**Title:** Novel Blood Biomarkers for an Earlier Diagnosis of Alzheimer's Disease: A Literature Review

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2. [illegible]
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**ABSTRACT**

Alzheimer's disease is a neurodegenerative condition associated with neurofibrillary tangles and cortical deposition of amyloid plaques. Clinical presentation of the disease involves manifestations such as memory loss, cognitive decline and dementia with some of the earliest reported deficits being episodic memory impairment and olfactory dysfunction. Current diagnostic approaches rely on autopsy characterization of gross brain pathology, or brain imaging of biomarkers late in the disease course. The aim of this literature review is to identify and compare novel blood-based biomarkers with the potential of making an earlier clinical diagnosis of Alzheimer's disease. Utilizing such techniques may allow for earlier therapeutic intervention, reduction of disability and enhancement of patients' quality of life. Literature review and analysis was performed by screening the PubMed database for relevant studies between July 1, 2014 and December 31, 2019. Sixteen studies were reviewed with biomarker candidates categorized under microRNAs (miRNAs), auto-antibodies, other blood-based proteins or circulating nucleic acids. Three biomarker candidates – serum neurofilament light chain, plasma β-secretase 1 activity and a panel of three miRNAs (miR-135a/193b/384) – reported statistically significant differences in testing between patients and controls, with high discriminative potential and high statistical power. In conclusion, certain blood biomarkers have shown promising results with high sensitivity and specificity, high discriminative potential for Alzheimer's disease early in its progression, and statistically significant results in larger study samples. Utilization of such diagnostic biomarkers could increase the efficacy of making an earlier clinical diagnosis of Alzheimer's disease.


**Key Words**: Alzheimer's disease; diagnosis; biomarkers; early diagnosis (Source: MeSH-NLM).

## INTRODUCTION

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases, first described by German psychiatrist Alois Alzheimer in 1906, and currently affecting millions of people on a global scale.[1] Approximately 5.8 million Americans are diagnosed with AD and by 2050 this number is expected to increase to 13.8 million.[2] An important characteristic to note is the propensity of the disease to cause dementia – an acquired syndrome resulting in declining memory, executive function and cognitive ability, sufficient to cause interference with daily life and functioning. Globally, an estimated 50 million people have dementia, of which 60-70% of cases are due to AD.[3] Total costs of health care and services in 2015 for all individuals with Alzheimer's or other dementias worldwide were estimated at US $818 billion, placing a substantial financial burden on many families.[3]

The best known risk factor for AD is increasing age, especially with people aged 65 and older, resulting in a subset of AD known as sporadic or late-onset Alzheimer's disease (LOAD). Old age, however, is not the defining requisite of the disease, as there is also a younger-onset (before age 65) pattern, referred to as early-onset Alzheimer's disease (EOAD), with about 13% associated with particular genes and familial inheritance.[4] One of the earliest cognitive deficits of either form of AD is episodic memory impairment, which presents as a reduced ability to recall events specific to a place and time.[5] As the disease progresses, it manifests as dementia, due to involvement of cortical association areas, with clinical manifestations including progressive memory impairment, deficits in executive functions and semantic memory, disorientation, behavioral changes and mood alterations. The diagnosis of AD is based on both clinical manifestations and gross morphological changes due to disease pathology and neurodegeneration in the brain. Consequently, AD (LOAD specifically) is detected quite late in the disease course, along with histopathological confirmation of neurodegeneration observable on autopsy, or in rare cases, biopsy.[6]

Since their discovery, neurofibrillary tangles (NFTs) and senile amyloid plaques have been the hallmark neuropathological features of AD.[7] Amyloid precursor protein (APP) is a highly conserved and integral membrane protein found in various tissues and is highly concentrated in neural synapses.[5] The sequential cleavage of APP by the enzymes β-secretase 1 (BACE1) and γ-secretase results in the formation of amyloid-β (Aβ). The pathogenesis of AD is hypothesized by some groups to be linked to an imbalance between amyloid-β (Aβ) production and clearance, resulting in the aggregation of Aβ predominantly as Aβ42 and Aβ40, which contribute to amyloid plaques and angiopathy respectively, although Aβ40 can also play a role in plaque formation.[8] The neurotoxicity of these plaques plays an important part in the preclinical phase of the disease.[5] Other groups hypothesize that the causative neuropathology of AD is related to tau, a protein expressed in neurons that plays an important role in the regulation of microtubules and their stability within axons. The functioning of tau is in turn regulated by several post-translational modifications to the protein itself. The most significant modification involves phosphorylation of serine and threonine residues, which can also result in hyperphosphorylation of tau protein, leading to the formation of NFTs.[5]

The strongest predisposing risk factor for LOAD is the genotype of Apolipoprotein E (APOE), a gene that encodes the ε2, ε3 and ε4 alleles. Of the three alleles, ε4 is inversely correlated with age of disease onset, as increased expression results in an earlier than usual disease onset. Additionally, one APOEε4 allele and two APOEε4 alleles are associated with a 3x and 12x increase in risk of developing LOAD, respectively.[9] The APOEε4 protein is an important regulator of lipoprotein metabolism and plays a significant role in the aggregation of Aβ as well as its clearance from the central nervous system. These underlying genetic changes ultimately give rise to the gross morphological features seen in the brains of patients with AD.

