

## Review on Role of Cerebrospinal Fluid and Blood Biomarkers in Alzheimer's Disease Diagnosis

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder characterized by progressive cognitive decline and memory impairment. Early and accurate diagnosis remains a pivotal challenge to improve patient outcomes and guide therapeutic interventions. In recent years, cerebrospinal fluid (CSF) and blood biomarkers have emerged as critical tools for the detection and monitoring of AD pathology. CSF biomarkers such as amyloid-beta 42 (A $\beta$ 42), total tau (t-tau), and phosphorylated tau (p-tau) directly reflect hallmark pathological changes, including amyloid plaque accumulation and neurofibrillary tangles, offering high diagnostic specificity. However, lumbar puncture's invasiveness limits routine use, prompting the development of blood-based biomarkers. Advances in ultrasensitive assay technologies have enabled reliable quantification of plasma A $\beta$ 42/40 ratios, phosphorylated tau isoforms (p-tau181, p-tau217), and neurofilament light chain (NFL), correlating strongly with AD pathology and clinical progression. Blood biomarkers provide a minimally invasive, accessible approach suitable for large-scale screening, early detection, and longitudinal monitoring. Integrating CSF and blood biomarkers with neuroimaging and cognitive assessments enhances diagnostic precision and supports personalized treatment strategies. Nonetheless, several challenges remain, including inter-assay variability, population heterogeneity, and standardization across laboratories, and limited validation in diverse clinical settings. Addressing these limitations is essential for translating biomarker discoveries into routine clinical practice. This review synthesizes current knowledge on CSF and blood biomarker advancements, evaluates their clinical applications, and discusses emerging challenges and future perspectives in biomarker-driven diagnosis and management of Alzheimer's disease.

**Keywords:** Alzheimer's disease; Amyloid-beta; Blood biomarkers; CSF biomarkers; Tau protein.

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Alzheimer's disease is a brain disorder that gradually deteriorates memory and thinking abilities, eventually leading to the failure to do even the most basic tasks. Memory loss with

initial symptoms that may differ from individual to individual. This is typically one of the earliest symptoms of Alzheimer's. There are typically four stages of Alzheimer's disease progression

preclinical, moderate, and severe also called as late stage of the disease. Alzheimer's disease is caused by damage to nerve cells in the brain. People suffering from Alzheimer's disease may experience changes in mood, personality, and behavior. The buildup of  $\beta$ -amyloid protein in brain cells may result in nerve cell injury.<sup>1</sup> The estimated prevalence rate in India is gradually increasing with an increase in the people's changes in lifestyle especially adults in India who are suffering from Alzheimer's disease. According to an Alzheimer's report in 2023 7.4% of the Indian population is suffering from Alzheimer's disease. Alzheimer's is mostly seen in women than men across Indian country.<sup>2</sup> Alzheimer's is a degenerative neurological disorder caused by the deposition of  $\beta$ -amyloid peptides in the brain. That tangle around the brain nerve cells which lead to damage in the brain cells.<sup>3</sup> Overall estimated amounts of Alzheimer's patients worldwide wide 50 million which may increase during the period by 2050.<sup>4</sup> The main purpose of this study is to understand Alzheimer's disease and its mechanisms and future methods of treatment by using biomarkers.

Early diagnosis of disease is crucial for effective therapy. Patients with Alzheimer's disease should have numerous neurological evaluations, as well as an MRI of their brain. Early detection can be accomplished by observing the major symptoms of the disease, such as memory loss, language impairment, and trouble doing daily chores. Clinical diagnosis and medical history of the patient play a key role in the diagnosis of Alzheimer's at the first stages of the treatment. During the clinical examination of the patient, the mini-mental state examination (MMSE) and the Montreal Cognitive Assessment (MoCA) are done to examine the cognitive condition of the patient.<sup>5</sup>

Biomarkers are the indications of the biological process and are also helpful in the diagnosis of the disease. Biomarkers are not only useful in disease diagnosis but also help in the treatment of the patient at all levels of the system.<sup>6</sup> Biomarkers are usually seen in the blood and CSF through brain imaging. Biomarkers enable us to identified targets of the disease. Biomarkers such as amyloid and tau neurofilament light chains and glial fibrillary acidic protein and a few biomarkers are used to measure Alzheimer's disease. Biomarkers play a vital role in the development of drug

discovery for long-term diseases such as cancer, heart and Alzheimer's, and certain neurological diseases.<sup>7</sup>

#### **Pathophysiology of alzheimers disease**

Alzheimer's disease is also caused due to the genetic factors in the patient mostly with the patients above 60 plus age. Genetically they are further categorized into two familial cases and sporadic cases.<sup>8</sup>

Familial cases are generally seen in families who have been affected with Alzheimer's disease in more than one generation, while sporadic cases are complex because they involve both genetic and environmental factors. In terms of pathological conditions, it is two different concepts; one is about the decline of acetylcholine (ACh) levels in the brain, which results from neuron loss and leads to an increase in the effects of Alzheimer's disease. The other concept which is most widely seen is the aggregation of  $\beta$ -amyloid proteins which accumulates in the brain neurones and leads to neuron loss.

Down syndrome, also known as trisomy 21, is a genetic disorder caused by the presence of an extra copy of chromosome 21. This additional genetic material affects the development and function of various organs and systems, including the brain. Among the genes located on chromosome 21 is the amyloid precursor protein (APP) gene. In individuals with Down syndrome, the presence of an extra chromosome 21 results in a duplication of the APP gene, leading to its overexpression. The APP gene plays a crucial role in the production of amyloid- $\beta$  (A $\beta$ ) protein. Under normal physiological conditions, the APP protein is cleaved by enzymes in a tightly regulated process. However, in the presence of excess APP—as seen in individuals with Down syndrome—this process becomes dysregulated, leading to increased production of amyloid- $\beta$  peptides. These peptides have a strong tendency to aggregate and form insoluble plaques in the brain tissue.

