



Early Diagnosis of Alzheimer's Disease with Blood Test; Tempting but Challenging

Fakhrossadat Farvadi,¹ Fatemeh Hashemi,^{2*} Azadeh Amini,³ Molood Alsadat Vakilinezhad⁴, Mohammad Javad Raei^{1*}

1. Center for Nanotechnology in Drug Delivery, Shiraz University of Medical Sciences, Shiraz, Iran.

2. School of Biomedical Sciences and Pharmacy, College of Health, Medicine and Wellbeing, the University of Newcastle, Newcastle, Australia.

3. Department of Pharmaceutical Biomaterials and Medical Biomaterials Research Center, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran.

4. Department of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran.

Article type: ABSTRACT

Review Article

The increasing prevalence of Alzheimer's disease (AD) has led to a health crisis. According to official statistics, more than 55 million people globally have AD or other types of dementia, making it the sixth leading cause of death. It is still difficult to diagnose AD and there is no definitive diagnosis yet; post-mortem autopsy is still the only definite method. Moreover, clinical manifestations occur very late in the course of disease progression; therefore, profound irreversible changes have already occurred when the disease manifests. Studies have shown that in the preclinical stage of AD, changes in some biomarkers are measurable prior to any neurological damage or other symptoms. Hence, creating a reliable, fast, and affordable method capable of detecting AD in early stage has attracted the most attention. Seeking clinically applicable, inexpensive, less invasive, and much more easily accessible biomarkers for early diagnosis of AD, blood-based biomarkers (BBBs) seem to be an ideal option. This review is an inclusive report of BBBs that have been shown to be altered in the course of AD progression. The aim of this report is to provide comprehensive insight into the research status of early detection of AD based on BBBs.

Received:

2022.12.03

Revised:

2023.11.25

Accepted:

2023.12.13

Keywords: Alzheimer's disease; blood-based biomarker; early diagnosis; multiplexing

Cite this article: Farvadi F, *et al.* Early Diagnosis of Alzheimer's Disease with Blood Test; Tempting but Challenging. *International Journal of Molecular and Cellular Medicine*. 2023; 12(2):172-210. **DOI:** 10.22088/IJMCM.BUMS.12.2.172

*Correspondings: Fatemeh Hashemi

Address: School of Biomedical Sciences and Pharmacy, College of Health, Medicine and Wellbeing, the University of Newcastle, Newcastle, Australia.

E-mail: fatemeh.hashemi@uon.edu.au

Mohammad Javad Raei

Address: Center for Nanotechnology in Drug Delivery, Shiraz University of Medical Sciences, Shiraz, Iran.

E-mail: raemohammadjavad@gmail.com



© The Author(s).

Publisher: Babol University of Medical Sciences

This work is published as an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by-nc/4>). Non-commercial uses of the work are permitted, provided the original work is properly cited.

The challenge of Alzheimer's disease diagnosis

There is no doubt that Alzheimer's disease (AD) is among the health priorities nowadays, especially in developed countries. According to latest World Health Organization (WHO) report, more than 55 million people worldwide have AD or other types of dementia and the number of new cases is about 10 million per year, although only one fourth of them have been diagnosed (1); a challenge that has turned AD into a health crisis, i.e. belated and uncertain diagnosis. Despite remarkable advances in diagnosis with up to 90% accuracy, there is no single and definite test to detect AD yet, and autopsy is still the only definitive method (2). Physicians need to assess several factors to confirm AD with more certainty including medical history and brain imaging, in addition to physical, neurological and mental status exams (genetic testing also can be done in the case of familial AD), while the differentiation of AD among other causes of dementia is still challenging (3). Clinical diagnostic guidelines undergo revision time to time according to recent scientific discoveries.

It is well recognized that AD starts at the molecular level many years, or even decades (20 years or more), prior to clinical manifestations (4). Therefore, profound irreversible changes have already occurred at the time of diagnosis (5). There are different stages proposed for the AD; from No Cognitive Impairment (NCI) to dementia. In the reports of a consensus panel of experts from the US and Europe regarding the improvement of AD diagnosis guidelines, it has been proposed to consider the second phase of disease (Subjective Cognitive Impairment (SCI)) as preclinical AD, before Mild Cognitive Impairment (MCI) and dementia phases (6). In this preclinical stage, changes in some biomarkers are measurable prior to any neurological damage or clinical manifestation. Incorporation of underlying biomarkers in diagnosis protocol, including the biomarkers of A- β accumulation, the biomarkers of neuronal degeneration or injury, along with imaging data, was another point stated in these reports (7).

Late indication of AD seems to be one of the main reasons of failure in effective treatment (8). Hence, creating a reliable, fast, and affordable method capable of detecting AD in early stage is of utmost importance. Vast endeavor is being done to find a new way of diagnosis at earlier stages based on novel AD biomarkers. In this regard, multiple biomarker categories are assumable including imaging, genetic, cerebrospinal fluid (CSF) and blood-based biomarkers, ocular and olfactory signals (visual and olfactory dysfunctions due to the impairment of neural networks) (9, 10), and very recently, digital biomarkers, which are defined as physiological and behavioral data that are collected and assessed non-invasively by means of mobile and wearable technologies (11); of these some are being evaluated in clinical trials (12).

Among these, peripheral biomarkers, e.g., blood-based ones are extensively under investigation and some of them are really promising. Being much easier to attain with considerably lower cost and difficulty for patients, and possibility of multiple sampling compared with other categories, blood-based biomarkers (BBBs) are an ideal option.

According to the literature, a high number of biomarkers have been shown to change in the plasma/serum/whole blood during neurodegeneration process as reported in tables 1-9. These biomarkers belong to various categories of biomolecules possessing different physiological functions. In this review, we provide a categorized comprehensive report of BBBs that have shown to be altered in AD or MCI patients.

We aim to provide readers a comprehensive insight of research status in early detection of AD based on BBBs.

Classification of Blood-Based Biomarkers (BBBs) of AD

A major challenge in utilizing BBBs for early detection of AD is the selection of a reliable biomarker or a combination of them (multiplex) that can show acceptable accuracy and precision. So far, several molecular mechanisms have been introduced for AD pathogenesis, such as amyloid β hypothesis, tau protein pathogenesis, cholinergic or glutamatergic neurotransmission pathways, the theory with emphasis on autophagy and the role of inflammatory processes, and oxidative stress (13, 14). In addition, neurodegeneration and cell loss leads in expression of biomarkers associated with cell-death including apoptosis biomarkers (15). These different proposed hypotheses with several involved molecules make a great chance for discovering potential AD biomarkers (Figure 1). Various methods, including immunoassays, omics technology, and nano-based techniques, have been employed to identify either a single biomarker or a multiplex of biomarkers to date. The authors intend to assess and discuss these techniques in a separate paper.

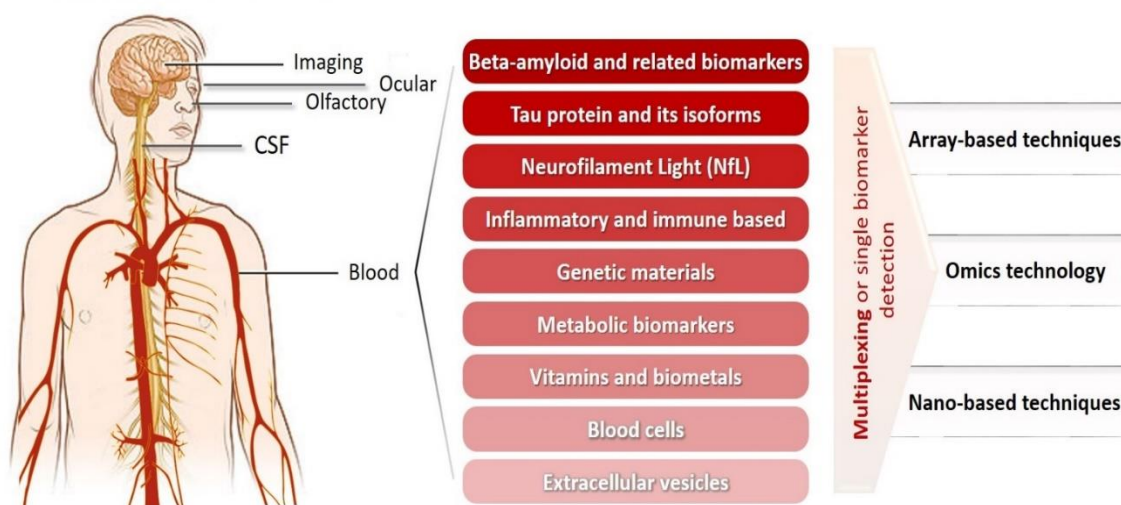


Fig.1. Alzheimer's disease biomarkers.

Beta-amyloid and Related Biomarkers

Although the theory of the role of β -amyloid protein in AD development is old, its application in early diagnosis is not. β -amyloid is perhaps the first and the main protein found to be associated with the conversion of MCI to AD (16). The Food and Drug Administration (FDA) approval of aducanumab, A β - monoclonal antibody, for the early AD treatment have encouraged the theory (17). Considering the results of numerous studies that have proved the role of β -amyloid in development and progression of AD, β -amyloid protein is suggested as one of the diagnostic entities in the updated framework of NIA-AA (National Institute on Aging and Alzheimer's Association), in which the AD diagnostic criteria have been shifted from clinical manifestation to a biological definition (18).

Amyloid precursor protein (APP) cleaves into various isoforms mainly $A\beta_{1-40}$ ($A\beta_{40}$) and $A\beta_{1-42}$ ($A\beta_{42}$). In healthy individuals, the first one is more abundant than the latter one (about 70% vs. 15%) (19). The abnormal cleavage of APP is known to form excessive insoluble $A\beta_{42}$, which aggregates in the brain in forms of oligomers, protofibrils, and fibrils. In early stage of AD, the elevation of these insoluble oligomers is the main reason of synaptic destruction (20).

Evidence has shown the abnormality of β -amyloid CSF levels in the earliest stages of AD (21). It is presumed that sequestration of β -amyloid inside the amyloid plaques decreases the amount of it in CSF. This is often observed several years before manifestation of clinical symptoms (22, 23). Although various amounts have been reported, about 50% reduction in $A\beta_{42}$ CSF level is considered as one part of the “AD signature” (24, 25).

The specificity of CSF- $A\beta_{42}$ was reported to be about 82% for discrimination of AD from healthy individuals, and about 67% for discrimination of AD from other types of dementia (26). These data varied from study to study leading to the general concept that $A\beta_{42}$ alone is not accurate for detection of AD. Some studies investigated the ratio of $A\beta_{42}/A\beta_{40}$ and vice versa (27). It is found that the CSF $A\beta_{42}/A\beta_{40}$ ratio could be correlated to the amyloid amount detected in the brain tissue by PET in the following 2-5 years (28).

It is now recognized that the only source of β -amyloid is not the brain itself. Peripheral β -amyloid originates from various tissues and body organs including, but not limited to, platelets, other blood cells, kidney, liver, and muscles. It has recently been shown that circulating β -amyloid can pass blood-brain barrier and may play an important role in AD pathologies (14, 29). Wang *et al.* have extensively reviewed the crosstalk between peripheral and central β -amyloid and their role in β -amyloid production and clearance (30).

Contrary to CSF level, as shown in table 1, plasma concentration of β -amyloid may not change in one direction, which could make it inconclusive. One of the reasons for this discrepancy might be due to the relatively low concentration of β -amyloid protein in plasma, which makes it difficult to be accurately quantified. Numerous detection methods have been developed so far to overcome this limitation offering various ranges of specification (31-33). Thanks to the development of more sensitive techniques, currently, $A\beta_{42}/A\beta_{40}$ is among the promising diagnostic plasma biomarkers along with p-tau217, p-tau181, and neurofilament light (NfL) (34).

To determine the early changes leading to AD, scientists took a step backward to investigate the process that results in creation of these two β -amyloid isoforms. Hence, studies were extended to the evaluation of various peptides, enzymes or genes involved in this pathway (35). The proteolysis of APP takes place by α - or β -secretase enzymes, which is subsequently followed by γ -secretase cleavage. Presenilins are components of multiprotein γ -secretase. It is presumed that the overexpression or mutation of β - or γ -secretases or presenilins could lead to AD (36, 37), however, the protective or pathological effects of α - and β -secretases or the APP intracellular domain peptide (AICD) fragments generated by γ -secretase are not defined clearly yet (38). Apart from β -amyloid peptides, APP, β -secretase (BACE), γ -secretase and AICD are among the β -amyloid relevant compounds which have been investigated the most (17). Table 1 summarizes the studies on β -amyloid and its related AD biomarkers in plasma.

Tau Protein and its Isoforms

The second main hallmark of AD is Tau pathology. Tau protein is predominantly found inside neurons. Stabilization of microtubules is one of the important functions of this protein. Tau, a member of microtubule-associated proteins family, consists of at least 30 phosphorylation sites. In pathological situations, specific tau phosphorylation increases its ability for self-assembly. In AD, the formation of β -amyloid aggregates, stimulates several kinases leading to the hyper-phosphorylation of tau protein which eventually forms neurofibrillary tangles (NFTs) (64).

Table 1. AD biomarkers related to β -Amyloid theory.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ A β 40 in plasma (~1.2-fold, p=0.005) ↔ A β 42 in plasma	78 probable AD & 61 controls	Sandwich ELISA	(39)
↓ A β 40 in plasma ↔ A β 42 in plasma	363 Dementia & MCI (from total 1045, 70-Year-Old men) & 196 Dementia & MCI (from total 680, 77-Year-Old men)	BNT77 ELISA	(40)
↓ A β 40 in plasma ↓ A β 42 in plasma	Swedish BioFINDER center: 513 CU & 265 MCI & 64 AD German Biomarker center: 34 CU & 109 MCI & 94 AD	Elecsys Immunoassays	(28)
↔ A β 40 in plasma ↓ A β 42 in plasma ↑ A β 40/A β 42 ratio in plasma ↔ APP 669–711 isoform in plasma ↑ (APP 669–711)/A β 42 in plasma	NCGG study (n=121) AIBL study (n=252) balanced number of CU, MCI, AD	IP–MS with MALDI–TOF mass spectrometry	(41)
↔ A β 40 in plasma (increase with age and familial AD, neither sensitive nor specific for MIC or sporadic AD) ↔ A β 40 in plasma	146 sporadic AD, 37 MIC, 96 PD, 92 control	BNT77 ELISA	(42)
↔ A β 40 in plasma	28 sporadic probable AD, 40 neurologic patients without dementia, 25 controls	ELISA	(43)
↑ A β 42 in plasma (in familial AD and in 10-20% of sporadic AD cases)	Study1: 12 Carrier & 31 non carrier of APP mutation Study2: 9 carrier PS1 mutation, 3 carrier PS2 mutation, 1 carrier APP mutation and 14 control Study3: 71 elderly patient & 75 control	BAN50/BA27 and BAN50/BC-05 sandwich ELISA	(44)
↑ A β 42 in plasma (p= 0.002)	105 CU & 64 AD	Double-antibody sandwich- ELISA	(45)
↑ A β 42 in plasma	128 participants (mutation carriers vs. non-carriers)	INNO-BIA Multiplex Assay, Innogenetics	(46)
↑ A β 42 in plasma (p=0.01) ↑ A β 42/ A β 40 ratio in plasma (p=0.001)	20 CU mutation carriers, 24 CU non-carriers (18-26 years old - matched for gender, age, educational level)	Multiplex Assay (Luminex xMAP bead-based methods (INNO-BIA AlzBio3™))	(47)
↑ A β 42 in plasma (p=0.031)	17 cases at risk for presenilin-1 and 4 cases at risk of amyloid precursor protein mutation	Sandwich ELISA	(48)
↔ A β 42 in plasma (p=0.079)	28 AD, 13 MCI, 6 CU (Taiwanese)	Immunoassays	(49)

Table 1. AD biomarkers related to β -Amyloid theory.