Autopsy findings in brains from AD patients are grossly characterized by widespread cortical atrophy, especially involving the entorhinal cortex (anterior portion of the parahippocampal gyrus) and the neighboring hippocampal formation.[10] The neuronal atrophic changes in these and other densely cholinergic areas are subsequently accompanied by sulcal widening and gyri narrowing in much of the cerebral cortex. The extensive cortical neuronal atrophy can also give rise to ventriculomegaly and hydrocephalus ex vacuo.[11] Microscopic examination of the affected tissue generally reveals senile plaques composed of Aβ as well as NFTs of hyperphosphorylated tau, as discussed. The disease is also characterized by whole brain reduction in acetylcholine while levels of other neurotransmitters remain relatively unaffected until late stages.[11]

The underlying pathology of AD is known to begin much earlier than the onset of clinical manifestations. As such, a set of new criteria for the staging of AD was proposed by the National Institute of Aging and Alzheimer's Association in 2012. The criteria define three distinct stages of AD: preclinical AD, mild cognitive impairment (MCI) and AD dementia.[12] The preclinical and early MCI stages would be those where AD pathology and possible memory deficits should be present, but cognition would be intact, and as such, disease-modifying therapeutics would be most efficacious in these stages.

Biomarkers have classically been used to characterize the pathology seen in several conditions including AD. The historic and most widely used biomarkers for AD are Aβ and tau, with AD patients having lower levels of Aβ in cerebrospinal fluid (CSF) due to accumulation in plaques, and higher levels of CSF tau.[13] When looking at these markers in blood, studies have reported marginally lower plasma Aβ42 levels,[14] and significantly higher plasma tau levels in AD patients as compared to control subjects.[15] Neuropathological markers such as Aβ and tau can be directly visualized by biopsy and immunohistochemistry. These diagnostic techniques are largely invasive and mostly utilized on autopsy or late during the disease course, once the patient develops cognitive decline and clinical interventions are warranted. Accumulation of Aβ in the brain is a very early event, starting at least a decade before symptoms appear. Well-established Aβ-biomarkers, such as Aβ-binding ligands for in-vivo positron imaging tomography (PET) imaging, can be utilized to measure the Aβ deposition.[16] Imaging methods like PET, however, may be prohibitively expensive. Nevertheless, there is ongoing research investigating plasma Aβ42/Aβ40, with a recent study providing Class II evidence that Aβ42/Aβ40 levels when combined with APOE ε4 status and age, can accurately determine amyloid PET status in cognitively normal individuals.[17]

The involvement of olfactory dysfunction early in the disease course of AD has been reported as early as 1974 and may possibly be one of the earliest manifestations of the disease.[18] The olfactory dysfunction seen in AD is associated with Aβ and NFT deposition in the olfactory bulb – the olfactory pathway's very first synaptic relay.[19] Early AD also affects portions of the olfactory cortex, and degeneration is known to be more pronounced in the left hemisphere of patients with AD. Techniques involving simple olfactory tests have been studied in the past, using stimuli such as a peanut butter. It has been previously reported that such a test can discriminate AD patients from those with MCI and controls with a sensitivity of 100% and specificity of 92%.[20]

Aside from Aβ and tau, there have been advances in the field of other blood-based biomarkers capable of discriminating between those with MCI and healthy controls, and several studies of novel biomarkers detectable in serum and plasma have emerged. Preclinical diagnosis of AD by using such biomarkers, can allow for early therapeutic interventions and new clinical trials, which may result in reduced disability and a better quality of life for patients. This literature review study explores the efficacy of utilizing novel blood-based biomarkers to detect AD earlier in the disease course.

## METHODOLOGY

### *Search Strategy*

The scientific literature used in this review was selected through screening of literature pertaining to the topic of interest by searching the PubMed database. The initial search parameters included combinations of Medical Subject Headings "Alzheimer's disease" or "Alzheimer disease" along with the words "biomarkers", "serum" and "plasma". This initial search yielded 372 articles. Only studies published in English and involving humans were considered for inclusion. A recent systematic review and meta-analysis on CSF and blood biomarkers for AD included studies from July 1, 1984 and June 30, 2014. Carrying forward from then, studies published between July 1, 2014 and December 31, 2019 were considered for inclusion in this review of novel blood-based AD biomarkers. Review articles were filtered out from the search. This yielded 162 articles for further screening and study selection. The abstracts of these articles were thoroughly reviewed to determine eligibility.

### *Eligibility Criteria*

Studies were selected by analyzing the abstracts of the studies for relevancy to the topic of interest. Given the focus of the review is on biomarkers with the potential to detect AD earlier in the disease course, studies not including mild AD or MCI patients in the study sample were excluded. Studies that focused on the ability of biomarkers to differentiate either AD patients, MCI patients, or AD and MCI patients from cognitively healthy individuals were selected. Studies that focused on the ability of biomarkers to discriminate between either AD patients, MCI patients, or AD and MCI patients from patients with other neurodegenerative conditions (such as Parkinson's disease, vascular dementia, etc.), were excluded. Studies that examined the utility of novel biomarkers for the initial diagnosis of MCI, or AD, or MCI and AD were selected; those studies in this subset examining only other effects of biomarker utility, such as AD progression or response to treatment, and not initial diagnosis, were also excluded. After thoroughly reviewing article abstracts and applying these eligibility criteria, sixteen articles were selected.

### *Data Extraction*

The following information was extracted from selected studies and analyzed for this review: Study objectives, sample size and classification, experimental methods, key findings, strengths, limitations and major conclusions.

## RESULTS

The sixteen reviewed studies investigated several different blood-based biomarker candidates for detecting AD earlier in the disease course. These candidates included microRNAs (miRNAs), autoantibodies, other proteins and circulating nucleic acids.