The accumulation of amyloid- $\beta$  plaques is one of the hallmark pathological features of Alzheimer's disease. These plaques disrupt cell-to-cell communication and activate immune responses that trigger inflammation and the eventual destruction of brain cells. As a result, individuals with Down syndrome are at a significantly higher risk of developing early-onset Alzheimer's

disease, often beginning in their 40s or 50s. The overexpression of the APP gene is therefore a key molecular link between Down syndrome and Alzheimer's disease, highlighting the critical role of genetic factors in neurodegenerative disease progression.

#### **Genetic Mutations and Alzheimer's disease: Role of A $\beta$ , PSEN1, and PSEN2 in A $\beta$ Aggregation**

In certain familial forms of Alzheimer's disease, mutations in specific genes have been found to play a critical role in the early onset and progression of the condition. Among these, mutations in the amyloid-beta (A $\beta$ ) region of the amyloid precursor protein (APP) gene, as well as in the presenilin 1 (PSEN1) and presenilin 2 (PSEN2) genes, are of particular importance. These genes are directly involved in the formation and functioning of the gamma-secretase enzyme complex, which is responsible for cleaving the APP protein to produce amyloid- $\beta$  peptides.

When mutations occur in PSEN1 or PSEN2, they alter the activity of the gamma-secretase complex. This abnormal activity results in the production of amyloid- $\beta$  peptides that are longer and more prone to aggregation—especially A $\beta$ 42, a highly aggregation-prone form. Similarly, mutations in the APP gene itself can lead to increased production or altered processing of A $\beta$ , further promoting plaque formation. These aggregated A $\beta$  peptides clump together to form toxic amyloid plaques, a major pathological hallmark of Alzheimer's disease.

The buildup of these plaques in the brain interferes with neuronal communication, triggers inflammation, and ultimately contributes to widespread neurodegeneration. Thus, mutations in the APP, PSEN1, and PSEN2 genes not only disrupt normal protein processing but also directly accelerate the aggregation of amyloid- $\beta$ , leading to early and aggressive forms of Alzheimer's disease, particularly in familial cases. Understanding these genetic mutations is crucial for developing targeted therapies and early diagnostic approaches. The presence of the APOE4 allele is considered one of the risk factors for Alzheimer's disease development in terms of genetic inheritance.<sup>9</sup>

#### **Amyloid plaques and tau tangles**

One of the critical events in the pathogenesis of Alzheimer's disease is the abnormal processing of the amyloid precursor protein (APP).

During its metabolism, a fragment called the APP C-terminal fragment can accumulate within neurons. This fragment serves as a precursor to amyloid- $\beta$  (A $\beta$ ) peptides, which, when produced in excess or abnormally cleaved, begin to aggregate. These aggregated A $\beta$  peptides progressively form amyloid fibrils, which are insoluble and toxic to brain tissue. As the amyloid fibrils accumulate, they lead to the formation of amyloid plaques—dense, extracellular deposits predominantly found in the grey matter of the brain. These plaques are often surrounded by dystrophic neuritis and activated glial cells, causing a chronic inflammatory response in the brain. The persistent inflammation, driven by microglial activation and cytokine release, further contributes to neuronal injury and degeneration.

Parallel to the amyloid pathway is the tauopathy pathway, which involves abnormalities in the microtubule-associated protein tau. In Alzheimer's disease, tau becomes abnormally hyperphosphorylated and starts forming paired helical filaments (PHFs). These filaments subsequently aggregate into neurofibrillary tangles (NFTs) within neurons. The presence of NFTs disrupts the normal microtubule structure, impairing axonal transport and cellular communication.

The combined effects of amyloid plaque deposition and tau-related neurofibrillary tangles culminate in neuronal loss and synaptic dysfunction. This neuronal degradation is a key contributor to the progressive cognitive decline observed in Alzheimer's disease. The interplay between these two pathological hallmarks—amyloid and tau—underpins the complex neurodegenerative cascade characteristic of this disorder.

APP accumulation leading to axonal traffic defects influences the formation of PHF & NFT propagation. Whereas amyloid accumulation at grey matter which causes inflammation triggers APOE4 Allele. Thus, the two pathways lead to the development of Alzheimer's disease.<sup>10</sup>

#### **Neuroinflammation and oxidative stress**

Neuroinflammation is one of the risk factors that initiate the inflammatory response in the brain by activating microglia which leads to proinflammatory reactions in the brain leading to cells or neuronal loss.<sup>11</sup> Due to the release of inflammatory agents like TNF- $\alpha$ , interleukins in the brain. The main reason for the release of proinflammatory agents in the brain is an

accumulation of the A $\beta$  cells and NFT leading to damage to the neuron cells and activation of the microglia cells by DAMP's the stimulation of proinflammatory molecules such as TNF $\alpha$ , IL-1 $\beta$ , IL-6, and IL-18 cause's Neuroinflammation in the brain.<sup>12</sup>

Oxidative stress is mainly caused by reactive oxygen species (ROS). ROS plays a key role in the phosphorylation. The accumulation of the A $\beta$  and NFT cells in the brain leads to an imbalance in the homeostasis in the brain.<sup>13</sup> It not only initiates the ROS generation but also obstructs the removal of the ROS the levels of Fe, Cu, and Zn are also responsible for neuron loss in the brain.<sup>14</sup> In Alzheimer's patients there is a significant increase in metals like Fe, Cu, and Zn these metals induce H<sub>2</sub>O<sub>2</sub> production.<sup>15</sup>