Biomarker alteration in body fluid	Studied group	Method	Ref.
\leftrightarrow Total β amyloid in plasma (p=0.409)			
\leftrightarrow A β 42 in plasma	28 sporadic probable AD, 40 neurologic patients without dementia, 25 CU	ELISA	(50)
\uparrow Pathogenic A β in plasma (\uparrow β -sheet content and shift of amide I band to lower wavenumbers, sensitivity: 90%)	39 AD & 61 control	Immuno-infrared sensor (A β secondary structure)	(51)
\uparrow A β 42/A β 40 in plasma	435 CU (follow-up for a median of 5 years)	Elecsys Immunoassays	(52)
\downarrow A β 42/ A β 40 ratio in plasma (No significant alteration with ELISA but significant differences via the INNO-BIA plasma A β assay, p= 0.001)	724 CU, 122 MCI, 186 AD	Commercial multiplex assay & ELISA	(53)
\downarrow APPs β isoform in plasma	80 probable AD, 14 bvFTD, 36 control	ELISA	(54)
\leftrightarrow APPs α isoform in plasma			
\uparrow APP (115 KD/ β -actin) in platelets (1.9-fold, p=0.0001)	30 MCI/AD, 23 normal control	ELISA & western blot	(55)
\downarrow APP (130/105 KD) in platelets (0.57-fold, p=0.0012)			
\downarrow APP (130/105 KD) in platelets (~0.5-fold for mAD & vmAD, p<0.001 - ~0.7-fold for MCI, p<0.001)	35 mAD, 21 vmAD, 30 MCI, 25 control	Immunoblotting	(56)
\leftrightarrow BACE1 in plasma (p = 0.41)	15 AD & 12 CU	ELISA	(57)
\downarrow BACE1 in platelets (0.88-fold, p=0.03)			
\downarrow Anti-amyloid antibodies in serum (0.4-fold, p<0.02)	39 AD & 39 CU	ELISA	(58)
\downarrow Anti-amyloid antibodies in serum	Study 1: 39 AD & 39 controls Study 2: 19 E4-negative AD & 33 E4-negative controls	ELISA	(59)
\downarrow Anti-amyloid antibodies in serum (p=0.001, independent of age, cognitive status, and ApoE ϵ 4 carrier status)	96 AD & 30 CU	Immunoassay	(60)
\uparrow ALZAS in plasma		ELISA	(61-63)
\uparrow ALZAS antibody in serum (up to 10-fold)			

AD (Alzheimer's disease), CU (cognitively unimpaired), MCI (mild cognitive impairment), APP (Amyloid precursor protein), bvFTD (behavioral variant frontotemporal dementia), mAD (mild Alzheimer's disease), vmAD (very mild Alzheimer's disease), BNT77 (IgA anti- A β 11-28), PD (Parkinson disease), BACE (β -secretase), ALZAS (Alzheimer's Associated protein, a novel gene/protein with a 79 amino acid sequence, containing A β -42 fragment, APP transmembrane signal and a 12 amino acid C-terminal that has been discovered on chromosome 21 within the APP region)

In the updated NIA-AA guideline for AD diagnosis, tau protein evaluation is included in the criteria (18). Flortaucipir the first diagnostic agent moreover, approved by FDA in 2020 to measure tau tangles on a brain PET scan. More conclusive than β -amyloid, CSF elevation of some forms of tau protein, such as total tau (t-tau), phosphorylated tau-181 (p-tau181) and phosphorylated tau-217 (p-tau217), is associated with

higher risk of AD (65). Although the elevation of t-tau concentration in CSF could also be associated with other disorders such as brain trauma or stroke, which may make it less specific for AD discrimination, it is still considered as one of the three core CSF biomarkers apart from p-tau and β -amyloid (66). The studies have shown about 300% increase in t-tau and 200% increase in p-tau in CSF of AD patients in comparison with the controlled individuals (24).

Although CSF levels of t-tau, p-tau181, and p-tau217 showed acceptable accuracy in detecting AD, their blood levels were not well associated with CSF levels (67). Very recently, several studies with exploiting techniques with higher sensitivity and biomarker enrichment strategies (Table 2), have shown a strong correlation between CSF and plasma levels of both p-tau181 and p-tau217, but not t-tau. These new findings seem promising especially for p-tau217, which performed even better than p-tau181, NfL and MRI in terms of diagnostic accuracy. P-tau217 biomarker not only diagnosed AD individuals, but also was able to discriminate AD from other neurodegenerative disorders. Moreover, it presented predicting capability, as its plasma level was higher in PSEN1 gene mutation carriers without any AD related symptoms, which are expected to develop AD about 20 years later (34, 68-70).

Table 2. AD biomarkers related to Tau protein theory

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ t-Tau in plasma (81 and 96% accuracy)	- BSHRI Site (United States): 16 CU, 16 AD (≥ 65 years) - NTUH Site (Taiwan): 63 CU, 31 AD	Ultra-sensitive Immune-magnetic reduction (IMR) assays	(71)
↑ t-Tau in plasma and CSF (associated with lower CSF A β 42)	Study 1: 179 AD, 195 MCI, 189 CU Study 2: 561 AD, 212 MCI, 174 cognitive decline, 274 CU	Multiplex xMAP Luminex platform (INNOBIA AlzBio3 kit) & Simoa HD-1 analyzer (Human Total Tau kit)	(67)
↑ t-Tau in plasma (p=0.078)	378 CU - 161 MCI	Quanterix Simoa HD-1 tau assay	(72)
↑ t-Tau in plasma (No correlation between plasma and CSF level)	54 AD - 75 MCI - 25 CU	Digital array technology	(73)
↑ t-Tau in plasma (Weak correlation between plasma and CSF level)	1453 CU (≥ 65 years, follow-up for a median of 6 years)	Single-molecule array technology	(74)
↑ t-Tau in plasma	71 bvFTD, 83 PPA, 22 controls	Human Total Tau kit with the Simoa HD-1 Analyser	(75)
↔ t-Tau in plasma (No correlation between plasma and CSF level)	111 SCD & 134 CU (age- and gender-matched)	Ultra-sensitive, single-molecule array (Simoa)	(76)
↔ t-Tau in plasma (p=0.227)	28 AD - 13 MCI - 6 CU (Taiwanese)	Immunoassays	(49)
↔ p-Tau-181 in plasma (p=0.531)			
↑ p-Tau-181 in Plasma (significant correlation between plasma and CSF level)	Study 1: 20 AD & 15 control Study 2: 20 DS & 22 controls Study 3: 8 AD & 3 other neurological diseases	Ultrasensitive immunoassay	(77)
↑ p-Tau-181 in Plasma ↑ p-Tau-217 in Plasma	Study1 (discovery cohort): 9 Young controls, 8 Aged controls, 2 Non-AD MCI, 5 Preclinical AD, 8 AD-MCI, 2 AD-moderate	Mass spectrometry	(78)

Table 2. AD biomarkers related to Tau protein theory

Biomarker alteration in body fluid	Studied group	Method	Ref.
	Study2 (validation cohort): 31 Aged controls, 11 Non-AD MCI, 20 Preclinical AD, 24 AD-MCI, 6 AD-moderate		
↑ p-Tau-181 in Plasma (3.5-fold)	Study 1 and 2: 69 CU, 47 MCI, 56 AD _{clin} , 39 CBS, 48 PSP, 50 bvFTD, 27 nfVPPA, 26 svPPA Study 3: 42 participants	Electrochemiluminescence	(69)
↑ p-Tau-181 in Plasma (16 years prior to estimated symptom onset, p= 0.050)	19 symptomatic FAD carriers, 24 pre-symptomatic FAD carriers, 27 non-FAD carriers	In-house single molecule array assay	(79)
↑ p-Tau-217 in Plasma (discriminated AD from other NDD)	Cross-sectional cohorts: Study1: 34 AD, 4 without AD Study2: 301 CU, 178 MCI, 121 AD, 99 other NDD Study3: 365 PSEN1 E280A mutation carriers, 257 mutation noncarriers	Immunoassays	(80)
↑ p-Tau-217 in Plasma	435 CU (followed for about 5 years)	Meso-Scale Discovery platform	(52)
↑ p-tau217 in CSF (6-fold) ↑ p-tau181 in CSF ↑ p-tau231 in CSF	109 CU, 20 MCI, 21 AD, 21 non-AD	Simoa single molecule array	(81)
↑ p-Tau181 in plasma (1.7-fold) ↑ tTau in plasma	Longitudinal study: 85 consisting of individuals with MCI (follow-up for 4 years) Cross-sectional study: 95 AD, 53 other NDD, 90 CU	Electrochemiluminescence immunoassays	(82)
↓ Tau-C in Serum ↓ Tau-A in Serum	5,309 postmenopausal Woman (median 13.7 years follow-up)	Solid phase competitive ELISA	(83)

AD (Alzheimer's disease), CU (cognitively unimpaired), MCI (mild cognitive impairment), NDD (neurodegenerative diseases), FAD (familial Alzheimer's disease), DS (Down syndrome), t-tau (total tau), p-tau181 (phosphorylated tau-181), p-tau217 (phosphorylated tau-217), Tau-C (caspase-3-generated cleavage site at Asp421), Tau-A (The antibodies detect an ADAM10-generated cleavage site at Ala152), bvFTD (behavioral variant frontotemporal dementia), PPA (primary progressive aphasia), SCD (subjective cognitive decline), AD_{clin} (clinically distinguished AD), PSP (progressive supranuclear palsy), CBS (corticobasal syndrome), bvFTD (behavioral variant frontotemporal dementia), nfVPPA (nonfluent variant primary progressive aphasia), svPPA (semantic variant PPA individuals).

Neurofilament Light (NfL)

Neurofilament light (NfL) protein is a structural protein found primarily in large-caliber myelinated axons in the brain. Following neuro-axonal damage, NfL releases to the environment and can be tracked in CSF. Neuronal loss is a normal part of aging and causes an annual increase in NfL baseline. However, longitudinal studies have shown that high NfL levels in the CSF are indicative of brain atrophy and a recognized biomarker for neurodegeneration (84, 85). Although CSF and serum NfL levels are demonstrated to be highly correlated, the very low concentration of peripheral NfL makes it undetectable by conventional methods. New advances in sensitive detection technologies enable scientists to detect low concentrations of NfL in peripheral blood hence studies are no longer limited to CSF. However, NfL is not a specific biomarker.

In a two multicenter cohorts with more than 2250 cases, done by Ashton and coworkers, plasma NfL was shown to be significantly increased in all cortical neurodegenerative disorders, amyotrophic lateral sclerosis, atypical parkinsonian disorders, and Down syndrome-associated dementia (84). NfL seems to be a promising biomarker of neurodegenerative disease progression, and can be used for monitoring treatment efficacy and assessment of clinical trials (86). Enzymes and other protein-based biomarkers that have been investigated for possible biomarker of AD are summarized in Table 3.

Table 3. Enzymes and other protein-based biomarkers for AD.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ NfL in Serum (association between serum NfL and brain volume changes in >60 y)	Population-based cohort study with a 5.9-year follow-up (n = 103)	Single molecule array (Simoa)	(85)
↑ NfL in Serum (p< 0.001, correlated with CSF biomarkers: low Aβ42 [p=0.001], high T-tau [p=0.02], high p-tau [p=0.02])	401 CU, 855 MCI, 327 AD	Ultrasensitive ELISA	(87)
↑ NfL in Serum (predict early pre-symptomatic familial AD)	187 CSF & 405 serum of individuals with 50% risk of carrying an autosomal-dominant AD mutation in one of three genes: APP, PSEN1, PSEN2	Ultrasensitive immunoassay	(86)
↑ NfL in Plasma	435 CU (followed for about 5 years)	Simoa-based assay	(52)
↑ NfL in plasma	Longitudinal study: 85 consisting of individuals with MCI (follow-up for 4 years) Cross-sectional study: 95 AD, 53 other NDD, 90 CU	Electrochemiluminescence immunoassays	(82)
↑ PTP in serum (1.54-fold, p<0.0001) ↑ Superoxide dismutase in plasma (1.16-fold, p<0.0001)	AIBL cohort: 754 controls & 207 AD ADNI cohort: 58 controls & 112 AD	151-analyte multiplex panel & sandwich ELISA & mass spectroscopy	(88)
↑ PTP in serum (1.33-fold) ↑ von Willebrand factor in serum (1.29-fold) ↓ Prostatic acid phosphatase in serum (0.78-fold)	197 AD and 203 controls [187 & 190 were white, respectively]	Multiplex fluorescent immunoassay	(89, 90)
↑ Superoxide dismutase in plasma (1.16-fold, p< 0.0001) ↓ Albumin in plasma (0.95-fold, p< 0.0001)	207 AD & 754 controls	Multiplex panel (Human DiscoveryMAP)	(88)
↑ Keratin type 2 in plasma (~ 2.5-fold (p < 0.001)) ↓ Albumin in plasma (> 2-fold (p < 0.01))	12 MCI & 12 control	Western blotting	(91)
↑ Albumin in plasma (1.1-fold (p<0.05))	25 AD (11 males, 14 females), 25 hospitalized patients as control (10 males, 15 females)	Immunonephelometric method	(92)
↓ Serum albumin precursor in plasma (0.09-fold, p=0.0250) ↑ Desmoplakin in plasma (1.78-fold, p=0.0003)	50 AD & 50 controls	Mass Spectrometry	(93)

Table 3. Enzymes and other protein-based biomarkers for AD.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↓ ADNP in serum (5-fold, $p < 0.05$) ↑ Actin in serum ↑ ITIH4 in serum ↑ ATIII in serum ($p < 0.05$)	45 early-stage AD (ages: 63–84 years with a mean of 77.2) & 20 control (ages: 55–84 years with a mean of 72.3)	Western blotting & ELISA & 2D gel electrophoresis combined with nano-HPLC-ESI-MS/MS	(94)
↓ REST in plasma	AddNeuroMed cohort: 65 healthy elderly, 36 stable MCI, 29 converter MCI, 65 AD	Sandwich enzyme-linked immunosorbent assay	(95)
↑ Hyperphosphorylated TDP-43 in plasma (in 46% of FTD vs. 22% of AD vs. 8% of control)	35 with FTD, 102 AD, 85 control subjects	ELISA	(96)
↑ Cystatin C in plasma ($p < 0.001$)	88 patients with dementia (43 AD, 45 VAD) & 45 controls	Latex-enhanced reagent, Behring BN ProSpec Analyzer	(97)
↓ AGT in plasma ↑ IGFBP-2 in plasma ↑ OPN in plasma ↓ SAP in plasma ↑ Cathepsin D in plasma	98 AD & 101 elderly controls	Luminex xMAP technology	(98)
Multiple CSF protein biomarker panels Synaptic and metabolic: ↑in CSF - ↓in brain Glial-enriched myelination and immunity: ↑in CSF - ↑in brain	Multiple studies	Mass spectrometry & Multiplex tandem mass tag (TMT)	(99)

NfL (Neurofilament light), PTP (pancreatic thread protein), ADNP (activity-dependent neuroprotector homeobox protein), ITIH4 (Inter-alpha-trypsin inhibitor heavy chain H4), ATIII (Anti-thrombin-III), REST (Repressor element 1-silencing transcription), FTD (frontotemporal dementia), AD (Alzheimer's disease), CU (cognitively unimpaired), MCI (mild cognitive impairment), VAD (Vascular dementia), TDP-43 (TAR DNA binding protein 43)

Inflammation and immune based biomarkers

In addition to β -amyloid and tau pathologies, some other pathological pathways at the molecular level have also been reported in the literature. As one of the key pathways, inflammation and inflammatory processes have attracted much attention in AD development and progression. Inflammation was assumed a subsequent of $A\beta$ deposition; nonetheless, clinical studies have shown that inflammatory responses are evident much earlier, in preclinical AD independently of $A\beta$ deposition. In fact, AD is also characterized by neuroinflammation, and it is believed that immune system contributes, at least in some part, to the pathogenesis and exacerbation of AD. For instance, brain's microglia drives the neuroinflammation which exacerbates with disease progression (100, 101).

