RESEARCH PAPER

The convergence of mechanisms and markers: Advances in pathogenesis insights and blood-based diagnostics for Alzheimer's Disease

Deepanshi Rana[1], Saumya Das[2*], Avijit Mazumder[3]

[1] Department of Pharmacology, Noida Institute of Engineering and Technology (Pharmacy Institute), 19, Knowledge Park-II, Greater Noida, 201306 Uttar Pradesh, India. Email: ranadeepanshi76@gmail.com

[2*] Department of Pharmacology, Noida Institute of Engineering and Technology (Pharmacy Institute), 19, Knowledge Park-II, Greater Noida, 201306 Uttar Pradesh, India.
Email: saumyadas.pharmacy@niet.co.in (Corresponding Author)

[3] Department of Pharmacology, Noida Institute of Engineering and Technology (Pharmacy Institute), 19, Knowledge Park-II, Greater Noida, 201306 Uttar Pradesh, India. Email: avijitmazum@yahoo.com

Received: 2nd Mar, 2026 | **Revised:** 14th Mar, 2026 | **Accepted:** 4th Apr, 2026 | **Available Online:** 20th Apr, 2026

ABSTRACT

Background: AD is a common neurodegenerative disorder that involves gradual neurocognitive deterioration follows complex pathological pathways. Various traditional methods, like PET and X-rays, were used to diagnose AD.

Objectives: This review emphasizes various pathological mechanisms involved in disease progression. This paper focuses on the traditional methods for the proactive screening of AD-related changes, along with the techniques that are being used nowadays, with their limitations in the current scenario. This review further explores blood-based biomarkers, neuroimaging modalities, and machine learning tools that serve as a promising way of detecting and disease monitoring, and further research into the mechanistic importance in AD development.

Material and methods: This review was executed through a comprehensive and systematic analysis of scholarly publications documented between 2000-2025, delving into molecular cascade, pathological routes, and advancing biomarker candidates related to AD. Search engines like PubMed, Scopus, Sci, Google Scholar, Bentham, and Web of Science were explored, implementing MeSH driven strategy combining keywords like "Alzheimer's disease", "calcium signalling", "neuroinflammation", and "biomarkers". Publications were screened and incorporated depending upon the clinical utility, novelty, and experimental accuracy, with priority on research elucidating molecular explanations with detection models. Ethical transparency was maintained by organized realignment and Vancouver style referencing configured with the mentioned DOIs.

Discussion: Early diagnosis of Alzheimer's disease is yet hindered by the current techniques of PET and X-ray diagnostics. Besides the issues on validation and availability, progress in blood-based biomarkers, neuroimaging, and machine learning could offer better sensitivity and monitoring. Mechanistic insights must be translated into models for the development of reliable, affordable methods for screening.

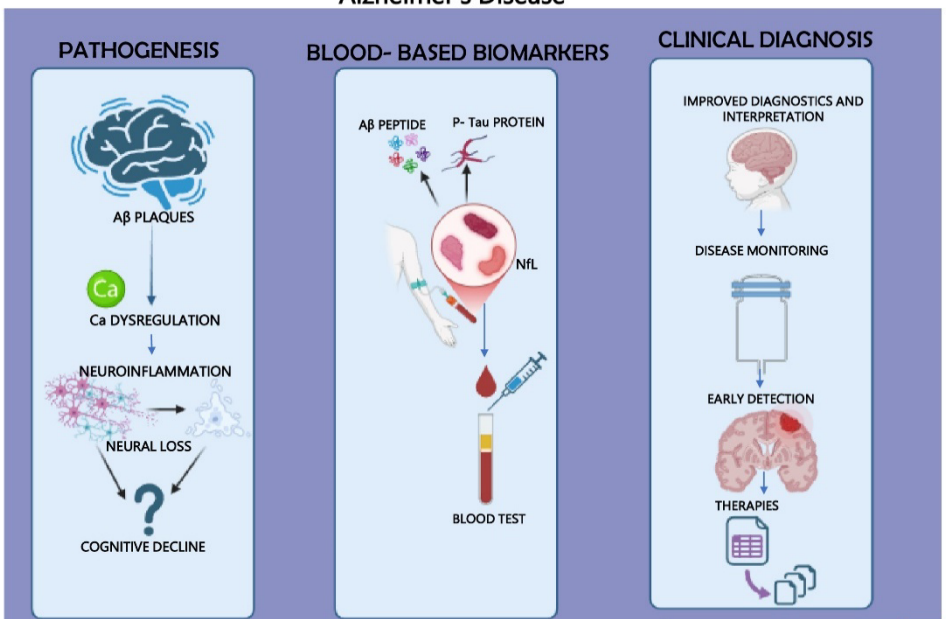
Conclusion: We explored the various pathological mechanisms having a causal relationship with AD. We also reviewed the potential biomarkers and techniques currently used in diagnosing AD.

Keywords: Alzheimer's disease, Amyloid cascade, Calcium signalling, Synaptic dysfunction, Biomarkers, Calpain.

How to cite this article: Rana D, Das S, Mazumder A. The convergence of mechanisms and markers: Advances in pathogenesis insights and blood-based diagnostics for Alzheimer's Disease. Int J Drug Deliv Technol. 2026;16(32s):656-681. DOI: 10.25258/ijddt.16.32s.74

Source of support: Nil.

Conflict of interest: The authors declare no conflict of interest.



Depiction of AD pathogenic mechanisms underscoring amyloid-β plaques, neuroinflammation, calcium imbalance, tau pathology, and neuronal loss, causing memory decline. Blood-based signatures encompassing Aβ peptide, total tau, and phosphorylated tau are shown as evolving modalities for preliminary diagnosing, disease tracking, and therapeutic segmentation, showcasing their translational applicability in clinical diagnostics.

***Graphical abstract*- Mechanisms and markers: Advances in pathogenesis insights and blood- based diagnostics for AD**

1. Introduction

The most commonly reported form of dementia - AD, is an incurable neurodegenerative illness that makes up more that 80% of all cortical degenerative ailment cases around the globe. Cognitive impairments in language, memory deficiencies, spatiotemporal orientations, along with behavioural changes, are the prodromes of AD [1]. Cognitive hallmarks of AD may include short-term amnesia, praxis, visuospatial, and executive dysfunctions. All of these factors are responsible for the progressive loss of personal autonomy [2].

The occurrence of AD is gradually escalating with advancing age, with 10% of the population ages 65 and older affected, and the prevalence rises to about 50% of people ages 85 and older. It is unusual to see this disorder in younger people; if it does occur, it may be due to genetic abnormalities. It is assumed that by 2050, over 131 million of the people will be impacted by AD, and the demographic groups that will be affected will be from the middle-income and low-income countries. Till now, there is no efficacious treatment of AD showing promising effects in reducing its progression [3].

Females show more propensity than men to receive an AD diagnosis, according to the epidemiological aggregated diagnostic data for AD. About 5.3 million adults 65 and older in the US are thought to have AD, with 3.3 million of them being female and up to 2.0 million being males. As caregiving responsibilities predominantly fall on women, of whom approximately one- third are daughters, Females shoulder a disproportionate share of responsibilities. The chance that an individual at a specific age will contract a disease during the remaining years of their life expectancy is known as the lifetime risk [4].

The principal contributor of AD in the population is the presence of two forms of abnormal misfolded proteins: amyloid plaques built from Amyloid-β [Aβ] peptide and neurofibrillary tangles (NFTs) assembled from hyperphosphorylated tau proteins. Distinct cerebral areas that govern knowledge encoding and retrieval, i.e., amygdala, hippocampus, and association cortices of parietal, frontal, and temporal lobes, are the unique,

robust pathological regions for the accumulation of both types of plaques. In addition, while not primary sites, the accumulation of plaques can also be detected within the microvasculature of the meninges and cerebral cortex; this condition is referred to as cerebral amyloid angiopathy [5] progress in a stereotypical manner, but advances through the isocortex and affects subcortical structures secondarily. Moreover, these plaques decrease the level of the entorhinal cortex and hippocampal formation [6].

AD is identified as two types: Familial and Sporadic. The term familial refers to conditions passed on in an autosomal dominant fashion and premature manifestation in an individual before age 65, and distinguished by alterations in specific genes, like the presenilin 1 gene. The sporadic form represents the late onset and is seen in individuals up to 62 years of age [7]. While age functions as a non- modifiable risk element, other emerging evidences suggest the connection between sporadic AD, including physical inactivity, depression, environmental pollution, traumatic brain injury, academic failure, and genetic mutations, decreased social interaction, and major genetic variation in the ε4 allele of apolipoprotein E (APOE, 19q13.32). Various factors are present that modify placebo baseline risks, like hypertension, insulin resistance, dyslipidaemia, inflammatory processes, midlife obesity, etc. The molecular cascade underlying AD initiates before 20 – 30 years, and the initial clinical symptoms become evident [8].

Significant efforts are being made by scientists to develop novel therapeutic agents to treat AD and reverse the progression through a thorough study of the potential drug targets.

There are two methods for figuring out how common AD is in a country. A longitudinal research initiative exploring the intersection of aging, memory, and sociodemographic variables, and other probability-based, nationally representative prevalence surveys serve as the foundation for the first strategy. The second approach, known as forward calculation, predicts the frequency of AD using estimates of death rates and demographic forecasts within a multistate framework, and AD incidence rates from epidemiological cohort [longitudinal] studies [9].

While there is presently limited strong scientific evidence to support any specific intervention to avoid memory impairment or the onset of dementia, a recent consensus report examining the evidence base for preventive and intervention approaches to cognitive decline and dementia concluded that control of blood pressure, cognitive engagement and training, and enhanced physical activity may have some benefit for prevention. A series of contemporary double-blind studies revealed that placebo-controlled trials of investigational drugs were unsuccessful in demonstrating therapeutic benefit for [ca]ses characterized by mild to intermediate clinical manifestations [10].

Methodology

Search strategy:

An extensive review of literature was carried out through a number of scientific databases encompassing PubMed, Google Scholar, Web of Science, and SciFinder. The search includes the literature from 2000 to 2025. A meSH- driven approach was applied using keywords like AD, Neuroinflammation, biomarkers, etc.

Study selection:

Publications were evaluated based on the abstract, title, and full text relevance. **Selection standards** were:

- Authentic publications focused on AD progression pathways or diagnostic biomarkers.
- Research, including clinical trials and proven results on animal models
- Articles containing molecular pathways, diagnostic tools, and biomarker validation

Exclusion criteria

- Non-English articles
- Research lacking methodologies adopted or relevance to humans
- Conference abstracts without full- texts

Data extraction and synthesis:

Key data was collected autonomously by the author using a benchmark template. Extraction data included:

- Study type and study design
- Population characteristic
- Validated biomarkers and diagnostic tools
- Reported disadvantages and future challenges

A narrative synthesis approach was used to integrate findings from different study types. Mechanistic insights were grouped according to the primary pathological hypotheses (e.g., calcium dysregulation, amyloid cascade, and tau hyperphosphorylation). Diagnostic modalities were divided into conventional and emerging techniques based on clinical utility, sensitivity, specificity, and translational potential. Tables and figures were created using the risk factors, diagnostic tools, and biomarker profiles.

2. Pathogenesis hypothesis

There are various assumptions that describe the progression of AD, such as the calcium-regulated mechanism amyloid cascade, tau phosphorylation, oxidative stress, etc. However, the underlying causes and treatments are still under ongoing research and difficult to find.

2.1. *Calcium-regulated pathogenesis*

A highly regulated second messenger, Ca2+ is involved in connectivity dynamics, neuronal morphogenesis and fate determination, action potential facilitation, and neurotransmitter release, among other functions. Neuronal calcium homeostasis is governed by localised control mechanisms within specialised compartments. Through diverse mechanisms, a number of elements, including the mitochondria, cell membrane, and ER, work to preserve Ca2+ equilibrium [11].

A pivotal element influencing the development of AD pathology is calcium dysregulation, which starts with the amyloid cascade. Aβ peptides activate the calcium-permeable channels such as CP- AMPAR and CALHM, causing the excessive influx of calcium ions. This damages the synaptic plasticity and stimulates Aβ production via NFAT/BACE1 signalling [12,13]. In addition, APP biotransformation is affected by store-operated calcium entry [SOCE], where its hindrance increases Aβ42 levels. Mutations in PS1 further contribute to enhancing Ca^{2+} imbalance, increasing intracellular Ca+ and Zn concentrations, thereafter arises β- and γ- secretase performance [14].

Tau pathology is also closely linked to calcium signalling. Increased calcium stimulates kinases such as GSK- 3β, CDK5, and CAMKII, which hyperphosphorylate Tau and are involved in the formation of neurofibrillary tangles [NFT] formation [15]. Calpain, a calcium-dependent protease, breaks Tau and its dependent kinases, which intensifies tau aggregation and phosphorylation [16]. Concurrently, Excessive Calcium inhibits phosphatases like PP2A, decreasing Tau dephosphorylation and further promoting its pathological accumulation [17].