#### **Genetic and environmental factors**

Frequent results in Alzheimer's disease with early onset. Only about 5% of familial Alzheimer's disease cases are caused by autosomal mutations in the APP, PSEN1, or PSEN2 genes of the  $\alpha$ -secretase. SAD is characterized by reduced hippocampus volume and increased disease prevalence, including diabetes, obesity, and poor A $\beta$  clearance. It is linked to late-onset Alzheimer's disease. Out of 70 individuals with AD have the gene allele in their DNA which is APOE which has APOE-2, APOE3, and APOE4. APOE-e4 is the gene that increases the risk of developing AD in individuals with an increase in their age.<sup>16-17</sup>

Environmental factors such as Pollution: pollution is also considered one of the risk factors in the present generations due to the gradual increase in pollution may lead to the initiation of inflammatory biomarkers in the brain which ultimately stimulates the accumulation of A $\beta$  concentration levels in the brain which leads to cognitive decline. Prolonged exposure to the pollution may also lead to Alzheimer's disease.<sup>18</sup>

Bad Nutrition: It is also considered a risk factor for developing Alzheimer's disease. Nowadays due to increasing consumption of fatty foods and high-calorie intake, alcohol consumption may lead to an increase in the risk of developing obesity, hypertension, diabetes, and neuronal diseases in the future. This can be prevented by increasing the intake of vitamin and mineral-rich foods and antioxidants, omega-3 fatty acids in their diet.<sup>19</sup>

Improper sleep: with the increase in age people also develop sleep disorders. Sleep is the only state where the brain cleanses the toxins through CSF. Insufficient sleep leads to the accumulation of A $\beta$  cells.<sup>20</sup> Ooms et.al found that short-term sleep deprivation significant rise in A $\beta$ -42 levels in CSF in healthy humans.<sup>21</sup>

These pathological processes form the foundation for identifying biomarkers, which are discussed in the following section on CSF biomarkers.<sup>22</sup>

#### **CSF Biomarkers**

##### **Overview of CSF Biomarkers**

CSF is the cerebrospinal fluid that circulates the sub-arachnoid space in the brain. Biomarkers are generally used to diagnose and find a particular disease.<sup>22</sup>

There are a few biomarkers that we usually see in every case of Alzheimer such as APOE, beta-amyloid, t-Tau protein, and p-Tau, and a few novel biomarkers such as Beta-site Amyloid.

##### **AMYLOID-BETA (A $\beta$ ) IN CSF**

The APP protein is the precursor to beta-amyloid in the cerebrospinal fluid. One of the most difficult components of researching AD initial changes is tracking them throughout time.<sup>23</sup> These changes in individuals with A $\beta$  plaque and NFT neuropathy could be challenging when they don't show symptoms over a while. A method to address these difficulties is to examine individuals who carry a genetically inherited AD mutation in the amyloid precursor protein (APP), presenilin 1 (PSEN1), or presenilin 2 (PSEN2) gene, that results to increased relative output of the A $\beta$ 42 peptide throughout life and early brain A $\beta$  plaque deposition.<sup>24</sup>

##### **TAU and phosphorylated TAU (P-TAU)**

Tau is not adequately phosphorylated, resulting in neuronal and synaptic loss in the brain. In Alzheimer's disease, tau phosphorylation increases threefold. Mutations of the MAPT gene alter tau's normal interaction to tubulin, causing a buildup of phosphorylated tau.<sup>25</sup> Tau can be phosphorylated at over 40 different locations. Tau phosphorylation at threonine 181 (p-tau181) increases in CSF in AD but not in other neurodegenerative disorders.<sup>26</sup>

##### **Neurofilament lightchain (NFL)**

Neurofilament heavy chain (NfH, 200-220 kDa), medium chain (NfM, 145-160 kDa),

light chain (NFL, 68-70 kDa), and  $\alpha$ -internexin (58-66 kDa in the central nervous system) or peripherin (57-59 kDa in the peripheral nervous system) are all subunits of neurofilaments, which are intermediate filament and class IV proteins.<sup>27</sup>

#### **Glial fibrillary acidic proteins (GFAP)**

Astrocytes are considered as the biomarkers of Alzheimer's disease. It is also considered one of the most sensitive biomarkers in Alzheimer's disease.<sup>28</sup>

#### **Limitations and challenges in CSK biomarkers**

The most important drawback of this invasive treatment is the necessity for a lumbar puncture to collect CSF. This can be painful for patients and may discourage some from having testing owing to perceived risk.<sup>29</sup> CSF biomarker concentrations might vary across people and over time, posing interpretation challenges.<sup>30</sup> Certain CSF markers are raised in certain neurodegenerative disorders like Alzheimer's, but they may also be high in other neurological disorders, limiting their diagnostic specificity.<sup>31</sup>

#### **Blood-based biomarkers**

##### **Overview of Blood Biomarkers**

Among the wide array of biomarkers currently studied in Alzheimer's disease, blood biomarkers have emerged as particularly valuable due to their accessibility, affordability, and growing diagnostic reliability. Unlike cerebrospinal fluid (CSF) biomarkers, which require lumbar puncture and are more invasive, blood-based biomarkers offer a non-invasive, cost-effective, and scalable solution for both clinical and research settings. As depicted in *Figure 1*, blood biomarkers are becoming indispensable tools in the early detection and monitoring of Alzheimer's disease, offering a promising alternative for routine screening and longitudinal tracking of disease progression.

