

Title: The Neurobiological Foundations and Translational Challenges of Current and New-Generation Alzheimer's Disease Biomarkers: A Review

Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative condition, and remains without a definitive cure despite decades of research. Biomarkers offer promising avenues for early diagnosis and disease monitoring, though their clinical adoption has proven to lag behind laboratory advances. This review synthesises recent literature on the neurobiological underpinnings of both well-known and novel AD biomarkers by drawing on 2015–2025, English, human-centered studies utilising assays, imaging, and assessments to highlight the mechanisms and limitations of current tools of diagnosis. This review also aims to analyse the key translational barriers involved in routine clinical diagnostics, and evaluate the clinical readiness and integration for molecular, imaging and functional biomarkers. Hence, this literature review investigates the neurobiological foundations and translational challenges of Alzheimer's disease biomarkers. Key findings include A β 42/40 being a strong biomarker for amyloid plaques, p-tau217 outperforming other plasma p-tau epitopes, and new technologies like second-gen tau PET being developed to reduce off-target issues. Other than certain technical limitations, AD biomarkers have strong neurobiological mechanistic evidence, but their translation is still impeded by practical and socioeconomic challenges, like barriers to access, standardisation and underrepresentation of communities in studies. Addressing and bridging these gaps would be essential for closing the bench-to-bedside divide in AD care, helping enable earlier intervention.

Keywords: Alzheimer's disease, biomarkers, neurobiology, translational neuroscience, diagnostics

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. It is characterised clinically by memory loss, impaired cognition, and behavioral changes. With prevalence rising sharply as populations age, AD poses mounting healthcare and socioeconomic challenges.

Despite being one of the most common forms of dementia and neurodegenerative disease, it remains an incurable illness. As of 2021, of the approximately 57 million people worldwide who have dementia, 60-70% have Alzheimer's (World Health Organisation, n.d.). This greatly affects their quality of life, and also places an enormous burden on caregivers and healthcare systems worldwide, costing economies US\$1.3 trillion globally, with 50% of these attributable to the care given by family members and close friends.

The pursuit of reliable biomarkers for AD has been driven by the need to detect the disease in its earlier preclinical stages, ideally before the onset of severe symptoms. If this is done, it could help facilitate the rapid development of an efficient treatment plan even

before the symptomatic clinical onset of the disease. Historically, the diagnosis of AD has relied on cognitive assessments and, ultimately, post-mortem histopathological confirmation. However, research in recent decades has witnessed rapid advancements in the identification of *in vivo* biomarkers, especially those reflecting amyloid-beta (A β) deposition, tau pathology, synaptic dysfunction and neuroinflammation, all of which are hallmarks of AD (Georgakas et al., 2023). Techniques such as positron emission tomography (PET), cerebrospinal fluid (CSF) assays, and blood-based biomarkers have shown increasing sensitivity and specificity in the past years. These are emphasised in research done on both animal (rat and primate) models and humans. Hansson et al. (2023), for example, states that blood-based biomarkers are an exceptionally promising avenue for revolutionizing the clinical practice of diagnostic and prognostic work-up of Alzheimer's disease. Anoop et al. (2010) explores the exciting recent advancements in CSF biomarkers for monitoring the early stages of AD before severe cognitive dysfunction is apparent. Márquez & Yassa (2019) also suggests a need for composite biomarkers in neuroimaging, as this can provide a deeper understanding of the pathophysiology of AD. Although the future of early diagnosis looks bright, it is undeniable that translational hurdles still persist, which have limited the availability of these diagnostics to certain communities due to factors like socioeconomic differences, standardisation, and education of clinical staff.

Methodology:

This literature review investigates how the neurobiological foundations of Alzheimer's disease biomarkers inform their utility, analysing the key translational challenges that hinder their integration into routine clinical practice.

The review is structured into four main sections. First, the molecular and cellular neurobiology underpinning key AD biomarkers will be discussed, with particular focus on the amyloid and tau hypotheses, neuroinflammation, mitochondrial dysfunction, and synaptic and neurotransmitter alterations. Second, it surveys the primary modalities used in biomarkers, their technical strength and mechanistic correlates. The third section examines translational challenges, including issues of socioeconomic accessibility, standardisation and technical limitations. Finally, the review evaluates the ongoing efforts in bridging the lab-to-clinic divide.

With a critical synthesis approach, this review analyses peer-reviewed literature across neuroscience, clinical neurology and translational medicine. It integrates findings from empirical studies, along with meta-analyses and systematic reviews to identify emerging consensus and unresolved tensions, such as the accuracies of specific biomarkers, within the field. A targeted narrative review of human and translational studies was conducted on Alzheimer's disease biomarkers published 2015 to 2025 in PubMed and Scopus, using terms combining *Alzheimer's disease* with *biomarker*, *blood*, *CSF*, *MRI*, *PET*, *retina*, *saliva*, *urine*, and *volatile organic compounds*. Inclusion prioritised peer-reviewed original research, along with systematic review and consensus statements. Seminal pre-2015 studies were

retained selectively to provide historical context. Non-peer-reviewed web sources were excluded except where clearly identified as illustrative, such as information about cost ranges.