Mitochondrial dysfunction in AD is tightly linked to calcium overload. Overabundant calcium in the mitochondria inhibits oxidative phosphorylation, which lowers membrane potential and promotes initiation of mitochondrial permeability transition events (mPTP) [18,19]. Calcium phosphate precipitates within mitochondria, inhibiting the electron chain (ETC) complex I. Misfolded pathogenic proteins, such as Aβ and tau, additionally contribute to deteriorating mitochondrial calcium handling by influencing VDAC and mPTP activity [20].

Oxidative stress is boosted by calcium- induced ROS generation, specifically via ETC complexes I and III. This causes lipid peroxidation and neuronal apoptosis [21,22]. Tau-mediated ROS production amplifies AMPAR and NMDAR, enhancing Ca+ influx and excitotoxicity. Moreover, SOCE dysregulation reduces mitochondrial Ca+ uptake, impairing ETC activity and energy metabolism [23,24].

The mitochondria- associated membrane [MAM] pathway smoothens the ER- Mitochondria Ca transfer, which is overregulated in AD. This fosters apoptosis and increases Aβ and Tau formation. APP fragments such as C99 situated at MAMs acts as a site for Aβ generation, linking Ca signalling to amyloid pathology [25,26].

Neuroinflammation is associated with Ca signalling through stimulation of microglial PKC, MAPK, and NF-κB pathways, marking the secretion of inflammation-inducing signalling, i.e., M1 phenotype and cytokine release. Increased calcium also marks NLRP3 inflammasome activation [27,28]. Astrocytic Ca fluctuation affects neuronal excitability and BBB integrity, adding to disease progression [29,30].

Autophagy dysfunction in AD is facilitated by Ca/CaMKII signalling, which influences autophagosome formation via TFEB and AMPK/m- TOR and cleaving ATG5 with Calpain [31,32]. Lysosomal Ca dysregulation disrupts acidification and autophagosome- lysosome fusion, decreasing clearance of misfolded proteins [33–35].

Additionally, the overactivation of Ca can lead to synaptic dysfunction by stimulating calcineurin, which weakens synaptic strength and causes LTD. Overstimulation of ryanodine receptors (RyRs) at synapses further imbalances neurotransmitter release and plasticity, causing cognitive decline [36].

Earlier calcium-mediated disruptions are associated with mitochondrial indicators such as GFAP, YKL-40, and IL-6, including oxidative stress. These proteins are now detectable in the blood and CSF, offering early markers of neuroinflammatory involvement and cell stress prior to the appearance of clinical manifestations [37]. As illustrated in Figure 3.

2.2. *Amyloid cascade hypothesis*

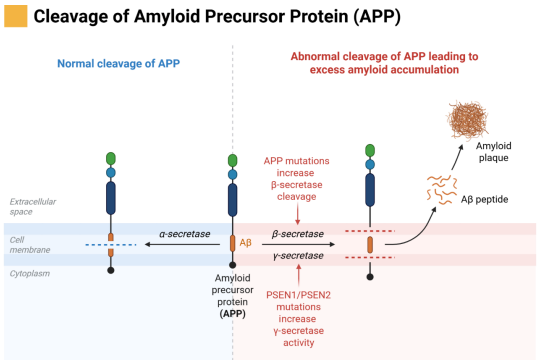


Fig. (1) Diagram showing the difference between pathological and normal APP hydrolysis. Under normal circumstances, α-secretase can cleave APP in a non-amyloidogenic manner, producing non-toxic products. However, pathological peptide bond disruption by β-secretase and γ-secretase leads to Aβ peptides that conglomerate into non-cellular Aβ plaques, a hallmark of AD. PSEN1/PSEN2 mutations cause inappropriate γ-secretase cleavage, which results in Aβ accumulation and neurotoxicity, whereas APP mutations increase β-secretase activity [37]

A two-stage enzymatic conversion of APP to Aβ occurs under the amyloidogenic pathway. The cytoplasmic tail fragment β [CTFβ] and soluble APPβ (sAPPβ) are first formed when βsecretase hydrolyses the N-terminal of an Aβ sequence. The A673T mutation, a genetic variant in APP known for its protective role against AD pathology, may stop the β-catalytic breakdown of APP and result in reducing the amount of Aβ synthesis by roughly 40%. Following γ-secretase's processing of the 99 amino acid CTFβ, several forms of Aβ with sequence lengths varying from 36 to 43 amino acids are produced, along with the APP intracellular domain (AICD). The two most prevalent types of Aβ are fragments with 40 or 42 amino acids. Because AD patients' Aβ levels are out of balance, Progressive Aβ burden is a cascade culminating in amyloid oligomers, protofilaments, aging, and disease. As illustrated in Fig. 1 [38].

In the sick state, there is a noticeable cleavage of the APP. Aβ is released when APP is serially fragmented by γ-secretase, a membrane-incorporated aspartyl protease complex comprising four integral subunits: Psen2, nicastrin, anterior pharynx defective 1 (Aph1), and presenilin, which are complexed together. BACE-1 is an aspartyl protease that spans membranes and has an active site in the lumen. Aβ fragments that are insoluble and neurotoxic are produced by the γ-secretase enzyme with the help of this complex. The βsecretase cleavage, which cuts at the N-terminus of Aβ, is the initial and reaction-limiting step. After the extracellular part of APP is mostly eliminated, only the C-terminal is left. This C-terminal is then further broken down at the Aβ C-terminus to create Aβ oligomers, which then polymerize to create aggregated plaques [39].

The current diagnostic model is amyloid-based and captured on amyloid imaging on PET scans and the CSF $A\beta_{42/4^1}$ ratio. The translational route of the CSF biomarkers to the use of blood-based biomarkers is evidenced by the existence of $A\beta_{42/40}$blood tests, which screen for amyloid disease through the plasma $A\beta_{42/40}$ ratio. These are now employed as minimally invasive screening tools [40]. As illustrated in Figure 3.

2.3. *Tau hyperphosphorylation hypothesis*

Microtubules make up the cytoskeleton, which preserves structural integrity and shape. When tau is dephosphorylated, which happens at the serine and threonine sites, microtubule-associated proteins promote the polymerization of microtubules and connect with tubulin, preventing their dissociation and maintaining microtubule assembly and stability. Ser-Pro and Thr-Pro motifs are present in at least 30 phosphorylation sites in tau [41]. As illustrated in Fig. 2.

Tau proteins that experience C-terminal hyperphosphorylation are more capable of self-assembling into paired helical filament [PHF] tangles. In the disease, these tangles build up into aggregates that cause the disintegration of microtubules and axonal or dendritic transport to be lost [42].

Levels of phosphorylated tau proteins pTau-217, pTau-181, pTau-231, and total tau tTau in the CSF and blood reflect the progression of tau-related pathology. These have now proved reliable markers of neurodegenerative disease due to tau. In addition, the above biochemical markers of tau-related disease have now been augmented by the use of tau-related imaging with the help of PET [43]. As illustrated in Figure 3.

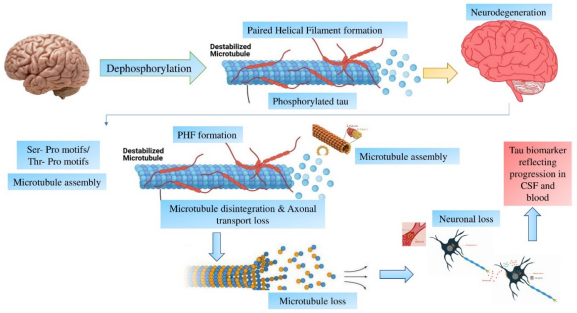


Fig (2). AD is characterized by tau-mediated microtubule instability. Tau proteins help sustain the neuronal architecture and functional stability in neurons by stabilizing microtubules. When tau is hyperphosphorylated in AD pathology, neurofibrillary tangles are created, which destabilize microtubules and promote neuronal death [44]

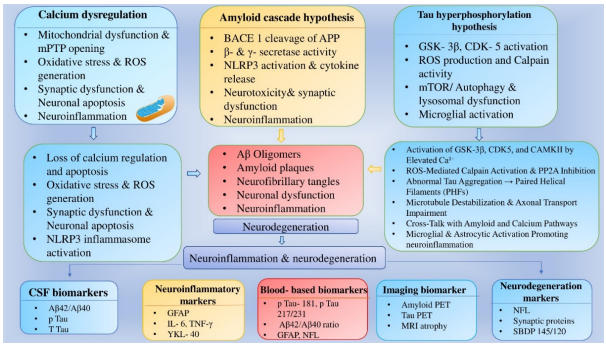


Fig (3). Integrated pathogenic mechanisms and biomarker trajectories in Alzheimer's disease.

3. Risk factors of AD:

Several frameworks have been constructed in recent years to elucidate the neurobiological basis of underlying diseases, notably the late-onset sporadic variant [LO-SAD]. Exacerbations caused by aging, genetics, aluminium exposure, head trauma, diet, immune system dysfunction, vascular disease, mitochondrial dysfunction, and infectious diseases are among them. There hasn't been a single, widely agreed-upon explanation, though, and it's now generally acknowledged that there are probably other risk factors at play [45]. Various risks reported are summarised in a tabular form in the Table 1:

Table 1. Risk factors of AD.

Grouping	Risk factors
Sociocultural and biological attributes	• Advancing age as a non-modifiable determinant • Lower educational attainment linked to reduced cognitive reserve • Gender based hormonal and neurobiological differences • Ethnic disparities in prevalence and access to care [46] • Socioeconomic status influencing lifestyle and healthcare access [47]
Hereditary and molecular influences	• Mutations in amyloidogenic genes • Presenilin 1 and 2 (PSEN1/2) • Apolipoprotein E (APOE) allele as a major genetic susceptibility factor [48] • Clusterin polymorphisms associated with neurodegeneration [CLU] [49] [47] • Estrogen receptor gene • Iron transport dysregulation via transferrin variants [50] • Vitamin D receptor gene variants affecting neuroprotection (VDR) [51]
Behavioural and habitual factors	• Alcohol consumption and sedentary behaviour have been implicated in accelerating neurodegenerative processes • Exposure to airborne pollutants and heavy metals (Al, Cu, Zn) may contribute to oxidative stress and neuroinflammation
Comorbid health conditions	• History of malignant tumours • CVS disease • CHF

	• Excessive body weight or Obesity • Stroke • Poorly managed type- 2 diabetes [52]
Psychological and emotional stressors	• Clinical depression • Exposure to early life psychological stress [53]
External and occupational exposures	• Long term exposure to air pollutants • Inadequate calcium intake • Geographic and climatic changes • Exposure to organic industrial solvents [54]
Pathogenic and microbial triggers	• Bacteria, e.g. *Chlamydophilia pneumonia, treponema* • Fungal colonization in neural tissues [53] • Viral infections with neuroinvasive potential

4. Diagnosis of AD

In practice, individuals with increasing memory loss lasting more than six months, accompanied by a corresponding impairment in self-care and social or occupational functioning, are diagnosed with AD. It's very important for individual affected by the conditions and their caregivers to get a reliable and timely recognition of AD. They help them prepare for the future and follow management options, while the patient can still participate in decision-making. Additionally, it is necessary to eliminate further ecological agents of memory impairment, including various types of dementia, throughout the diagnostic process [52]

A significant clinical distinction can be made between dementia, denotes a substantial deterioration in cognition severe enough to affect individuals capacity to manage routine life activities, full syndrome of mild cognitive impairment [MCI], which is recognized as degradation in one or multiples cognitive domains with preserved overall intellectual capacity and preserved day-to-day functioning, and perceived impairment in memory and thinking in, which is defined as cognitive complaints without deficits in cognitive test performance. Though MCI and subjective cognitive decline are acknowledged as potential variables for the emergence of memory impairment in individuals, the majority of countries do not support specific pharmaceutical therapies outside of research trials [55]. Root cause analysis is difficult since autopsy findings indicate that concurrent involvement of two or more pathogenic protein aggregates is frequently reported in neuropathological assessments. Standard laboratory tests, structural brain imaging with MRI or CT, and thorough neuropsychological and neurologic evaluations are necessary for the diagnosis. An extra diagnostic procedure may encompass circulating molecular indicators from peripheral blood, PET brain imaging, and CSF [in the future [56]. The conventional method for the detection of AD is summarized in Table 2:

Table 2. Conventional method for AD.

