau181 in peripheral blood were higher in the AD group compared to the aMCI group and controls. The diagnostic efficacy of combining these three biomarkers was generally consistent with those in cerebrospinal fluid.[86] Later, the same team found that the levels of four proteins reflecting synaptic function, namely growth associated protein 43, neurogranin, synaptosome associated protein 25 and synaptotagmin 1, were lower in the AD group than in the aMCI group or controls, and these levels were significantly correlated with cognitive function. The combination of these four proteins could predict AD, further broadening the application of exosomes in the AD diagnostic model.[87] Additionally, studies detecting micro-RNA (miRNA) content in blood exosomes have shown that miRNAs related to AD pathological processes, such as A-beta degradation, A-beta aggregation and tau phosphorylation, were significantly differentially expressed between AD patients and controls.[88–91] Thus, a combination of miRNAs can also serve as a promising biomarker to distinguish AD from controls.

Regarding exosome detection technology, the immunomagnetic exosomal polymerase chain reaction (iMEP) platform developed by Hu et al, has enhanced operability and precision.[92] Furthermore, the graphene electrolyte-gated transistor biosensor developed by Li et al, specifically detects serum neuronal-derived exosomal Abeta1-42 with 100% accuracy in

identifying AD from controls.[93] These development and application of new technologies provide stronger support for blood exosome detection.

Other Potential Biomarkers in Peripheral Blood

Beyond the classic ATN framework, there are various blood-derived biomarkers that are considered valuable for diagnosing and distinguishing AD. The diagnostic efficacy of these biomarkers in combination may even surpass that of blood Abate and tau proteins. Banerjee et al, found that levels of IL-6 and TNF-α in the peripheral blood of AD patients were significantly higher compared to the controls, while the level of 25-hydroxyvitamin D was significantly lower.[94] Reduced vitamin D level is thought to be associated with the progression of AD,[94] and IL-6 is related to neurodegeneration and apoptosis.[95] Leptin can promote long-term potentiation and synaptic plasticity in the hippocampus and facilitate Abeta clearance.[96] Baranowska-Bik et al, found that plasma leptin levels in female AD patients were significantly lower than the controls, while soluble leptin receptor levels were significantly higher, although these levels were not correlated with MMSE scores.[97] Additionally, a prospective study showed that higher leptin levels were associated with a lower risk of AD.[97] Proteomics also has extensive applications in the field of AD biomarkers.[98] Jiang et al, selected 21 proteins related to neurodegeneration, immune response, inflammation, metabolism, and cardiovascular function. The combination of these proteins can accurately distinguish AD from MCI patients and assess the CNS Abeta load.[98] Findings from bioinformatics research suggested that immune response, glucose metabolism, and lysosomal dysfunction may be potential targets for identifying AD.[99] Overall, these biomarkers can broaden the understanding of AD from multiple perspectives. Exploring their pathogenic or protective mechanisms in the development and progression of AD can also provide new insights for the diagnosis and treatment of the disease.

Multi-Target Detection

Combining multiple biomarkers into a panel may represent a practical approach to enhance the screening capabilities for AD in clinical applications. Various studies have demonstrated that the combination of biomarkers yields high credibility and accuracy in diagnosis.[57,100–102] For instance, research conducted by Oskar Hansson and his team within the BioFINDER and ADNI cohorts has confirmed that the solitary use of plasma p-Tau can accurately predict the occurrence of AD dementia within four years (AUC = 0.83). However, when combined with brief cognitive tests of memory, executive function, and APOE genotype, the accuracy of diagnosing and predicting AD significantly improved (AUC = 0.91). The accuracy was further enhanced by including cortical thickness and plasma NfL as variables. Notably, replacing plasma biomarkers with CSF biomarkers did not show a significant difference in accuracy.[103]