Although still controversial, several investigations' results claimed that use of nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of AD (102). The results were also supported by a recent meta-analysis undertaken by Zhang et al. (103). However, the efficacy of NSAIDs still needs further investigation. Genomics studies also have revealed the role of immune system in predisposing to AD (104).

According to our comprehensive literature survey, more than hundred inflammatory cytokines, in total, have been reported to change in blood levels of individuals with AD (Table 4). Some of these biomarkers

are anti-inflammatory (e.g., TGF- β , IFN- γ , and IL-10) while most of them are pro-inflammatory cytokines (e.g., TNF- α , IL-2, IL-4, IL-6, CRP, complement factors, and β 2-microglobulin).

Aside from whether the inflammatory responses are “deleterious or beneficial” in AD pathology, the analytes can provide valuable insights into the state of the disease. Pillai et al. used a multiplex system for investigation of inflammatory pathways in AD and showed that inflammation-based biomarkers correlate well with neurodegeneration state and cognition decline. TNF signaling pathway, complement and coagulation cascade correlated with tau and A β ₄₂ levels, respectively. The post-mortem brain tissue transcriptomics also revealed the overexpression of correlated genes (105). Morgan and co-workers, reported ten plasma biomarkers (including factor H, factor B, C3, C4, C5, MCP-1, CRP, sCR1, Eotaxin-1, and MIP-1b) involved in inflammatory pathways which can discriminate AD from non-AD individuals. sCR1, MCP-1, and Eotaxin-1 could even optimally differentiate AD and MCI patients (106).

In most studies plasma level of C-reactive protein (CRP) was used for differentiating AD from control group since it decreases as AD progresses (106, 107). Increased plasma level of glial fibrillary acidic protein (GFAP) was also detected in MCI patients developing AD during the follow up studies (82, 108).

According to subtypes and stages of AD, contribution of inflammatory and immune-related biomarkers may be different, accompanying various hallmarks and risk factors (109). Nevertheless, multiplex detection of inflammatory biomarkers might be a doorway towards early diagnosis of AD. Table 4 shows the Inflammatory and immune based AD biomarkers.

Table 5. Inflammatory and immune based AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
TNF-related biomarkers			
TNF- α			
↑ in peripheral blood (p= 0.01)	680 AD & 447 controls (Pooled from 14 studies)	A Meta-Analysis of Cytokines in AD	(110)
↑ in serum (~ 1.65-fold in severe AD, p<0.001)	11 severe AD & 25 mild AD	ELISA	(111)
↓ in plasma (2.6-fold)	85 AD, 47 MCI, 11 other dementias, 21 other neurological diseases, 79 control	Filter-based, arrayed sandwich ELISA (microarray)	(112)
↓ in Serum (0.74-fold)	197 AD & 203 controls	Multiplex immunoassay	(89, 90)
↔ in Serum	34 AD, 33 VAD, 30 control	ELISA	(113)
↔ in Plasma (p=0.41))	28 AD, 13 MCI, 60 control (Taiwanese)	Immunoassays	(49)
TNF- β			
↑ in Serum (1.37-fold)	197 AD & 203 controls	Multiplex immunoassay	(89, 90)
↓ in Serum (0.65-fold)	32 AD & 19 Controls	ELISA	(114)
TRAIL-R4			
↑ in plasma (1.8-fold)	85 AD, 47 MCI, 11 other dementias, 21 other neurological diseases, 79 control	Filter-based, arrayed sandwich ELISA (microarray)	(112)
TNF receptor like 2			

Table 5. Inflammatory and immune based AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ in Plasma (1.27-fold, p= 0.0002)	207 AD & 754 controls	Multiplex panel (Human DiscoveryMAP)	(88)
sTNFR-II			
↓ in Serum (0.7-fold)	32 AD & 19 Controls	ELISA	(114)
TGF-β			
↑ in peripheral blood (p=0.0006)	190 AD & 158 controls (Pooled from 5 studies)	A Meta-Analysis of Cytokines in AD	(110)
↓ in Serum (0.77-fold)	32 AD & 19 Controls	ELISA	(114)
↔ in Serum	30 Probable AD & 25 control	Radioimmunoassay	(115)
Interferons			
IFN-α			
↔ in Plasma	20 AD & 17 controls	ELISA	(116)
IFN-γ			
↔ in peripheral blood (p=0.55)	113 AD & 102 controls (Pooled from 4 studies)	A Meta-Analysis of Cytokines in AD	(110)
↔ in Serum	30 Probable AD & 25 control	Radioimmunoassay	(115)
↔ Plasma	20 AD & 17 controls	ELISA	(116)
Interleukins			
IL-1β			
↑ in peripheral blood (p=0.00001)	574 AD & 370 controls (Pooled from 10 studies)	A Meta-Analysis of Cytokines in AD	(110)
↑ in plasma (~ 10-fold, p<0.001-Detectable in 17 cases of AD but only in 1 control)	145 AD & 51 control	ELISA	(117)
↔ in Plasma	11 Sporadic AD, 22 PD, 12 control	ELISA	(118)
↔ in Serum	34 AD, 33 VAD, 30 control	ELISA	(113)
↔ in serum (p=0.95)	11 severe AD & 25 mild AD	ELISA	(111)
↔ in Serum	40 AD, 20 PD, 42 control	ELISA	(119)
IL-1α			
↓ in plasma (2.9-fold)	85 AD, 47 MCI, 11 other dementias, 21 other neurological diseases, 79 control	Filter-based, arrayed sandwich ELISA (microarray)	(112)
IL-1ra			
↓ in Serum (0.81-fold)	197 AD & 203 controls	Multiplex fluorescent immunoassay	(89, 90)
IL-2			
↔ in Plasma	11 Sporadic AD, 22 PD, 12 control	ELISA	(118)
IL-3			
↓ IL-3 in plasma (2.0-fold)	85 AD, 47 MCI, 11 other dementias, 21 other neurological diseases, 79 control	Filter-based, arrayed sandwich ELISA (microarray)	(112)
IL-4			
↔ in peripheral blood (p=0.59)	68 AD & 66 controls (Pooled from 4 studies)	A Meta-Analysis of Cytokines in AD	(110)
IL-6			

Table 5. Inflammatory and immune based AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ in peripheral blood (p=0.00001)	985 AD & 680 controls (Pooled from 14 studies)	A Meta-Analysis of Cytokines in AD	(110)
↑ in Plasma (5-fold)	128 AD & 83 controls (Japanese)	ELISA	(120)
↑ in Plasma (6-fold - Detected in 53% of AD and 27% of controls)	145 AD & 51 control	ELISA	(117)
↑ in Plasma (~5.5-fold, p=0.032)	20 AD & 17 controls	ELISA	(116)
↑ in Serum (~ 6.4-fold in severe AD, no change in mild-moderate AD)	26 Severe AD, 15 mild-moderate AD & 24 control	ELISA	(121)
↑ in Serum	34 AD, 33 VAD, 30 control	ELISA	(113)
↔ in Plasma (p=0.65)	28 AD, 13 MCI, 60 control (Taiwanese)	Immunoassays	(49)
↔ in Plasma	11 Sporadic AD, 22 PD, 12 control	ELISA	(118)
↔ in Serum	41 AD & 32 control	ELISA	(122)
↔ IL-6 in Serum	97 AD & 79 control (age- and sex-matched)	Bioassay	(123)
sIL-6R			
↓ in Serum (~0.84-fold, p<0.001)	41 AD & 32 control	ELISA	(122)
IL-8 (CXCL8)			
↑ in plasma (1.7-fold)	85 AD, 47 MCI, 11 other dementias, 21 other neurological diseases, 79 control	Filter-based, arrayed sandwich ELISA (microarray)	(112)
↑ in Serum (1.15-fold)	197 AD & 203 controls	Multiplex fluorescent immunoassay	(89, 90)
↔ in peripheral blood (p=0.36)	69 AD & 64 controls (Pooled from 3 studies)	A Meta-Analysis of Cytokines in AD	(110)
IL-10			
↑ in Plasma (1.10-fold, p< 0.0001)	207 AD & 754 controls	Multiplex panel (Human DiscoveryMAP)	(88)
↓ in Serum (0.76-fold)	197 AD & 203 controls	Multiplex fluorescent immunoassay	(89, 90)
↔ in Serum	30 Probable AD & 25 control	Radioimmunoassay	(115)
↔ in peripheral blood (p=0.90)	156 AD & 123 controls (Pooled from 4 studies)	A Meta-Analysis of Cytokines in AD	(110)
IL-11			
↑ in plasma (2.1-fold)	85 AD, 47 MCI, 11 other dementias, 21 other neurological diseases, 79 control	Filter-based, arrayed sandwich ELISA (microarray)	(112)
IL-12			
↑ in peripheral blood (p < 0.00001)	148 AD & 106 controls (Pooled from 5 studies)	A Meta-Analysis of Cytokines in AD	(110)
↔ in Plasma	20 AD & 16 controls	ELISA	(116)
↔ in Serum	30 Probable AD & 25 control	Radioimmunoassay	(115)
IL-15			
↑ in Serum (1.26-fold)	197 AD & 203 controls	Multiplex fluorescent immunoassay	(89, 90)
IL-17			

Table 5. Inflammatory and immune based AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↓ in Plasma (0.87-fold, $p < 0.0001$)	207 AD & 754 controls	Multiplex panel (Human DiscoveryMAP)	(88)
IL-18			
↑ in peripheral blood ($p=0.03$)	131 AD & 94 controls (Pooled from 4 studies)	A Meta-Analysis of Cytokines in AD	(110)
Other biomarkers			
↔ CRP in peripheral blood ($p=0.67$)	253 AD & 155 controls (Pooled from 5 studies)	A Meta-Analysis of Cytokines in AD	(110)
↓ CRP in plasma	Plasma test: 112 AD & 52 control	Multiplex fluorescent	(107)
↓ CRP in serum	Serum test: 197 AD & 199 control		
↓ CRP in Plasma (Differentiate AD from control)	262 AD, 199 MCI, 259 control	Commercial and In-house Singleplex and Multiplex assays	(106)
↑ Complement C4 in Plasma (Differentiate AD from control)			
↑ Complement C3 in Plasma (Differentiate AD from control)			
↑ Complement C5 in Plasma (Differentiate MCI from control)			
↑ CCL2 in Plasma (Differentiate AD from control)			
↑ Factor H in Plasma (Differentiate MCI from control)			
↓ Factor B in Plasma (Differentiate MCI from control)			
↓ sCR1 in Plasma (Differentiate AD from control)			
↔ CRP in Plasma	145 AD & 51 control	Radial immune-diffusion kit & ELISA	(117)
↑ ACT in Plasma (~ 1.25-fold, $p < 0.001$)			
↔ Neopterin in Plasma			
↓ CRP in Serum (0.86-fold)	197 AD & 203 controls	Multiplex fluorescent immunoassay	(89, 90)
↑ NTP in Serum (1.33-fold)			
↑ $\alpha 2M$ (α -2-macroglobulin) in Serum (2.45-fold)			
↑ B-2-microglobulin in Serum (1.36-fold)			
↓ CCL3 in Serum (0.70-fold)			
↓ Creatine kinase MB in Serum (0.80-fold)			
↓ G-CSF in serum (0.7-fold)			
↑ IGFBP-2 in Serum (1.23-fold)			
↑ TPO in Serum (2.18-fold)			
↑ Eotaxin 3 in Serum (1.26-fold)			
↑ Tenascin C in Serum (1.60-fold)			
↑ Fas ligand in Serum (1.03-fold)			
↑ Prolactin in Serum (1.21-fold)			
↑ Resistin in Serum (1.17-fold)			

Table 5. Inflammatory and immune based AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↓ SCF in Serum (0.74-fold) ↓ S-100B in Serum (0.72-fold) ↓ Prostatic acid phosphatase in Serum (0.78-fold) ↔ CRP in Serum ↔ SAP in Serum	140 AD & 30 control	ELISA	(124)
↓ Complement C4 precursor in Plasma (0.49-fold, p=0.0206) ↓ CD5 antigen-like precursor in Plasma (0.62-fold, p=0.0290) ↑ α2M precursor in Plasma (8.83-fold, p=0.0060) ↑ CFH precursor in Plasma (13.7-fold, p=0.0014) ↑ Ig kappa chain C region in Plasma (2.11-fold, p=0.0013) ↑ Ig lambda chain C regions in plasma (2.43-fold, p=0.0054) ↓ ITIH4 precursor in Plasma (0.24-fold, p=0.0155)	50 AD & 50 controls	Mass Spectrometry	(93)
↑ Complement C4 in plasma ↑ β-2-microglobulin in Plasma ↑ BDNF in plasma	98 AD & 101 CU controls	Luminex xMAP technology	(98)
↑ CD40 in Plasma (1.21-fold, p=0.040) ↑ AAT in Plasma (1.11-fold, p=0.0003) ↑ NTP in Plasma (1.54-fold, p<0.0001) ↑ β-2-microglobulin in Plasma (1.24-fold, p=0.006) ↑ CCL3 in Plasma (1.12-fold, p=0.006) ↑ B lymphocyte chemoattractant in Plasma (1.45-fold, p=0.002) ↑ Carcinoembryonic antigen in Plasma (1.4-fold, p=0.001) ↑ MMP-2 in Plasma (1.13-fold, p=0.001) ↑ MMP-9 in Plasma (1.19-fold, p=0.001) ↑ TIMP-1 in Plasma (1.18-fold, p=0.0003) ↑ VCAM-1 in Plasma (1.18-fold, p<0.0001) ↓ EGFR in Plasma (0.89-fold, p=0.012) ↑ IGFBP-2 in Plasma (1.61-fold, p<0.0001) ↑ Angiopoietin 2 in Plasma (1.23-fold, p=0.003) ↑ Homocysteine in plasma (1.23-fold, p=0.002)	207 AD & 754 controls	Multiplex panel (Human DiscoveryMAP)	(88)

Table 5. Inflammatory and immune based AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ Erythrocyte sedimentation rate in plasma (1.46-fold, p=0.002)			
↑ Cortisol in Plasma (1.28-fold, p<0.0001)			
↑ Lcn2 in plasma	38 Controls, 41 MCI, 62 AD	ELISA	(125)
↓ Lcn2 in CSF	26 AD, 28 MCI, 26 Control	ELISA	(126)
↔ Lcn2 in serum			
↔ ACT in Serum	40 AD, 20 PD, 42 control	ELISA	(119)
↑ AAT in serum (~2-fold)	45 early-stage AD, 20 control	ELISA & 2D gel electrophoresis combined with nano-HPLC-ESI-MS/MS & western blotting	(94)
↑ ITIH4 in Serum			
↑ NTP in Serum (40-fold than CSF, correlation between serum and CSF and between the CSF/serum albumin ratio (BBB damage marker))	-	Microparticle enzyme immunoassay	(127)
↑ CCL2 in CSF (p < 0.005)	21 AD		(128)
↑ CCL5 in Plasma	96 AD, 44 MCI, 42 depression with or without cognitive impairment, 19 controls	Searchlight Multiplex ELISA	(129)
↑ CCL15 in Plasma			
↑ CCL18 in Plasma			
↑ EGF in Plasma			
↑ GDNF in Plasma (~22-fold)			
↑ GFAP in plasma	-	Simoa technology & MS.	(108)
↔ TREM2 in plasma			
↑ GFAP in plasma (1.6- fold)	Longitudinal study: 85 consisting of individuals with MCI (follow-up for 4 years) Cross-sectional study: 95 AD, 53 other NDD, 90 CU	Electrochemiluminescence immunoassays	(82)
↓ CCL5 in Plasma (2.9-fold)	85 AD, 47 MCI, 11 other dementias, 21 other neurological diseases, 79 control	Filter-based, arrayed sandwich ELISA (microarray)	(112)
↓ CCL7 in Plasma (1.7-fold)			
↓ CCL15 in Plasma (1.6-fold)			
↑ CCL18 in Plasma (1.9-fold)			
↑ ICAM-1 in Plasma (2.2-fold)			
↓ EGF in Plasma (2.7-fold)			
↓ G-CSF in Plasma (1.9-fold)			
↓ M-CSF in Plasma (2.4-fold)			
↓ PDGF-BB in Plasma (3.4-fold)			
↓ GDNF in Plasma (1.8-fold)			
↑ IGFBP-6 in Plasma (1.5-fold)			
↑ Angiopoietin 2 in Plasma (2.1-fold)			
↓ CCL5 in Plasma	95 AD & 88 Controls	Sandwich ELISA	(114)
↓ BDNF in Plasma			
↓ Leptin in Plasma			
↓ MSP-α in Serum (0.76-fold)	32 AD & 19 Controls	ELISA	(114)
↓ NAP-2 in Serum (0.67-fold)			
↓ NT-3 in Serum (0.7-fold)			