Section 1: Molecular and cellular neurobiology and pathophysiology of AD

Alzheimer's disease is characterised by the slow, progressive neurodegeneration brought on by neuronal cell death and activity dysfunction, usually starting in the entorhinal cortex inside the hippocampus, an area crucial for various forms of explicit memory formation, particularly spatial memory (Karimani et al., 2024). The pathophysiological hallmarks of AD are accumulations of abnormal neuritic plaques and neurofibrillary tangles (NFTs). With these changes, there is also a loss of neurons, specifically cholinergic neurons in the basal forebrain and neocortex (Kumar et al., 2024). There are two main hypotheses that are popular within the scientific community, the first of which is the cholinergic hypothesis and the amyloid hypothesis. This literature review focuses on the amyloid hypothesis, as it is currently the most widely accepted pathophysiological mechanism for AD. However, it should be noted that this hypothesis has also faced challenges and criticisms, and it is believed that there are alternative or complementary mechanisms in the pathogenesis of AD (Nasb et al., 2024).

Amyloid beta

The peptide amyloid beta ($A\beta$) is derived from a protein, called the amyloid precursor protein (APP), through the abnormal activity of β - and γ -secretase enzymes responsible for breaking down proteins (Mucke & Selkoe, 2012). Typically, APP is cleaved by either α - or β -secretase and the smaller fragments formed are not neurotoxic. However, the cleavage by β - followed by γ -secretase results in peptides of 42 amino acids, known as $A\beta_{42}$, which is a long peptide more prone to aggregation than shorter species. $A\beta$ oligomers, which are smaller aggregates of these $A\beta$ peptides, are considered especially neurotoxic. Increase in $A\beta_{42}$ levels leads to the aggregation of amyloid, creating the commonly seen abnormal neuritic plaques. These are spherical microscopic lesions made of an extracellular $A\beta$ peptide surrounded by enlarged axonal endings, and are usually found within the cortical gray matter or around the meningeal and cerebral vessels of the brain. These deposits initially form in different areas, but grow and merge to comprise the larger more recognisable plaque structures, which then lead to neuronal toxicity. This is caused by $A\beta$ plaques damaging mitochondria as they accumulate inside neuronal cells, impairing its ability to generate ATP. Without sufficient energy, the neuron cannot maintain its normal function, leading to the initiation of apoptosis (programmed cell death). Furthermore, $A\beta$ oligomers can insert themselves into the cell membrane of neurons, forming pores that allow for the unregulated influx of calcium ions, disrupting the balance of calcium concentrations which is critical for signalling and cell survival. These high calcium levels within the cell can activate enzymes that degrade proteins and lipids, leading to the breakdown of the neuronal cytoskeleton, and further mitochondrial dysfunction.

Certain mutations, specifically those in the genes encoding APP and secretases (e.g. presenilin 1 and 2, shortened to PSEN1 and PSEN2) have been linked to cases of familial AD, lending support to the A β hypothesis. This is because these mutations increase the production of longer and more aggregation-prone isoforms of A β (Lanoiselée et al., 2017). Another gene is the apolipoprotein E (APOE) gene, which is the “strongest genetic risk factor for late-onset AD”, according to Kamatham et al. (2024), as it is associated with producing proteins that lead to higher levels of A β aggregation and reduced clearance.

Tau tangles

The aggregation of A β leads to a cascade of events. One of these events is the hyperphosphorylation of tau (the addition of too many phosphate groups which modifies the shape of tau) through the activation of kinases like GSK β , causing the protein that stabilises axonal microtubules vital for intracellular transport to become misfolded and aggregate within neurons (Kumar et al., 2024). With this, these tau molecules become prone to self-aggregation and form insoluble twisted paired helical filaments. Known as neurofibrillary tangles, they first appear in the hippocampus. Recent research has supported the idea that tau aggregates have prion-like properties, allowing their propagation throughout the brain by inducing the misfolding of normal tau in neighbouring neurons. This may explain why tau is often seen spreading throughout the cerebral cortex as AD progresses, contributing to cognitive decline in affected individuals.

Inflammation

Both A β peptides and NFTs can trigger inflammatory responses within the brain by activating microglia and astrocytes (Kamatham et al., 2024). These cells then become ramified and abnormally enlarged (hypertrophied) in NFT-rich brain regions. These immune responses are triggered to clear these harmful substances by the release of pro-inflammatory molecules. However, while this process is meant to protect the brain, chronic and dysregulated inflammation produces proinflammatory cytokines that lead to neuronal damage and exacerbate the progression of the disease. This sustained state of inflammation can exacerbate neuronal damage and further promote the spread of tau pathology.

Mitochondrial Dysfunction

A β peptides have also been shown to impair mitochondrial function by accumulating within the inter-mitochondrial membrane space and disrupting their normal function (Aran & Singh, 2023). This causes a reduction in ATP production and an increase in ROS generation, leading to oxidative stress and eventual damage to the organelle and its normal activity. This then triggers a positive feedback loop, as impaired mitochondrial function further promotes A β production and aggregation. In addition to this, abnormally phosphorylated and aggregated tau proteins have also been implicated in causing mitochondrial dysfunction by impairing mitochondrial transport along neuronal axons, which consequently leads to local energy deficits. It also disrupts fission and fusion processes crucial for maintaining a healthy mitochondrial network. This also triggers a

cycle, where mitochondrial dysfunction results in the formation of even more tau aggregates.

Synaptic and neurotransmitter alterations

Both protein aggregates of A β and tau prevent regular synaptic function by hindering the release of neurotransmitters (including acetylcholine, which plays an important role in memory and cognition), which reduces synaptic density and interferes with synaptic plasticity, particularly in cholinergic neurons (Kamatham et al., 2024). With this, there is a deficiency in signalling, contributing to the memory impairment and cognitive decline associated with AD. Glutamate neurotransmission is also affected, where it excessively activates glutamate receptors, contributing to excitotoxicity, which consequently leads to neuronal damage and cell death. As the membranes of these axonal cells are disrupted and the cells die, the intracellular contents, which contain essential structural components like neurofilaments (NFLs), leak out into the surrounding fluid.