*microRNA levels*

Five studies, summarized in **Table 1,** focused on investigating the utility of miRNAs as biomarkers for earlier detection of AD.[21-25] Four studies included patients with AD and MCI, and one study included patients with mild and moderate AD. All five studies utilized quantitative real time polymerase chain reaction for quantification of differentially expressed miRNAs. Levels of seven miRNAs (miR-135a, -384, -4668-5p, -483-5p, -200a-3p, -93 and 146a) were found to be significantly higher in MCI patients as compared to controls. Levels of three other miRNAs (miR-193b, -222 and-143) were found to be significantly lower in patients with MCI or mild AD as compared to controls.

*Serum autoantibodies*

Two studies, summarized in **Table 2,** focused on investigating serum autoantibodies as diagnostic biomarkers for early detection of AD. The studies included patients with MCI or mild AD and utilized the enzyme-linked immunosorbent assay technique for antibody detection. Levels of anti-phosphatidylserine-dependent antibody (aPSd), anti-phosphatidylethanolamine-dependent antibody (aPEd) and anti-phosphatidylcholine-independent antibody (aPCi) were found to be significantly elevated in the serum of MCI patients as compared to controls.[26] Antibodies against the angiotensin 2 type 1 receptor (anti-ATR1) were found to be significantly higher in mild AD patients without hypertension or diabetes.[27]

*Other blood-based proteins*

Eight studies, summarized in **Table 3,** focused on other blood-based proteins as biomarkers for earlier detection of AD. Dynamics of neurofilament light chain (NfL) have been found to predict neurodegeneration and clinical progression in presymptomatic AD.[28] Serum NfL rates of change were significantly elevated in individuals carrying highly penetrant autosomal-dominant mutations in the amyloid beta precursor protein (*APP*), presenilin 1 (*PSEN1*) or presenilin 2 (*PSEN2)* genes, as compared to non-carriers. Furthermore, the rates of change of serum NfL in symptomatic mutation carriers were significantly associated with rates of cortical thinning in the precuneus.[28]

Keratin type-2 expression, neuronal pentraxin 1 (NP1) levels and BACE1 activity were all found to be significantly elevated in MCI patients compared to controls.[29-31] Angiotensin converting enzyme (ACE) serum activity was significantly higher in AD patients as compared to controls and MCI patients, but no significant difference existed between MCI patients and controls.[32] Levels of soluble endothelial protein C receptor (sEPCR) and Galectin-3 (Gal-3) were found to be significantly elevated in AD patients compared to controls, but no significant difference existed between MCI patients and controls.[33,34] Expression of albumin was significantly decreased in MCI patients compared to controls.[29]

Mean exosomal levels of extracted phospho-serine-type 1 insulin receptor substrate (P-S312-IRS-1) were significantly higher in early AD patients compared to controls.[35] Mean exosomal levels of extracted phospho-tyrosine-type 1 insulin receptor substrate (P-panY-IRS-1) were significantly lower in early AD patients compared to controls. The ratio of P-S312-IRS-1 to P-panY-IRS-1 (Insulin Resistance Index, R) was significantly higher in early AD patients. Insulin resistance reflected by R values could accurately predict development of AD up to 10 years prior to symptom onset.[35]

*Circulating Nucleic Acids*

One study, summarized in **Table 4,** focused on circulating nucleic acids (CNAs) as diagnostic biomarkers for AD. Patients with probable-AD were found to have higher CNA concentrations compared to controls. DNA methylation of the *LHX2* gene was also found to be significantly higher in these patients.[36] Furthermore, upon subclassifying probable-AD patients by MMSE scores, CNA concentrations peaked in the MCI subclass (significantly higher compared to controls).[36]

## DISCUSSION

A novel biomarker, which is sensitive and specific to the development of AD pathology would be an ideal candidate for the preclinical detection of the disease. As such, an ideal biomarker for early AD diagnosis should distinguish between cognitively normal elderly controls and patients with MCI, with great accuracy, sensitivity and specificity. The ideal biomarker should also reasonably predict conversion from cognitively healthy individuals to MCI, and progression from MCI to AD. For each biomarker reviewed, the discriminative potential quantified by measures of diagnostic accuracy, if available, is summarized in **Table 5.**

Over the last decade, researchers have focused on developing non-invasive tests for AD based on detection of miRNAs in the blood. These non-coding, small nucleotide molecules have been found to be differentially regulated in the blood, CSF and even brain tissue of patients with AD.[37] The panel of three miRNAs (miR-135a, -193b, -384) studied by Yang et al. (2018) showed the most promising results, with high discriminative potential and study power.[21] Another biomarker candidate, miR-483-5p, studied by Nagaraj et al. (2017), also revealed high discriminative potential for both AD and MCI, as well as statistically significant results in both pilot and verification studies.[24] This study, however, was limited by the small sample size and consequently, low study power. The other miRNA studies, which presented statistically significant results, either failed to investigate discriminative potential or had low statistical power.

Autoantibodies are another area of focus when looking for non-invasive blood-based biomarker candidates for AD. The study by McIntyre et al. (2015) identified redox reactive antiphospholipid antibodies as serum autoantibodies detectable upon exposure to oxidizing agents, and potential biomarkers of early AD.[26] A limitation of the study lied in the redox reactive oxidizing reagents required for detection, due to cost and limited availability of reagent in different areas. Nevertheless, there is increased ability to generalize the technique to patients having early and late LOAD, due to inclusion of MCI patients in the sample. The resulting discriminative potential was also quite high for MCI patients. Giil et al. (2015) studied anti-ATR1 as a biomarker candidate, which yielded statistically significant differences in antibody levels for mild AD patients.[27] The utility of this biomarker can be severely limited, as the significant results were only applicable to patients without hypertension or diabetes, two highly prevalent systemic diseases. Future steps in evaluating autoantibodies include performing studies on a larger scale to increase statistical power and checking for accuracy of discriminating values.