Table 5. Inflammatory and immune based AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↓ TIMP-1 in Serum (0.72-fold) ↓ TIMP-2 in Serum (0.75-fold) ↓ FGF in Serum (Basic FGF: 0.67-fold - FGF-6: 0.66-fold) ↓ TPO in Serum (0.71-fold)			
↓ FGF21 in plasma ↔ GDF15 in plasma (↑ DM2) ↓ Humanin (HN) in plasma	102 centenarian offspring (OFF), 92 controls, 162 DM2 without complications, 93 DM2 with complications, 120 AD	ELISA	(130)
↑ CCL15 (1.2-fold) ↔ CCL18 in Plasma ↔ ICAM-1 in Plasma ↑ EGF in Plasma (1.3-fold) ↑ PDGF-BB in Plasma (1.3-fold) ↔ Angiotensin in Plasma	142 AD, 174 control, 88 other dementias	Antibody array	(131)
↓ IgG anti-Aβ42 in Serum (0.4-fold, p<0.01) ↔ Isoprostanes in Plasma	39 AD & 39 controls 12 AD, 25 probable AD, 5 dementia with Lewy bodies and HD as a control group	ELISA	(59) (132)
↑ ACE in CSF ↑ Angiotensinogen in CSF ↑ Angiotensin in CSF	20-21 AD, 8-10 MCI, 25-28 control	ELISA	(133)
↑ Homocysteine in Plasma (>14 μmol/L almost doubled the risk of AD)	1092 CU among whom 83 developed to AD over a median follow-up period of 8 years	HPLC	(134)
↓ Eotaxin-1 in Plasma (Differentiate AD from control)	262 AD, 199 MCI, 259 control	Commercial and In-house Singleplex and Multiplex assays	(106)

AD (Alzheimer's disease), CU (cognitively unimpaired), MIC (mild cognitive impairment), VAD (Vascular dementia), PD (Parkinson's disease), HD (Huntington's disease), AAT (α -1-antitrypsin), ACE (angiotensin-converting enzyme), ACT (α -1-antichymotrypsin), BDNF (brain-derived neurotrophic factor), BNT77 (IgA anti- A β 11-28), CCL15 (MIP-1- δ , Leukotactin-1, MIP-5, HCC-2, NCC-3), CCL18 (MIP4, PARK, PARC), CCL2 (MCP-1: monocyte chemoattractant protein-1), CCL3 (MIP1 α macrophage inflammatory protein 1- α), CCL5 (RANTES), CCL7 (MCP-3), CRP (C-reactive protein), FGF (Fibroblast growth factor), GDNF (glial-derived neurotrophic factor), GFAP (glial fibrillary acidic protein), ICAM-1 (intercellular adhesion molecule-1), IGFBP (Insulin like growth factor binding protein), ITIH4 (Inter-alpha-trypsin inhibitor heavy chain H4), Lcn2 (Lipocalin), MMP (Matrix metalloproteinase), MSP- α (Macrophage stimulating protein- α), NAP-2 (Neutrophil activating peptide-2), NT-3 (Neurotrophin-3), NTP (neuronal thread protein or pancreatic thread protein), PDGF-BB (platelet-derived growth factor BB), S-100B (pro-inflammatory agent), SAP (glycoprotein serum amyloid P), SCF (Stem cell factor), sCR1 (Plasma soluble complement receptor 1), sIL-6R (Soluble IL-6 receptor), sTNFR-II (soluble TNF receptor II), sTREM2 (soluble triggering receptor expressed on myeloid cells 2), TIMP (Tissue inhibitor of metalloproteinase), TRAIL-R4 (TNF-related apoptosis-inducing ligand receptor-4), TPO (Thrombopoietin), VCAM-1 (vascular cell adhesion molecule 1), GDF (Growth Differentiation Factor 15), DM2 (type 2 diabetes mellitus)

Genetic materials

The role of genetic factors in predisposition to AD is evident now. About 25% of AD is familial (135) and the heritability of the disease is estimated to be up to 80% (136). Since the discovery of three mutations (in Amyloid precursor protein, Presenilin 1, and Presenilin 2) causing autosomal dominant AD (with a prevalence of <1%), the search for other genetic risk loci for the more common form of AD (the late-onset) was massively started. To date, more than 25 established risk genes involved in AD susceptibility have been

discovered (136-138). Genome-Wide Association Studies (GWAS) and the International Genomics of Alzheimer's Project (IGAP) consortium have had a considerable role in advancing polygenic basis of AD. Although "neither necessary nor sufficient for the disease" (135), $\epsilon 4$ allele of ApoE has the most genetic risk for both early- and late-onset AD (139). Recently, the ApoE status and polygenic risk scores (PRS) have attracted much attention as genetic biomarkers for AD prediction (140).

Genetic alterations may also be revealed through transcriptomics, i.e., at RNA level. Indeed, genetic variants are not always affecting the pathophysiology directly. Sometimes the alteration is at the gene regulation and/or post translational modifications (PTM) levels (136). Gene dysregulations that contribute to AD occur early in the course of the disease pathology process. Transcriptomics can be applied to identify ill gene expressions in (preclinical stage of) AD (141) and therefore is useful for diagnosis/prediction of the disease through body fluids. We summarized investigated transcriptome in Table 5.

Table 6. AD biomarkers related to genetic materials

Biomarker alteration in body fluid	Studied group	Method	Ref.
7-miRNA signature in plasma (95% accuracy): ↓ hsa-let-7d-5p ↓ hsa-let-7g-5p ↓ hsa-miR-15b-5p ↓ hsa-miR-142-3p ↓ hsa-miR-191-5p ↓ hsa-miR-301a-3p ↓ hsa-miR-545-3p	Cohort 1: 11 AD, 9 MCI and 20 CU Cohort 2: 20 AD, 17 CU	TaqMan qPCR	(142)
12-miRNA signature in plasma (93% accuracy): ↓ hsa-let-7f-5p ↓ hsa-miR-1285-5p ↓ hsa-miR-107 ↓ hsa-miR-103a-3p ↓ hsa-miR-26b-3p ↓ hsa-miR-26a-3p ↓ hsa-miR-532-3p ↑ hsa-miR-151a-3p ↑ brain-mir-161 ↑ hsa-let-7d-3p ↑ brain-miR-112 ↑ hsa-miR-5010-3p	Study1: 48 AD & 22 control Study2: 94 AD & 18 control	RT-qPCR	(143)
16-miRNA signatures with ApoE $\epsilon 4$ in plasma: ↓ hsa-miR-1306-5p ↓ hsa-miR-342-3p ↓ hsa-miR-15b-3p ↑ hsa-miR-361-5p ↑ hsa-miR-30e-5p ↑ hsa-miR-93-5p ↑ hsa-miR-15a-5p	16 AD & 36 control	qRT-PCR	(144)

Table 6. AD biomarkers related to genetic materials

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ hsa-miR-143-3p			
↑ hsa-miR-335-5p			
↑ hsa-miR-106b-5p			
↑ hsa-miR-101-3p			
↑ hsa-miR-424-5p			
↑ hsa-miR-106a-5p			
↑ hsa-miR-18b-5p			
↑ hsa-miR-3065-5p			
↑ hsa-miR-20a-5p			
↑ hsa-miR-582-5p			
↑ miRNA-34a in plasma and PBMCs	78 AD & 85 control	TAQMAN	(145)
↑ miRNA-34c in plasma and PBMCs	25 AD & 27 control	MicroRNA REAL TIME qPCR	
↓ miR-146b-5p in Peripheral blood	40 AD & 31 CU	PAXgene Blood miRNA Kit (Qiagen, Germany)	(146)
↓ miR-15b-5p in Peripheral blood			
↑ miR-92a-3p in plasma (p=0.0442)	38 CU, 26 MCI, 56 AD, 27 FTD	RT-qPCR	(147)
↑ miR-181c-5p in plasma (p=0.0024)			
↑ miR-210-3p in plasma (p=0.0006)			
↓ miR-9 in whole blood (3-fold, p=0.001)	36 probable AD & 38 controls (women)	RT-qPCR	(148)
↓ Heme oxygenase-1 (HO-1) mRNA in plasma	46 AD & 25 controls, 13 PD, 30 MCI, 9 control	Northern blotting	(149)
RNA signature in whole-blood (170 oligonucleotide probe sets associated with 133 genes)	80 AD & 70 control	RT-PCRs	(150)

AD (Alzheimer's disease), CU (cognitively unimpaired), MCI (mild cognitive impairment), FTD (frontotemporal dementia)

Metabolic biomarkers

The idea that AD is basically a metabolic disorder in which different biochemical metabolic pathways run in a pathologic way is growing (151). Having immediate correlation with cell functions, metabolic pathways are considered as the “last avenue to explore” and the most straightforward indicative of pathophysiological conditions (152).

Metabolomics of AD vs. non-AD subjects revealed multiple metabolic pathways are affected. Lipid biosynthesis and metabolism (153-156), cholesterol, sphingolipids, and glycerophospholipids metabolism and transport (155, 157), neurotransmitter metabolism and signaling (151, 155, 158), amino acid metabolism (151, 155, 158), transmethylation (151), polyamine synthesis and catabolism (151), glucose metabolism (159), energy metabolism (155, 160, 161), Krebs cycle (155), urea cycle (151), mitochondrial function (151, 155), aminoacyl-tRNA biosynthesis (155), glutathione synthesis and oxidative stress (151, 160), and hyperammonemia (160) are examples of affected metabolic pathways in AD patients vs. controls. It seems that abnormality in basic metabolic pathways such as glycolysis and transmethylation may upset other related metabolic pathways (151).

It has been shown that the metabolic abnormalities may start at pre-clinical stage (159) and that the severity and the progression of the disease is associated with the metabolite concentrations (151, 157). Sphingolipids (157), desmosterol and desmosterol/cholesterol ratio (162) have been suggested as specific biomarkers for early diagnosis of AD (Table 6).

In a metabolomics screening of 73 CSF samples, diagnostic accuracy based on the proposed biomarkers was 83%, which is claimed to be more accurate compared with using classical biomarkers ($A\beta_{42}$ and tau) (158).

Table 7. Metabolic AD biomarkers

Biomarker alteration in body fluid	Studied group	Method	Ref.
Lipids			
↓ ApoE in plasma (0.97-fold, $p=0.042$)	207 AD & 754 controls	ELISA (commercial assay)	(88)
↓ ApoE in plasma ↑ ApoCIII in serum ↑ Lipoprotein (a) in serum	203 off-springs of AD & 197 non-AD parents (Texas Alzheimer's Research Consortium)	Multiplex fluorescent immunoassay	(89, 90)
↓ ApoA1 in serum (0.8-fold, $p=10^{-7}$) ↓ HDL in serum (0.7-fold, $p=10^{-7}$)	98 AD & 59 controls	Immunonephelometry on Behring Nephelometer Analyzer, with Behring reagents	(165)
↑ ApoA-IV in serum ($p<0.05$)	45 early-stage AD & 20 control	ELISA & 2D gel electrophoresis combined with nano-HPLC-ESI-MS/MS	(94)
↑ Cholesterol in serum	411 AD or normal, 87 having both the ApoE genotype and TC	-	(166)
↓ Cholesterol in serum (0.92-fold, $p<0.05$) ↔ TG (triglycerides) in serum (0.7-fold, $p<0.0000001$)	80 AD & 59 controls	Enzymatic methods	(165)
↔ Cholesterol in plasma ($p<0.31$) ↑ 24S-Hydroxycholesterol in plasma (1.25-fold, $p<0.001$)	30 AD & 30 controls	Enzymatic methods & Isotope dilution-mass spectrometry	(167)
↔ Cholesterol in plasma ($p>0.05$) ↓ 24S-Hydroxycholesterol in plasma ($p<0.05$) ↓ 27-Hydroxycholesterol (0.78-fold, $p<0.01$) ↔ HDL in plasma ($p>0.05$)	20 AD, 8 MCI, 25 controls	Isotope dilution-mass spectrometry	(133)
↓ HDL in plasma	43 AD, 45 VAD, 45 controls	Enzymatic methods (commercial kits)	(97)
↓ HDL in plasma	103 women (age 97.7 ± 0.2) & 37 men (age 97.6 ± 0.4)	-	(168)
↑ Cortisol in plasma (1.28-fold, $p<0.0001$)	AIBL cohort: 754 controls & 207 AD ADNI cohort: 58 controls & 112 AD	ICP-MS & 151-analyte multiplex panel & sandwich ELISA	(88)

Table 7. Metabolic AD biomarkers

Biomarker alteration in body fluid	Studied group	Method	Ref.
↓ Progranulin in plasma	70 control, 72 early-onset probable AD, 9 symptomatic and 18 asymptomatic relatives of <i>GRN</i> mutation families	ELISA	(169)
↑ Clusterin (Apo J) in plasma	Discovery cohort: 95 Validation cohort: 689 (additional 60 subjects)	Sandwich ELISA & Two-dimensional gel electrophoresis and LC-MS-MS	(170)
↑ Clusterin (Apo J) in plasma (p<0.15)	16 MCI among 139 non-demented (with longitudinal monitoring)	ELISA	(171)
↑ Clusterin (Apo J) in plasma	98 AD & 101 controls	Luminex technology	xMAP (98)
↔ Clusterin (Apo J) in plasma	171 control, 127 AD, 82 with other dementias, 30 with depression	ELISA	(172)
Others			
↑ 2,4-dihydroxybutanoic acid in serum (p=0.0048)	46 controls, 143 MCI, 47 AD	GC-TOFMS & UPLC/MS	(173)
↑ Glutamine in plasma	Study1: 43 AD, 45 MCI, 41 controls	LC-MS	(174)
↓ Piperine in plasma	Study2: 50 AD, 18 controls		
↓ 3 acylcarnitines: Decanoylcarnitine Pimelylcarnitine Tetra decadienylcarnitine	longitudinal clinical-pathologic cohort studies of aging and dementia: 436 Non-Converter & 85 Converter	Biocrates AbsoluteIDQ® p180 Kit & FIA-MS/MS & UPLC-MS/MS	(175)

AD (Alzheimer's disease), CU (cognitively unimpaired), MCI (mild cognitive impairment), VAD (Vascular Alzheimer's disease), TC (Total serum cholesterol)

ApoE genotype may affect metabolic pathways (163). A metabolomics analysis revealed that ApoE4 genotype might changes lipid metabolism and increases lysophosphatidylcholine and glycerophospholipids in early stages of AD. Based on these metabolites the authors reported the satisfactory discrimination of early AD patients from healthy controls with $R^2=0.738$ (164).

Vitamins and bio-metals

The role of vitamins contributing to AD was investigated by several studies. Vitamin D, E, A, C, B₁₂, and folate are the most prominent ones. Hyperhomocysteinemia is also shown to be prevalent in AD patients (Table 7).