#### **Plasma Amyloid $\beta$ and Tau**

Plasma levels of amyloid- $\beta$  (A $\beta$ ) and tau proteins—specifically phosphorylated tau—are increasingly being used to reflect the core pathological hallmarks of Alzheimer's disease. The cholinergic system, which plays a vital role in cognitive function and memory, relies on neurotransmitters such as acetylcholine (ACh), dopamine, adrenaline, and noradrenaline for signal transmission. Dysregulation in these systems, often mirrored by altered levels of A $\beta$  and tau in the blood, provides important insights into the

ongoing neurodegenerative processes and helps guide therapeutic interventions.<sup>32</sup>

#### **Neurofilament Light Chain in Blood**

The neurofilament light chain (NFL) is a structural protein of neurons that is released into the bloodstream during axonal damage. Elevated NFL levels in blood serve as a sensitive marker of neurodegeneration, correlating with disease severity and progression. Its growing diagnostic and prognostic utility across various neurodegenerative disorders, including Alzheimer's disease, has made it a key focus in blood-based biomarker research.

#### **Emerging Blood Biomarkers**

Beyond A $\beta$ , tau, and NFL, several emerging blood biomarkers are under active investigation. These include markers related to inflammation, oxidative stress, synaptic function, and glial activity. Advances in proteomic and transcriptomic technologies have allowed for the discovery of novel molecules that may enhance the specificity and sensitivity of Alzheimer's diagnosis and contribute to a more comprehensive understanding of disease pathology.

#### **Advantages and Challenges of Blood Biomarkers**

Blood biomarkers offer several compelling advantages: they are easily accessible, minimally invasive, cost-effective, and time-efficient. These features make them particularly attractive for use in population-wide screening, repeated assessments, and early disease detection. However, challenges remain in terms of standardization, sensitivity to early-stage pathology, and distinguishing Alzheimer's disease from other neurodegenerative conditions. Continued research and technological refinement are essential to overcome these limitations and fully harness the potential of blood biomarkers in clinical practice.<sup>33</sup>

#### **Technological Advances in Biomarkers Discovery**

##### **Mass spectroscopy and proteomics**

There are many advantages of using mass spectrometry-based proteomics to investigate the pathophysiology of Alzheimer's disease. Technical and economic restrictions have previously hampered mass-spectrometry-based proteomic investigations; nevertheless, these variables have lately become less limiting, increasing the number of proteomics employing AD brain tissue. Proteomics is used to compare protein expression in AD and control brain homogenates, which are often confined to one susceptible brain area.<sup>34</sup>

This method's ability to be used with formalin-fixed paraffin-embedded (FFPE) tissue is one of its main advantages. Most human tissue specimens for research are FFPE blocks from autopsies. One example protein that we discovered using proteomics and have since followed up on in a targeted study is secernin-1. Phosphorylated tau interacts with proteins involved in two major protein breakdown processes in the cell: the ubiquitin-proteasome system, TEM, and the phagosome-lysosome system.

#### **Imaging Technologies and Biomarkers**

The two main approaches for getting structure information on the brains of Alzheimer's patients are computed tomography (CT) and magnetic resonance imaging (MRI).<sup>35</sup> Guidelines propose utilizing one of them to assess individuals in the clinical setting who have cognitive/dementia syndrome (CDS).<sup>36</sup> Because of its excellent spatial resolution, MRI is the most often used technology in diagnosing Alzheimer's disease, which allows the difference between two arbitrarily similar but not identical tissues and multiparametric acquisitions to be recognized. Diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS) approaches have shown to define a spectrum of alterations associated with vascular diseases and white matter disorders in the brain, as well as to identify microstructure and metabolic abnormalities.<sup>37</sup>

#### **Liquid biopsy for alzheimer's disease**

A liquid biopsy is a test that is conducted on a sample of blood, urine, or other bodily fluid to check for cancer cells from a tumors or tiny pieces of RNA, DNA, or any other components. To understand the genetic and molecular changes in the DNA Blood-based liquid biopsy serves as a best alternative to CSF-based liquid biopsy due to its non-invasive nature and cost-effectiveness.<sup>38</sup> Each biological cluster's clinical trajectory will then show, producing a novel liquid biopsy-based risk prediction matrix for the pathophysiology of AD in at-risk patients who do not exhibit symptoms. Following that, the clinical trajectories of each biological cluster will be plotted, producing a novel liquid biopsy-based risk prediction matrix for the pathophysiology of AD in at-risk individuals who do not exhibit symptoms.<sup>39</sup> Vitamin B5 serves as a precursor for the enzyme PANK3,

which is involved in the synthesis of Coenzyme A. This discovery bolsters earlier studies linking metabolic deregulation to Alzheimer's and raises the possibility that food may contribute to the pathophysiology of the illness.<sup>40</sup>

#### **Artificial intelligence and machine learning in alzheimers disease**

MRI, PET, and CT scans are key methods for finding Alzheimer's disease. MRI provides detailed pictures of the brain's anatomy, making it possible to identify shrinkage in areas like the hippocampus, which is frequently impacted in AD. By measuring metabolic activity and amyloid beta deposition, PET scans provide information on the disease's pathological and functional features. AI methods, particularly machine learning (ML), have been successfully employed to evaluate a wide range of data modalities for dementia-related disorders and to investigate various biomarkers. An open-source cohort called PREVENT-AD (Presymptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's disease) was created to study AD before symptoms appear. The Deep and Frequent Phenotyping cohort gathers information from imaging, digital wearable's, fluid biomarkers, and clinical assessments that regularly measure cognition across time.<sup>41</sup>

When it comes to dementia, "early diagnosis" means detecting the illness early on, usually when cognitive decline is mild, enabling earlier intervention and management. "Difference from another dementia" means differentiating between dementia types such as Alzheimer's, vascular dementia, or Lewy body dementia, which can show distinct symptoms and patterns of cognitive decline even in their initial stages.<sup>42</sup> Are no major contributing factors that could be caused by anything could be delirium or neurological or systemic conditions apart from AD. There are several recognized phenotypic types of AD dementia, including atypical non-amnesic (mostly difficulties in word-finding, spatial cognition, or executive abilities) and typical amnesic (mainly impairments in learning and recall of newly learnt formation).<sup>43</sup>

Lewy body dementia range of conditions involving alterations in mobility, cognition, and autonomic functions. Dementia with Lewy bodies and dementia caused by Parkinson's illness.