Screening tool	**Assessment type**	**Technique applied**	**Technological interface**
Multimodal MRI protocol [57]	Non-cognitive	Structural and functional brain imaging	MRI systems and clinical imaging hardware
Ambulatory gait analysis [58]	Non-cognitive	Movement pattern analysis	wearable foot-mounted sensors
In-home behavioral tracking [59]	Non-cognitive	Daily activity analysis	Ambient sensors installed in residential settings
loT-integrated M2M framework [60]	Non-cognitive	Remote behavioral surveillance	Smart home sensors linked to cloud-based platforms
Eye movement assessment via paired visual stimuli [61]	Non-cognitive	Oculomotor response evaluation	Precision eye tracking instrumentation

Computer-based visual search task	Non-cognitive	Interactive Behavioral testing	High-resolution display systems
Mini mental state examination [62]	Cognitive	Standardized cognitive challenge	Everyday objects for task execution
Dichotic listening task [60]	Cognitive	Auditory processing evaluation	Stereo headphones
Emotion recognition paradigm [61]	Cognitive	Affective response identification	Curated image sets
Visual environment-based memory assessment	Cognitive	Stimulated memory and problem-solving task	Immersive VR head-mounted
Cognitive training via serious gaming [62]	Cognitive	Interactive memory and reasoning modules	VR- enabled gaming headsets
Object recognition and perception	Cognitive	Visual discrimination exercise	Image-based stimuli
Mini-Cog screening tool [63]	Cognitive	Brief cognitive and recall test	Paper- based material and writing tools

4.1.2. Limitations of conventional methods of AD

- When methods like skull X-ray, ordinary MRI, and CT examination are unable to observe degeneration, damage, corrosion, etc., skull X-ray examination is more important in assessing the aging of the skull [64]
- While cranial CT scans do not reveal abnormal brain findings. cerebral atrophy, ventricular dilatation, enlarged sulci, cortical atrophy, white matter osteoporosis, and other possible symptoms in patients in the medium and severe stages [65]
- Head MRI data have poor specificity and sensitivity for diagnosing AD, and they don't show many physiological and biochemical alterations linked to the disease [66]

Various Newer methods of diagnosing AD are summarized in Table 3.

Table 3. Newer Technologies for the Detection of AD.

Technology	**Key biomarker**	**Function**	**Advantage**	**Challenges**	**Sensitivity / Specificity**
CSF Biomarker analysis	Aβ, t-tau, p-tau, NfL [67]	Detect biochemical changes in CSF linked to AD pathogenesis	High sensitivity and specificity; early detection of degeneration of nerves [68]	Invasive [Lumbar puncture]; limited access to data	85-95%/ 80-90%
Amyloid PET imaging	Radiotracers: PiB, florbetapir, florbetaben, flutemetamol [69]	Detects beta-amyloid deposition in the brain	Enhanced diagnostic accuracy; differentiates AD from other dementias[70]	High cost, Radiation exposure	90-95%/ 80-90%
Tau PET imaging [71]	Radiotracers: 18F-AV-1451 [T807], 18F-MK-6240	Identifies tau protein aggregates and neurofibrillary	Tracks disease progression; complements amyloid PET	Emerging technology; limited clinical access	70-75%/ 80%

		tangles			
Blood-based biomarker [72]	Aβ42, Aβ40, p- tau, t- tau, NfL	Non-invasive detection of AD-related proteins in blood	Accessible; cost-effective, potential for routine screening	Lower sensitivity than CSF, requires assay standardization	Emerging
Liquid biopsies [73]	Cell-free DNA/ RNA, exosomal markers	Identifies genetic/ epigenetic changes and protein markers from body fluids	Non-invasive; multifluid applicability [CSF, saliva, urine, blood]	Still under research, need validation	Emerging
Neuroimaging + machine learning [74,75]	fMRI, graph-based methods, support vector machines	Analyse brain connectivity and activity patterns to identify AD	Detects subtle functional changes; enables predictive modelling	Requires large databases, interpretability of models are needed	Emerging

- The diagnostic use of head CT scans for evaluating AD patients is limited since they are unable to differentiate between moderate cerebral atrophy and hippocampus atrophy [66]. The Table 3 above depicts the newer techniques for diagnosing AD.

4.1.3. Critical analysis

Classic neuroimaging such as CT and MRI provide anatomic information, but these techniques lack the sensitivity to detect early biochemical changes associated with Alzheimer's disease. Advanced techniques include PET and CSF biomarkers, which demonstrate sensitivity greater than 90% and specificity greater than 85% in differentiating AD from other dementias. However, therapeutic applications remain limited due to restricted accessibility, high costs, and invasiveness of these approaches [76].

New blood-based biomarkers, like pTau181, Aβ42/40, and NfL, might be promising candidates for non-invasive, inexpensive diagnoses with increasing accuracy, at about 80-90%. Predictive capacity can be further improved by combining machine learning methods with multimodal imaging, while repeatability and interpretability remain issues [77].

In the future, biochemical neuroimaging, and computational techniques will all be included in diagnosis to reach an early, accurate, and clinical diagnosis of AD.

5. Integrated Multimodal Diagnostic Approaches Combining Biomarkers and Machine Learning

The use of multimodal data, namely neuroimaging, biochemical, and clinical data, using machine learning-based models, was made possible through recent advancements in the field of computational neuroscience. Thus, this led to an improvement in the diagnostic accuracy and early prediction of Alzheimer's disease. The use of traditional methods, which rely solely on single-modality analysis, such as analysis using CSD biomarkers or MRI, often only reflects a fraction of the pathophysiology of a particular disease [78]. On the other hand, multimodal analysis, which includes fluid biomarkers (pTau isoforms, Aβ42/40, NfL, GFAP), imaging biomarkers (MRI, PET), as well as scores of cognition, reflects a more comprehensive manifestation of a particular disease. For the differentiation of Alzheimer's disease, mild cognitive impairment, and healthy controls, machine learning methods, including vector machines, convolutional neural

networks, and ensemble classifiers, were shown to outperform traditional methods with regards to accuracy and precision.

Integrative approaches now allow for the detection of early disease progression, even before clinical symptoms occur, by making use of combining features and predictive modeling in order to identify minor nonlinear correlations across datasets. Latent imaging biomarkers from PET and MRI may also be extracted by means of deep learning-driven radiomics [79]. These biomarkers are well-correlated with plasma and CSF measurements, thus connecting molecular and structural disease. However, demographic bias, model interpretability, and data harmonization remain problematic, which raises the need for standardized datasets and open validation frameworks. Cooperative projects such as the European Prevention of Alzheimer's Dementia (EPAD) and the Alzheimer's Disease Neuroimaging Initiative (ADNI) pioneer large-scale, multimodal archives. These repositories will become indispensable in creating clinically deployable AI-assisted diagnostic algorithms [80].

6. Blood-based biomarkers in the detection of AD

There are limited neuroimaging biomarkers available and affordable for prognostic and diagnostic evaluation. Furthermore, determining CSF biomarkers necessitates a CSF sampling that relies on lumbar puncture, possessing procedural invasiveness, and is linked to a number of additional difficulties in tracking the trajectory of disease progression and creating medical interventions that alter it [81]. In light of this, a wide spectrum of clinical biomarkers and biomarker combinations has been the focus of in-depth research in recent years, and initiatives are being made to create quantitative and non-invasive biomarker testing methods. t-Tau, p-Tau181, and Aβ1-42 have been consistently incorporated into CSF profiling strategies for AD detection. Moreover, the NFL has gained notice lately as a sign of neuroaxonal damage [82]. The multimodal classification method known as the ATN system [amyloid, tau, and neurodegeneration] has been developed. The NFL's possible use as a forward-looking diagnostic tool for monitoring neuronal decay in AD is highlighted by how easily its concentration can be detected in plasma and CSF. According to a longitudinal study, plasma NFL may support longitudinal observation of AD pathology through non-invasive assessment for neurodegeneration in AD patients [83].

Recent progress has highlighted the potential applications of blood-based biomarkers as the least invasive diagnostic tools for AD. Plasma p-Tau181, p-Tau217, and p-Tau231 have allowed the prediction of a transition from MCI to AD, showing a good association with CSF and PET findings. Although its specificity is limited by an increase in other neurodegenerative conditions, NfL, an axonal damage marker, is measurable in both CSF and plasma and reflects disease progression [84].

Recent progress has highlighted the potential applications of blood-based biomarkers as the least invasive diagnostic tools for AD. Plasma p-Tau181, p-Tau217, and p-Tau231 have allowed the prediction of a transition from MCI to AD, showing a good association with CSF and PET findings. Although its specificity is limited by an increase in other neurodegenerative conditions, NfL, an axonal damage marker, is measurable in both CSF and plasma and reflects disease progression [85].

5.1. Neurodegeneration biomarkers

Neurofilament chains [NfL]

Neurofilaments are crucial structural elements of the axonal framework and are specifically prevalent in myelinated large-calibre axons. Each of their five subunits—medium chain, peripherin, α-internexin, neurofilament light chain [NfL], and heavy chain—has a distinct domain structure and function [86]. NfL is poured into the blood and CSF along with neuronal dysfunction and is a sensitive marker of neuroaxonal degeneration. While healthy middle-aged to older adults can have NfL levels, their rate of growth over time is significantly lower than that of people with neurodegenerative diseases [87]. It may be more challenging to differentiate between pathogenic and non-pathological states when NfL levels vary with age, as this could reflect subclinical diseases. As depicted in Fig. 4.

Importantly, baseline blood NfL levels are a better predictor of future neurodegeneration than of current neurodegeneration in people without cognitive impairment, particularly in those with elevated amyloid-β [Aβ] load. This bolsters blood NfL's function as a vulnerability-associated tool for determining who is more likely to suffer from the anatomical and physiological brain alterations associated with AD, as well as a predictive biomarker for the disease's progression [88].

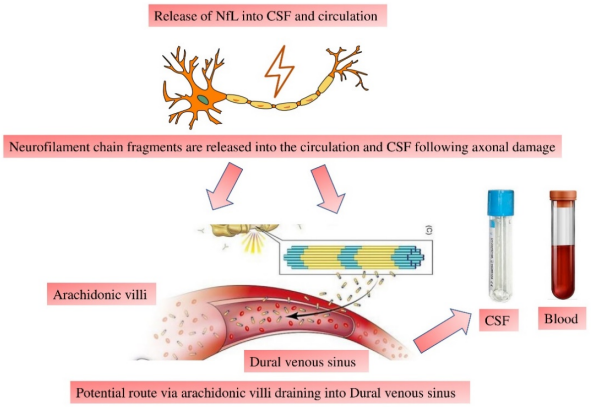


Fig (3). Release of NfL into the CSF and circulation along with axonal detoriation. Neurofilament chain fragments are released into the circulation and CSF following axon damage. It is currently unknown how NfL enters the bloodstream and cerebrospinal fluid. One possible route for NfL fragments to enter the bloodstream is through the arachnoid villi, which may permit NfL-containing CSF to drain to the dural venous sinuses.

5.2. *Astroglia activation*

Astrocytes are expressed throughout the CNS. Astrocytes sustain the blood-brain barrier [BBB], support neurons metabolically, trophically, and antioxidantly, control neurotransmitter levels, regulate synaptogenesis and synaptic transmission, and contribute to the pathophysiology of neurodegenerative diseases. Astrocytes are important and involved in neurodegenerative diseases [89].

In the central nervous system, microglia cells—which make up about 5–20% of all glial cells—are the most common kind of macrophage. Their primary role is to preserve neuronal circuits and routinely check brain areas for foreign objects and cellular debris.

Significant loss of synaptic connections is one of the most recent neurobiological events that accounts for the clinical features of dementia. Cognitive impairment and dementia's hallmark lesions, such as amyloid-β, have a nonlinear relationship, but synaptic loss and dementia clinical symptoms are consistently correlated [90].

5.2.1. *Glial fibrillary acidic protein [GFAP]*

GFAP, a brain-specific structural filament protein primarily localized in astrocytes, has arisen as a promising systemic biomarker in AD research. GFAP ranges are low in healthy people, but they are greatly increased in AD because of gliosis and astrocyte activation close to amyloid-β [Aβ] plaques. GFAP leakage into the bloodstream is facilitated by astrocyte disruption and weakened blood–brain barrier integrity [91,92]. The diagnostic potential of blood GFAP has been highlighted by studies that show its ability to distinguish between people who are Aβ PET-positive and those who are not, with area AUC values ranging from 0.69 to 0.86. Furthermore, GFAP might serve as a useful indicator of the disease's severity and the efficacy of treatment, especially when it comes to neuroinflammation in AD regions that are susceptible [93]. These findings support GFAP's value as a predictive and diagnostic biomarker and its importance in guiding anti-inflammatory treatment strategies for AD.

5.2.2. *Soluble triggering receptor expressed on myeloid cells 2 [sTREM2]*

The majority of cells that express the transmembrane receptor TREM2 are microglial cells. Its primary functions include enhancing macrophage and microglia phagocytic capabilities and controlling inflammatory signals. Variants of the TREM2 gene have shown enhance the likelihood of progressing towards AD by interfering with the normal proinflammatory response of microglia cells and by affecting their ability to efficiently eliminate Aβ. Currently, more than fifty TREM2 gene variations are investigated that exhibit a connection with AD [94,95].

A decrease in microglia at the site of Aβ accumulation and an escalation in neuritic dystrophy are thought to result from the R47H variant's contribution to a functional decline in TREM2 mediated immune regulation. Another mutation at the TREM2 location, H157Y (rs2234255), is hydrolysed by two α-secretases, a metalloproteinase 10 and 17, and disintegrin (ADAM-10 and ADAM-17). TREM2 shedding elevates as a result, and the receptor's cell surface expression declines

[96]. Compared to the R47H and R62H isoforms, H157Y is much more common among the Asian population, with notable emphasis on being linked to a higher susceptibility towards AD in the Han Chinese community. There was no discernible link between this variation and AD in previous research on African-American, Japanese, and Caucasian populations [97,98].