Nevertheless, the effect size of these elements is not that significant, and the findings are controversial. While some researchers say that concurrent use of vit. C and E are associated with a reduced risk of AD (176), there are studies which showed that neither intake of antioxidant vitamins such as carotenes, vit. C, and vit. E has any effect on lowering the risk of AD development (177) nor high doses of vit. B and folate has any effect on slowing cognitive decline (178). Vit. D is another vitamin that its contribution in AD is controversial. A meta-analysis showed that the risk of AD development is not associated with the level of vit. D (179). More details can be found in Table 7.

The investigations have revealed the dyshomeostasis of metal ions in AD. Findings show modified expression levels and distribution of bio-metal transporters in AD, as well as accumulation of transition

metals such as zinc, iron, and copper, in the brain of patients (180, 181). Although the exact pathological role of the transition metals has not been determined, some studies suggest the accumulation of these metals may be associated to AD (182) via acceleration of A β deposition (183) and microtubule anomaly induction (184). The co-measurement of plasma metals has been suggested as potential BBBs for AD diagnosis as shown in table 7 (185-187).

Table 8. Vitamin and bio-metal AD biomarkers

Biomarker alteration in body fluid	Studied group	Method	Ref.
Vitamins			
↓ Vitamin B12 in serum ($p < 0.001$)	108 Control, 164 Clinically diagnosed AD, 76 Histologically confirmed AD	Radioimmunoassay	(188)
↓ Vitamin C in plasma (AD ($p < 0.001$), VAD ($p < 0.001$), PD & dementia ($p < 0.01$)) ↓ Vitamin A in plasma (AD ($p < 0.01$), VAD ($p < 0.001$)) ↓ Vitamin E in plasma (AD ($p < 0.01$), VAD ($p < 0.001$))	79 AD, 37 VAD, 18 PD & dementia, 58 Matching control & 41 PD, 41 Matching controls	Fluorimetric assay & HPLC	(189)
↓ 25-hydroxy vitamin D in plasma	10186 (white Danish general population- During 30 years of follow-up, 418 developed AD and 92 developed VAD)	DiaSor in Liaison 25(OH)D TOTAL assay	(190)
Biometals			
↑ Zinc in serum (1.9-fold)	9 AD & 8 controls	Flame atomic absorption spectrometry	(191)
↓ Zinc in plasma (0.91-fold, $P < 0.0001$)	207 AD & 754 controls	ICP-MS	(88)
↓ Zinc in serum ($p = 0.043$) ↔ Copper in serum ($p = 0.560$)	28 AD, 13 MCI, 6 control (Taiwanese)	Flame atomic absorption spectrometry	(49)
↓ Zinc in serum (0.89-fold, $p = 0.0007$) ↑ Arsenic in serum ($p < 0.0001$) ↑ Aluminum in serum (1.6-fold) ↑ Chromium in serum (1.3-fold) ↓ Iron in serum (0.74-fold)	44 AD & 41 control	ICP-MS	(192)
↓ Zinc in serum (0.77-fold) ↑ Chromium in serum (2.6-fold) ↓ Cobalt in serum ↓ Nickle in serum ↓ Iron in serum (0.47-fold)	50 AD & 50 control	ICP-MS	(193)
↔ Zinc in plasma ($p = 0.36$) ↓ Copper in plasma (0.87-fold, $p = 0.018$)	16 AD & 13 control	Atomic Absorption Spectroscopy	(194)
↑ Copper in serum ($p < 0.001$) ↑ Ceruloplasmin in Serum ($p = 0.052$)	47 AD, 24 VAD, 44 controls	Atomic Absorption Spectroscopy	(195)
↔ Free Copper in serum ($p = 0.24$) ↑ Cu/Ceruloplasmin in serum ($p = 0.01$)	28 AD & 29 controls	Atomic Absorption Spectroscopy & enzymatic (eCp) and immunologic (iCp) methods	(196)

Table 8. Vitamin and bio-metal AD biomarkers

Biomarker alteration in body fluid	Studied group	Method	Ref.
↓ Calcium in plasma (0.94-fold, $p < 0.0001$) ↓ Hemoglobin in plasma (0.95-fold)	AIBL cohort: 754 controls & 207 AD ADNI cohort: 58 controls & 112 AD	ICP-MS & 151-analyte multiplex panel & sandwich ELISA	(88)
↔ Iron in serum ($p = 0.428$)	28 AD, 13 MCI, 6 control (Taiwanese)	Flame atomic absorption spectrometry	(49)
↑ Heme oxygenase suppressor-1 (HOS) in plasma (AD: 2.7-fold, $p < 0.001$) ↑ $\alpha 1$ -antitrypsin (AAT) in plasma (AD: $p < 0.05$)	46 AD, 13 PD, 30 MCI, 25 controls >60year, 9 control <60year	Glial bioassay for HOS activity & Turbidometric assay	(149)
↑ $\alpha 1$ -antitrypsin (AAT) in plasma (~107-fold, $p < 0.001$) ↑ Transferrin in plasma (~5-fold, $p < 0.001$) ↑ Hemopexin in plasma (~6.5-fold, $p < 0.001$)	10 sporadic AD & 9 controls	Affinity chromatography & SDS-PAGE & Western blotting & MALDI-TOF MS	(197)
↑ $\alpha 1$ -antitrypsin (AAT) in serum ($p < 0.05$)	45 early-stage AD & 20 control	ELISA & 2D gel electrophoresis combined with nano-HPLC-ESI-MS/MS	(94)
↓ Transferrin in serum ($p < 0.05$) ↑ Ferritin in plasma ($p \leq 0.01$) ↑ Ferritin in serum ($p < 0.05$)	32 high NAL & 62 low NAL	Sandwich immunoassay using direct chemiluminometric	(198)
Positively associated with CSF A β 1–42, p-tau, t-tau biomarker: Heavy metals (As, Cd, Hg, Ni, Pb, Tl) Essential metals (Ca, Co, Cu, Fe, Mg, Mn, Mo, Na, K, Zn) Essential non-metals (P, S, Se)	124 AD, 50 MCI, 19 control	ICP-MS	(187)

AD (Alzheimer's disease), CU (cognitively unimpaired), MCI (mild cognitive impairment), PD (Parkinson disease), ICP-MS (Induction-coupled plasma mass spectrometry), NAL (neocortical amyloid- β load)

Blood cells

Blood cells are another matrix showed to reflect the health status of the brain. For example, the activated form of cyclic-AMP response element-binding protein (pCREB), which is involved in the formation of memories, reduces in the brain of AD patients. Bartolotti et al. have shown that the expression of this protein in peripheral blood mononuclear cells (PBMC) is correlated with its brain level according to the postmortem studies. It was claimed that the PBMC concentration of this protein can serve as a possible indicator of its expression level in the brain and thus “as a biomarker of cognitive function and disease progression in AD” (199). Seeking for biomarkers in blood cells has the advantage of less inter-individual variability due to dietary differences (in comparison to serum or plasma samples) and also less contamination (200, 201). Ren et al. investigated the PBMC miRNA changes in AD patients and proposed two miRNAs (miR-339 and miR-425) as potential biomarkers for AD with sensitivity and specificity of up to 80% (200). Using a whole platelet miRNA transcriptomics, Beyer and coworkers could have discriminated AD from dementia with Lewy bodies (202).

As mentioned earlier, A β production is not limited to the CNS, peripheral cells like blood cells can also produce A β . The A β produced by platelets, is shown to induce AD phenotype in mice (29). The AD related biomarkers (APP, PS1 and PS2 mRNA; and PS1 and PS2 protein) are found in PBMC of obese subjects (203). The platelet APP form ratio is considered as a potential early diagnostic biomarker for AD with sensitivity and specificity of 88.2% of 89.4%, respectively (204). Neutral lipid accumulation in PBMCs (205), elevated coated-platelet levels (206), higher platelet-serotonin content (207), and increased platelet volume (208) are among the other blood cell changes reported in AD patients (Table 8).

Extracellular vesicles

Extracellular vesicles (EVs) including exosomes can originate from most cell types and can be isolated from multiple biofluids including urine, blood, or CSF (212). Some studies have introduced peripheral biomarkers based on EVs and their cargos that can be used for AD diagnosis and monitoring (Table 9) (213). For example, the exosomal level of A β ₄₂, p-S396-tau, and p-tau181 has been shown to significantly increase in blood samples of AD patients (214-216).

Reduction of several exosomal synaptic proteins such as synaptophysin, synaptopodin, and neurogranin occurs early in normal aging and senile dementias, which can be used as early diagnostic marker for AD (215, 217). A decrease in some exosomal transcription factors which promote neuronal resistance against stresses is also reported in AD (216). Goetzl and co-workers showed that these factors could predict AD up to 10 years before clinical diagnosis (218).

Table 9. Blood cells-extracted AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
RBC			
↓ Mean cell hemoglobin concentration in RBC (0.99-fold, p< 0.0001)	207 AD & 754 controls	Multiplex panel (Human DiscoveryMAP)	(88)
Peripheral Blood Mononuclear Cells (PBMCs)-derived			
↓ pCREB in PBMCs (p=0.05)	> 1200 participants and > 600 autopsies (Only female)	Western blot	(199)
↑ fatty acid amide hydrolase (faah) in PBMCs (p<0.05)	LOAD (23 females/ 9 males for DNA methylation studies and protein level detection, 13 for gene expression analysis), age- and sex-matched control (33 for DNA methylation studies and protein level detection, 12 for gene expression analysis)	HPLC	(209)
↓ DNA methylation activity at faah gene promoter (p<0.05)			
↑ Neutral lipids & ACAT-1 protein in PBMCs	93 probable AD and 91 their first-degree relatives vs. 57 CU and 113 blood donors as control	Oil red O (ORO) staining	(205)
↓ HDL cholesterol in plasma			
↑ APP, PS1 and PS2 mRNA in PBMCs	45 middle-aged men	Real-time RT-PCR	(203)
↑ miR-339 in PBMCs	4 mild sporadic LOAD, 4 severe sporadic LOAD, 4 control	Microarray & Q-PCR	(200)
↑ miR-425 in PBMCs			

Table 9. Blood cells-extracted AD biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ Pin1 mRNA in PBMCs (+74%; p = 0.018) ↓ Ser ¹⁶ phosphorylation (−30%; p = 0.041) ↓ promoter methylation (−8%; p = 0.001)	32 LOAD (23 females/9 males), 28 age- and gender-matched control (19 females/9 males)	Chomczynski and Sacchi's modified method & real-time PCR	(209)
↑ Chitotriosidase mRNA in macrophages (~ 19-fold, p < 0.0001) ↑ IL-16 mRNA in macrophages (~21-fold, p < 0.0001) ↑ IL-18 mRNA in macrophages (~19-fold, p < 0.0001) ↑ TGF-β1 mRNA in macrophages (~32-fold, p < 0.0001) ↑ superoxide anion release from macrophages (~14-fold, p < 0.0001)	40 AD, 40 ischemic cerebrovascular dementias, 40 controls	Quantitative real-time polymerase chain reaction & Spectrophotometric measurement of ferricytochrome c reduction	(210)
↔ IL-1α in PBMCs ↑ IL-3 in PBMCs during MCI phase (p < 0.05) ↑ IL-11 in PBMCs during MCI phase (p < 0.01) ↑ EGF in PBMCs during MCI phase (p < 0.05) ↔ G-CSF in PBMCs ↔ MCP-3 in PBMCs ↔ IL-6 in PBMCs	37 AD, 20 MCI, 9 Control	Searchlight mutiplex ELISA & Commercial single ELISA	(211)

AD (Alzheimer's disease), CU (cognitively unimpaired), MCI (mild cognitive impairment), pCREB (cyclic-AMP response element-binding protein), LOAD (Late-Onset Alzheimer's Disease)

The onset and stage of AD may be reflected by miRNAs which are primarily found in nervous system. Recent studies have suggested that the brain miRNAs can cross the blood-brain barrier by transcytosis of exosomes and may be detected in biological fluids. Exosomes provide a protective shield against RNase-rich environments in circulatory system. Therefore isolating the enriched exosomal miRNA can enhance the chance of AD diagnosis (212).

Several laboratories have sought for differences in exosomal miRNAs expression between AD and non-AD subjects. Hill laboratory conducted a screening for miRNA sets to make a fingerprint for AD diagnosis. They identified a 16-miRNA signature extracted from serum exosomes which was specific for AD (144). Lugli et al. showed twenty miRNAs which were different in the AD group, among which a panel of seven miRNAs was highly informative (219). The most important miRNA was miR-342-3p that was reported in both studies (144, 219).

Insulin resistance is a disorder that is common between AD and type 2 diabetes mellitus. It is caused by abnormality in insulin receptor function, which is associated with more phospho-serine-type 1 insulin receptor substrate (P-serine 312-IRS-1) and less P-tyrosine-IRS-1. Kapogiannis et al. measured the ratio of these two proteins in plasma exosomes as AD biomarker. They showed that the ratio was significantly higher for AD patients compared with normal and even diabetes patient, and could 100% classify them (220).

Despite evidence for blood based exosomal biomarkers to be a promising and complementary tool for AD prediction (144, 212), there is a concern about the relevance of blood extracted exosomes that can originate from all others organs instead of CNS. Goetzl and co-workers offer a novel approach to increase specificity of these biomarkers. They extract brain-derived exosomes in blood samples using anti L1 cell adhesion molecule (L1-CAM) antibodies (217, 218), and could remove the noises generated from whole bloodstream exosomes. Although this approach was developed in order to be brain-specific, L1-CAM is also expressed in the renal system; therefore the interpretation of the results should be done with caution (221). Fiandaca et al. compared Neural Cell Adhesion Molecule 1 (NCAM-1) to L1-CAM for enrichment of neuron-derived exosomes in blood samples. These cell adhesion molecules are differently distributed in the nervous system, however the difference between extracted exosomal p-tau181, P-S396-tau, total tau, and A β ₄₂ were statistically insignificant whether enriched with anti-NCAM-1 or anti-L1CAM antibodies (214).

In another study, Goetzl et al. used astrocyte cell surface antigen-1 (ACSA-1) to isolate astrocyte-derived exosomes. They observed that the levels of A β ₄₂-generating system factors (BACE-1, sAPPb and septin-8) and neuronal trophic-survival factor (GDNF), a small protein involved in neuron survival, can significantly be altered in astrocyte-derived exosomes (but not in neuron-derived ones) of AD patients in comparison to controls (222).

The effect of autophagocytic-lysosomal dysfunction in pathophysiology of AD has been emphasized in recent years. Goetzl et al. suggested that the enhancement of lysosomal components (cathepsin D, LAMP-1 and ubiquitinated proteins) and reduction of HSP-70 can be biomarker candidates for AD diagnosis (223).

Table 10. Blood-extracted Extracellular Vesicles-related AD Biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
↑ A β 1-42 in Blood	57 AD, 16 FTD, 24 CU that 1-10 years later diagnosed with AD	Enrichment of Neuronal derived exosome by NCAM-1 and L1CAM	(214)
↑ P-S396-tau in Blood			
↑ P-T181-tau in Blood			
↑ A β 1-42 in plasma	10 CU, 10 AD, 20 stable MCI, 20 transitioned from MCI to AD within 36 months	Enrichment of Neuronal derived exosome by L1CAM	(215)
↑ P-S396-tau in plasma			
↑ P-T181-tau in plasma			
↓ neurogranin in plasma			
↑ REST in plasma	20 volunteers (≥ 60) (samples collected at 3- to 11-year intervals)	Enrichment of Neuronal derived exosome by L1CAM	(216)
↑ A β 1-42 in plasma			
↑ P-T181-tau in plasma			
↑ Cathepsin D (Lysosomal proteins) in plasma			
↓ neurogranin in plasma	12 AD, 14 FTD, 20 control	Enrichment of Neuronal derived exosome by ACSA-1	(222)
↑ BACE-1 in plasma			
↑ sAPPb in plasma			
↓ septin-8 in plasma			
↓ GDNF (Neurotropic factors) in plasma			
Lysosomal proteins:	26 AD, 16 FTD, 20 control which 1 to 10 years later diagnosed with AD	Enrichment of Neuronal derived exosome by L1CAM	(223)
↑ Cathepsin D in Blood			
↑ LAMP-1 in Blood			
↑ Ubiquitinated proteins in Blood			
↓ HSP 70 in Blood			

Table 10. Blood-extracted Extracellular Vesicles-related AD Biomarkers.