Environmental Factors

Although novel, it has been posited that bacterial and viral infections may be implicated in the process of AD pathogenesis, as different types of microbes have been shown to stimulate A β aggregation and deposition. Therefore, there may be a pathogenic factor in the onset of AD as well (González-Sánchez et al., 2020).

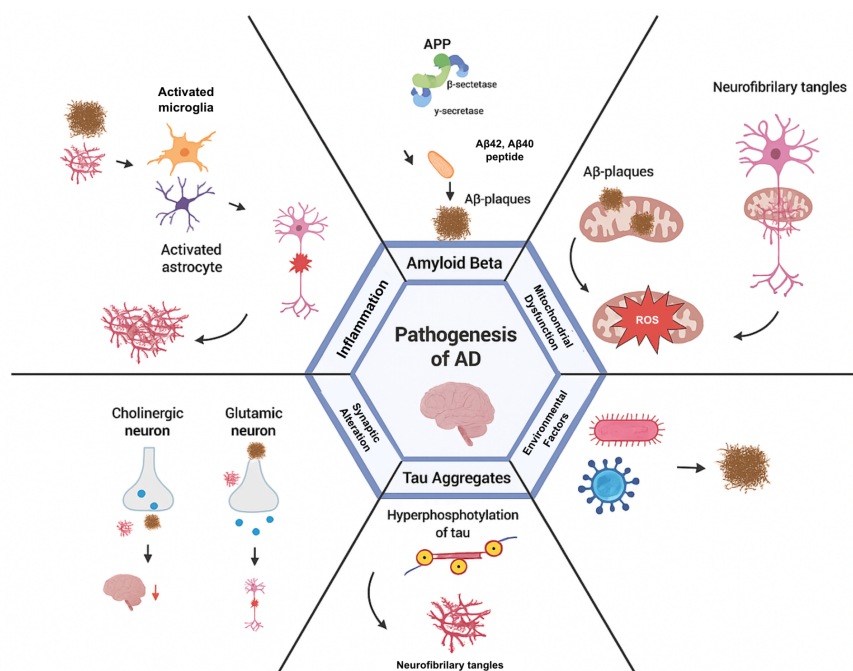


Figure 1. Diagram of the proposed forms of pathogenesis of AD

All the different pathologies of AD are largely thought to be dynamically interacting rather than isolated, suggesting that biomarkers that are able to capture multiple axes of degeneration may be most effective in diagnosing the disease.

Section 2: Types of Biomarkers and How They Work: Established

Biomarkers are defined as being naturally occurring molecules, genes, or characteristics by which a particular pathological or physiological process or disease can be identified. This literature review identifies three types of biomarkers typically employed in the diagnosis of AD: molecular, imaging and functional. Molecular biomarkers include proteins or other biological compounds found through the testing of substances like blood, CSF and other bodily fluids. Imaging biomarkers include scans like PET, MRI and CT, while functional biomarkers are measured through performance on cognitive or motor assessments and tasks. Usually, biomarkers are employed to determine the cause of mild cognitive impairment (MCI) in the initial stage of dementia. This literature review further investigates promising new biomarkers that have shown potential in predicting AD.

MOLECULAR

CSF

Cerebrospinal fluid is an ideal candidate for reflecting the molecular events of the brain, as it is in direct contact with the brain's extracellular space. Hence, experts agree that it is the best way to identify AD biomarkers that reflect its pathogenesis.

Because A β 42 is a large component of typical AD plaques, the amount of total A β in CSF is well-correlated with AD diagnosis. There is a positive correlation between lower CSF A β 42 levels and plaque burden, due to plaques acting as “amyloid sinks”, where the peptide is deposited and accumulates (Anoop et al., 2010). There has also been a correlation between a rise in A β 38 levels and a fall in A β 42, which supports the idea that the ratio A β 42:A β 38 may be more useful and precise in diagnosing AD. This reduced A β 42 has also been observed in other types of dementia, and many works and wider literature agrees that A β 42 is the most accurate CSF biomarker of AD in patients with MCI. However, there was no significant difference observed in the *total* CSF A β levels in AD patients and non-AD controls. Therefore, it seems that only A β 42 is a relatively accurate predictor of the disease.

Increased levels of tau and p-tau in CSF has been shown to be correlated with AD onset, with several studies suggesting that total tau (t-tau) concentration in AD patients' CSF is higher than the control (Papaliagkas et al., 2023). However, it is noted that this biomarker, although sensitive, is limited in its ability to differentiate AD from other forms of dementia, as an increase in t-tau is not a feature unique to Alzheimer's. Hence, p-tau is the more accurate biomarker, as it is more closely related to the pathophysiology of AD as compared to simply being a marker of axonal damage as t-tau is

Levels of GFAP (glial fibrillary acidic protein) tend to be increased in individuals with early A β pathology, as it is hypothesised to reflect the increased reactive astrocytes characteristic of AD (Marksteiner & Humpel, 2025). This can be measured reliably in the CSF. Plasma NfL levels also correlate well with the levels found in CSF, as it leaks from cells due to injury.

Another potential biomarker is neuronal threat proteins (NTPs). These are expressed in the brain during AD-related neurodegeneration, specifically abnormal AD7c-NTP. This has been found to be higher in postmortem CSF of AD individuals compared to controls (Kang & Wang, 2022).