5.3. Inflammation markers

5.3.1. *YKL- 40*

Glycoprotein YKL-40, also called chitinase-3-like protein 1 [CHI3L1], is found in macrophages, fibroblasts, vascular smooth muscle cells, endothelial cells, chondrocytes, and even specific malignant cell types [99]. Transcription level is highest in injury-responsive glial cells. While there is ongoing discussion regarding the precise physiological function of YKL-40, learned that YKL-40 is a pathological biomarker indicative of aberrant tau aggregation that exhibits glial cell activity, including astrocytes and microglia [100]. In a variety of neurodegenerative diseases, CSF gradients of YKL-40 have also been connected to detectors of neurodegeneration [total tau, t-tau], synaptic damage [neurogranin, SNAP-25], tau-mediated toxicity [p-tau], neurodegeneration large-caliber myelinated axon damage [NfL], and synaptic damage [neurogranin, SNAP-25] [101]. Reactive astrocytes that coexistence of neuritic plaque and CAA affected vasculature in AD progression express YKL-40 [102]. Some theories suggest that proinflammatory cytokines generated by phagocytic immune cells as a result of cytokine- driven inflammation in the peripheral or central nervous systems may activate YKL-40 transcription in astrocytes [103].

5.3.2. *Progranulin*

According to research, progranulin, a growth protein that encourages the growth and survival of neurons, could be used as a marker for early patient prediction [104,105]. By participating in the regulation of neuroinflammatory processes, this growth driver, which is localised in microglia and neurons, reduces microgliosis and astrogliosis. In the biofluid profiles of sufferers with familial AD and LOS AD, progranulin levels [1082 pg/ml] rise during the asymptomatic window preceding diagnosis [106].

5.3.3. *Vascular cell adhesion molecule-1 [VCAM-1] and intercellular adhesion molecule 1 [ICAM-1]*

The glycoproteins VCAM-1 and ICAM-1 on the outer cellular membrane of immune and vascular lining cells mediate leucocyte- endothelium interaction to cells of vascular endothelium and subsequent translocation across the BBB. They make up another intriguing set of AD inflammatory markers [107]. Higher ranges of VCAM-1 and ICAM-1 are linked to vasodilatory response governed by microvascular endothelium in older adults and higher levels of C-reactive protein [CRP] [108].

Compared to both healthy controls and people with non-inflammatory neurological disorders [NINDs], AD patients were shown to have higher serum soluble ICAM-1 levels. An elevated CSF amount of ICAM-1 was associated with the intensity of executive dysfunction in AD patients at the early, preclinical, and MCI stages [109].

5.4. Tau-related biomarkers

Currently, PET and MRI are the two most commonly used technologies used in diagnosing AD. Notwithstanding their benefits, these technologies are expensive and exclusive to the wealthy. In the beginning stages of AD, they also show reduced sensitivity [110,111]. It can reliably detect biomarkers like t-tau and p-tau, but doing so necessitates needle- guided extractions from CSF, which makes it inefficient for routine or extensive assessment [112,113]. Blood-based biomarkers, especially p-tau types such as p-tau181 and p-tau217, have become viable, affordable, and nonintrusive options for nascent detection of AD [114,115].

5.4.1. *p- tau 181*

Amyloid PET imaging and CSF tau levels are strongly correlated with plasma p-tau181, a well-known biomarker for AD [116,117]. It is higher when AD is in its preclinical stages [healthy group: ~2.46 pg/mL] and rises even more as the illness progresses [mild AD patients: ~6.14 pg/mL]. The biomarker productively distinguishes AD from MCI, cognitively normal people, and other neurodegenerative diseases [118].

Tau neuropathology and P-tau181 are related, and elevated plasma P-tau181 in AD dementia with neuropathological confirmation shows that plasma P-tau181 is correlated with pathogenic tau assemblies in AD [119,120]. Across the cognitive spectrum, people showed that Plasma P-tau181 showed a relationship with tau PET in every region examined, with the exception of Braak I–II [entorhinal cortex]. Due to its precedence in pathological involvement, the entorhinal cortex is a region where tau pathology in AD occurs and one of the major regions where tau PET is elevated; it is possible

that Tau PET is drenched in the majority of patients with observable clinical features. The possible mechanisms causing elevated fluid P-tau181 in AD have been clarified by newer studies employing stable isotope-labelling kinetics24, which also demonstrated that neurons exposed to Aβ or afflicted by AD produce and secrete more Tau. These neurons could deteriorate and acquire tangle disease over time [121]. Rather than tangle disease being the immediate trigger of the escalation in P-tau181, it is a reasonable explanation for the favourable relation between tau PET signal and plasma P-tau181 concentration.

5.4.2. ***p- tau 217***

Given its stronger link with the extent of symptom expression of AD, emerging data imply that p-tau217 serves as a more optimized biomarker than p-tau181 [122]. Its function in differential diagnosis is supported by the fact that patients with AD have higher levels of p-tau217 in their brain tissue than people with age-related tauopathies or other tau disorders that are not Alzheimer's. Furthermore, circulating p-tau217 may act as an indirect indicator of Aβ deposition because plasma concentrations of the protein before death exhibit a strong spatial correlation with its distribution in post-mortem brain samples among people with amyloid plaque pathology. p-tau217 stands out among tau-related biomarkers because of its frequent association with the hippocampal CA1 subfield, where it is primarily found in granulovacuolar bodies and neurofibrillary tangles [123]. It is common to see granulovacuolar bodies [GVBs] in hippocampus pyramidal neurons. As tau-related abnormalities and amyloid-β deposition progress, they are distributed from the entorhinal cortex toward areas like the frontal and parietal lobes, neocortex, hypothalamus, and amygdala, reflecting the worsening of neurodegenerative pathology [124]. The onset of intraneural tau inclusion and neuronal dysfunctional changes has been strongly associated with GVBs, highlighting their role in tau-mediated cellular pathology [125].

5.4.3. ***p- tau 231***

Excellent analytical performance and high precision were demonstrated by the plasma p-tau231 test both within and between clinical studies. The test specifically detects tau fragments covering residues from N- terminus to mid domain, particularly those altered by phosphorylation at threonine-231; according to immunoprecipitation and mass spectrometry [126].

According to recent research, plasma p-tau231 is a candidate marker with translational relevance for assessing AD pathology in its prodromal period and may find use in therapeutic research settings. Amyloid PET imaging can detect mild Aβ accumulation when plasma levels of p-tau231 rise before those of p-tau181 [127]. There is potential for using plasma p-tau231 to detect AD in its initial biomarker-positive stage since it seems to reflect the tau seeding phase in the brain. Nonetheless, its diagnostic capabilities are relatively restricted; it exhibits lower concordance with amyloid PET positivity than CSF p-tau231 and lower precision in characterizing AD from other different neurodegenerative diseases. According to the data, while plasma p-tau231 might be able to detect early tau-related alterations in Alzheimer's disease, isoforms such as p-tau181 seem to have a higher predictive value for long-term cognitive decline. This difference highlights p-tau181's potential as a more trustworthy biomarker for monitoring the course of disease after the early pathological phase [127–129].

5.4.4. ***MTBR-tau243***

The cerebrospinal fluid biomarker MTBR-tau243 showed a strong correlation with tau-related pathology in two large human based study of sporadic AD, but it showed little correlation with amyloid abnormalities. In contrast to traditional phosphorylated tau markers like pT181 and pT217, MTBR-tau243 demonstrated a noteworthy degree of alignment with cognitive performance metrics, suggesting its potential utility in therapeutic settings. In addition, MTBR-tau243 increased the fastest in those with both tau and amyloid positivity, indicating its potential for monitoring disease progression in later clinical phases. For continuous tau-PET metrics, cerebrospinal fluid MTBR-tau243 exhibits predictive strength comparable to pT205/T205 and holds promise as a reliable measure of cognitive performance tests associated with tau pathology. With these results, MTBR-tau243 could be a useful counterpart to tau-PET in human-based studies, providing a reliable diagnostic marker and a possible screening tool for tau-related endpoints [130].

5.4. Amyloid-related biomarkers

Research on blood amyloid-related marker detection has supported the idea that amyloid formation exerts a critical influence on the development of AD.

5.4.1. ***Aβ42***

Before amyloid-PET abnormalities and neurodegeneration are discovered early in the pathophysiology of AD, CSF Aβ42 is abnormally low [131]. When the amyloidogenic Aβ42, the soluble fraction of Aβ, varies, and the CSF Aβ42 levels decrease as brain amyloid levels rise [132]. While elevated brain amyloid is not always associated with AD, Aβ42 is [no one with AD has high Aβ42 levels]. By the age of 85, approaching the end of a normal lifespan, four out of five amyloid-positive individuals have normal cognition [133].

The 42-residue form of β-amyloid, called Aβ42, is corelated to AD- related cerebral amyloid angiopathy and is essential in the development of senile plaques. Although the cleavage fragment extracted from the type I transmembrane APP has pathological significance, its biological function is still unknown. β- and γ-secretases in secretory vesicles involved in neuronal signalling, enzymatically cleave amyloid precursor protein [APP], which causes neurons to release it. Neuronal cells secrete Aβ42 in greater quantities than other cell types, despite the fact that multiple cell types contribute to APP metabolism. This process appears to be influenced by synaptic transmission dynamics [134]. Aβ42 concentrations in peripheral blood and cerebrospinal fluid have a weak correlation with one another, suggesting that there is little agreement between these biological compartments [135]. When Down syndrome or familial inheritance is present, the difference between the serum concentrations of Aβ1-42 in AD patients and healthy people is more pronounced. Reduced Aβ40 levels, elevated plasma Aβ42, and a decreased Aβ42/Aβ40 ratio in aging populations may all point to an early transition from baseline cognitive aging process toward MCI or AD [136,137].

5.4.2. *Aβ42/Aβ40*

Cerebrospinal fluid Aβ42/Aβ40 is a reliable surrogate marker of amyloidogenic burden. This biomarker exhibits a dual-peaked anatomical allocation, with only a narrow transitional area close to the diagnostic threshold, according to numerous clinical and scholarly studies. Aβ accumulation in the brain, an indicator of AD, is closely bridged to a noticeably lower Aβ42/Aβ40 ratio, which is suggestive of a selective decline in cerebrospinal Aβ42 [138]. Thanks to recent technological developments, comparable decreases in Aβ42/Aβ40 in plasma can now be measured [139]. The slight difference between people with and without amyloid build-up makes clinical differentiation difficult, which is one of the main drawbacks of using plasma Aβ42/Aβ40 ratios to represent amyloid pathology. Aβ42/Aβ40 is the only order that is reduced. While plasma readings range from 8% to 15% and CSF from 40% to 60%, the majority of amyloid-positive people have plasma readings that fall within the positive/negative cut-off [140] .

5.4.3. MicroRNAs and Genetic Signatures

miRNAs are a new class of biomarkers with great potential for diagnosis. Dysregulated miRNA panels, such as miR-125b, miR-181c, miR-29c, miR-34a, and miR-132, have been implicated in the pathogenesis of AD, and some studies suggest that they are reliable for predictions even decades before the beginning of symptoms. These small non-coding RNAs regulate gene expression and may shed light on the molecular processes underlying dementia. The integration of miRNA profiling with other genetic risk factors, like the APOE ε4 allele, enhances risk stratification and supports personalized treatment strategies [141].

5.4.4. Ocular biomarkers

In AD diagnosis, there is a non-invasive frontier in the field of ocular imaging. Neurodegeneration is associated with the thinning of the retinal nerve fibre layer and the loss of ganglion cells, which can be detected by optical coherence tomography (OCT) and spectral-domain OCT (SD-OCT). Importantly, abnormalities in the retina have been acknowledged to exist prior to the onset of intellectual impairment, suggesting that biomarkers found in the eyes may precede AD disease [142].

5.5.5. Advanced imaging modalities

Apart from tau and amyloid PET, other biomarkers are being studied. Due to unique binding profiles for posterior cerebral atrophy, logopen variant aphasia, and typical amnestic AD, tau PET ligands such as flortaucipir enable staging and classification. Neuroinflammatory PET (TSPO ligands) and Synaptic Density PET (SV2A ligands) have shown promise as staging and prognostic methods, albeit only as investigational modalities at this point. These could complement fluid biomarkers as part of comprehensive diagnosing methods, potentially providing a mechanistic perspective as well [143].

5.5.6. Multi- Omics and Artificial Intelligence

The area related to biomarkers is being augmented by research related to proteomics, metabolomics, or integrated multi-omics approaches. Scientists are also attempting to develop multiplexed panels for biomarkers with enhanced diagnostic accuracy by combining CSF, plasma, imaging, and genetic information. Predictive models, risk estimation, as well as personalized treatment

monitoring can also now be achieved because of increasing applications of AI models for complicated data analysis. This is how AD precision diagnostics might evolve as an area in the future [144].