Additionally, two studies have explored the diagnostic value of combining plasma p-Tau181 and NfL. In the AD severity continuum, plasma p-Tau181 and NfL concentrations increased and showed distinct temporal dynamics and longitudinal associations with cognitive abilities, Tau-PET, and A-beta-PET. These biomarkers can provide complementary information, and their combined use may offer additional value for predicting, diagnosing, and differentiating AD from other neurodegenerative diseases.[104] Another study indicated that plasma p-Tau181 has high accuracy in predicting amyloid status, potentially avoiding CSF/amyloid PET tests in approximately 60% of participants in a memory clinic setting. NfL is useful in distinguishing frontotemporal dementia (FTD) due to non-neurodegenerative causes but performed less effectively than p-Tau181 in all other comparisons. The combined use of p-Tau181 and NfL enhanced the diagnostic efficacy for FTD and non-neurodegenerative diagnoses.[105] Within the AT(N) framework, being positive for both A and T (regardless of N status) classifies as AD, while being only positive for A, with T and N negative, is considered probable AD. Being positive for A and N may indicate early-stage AD. Patients negative for A are considered non-AD, whereas being positive for N, regardless of T status, is classified as non-AD neurodegenerative changes.[106] Although diagnosing AD accurately using only plasma biomarkers remains challenging, the combination of various blood biomarkers can identify potential AD patients from control groups, promising to become an effective method for pre-screening AD (Table 2).

Table 2 Combined Detection Based on A+T or A+T+N

Year	Study	Biomarkers	Assays	Main Results
2023	Joshua Stevenson-Hoare	Aβ40, Aβ42, GFAP, NfL	Simoa	The prediction accuracy of Alzheimer's disease clinical diagnosis by the combination of all biomarkers, APOE and polygenic risk score reached area under receiver operating AUC=0.81, with the most significant contributors being ϵ4, Aβ40 or Aβ42, GFAP and NfL. All biomarkers were significantly associated with age in cases and controls.
2023	Christophe Hirtz	Aβ42, Aβ40, t-tau APP669-711	IPMS AND Simoa	The amyloid IPMS-Shim composite biomarker (combining APP669-711/Aβ42 and Aβ40/Aβ42 ratios) discriminated AD from SCI (AUC: 0.91), OND (0.89), and NDD (0.81). The IPMS-Shim Aβ42/40 ratio also discriminated AD from MCI (0.78). IPMS-Shim biomarkers have similar relevance to discriminate between amyloid-positive and amyloid-negative individuals (0.73 and 0.76 respectively) and A-T-N-/A+T+N+ profiles (0.83 and 0.85). Performances of the Simoa 3-PLEX Aβ42/40 ratio were more modest. Pilot longitudinal analysis on the progression of plasma biomarkers indicates that IPMS-Shim can detect the decrease in plasma Aβ42 that is specific to AD patients.
2021	Joel Simrén	Tau181, Aβ42, Aβ40, Tau, GFAP	Simoa	P-tau181, neurofilament light, amyloid-β (Aβ42/40), Total-tau and Glial fibrillary acidic protein were altered in AD dementia but P-tau181 significantly outperformed all biomarkers in differentiating AD dementia from CU. P-tau181 was increased in MCI converters compared to non-converters. Higher P-tau181 was associated with steeper cognitive decline and gray matter loss in temporal regions. Longitudinal change of P-tau181 was strongly associated with gray matter loss in the full sample and with Aβ measures in CU individuals.
2023	Feng Gao	P-Tau181, Aβ42, Aβ40, T-Tau, GFAP, NFL	Simoa	A combination of the APOE genotype with plasma pTau and serum GFAP demonstrated exceptional performance in distinguishing Aβ status. Furthermore, baseline GFAP levels exhibited a strong association with cognitive decline over time and brain atrophy, with higher GFAP levels predicting a faster rate of neurodegeneration.
2023	Anna Lidia Wojdała	Aβ42/40, p-tau181, p-tau231, t-tau, NFL, GFAP, UCHL-1	Simoa	Among plasma biomarkers, we found Aβ42/Aβ40, GFAP, and p-tau231 to show the largest rate of change at the CSF biomarker-defined cut-offs for amyloidosis and tauopathy. GFAP, NF-L, and p-tau181 as the biomarkers most significantly associated with disease progression in both CSF and plasma.
2023	Zachary Winder	Aβ42, Aβ40, p-Tau-181, T-Tau, NFL GFAP, TNFa, IL-6, IL-8, IL-10, PLGF, VEGF, MMP9, TGFb	Simoa	Included cases (N = 90) showed increased tau/amyloid beta (Aβ)42 ratio, GFAP, VEGF-A, and PlGF were positively associated with higher level of AD neuropathological change, while higher Aβ42/Aβ40 ratio was inversely associated. Higher PlGF, VEGF-A, and interleukin 6 were inversely associated with chronic cerebrovascular disease, while Aβ42/Aβ40 ratio was positively associated.
2023	Xin Wang	Aβ42, Aβ40, p-Tau-181, T-Tau	Simoa	A decrease in the Aβ42/40 ratio was associated with a faster decline in the DSB score. A higher p-tau181 concentration was associated with a faster decline in the SDMT score.