Biomarker alteration in body fluid	Studied group	Method	Ref.
Insulin resistance factor (R): ↑ P-serine 312-IRS-1/ P-pan-t yrosine-IRS-1 ratio in plasma	26 AD, 20 DM2, 16 FTD, 22 control which 1 to 10 years later diagnosed with AD	Enrichment of Neuronal derived exosome by L1CAM	(220)
Synaptic proteins: ↓ Synaptophysin in plasma ↓ Synaptopodin in plasma ↓ synaptotagmin-2 in plasma ↓ neurogranin in plasma ↓ growth-associated protein 43 in plasma ↓ synapsin 1 in plasma	Cross-sectional study: 12 AD, 16 FTD, 28 controls Longitudinal study: 9 AD, 10 FTD, 19 controls	Enrichment of Neuronal derived exosome by L1CAM	(217)
Transcription factors: ↓ LRP6 ↓ HSF1 ↓ REST	24 (cross-sectional studies), 16 (longitudinal studies), 10 FTD	Enrichment of Neuronal derived exosome by L1CAM	(218)
↑ MicroRNA in serum: has-MiR-101.3p, has-MiR-106.a5p, has-MiR-106b.5p, has-MiR-1306.5p, has-MiR-143.3p, has-MiR-15a.5p, has-MiR-15b.3p, has-MiR-18b.5p, has-MiR-20a.5p, has-MiR-30e.5p, has-MiR-335.5p, has-MiR-342.3p, has-MiR-361.5p, has-MiR-424.5p, has-MiR-582.5p, has-MiR-93.5p	Discovery test: 23 CU, 3 MCI, 23 AD Validation test: 36 CU, 8 MCI, 16 AD	-	(144)
↑ MicroRNA in plasma miR-23b-3p, miR-24-3p, miR-29b-3p, miR-125b-5p, miR-138-5p, miR-139-5p, miR-141-3p, miR-150-5p, miR-152-3p, miR-185-5p, miR-338-3p, miR-342-3p, miR-342-5p, miR-548-5p, miR-659-5p, miR-306-5p, miR-3613-3p, miR-3916, miR-4772-3p, miR-5001-3p	35 AD & 35 control	-	(219)

AD (Alzheimer's disease), CU (cognitively unimpaired), MCI (mild cognitive impairment), FTD (frontotemporal dementia), REST (repressor element 1-silencing transcription factor), DM2 (type 2 diabetes mellitus), LRP6 (low-density lipoprotein receptor-related protein 6), HSF1 (heat-shock factor-1),

Conclusion

The importance of early diagnosis of AD in the prevention of disease progression and treatment success is evident today and thanks to technological advances, the role of biomarkers is expanding in this field. Among the different categories of biomarkers, blood-based biomarkers (BBBs) are promising due to their ease of access that provides the possibility of multiple sampling with low cost and less invasion.

However, no single biomarker has shown to be robust enough to diagnose AD with reliable sensitivity and specificity. Considering the complexity and heterogeneous nature of AD, simultaneous assessment of multiple biomarkers, not necessarily interrelated, may enhance the possibility of attaining a more reliable sensor array. Currently, p-tau218 is among the most promising diagnostic plasma biomarkers, in addition to, neurofilament light (NfL), p-tau181, and A β ₄₂/A β ₄₀ ratio. It seems that application of these BBBs would be a big step towards detection of AD in early stages.

Despite the efforts made so far, inconsistency of results among various studies remains a main challenge to be addressed. In addition to demographic characteristics, diet, health condition, and comorbidities, the profile of BBBs may also be affected to some extent by pre-clinical and analytical steps. Multiplexing as a method for simultaneous detection of an array of suitable biomarkers from different categories may overcome the inter-individual variability and enhance the chance of early diagnosis.

Acknowledgments

This work was financially supported by the Shiraz University of Medical Sciences.

References

1. Dementia: World Health Organization. 2023; Available from: <https://www.who.int/news-room/fact-sheets/detail/dementia>.
2. Fearing MA, Bigler ED, Norton M, et al. Autopsy-confirmed Alzheimer's disease versus clinically diagnosed Alzheimer's disease in the Cache County Study on Memory and Aging: a comparison of quantitative MRI and neuropsychological findings. *J Clin Exp Neuropsychol* 2007;29:553-60.
3. Dementia As. Medical Tests: Alzheimer's Association. Available from: https://www.alz.org/alzheimers-dementia/diagnosis/medical_tests.
4. Knopman DS. Alzheimer disease biomarkers and insights into mild cognitive impairment. *Neurology* 2013;80:978-80.
5. Burnham SC, Faux NG, Wilson W, et al. A blood-based predictor for neocortical Abeta burden in Alzheimer's disease: results from the AIBL study. *Mol Psychiatry* 2014;19:519-26.
6. Hoag. Cognitive Severity Stages (Normal Aging - Dementia) [Available from: <https://www.hoag.org/specialties-services/neurosciences/programs/memory-cognitive-disorders/types-of-memory-cognitive-disorders/cognitive-severity-stages/>. Available from: <https://www.hoag.org/specialties-services/neurosciences/programs/memory-cognitive-disorders/types-of-memory-cognitive-disorders/cognitive-severity-stages/>.
7. Jack CR, Jr., Albert MS, Knopman DS, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:257-62.
8. Blennow K, Zetterberg H, Haass C, et al. Semagacestat's fall: where next for AD therapies? *Nat Med* 2013;19:1214-5.
9. Chaitanuwong P, Singhanetr P, Chainakul M, et al. Potential Ocular Biomarkers for Early Detection of Alzheimer's Disease and Their Roles in Artificial Intelligence Studies. *Neurol Ther* 2023;12:1517-32.
10. Yan Y, Aierken A, Wang C, et al. A potential biomarker of preclinical Alzheimer's disease: The olfactory dysfunction and its pathogenesis-based neural circuitry impairments. *Neurosci Biobehav Rev* 2022;132:857-69.
11. Harms RL, Ferrari A, Meier IB, et al. Digital biomarkers and sex impacts in Alzheimer's disease management - potential utility for innovative 3P medicine approach. *EPMA J* 2022;13:299-313.
12. Cummings J, Zhou Y, Lee G, et al. Alzheimer's disease drug development pipeline: 2023. *Alzheimers Dement (N Y)* 2023;9:e12385.
13. Sanabria-Castro A, Alvarado-Echeverria I, Monge-Bonilla C. Molecular Pathogenesis of Alzheimer's Disease: An Update. *Ann Neurosci* 2017;24:46-54.
14. Kurkinen M, Fulek M, Fulek K, et al. The Amyloid Cascade Hypothesis in Alzheimer's Disease: Should We Change Our Thinking? *Biomolecules* 2023;13.
15. Ankarcrona M, Winblad B. Biomarkers for apoptosis in Alzheimer's disease. *Int J Geriatr Psychiatry* 2005;20:101-5.

16. Pais M, Martinez L, Ribeiro O, et al. Early diagnosis and treatment of Alzheimer's disease: new definitions and challenges. *Braz J Psychiatry* 2020;42:431-41.
17. Imbimbo BP, Ippati S, Watling M, et al. Role of monomeric amyloid-beta in cognitive performance in Alzheimer's disease: Insights from clinical trials with secretase inhibitors and monoclonal antibodies. *Pharmacol Res* 2023;187:106631.
18. Jack CR, Jr., Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimers Dement* 2018;14:535-62.
19. Dolev I, Fogel H, Milshtein H, et al. Spike bursts increase amyloid-beta 40/42 ratio by inducing a presenilin-1 conformational change. *Nat Neurosci* 2013;16:587-95.
20. Kaye R, Head E, Thompson JL, et al. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. *Science* 2003;300:486-9.
21. Panza F, Lozupone M, Loggoscino G, et al. A critical appraisal of amyloid-beta-targeting therapies for Alzheimer disease. *Nat Rev Neurol* 2019;15:73-88.
22. Fjell AM, Amlie IK, Westlye LT, et al. CSF biomarker pathology correlates with a medial temporo-parietal network affected by very mild to moderate Alzheimer's disease but not a fronto-striatal network affected by healthy aging. *Neuroimage* 2010;49:1820-30.
23. Khoury R, Ghossoub E. Diagnostic biomarkers of Alzheimer's disease: a state-of-the-art review. *Biomarkers in Neuropsychiatry* 2019;1:100005.
24. Forlenza OV, Radanovic M, Talib LL, et al. Cerebrospinal fluid biomarkers in Alzheimer's disease: Diagnostic accuracy and prediction of dementia. *Alzheimers Dement (Amst)* 2015;1:455-63.
25. Zetterberg H, Bendlin BB. Biomarkers for Alzheimer's disease-preparing for a new era of disease-modifying therapies. *Mol Psychiatry* 2021;26:296-308.
26. Bloudek LM, Spackman DE, Blankenburg M, et al. Review and meta-analysis of biomarkers and diagnostic imaging in Alzheimer's disease. *J Alzheimers Dis* 2011;26:627-45.
27. Cummings J. The Role of Biomarkers in Alzheimer's Disease Drug Development. *Adv Exp Med Biol* 2019;1118:29-61.
28. Palmqvist S, Janelidze S, Stomrud E, et al. Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease-Related beta-Amyloid Status. *JAMA Neurol* 2019;76:1060-9.
29. Sun HL, Chen SH, Yu ZY, et al. Blood cell-produced amyloid-beta induces cerebral Alzheimer-type pathologies and behavioral deficits. *Mol Psychiatry* 2021;26:5568-77.
30. Wang J, Gu BJ, Masters CL, et al. A systemic view of Alzheimer disease - insights from amyloid-beta metabolism beyond the brain. *Nat Rev Neurol* 2017;13:612-23.
31. Veerabhadrapa B, Delaby C, Hirtz C, et al. Detection of amyloid beta peptides in body fluids for the diagnosis of Alzheimer's disease: Where do we stand? *Crit Rev Clin Lab Sci* 2020;57:99-113.
32. Gao H, Liu M, Zhao Z, et al. Diagnosis of Mild Cognitive Impairment and Alzheimer's Disease by the Plasma and Serum Amyloid-beta 42 Assay through Highly Sensitive Peptoid Nanosheet Sensor. *ACS Appl Mater Interfaces* 2020;12:9693-700.
33. Chong JR, Ashton NJ, Karikari TK, et al. Blood-based high sensitivity measurements of beta-amyloid and phosphorylated tau as biomarkers of Alzheimer's disease: a focused review on recent advances. *J Neurol Neurosurg Psychiatry* 2021;92:1231-41.
34. Fyfe I. Tau species has potential for Alzheimer disease blood test. *Nat Rev Neurol* 2020;16:521.
35. Nizzari M, Thellung S, Corsaro A, et al. Neurodegeneration in Alzheimer disease: role of amyloid precursor protein and presenilin 1 intracellular signaling. *J Toxicol* 2012;2012:187297.

36. Golde TE, Eckman CB. Physiologic and pathologic events mediated by intramembranous and juxtamembranous proteolysis. *Sci STKE* 2003;2003:RE4.
37. Heilig EA, Xia W, Shen J, et al. A presenilin-1 mutation identified in familial Alzheimer disease with cotton wool plaques causes a nearly complete loss of gamma-secretase activity. *J Biol Chem* 2010;285:22350-9.
38. Neve RL, McPhie DL. The cell cycle as a therapeutic target for Alzheimer's disease. *Pharmacol Ther* 2006;111:99-113.
39. Mehta PD, Pirttila T, Mehta SP, et al. Plasma and cerebrospinal fluid levels of amyloid beta proteins 1-40 and 1-42 in Alzheimer disease. *Arch Neurol* 2000;57:100-5.
40. Sundelof J, Giedraitis V, Izarry MC, et al. Plasma beta amyloid and the risk of Alzheimer disease and dementia in elderly men: a prospective, population-based cohort study. *Arch Neurol* 2008;65:256-63.
41. Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid-beta biomarkers for Alzheimer's disease. *Nature* 2018;554:249-54.
42. Fukumoto H, Tennis M, Locascio JJ, et al. Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels. *Arch Neurol* 2003;60:958-64.
43. Tamaoka A, Fukushima T, Sawamura N, et al. Amyloid beta protein in plasma from patients with sporadic Alzheimer's disease. *J Neurol Sci* 1996;141:65-8.
44. Scheuner D, Eckman C, Jensen M, et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 1996;2:864-70.
45. Mayeux R, Tang MX, Jacobs DM, et al. Plasma amyloid beta-peptide 1-42 and incipient Alzheimer's disease. *Ann Neurol* 1999;46:412-6.
46. Bateman RJ, Xiong C, Benzinger TL, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med* 2012;367:795-804.
47. Reiman EM, Quiroz YT, Fleisher AS, et al. Brain imaging and fluid biomarker analysis in young adults at genetic risk for autosomal dominant Alzheimer's disease in the presenilin 1 E280A kindred: a case-control study. *Lancet Neurol* 2012;11:1048-56.
48. Ringman JM, Younkin SG, Pratico D, et al. Biochemical markers in persons with preclinical familial Alzheimer disease. *Neurology* 2008;71:85-92.
49. Huang CW, Wang SJ, Wu SJ, et al. Potential blood biomarker for disease severity in the Taiwanese population with Alzheimer's disease. *Am J Alzheimers Dis Other Dement* 2013;28:75-83.
50. Tamaoka A, Sawamura N, Fukushima T, et al. Amyloid beta protein 42(43) in cerebrospinal fluid of patients with Alzheimer's disease. *J Neurol Sci* 1997;148:41-5.
51. Nabers A, Ollesch J, Schartner J, et al. Amyloid-beta-Secondary Structure Distribution in Cerebrospinal Fluid and Blood Measured by an Immuno-Infrared-Sensor: A Biomarker Candidate for Alzheimer's Disease. *Anal Chem* 2016;88:2755-62.
52. Cullen NC, Leuzy A, Janelidze S, et al. Plasma biomarkers of Alzheimer's disease improve prediction of cognitive decline in cognitively unimpaired elderly populations. *Nat Commun* 2021;12:3555.
53. Lui JK, Laws SM, Li QX, et al. Plasma amyloid-beta as a biomarker in Alzheimer's disease: the AIBL study of aging. *J Alzheimers Dis* 2010;20:1233-42.
54. Perneczky R, Guo LH, Kagerbauer SM, et al. Soluble amyloid precursor protein beta as blood-based biomarker of Alzheimer's disease. *Transl Psychiatry* 2013;3:e227.
55. Jelic V, Hagman G, Yamamoto NG, et al. Abnormal platelet amyloid-beta protein precursor (AβetaPP) metabolism in Alzheimer's disease: identification and characterization of a new AβetaPP isoform as potential biomarker. *J Alzheimers Dis* 2013;35:285-95.