Sleep disruption, muscle stiffness, and early visual hallucinations. Neurons have deposits of  $\alpha$ -synuclein.<sup>44</sup>

Lobar degeneration in the front temporal region Frontal and temporal lobe focal atrophy; MRI shows knife-edge atrophy. Early onset, behavioral and personality abnormalities, linguistic difficulty, and a significant familial par.<sup>45</sup>

Vascular dementia focuses on neurologic symptoms and a stepwise progression. Moderate motor symptoms in sub cortical vascular dementia, depression, retrieval issues, dysexecutive syndrome, and reduced processing speed. AD symptoms are similar.<sup>46</sup>

### **Predicting disease progression and cognitive decline**

Diagnostic and Prognostic Utility of Biomarkers have appeared as crucial tools in the early diagnosis of dementia, offering a means to detect pathophysiological changes before the onset of clinical symptoms. Anticipating cognitive symptoms can be aided by identifying biomarkers of Alzheimer's disease (AD) pathology and implement preventative strategies. Early detection leads to personalized treatment solutions, needing continued study and cross-disciplinary collaboration to have a thorough understanding of AD pathophysiology. The integration of wearable devices and sensors for detecting digital biomarkers is rapidly advancing the field of Alzheimer's disease (AD) research. Cognitive, behavioral, and motor changes often precede clinical symptoms, making early detection crucial for intervention. Researchers are increasingly leveraging consumer-grade mobile and wearable technologies to find digital biomarkers that can aid in large-scale screening and patient monitoring.<sup>47</sup> A key breakthrough in this area is the use of Smartphone and tablet-based digital biomarker tasks, such as AlodiaiADL tasks, which assess spatial memory and navigation. Additionally, episodic memory tasks like the Gallery Game have shown efficacy in finding preclinical stages of AD, improving recruitment for clinical trials, and disease tracking<sup>48</sup>. Sensor-based biomarkers are also gaining traction in early AD detection. These sensors, capable of monitoring eye movements, pupillary reflexes, and speech, offer valuable insights into disease onset. Camera and light sensors capture early-stage deficits in eye movements, a hallmark of AD.<sup>49</sup> Similarly,

speech analysis using machine learning tools such as the open SMILE v2.1 toolkit has shown promising results in diagnosing AD through acoustic feature extraction<sup>50</sup>. These approaches offer a non-invasive, cost-effective alternative to traditional biomarkers like imaging or blood tests. Blood-based biomarkers, although present in lower concentrations due to the blood-brain barrier, hold enormous potential for AD screening when paired with advanced analytical methods. Plasma-based screening of biomarkers such as amyloid-beta (A $\beta$ 1-42), tau proteins, and neurofilament light chain (NFL) has proved high diagnostic accuracy.<sup>51</sup> The use of mass spectrometry and immunoassays to measure these markers significantly enhances early diagnosis. Notably, longitudinal studies have confirmed that plasma biomarkers like p-tau181 and NFL can effectively track disease progression and cognitive decline, making them powerful tools for monitoring AD<sup>52</sup>. Biosensors are another promising frontier in AD research. Recent advancements include a label-free electrochemical biosensor for detecting amyloid-beta oligomers and a graphene oxide-based sensor for miRNA-34a, both of which could play a significant role in early AD diagnosis<sup>53</sup>. The diagnosis of AD still heavily relies on medical imaging methods, especially positron emission tomography (PET) and magnetic resonance imaging (MRI). These methods enable non-invasive visualization of brain changes associated with AD. Deep learning models, trained on PET and MRI scans, have shown superior performance in finding AD-related abnormalities compared to traditional radiology assessments. AI-driven models, such as InceptionV3 and PETNet, offer highly correct and computationally efficient approaches for early AD diagnosis<sup>54</sup>. The autonomic nervous system (ANS) also undergoes pathological changes in AD, with heart rate variability (HRV) serving as a potential biomarker for cognitive impairment<sup>55</sup>. Studies suggest that reduced parasympathetic activity correlates with AD progression. Photoplethysmography (PPG) technology integrated into smartwatches and pulse oximeters has been used to measure HRV, offering an accessible and non-invasive method for AD detection<sup>56</sup>. Multi-sensor approaches combining HRV monitoring with gait analysis have deproteinising results in distinguishing mild cognitive impairment from normal aging.

The integration of digital biomarkers, wearable technologies, biosensors, blood-based markers, and advanced imaging techniques is revolutionizing AD research. These innovative approaches not only fasciate detection but also enhance disease monitoring, treatment response assessment, and clinical trial recruitment. As technological advancements continue, the potential for non-invasive, cost-effective, and scalable solutions in AD diagnostics becomes increasingly practical, laying the path for more focused treatment approaches and better patient outcomes.

### **Biomarkers Used in Clinical Trials for Alzheimer's disease**

In clinical trials, biomarkers play a crucial role in assisting with early diagnosis and monitoring the course of disease, and evaluating therapeutic responses. These biomarkers are derived from cerebrospinal fluid (CSF), blood, imaging modalities, and genetic or epigenetic markers. In AD clinical trials, biomarkers are categorized based on their involvement in key pathological processes: amyloid pathology, tau pathology, neurodegeneration, neuroinflammation, synaptic dysfunction, and genetic predisposition. While CSF and imaging biomarkers stay the gold

standard, emerging blood-based biomarkers are becoming increasingly relevant for early and non-invasive diagnosis.<sup>57</sup> The following table presents a comprehensive overview of validated and emerging biomarkers used in Alzheimer's disease clinical trials, detailing their category, sample type, clinical utility, and supporting references.