Abbreviations: AD, Alzheimer's disease; APOE, Apolipoprotein E; Ab40, Amyloid-beta40; Ab42, Amyloid-beta42; CSF, Cerebrospinal fluid; CU, Cognitively unimpaired; AUC, area under the curve; GFAP, Glial fibrillary acidic protein; NfL, Neurofilament light; IPMS, Immunoprecipitation-mass spectrometry; MMP, Metallo-proteinase; VEGF-A, vascular endothelial growth factor A; PlGF, placental growth factor; DSB, Digit Span Backward; SDMT, Symbol Digit Modalities Test; P-tau181, Phosphorylated-tau181; NfL, Neurofilament light.

Plasma Immunological Marker Detection: From Conventional to High Sensitivity

Traditionally, early laboratory techniques such as ELISA have been employed to detect biomarkers for AD. However, these conventional methods have limitations primarily due to their insufficient detection sensitivity and the extremely low concentration of AD biomarkers in plasma. Consequently, the research outcomes derived from these methods are often inconsistent or even contradictory, and the lack of consistency between the concentrations of biomarkers in plasma and CSF reduces their reliability for early warning of AD.

In recent years, significant advances have been made in the field of technology to achieve higher detection sensitivities. Newly developed ultra-sensitive detection platforms, such as Simoa, Immunomagnetic Reduction (IMR), Meso Scale Discovery (MSD), and Elecsys immunoassays, have successfully addressed the issues of interference by blood proteins and heterophilic antibodies, enabling effective detection of low-concentration target biomarkers. These advancements offer new possibilities for enhancing the accuracy and reliability of AD biomarker detection.

Simoa

In 2010, a significant advancement was made in the field of biomarker detection for AD with the development of a novel assay technique known as single-molecule enzyme-linked immunosorbent assay (digital ELISA).[107] This method incorporates the utilization of Simoa arrays (Quanterix, Billerica, MA, USA), which consist of femtoliter-sized reaction chambers, thereby enhancing the sensitivity and specificity of AD biomarker detection.[108] Central to this technology is the innovative employment of sandwich antibody complexes formed on microbeads (diameter: 2.7 μm), which are subsequently enzymatically labeled, mirroring the methodology of traditional bead-based ELISAs.

When the sample presents proteins at exceedingly low concentrations, the proportional relationship between protein molecules (and, by extension, the enzymatically labeled complexes) and the microbeads is notably diminished, typically maintaining a 1:1 ratio. This condition results in a distinctive distribution pattern where the likelihood of microbeads bearing labeled immune complexes adheres to a Poisson distribution. In the context of such low protein concentrations, the Poisson distribution reveals that microbeads are predominantly found to either harbor a singular immune complex or none. For example, the process of sequestering 50 aM of protein from a sample volume of 0.1 mL (corresponding to approximately 3000 molecules) and affixing these to 200,000 beads manifests in merely 1.5% of the beads presenting a singular protein molecule, with the remaining 98.5% devoid of protein molecules.

Subsequent steps involve loading these beads into an array configured with wells of femtoliter dimensions; the specific array utilized was 2mm in breadth, composed of approximately 50,000 wells, each with a diameter of 4.5 μm and a depth of 3.25 μm. Upon introducing fluorescent enzyme substrate droplets, the array is hermetically sealed using a rubber gasket, effectively isolating each bead within an individual femtoliter volume reaction chamber. This isolation facilitates the microbeads, each bearing a single enzyme-labeled immune complex, to generate a concentrated fluorescent signal confined solely within the 50 fl reaction chambers. Acquiring delayed fluorescence imagery of the array, facilitated by conventional microscope optics, permits the concurrent detection of myriad singular immune complexes. The quantitative protein concentration analysis within the test sample determines the ratio of wells manifesting both beads and fluorescent signals against the aggregate number of wells containing beads. This innovative platform demonstrates a superior capability in detecting protein concentrations at levels significantly lower than those detectable by traditional ELISA or multiplex assay methodologies (Figure 1).