Preische et al. (2019) conducted an extensive study on serum NfL in relation to the onset and progression of AD.[28] Longitudinal analysis of rates of change in serum NfL yielded significant elevations in subclasses of carriers of mutations in *APP, PSEN1* or *PSEN2* genes, which contribute to the heritability of EOAD.[38,39] The strong association between NfL changes in CSF and blood is indicative of blood-based NfL changes reflecting changes in the brain in AD.[40] The significant association between serum NfL rates of change and rates of precuneus cortical thinning is a noteworthy finding, since this area has been shown to be most sensitive to AD progression.[41,42] The study results coupled with high statistical power show that longitudinal measures of serum NfL are a relatively cheap, non-invasive and reliable method of evaluating neurodegeneration and clinical progression. Future direction for longitudinal NfL studies warrants closer follow-up intervals to determine the association between the time period of rate of change and clinical predictability. Future work should also address translation of these findings to sporadic AD.

Another promising blood-based protein studied was BACE1, which showed statistically significant increases in activity in AD patients, and those with MCI who eventually converted to AD.[31] With a larger sample, the study had relatively high statistical power, and was also highly generalizable since patient groups were recruited from different populations in multiple countries. ACE activity was also similarly studied in a large population but revealed no statistically significant differences between MCI patients and controls. Nevertheless, the study reported significant data and utility of ACE activity pertaining to progression from MCI to AD.[32] NP1, keratin type-2 and albumin had significantly different levels in MCI patients compared to controls, but discriminative potential of these biomarkers was not investigated and the studies had low statistical power and poor generalizability.[29,30] Future studies warrant replication in larger, more representative study samples for validation of results.

The studies investigating sEPCR and Gal-3 showed statistically significant differences in levels of each serum protein for AD patients, but there were no significant differences between MCI patients and controls.[33,34] Further study is warranted in larger study samples to validate these results and determine discriminative potential. Pai et al. (2018) reported significantly elevated CNA concentrations in patients with MCI compared to controls, but the study had low statistical power.[36] The marker may show promise but warrants future work in larger MCI patient samples and investigation of discriminative potential for MCI patients specifically.

Brain tissues from AD patients are noted to have abnormal expression of insulin receptors, as well as an alteration in the phosphorylation pattern of IRS-1, as is seen in patients with type II diabetes mellitus.[43]

Kapogiannis et al. (2014) investigated differential phosphorylation of the serine and tyrosine type-1 insulin receptor substrate (IRS-1) secondary to insulin resistance and reported statistically significant differences in these proteins in MCI as well as AD patients, as compared to controls.[35] Furthermore, significant longitudinal study findings supported this biomarker candidate's ability to accurately predict AD development, up to 10 years prior to onset of clinical symptoms. The power of the study was a significant limitation due to the low sample size. Future work with IRS-1 should focus on replication in larger study samples, with a focus on subjects with MCI and determination of discriminative potential when differentiating MCI from controls.

This review summarizes sixteen journal articles investigating various novel biomarkers potentially capable of aiding in the earlier diagnosis of AD. The studies were conducted in several different countries, giving a global perspective on the issue, but several studies had low statistical power due to relatively small sample sizes. Nevertheless, certain biomarkers such as NfL, BACE1 activity and the panel of miR-135a/193b/384 showed promising results with high relevance towards development of a non-invasive, clinically applicable AD diagnostic biomarker.

The articles included in this study were limited to publications in English, which may have potentially excluded important and relevant manuscripts pertinent to the topic. Additionally, several reviewed articles used study samples from specific populations, leading to selection bias. Another limitation includes one database being used to search and identify publications relevant to the topic. The search was conducted by one investigator and selection of relevant articles depended on a single investigator's judgement, potentially allowing for selection and reporting bias.

**Conclusion**

The blood-based biomarkers for an earlier AD diagnosis presented in this review encompassed microRNAs, autoantibodies, other proteins and circulating nucleic acids. Some of the novel biomarkers reviewed will require future studies for validation of results in larger study samples, or for determination of discriminative values. Further work, in terms of validation of these study results in larger samples and careful evaluation of the diagnostic technique, is warranted to identify the strongest diagnostic biomarkers with high potential and applicability to a clinical setting. A combinatorial approach is also possible and should be considered. Certain biomarkers – such as NfL, BACE1 activity and the panel of miR-135a/193b/384 – have shown promising results with high sensitivity and specificity, high discriminative potential for early AD (MCI patients vs. control subjects) and valid, statistically significant results. Utilization of such biomarkers will increase the efficacy of making an early clinical diagnosis of Alzheimer's disease and begin interventions sooner. Such interventions could potentially reduce disability, delay severe disability, and enhance patients' quality of life.