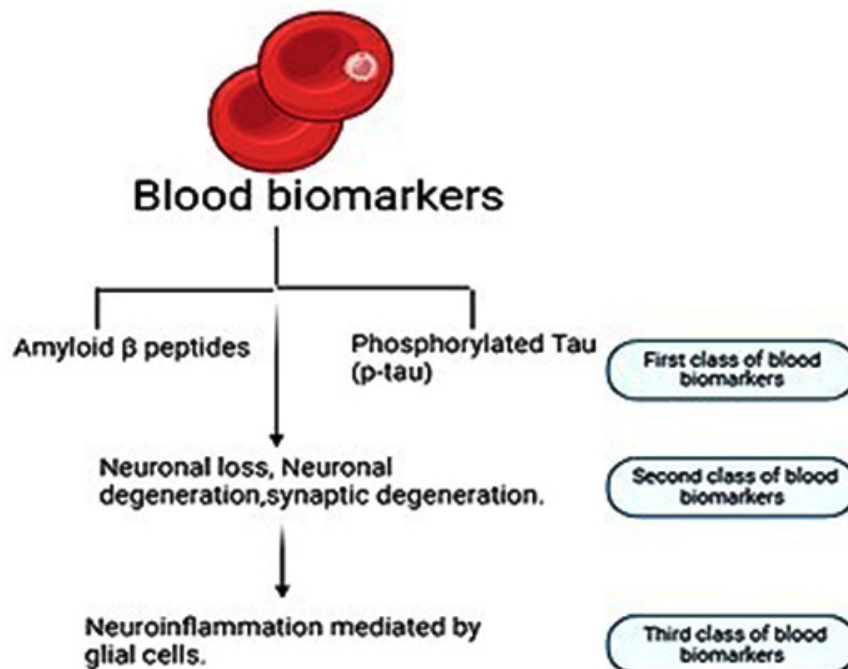
### **Standardization and validation of biomarkers**

For Alzheimer's disease (AD) biomarkers to be successfully incorporated into clinical practice and research, they must be standardized and validated. Standardization ensures consistency and reliability across different laboratories and studies, while validation confirms that these biomarkers accurately reflect the disease processes, they are intended to measure.<sup>73</sup>

### **Standardization Efforts**

Several initiatives have been set up to promote the standardization of AD biomarkers

- Global Biomarker Standardization Consortium (GBSC): Founded by the Alzheimer's Association, the GBSC brings together experts from academia, industry, and regulatory agencies to develop consensus on standardizing biomarker tests for global clinical application.



**Fig. 1.** Blood biomarkers in diagnosis of Alzheimer's disease

**Table 1.** Overview of Biomarkers Used in Clinical Trials for Alzheimer’s disease

| Biomarker  | Type       | Clinical Utility                                   | Sample Type | Reference |
|--|------------|--|-------------|-----------|
| A $\beta$ 42/A $\beta$ 40 ratio                      | Fluid      | Diagnosis, disease staging                         | CSF, Plasma | 58        |
| PET Amyloid Imaging                                  | Imaging    | Early detection, clinical trial inclusion criteria | PET Scan    | 59        |
| Amyloid PET (Florbetapir, Flutemetamol, Florbetaben) | Imaging    | Outcome measure, disease progression tracking      | PET Scan    | 60        |
| CSF Total Tau (t-tau)                                | Fluid      | Disease progression, neuronal damage indicator     | CSF         | 61        |
| CSF Phosphorylated Tau (p-tau181, p-tau217)          | Fluid      | Diagnosis, response to anti-tau therapy            | CSF         | 62        |
| Neurofilament Light Chain (NFL)                      | Fluid      | Synaptic damage and neurodegeneration marker       | CSF, Blood  | 63        |
| Glial Fibrillary Acidic Protein (GFAP)               | Fluid      | Astrocytic activation, disease progression         | Plasma      | 64        |
| MRI-based Hippocampal Atrophy                        | Imaging    | Structural biomarker for disease staging           | MRI         | 65        |
| Synaptosome-Associated Protein 25 (SNAP-25)          | Fluid      | Synaptic degeneration marker                       | CSF         | 66        |
| SV2A PET Imaging                                     | Imaging    | Synaptic density marker                            | PET Scan    | 67        |
| Plasma p-tau217                                      | Fluid      | Non-invasive diagnostic biomarker                  | Plasma      | 68        |
| Plasma A $\beta$ 42/A $\beta$ 40 ratio               | Fluid      | Screening and disease progression                  | Plasma      | 69        |
| Apolipoprotein (APOE $\epsilon$ 4)                   | Genetic    | Risk stratification, clinical trial inclusion      | Blood (DNA) | 70        |
| Polygenic Risk Scores (PRS)                          | Genetic    | Risk prediction model                              | Blood (DNA) | 71        |
| DNA Methylation (e.g., 5-hmC in APP, PSEN1)          | Epigenetic | Early diagnosis                                    | Blood, CSF  | 72        |

• Standardization of Alzheimer's Blood Biomarkers (SABB) Workgroup: This collaborative project aims to create standardized operating procedures (SOPs) for blood-based biomarker measurements, helping their best use in clinical studies and practice.<sup>74</sup>

#### **Validation Methodologies**

The validation of AD biomarkers involves several key steps:

1. Analytical Validation: Assessing the precision, accuracy, and reproducibility of biomarker assays across different platforms and laboratories.
2. Clinical Validation: Setting up the biomarker's ability to detect or predict clinical outcomes associated with AD, such as cognitive decline or disease progression.
3. Cutoff Determination: Defining threshold values that distinguish between normal and pathological states, which is crucial for diagnostic decision-making.