56. Padovani A, Borroni B, Colciaghi F, et al. Abnormalities in the pattern of platelet amyloid precursor protein forms in patients with mild cognitive impairment and Alzheimer disease. *Arch Neurol* 2002;59:71-5.
57. Decourt B, Walker A, Gonzales A, et al. Can platelet BACE1 levels be used as a biomarker for Alzheimer's disease? Proof-of-concept study. *Platelets* 2013;24:235-8.
58. Reddy MM, Wilson R, Wilson J, et al. Identification of candidate IgG biomarkers for Alzheimer's disease via combinatorial library screening. *Cell* 2011;144:132-42.
59. Weksler ME, Relkin N, Turkenich R, et al. Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals. *Exp Gerontol* 2002;37:943-8.
60. Brettschneider S, Morgenthaler NG, Teipel SJ, et al. Decreased serum amyloid beta(1-42) autoantibody levels in Alzheimer's disease, determined by a newly developed immuno-precipitation assay with radiolabeled amyloid beta(1-42) peptide. *Biol Psychiatry* 2005;57:813-6.
61. Jellinger KA, Janetzky B, Attems J, et al. Biomarkers for early diagnosis of Alzheimer disease: 'ALZheimer ASsociated gene'--a new blood biomarker? *J Cell Mol Med* 2008;12:1094-117.
62. Kienzl E, Jellinger K, Janetzky B, et al. A broader horizon of Alzheimer pathogenesis: ALZAS--an early serum biomarker? *J Neural Transm Suppl* 2002;87-95.
63. Bibl M, Esselmann H, Wiltfang J. Neurochemical biomarkers in Alzheimer's disease and related disorders. *Ther Adv Neurol Disord* 2012;5:335-48.
64. Barbier P, Zejneli O, Martinho M, et al. Role of Tau as a Microtubule-Associated Protein: Structural and Functional Aspects. *Front Aging Neurosci* 2019;11:204.
65. Shen XN, Li JQ, Wang HF, et al. Plasma amyloid, tau, and neurodegeneration biomarker profiles predict Alzheimer's disease pathology and clinical progression in older adults without dementia. *Alzheimers Dement (Amst)* 2020;12:e12104.
66. Lee JC, Kim SJ, Hong S, et al. Diagnosis of Alzheimer's disease utilizing amyloid and tau as fluid biomarkers. *Exp Mol Med* 2019;51:1-10.
67. Mattsson N, Zetterberg H, Janelidze S, et al. Plasma tau in Alzheimer disease. *Neurology* 2016;87:1827-35.
68. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol* 2020;19:422-33.
69. Thijssen EH, La Joie R, Wolf A, et al. Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration. *Nat Med* 2020;26:387-97.
70. Janelidze S, Mattsson N, Palmqvist S, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. *Nat Med* 2020;26:379-86.
71. Lue LF, Sabbagh MN, Chiu MJ, et al. Plasma Levels of Abeta42 and Tau Identified Probable Alzheimer's Dementia: Findings in Two Cohorts. *Front Aging Neurosci* 2017;9:226.
72. Dage JL, Wennberg AMV, Airey DC, et al. Levels of tau protein in plasma are associated with neurodegeneration and cognitive function in a population-based elderly cohort. *Alzheimers Dement* 2016;12:1226-34.
73. Zetterberg H, Wilson D, Andreasson U, et al. Plasma tau levels in Alzheimer's disease. *Alzheimers Res Ther* 2013;5:9.
74. Pase MP, Beiser AS, Himali JJ, et al. Assessment of Plasma Total Tau Level as a Predictive Biomarker for Dementia and Related Endophenotypes. *JAMA Neurol* 2019;76:598-606.
75. Foiani MS, Woollacott IO, Heller C, et al. Plasma tau is increased in frontotemporal dementia. *J Neurol Neurosurg Psychiatry* 2018;89:804-7.

76. Muller S, Preische O, Gopfert JC, et al. Tau plasma levels in subjective cognitive decline: Results from the DELCODE study. *Sci Rep* 2017;7:9529.
77. Tatebe H, Kasai T, Ohmichi T, et al. Quantification of plasma phosphorylated tau to use as a biomarker for brain Alzheimer pathology: pilot case-control studies including patients with Alzheimer's disease and down syndrome. *Mol Neurodegener* 2017;12:63.
78. Barthelemy NR, Horie K, Sato C, et al. Blood plasma phosphorylated-tau isoforms track CNS change in Alzheimer's disease. *J Exp Med* 2020;217.
79. O'Connor A, Karikari TK, Poole T, et al. Plasma phospho-tau181 in presymptomatic and symptomatic familial Alzheimer's disease: a longitudinal cohort study. *Mol Psychiatry* 2021;26:5967-76.
80. Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA* 2020;324:772-81.
81. Ashton NJ, Benedet AL, Pascoal TA, et al. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. *EBioMedicine* 2022;76:103836.
82. Kivisakk P, Carlyle BC, Sweeney T, et al. Plasma biomarkers for diagnosis of Alzheimer's disease and prediction of cognitive decline in individuals with mild cognitive impairment. *Front Neurol* 2023;14:1069411.
83. Neergaard JS, Dragsbaek K, Christiansen C, et al. Two novel blood-based biomarker candidates measuring degradation of tau are associated with dementia: A prospective study. *PLoS One* 2018;13:e0194802.
84. Ashton NJ, Janelidze S, Al Khleifat A, et al. A multicentre validation study of the diagnostic value of plasma neurofilament light. *Nat Commun* 2021;12:3400.
85. Khalil M, Pirpamer L, Hofer E, et al. Serum neurofilament light levels in normal aging and their association with morphologic brain changes. *Nat Commun* 2020;11:812.
86. Preische O, Schultz SA, Apel A, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med* 2019;25:277-83.
87. Mattsson N, Cullen NC, Andreasson U, et al. Association Between Longitudinal Plasma Neurofilament Light and Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurol* 2019;76:791-9.
88. Doecke JD, Laws SM, Faux NG, et al. Blood-based protein biomarkers for diagnosis of Alzheimer disease. *Arch Neurol* 2012;69:1318-25.
89. O'Bryant SE, Xiao G, Barber R, et al. A serum protein-based algorithm for the detection of Alzheimer disease. *Arch Neurol* 2010;67:1077-81.
90. O'Bryant SE, Xiao G, Barber R, et al. A blood-based algorithm for the detection of Alzheimer's disease. *Dement Geriatr Cogn Disord* 2011;32:55-62.
91. Kumar A, Singh S, Verma A, et al. Proteomics based identification of differential plasma proteins and changes in white matter integrity as markers in early detection of mild cognitive impaired subjects at high risk of Alzheimer's disease. *Neurosci Lett* 2018;676:71-7.
92. Frolich L, Kornhuber J, Ihl R, et al. Integrity of the blood-CSF barrier in dementia of Alzheimer type: CSF/serum ratios of albumin and IgG. *Eur Arch Psychiatry Clin Neurosci* 1991;240:363-6.
93. Hye A, Lynham S, Thambisetty M, et al. Proteome-based plasma biomarkers for Alzheimer's disease. *Brain* 2006;129:3042-50.
94. Yang MH, Yang YH, Lu CY, et al. Activity-dependent neuroprotector homeobox protein: A candidate protein identified in serum as diagnostic biomarker for Alzheimer's disease. *J Proteomics* 2012;75:3617-29.

95. Ashton NJ, Hye A, Leckey CA, et al. Plasma REST: a novel candidate biomarker of Alzheimer's disease is modified by psychological intervention in an at-risk population. *Transl Psychiatry* 2017;7:e1148.
96. Foulds P, McAuley E, Gibbons L, et al. TDP-43 protein in plasma may index TDP-43 brain pathology in Alzheimer's disease and frontotemporal lobar degeneration. *Acta Neuropathol* 2008;116:141-6.
97. Yoo YK, Kim J, Kim G, et al. A highly sensitive plasma-based amyloid-beta detection system through medium-changing and noise cancellation system for early diagnosis of the Alzheimer's disease. *Sci Rep* 2017;7:8882.
98. Cheng Z, Yin J, Yuan H, et al. Blood-Derived Plasma Protein Biomarkers for Alzheimer's Disease in Han Chinese. *Front Aging Neurosci* 2018;10:414.
99. Higginbotham L, Ping L, Dammer EB, et al. Integrated proteomics reveals brain-based cerebrospinal fluid biomarkers in asymptomatic and symptomatic Alzheimer's disease. *Sci Adv* 2020;6.
100. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci* 2015;16:358-72.
101. Heneka MT, Carson MJ, El Khoury J, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 2015;14:388-405.
102. Deardorff WJ, Grossberg GT. Targeting neuroinflammation in Alzheimer's disease: evidence for NSAIDs and novel therapeutics. *Expert Rev Neurother* 2017;17:17-32.
103. Zhang C, Wang Y, Wang D, et al. NSAID Exposure and Risk of Alzheimer's Disease: An Updated Meta-Analysis From Cohort Studies. *Front Aging Neurosci* 2018;10:83.
104. Bradshaw EM, Chibnik LB, Keenan BT, et al. CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nat Neurosci* 2013;16:848-50.
105. Pillai JA, Maxwell S, Bena J, et al. Key inflammatory pathway activations in the MCI stage of Alzheimer's disease. *Ann Clin Transl Neurol* 2019;6:1248-62.
106. Morgan AR, Touchard S, Leckey C, et al. Inflammatory biomarkers in Alzheimer's disease plasma. *Alzheimers Dement* 2019;15:776-87.
107. O'Bryant SE, Xiao G, Barber R, et al. A blood-based screening tool for Alzheimer's disease that spans serum and plasma: findings from TARC and ADNI. *PLoS One* 2011;6:e28092.
108. Alawode DOT, Fox NC, Zetterberg H, et al. Alzheimer's Disease Biomarkers Revisited From the Amyloid Cascade Hypothesis Standpoint. *Front Neurosci* 2022;16:837390.
109. Wyss-Coray T. Inflammation in Alzheimer disease: driving force, bystander or beneficial response? *Nat Med* 2006;12:1005-15.
110. Swardfager W, Lanctot K, Rothenburg L, et al. A meta-analysis of cytokines in Alzheimer's disease. *Biol Psychiatry* 2010;68:930-41.
111. Paganelli R, Di Iorio A, Patricelli L, et al. Proinflammatory cytokines in sera of elderly patients with dementia: levels in vascular injury are higher than those of mild-moderate Alzheimer's disease patients. *Exp Gerontol* 2002;37:257-63.
112. Ray S, Britschgi M, Herbert C, et al. Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins. *Nat Med* 2007;13:1359-62.
113. Tarkowski E, Blennow K, Wallin A, et al. Intracerebral production of tumor necrosis factor-alpha, a local neuroprotective agent, in Alzheimer disease and vascular dementia. *J Clin Immunol* 1999;19:223-30.
114. Ray S, Wyss-coray A, inventors; Method for diagnosis and monitoring of Alzheimer's disease 2013.

115. Rota E, Bellone G, Rocca P, et al. Increased intrathecal TGF-beta1, but not IL-12, IFN-gamma and IL-10 levels in Alzheimer's disease patients. *Neurol Sci* 2006;27:33-9.
116. Singh VK, Guthikonda P. Circulating cytokines in Alzheimer's disease. *J Psychiatr Res* 1997;31:657-60.
117. Licastro F, Pedrini S, Caputo L, et al. Increased plasma levels of interleukin-1, interleukin-6 and alpha-1-antichymotrypsin in patients with Alzheimer's disease: peripheral inflammation or signals from the brain? *J Neuroimmunol* 2000;103:97-102.
118. Blum-Degen D, Muller T, Kuhn W, et al. Interleukin-1 beta and interleukin-6 are elevated in the cerebrospinal fluid of Alzheimer's and de novo Parkinson's disease patients. *Neurosci Lett* 1995;202:17-20.
119. Pirttila T, Mehta PD, Frey H, et al. Alpha 1-antichymotrypsin and IL-1 beta are not increased in CSF or serum in Alzheimer's disease. *Neurobiol Aging* 1994;15:313-7.
120. Shibata N, Ohnuma T, Takahashi T, et al. Effect of IL-6 polymorphism on risk of Alzheimer disease: genotype-phenotype association study in Japanese cases. *Am J Med Genet* 2002;114:436-9.
121. Kalman J, Juhasz A, Laird G, et al. Serum interleukin-6 levels correlate with the severity of dementia in Down syndrome and in Alzheimer's disease. *Acta Neurol Scand* 1997;96:236-40.
122. Angelis P, Scharf S, Mander A, et al. Serum interleukin-6 and interleukin-6 soluble receptor in Alzheimer's disease. *Neurosci Lett* 1998;244:106-8.
123. van Duijn CM, Hofman A, Nagelkerken L. Serum levels of interleukin-6 are not elevated in patients with Alzheimer's disease. *Neurosci Lett* 1990;108:350-4.
124. Mulder SD, Hack CE, van der Flier WM, et al. Evaluation of intrathecal serum amyloid P (SAP) and C-reactive protein (CRP) synthesis in Alzheimer's disease with the use of index values. *J Alzheimers Dis* 2010;22:1073-9.
125. Choi J, Lee HW, Suk K. Increased plasma levels of lipocalin 2 in mild cognitive impairment. *J Neurol Sci* 2011;305:28-33.
126. Naude PJ, Nyakas C, Eiden LE, et al. Lipocalin 2: novel component of proinflammatory signaling in Alzheimer's disease. *FASEB J* 2012;26:2811-23.
127. Blennow K, Wallin A, Chong JK. Cerebrospinal fluid 'neuronal thread protein' comes from serum by passage over the blood-brain barrier. *Neurodegeneration* 1995;4:187-93.
128. Galimberti D, Schoonenboom N, Scarpini E, et al. Chemokines in serum and cerebrospinal fluid of Alzheimer's disease patients. *Ann Neurol* 2003;53:547-8.
129. Marksteiner J, Kemmler G, Weiss EM, et al. Five out of 16 plasma signaling proteins are enhanced in plasma of patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging* 2011;32:539-40.
130. Conte M, Sabbatinelli J, Chiariello A, et al. Disease-specific plasma levels of mitokines FGF21, GDF15, and Humanin in type II diabetes and Alzheimer's disease in comparison with healthy aging. *Geroscience* 2021;43:985-1001.
131. Bjorkqvist M, Ohlsson M, Minthon L, et al. Evaluation of a previously suggested plasma biomarker panel to identify Alzheimer's disease. *PLoS One* 2012;7:e29868.
132. Montine TJ, Shinobu L, Montine KS, et al. No difference in plasma or urinary F2-isoprostanes among patients with Huntington's disease or Alzheimer's disease and controls. *Ann Neurol* 2000;48:950.
133. Mateos L, Ismail MA, Gil-Bea FJ, et al. Upregulation of brain renin angiotensin system by 27-hydroxycholesterol in Alzheimer's disease. *J Alzheimers Dis* 2011;24:669-79.
134. Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 2002;346:476-83.
135. Bird TD. Genetic aspects of Alzheimer disease. *Genet Med* 2008;10:231-9.