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**FIGURES AND TABLES.**

**Table 1.** MicroRNA biomarkers for Alzheimer's disease.

| Author, Title, Objective | Study Sample, Selection Criteria | Methods | Key Findings |
|---|---|---|---|
| Yang et al. (2018)<br><br>The Serum Exosome Derived MicroRNA-135a, -193b, and -384 Were Potential Alzheimer's Disease Biomarkers.<br><br>Objective: To explore the potential value of serum exosomal microRNAs as biomarkers for diagnosing AD. | 107 AD; 101 MCI; 228 controls<br><br>Patients admitted to Xuanwu Hospital of Capital Medical University (Beijing, China) between September 2015 and December 2016 were enrolled in the study. | Serum levels of three exosomal miRNAs (miR-135a, miR-193b and miR-384) were measured through exosome isolation, Western blotting and qRT-PCR analysis. | *Serum miR-135a level*<br>Compared to controls:<br>Significantly increased in AD ($P < 0.05$)<br>Significantly increased in MCI ($P < 0.05$)<br><br>*Serum miR-193b level*<br>Compared to controls:<br>Significantly reduced in AD ($P < 0.01$)<br>Significantly reduced in MCI ($P < 0.05$)<br><br>*Serum miR-384 level*<br>Compared to MCI:<br>Significantly higher in AD ($P < 0.05$)<br>Significantly lower in controls ($P < 0.05$) |
| Kumar et al. (2017)<br><br>MicroRNA-455-3p as a potential peripheral biomarker for Alzheimer's disease.<br><br>Objective: To identify microRNAs as early detectable peripheral biomarkers in AD. | 10 AD; 16 MCI; 14 controls<br><br>Sera and DNA samples obtained from patients under the FRONTIERS project (Texas Tech University Health Sciences Center).<br><br>Inclusion criteria: Age ≥ 45 years; rural community-based West Texas individuals; assessed for cognitive functions.<br><br>Exclusion criteria: On strong medications; many health complications | After miRNA extraction, primary screening was performed by microarray analysis. Differentially expressed miRNAs were validated by qRT-PCR.<br><br>miRNA data was further validated by using AD postmortem brains. | *miR-455-3p expression*<br>Compared to controls:<br>Significantly upregulated in AD ($P = 0.007$)<br><br>*miR-4668-5p expression*<br>Compared to controls:<br>Significantly upregulated in MCI ($P = 0.016$)<br><br>*Postmortem AD brains*<br>Significant upregulation of miR-455-3p ($P = 0.016$) |
| Zeng et al. (2017)<br><br>Expression of microRNA-222 in serum of patients with Alzheimer's disease.<br><br>Objective: To determine the association between AD and serum microRNA-222 in patients with AD. | 30 moderate AD; 30 mild AD; 30 controls<br><br>Patients were categorized into groups according to MMSE: mild (15< MMSE ≤26) and moderate (10≤ MMSE ≤15)<br><br>Exclusion criteria: History of cerebral vascular disease; TBI; toxic/metabolic/other brain disorders; drug therapy prior to diagnosis; blood system disease; dementia by vascular or other causes; no signed informed consent. | After miRNA extraction, primary screening was performed by microarray analysis. Differentially expressed miRNAs were validated by qRT-PCR. | *microRNA-222 expression*<br><br>Compared to controls:<br>Significantly lower in mild AD ($P < 0.05$)<br><br>Compared to controls:<br>Significantly lower in moderate AD ($P < 0.05$)<br><br>Compared to mild AD:<br>Significantly lower in moderate AD ($P < 0.05$) |
| Nagaraj et al. (2017)<br><br>Profile of 6 microRNA in blood plasma distinguish | 20 AD; 15 MCI; 15 controls<br><br>All study subjects were Caucasian individuals from | The study sample was divided into two groups: a pilot experiment (20 subjects) and a verification | *miR-483-5p level*<br>Compared to controls:<br>Significantly increased in MCI ($P < 0.01$ in pilot; $P <$ |

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| early stage Alzheimer's disease patients from non-demented subjects.<br><br>Objective: To investigate the utility of plasma microRNAs as biomarkers for detecting early AD. | Poland. Blood samples were taken from patients enrolled in the Alzheimer's ward of Central Clinical Hospital of the Ministry of Interior in Warsaw. | experiment (30 subjects).<br><br>After miRNA isolation, qRT-PCR was performed for both experiments (179 miRNAs for pilot and 15 miRNAs for verification). | 0.001 in verification)<br><br>*miR-200a-3p level*<br>Compared to controls: Significantly increased in MCI ($P < 0.01$ in both pilot and verification) |
| Dong et al. (2015)<br><br>Serum MicroRNA Profiles Serve as Novel Biomarkers for the Diagnosis of Alzheimer's Disease.<br><br>Objective: To identify and validate the potential of circulating miRNAs as novel biomarkers for AD. | 127 AD; 30 MCI; 123 controls<br><br>Study subjects comprised of patients being treated at Shanghai Mental Health Center, Nanjing Brain Hospital and Guangxi Jiangbin Hospital. | After miRNA extraction, quantification of miRNAs was performed by qRT-PCR. | *miR-93 concentration*<br>Compared to controls: Significantly higher in MCI ($P < 0.001$)<br><br>*miR-143 concentration*<br>Compared to controls: Significantly lower in MCI ($P < 0.01$)<br><br>*miR-146a concentration*<br>Compared to controls: Significantly higher in MCI ($P < 0.01$) |

**AD:** Alzheimer's Disease; **MCI:** Mild Cognitive Impairment; **miRNA:** microRNA; **qRT-PCR:** Quantitative Real Time Polymerase Chain Reaction; **MMSE:** Mini-Mental State Examination; **TBI:** Traumatic Brain Injury