6. Translational hurdles

6.1. Assay standardization

Lack of assay variability standardization among labs and among diagnostic kits is one of the challenges for the use of clinical research of biomarkers. Specifically, for pTau isoforms, ratios of Aβ42 to Aβ40, as well as NfL concentrations, variations in sampling, processing, and analysis could drastically impact biomarker assessment. There are now variations in the methods of detection for SIMOA, mass spectrometry, and ELISA in terms of the threshold values for the classification of illness. International standards for calibration, reference materials, and quality controls need to developed to ensure that there is reproducibility, as well as multi-center validation for diagnostics based on biomarkers [145].

6.2. Ethical concerns

There are serious ethical and legal concerns with the growing intersection of AI-related and biomarker-related diagnostics.The issues of informed consent, psychological impact, and patient autonomy come into conflict with the challenge presented by early biomarker discoveries in asymptomatic individuals, particularly in the context of the lack of treatment modalities.Moreover, the privacy issues associated with data governance come into play where genetic/molecular data is employed in comprehensive testing/diagnostic screenings [146].On the positive side of early identification, the possible costs of misidentification and stigma and insurance bias in ethical principles need to be considered.Involvement of biomarker assays within standard clinical practice emphasizes the need for established clinical thresholds and transparent risk communications by regulation bodies such as the FDA and EMA.

6.3. Population variability

Population heterogeneity is another major translational challenge in Alzheimer's disease. Many factors can influence biomarker concentrations, including age, sex, ethnicity, concomitant diseases (e.g., diabetes or vascular dementia), and genetic background (e.g., APOE ε4 status). Most of the current biomarker research comes from cohorts in Western or wealthy nations; therefore, the generalizability to a number of global populations is limited.

Thus, the creation of normative databases and population-specific reference ranges is essential to avoid diagnostic bias and to provide equal opportunity for healthcare among different demographic groups [146].

7. Future Prospective

Many obstacles still exist in spite of these developments. To guarantee repeatability and comparability, tests must be standardized across labs. Establishing usefulness in a variety of groups requires clinical validation in sizable, multicenter cohorts. Widespread use is hampered by the expense and accessibility of PET imaging and high-sensitivity tests, especially in environments with limited resources. It's also necessary to address privacy and ethical issues related to genomic and miRNA profiling. In the end, multiplexed biomarker panels that incorporate neurogranin, plasma p-Tau, NfL, Aβ42/40 ratio and miRNA signatures—supported by imaging and AI analytics—will be the key to the future of AD diagnoses. Pre-symptomatic identification, accurate disease staging, and tailored therapy monitoring are all possible with these advancements.

7.1. *Future challenges*

Besides rapid advancements in biomarker technologies, several serious challenges remain that may hinder their widespread clinical adoption and impact:

- ***Lack of outcome-based evidence***
  Blood markers like GFAP, Aβ42/40, and p-tau181 are being used for recognition of AD, yet their impact on patient centred outcome doesn't remain clear.
- ***Ethical and clinical confusions in asymptomatic diagnosis***
  The ability to detect AD pathology before the hallmarks arise raises a serious concern. Some scientists highlighted the psychological burden and clinical uncertainty of diagnosing asymptomatic patients based on biomarkers alone.
- ***Standardization and validation***
  Technologies like SIMOA and MS complicate the interpretation of biomarkers. FDA's 2025 guidance emphasizes the need for context of

use-driven validation, but lacks clarity on clinical utility and standards

- ***Population variability and generalizability***
  Most of the biomarker studies are conducted in a homogeneous cohort population. AD/PD data concluded that comorbidities like diabetes and ethnicity increasingly influence biomarker expression, marking the need for inclusive validation.
- ***Integrated primary care and Real-world settings***
  While blood biomarkers offer accessibility to data and early diagnosis, their integration into primary care remains limited. The need for training, infrastructure, and decision- support tools to enable early detection of diseases and equitable access in diverse clinical environments.

7. Result and discussion

Blood-based biomarkers like amyloid, tau, neurodysfunctional, and inflammatory biomarkers serve as a robust modality for prodromal detection and disorder monitoring. These indicators demonstrate fundamental disease related process involving tau accumulation, neuroinflammation, axonal trauma, etc., providing non- surgical options to CSF and PET visualization. Nonetheless, obstacles remain in unifying assays and certifying cross- group comparison dependability. New biomarkers, such as dysregulated microRNAs, including miR-125b, miR-181c, miR-29c, miR-34a, and miR-132, and genetic risk variables, including APOE ε4, have the potential for the detection of AD decades before the appearance of symptoms. Advanced PET modalities, including tau ligands of flortaucipir, TSPO-based neuroinflammatory tracers, and synaptic density markers of SV2A, yield valuable mechanistic insights but, to date, remain largely investigational, whereas non-invasive ocular imaging with OCT and SD-OCT shows retinal changes that may presage cognitive decline.

Multiplexed biomarker strips that integrate the data from fluid, imaging, and genetic sources towards enhanced diagnosis sensitivity and personalized monitoring are being pushed forward by the use of multi-omics tools and AI analysis. The future for AD diagnosis is in proven multi-parametric panels that involve neurogranin, NfL, Aβ42/40 ratio, plasma p-Tau, and miRNA. These panels would facilitate pre-symptomatic testing, accurate staging, and personalized therapeutic strategies, irrespective of challenges regarding cost, accessibility, and standardization. Problems such as standardization of assays, bioethical issues associated with the early diagnosis of the disease, as well as interpopulation variability that influences the precision of the results, have until now represented barriers to the translation of AD biomarkers. Solutions to such challenges are essential in ensuring accurate application.

8. Conclusions

AD is currently the most common disorder that causes neural brain damage marked by a continuous decline in cognitive memory and pathogenetic pathways. This review stresses the potential role of Ca^{+2} imbalance, synaptic loss, amyloid pathways, tau hyperphosphorylation, and nerve loss. Time- tested diagnostic tools, despite being instructive, are bound by procedural intensity, money, and physical access.

Modern breakthroughs on blood- based biomarkers- like GFAP, Nfl, p- tau isoforms, etc. serves hopeful approach for preliminary and non- intrusive diagnosing of disorders and study of pathogenic progression. Unification of these clinical signatures within the ATN model, along with neurological mapping and computational learning systems, increases evaluative accuracy and provides easy segmentations of various phases of illness. Jointly, these evidences aid an evolutionary change towards more reachable, multi- sourced and mechanistic detection methods.

Data availability statement

This review is based on a thorough study and review of literature that is referenced for publicly reachable databases like PubMed and Google Scholar. All the data in this review are supported and cited within the article through the sources. No new data were generated during the preparation of this manuscript.

Author's contributions:

D was responsible for conducting the literature search and prepare draft of the manuscript. A M contributed to the conceptualization of the study and provided critical revisions to refine the manuscript. S D thoroughly reviewed the manuscript and granted the final approval for submission.

List of abbreviations:

Abbreviation	Full form
AD	Alzheimer's Disease
Aβ	Amyloid-beta
NFTs	Neurofibrillary Tangles
APP	Amyloid Precursor Protein
CP-AMPAR	Calcium-Permeable AMPA Receptor
CALHM	Calcium Homeostasis Modulator
NFAT	Nuclear Factor of Activated T-cells
BACE1	Beta-Site APP-Cleaving Enzyme 1
SOCE	Store-Operated Calcium Entry
PS1	Presenilin 1
GSK-3β	Glycogen Synthase Kinase 3 Beta
CDK5	Cyclin-Dependent Kinase 5
CAMKII	Calcium/Calmodulin-Dependent Protein Kinase II
PP2A	Protein Phosphatase 2A
mPTP	Mitochondrial Permeability Transition Pore
ETC	Electron Transport Chain
VDAC	Voltage-Dependent Anion Channel
ROS	Reactive Oxygen Species
MAM	Mitochondria-Associated Membrane
PKC	Protein Kinase C
MAPK	Mitogen-Activated Protein Kinase
NF-κB	Nuclear Factor Kappa B
BBB	Blood-Brain Barrier
TFEB	Transcription Factor EB
AMPK	AMP-Activated Protein Kinase
mTOR	Mechanistic Target of Rapamycin
ATG5	Autophagy Related 5
RyRs	Ryanodine Receptors
LTD	Long-Term Depression
sAPPβ	Soluble APP Beta
CTFβ	C-Terminal Fragment Beta
AICD	APP Intracellular Domain
Aph1	Anterior Pharynx Defective 1
MCI	Mild Cognitive Impairment
CSF	Cerebrospinal Fluid
PET	Positron Emission Tomography
MRI	Magnetic Resonance Imaging
VR	Virtual Reality
DLT	Dichotic Listening Test
MMSE	Mini-Mental State Examination
VEs	Virtual Environments
NfL	Neurofilament Light Chain
sTREM2	Soluble Triggering Receptor Expressed on Myeloid Cells 2
SNAP-25	Synaptosomal-Associated Protein 25
CHI3L1	Chitinase-3-Like Protein 1
CRP	C-Reactive Protein
MTBR-tau243	Microtubule Binding Region Tau 243
GVBs	Granulovacuolar Bodies
SIMOA	Single Molecule Array
EPAD	European Prevention of Alzheimer's Dementia
ADNI	Alzheimer's Disease Neuroimaging Initiative

Consent for publication

Not applicable

Funding

None.

Conflict of interest

The authors declare no conflict of interest, financially or otherwise.

Acknowledgements

The authors would like to express gratitude to the management of Noida Institute of Engineering and

Technology [Pharmacy Institute], India, for their persistent assistance, facilitation, passion, and immense expertise and knowledge.

REFERENCES

[1] Andrade-Guerrero J, Santiago-Balmaseda A, Jeronimo-Aguilar P, Vargas-Rodríguez I, Cadena-Suárez AR, Sánchez-Garibay C, et al. Alzheimer's Disease: An Updated Overview of Its Genetics. Int J Mol Sci 2023;24:3754–9. https://doi.org/10.3390/ijms24043754.

[2] Asuku AO, Ayinla MT, Gbonjubola OO, Babatunde SS, Olajide TS, Oyerinde TO. Neurocognitive Aspects of Dementia, 2024, p. 109–30. https://doi.org/10.1007/978-981-97-4117-5_5.

[3] Zvěřová M. Clinical aspects of Alzheimer's disease. Clin Biochem 2019;72:3–6. https://doi.org/10.1016/j.clinbiochem.2019.04.015.

[4] Aggarwal NT, Mielke MM. Sex Differences in Alzheimer's Disease. Neurol Clin 2023;41:343–58. https://doi.org/10.1016/j.ncl.2023.01.001.

[5] Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. Neuropathological Alterations in Alzheimer Disease. Cold Spring Harb Perspect Med 2011;1:a006189–a006189. https://doi.org/10.1101/cshperspect.a006189.

[6] Uddin MdS, Al Mamun A, Rahman MdA, Behl T, Perveen A, Hafeez A, et al. Emerging Proof of Protein Misfolding and Interactions in Multifactorial Alzheimer's Disease. Curr Top Med Chem 2020;20:2380–90. https://doi.org/10.2174/1568026620666620060116 1703.

[7] Valdez-Gaxiola CA, Rosales-Leycegui F, Gaxiola-Rubio A, Moreno-Ortiz JM, Figuera LE. Early- and Late-Onset Alzheimer's Disease: Two Sides of the Same Coin? Diseases 2024;12:110. https://doi.org/10.3390/diseases12060110.

[8] Ezkurdia A, Ramírez MJ, Solas M. Metabolic Syndrome as a Risk Factor for Alzheimer's Disease: A Focus on Insulin Resistance. Int J Mol Sci 2023;24:4354. https://doi.org/10.3390/ijms24054354.

[9] Gao L, Zhang Y, Sterling K, Song W. Brain-derived neurotrophic factor in Alzheimer's disease and its pharmaceutical potential. Transl Neurodegener 2022;11:4. https://doi.org/10.1186/s40035-022-00279-0.

[10] Livingston G, Huntley J, Liu KY, Costafreda SG, Selbæk G, Alladi S, et al. Dementia prevention, intervention, and care: 2024 report of the Lancet standing Commission. The Lancet 2024;404:572–628. https://doi.org/10.1016/S0140-6736(24)01296-0.

[11] Heck J, Palmeira Do Amaral AC, Weißbach S, El Khallouqi A, Bikbaev A, Heine M. More than a pore: How voltage-gated calcium channels act on different levels of neuronal communication regulation. Channels 2021;15:322–38. https://doi.org/10.1080/19336950.2021.1900024.

[12] Ludewig S, Herrmann U, Michaelsen-Preusse K, Metzdorf K, Just J, Bold C, et al. APPsα rescues impaired Ca 2+ homeostasis in APP- and APLP2-deficient hippocampal neurons. Proceedings of the National Academy of Sciences 2021;118. https://doi.org/10.1073/pnas.2011506118.

[13] Scremin E, Agostini M, Leparulo A, Pozzan T, Greotti E, Fasolato C. ORAI2 Down-Regulation Potentiates SOCE and Decreases Aβ42 Accumulation in Human Neuroglioma Cells. Int J Mol Sci 2020;21:5288–90. https://doi.org/10.3390/ijms21155288.