Blood

As A β 42 and A β 40 peptides accumulate within the brain, plasma levels of individuals with AD are typically indicative of this. The plasma A β 42:A β 40 ratio is usually lower in A β 42-positive groups than in A β 42-negative groups, as has been demonstrated by different immunoassays and mass spectrometry-based assays. However, the performance of these different assays have been shown to vary substantially, with a recent comparison showing that certain mass spectrometry-based assays were far better at identifying A β pathology than more commonly used immunoassays. Regardless, these higher-performing A β 42:A β 40 assays still only exhibit modest correlations between the levels of A β and that found in CSF, likely due to the fact that A β in plasma is derived from peripheral sources, as posited by Hansson et al. (2023). Hence, it is agreed that plasma A β 42 levels alone cannot be considered an AD marker.

On the other hand, researchers have recently developed several high-sensitivity assays for the detection of different phosphorylated tau (p-tau) protein variants, or isoforms, including specific ones like p-tau181, p-tau231 and p-tau217 (Janelidze et al., 2025). When compared, it seems that assays quantifying plasma p-tau217 have been shown to be better at detecting AD pathology and predicting the development of AD dementia. However, it can be argued that p-tau231 is a good marker of AD in its early stages, as it starts increasing at very low A β levels. Hence, when combining plasma A β 42 and A β 40 with p-tau, early-stage amyloid positivity detection could be improved.

Similarly, GFAP, can also be measured reliably in the blood as well as the CSF. Plasma NfL levels also correlate well with the levels found in CSF, although it seems this correlation is not as strong as that of p-tau217 (Garcia-Escobar et al., 2024). There is also little to no correlation between levels of NfL and A β 42 in the blood, which has led researchers to believe that NfL suggests neurodegeneration independently of the pathology of A β .

Janelidze et al. (2022)'s study has shown that the combination of plasma p-tau231 and A β 42:A β 40 could be used to detect very early stage AD, and it is supported by the fact that it was able to detect amyloid pathology in cognitively unimpaired individual.

Saliva

Saliva is not only an easily accessible body fluid, its secretion and composition might also be affected by various diseases (Surdu et al., 2025). It is posited that AD can influence these salivary parameters, as there seems to be a relationship between the brain and saliva. This occurs through six pathways communicating brain molecules with the saliva and vice versa. However, for both A β and tau, concentrations are considerably lower in non-plasma fluids.

From a review conducted by Nijakowski et al. (2024), it was seen that there was a statistically significant number of studies that have examined the saliva of AD patients and found elevated levels of A β . However, it was noted that in three studies, neither A β 40 or A β 42 were detected in AD patients. This suggests that there is some degree of reliability of correlating A β levels with AD.

Based on the meta-analysis performed by Nijakowski et al., it seems that the reliability of tau as a biomarker for AD is inconsistent and unreliable when considered alone. There is no consensus on whether t-tau, p-tau and their ratio are reliable indicators of AD. The studies examined by Nijakowski have reported conflicting results, and suggest that while tau may be part of the AD pathology, its biomarker use in saliva cannot be considered a standalone reliable biomarker.

Nijakowski et al. also explores lactoferrin (Lf), a protein with anti-inflammatory properties, and proposes that it may have neuroprotective effects in neurodegenerative diseases like AD. According to González-Sánchez et al. (2020), biomarkers that reflect the integrity of the immune system could be useful. Lf has previously been detected in senile plaques, neurofibrillary tangles and microglia in AD brains. Although it was particularly useful in differentiating AD and MCI patients from healthy individuals, it appears to be a promising, but not fully conclusive biomarker for AD. While several studies analysed by Nijakowski et al.'s paper showed a clear link between decreased salivary lactoferrin levels and AD, there are also conflicting findings that appear to warrant further investigation.

Urine

Both A β and tau are possible to detect in urine, because they can be eliminated from the organism by action of the kidneys (Armenta-Castra et al., 2024). These are detected through methods like ELISA and Western blot. Several studies show a correlation between the presence of these proteins in urine and AD, and it appears that A β oligomers are better indicators of AD progression, instead of the monomers A β 40 and A β 42. Both p-tau and tau were found to be significantly higher in AD patients compared to control individuals, but as was with saliva, these concentrations were much lower in urine than in plasma for both groups.

NTPs have been found in urine, as well as the CSF. Evidence for urinary AD7c-NTP is preliminary and replication in contemporary cohorts is limited, leaving clinical utility

uncertain. One such piece of evidence is the ELISA results from Ghanbari et al.'s 1998 study utilised ELISA to show that results in urine showed similar sensitivity and specificity to CSF, and that urinary AD7c-NTP was significantly higher than in the non-AD group.

IMAGING

Neuroimaging now allows for diagnosis of the AD pathophysiological process during life, even though neuropathology remains the definitive standard. Clinicians can begin to assess cases in vivo, with several modalities having been researched, including Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET).

Neurodegeneration leads to cerebral atrophy, which is most notable in the hippocampus and entorhinal cortex of patients with AD (Tabatabaei-Jafari et al., 2015). The level of atrophy in the medial temporal lobe is strongly correlated with scores on cognitive testing, and is therefore associated with disease progression. As this degeneration progresses to other cortical structures, lateral ventricles become enlarged in the later stages of the disease. In addition to this, limbic structures (such as the amygdala, cingulate gyrus, thalamus, and olfactory bulb tract) are impacted, and the atrophy leads to volumetric reductions in the frontal, parietal and temporal brain areas.

MRI

MRI is an imaging technique that is able to generate high-resolution images of water-containing tissue. This is done by applying a strong magnetic field and pulses of radio waves that manipulate electromagnetic fields and energy states of protons within the body. These protons emit energy, which are then converted to signals and images.

Structural MRIs are used to provide highly detailed images of anatomy as well as a strong gray/white matter contrast. These scans are able to display white matter hyperintensities that indicate demyelination and axonal loss (Meng et al., 2022). Compared to patients without AD, patients with AD have greater white matter hyperintensities in the frontal lobe that index small-vessel disease burden and help in differential diagnosis and staging, but are not necessarily specific to AD.