Multiplex Analyte Profiling and Cytometric Bead Array

The multiplex analyte profiling (xMAP) technology, developed by Luminex Corporation, is considered an advanced technique capable of substituting traditional ELISA methods. Characterized by its high flexibility, efficiency, and sensitivity, xMAP technology has been extensively applied in diagnosing AD and a wide range of biomedical research.[109] The technique utilizes polymer beads with distinct optical properties encoded with different colors to represent various analytes. Specific antibodies or probes can be uniquely attached to each type of microbead, allowing for the capture of particular proteins or molecules in blood, saliva, or other biological samples. xMAP technology can

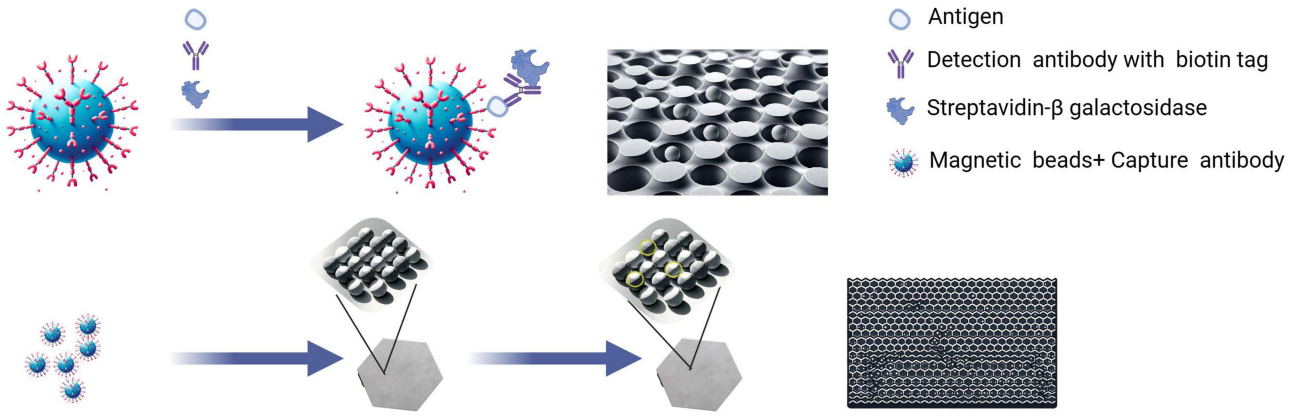

Figure 1 Single Molecule Array (Simoa) technology is a highly sensitive bio-detection method used to detect extremely low concentrations of biomarkers associated with Alzheimer's Disease (AD). Prepare samples containing target AD biomarkers, like blood or cerebrospinal fluid, and employ Simoa chips or kits equipped with highly specific antibodies to capture and detect specific proteins within the samples. Enhance the signal of the antibodies bound to these proteins using signal amplification techniques, such as enzyme labeling or fluorescent tagging, allowing for the detection of proteins at the single-molecule level. Use the Simoa analyzer to read and analyze the samples, providing quantitative data on biomarker concentrations. By Figdraw.

simultaneously detect multiple biomarkers associated with AD, such as A-beta and tau proteins, making it particularly suited for the early diagnosis and monitoring of the disease's progression.[109] However, despite the potential for xMAP detection to surpass traditional ELISA methods in terms of sensitivity, current research indicates a lack of significant correlation between the concentrations of AD biomarkers measured in plasma and cerebrospinal fluid using the xMAP technique. Moreover, the reproducibility of results across different laboratories is poor.[110–112] This suggests that while xMAP technology holds promise, its application in detecting AD biomarkers requires further study and validation.

The Cytometric Bead Array (CBA) method represents a sophisticated assay technique for the simultaneous quantitative analysis of multiple biomarkers. This method has been extensively applied to detect various components in food, agricultural products, and environmental samples. The technique combines flow cytometry with diverse sets of detection microspheres, each coated with antibodies specific to particular AD biomarkers. When exposed to samples containing these biomarkers, they bind to their respective antibodies on the beads. A detection antibody, typically labeled with a fluorescent tag, is added to the mixture. This antibody binds to the biomarkers, enabling the identification and quantification of each bead set (and thus each biomarker) based on its fluorescence intensity during flow cytometric analysis. The CBA methodology, distinguished by its pronounced sensitivity, specificity, and proficiency to simultaneously evaluate multiple biomarkers from an individual sample, demonstrates considerable promise for application in AD diagnostics.