[14] Kim M, Bezprozvanny I. Conformational Models of APP Processing by Gamma Secretase Based on Analysis of Pathogenic Mutations. Int J Mol Sci 2021;22:13600–10. https://doi.org/10.3390/ijms222413600.

[15] Stefanoska K, Gajwani M, Tan ARP, Ahel HI, Asih PR, Volkerling A, et al. Alzheimer's disease: Ablating single master site abolishes tau hyperphosphorylation. Sci Adv 2022;8:56–75. https://doi.org/10.1126/sciadv.abl8809.

[16] Brocard F, Dingu N. Calpains at the Crossroads of Spinal Cord Physiology, Plasticity, and Pathology. Cells 2025;14:1503–20. https://doi.org/10.3390/cells14191503.

[17] Arnsten AFT, Datta D, Del Tredici K, Braak H. Hypothesis: Tau pathology is an initiating factor in sporadic Alzheimer's disease. Alzheimer's & Dementia 2021;17:115–24. https://doi.org/10.1002/alz.12192.

[18] Moawad MHED, Serag I, Alkhawaldeh IM, Abbas A, Sharaf A, Alsalah S, et al. Exploring the Mechanisms and Therapeutic Approaches of Mitochondrial Dysfunction in Alzheimer's Disease: An Educational Literature Review. Mol Neurobiol 2025;62:6785–810. https://doi.org/10.1007/s12035-024-04468-y.

[19] Wu AJ, Tong BC-K, Huang AS, Li M, Cheung K-H. Mitochondrial Calcium Signaling as a Therapeutic Target for Alzheimer's Disease. Curr Alzheimer Res 2020;17:329–43. https://doi.org/10.2174/1567205016666191210091302.

[20] Sharma C, Kim S, Nam Y, Jung UJ, Kim SR. Mitochondrial Dysfunction as a Driver of Cognitive Impairment in Alzheimer's Disease. Int J Mol Sci 2021;22:4850–952. https://doi.org/10.3390/ijms22094850.

[21] Calvo-Rodriguez M, Hou SS, Snyder AC, Kharitonova EK, Russ AN, Das S, et al. Increased mitochondrial calcium levels associated with neuronal death in a mouse model of Alzheimer's disease. Nat Commun 2020;11:2146–50. https://doi.org/10.1038/s41467-020-16074-2.

[22] Üremiş N, Üremiş MM. Oxidative/Nitrosative Stress, Apoptosis, and Redox Signaling: Key Players in Neurodegenerative Diseases. J Biochem Mol Toxicol 2025;39:1–12. https://doi.org/10.1002/jbt.70133.

[23] Esteras N, Abramov AY. Mitochondrial Calcium Deregulation in the Mechanism of Beta-Amyloid and Tau Pathology. Cells 2020;9:2135. https://doi.org/10.3390/cells9092135.

[24] Britti E, Ros J, Esteras N, Abramov AY. Tau inhibits mitochondrial calcium efflux and makes neurons vulnerable to calcium-induced cell death. Cell Calcium 2020;86:102150. https://doi.org/10.1016/j.ceca.2019.102150.

[25] Li Z, Cao Y, Pei H, Ma L, Yang Y, Li H. The contribution of mitochondria-associated endoplasmic reticulum membranes (MAMs) dysfunction in Alzheimer's disease and the potential countermeasure. Front Neurosci 2023;17:1–18. https://doi.org/10.3389/fnins.2023.1158204.

[26] Bhattacharyya R, Black SE, Lotlikar MS, Fenn RH, Jorfi M, Kovacs DM, et al. Axonal generation of amyloid-β from palmitoylated APP in mitochondria-associated endoplasmic reticulum membranes. Cell Rep 2021;35:109134–15. https://doi.org/10.1016/j.celrep.2021.109134.

[27] Yu W, Jin H, Huang Y. Mitochondria-associated membranes (MAMs): a potential therapeutic target for treating Alzheimer's disease. Clin Sci 2021;135:109–26. https://doi.org/10.1042/CS20200844.

[28] Huang Y, Wei F, Cheng X, Shi Y, Liu Z, Ye L. Neurotropin alleviates Alzheimer's disease pathology by inhibiting FUS-mediated Calhm2 transcription, blocking the Calhm2/EFhd2 interaction, to improve mitochondrial dysfunction-associated microglia polarization. Biosci Trends 2025;19:2025.01220. https://doi.org/10.5582/bst.2025.01220.

[29] Syvänen V, Koistinaho J, Lehtonen Š. Identification of the abnormalities in astrocytic functions as potential drug targets for neurodegenerative disease. Expert Opin Drug Discov 2024;19:603–16. https://doi.org/10.1080/17460441.2024.2322988.

[30] Santiago-Balmaseda A, Aguirre-Orozco A, Valenzuela-Arzeta IE, Villegas-Rojas MM, Pérez-Segura I, Jiménez-Barrios N, et al. Neurodegenerative Diseases: Unraveling the Heterogeneity of Astrocytes. Cells 2024;13:921. https://doi.org/10.3390/cells13110921.

[31] Paumier A, Boisseau S, Jacquier-Sarlin M, Pernet-Gallay K, Buisson A, Albrieux M. Astrocyte–neuron interplay is critical for Alzheimer's disease pathogenesis and is rescued by TRPA1 channel blockade. Brain 2022;145:388–405. https://doi.org/10.1093/brain/awab281.

[32] Hou M, Zhang Z, Fan Z, Huang L, Wang L. The mechanisms of Ca2+ regulating autophagy and its research progress in neurodegenerative diseases: A review. Medicine 2024;103:e39405. https://doi.org/10.1097/MD.0000000000039405.

[33] Benito-Cuesta I, Ordóñez-Gutiérrez L, Wandosell F. AMPK activation does not enhance autophagy in neurons in contrast to MTORC1 inhibition: different impact on β-amyloid clearance. Autophagy 2021;17:656–71.

https://doi.org/10.1080/15548627.2020.1728095.

[34] Nixon RA. Autophagy–lysosomal-associated neuronal death in neurodegenerative disease. Acta Neuropathol 2024;148:42. https://doi.org/10.1007/s00401-024-02799-7.

[35] Hasan AR, Tasnim F, Aktaruzzaman Md, Islam MdT, Rayhan R, Brishti A, et al. The Alteration of Microglial Calcium Homeostasis in Central Nervous System Disorders: A Comprehensive Review. Neuroglia 2024;5:410–44. https://doi.org/10.3390/neuroglia5040027.

[36] Pikor D, Hurła M, Słowikowski B, Szymanowicz O, Poszwa J, Banaszek N, et al. Calcium Ions in the Physiology and Pathology of the Central Nervous System. Int J Mol Sci 2024;25:13133. https://doi.org/10.3390/ijms252313133.

[37] Verma M, Lizama BN, Chu CT. Excitotoxicity, calcium and mitochondria: a triad in synaptic neurodegeneration. Transl Neurodegener 2022;11:3. https://doi.org/10.1186/s40035-021-00278-7.

[38] Zhou R, Yang G, Guo X, Zhou Q, Lei J, Shi Y. Recognition of the amyloid precursor protein by human γ-secretase. Science (1979) 2019;363:1157–62. https://doi.org/10.1126/science.aaw0930.

[39] Wu T, Lin D, Cheng Y, Jiang S, Riaz MW, Fu N, et al. Amyloid Cascade Hypothesis for the Treatment of Alzheimer's Disease: Progress and Challenges. Aging Dis 2022;13:1745. https://doi.org/10.14336/AD.2022.0412.

[40] Tiwari S, Atluri V, Kaushik A, Yndart A, Nair M. Alzheimer's disease: pathogenesis, diagnostics, and therapeutics . Int J Nanomedicine 2019;Volume 14:5541–54. https://doi.org/10.2147/IJN.S200490.

[41] Rawat P, Sehar U, Bisht J, Selman A, Culberson J, Reddy PH. Phosphorylated Tau in Alzheimer's Disease and Other Tauopathies. Int J Mol Sci 2022;23:12841. https://doi.org/10.3390/ijms232112841.

[42] Pei J-J, Khatoon S, An W-L, Nordlinder M, Tanaka T, Braak H, et al. Role of protein kinase B in Alzheimer's neurofibrillary pathology. Acta Neuropathol 2003;105:381–92. https://doi.org/10.1007/s00401-002-0657-y.

[43] Yin X, Qiu Y, Zhao C, Zhou Z, Bao J, Qian W. The Role of Amyloid-Beta and Tau in the Early Pathogenesis of Alzheimer's Disease. Medical Science Monitor 2021;27. https://doi.org/10.12659/MSM.933084.

[44] Hong X, Huang L, Lei F, Li T, Luo Y, Zeng M, et al. The Role and Pathogenesis of Tau Protein in Alzheimer's Disease. Biomolecules 2025;15:824. https://doi.org/10.3390/biom15060824.

[45] Zhou S, Wang K. Childhood Secondhand Smoke Exposure and Risk of Dementia, Alzheimer's Disease and Stroke in Adulthood: A Prospective Cohort Study. J Prev Alzheimers Dis 2021;8:345–50. https://doi.org/10.14283/jpad.2021.10.

[46] Perez MA, Reyes-Esteves S, Mendizabal A. Racial and Ethnic Disparities in Neurological Care in the United States. Semin Neurol 2024;44:178–92. https://doi.org/10.1055/s-0043-1778639.

[47] Wang P, Gao X, Willett WC, Giovannucci EL. Socioeconomic Status, Diet, and Behavioral Factors and Cardiometabolic Diseases and Mortality. JAMA Netw Open 2024;7:e2451837. https://doi.org/10.1001/jamanetworkopen.2024.51837.

[48] Neuffer J, Wagner M, Moreno E, Le Grand Q, Mishra A, Trégouët D, et al. Association of LIfestyle for BRAin health risk score (LIBRA) and genetic susceptibility with incident dementia and cognitive decline. Alzheimer's & Dementia 2024;20:4250–9. https://doi.org/10.1002/alz.13801.

[49] Yu Y, Wang C, Wang B, Wang X, Zhao Q, Yan Y, et al. Clusterin Regulates the Mechanisms of Neuroinflammation and Neuronal Circuit Impairment in Alzheimer's Disease. Int J Mol Sci 2025;26:7271. https://doi.org/10.3390/ijms26157271.

[50] Khan MA. Iron-Mediated Overexpression of Amyloid Precursor Protein via Iron Responsive mRNA in Alzheimer's Disease. Int J Mol Sci 2025;26:5283. https://doi.org/10.3390/ijms26115283.

[51] Sailike B, Onzhanova Z, Akbay B, Tokay T, Molnár F. Vitamin D in Central Nervous System: Implications for Neurological Disorders. Int J Mol Sci 2024;25:7809. https://doi.org/10.3390/ijms25147809.

[52] Zhu L, Tang M, Cai Y, Wang P. Association between exposure to environmental pollutants and increased oral health risks, a comprehensive review. Front Public Health 2025;12. https://doi.org/10.3389/fpubh.2024.1482991.

[53] Holstege H, Hulsman M, Charbonnier C, Grenier-Boley B, Quenez O, Grozeva D, et al. Exome sequencing identifies rare damaging variants in ATP8B4 and ABCA1 as risk factors for Alzheimer's disease. Nat Genet 2022;54:1786–94. https://doi.org/10.1038/s41588-022-01208-7.

[54] Li X, Feng X, Sun X, Hou N, Han F, Liu Y. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2019. Front Aging Neurosci 2022;14. https://doi.org/10.3389/fnagi.2022.937486.

[55] Teipel S, Gustafson D, Ossenkoppele R, Hansson O, Babiloni C, Wagner M, et al. Alzheimer Disease: Standard of Diagnosis, Treatment, Care, and Prevention. Journal of Nuclear Medicine 2022;63:981–5. https://doi.org/10.2967/jnumed.121.262239.

[56] Knudsen LV, Gazerani P, Duan Y, Michel TM, Vafaee MS. The role of multimodal MRI in mild cognitive impairment and Alzheimer's disease. Journal of Neuroimaging 2022;32:148–57. https://doi.org/10.1111/jon.12940.

[57] Hebling Vieira B, Liem F, Dadi K, Engemann DA, Gramfort A, Bellec P, et al. Predicting future cognitive decline from non-brain and multimodal brain imaging data in healthy and pathological aging. Neurobiol Aging 2022;118:55–65. https://doi.org/10.1016/j.neurobiolaging.2022.06.008.

[58] Farabolini G, Baldini N, Pagano A, Andrenelli E, Pepa L, Morone G, et al. Continuous Movement Monitoring at Home Through Wearable Devices: A Systematic Review. Sensors 2025;25:4889. https://doi.org/10.3390/s25164889.

[59] Zhao Z, Chuah JH, Lai KW, Chow C-O, Gochoo M, Dhanalakshmi S, et al. Conventional machine learning and deep learning in Alzheimer's disease diagnosis using neuroimaging: A review. Front Comput Neurosci 2023;17. https://doi.org/10.3389/fncom.2023.1038636.