**Table 2.** Autoantibodies as biomarkers for Alzheimer's disease.

| Author, Title, Objective | Study Sample, Selection Criteria | Methods | Key Findings |
|---|---|---|---|
| McIntyre et al. (2015)<br><br>Antiphospholipid autoantibodies as blood biomarkers for detection of early stage Alzheimer's disease.<br><br>Objective: To investigate redox-reactive antiphospholipid autoantibodies as a diagnostic tool for mild pre-AD. | 30 AD; 30 MCI; 30 controls<br><br>Coded serum samples assigned to the three study groups by the Alzheimer's Disease Neuroimaging Initiative were used. | aPLs dependent on plasma-protein binding before binding epitopes on PLs were designated as aPLd, and those directly to epitopes on PLs were designated as aPLi. Four different types of R-RAA aPLs were quantified in each group using ELISA, each with dependent and independent subtypes: (aPSd and aPSi), (aCLd and aCLi), (aPCd and aPCi) and (aPEd and aPEi).<br><br>Quantitative ELISA was run on coded serum samples and R-RAA aPL activity was expressed as the difference in optical density between buffer-controlled samples and those treated with hemin (a redox reactive reagent which would unmask the aPLs and allow their detection). | *Serum IgG R-RAA aPSd*<br>Compared to controls: Significantly elevated in MCI (P = 0.011)<br>*Serum IgG R-RAA aPEd*<br>Compared to controls: Significantly elevated in MCI (P = 0.005)<br><br>*Serum IgG R-RAA aPCi*<br>Compared to controls: Significantly elevated in MCI (P = 0.001) |
| Giil et al. (2015)<br><br>Autoantibodies Toward the Angiotensin 2 Type 1 Receptor: A Novel Autoantibody in Alzheimer's Disease.<br><br>Objective: To investigate the association between anti-AT1R and AD, and to investigate the association between clinical/biomarker features of anti-ATR1 and AD. | 92 mild AD; 102 controls<br><br>Study subjects were recruited from the Dementia Study in Western Norway during 2005-2007 from three participating hospitals.<br><br>Exclusion criteria: acute delirium/confusion, terminal illness, recently diagnosed major somatic illness, previous bipolar/psychotic disorder. | Measurement of serum anti-ATR1 antibodies was done in duplicates by using a solid-phase sandwich ELISA.<br><br>Absorbance was measured using an ELISA plate reader. | *Serum anti-ATR1 level*<br><br>Compared to controls: Significantly higher in mild AD patients without hypertension (*p* = 0.04)<br><br>Compared to controls: Significantly higher in mild AD patients without diabetes (*p* = 0.008) |

**AD:** Alzheimer's Disease; **MCI:** Mild Cognitive Impairment; **aPLs**: anti-phospholipid antibodies; **aPSd:** anti-Phosphatidylserine-dependent Antibody; **aPEd:** anti-Phosphatidylethanolamine-dependent Antibody; **aPCi:** anti-Phosphatidylcholine-independent Antibody; **R-RAA:** Redox-Reactive Auto-Antibodies; **ELISA:** Enzyme-Linked Immunosorbent Assay; **anti-ATR1:** anti-angiotensin 2 type 1 receptor antibody

**Table 3.** Other blood-based protein biomarkers for Alzheimer's Disease.

| Author, Title, Objective | Study Sample, Selection Criteria | Methods | Key Findings |
|---|---|---|---|
| Preische et al. (2019)<br><br>Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease.<br><br>Objective: To demonstrate that NfL levels in CSF and serum are correlated with each other and are elevated at the presymptomatic stages of familial AD. | 243 mutation carriers; 162 non-carriers<br><br>DIAN Data and biospecimens were used in the study. DIAN participants were members of families carrying autosomal-dominant mutations in *APP, PSEN1 or PSEN2.* Family members not carrying the mutations served as controls. | Single-molecule array immunoassay technology was used to measure NfL in CSF and serum of 405 participants at the initial visit.<br><br>196 participants returned for another 1-5 visits over a median observation time of 3 years from initial visit. Among these, mutation carriers were further subdivided into pre-symptomatic (CDR=0 across all visits), converter (initially CDR=0, and CDR>0 at subsequent visits) or symptomatic (CDR>0 across all visits).<br><br>Serum NfL rates of change were determined for these participants. Additionally, regression analysis was performed between NfL rates of change and rates of change in brain imaging. | *Serum NfL rates of change*<br><br>Significantly elevated in pre-symptomatic carriers compared to non-carriers ($P = 0.000671$)<br><br>Significantly elevated in converters compared to:<br>Non-carriers ($P = 3.05 \times 10^{-7}$) and<br>Pre-symptomatic mutation carriers ($P = 0.00119$)<br><br>Significantly elevated in symptomatic mutation carriers compared to:<br>Non-carriers ($P = 8.78 \times 10^{-12}$) and<br>Pre-symptomatic mutation carriers ($P = 0.000151$)<br><br>*Rates of precuneus cortical thinning*<br>Significantly associated with rate of change of serum NfL in symptomatic mutation carriers ($P=0.018$) |
| Kumar et al. (2018)<br><br>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.<br><br>Objective: To identify and quantify differentially regulated plasma proteins in MCI subjects vs healthy controls. | 50 MCI<br>50 controls<br><br>Inclusion criteria: Ability to converse in Hindi/English; age > 50; memory complaint for > 6 months; stable and controlled medical conditions such as HTN, DM, hyperlipidemia.<br><br>Exclusion criteria: Having other neurological diseases (stroke, severe small vessel disease, any other systemic problem). | 2D-PAGE of plasma protein in MCI (n=50) and controls (n=50), and identification of differentially regulated proteins with MALDI-TOF and MS-MS.<br><br>Western blotting for quantification of Keratin 2 and Albumin expression in serum of MCI (n=12) vs controls (n=12). | *Serum expression of Keratin type-2 protein*<br>Significantly increased in MCI compared to controls ($p \leq 0.001$)<br><br>*Serum expression of Albumin*<br>Significantly decreased in MCI compared to controls ($p \leq 0.01$) |
| Ma et al. (2018)<br><br>Neuronal pentraxin 1: A synaptic-derived plasma biomarker in Alzheimer's disease.<br><br>Objective: To evaluate NP1, a potential CNS-plasma derived biomarker of excitatory synaptic pathology. | 33 MCI; 31 controls<br><br>Human plasma samples were obtained from APOE-genotyped controls and patients from the ImaGene Study conducted through the UCLA Easton Alzheimer's Center. | Quantification of plasma NP1 by sandwich ELISA. | *Plasma NP1 level*<br>Compared to controls:<br>Significantly higher in MCI ($p < 0.05$) |
| Shen et al. (2018)<br><br>Increased Plasma BACE1 May Predict Conversion to Alzheimer's Disease Dementia in Individuals With Mild Cognitive | 75 probable AD; 96 MCI; 53 controls<br><br>Study subjects were recruited from three independent international academic AD research | Plasma BACE1 activity was measured by a synthetic fluorescence substrate ELISA.<br><br>Protein expression of BACE1 was assessed by | *Plasma BACE1 activity* (*$V_{max}$*)<br>Compared to controls:<br>Significantly increased by 62.8% ($p = 0.001$) in MCI converters<br>Significantly increased by |