Currently, methodologies for investigating neurodegenerative diseases such as Alzheimer's and Parkinson's, based on the ELISA experimental approach, encompass the utilization of BioLegend-developed LEGEND MAX™ Human A-beta1-42 and A-beta1-40 ELISA Kits, as well as the Tau Antibody Sampler Kit. These tools facilitate the detection of full-length APP and its various cleaved segments, including the A-beta1-40 and A-beta1-42 peptides, in addition to both phosphorylated and non-phosphorylated forms of tau. Recently, BioLegend has advanced the field by introducing a new multiplex detection system, LEGENDplex, which enables the simultaneous measurement of α-Synuclein, Tau, A-beta1-40, A-beta1-42, and NfL, thereby achieving greater sensitivity in detection. This technique facilitates an all-encompassing analysis via diverse biomarkers, significantly aiding in the early detection, continuous monitoring of disease evolution, and the formulation of tailored therapeutic interventions (Figure 2).

Immunoprecipitation Mass Spectrometry Assays

Immunoprecipitation-mass spectrometry (IP/MS) technology integrates immunoprecipitation (IP) and mass spectrometry (MS) analyses, primarily utilized for investigating protein interactions and functions and identifying biomarkers related to diseases. This method employs specific antibodies to capture and isolate target proteins or protein complexes for subsequent mass spectrometry analysis. For instance, in the context of AD, immunoprecipitation using magnetic beads

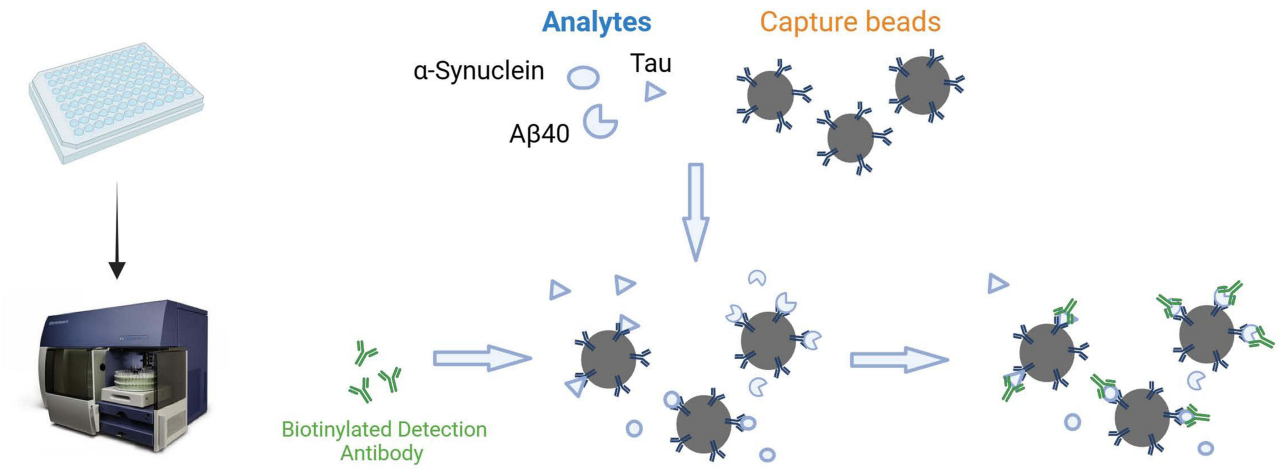

Figure 2 Differentiate beads by size and fluorescence and conjugate with specific antibodies. Mix bead panel with target analyte sample, bind, then wash. Add biotinylated detection antibodies to form bead-analyte-antibody complexes. Add Streptavidin-phycoerythrin (SA-PE) for fluorescence. Use flow cytometry to segregate and quantify bead populations. Determine analyte concentrations using the assay's standard curve. By Figdraw.

coated with specific antibodies against A-beta enriches A-beta in plasma. These antibodies specifically target the central region of A-beta.[57,113,114] Subsequently, synthetic A-beta peptides marked with stable isotopes, such as A-beta1-42 and A-beta1-40, serve as quantitative standards in mass spectrometry. In the studies conducted by Nakamura et al. Moreover, some MS-based methods can be integrated with liquid chromatography (LC/MS) to enhance the accuracy of the analysis. Recent studies, through head-to-head comparisons, have demonstrated that the LC/MS approach significantly surpasses traditional ELISA methods in terms of accuracy. Currently, the immunoprecipitation-mass spectrometry technology has been extensively applied in the research and diagnosis of A-beta, mainly due to its high specificity and sensitivity, enabling the detection of low-abundance biomarkers.[101,115] However, this technology demands high-quality antibodies and involves complex sample processing and analytical procedures. As for other biomarkers in the blood, such as tau protein and NfL, precise detection methods are still lacking. Consequently, further development and validation are required to quantify different plasma biomarkers (p-Tau species).[116]