[60] Elgandelwar SM, Bairagi V, S. Vasekar S, Nanthaamornphong A, Tupe-Waghmare P. Analyzing electroencephalograph signals for early Alzheimer's disease detection: deep learning vs. traditional machine learning approaches. International Journal of Electrical and Computer Engineering (IJECE) 2024;14:2602. https://doi.org/10.11591/ijece.v14i3.pp2602-2615.

[61] Ghosh N, Sinha K, Sil PC. A review on the new age methodologies for early detection of Alzheimer's and Parkinson's disease. Basic Clin Pharmacol Toxicol 2024;134:602–13. https://doi.org/10.1111/bcpt.14003.

[62] Hu S, Yang C, Luo H. Current trends in blood biomarker detection and imaging for Alzheimer's disease. Biosens Bioelectron 2022;210:114278. https://doi.org/10.1016/j.bios.2022.114278.

[63] Shukla A, Tiwari R, Tiwari S. Review on Alzheimer Disease Detection Methods: Automatic Pipelines and Machine Learning Techniques. Sci 2023;5:13–20. https://doi.org/10.3390/sci5010013.

[64] Kim Y, Jiang X, Giancardo L, Pena D, Bukhbinder AS, Amran AY, et al. Multimodal Phenotyping of Alzheimer's Disease with Longitudinal Magnetic Resonance Imaging and Cognitive Function Data. Sci Rep 2020;10:5527. https://doi.org/10.1038/s41598-020-62263-w.

[65] Bonakdarpour B, Takarabe C. Brain Networks, Clinical Manifestations, and Neuroimaging of Cognitive Disorders. Clin Geriatr Med 2023;39:45–65. https://doi.org/10.1016/j.cger.2022.07.004.

[66] Risacher SL, Apostolova LG. Neuroimaging in Dementia. Continuum (N Y) 2023;29:219–54. https://doi.org/10.1212/CON.0000000000001248.

[67] Chimthanawala NMA, Haria A, Sathaye S. Non-invasive Biomarkers for Early Detection of Alzheimer's Disease: a New-Age Perspective. Mol Neurobiol 2024;61:212–23. https://doi.org/10.1007/s12035-023-03578-3.

[68] Bouwman FH, Frisoni GB, Johnson SC, Chen X, Engelborghs S, Ikeuchi T, et al. Clinical application of CSF biomarkers for Alzheimer's disease: From rationale to ratios. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring 2022;14. https://doi.org/10.1002/dad2.12314.

[69] Benjamin SR, Lima F de, Nunes PIG, Dutra RF, Andrade GM de, Oriá RB. Advanced Biosensing Technologies: Leading Innovations in Alzheimer's Disease Diagnosis. Chemosensors 2025;13:220–5. https://doi.org/10.3390/chemosensors13060220.

[70] Helaly HA, Badawy M, Haikal AY. Deep Learning Approach for Early Detection of Alzheimer's Disease. Cognit Comput 2022;14:1711–27. https://doi.org/10.1007/s12559-021-09946-2.

[71] Ioannou K, Bucci M, Tzortzakakis A, Savitcheva I, Nordberg A, Chiotis K. Tau PET positivity predicts clinically relevant cognitive decline driven by Alzheimer's disease compared to comorbid cases; proof of concept in the ADNI study. Mol Psychiatry 2025;30:587–99. https://doi.org/10.1038/s41380-024-02672-9.

[72] Varesi A, Carrara A, Pires VG, Floris V, Pierella E, Savioli G, et al. Blood-Based Biomarkers for Alzheimer's Disease Diagnosis and Progression: An Overview. Cells 2022;11:1367. https://doi.org/10.3390/cells11081367.

[73] Gong X, Zhang H, Liu X, Liu Y, Liu J, Fapohunda FO, et al. Is liquid biopsy mature enough for the diagnosis of Alzheimer's disease? Front Aging Neurosci 2022;14:52–65. https://doi.org/10.3389/fnagi.2022.977999.

[74] Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: Evidence from functional MRI. Proceedings of the National Academy of Sciences 2004;101:4637–42. https://doi.org/10.1073/pnas.0308627101.

[75] Stam C, Jones B, Nolte G, Breakspear M, Scheltens P. Small-World Networks and Functional Connectivity in Alzheimer's Disease. Cerebral Cortex 2006;17:92–9. https://doi.org/10.1093/cercor/bhj127.

[76] Chouliaras L, O'Brien JT. The use of neuroimaging techniques in the early and differential diagnosis of dementia. Mol Psychiatry 2023;28:4084–97. https://doi.org/10.1038/s41380-023-02215-8.

[77] Doecke JD, Bellomo G, Vermunt L, Alcolea D, Halbgebauer S, in 't Veld S, et al. Diagnostic performance of plasma Aβ42/40 ratio, p-tau181, GFAP, and NfL along the continuum of Alzheimer's disease and non-AD dementias: An international multi-center study. Alzheimer's & Dementia 2025;21. https://doi.org/10.1002/alz.14573.

[78] Wang Y, Chen H-J, Cheng Y, Xie Y, Cheng Y, Zhao S, et al. Multimodal integration of plasma biomarkers, MRI, and genetic risk to predict cerebral amyloid burden in Alzheimer's disease. Neuroimage 2025;322:121550. https://doi.org/10.1016/j.neuroimage.2025.121550.

[79] Malik I, Iqbal A, Gu YH, Al-antari MA. Deep Learning for Alzheimer's Disease Prediction: A Comprehensive Review. Diagnostics 2024;14:1281. https://doi.org/10.3390/diagnostics14121281.

[80] Lorenzini L, Ingala S, Wink AM, Kuijer JPA, Wottschel V, Dijsselhof M, et al. The Open-Access European Prevention of Alzheimer's Dementia (EPAD) MRI dataset and processing workflow. Neuroimage Clin 2022;35:103106–10. https://doi.org/10.1016/j.nicl.2022.103106.

[81] Riviere-Cazaux C, Keough MB, Zuccato JA, Kumar R, Schulz SC, Warrington AE, et al. A hitchhiker's guide to cerebrospinal fluid biomarkers for neuro-oncology. Neuro Oncol 2025;27:1165–79. https://doi.org/10.1093/neuonc/noae276.

[82] Karantali E, Kazis D, Chatzikonstantinou S, Petridis F, Mavroudis I. The role of neurofilament light chain in frontotemporal dementia: a meta-analysis. Aging Clin Exp Res 2021;33:869–81. https://doi.org/10.1007/s40520-020-01554-8.

[83] Park JE, Gunasekaran TI, Cho YH, Choi S-M, Song M-K, Cho SH, et al. Diagnostic Blood Biomarkers in Alzheimer's Disease. Biomedicines 2022;10:169. https://doi.org/10.3390/biomedicines10010169.

[84] Cano A, Capdevila M, Puerta R, Arranz J, Montrreal L, de Rojas I, et al. Clinical value of plasma pTau181 to predict Alzheimer's disease pathology in a large real-world cohort of a memory clinic. EBioMedicine 2024;108:105345. https://doi.org/10.1016/j.ebiom.2024.105345.

[85] Grande G, Valletta M, Rizzuto D, Xia X, Qiu C, Orsini N, et al. Blood-based biomarkers of Alzheimer's disease and incident dementia in the

community. Nat Med 2025;31:2027–35. https://doi.org/10.1038/s41591-025-03605-x.

[86] Gafson AR, Barthélemy NR, Bomont P, Carare RO, Durham HD, Julien J-P, et al. Neurofilaments: neurobiological foundations for biomarker applications. Brain 2020;143:1975–98. https://doi.org/10.1093/brain/awaa098.

[87] Milà-Alomà M, Salvadó G, Gispert JD, Vilor-Tejedor N, Grau-Rivera O, Sala-Vila A, et al. Amyloid beta, tau, synaptic, neurodegeneration, and glial biomarkers in the preclinical stage of the Alzheimer's *continuum*. Alzheimer's & Dementia 2020;16:1358–71. https://doi.org/10.1002/alz.12131.

[88] Jung Y, Damoiseaux JS. The potential of blood neurofilament light as a marker of neurodegeneration for Alzheimer's disease. Brain 2024;147:12–25. https://doi.org/10.1093/brain/awad267.

[89] Habib N, McCabe C, Medina S, Varshavsky M, Kitsberg D, Dvir-Szternfeld R, et al. Disease-associated astrocytes in Alzheimer's disease and aging. Nat Neurosci 2020;23:701–6. https://doi.org/10.1038/s41593-020-0624-8.

[90] Edison P. Astroglial activation: Current concepts and future directions. Alzheimer's & Dementia 2024;20:3034–53. https://doi.org/10.1002/alz.13678.

[91] Lista S, Imbimbo BP, Grasso M, Fidilio A, Emanuele E, Minoretti P, et al. Tracking neuroinflammatory biomarkers in Alzheimer's disease: a strategy for individualized therapeutic approaches? J Neuroinflammation 2024;21:187. https://doi.org/10.1186/s12974-024-03163-y.

[92] Benedet AL, Milà-Alomà M, Vrillon A, Ashton NJ, Pascoal TA, Lussier F, et al. Differences Between Plasma and Cerebrospinal Fluid Glial Fibrillary Acidic Protein Levels Across the Alzheimer Disease Continuum. JAMA Neurol 2021;78:1471. https://doi.org/10.1001/jamaneurol.2021.3671.

[93] Karlsson L, Vogel J, Arvidsson I, Åström K, Strandberg O, Seidlitz J, et al. Machine learning prediction of tau-PET in Alzheimer's disease using plasma, MRI, and clinical data. Alzheimer's & Dementia 2025;21. https://doi.org/10.1002/alz.14600.

[94] Wang X, Wang Y, Yang L, Zhang Y, Yang L. TREM2+ macrophages: a key role in disease development. Front Immunol 2025;16:52–6. https://doi.org/10.3389/fimmu.2025.1550893.

[95] Liu W, Taso O, Wang R, Bayram S, Graham AC, Garcia-Reitboeck P, et al. *Trem2* promotes anti-inflammatory responses in microglia and is suppressed under pro-inflammatory conditions. Hum Mol Genet 2020;29:3224–48. https://doi.org/10.1093/hmg/ddaa209.

[96] Meilandt WJ, Ngu H, Gogineni A, Lalehzadeh G, Lee S-H, Srinivasan K, et al. Trem2 Deletion Reduces Late-Stage Amyloid Plaque Accumulation, Elevates the Aβ42:Aβ40 Ratio, and Exacerbates Axonal Dystrophy and Dendritic Spine Loss in the PS2APP Alzheimer's Mouse Model. The Journal of Neuroscience 2020;40:1956–74. https://doi.org/10.1523/JNEUROSCI.1871-19.2019.

[97] Fucci A, Giacobbe S, Guerriero I, Suzumoto Y, D'Andrea EL, Scrima M, et al. The DiaCoVAb Study in South Italy: Immune Response to SARS-CoV-2 Vaccination in Dialysis Patients. Kidney Blood Press Res 2022;47:467–74. https://doi.org/10.1159/000524034.

[98] Li Y, Laws SM, Miles LA, Wiley JS, Huang X, Masters CL, et al. Genomics of Alzheimer's disease implicates the innate and adaptive immune systems. Cellular and Molecular Life Sciences 2021;78:7397–426. https://doi.org/10.1007/s00018-021-03986-5.

[99] Mielke MM, Aggarwal NT, Vila-Castelar C, Agarwal P, Arenaza-Urquijo EM, Brett B, et al. Consideration of sex and gender in Alzheimer's disease and related disorders from a global perspective. Alzheimer's & Dementia 2022;18:2707–24. https://doi.org/10.1002/alz.12662.

[100] Jin Z, Lu Y, Tang H, Cui H. Integrating neuroinflammation biomarkers into the ATN(X) framework: Advances in Alzheimer's pathogenesis, diagnosis, and insights from non-human primate models. Alzheimer's & Dementia 2025;21:45–56. https://doi.org/10.1002/alz.70472.

[101] Ceylan G, Karagöz Sakallı N, Eroğlu İçli H, Küçükgergin C, Doğru Abbasoğlu S, Vural P. The Evaluation of Serum Total Tau, NFL, Neurogranin, YKL-40, and FABP-3 as Screening Biomarkers for Alzheimer's Disease. Experimed

2025;15:77–85. https://doi.org/10.26650/experimed.1587358.

[102] Blanco-Palmero VA, Rubio-Fernández M, Antequera D, Villarejo-Galende A, Molina JA, Ferrer I, et al. Increased YKL-40 but Not C-Reactive Protein Levels in Patients with Alzheimer's Disease. Biomedicines 2021;9:1094. https://doi.org/10.3390/biomedicines9091094.

[103] Ebrahimi R, Shahrokhi Nejad S, Falah Tafti M, Karimi Z, Sadr SR, Ramadhan Hussein D, et al. Microglial activation as a hallmark of neuroinflammation in Alzheimer's disease. Metab Brain Dis 2025;40:207. https://doi.org/10.1007/s11011-025-01631-9.