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| Impairment.<br><br>Objective: To identify the presence of BACE1 activity and determine potential BACE1 activity alterations in subjects with MCI. | centers and memory clinics (Munich, Sweden and USA). This included patients with cognitively stable MCI (non-converters) and those with MCI who converted to AD (converters). | Western blotting. | 68.9% ($p < 0.001$) in AD<br><br>*Plasma BACE1 concentration*<br>Compared to controls: Significantly increased in AD ($p < 0.05$) |
| Zhuang et al. (2016)<br><br>Angiotensin converting enzyme serum activities: Relationship with Alzheimer's disease.<br><br>Objective: To determine serum activities of ACE as a marker in diagnosis of AD. | 59 moderate-severe AD; 19 mild AD; 45 aMCI; 39 controls<br><br>Study subjects were recruited from patients enrolled in Qingdao Municipal Hospital and through advertisements at senior clubs (2013-2014).<br><br>Exclusion criteria: non-AD dementia; severe CHF; severe liver or kidney disease; severe COPD; cancer; symptoms of depression/anxiety/OCD; taking ACEi, ARB or other medication that could influence cognition. | ACE activity was measured by sandwich ELISA. | *Serum ACE activity*<br><br>Compared to aMCI: Significantly higher in AD, considering different stages altogether ($P = 0.03$)<br><br>Compared to aMCI: Significantly higher in moderate-severe AD ($P = 0.02$)<br><br>Compared to controls: Significantly higher in AD, considering different stages altogether ($P = 0.01$)<br><br>Compared to controls: Significantly higher in moderate-severe AD ($P = 0.01$) |
| Zhu et al. (2015)<br><br>Serum sEPCR Levels Are Elevated in Patients With Alzheimer's Disease.<br><br>Objective: To examine serum sEPCR levels in patients with AD, MCI and controls, and to determine its association with the degree of cognitive impairment (measured by MMSE). | 45 AD; 36 MCI; 42 controls<br><br>Study subjects were recruited from the Department of Gerontology at the Huangshi Central Hospital Affiliated to Hubei Polytechnic University. | Serum sEPCR levels were measured by ELISA. | *Serum sEPCR level*<br><br>Compared to controls: Significantly higher in AD ($P = 0.0005$) |
| Wang et al. (2015)<br><br>Elevated Galectin-3 Levels in the Serum of Patients With Alzheimer's Disease.<br><br>Objective: To compare serum Gal-3 levels in patients with AD, MCI and controls, and to evaluate its association with the clinical features of the disease. | 41 AD; 32 MCI; 46 controls<br><br>Study subjects were recruited from the Department of Neurology in Yuhuangding Hospital and Qilu hospital of Shandong University. | Serum Gal-3 levels were measured by ELISA. | *Serum Gal-3 level*<br><br>Compared to controls: Significantly higher in AD ($P = 0.017$) |
| Kapogiannis et al. (2014)<br><br>Dysfunctionally phosphorylated type 1 insulin receptor substrate in neural-derived blood exosomes of preclinical Alzheimer's disease.<br><br>Objective: To investigate IRS-1 and its phosphorylated forms in neurally derived plasma | 32 AD; 16 aMCI; 81 controls<br><br>Study subjects (aMCI, n=16; mild/moderate dementia, n=10) included identified patients who had donated blood once in the CRU-NIA of Harbor Hospital (Baltimore, MD) or at Jewish Home of San Francisco (San Francisco, CA). | After isolation of exosomes from plasma, quantification of exosome proteins was performed by ELISA. | *P-S312-IRS-1 level*<br>Compared to controls: Significantly higher in AD ($P < 0.0001$)<br><br>*P-panY-IRS-1 level*<br>Compared to controls: Significantly lower in AD ($P < 0.0001$)<br><br>*Insulin Resistance Index, R*<br>Compared to controls: Significantly higher in AD (*P* |