136. Verheijen J, Sleegers K. Understanding Alzheimer Disease at the Interface between Genetics and Transcriptomics. *Trends Genet* 2018;34:434-47.
137. Andrade-Guerrero J, Santiago-Balmaseda A, Jeronimo-Aguilar P, et al. Alzheimer's Disease: An Updated Overview of Its Genetics. *Int J Mol Sci* 2023;24.
138. Andrews SJ, Renton AE, Fulton-Howard B, et al. The complex genetic architecture of Alzheimer's disease: novel insights and future directions. *EBioMedicine* 2023;90:104511.
139. Bertram L, McQueen MB, Mullin K, et al. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. *Nat Genet* 2007;39:17-23.
140. Creese B, Arathimos R, Brooker H, et al. Genetic risk for Alzheimer's disease, cognition, and mild behavioral impairment in healthy older adults. *Alzheimers Dement (Amst)* 2021;13:e12164.
141. Navarro JF, Croteau DL, Jurek A, et al. Spatial Transcriptomics Reveals Genes Associated with Dysregulated Mitochondrial Functions and Stress Signaling in Alzheimer Disease. *iScience* 2020;23:101556.
142. Kumar P, Dezso Z, MacKenzie C, et al. Circulating miRNA biomarkers for Alzheimer's disease. *PLoS One* 2013;8:e69807.
143. Leidinger P, Backes C, Deutscher S, et al. A blood based 12-miRNA signature of Alzheimer disease patients. *Genome Biol* 2013;14:R78.
144. Cheng L, Doecke JD, Sharples RA, et al. Prognostic serum miRNA biomarkers associated with Alzheimer's disease shows concordance with neuropsychological and neuroimaging assessment. *Mol Psychiatry* 2015;20:1188-96.
145. Bhatnagar S, Chertkow H, Schipper HM, et al. Increased microRNA-34c abundance in Alzheimer's disease circulating blood plasma. *Front Mol Neurosci* 2014;7:2.
146. Wu HZY, Thalamuthu A, Cheng L, et al. Differential blood miRNA expression in brain amyloid imaging-defined Alzheimer's disease and controls. *Alzheimers Res Ther* 2020;12:59.
147. Siedlecki-Wulich D, Catala-Solsona J, Fabregas C, et al. Altered microRNAs related to synaptic function as potential plasma biomarkers for Alzheimer's disease. *Alzheimers Res Ther* 2019;11:46.
148. Souza VC, Morais GS, Jr., Henriques AD, et al. Whole-Blood Levels of MicroRNA-9 Are Decreased in Patients With Late-Onset Alzheimer Disease. *Am J Alzheimers Dis Other Dement* 2020;35:1533317520911573.
149. Maes OC, Kravitz S, Mawal Y, et al. Characterization of alpha1-antitrypsin as a heme oxygenase-1 suppressor in Alzheimer plasma. *Neurobiol Dis* 2006;24:89-100.
150. Fehlbauer-Beurdeley P, Jarrige-Le Prado AC, Pallares D, et al. Toward an Alzheimer's disease diagnosis via high-resolution blood gene expression. *Alzheimers Dement* 2010;6:25-38.
151. Mahajan UV, Varma VR, Griswold ME, et al. Dysregulation of multiple metabolic networks related to brain transmethylation and polyamine pathways in Alzheimer disease: A targeted metabolomic and transcriptomic study. *PLoS Med* 2020;17:e1003012.
152. Ibrahim SM, Gold R. Genomics, proteomics, metabolomics: what is in a word for multiple sclerosis? *Curr Opin Neurol* 2005;18:231-5.
153. Snowden SG, Ebshiana AA, Hye A, et al. Association between fatty acid metabolism in the brain and Alzheimer disease neuropathology and cognitive performance: A nontargeted metabolomic study. *PLoS Med* 2017;14:e1002266.
154. Gonzalez-Dominguez R, Garcia-Barrera T, Gomez-Ariza JL. Combination of metabolomic and phospholipid-profiling approaches for the study of Alzheimer's disease. *J Proteomics* 2014;104:37-47.
155. Trushina E, Dutta T, Persson XM, et al. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. *PLoS One* 2013;8:e63644.

156. Han X, Rozen S, Boyle SH, et al. Metabolomics in early Alzheimer's disease: identification of altered plasma sphingolipidome using shotgun lipidomics. *PLoS One* 2011;6:e21643.
157. Varma VR, Oommen AM, Varma S, et al. Brain and blood metabolite signatures of pathology and progression in Alzheimer disease: A targeted metabolomics study. *PLoS Med* 2018;15:e1002482.
158. Ibanez C, Simo C, Martin-Alvarez PJ, et al. Toward a predictive model of Alzheimer's disease progression using capillary electrophoresis-mass spectrometry metabolomics. *Anal Chem* 2012;84:8532-40.
159. An Y, Varma VR, Varma S, et al. Evidence for brain glucose dysregulation in Alzheimer's disease. *Alzheimers Dement* 2018;14:318-29.
160. Gonzalez-Dominguez R, Garcia-Barrera T, Gomez-Ariza JL. Metabolite profiling for the identification of altered metabolic pathways in Alzheimer's disease. *J Pharm Biomed Anal* 2015;107:75-81.
161. Dharshini SAP, Taguchi YH, Gromiha MM. Investigating the energy crisis in Alzheimer disease using transcriptome study. *Sci Rep* 2019;9:18509.
162. Sato Y, Suzuki I, Nakamura T, et al. Identification of a new plasma biomarker of Alzheimer's disease using metabolomics technology. *J Lipid Res* 2012;53:567-76.
163. Christensen A, Pike CJ. APOE genotype affects metabolic and Alzheimer-related outcomes induced by Western diet in female EFAD mice. *FASEB J* 2019;33:4054-66.
164. Pena-Bautista C, Roca M, Lopez-Cuevas R, et al. Metabolomics study to identify plasma biomarkers in alzheimer disease: ApoE genotype effect. *J Pharm Biomed Anal* 2020;180:113088.
165. Merched A, Xia Y, Visvikis S, et al. Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concentrations are highly correlated with the severity of Alzheimer's disease. *Neurobiol Aging* 2000;21:27-30.
166. Evans RM, Emsley CL, Gao S, et al. Serum cholesterol, APOE genotype, and the risk of Alzheimer's disease: a population-based study of African Americans. *Neurology* 2000;54:240-2.
167. Lutjohann D, Papassotiropoulos A, Bjorkhem I, et al. Plasma 24S-hydroxycholesterol (cerebrosterol) is increased in Alzheimer and vascular demented patients. *J Lipid Res* 2000;41:195-8.
168. Atzmon G, Gabrieli I, Greiner W, et al. Plasma HDL levels highly correlate with cognitive function in exceptional longevity. *J Gerontol A Biol Sci Med Sci* 2002;57:M712-5.
169. Finch N, Baker M, Crook R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. *Brain* 2009;132:583-91.
170. Thambisetty M, Simmons A, Velayudhan L, et al. Association of plasma clusterin concentration with severity, pathology, and progression in Alzheimer disease. *Arch Gen Psychiatry* 2010;67:739-48.
171. Thambisetty M, An Y, Kinsey A, et al. Plasma clusterin concentration is associated with longitudinal brain atrophy in mild cognitive impairment. *Neuroimage* 2012;59:212-7.
172. Silajdzic E, Minthon L, Bjorkqvist M, et al. No diagnostic value of plasma clusterin in Alzheimer's disease. *PLoS One* 2012;7:e50237.
173. Oresic M, Hyotylainen T, Herukka SK, et al. Metabolome in progression to Alzheimer's disease. *Transl Psychiatry* 2011;1:e57.
174. Niedzwiecki MM, Walker DI, Howell JC, et al. High-resolution metabolomic profiling of Alzheimer's disease in plasma. *Ann Clin Transl Neurol* 2020;7:36-45.
175. Huo Z, Yu L, Yang J, et al. Corrigendum to brain and blood metabolome for Alzheimer's dementia: findings from a targeted metabolomics analysis [Neurobiology of Aging Volume 86, February 2020, Pages 123-133]. *Neurobiol Aging* 2020;91:169.

176. Zandi PP, Anthony JC, Khachaturian AS, et al. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. *Arch Neurol* 2004;61:82-8.
177. Luchsinger JA, Tang MX, Shea S, et al. Antioxidant vitamin intake and risk of Alzheimer disease. *Arch Neurol* 2003;60:203-8.
178. Aisen PS, Schneider LS, Sano M, et al. High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. *JAMA* 2008;300:1774-83.
179. Yang K, Chen J, Li X, et al. Vitamin D concentration and risk of Alzheimer disease: A meta-analysis of prospective cohort studies. *Medicine (Baltimore)* 2019;98:e16804.
180. Wang T, Wang Z-Y, editors. Metal chelators inhibits Alzheimer neuropathologies in APP/PS1 transgenic mouse brain. Proceedings of the 12th National Academic Conference of the Chinese Society for Neuroscience; 2017; China.
181. Fasae KD, Abolaji AO, Faloye TR, et al. Metallobiology and therapeutic chelation of biometals (copper, zinc and iron) in Alzheimer's disease: Limitations, and current and future perspectives. *J Trace Elem Med Biol* 2021;67:126779.
182. Shcherbatykh I, Carpenter DO. The role of metals in the etiology of Alzheimer's disease. *J Alzheimers Dis* 2007;11:191-205.
183. Asili E, Yarahmadian S, Khani H, et al. A Mathematical Model for Amyloid-. *Bull Math Biol* 2019;81:1943-64.
184. Shevtsov PN, Shevtsova EF, Bachurin SO. Influence of Metal Ions on Microtubules as a Possible Mechanism of Pathogenesis of Alzheimer's Disease. *Biomed chem: res methods* 2018;1:e00050-e.
185. Xu J, Church SJ, Patassini S, et al. Plasma metals as potential biomarkers in dementia: a case-control study in patients with sporadic Alzheimer's disease. *Biometals* 2018;31:267-76.
186. Puentes-Diaz N, Chaparro D, Morales-Morales D, et al. Role of Metal Cations of Copper, Iron, and Aluminum and Multifunctional Ligands in Alzheimer's Disease: Experimental and Computational Insights. *ACS Omega* 2023;8:4508-26.
187. Babic Leko M, Langer Horvat L, Spanic Popovacki E, et al. Metals in Alzheimer's Disease. *Biomedicines* 2023;11.
188. Clarke R, Smith AD, Jobst KA, et al. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol* 1998;55:1449-55.
189. Foy CJ, Passmore AP, Vahidassr MD, et al. Plasma chain-breaking antioxidants in Alzheimer's disease, vascular dementia and Parkinson's disease. *QJM* 1999;92:39-45.
190. Afzal S, Bojesen SE, Nordestgaard BG. Reduced 25-hydroxyvitamin D and risk of Alzheimer's disease and vascular dementia. *Alzheimers Dement* 2014;10:296-302.
191. Rulon LL, Robertson JD, Lovell MA, et al. Serum zinc levels and Alzheimer's disease. *Biol Trace Elem Res* 2000;75:79-85.
192. Baum L, Chan IH, Cheung SK, et al. Serum zinc is decreased in Alzheimer's disease and serum arsenic correlates positively with cognitive ability. *Biometals* 2010;23:173-9.
193. Azhdarzadeh M, Noroozian M, Aghaverdi H, et al. Serum multivalent cationic pattern: speculation on the efficient approach for detection of Alzheimer's disease. *Sci Rep* 2013;3:2782.
194. Kessler H, Pajonk FG, Meisser P, et al. Cerebrospinal fluid diagnostic markers correlate with lower plasma copper and ceruloplasmin in patients with Alzheimer's disease. *J Neural Transm (Vienna)* 2006;113:1763-9.
195. Squitti R, Pasqualetti P, Dal Forno G, et al. Excess of serum copper not related to ceruloplasmin in Alzheimer disease. *Neurology* 2005;64:1040-6.
196. Brewer GJ, Kanzer SH, Zimmerman EA, et al. Copper and ceruloplasmin abnormalities in Alzheimer's disease. *Am J Alzheimers Dis Other Dement* 2010;25:490-7.

197. Yu HL, Chertkow HM, Bergman H, et al. Aberrant profiles of native and oxidized glycoproteins in Alzheimer plasma. *Proteomics* 2003;3:2240-8.
198. Goozee K, Chatterjee P, James I, et al. Elevated plasma ferritin in elderly individuals with high neocortical amyloid-beta load. *Mol Psychiatry* 2018;23:1807-12.
199. Bartolotti N, Bennett DA, Lazarov O. Reduced pCREB in Alzheimer's disease prefrontal cortex is reflected in peripheral blood mononuclear cells. *Mol Psychiatry* 2016;21:1158-66.
200. Ren RJ, Zhang YF, Dammer EB, et al. Peripheral Blood MicroRNA Expression Profiles in Alzheimer's Disease: Screening, Validation, Association with Clinical Phenotype and Implications for Molecular Mechanism. *Mol Neurobiol* 2016;53:5772-81.
201. Mitrea L, Nemes SA, Szabo K, et al. Guts Imbalance Imbalances the Brain: A Review of Gut Microbiota Association With Neurological and Psychiatric Disorders. *Front Med (Lausanne)* 2022;9:813204.
202. Gamez-Valero A, Campdelacreu J, Vilas D, et al. Platelet miRNA Biosignature Discriminates between Dementia with Lewy Bodies and Alzheimer's Disease. *Biomedicines* 2021;9.
203. Lei T, Yu L, Qin L, et al. Stress kinases, endoplasmic reticulum stress, and Alzheimer's disease related markers in peripheral blood mononuclear cells from subjects with increased body weight. *Sci Rep* 2016;6:30890.
204. Borroni B, Agosti C, Marcello E, et al. Blood cell markers in Alzheimer Disease: Amyloid Precursor Protein form ratio in platelets. *Exp Gerontol* 2010;45:53-6.
205. Pani A, Mandas A, Diaz G, et al. Accumulation of neutral lipids in peripheral blood mononuclear cells as a distinctive trait of Alzheimer patients and asymptomatic subjects at risk of disease. *BMC Med* 2009;7:66.
206. Prodan CI, Ross ED, Stoner JA, et al. Coated-platelet levels and progression from mild cognitive impairment to Alzheimer disease. *Neurology* 2011;76:247-52.
207. Milovanovic M, Eriksson K, Winblad B, et al. Alzheimer and platelets: low-density platelet populations reveal increased serotonin content in Alzheimer type dementia. *Clin Biochem* 2014;47:51-3.
208. Koc ER, Uzar E, Cirak Y, et al. The increase of mean platelet volume in patients with Alzheimer disease. *Turk J Med Sci* 2014;44:1060-6.
209. D'Addario C, Di Francesco A, Arosio B, et al. Epigenetic regulation of fatty acid amide hydrolase in Alzheimer disease. *PLoS One* 2012;7:e39186.
210. Di Rosa M, Dell'Ombra N, Zambito AM, et al. Chitotriosidase and inflammatory mediator levels in Alzheimer's disease and cerebrovascular dementia. *Eur J Neurosci* 2006;23:2648-56.
211. Olson L, Humpel C. Growth factors and cytokines/chemokines as surrogate biomarkers in cerebrospinal fluid and blood for diagnosing Alzheimer's disease and mild cognitive impairment. *Exp Gerontol* 2010;45:41-6.
212. Vella LJ, Hill AF, Cheng L. Focus on Extracellular Vesicles: Exosomes and Their Role in Protein Trafficking and Biomarker Potential in Alzheimer's and Parkinson's Disease. *Int J Mol Sci* 2016;17:173.
213. Gomes P, Tzouanou F, Skolariki K, et al. Extracellular vesicles and Alzheimer's disease in the novel era of Precision Medicine: implications for disease progression, diagnosis and treatment. *Exp Neurol* 2022;358:114183.
214. Fiandaca MS, Kapogiannis D, Mapstone M, et al. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. *Alzheimers Dement* 2015;11:600-7 e1.
215. Winston CN, Goetzl EJ, Akers JC, et al. Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. *Alzheimers Dement (Amst)* 2016;3:63-72.

216. Abner EL, Jicha GA, Shaw LM, et al. Plasma neuronal exosomal levels of Alzheimer's disease biomarkers in normal aging. *Ann Clin Transl Neurol* 2016;3:399-403.
217. Goetzl EJ, Kapogiannis D, Schwartz JB, et al. Decreased synaptic proteins in neuronal exosomes of frontotemporal dementia and Alzheimer's disease. *FASEB J* 2016;30:4141-8.
218. Goetzl EJ, Boxer A, Schwartz JB, et al. Low neural exosomal levels of cellular survival factors in Alzheimer's disease. *Ann Clin Transl Neurol* 2015;2:769-73.
219. Lugli G, Cohen AM, Bennett DA, et al. Plasma Exosomal miRNAs in Persons with and without Alzheimer Disease: Altered Expression and Prospects for Biomarkers. *PLoS One* 2015;10:e0139233.
220. Kapogiannis D, Boxer A, Schwartz JB, et al. Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease. *FASEB J* 2015;29:589-96.
221. Soria FN, Pampliega O, Bourdenx M, et al. Exosomes, an Unmasked Culprit in Neurodegenerative Diseases. *Front Neurosci* 2017;11:26.
222. Goetzl EJ, Mustapic M, Kapogiannis D, et al. Cargo proteins of plasma astrocyte-derived exosomes in Alzheimer's disease. *FASEB J* 2016;30:3853-9.
223. Goetzl EJ, Boxer A, Schwartz JB, et al. Altered lysosomal proteins in neural-derived plasma exosomes in preclinical Alzheimer disease. *Neurology* 2015;85:40-7.