#### **Regulatory Considerations**

For biomarkers to transition from research settings to routine clinical use, they must undergo rigorous evaluation by regulatory bodies to ensure their safety and efficacy. This process includes Compliance with Guidelines: Adhering to set up protocols for biomarker development and validation. Demonstrating Clinical Utility: Providing evidence that the biomarker improves patient outcomes or aids in clinical decision-making.<sup>75</sup>

#### **Clinical guidelines and biomarker integration**

The integration of biomarkers into clinical guidelines for Alzheimer's disease (AD) has significantly enhanced diagnostic precision and patient management. Traditionally, AD diagnosis relied heavily on clinical assessments and cognitive evaluations. However, the advent of biomarkers has introduced objective measures that reflect underlying neuropathological changes, enabling earlier and more correct detection.<sup>76</sup>

#### **Incorporation into Diagnostic Criteria**

Recent revisions to AD diagnostic criteria emphasize a biological definition of the disease, incorporating biomarkers as central components. According to the 2024 criteria, a Core 1 biomarker test that is abnormal is enough to establish an AD diagnosis and guide clinical judgment across the disease spectrum. Amyloid positron emission tomography (PET), authorized

cerebrospinal fluid (CSF) biomarkers, and accurate plasma biomarkers—particularly phosphorylated tau 217—are examples of core 1 biomarkers. These biomarkers indicate the existence of AD neuropathological alterations by mapping onto either the amyloid beta or AD tauopathy pathway.<sup>77</sup>

#### **Biomarkers derived from blood in clinical practice**

The development of blood-based biomarkers (BBMs) provides a more accessible and less intrusive substitute for conventional diagnostic techniques such as CSF analysis and amyloid PET. BBMs are increasingly used in clinical practice to support AD diagnosis, offering a cost-effective choice for biomarker testing. Their integration into clinical guidelines is underway, with recommendations focusing on their proper use and interpretation in various clinical contexts.<sup>78</sup>

#### **Guideline Development and Standardization Efforts**

Organizations such as the Alzheimer's Association are at the forefront of developing clinical practice guidelines that incorporate biomarkers. Utilizing rigorous methodologies like the GRADE framework, these guidelines distill the best available evidence to support decision-making in patient care. Efforts are also directed toward standardizing biomarker assays and setting up clear protocols for their clinical application, ensuring consistency and reliability across different healthcare settings.<sup>79</sup>

#### **Challenges and Future Directions**

While the integration of biomarkers into clinical guidelines marks considerable progress, challenges stay. Ensuring the widespread availability and affordability of biomarker testing, particularly in resource-limited settings, is crucial. Additionally, continuous research is needed to confirm emerging biomarkers and refine their application in diverse populations. Future guidelines will likely evolve to incorporate novel biomarkers and advanced diagnostic technologies, further enhancing the precision of AD diagnosis and treatment.<sup>80</sup>

#### **Ethical and practical considerations**

Numerous moral and practical difficulties arise while providing care for people with Alzheimer's disease (AD) that healthcare providers, caregivers, and families must navigate to ensure compassionate and effective care.

### Ethical Considerations

- **Respecting Patient Autonomy:** As AD progresses, patients experience diminishing decisional ability. It's imperative to honour their autonomy by involving them in care decisions as much as possible and respecting their previously expressed wishes.
- **Informed Consent:** Obtaining informed consent for treatments and participation in research becomes complex as cognitive functions decline. Ensuring that patients understand the implications of medical decisions is essential, and when they cannot, involving legally authorized representatives is necessary.
- **Early Diagnosis Implications:** While early diagnosis can ease planning and intervention, it also raises concerns about the patient's psychological well-being, potential stigma, and the impact on personal identity.
- **Use of Emerging Therapies:** The advent of treatments like anti-amyloid immunotherapy introduces ethical dilemmas related to beneficence and nonmaleficence. In order to ensure that patients and their families are informed, clinicians must balance the possible advantages against risks and adverse consequences.<sup>81</sup>

### Practical Considerations

- **Caregiver Support:** Caregivers often face emotional and physical burdens, including depression and anxiety. Providing them with resources, respite care, and support networks is crucial to keeping their well-being and sustaining the quality of care.
- **Behavioural Management:** Individuals with AD may show challenging behaviours. Implementing routines, creating safe environments, and addressing underlying causes like pain or overstimulation can mitigate these issues.
- **Access to Care:** Barriers such as excessive costs, limited availability of specialized providers, and logistical challenges can impede access to quality care. Addressing these issues requires systemic changes and advocacy for better resources and support.
- **Care Planning:** Developing comprehensive care plans that adapt to the evolving needs of the patient ensures continuity and quality of care. This includes medical management, daily living aid, and end-of-life considerations.<sup>82</sup>

### Future Directions in Alzheimer's disease biomarker research (AD)

#### Challenges in Translating Biomarkers into Clinical Use

Challenges stay one of the most complex and challenging neurodegenerative disorders, with early and correct diagnosis being a critical yet unresolved issue. Biomarker research has made significant strides in recent years, with cerebrospinal fluid (CSF) and imaging-based markers offering valuable insights into disease pathology. However, translating these biomarkers into routine clinical practice faces multiple challenges, needing further advancements to refine their utility, improve diagnostic accuracy, and enable personalized treatment strategies. One of the foremost challenges in translating AD biomarkers into clinical use is the variability in biomarker performance across different patient populations. While biomarkers in CSF and positron emission tomography (PET) imaging, such as tau protein and amyloid- $\beta$  (A $\beta$ ), provide robust disease indicators, their accessibility and cost remain barriers to widespread adoption. Additionally, inter-individual variability in biomarker expression complicates standardization, making it difficult to define universally applicable diagnostic thresholds.<sup>83</sup>