Diffusion Tensor Imaging (DTI) uses the displacement of water molecules to measure the integrity of the white matter tract. The direction of diffusion allows scientists to better understand tracts and specific fibre bundles that may be impacted by neurodegeneration (Esrael et al., 2021). The major measures of DTI are mean diffusivity (MD), which is the average rate of water molecule diffusivity, and fractional anisotropy (FA), which is the variability associated with diffusion. In AD brains, MD can be seen in all four areas of the brain (frontal, occipital, parietal and temporal), including the hippocampus. This is a clear difference from MCI, where these increases are not seen in the frontal and occipital regions. AD also presents as decreased FA localised to the cingulum, corpus callosum, uncinate fasciculus and superior lateral fasciculus, as well as throughout the temporal, occipital and frontal white matter. Patients with MCI have no irregularities of FA in

occipital and parietal areas, which sets MCI scans apart from AD. According to a study done by Magalhães et al. (2023), there was a significant correlation between white matter tracts and both p- and t-tau.

Functional MRI (fMRI) produces dynamic representations of brain activity through blood oxygen level-dependent (BOLD) signals (Chandra et al., 2018). This measures changes in blood volume and flow. When asked to perform memory tasks, AD patients showed little to no activation of hippocampal and other medial temporal structures as compared to non-AD individuals. Resting-state fMRIs of the AD brain also show abnormal coactivation of the medial temporal structures and the posterior cingulate cortex, which differs from those of normal brains.

PET

PET is an imaging modality that allows for the in vivo measurement of disease pathologies using radioligands to detect compounds of interest. In the case of AD, that would be neurofibrillary tangles and A β plaques.

FDG-PET uses 18-F-fluorodeoxyglucose (FDG), which allows researchers to estimate glucose metabolism in different brain areas and identify which ones may have abnormal metabolic activity (Bouter et al., 2019). From studies done, these scans show temporoparietal hypometabolism in patients with AD, which is characteristic of the disease. Furthermore, there was an observed decrease in metabolism in the posterior cingulate cortex and precuneus, which is thought to be related to the episodic memory deficits typical of AD.

Because of accumulated A β , there is known to be increased amyloid tracer binding in areas of the brain affected by the disease (more specifically, temporal parietal and frontal regions) (Villa et al., 2020). Tracers such as Pittsburgh Compound B (PiB) are able to bind to misfolded amyloid plaques, therefore helping to map distribution and quantify amyloid burden. Other tracers include florbetapir, 18-F-labeled flutemetamol and 18-F-labeled florbetaben.

Part of the underlying pathophysiology of AD is the neuroinflammatory cascades leading to an increase in microglial activation. Radiotracers that bind to TSPO (translocator protein-18 kDa) have been utilised as biomarkers of AD neuroinflammatory cascades (Georgakas et al., 2023). Since TSPO is a peripheral benzodiazepine receptor found on the membrane of the mitochondria, increased expression of this protein can be used as an estimate for increased inflammation, as the expression of this protein is upregulated by activated microglial cells. Studies done have found TSPO expression increased when compared to healthy controls.

Machine Learning and Artificial Intelligence

With the rise of better artificial intelligence algorithms, AI is being increasingly utilised in the diagnosis of AD (Kamatham et al., 2024). AI models are capable of analysing large, complex datasets containing neuroimaging data and biomarker profiles to identify patterns. MRI and PET generate vast amounts of data that can be analysed using AI to detect both structural and functional brain changes associated with AD. Biomarker data can also be incorporated in order to develop predictive models for risk assessment. This holds great potential for improving efficiency and accuracy, since AI models can therefore aid healthcare providers in making increasingly informed diagnostic decisions, facilitating the early detection of AD and the generation of personalised treatment plans based on individual profiles.

BEHAVIOURAL

Functional

The progressive loss of ability to perform activities of daily living unassisted is a primary characteristic of dementia (Chaves et al., 2011). These activities can be divided into basic (BADL) and instrumental (IADL). BADL includes the ability to carry out tasks necessary for personal hygiene, sphincter control and feeding. IADL, on the other hand, are more complex and are activities such as preparing meals, carrying out domestic chores, and managing finances and correspondence.

An example of an assessment used to evaluate changes in functional performance is through an assessment measure known as Functional Assessment Staging (FAST). This allows healthcare practitioners to specifically evaluate these changes throughout the entire course of AD (Sclan & Reisberg, 1992). Another such assessment measure is the Alzheimer's Disease Cooperative Study – Activities of Daily Living for Mild Cognitive Impairment, otherwise known as ADCS-MCI-ADL. This assesses the competence of AD patients in both basic and instrumental ADLs, and can be completed by a caregiver or administered by a clinician as a structured interview with a caregiver (Fish et al., 2011).

Cognitive

The initial and most common presenting symptom of AD is episodic short-term memory loss (Kumar et al., 2024). This includes difficulty retaining new information, and experiencing impairments in problem-solving, executive functioning, judgement, and organisational skills. Because of this, they may struggle with multitasking and abstract thinking, which affects their ability to carry out ADLs like driving, cooking, and activity planning. Following this, there are often language and visuospatial skill impairments, along with neuropsychiatric symptoms like changes in social behaviour.

A common example of a cognitive assessment is the Mini-Mental State Examination (MMSE) (Arevalo-Rodriguez et al., 2015). This is a common test for assessing changes in cognition, and evaluates functions such as orientation, language, memory and other functions. This is done through tasks that test reading, writing and short-term memory,

including tasks like subtraction, remembering a series of unconnected words or copying a diagram on paper.