Immunomagnetic Reduction

Immunomagnetic Reduction (IMR) is a biotechnological detection method that integrates magnetic nanotechnology with immunological principles and has demonstrated significant potential for early diagnosis of AD in recent years.[117] This technique is particularly adept at detecting low-concentration biomarkers in peripheral blood, offering high sensitivity while avoiding invasive procedures. The operational mechanism of IMR technology is based on specific biological probes or antibodies tagged on the surface of magnetic nanoparticles. When these antibody-laden magnetic nanoparticles bind with target biomolecules in the sample, such as AD-related A-beta or tau proteins, this leads to the aggregation or enlargement of the nanoparticles, thereby altering their original magnetic properties. More target biomolecules in the sample result in more magnetic nanoparticles participating in this binding and aggregation process, leading to a more significant reduction in magnetism (a phenomenon known as magnetic reduction). The concentration of target biomarkers in the sample can be quantitatively analyzed by measuring these changes in magnetism. IMR technology, through the precise detection of changes in magnetic nanoparticles, provides a highly sensitive detection method for various low-concentration biological markers in peripheral blood, making it particularly suitable for the early diagnosis of diseases such as AD (Figure 3).

The Overlooked Importance of Pre-Analytical Sample Processing

Numerous studies indicate that different sample processing procedures, from biofluid collection to analysis during the pre-analytical phase, can lead to variability in results.[118] Similarly, in the detection of AD biomarkers, pre-treatment evaluations of samples demonstrate that the use of different anticoagulants results in variations in the levels of A-beta1-

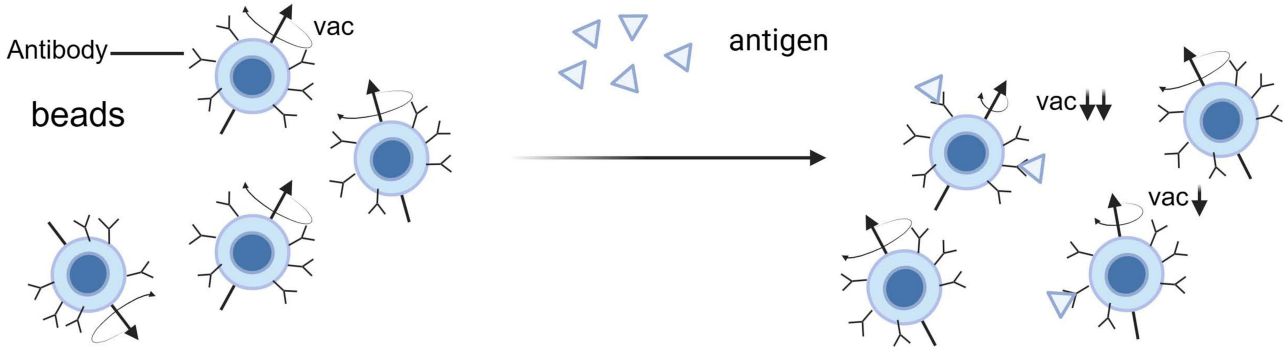

Figure 3 This detection method predates the high-specificity binding between select antibodies or antigens and target biomolecules. Under an applied alternating magnetic field, these magnetic particles rotate, leading to variations in the magnetic signal. The binding of target biomolecules reduces the number of magnetic particles that can rotate, thus diminishing the magnetic signal (magnetic reduction). The extent of magnetic reduction is directly proportional to the concentration of biomolecules in the sample, allowing for the quantitative analysis of biomolecules by measuring the reduction in the magnetic reagent's signal. The functionalities of the analyzer include generating an alternating magnetic field and detecting changes in the magnetic signal to ascertain the concentration of biomolecules. By Figdraw.