#### Biomarker Combinations for Improved Diagnostics

Combining blood-based biomarkers like neurofilament light chain (NFL) and phosphorylated tau (p-tau), offers a promising alternative, but their validation across diverse cohorts is essential before they can replace invasive and expensive techniques. Given the limitations of single biomarkers, a shift toward biomarker combinations is appearing as a key strategy to improve diagnostic accuracy. Multimodal approaches that integrate fluid biomarkers, neuroimaging, and digital cognitive assessments are being explored to enhance sensitivity and specificity. For instance, combining plasma p-tau with amyloid PET scans may allow for earlier detection of AD pathology, even before the onset of clinical symptoms. Similarly, machine learning algorithms applied to multi-omics data, including proteomics, transcriptomics, and metabolomics, can help uncover novel biomarker panels that offer a more holistic view of disease progression. Such combinations not only improve diagnostic precision

but also ease the staging of AD, enabling early intervention strategies. Another crucial avenue in AD biomarker research is the incorporation of personalized medicine approaches.<sup>84</sup>

### **Methods of Personalized Medicine in Alzheimer's disease**

Given heterogeneity AD, biomarkers must be tailored to individual patient profiles to predict disease trajectory and treatment response more effectively. Genetic factors, such as APOE  $\epsilon$ 4 status, influence biomarker expression, and disease progression, underscoring the need for stratified diagnostic models. Furthermore, emerging technologies like Spatial transcriptomics and single-cell RNA sequencing are opening the door to a better comprehension of specific disease pathways. This precision-medicine paradigm could ultimately lead to targeted therapeutic interventions, improving patient outcomes by addressing specific molecular pathways involved in disease pathology. As the field advances, to close the gap between biomarker discovery and clinical use, these issues must be resolved. Biomarker assay standardization, cross-population validation, and incorporation of AI-powered analytical instruments will be a key in shaping the future of AD diagnostics and treatment. With ongoing progress in biomarker research, the vision of early, precise, and personalized Alzheimer's disease management is becoming an attainable reality.<sup>85</sup>

### **DISCUSSION**

Although cerebrospinal fluid (CSF) biomarkers such as A $\beta$ 42, t-tau, and p-tau remain the gold standard for Alzheimer's disease (AD) diagnosis, their invasive collection limits routine application. Blood-based biomarkers, particularly plasma p-tau isoforms and the A $\beta$ 42/40 ratio, have demonstrated strong correlations with AD pathology and clinical progression, yet variability across studies raises questions about their reliability in diverse populations. For example, while p-tau<sub>217</sub> is reported to outperform p-tau<sub>181</sub> in distinguishing AD from other dementias, conflicting evidence suggests assay- and cohort-specific differences, indicating that no single biomarker can be universally relied upon. Similarly, neurofilament light chain (NfL) is a sensitive marker of neuronal

damage but lacks specificity, as it is elevated in other neurodegenerative disorders. These inconsistencies highlight the need to interpret biomarker data in context and consider alternative explanations for abnormal values.

Another important consideration is whether biomarkers merely reflect ongoing pathology or can truly predict disease onset before symptoms appear. This debate underscores the importance of integrating biomarkers with imaging modalities, cognitive testing, and emerging digital biomarkers to improve diagnostic accuracy. Furthermore, environmental and lifestyle factors such as sleep deprivation, pollution, and nutrition may influence biomarker levels, suggesting that interpretation should extend beyond neuropathological mechanisms alone.

Overall, while significant progress has been made, translation into clinical practice requires overcoming challenges in assay standardization, validation across populations, and accessibility in resource-limited settings. Biomarker-driven diagnosis must therefore be viewed as part of a multi-modal strategy rather than a standalone solution.

### **CONCLUSION**

Alzheimer's disease remains one of the most challenging neurodegenerative disorders, where early and accurate diagnosis is crucial for effective intervention. Cerebrospinal fluid (CSF) biomarkers, particularly A $\beta$ 42, total tau, and phosphorylated tau, provide robust insights into underlying pathology and remain the current gold standard. However, their invasive collection limits widespread use. Recent advances in blood-based biomarkers, especially plasma p-tau isoforms, A $\beta$ 42/40 ratios, and neurofilament light chain (NfL), offer promising non-invasive alternatives that strongly correlate with disease progression. Despite these advancements, significant challenges remain. Variability across assays, lack of standardization, and limited validation in diverse populations restrict their translation into routine clinical settings. Moreover, some biomarkers, such as NfL, reflect general neurodegeneration rather than AD-specific pathology, underscoring the importance of multimodal approaches. Integrating fluid

biomarkers with neuroimaging, genetic profiling, and digital health technologies may provide the most reliable diagnostic framework.

Overall, the evidence supports that biomarkers have the potential to shift AD diagnosis from symptom-based recognition to biologically informed, earlier detection. Yet, careful standardization, large-scale validation, and cost-effective implementation strategies are essential before these tools can be fully embedded in clinical practice. Future research should focus on combining complementary biomarkers into diagnostic panels and aligning discovery with patient-centered applications to achieve precision medicine in Alzheimer's disease.

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#### Author's contribution

Nagaraju Bandaru - Contributed to conceptualization and methodology; Pydiraju Kondrapu, Pydikondala Nehitha Purna, Shashikala Metri: Performed the literature research, analyzed the data and drafted the manuscript; Alekya Ketha:

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