[104] Rhinn H, Tatton N, McCaughey S, Kurnellas M, Rosenthal A. Progranulin as a therapeutic target in neurodegenerative diseases. Trends Pharmacol Sci 2022;43:641–52. https://doi.org/10.1016/j.tips.2021.11.015.

[105] Yang K-D, He Y, Xiao S, Ai Q, Yu J-L. Identification of progranulin as a novel diagnostic biomarker for early-onset sepsis in neonates. European Journal of Clinical Microbiology & Infectious Diseases 2020;39:2405–14. https://doi.org/10.1007/s10096-020-03981-x.

[106] Han J, Zhang Z, Zhang P, Yu Q, Cheng Q, Lu Z, et al. The roles of microglia and astrocytes in neuroinflammation of Alzheimer's disease. Front Neurosci 2025;19:1574342. https://doi.org/10.3389/fnins.2025.1575453.

[107] Zhang Q, Yang G, Luo Y, Jiang L, Chi H, Tian G. Neuroinflammation in Alzheimer's disease: insights from peripheral immune cells. Immunity & Ageing 2024;21:38. https://doi.org/10.1186/s12979-024-00445-0.

[108] Beran M, Jansen WJ, Oomens JE, Moonen JEF, Slagboom PE, Huisman M, et al. Biomarkers of endothelial dysfunction and cognition: A two-step IPD meta-analysis. Alzheimer's & Dementia 2024;20:8402–11. https://doi.org/10.1002/alz.14272.

[109] Janelidze S, Mattsson N, Stomrud E, Lindberg O, Palmqvist S, Zetterberg H, et al. CSF biomarkers of neuroinflammation and cerebrovascular dysfunction in early Alzheimer disease. Neurology 2018;91:e867–77. https://doi.org/10.1212/WNL.0000000000006082.

[110] Aramadaka S, Mannam R, Sankara Narayanan R, Bansal A, Yanamaladoddi VR, Sarvepalli SS, et al. Neuroimaging in Alzheimer's Disease for Early Diagnosis: A Comprehensive Review. Cureus 2023;15:e38544. https://doi.org/10.7759/cureus.38544.

[111] Gao F. Integrated Positron Emission Tomography/Magnetic Resonance Imaging in clinical diagnosis of Alzheimer's disease. Eur J Radiol 2021;145:110017. https://doi.org/10.1016/j.ejrad.2021.110017.

[112] Kang JH, Korecka M, Lee EB, Cousins KAQ, Tropea TF, Chen-Plotkin AA, et al. Alzheimer Disease Biomarkers: Moving from CSF to Plasma for Reliable Detection of Amyloid and tau Pathology. Clin Chem 2023;69:1247–59. https://doi.org/10.1093/clinchem/hvad139.

[113] Arastoo M, Lofthouse R, Penny LK, Harrington CR, Porter A, Wischik CM, et al. Current Progress and Future Directions for Tau-Based Fluid Biomarker Diagnostics in Alzheimer's Disease. Int J Mol Sci 2020;21:8673. https://doi.org/10.3390/ijms21228673.

[114] Lai R, Li B, Bishnoi R. P-tau217 as a Reliable Blood-Based Marker of Alzheimer's Disease. Biomedicines 2024;12:1836. https://doi.org/10.3390/biomedicines12081836.

[115] Assfaw AD, Schindler SE, Morris JC. Advances in blood biomarkers for Alzheimer disease (<scp>AD</scp>): A review. Kaohsiung J Med Sci 2024;40:692–8. https://doi.org/10.1002/kjm2.12870.

[116] Janelidze S, Mattsson N, Palmqvist S, Smith R, Beach TG, Serrano GE, et al. Plasma P-tau181 in Alzheimer's disease: relationship to other biomarkers, differential diagnosis, neuropathology and longitudinal progression to Alzheimer's dementia. Nat Med 2020;26:379–86. https://doi.org/10.1038/s41591-020-0755-1.

[117] Martínez-Dubarbie F, Guerra-Ruiz A, López-García S, Lage C, Fernández-Matarrubia M, Infante J, et al. Accuracy of plasma Aβ40, Aβ42, and p-tau181 to detect CSF Alzheimer's pathological changes in cognitively unimpaired subjects using the Lumipulse automated platform. Alzheimers Res Ther 2023;15:163. https://doi.org/10.1186/s13195-023-01319-1.

[118] Shen X-N, Huang Y-Y, Chen S-D, Guo Y, Tan L, Dong Q, et al. Plasma phosphorylated-tau181 as a predictive biomarker for Alzheimer's amyloid, tau and FDG PET status. Transl Psychiatry 2021;11:585. https://doi.org/10.1038/s41398-021-01709-9.

[119] Teunissen CE, Kolster R, Triana-Baltzer G, Janelidze S, Zetterberg H, Kolb HC. Plasma p-tau immunoassays in clinical research for Alzheimer's disease. Alzheimer's & Dementia 2025;21:e14397. https://doi.org/10.1002/alz.14397.

[120] Kac PR, González-Ortiz F, Emeršič A, Dulewicz M, Koutarapu S, Turton M, et al. Plasma p-tau212 antemortem diagnostic performance and prediction of autopsy verification of Alzheimer's disease neuropathology. Nat Commun 2024;15:2615. https://doi.org/10.1038/s41467-024-46876-7.

[121] Schöll M, Maass A, Mattsson N, Ashton NJ, Blennow K, Zetterberg H, et al. Biomarkers for tau pathology. Molecular and Cellular Neuroscience 2019;97:18–33. https://doi.org/10.1016/j.mcn.2018.12.001.

[122] Janelidze S, Stomrud E, Smith R, Palmqvist S, Mattsson N, Airey DC, et al. Cerebrospinal fluid p-tau217 performs better than p-tau181 as a biomarker of Alzheimer's disease. Nat Commun 2020;11:1683. https://doi.org/10.1038/s41467-020-15436-0.

[123] Jarek DJ, Mizerka H, Nuszkiewicz J, Szewczyk-Golec K. Evaluating p-tau217 and p-tau231 as Biomarkers for Early Diagnosis and Differentiation of Alzheimer's Disease: A Narrative Review. Biomedicines 2024;12:786. https://doi.org/10.3390/biomedicines12040786.

[124] Hu L, Tao Y, Jiang Y, Qin F. Recent progress of nanomedicine in the treatment of Alzheimer's disease. Front Cell Dev Biol 2023;11:45–63. https://doi.org/10.3389/fcell.2023.1228679.

[125] Koper MJ, Van Schoor E, Ospitalieri S, Vandenberghe R, Vandenbulcke M, von Arnim CAF, et al. Necrosome complex detected in granulovacuolar degeneration is associated with neuronal loss in Alzheimer's disease. Acta Neuropathol 2020;139:463–84. https://doi.org/10.1007/s00401-019-02103-y.

[126] Ashton NJ, Pascoal TA, Karikari TK, Benedet AL, Lantero-Rodriguez J, Brinkmalm G, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. Acta Neuropathol 2021;141:709–24. https://doi.org/10.1007/s00401-021-02275-6.

[127] Ashton NJ, Benedet AL, Pascoal TA, Karikari TK, Lantero-Rodriguez J, Brum WS, et al. Cerebrospinal fluid p-tau231 as an early indicator of emerging pathology in Alzheimer's disease. EBioMedicine 2022;76:103836. https://doi.org/10.1016/j.ebiom.2022.103836.

[128] Mattsson-Carlgren N, Janelidze S, Palmqvist S, Cullen N, Svenningsson AL, Strandberg O, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. Brain 2020;143:3234–41. https://doi.org/10.1093/brain/awaa286.

[129] Therriault J, Servaes S, Tissot C, Rahmouni N, Ashton NJ, Benedet AL, et al. Equivalence of plasma p-tau217 with cerebrospinal fluid in the diagnosis of Alzheimer's disease. Alzheimer's & Dementia 2023;19:4967–77. https://doi.org/10.1002/alz.13026.

[130] Horie K, Salvadó G, Barthélemy NR, Janelidze S, Li Y, He Y, et al. CSF MTBR-tau243 is a specific biomarker of tau tangle pathology in Alzheimer's disease. Nat Med 2023;29:1954–63. https://doi.org/10.1038/s41591-023-02443-z.

[131] Chauhan A, Devi R, Singh S, Bhutani K, Prasad S, Singh A, et al. Exploring Alzheimer's diseases with focus on diagnostic strategies targeting amyloid beta 42 (Aβ42). Discover Neuroscience 2025;20:18. https://doi.org/10.1186/s13064-025-00217-6.

[132] Andersson E, Lindblom N, Janelidze S, Salvadó G, Gkanatsiou E, Söderberg L, et al. Soluble cerebral Aβ protofibrils link Aβ plaque pathology to changes in CSF Aβ42/Aβ40 ratios, neurofilament light and tau in Alzheimer's disease model mice. Nat Aging 2025;5:366–75. https://doi.org/10.1038/s43587-025-00810-8.

[133] Zecca C, Pasculli G, Tortelli R, Dell'Abate MT, Capozzo R, Barulli MR, et al. The Role of Age on Beta-Amyloid1–42 Plasma Levels in Healthy Subjects. Front Aging Neurosci 2021;13. https://doi.org/10.3389/fnagi.2021.698571.

[134] Azargoonjahromi A. The duality of amyloid-β: its role in normal and Alzheimer's disease states. Mol Brain 2024;17:44. https://doi.org/10.1186/s13041-024-01118-1.

[135] Morató X, Puerta R, Cano A, Orellana A, de Rojas I, Capdevila M, et al. Associations of plasma SMOC1 and soluble IL6RA levels with the progression from mild cognitive impairment to dementia. Brain Behav Immun Health 2024;42:100899. https://doi.org/10.1016/j.bbih.2024.100899.

[136] Dhauria M, Mondal R, Deb S, Shome G, Chowdhury D, Sarkar S, et al. Blood-Based Biomarkers in Alzheimer's Disease: Advancing Non-Invasive Diagnostics and Prognostics. Int J Mol Sci 2024;25:10911. https://doi.org/10.3390/ijms252010911.

[137] Soni H, Goyal MK, Sarma P, Singh H, Modi M, Sharma A, et al. Evaluation of Plasma Amyloid Peptides Aβ1-40 and Aβ1-42 as Diagnostic Biomarker of Alzheimer's Disease, its Association with Different Grades of Clinical Severity and 18F-Fluorodeoxyglucose Positron Emission Tomography Z score in the Indian Population. Indian Journal of Nuclear Medicine 2021;36:391–7. https://doi.org/10.4103/ijnm.ijnm_50_21.

[138] Udeh-Momoh C, Zheng B, Sandebring-Matton A, Novak G, Kivipelto M, Jönsson L, et al. Blood Derived Amyloid Biomarkers for Alzheimer's Disease Prevention. J Prev Alzheimers Dis 2022;9:12–21. https://doi.org/10.14283/jpad.2021.70.

[139] Budelier MM, Bateman RJ. Biomarkers of Alzheimer Disease. J Appl Lab Med 2020;5:194–208. https://doi.org/10.1373/jalm.2019.030080.

[140] Doecke JD, Pérez-Grijalba V, Fandos N, Fowler C, Villemagne VL, Masters CL, et al. Total Aβ 42 /Aβ 40 ratio in plasma predicts amyloid-PET status, independent of clinical AD diagnosis. Neurology 2020;94. https://doi.org/10.1212/WNL.0000000000009240.

[141] Li Y-B, Fu Q, Guo M, Du Y, Chen Y, Cheng Y. MicroRNAs: pioneering regulators in Alzheimer's disease pathogenesis, diagnosis, and therapy. Transl Psychiatry 2024;14:367. https://doi.org/10.1038/s41398-024-03075-8.

[142] Yuan A, Lee CS. Retinal Biomarkers for Alzheimer Disease: The Facts and the Future. Asia-Pacific Journal of Ophthalmology 2022;11:140–8. https://doi.org/10.1097/APO.0000000000000505.

[143] Dubois B, von Arnim CAF, Burnie N, Bozeat S, Cummings J. Biomarkers in Alzheimer's disease: role in early and differential diagnosis and recognition of atypical variants. Alzheimers Res Ther 2023;15:175. https://doi.org/10.1186/s13195-023-01314-6.

[144] Meng L, Jin H, Yulug B, Altay O, Li X, Hanoglu L, et al. Multi-omics analysis reveals the key factors involved in the severity of the Alzheimer's disease. Alzheimers Res Ther 2024;16:213. https://doi.org/10.1186/s13195-024-01578-6.

[145] Dong Y, Song X, Wang X, Wang S, He Z. The early diagnosis of Alzheimer's disease: Blood-based panel biomarker discovery by proteomics and metabolomics. CNS Neurosci Ther 2024;30. https://doi.org/10.1111/cns.70060.

[146] Ursin F, Timmermann C, Steger F. Ethical Implications of Alzheimer's Disease Prediction in Asymptomatic Individuals through Artificial Intelligence. Diagnostics 2021;11:440. https://doi.org/10.3390/diagnostics11030440.