The Alzheimer’s Disease Assessment Scale – Cognitive, or ADAS-Cog, is generally more thorough (Dementia Australia), and is considered the gold standard for assessing AD (Kueper et al., 2018). This includes 11 tasks, including both tests completed by the subject and assessments completed by the observer. When put together, these tasks assess memory, language, and praxis. Specific tasks, like the MMSE, include word recall, naming objects, commands, and word recognition among others.

As can be seen by this, there already exists a plethora of widely acknowledged and effective biomarkers, whether they be molecular, imaging or functional. A summary of the above information can be found in Table 1.

Table 1. Summary of Key Biomarkers and Diagnostic Modalities for Alzheimer’s Disease (AD)

Category	Subcategory	Biomarker / Method	Key Findings	Work Cited
Molecular	CSF	Aβ42	Lower levels correlate with plaque burden; most promising CSF biomarker for AD in MCI	Anoop et al., 2010
		Aβ42:Aβ38 ratio	May improve diagnostic precision	
		t-tau	Elevated in AD; sensitive for neurodegeneration	Papaliagkas et al., 2023
		p-tau	More closely related to AD pathology than t-tau	
		GFAP	Increased with early Aβ pathology; reflects reactive astrocytes	Marksteiner & Humpel, 2025
		NfL	Correlates with CSF levels; marker of axonal injury	
		AD7c-NTP	Higher in postmortem CSF of AD patients (still relatively new and emerging)	Kang & Wang, 2022
	Blood	Aβ42:Aβ40 ratio	Lower in Aβ-positive groups; better with mass spectrometry	Hansson et al., 2023
		p-tau217	Strong predictor of AD pathology and progression	Janelidze et al., 2025
		p-tau231	Early-stage AD marker (increases at low Aβ levels)	

Imaging	Saliva	p-tau181	Widely adopted, earlier generation with less specific indication of AD	
		GFAP	Detectable in blood; reflects astrocyte activation	Garcia-Escobar et al., 2024
		NfL	Detectable; less correlated with CSF than p-tau217	Janelidze et al., 2022
		Combined p-tau231 + Aβ42:Aβ40	Detects very early-stage AD	Hansson et al., 2023 Janelidze et al., 2025
		Aβ	Some studies show elevated levels in AD	Nijakowski et al., 2024
		t-tau, p-tau	Conflicting results; unreliable alone	
		Lactoferrin	Lower in AD; potential immune biomarker	González-Sánchez et al., 2020
	Urine	Aβ oligomers	Better indicators of progression than monomers	Armenta-Castro et al., 2024
		p-tau, tau	Higher in AD patients than controls	
		AD7c-NTP	Elevated; similar sensitivity/specificity to CSF	Ghanbari et al., 1998
	MRI	Structural MRI	Hippocampal/entorhinal atrophy; white matter hyperintensities	Tabatabaei-Jafari et al., 2015 Meng et al., 2022
		DTI	Increased MD and reduced FA in specific tracts; correlates with tau	Esrael et al., 2021
		fMRI	Reduced hippocampal activation; altered resting-state connectivity	Chandra et al., 2018
	PET	FDG-PET	Temporoparietal hypometabolism; PCC/precuneus deficits	Bouter et al., 2019
Amyloid PET		PiB and other tracers map amyloid burden	Villa et al., 2020	
TSPO PET		Detects neuroinflammation via microglial activation	Georgakas et al., 2023	
	AI/ML Integration	AI models	Detect structural/functional changes; integrate biomarkers	Kamatham et al., 2024
Functional	Behavioural	BADL/IADL assessments (FAST, ADCS-MCI-A DL)	Measures decline in daily functioning	Chaves et al., 2011 Sclan & Reisberg, 1992 Fish et al., 2011

Cognitive	MMSE/ADAS -Cog	Screens orientation, memory, language	Kumar et al., 2024 Arevalo-Rodriguez et al., 2015 Kueper et al., 2018
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Section 3: Types of Biomarkers and How They Work: Novel

While traditional biomarkers have focused on the more established hallmarks of AD, such as A β and tau, recent research has expanded into a more diverse spectrum of novel biomarkers that may serve to capture alternative aspects of the disease's biology. These include new developments in both molecular and imaging biomarkers as technological advancements occur.

MOLECULAR

MicroRNA

MicroRNAs (miRNAs) are a type of short-length, non-coding, and single-stranded endogenous RNAs (also known as sncRNAs) found in a stable form in the blood, CSF, and various body fluids (Chimthanawala et al., 2023). By controlling the expression of genes and pathways, miRNAs by suppressing the translation of target mRNAs and facilitating their degradation, contributing to APP degradation and A β metabolism. AD patients had higher levels of miRNA-4722-5p and miRNA-615-3p, and combining these two AD-associated miRNAs provided a better diagnostic score than either miRNA alone. miRNA-20b-5p also downregulated APP levels, hence reducing the availability of A β . However, further research in this domain needs to be carried out, especially on three miRNAs that strongly correlated with the early stages of AD, namely miRNA-92a-3p, miRNA-486-5p, and miRNA-29a. Levels of miRNA-92a-3p and miRNA-486-5p were lower in AD patients compared to controls, but higher levels of miRNA-29a-3p were found in the plasma of AD and MCI patients. Plasma levels of miRNA-483-5p levels were higher in AD patients compared to healthy controls, and it was concluded that increased levels of miRNA-483-5p in plasma can reliably distinguish both MCI and AD groups from the healthy controls. The above all suggests that these miRNAs could be potential biomarkers in early AD diagnosis.