42, A-beta1-40, GFAP, NfL, t-Tau, and p-Tau181, with A-beta levels possibly being unstable before and after centrifugation in EDTA-treated blood, although the detected levels of t-Tau and NfL appear to be unaffected by anticoagulant factors, some contradictory results exist.[119,120]

Research by Teunissen et al indicates that the timing and temperature of centrifugation and storage conditions are critical pre-analytical variables. They found that A-beta detection values decrease when whole blood and separated plasma are maintained at room temperature for 24 hours but not when stored in the refrigerator, and there is no decrease when stored at −20°C for two weeks. Studies also show that GFAP, NfL, and p-Tau181 remain stable under delayed centrifugation and storage conditions, but delayed centrifugation has a significant impact on t-Tau results; t-Tau levels decrease when whole blood is maintained at room temperature for ≥3 hours, whereas they increase when whole blood is kept in the refrigerator for ≥3 hours.[121] However, studies also show that t-Tau can remain stable at room temperature for 6–8 hours.[119,122] Currently, there is limited research on the pre-analytical stability of foundational blood A-beta1-42, A-beta1-40, t-Tau, GFAP, NfL, and p-Tau181. Another study using the fully automatic Elecsys plasma prototype immunoassay showed similar results, recommending that whole blood and EDTA anticoagulated plasma should be placed in a 4°C refrigerator as soon as possible to avoid being left at room temperature, with results within 24 hours having strong credibility. For samples requiring longer storage, freezing at −20 or −80 °C is recommended.[123] Although there is not much research on the pre-treatment of AD plasma biomarkers currently, and the detection platforms are inconsistent, in the AT(N) framework of detection indicators, most studies show that A-beta1-40 and A-beta1-42 seem to be the most unstable. However, some studies suggest that A-beta1-42/1-40 ratios can offset this downward trend; others indicate that A-beta1-42 decreases more rapidly than A-beta1-40. In summary, placing samples in a 4°C refrigerator as soon as possible and testing within 24 hours appears to be a recommended method applicable to primary healthcare units and simplifying sample processing steps.

On the other hand, studies have found that postprandial food intake can change the levels of AD blood biomarkers. Huber et al investigated the impact of pre-test food intake on the pathology of A-beta and tau, NfL, and glial cell activity biomarkers in the plasma of cognitively normal adults. Multiple blood samples were taken from 111 participants within 3 hours after a standardized meal (postprandial group, PG). In contrast, blood was drawn from a fasting subgroup within 3 hours (fasting group, FG). Results showed significant differences between FG and PG in NfL, GFAP, A-beta1-42/1-40, p-Tau181, and p-Tau231. The most remarkable changes relative to baseline for GFAP and p-Tau181 occurred within 120 minutes after the meal ($p < 0.0001$).[124] These results suggest that AD-related plasma biomarkers may change after meals. However, more research is needed in the future using different manufacturers' plasma biomarker immunoassay methods so that comparisons can be made between different platforms.

Conclusion

Over the past decade, the discovery of novel biomarkers has significantly advanced our understanding of the complex pathophysiology of AD, enhancing research cognition towards the disease. On the other hand, the application of ultra-sensitive detection methods, exemplified by Simoa, in the detection of plasma AD biomarkers has further propelled the utilization of blood biomarkers in the early diagnosis and prognosis assessment of AD within both specialty clinics and primary healthcare settings. The utility of these biomarkers at the individual patient level remains incomprehensive. Given the high costs associated with traditional AD detection methods such as PET-CT and the invasiveness of cerebrospinal fluid testing, blood-based biomarkers for AD are expected to enter broad clinical application in the future. New detection techniques and methodologies also bring hope for developing new neuro-specific protein biomarkers. However, the notion that biomarkers alone can more accurately predict AD still requires comprehensive validation. Longitudinal studies that combine genetics with various plasma biomarker such as A-beta1-42, p-Tau181, NFL, GFAP, et al diagnostic predictive models could provide more accurate analyses, transitioning from the diagnosis to the prediction of AD. The validation and implementation of blood biomarkers will facilitate the development of precision medicine. Another critical factor is that environmental and pre-analytical factors such as sample collection timing, storage temperature, and detection time could significantly affect the detection results of plasma biomarkers including A-beta1-40, A-beta1-42, p-Tau181, et al. Therefore, establishing a standardized AD plasma biomarker detection workflow and protocol might be an urgent issue to address for the future application of AD blood biomarkers in clinical diagnosis and prognosis.

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Disclosure

The authors declare no conflicts of interest in this work.

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