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| exosomes of patients with AD. | For longitudinal studies, 22 additional AD patients were identified, who had given blood twice at Mayo Clinic of University of Kentucky (first when cognitively normal, second when diagnosed with AD). | | < 0.0001)<br><br>*Longitudinal Analysis of R*<br>Accurately predicted development of AD up to 10 years prior to symptom onset |

**NfL:** Neurofilament Light Chain; **CSF:** Cerebrospinal Fluid; **AD:** Alzheimer's Disease; **DIAN:** Dominantly Inherited Alzheimer Network; **APP:** Amyloid Precursor Protein; **PSEN1:** Presenilin 1; **PSEN2:** Presenilin 2; **CDR:** Clinical Dementia Rating; **MCI:** Mild Cognitive Impairment; **HTN:** Hypertension; **DM:** Diabetes Mellitus; **2D-PAGE:** Two-Dimensional Polyacrylamide Gel Electrophoresis; **MALDI-TOF:** Matrix Assisted Laser Desorption/Ionization Time of Flight; **MS-MS:** Mass Spectrometry; **NP1:** Neuronal Pentraxin 1; **CNS:** Central Nervous System; **APOE:** Apolipoprotein E; **UCLA:** University of California Los Angeles; **ELISA:** Enzyme-Linked Immunosorbent Assay; **BACE1:** Beta-Secretase 1; **ACE:** Angiotensin Converting Enzyme; **aMCI:** Amnestic Mild Cognitive Impairment; **CHF:** Congestive Heart Failure; **COPD:** Chronic Obstructive Pulmonary Disease; **OCD:** Obsessive-Compulsive Disorder; **ACEi:** Angiotensin Converting Enzyme Inhibitor; **ARB:** Angiotensin II Receptor Blocker; **sEPCR:** Soluble Endothelial Protein C Receptor; **MMSE:** Mini-Mental State Examination; **Gal-3:** Galectin-3; **IRS-1:** Type 1 Insulin Receptor Substrate; **CRU-NIA:** Clinical Research Unit of the National Institute on Aging

**Table 4.** Circulating Nucleic Acids as biomarkers for Alzheimer's disease.

| Author, Title, Objective | Study Sample, Selection Criteria | Methods | Key Findings |
|---|---|---|---|
| Pai et al. (2018)<br><br>The Role of Methylated Circulating Nucleic Acids as a Potential Biomarker in Alzheimer's Disease.<br><br>Objective: To explore the role of methylated CNAs as potential biomarkers for diagnosing AD. | 27 probable-AD; 9 controls<br><br>Study subjects were recruited from National Cheng Kung University Hospital (cases) and outpatient clinics (controls).<br><br>Exclusion criteria: Evidence of stroke; diabetes; trauma; autoimmune disorders; known malignancy. | CNAs were extracted using the QIAamp CNA Kit. Purified CNAs were quantified by qRT-PCR for the human β-globin gene.<br><br>DNA methylation of the LHX2 gene was analyzed by pyrosequencing after performing genome-wide amplification of the plasma CNAs. | *CNA concentrations*<br>Compared to controls: Significantly higher in probable-AD group ($p < 0.01$)<br><br>Peaked in probable-AD patients classified as mild cognitive impairment, by MMSE ($p< 0.05$)<br><br>*LHX2 methylation*<br>Compared to controls: Significantly higher methylation of CpG sites 1 and 5 in probable-AD group. |

**CNA:** Circulating Nucleic Acid; **AD:** Alzheimer's Disease; **qRT-PCR:** Quantitative Real Time Polymerase Chain Reaction; **LHX2:** LIM Homeobox 2; **CpG:** Cytosine-phosphate-Guanine

**Table 5.** Discriminative potential of novel biomarkers, quantified by measures of diagnostic accuracy

| Study | Diagnostic marker | Control vs AD | | Control vs MCI | |
|---|---|---|---|---|---|
| | | Sensitivity | Specificity | Sensitivity | Specificity |
| **Yang et al.** | miR-135a, miR193b, miR-384 | - | - | 99% | 95% |
| | miR-135a | - | - | 90% | 95% |
| | miR-193b | - | - | 78% | 77% |
| | miR-384 | - | - | 85% | 90% |
| **Nagaraj et al.** | miR-483-5p (pilot study) | 80.0% | 100% | 83% | 100% |
| | miR-483-5p (verification study) | 92.0% | 100% | 87% | 100% |
| **McIntyre et al.** | aPSd, aPEd and aPCi | - | - | 80% | 83.3% |
| **Shen et al.** | BACE1 activity | 64 – 84% | 86 – 88% | 66 – 70% | 86 – 88% |
| **Pai et al.** | CNA concentration | 67% | 89% | - | - |

| Study | Diagnostic marker | Control vs presymptomatic MC | | Control vs symptomatic MC | |
|---|---|---|---|---|---|
| | | Sensitivity | Specificity | Sensitivity | Specificity |
| **Preische et al.** | Serum NfL (baseline) | 92.0% | 14.0% | 85.0% | 75.0% |
| | Serum NfL (rate of change) | 58.0% | 78.0% | 82.0% | 89.0% |

**AD:** Alzheimer's Disease; **MCI:** Mild Cognitive Impairment; **miR:** microRNA; **aPSd:** anti-Phosphatidylserine-dependent Antibody; **aPEd:** anti-Phosphatidylethanolamine-dependent Antibody; **aPCi:** anti-Phosphatidylcholine-independent Antibody; **BACE1:** Beta-Secretase 1; **CNA:** Circulating Nucleic Acid; **NfL:** Neurofilament Light Chain; **MC:** Mutation Carrier