Long non-coding RNA

A second type of RNA that can be used is long non-coding RNAs (LncRNAs). These resemble mRNA (messenger RNA) molecules, but are not responsible for encoding proteins (Black et al., 2024). They typically contribute to the regulation of several processes by regulating chromatin remodelling, and providing alternative mRNA splicing. This affects gene expression, and has been shown to “directly or indirectly regulate the key features of AD including A β deposition, aberrant tau formation, oxidative stress, neuroinflammation, and neuronal death”. LncRNAs also interact with miRNAs, thereby

influencing AD-related neuropathology. The expression of numerous types of these molecules have been detected in plasma and CSF samples from AD patients, which indicates that lncRNAs could function as a likely biomarker in the diagnosis and progression of AD.

Apolipoprotein C3

A study done by Watanabe et al. (2020) suggests that Apolipoprotein C3 (ApoC3) may be a potential urinary biomarker for AD, although further analysis of this is warranted (Watanabe et al., 2020). ApoC3 is an abundant apolipoprotein mainly present in triglyceride-rich lipoproteins (chylomicrons and VLDL), as well as cholesterol particles. Watanabe et al. found that studies have reported people with AD had lower plasma levels of ApoC3 compared to healthy individuals, and that higher ApoC3 was associated with reduced grey matter volume in the brain. The paper also pointed to recent research suggesting that ApoC3 acts as a trigger for inflammation by activating the NLRP3 inflammasome. This is significant because the NLRP3 inflammasome is known to be activated in the brains of AD patients by amyloid beta. The result of Watanabe et al.'s experiment was that all samples revealed ApoC3 levels to be significantly higher in the AD group relative to the control group.

MCP-1

Monocyte Chemoattractant Protein 1 (MCP-1, also known as CCL2) is a protein that belongs to a family of signalling molecules called chemokines (Huang et al., 2023). They play key roles in the body's inflammatory response by attracting immune cells to sites of inflammation. As a biomarker, MCP-1 can be measured in both blood and urine. In AD, chronic inflammation in the brain (neuroinflammation) contributes to neuronal damage. It is thought that it is because of this that elevated levels of MCP-1 have been found in the blood and CSF of AD patients. However, this is not consistent across different studies, which is why Huang et al. hypothesised that MCP-1 levels impact individuals who are genetically at risk of developing AD.

Molecules Associated with Oxidative Stress

A β induces oxidative stress, the production of harmful reactive oxygen species (ROS), which is thought to be a major contributor to cellular damage in the brain (Gella & Durany, 2009). ROS are too unstable to measure directly, so measuring their byproducts is a method used to quantify oxidative stress. Several products, specifically 4-hydroxy-2,3-noneal (HNE), acrolein, F2-isoprostanes and malondialdehyde have been observed to exist in higher levels in AD brains. However, like many other biomarkers, oxidative stress is a general sign of cellular damage and is not necessarily specific to AD. It's a feature of many neurodegenerative disorders and is also affected by ageing and environmental factors.

Volatile Organic Compounds

Exhaled breath contains hundreds of different byproducts of metabolism, also known as volatile organic compounds (VOCs). In AD, metabolic changes in the brain could produce a unique “breathprint” (Zhang et al., 2025). Using sophisticated sensors or mass spectrometers, analysis of this breathprint can be carried out to identify patterns that correlate with this disease. However, this research is still in its early stages, and whether it can be used for AD diagnosis is unclear.

IMAGING

Retinal Imaging

In the last decade, there has been rapid progress in using retinal imaging to find markers of AD. Techniques like optical coherence tomography (OCT) are shown to be especially promising because of their non-invasive, affordable and accessible nature as compared to traditional brain imaging (Georgakas et al., 2023). Research has shown structural, vascular and neuropathological changes in the retinas of both mice and humans with AD. Structural markers, such as the thinning of the retinal nerve fibre layer (RNFL) are consistently seen in older adults with AD. This thinning may start early in disease progression, as it has been linked to levels of A β and tau in CSF, as well as to hippocampal volume in cognitively normal adults. This suggests its usefulness as a tool for early AD screening. Vascular changes are also being explored, as a 2013 study found that asymptomatic adults with high cerebral A β had less-branched retinal blood vessels. Another study found that a larger foveal avascular zone (FAZ), which is the area of the retina without blood vessels, was typically associated with higher cerebral A β in cognitively normal adults. This suggests that the FAZ area, along with retinal imaging in general, may be an effective marker for detecting preclinical AD. However, this research has yielded mixed results, with some finding strong correlations and others not.

CT

A CT scan uses a series of X-rays from different angles to generate cross-sectional images of the brain. While it cannot visualise amyloid plaques or tau tangles directly, it is able to detect brain atrophy, or shrinkage, of regions like the hippocampus (Pasi et al., 2011). However, it is only really supportive and non-specific with the typical patterns of AD. It may also prove more useful only for later-stage AD, as brain atrophy usually cannot be seen until patients begin to exhibit behavioural and cognitive changes. In addition to this, CT scans involve using ionizing radiation which, though at a low dose, carries a small risk, especially for repeated scans.

Tau PET

Tau PET molecular imaging is a relatively new technology. So far, there is a first-generation FDA-approved tracer, known as 18-F-flortaucipir, and has been shown to have high affinity for detecting the tau aggregates that are characteristic of AD (Petersen et al., 2022). However, off-target binding happens, where tracers bind to molecules other than tau, which produces unreliable results in a PET scan. This is why there are ongoing

efforts to develop second-generation tracers, which are being designed to have improved binding specificity.

Most non-CSF, non-blood matrices remain exploratory, but from current research, it's clear that many of them warrant significant potential to become alternative biomarkers. Some may even prove to be more advantageous than traditional biomarkers due to their cost-effectiveness and accessibility, as well as their specificity or accuracy.

Section 4: Translational challenges and barriers

Data standardization and comparability represent a critical challenge in both imaging data collection and biomarker fluid acquisition protocols that can vary considerably across different Alzheimer's Disease Research Centers (ADRCs) (Braudeau et al., 2025). This variability complicates the establishment of universal diagnostic thresholds and hinders the direct comparability and integration of findings from diverse research cohorts, making it difficult to generalize results and apply them uniformly in clinical practice. Consequently, even if a biomarker demonstrates exceptional scientific efficacy, its practical clinical utility is severely constrained if it cannot be widely deployed across diverse healthcare settings. This accessibility-utility gap highlights pressing health equity concerns in AD diagnosis, driving the urgent need for less invasive and more affordable alternatives, even if those alternatives present their own set of challenges.

IMAGING

High cost and limited accessibility are paramount challenges. Imaging costs can vary widely across settings, but illustrative ranges suggest substantial patient-level expense, with an MRI costing up to \$8,400, and a PET scan up to \$3,000 in some countries (Imaging Technology News, 2018). This substantial cost, coupled with the requirement for specialized equipment, trained personnel, and dedicated imaging facilities, restricts their availability, particularly in underserved regions and low-resource settings. This creates a significant barrier to widespread clinical use, contributing to health inequities in AD diagnosis.

Patient burden and contraindications also pose practical limitations (Decazes et al., 2021). Since PET scans involve a small dose of radiation, patients may be advised to avoid this scan if they are pregnant, and this radiation carries its own risks as well. Both MRI and PET scans require patients to remain still in enclosed spaces for extended periods, which can cause discomfort or claustrophobia. Furthermore, patients with certain medical devices or metal implants, such as pacemakers, may have contraindications for MRI due to the powerful magnetic fields involved .

PET

Cost and reimbursement represent major practical barriers to the widespread clinical adoption of amyloid PET (Lee et al., 2021). These scans are expensive, typically costing between \$3,000 and \$6,000. In the USA, third-party payers often do not reimburse for amyloid PET, citing a need for additional data demonstrating that the scan results directly impact clinical care and patient-based outcomes. This lack of coverage significantly restricts access for many patients.

Pittsburgh Compound B (PIB) utilises carbon-11, which has a very short half-life of 20 minutes, which limits its clinical applicability due to logistical challenges (Wolk et al., 2012). While more clinically viable F18-labeled tracers with a longer half-life of 110 minutes have since been developed, the cost and accessibility issues persist. Additionally, U.S. tracer labels and clinical practice typically use a binary (positive or negative) interpretation for amyloid PET results, which may oversimplify the continuous biological data and potentially obscure nuanced information about amyloid burden.

The designation of amyloid PET as a “gold standard” for detecting amyloid pathology is important to consider in context. While it excels at identifying the presence of amyloid plaques, a necessary component of AD, the presence of these plaques alone is not sufficient for a definitive AD diagnosis (Roberts et al., 2015). Amyloid plaques are found in other conditions and in a substantial proportion of cognitively normal individuals. Furthermore, the overall amyloid burden, as assessed by PET, does not consistently correlate strongly with cognitive deficits. This implies that a positive amyloid PET scan indicates the presence of amyloid pathology, but it does not definitively confirm symptomatic AD or establish amyloid as the sole cause of cognitive impairment.

MRI

In the very early stages of AD, an MRI scan of the brain may appear normal, making it less sensitive for preclinical detection compared to molecular imaging techniques (van Oostveen & de Lange, 2021). While it provides detailed structural changes, it cannot solely confirm the presence of AD pathology; comprehensive assessments combining MRI findings with clinical evaluations, cognitive testing, and patient history are essential for accurate diagnosis. Fundamentally, MRI primarily reflects neuronal loss and brain atrophy, which are consequences of amyloid and tau pathology, rather than directly visualizing these protein aggregates themselves, meaning it is less sensitive to preclinical pathology than molecular biomarkers. Emerging techniques like fMRI and DTI hold significant promise, but their widespread standardization and established clinical utility for routine AD diagnosis are still under development.

MOLECULAR

CSF

Despite how thoroughly characterised and well-established CSF markers for AD are, there are still significant limitations associated with these biomarkers, primarily revolving around the invasiveness of the lumbar puncture procedure used to procure the fluid

(Inekci et al., 2015). The discomfort and potential risks, although rare, including nerve damage, infection and herniation, are currently primary drivers for the ongoing search for less invasive diagnostic alternatives.

Blood

Compared to CSF, blood-based biomarkers are more appealing because they tend to be significantly less invasive in nature and more cost-effective extraction methods (Mielke et al., 2024). However, as aforementioned in this literature review, the challenges include lower biomarker concentrations in blood compared to CSF, and also less achievable accuracy due to complex matrix interference in blood. In addition to this, peripheral metabolism can significantly affect blood biomarker levels, making them less direct reflections of brain pathology.

Other Bodily Fluids

With research exploring the potential of other bodily fluids like saliva and urine, these fluids offer a theoretical advantage of maximal non-invasiveness, which makes them highly attractive for broader-scale screening (Kumari et al., 2023). However, this research is still in its nascent stages. Low concentrations of relevant analytes, high biological variability coupled with a significant lack of robust validation in studies limit their establishment for clinical use in AD diagnosis.

OVERALL

Specificity

Even with seemingly AD-specific biomarkers like tau and NfLs, there still exist difficulties in differential diagnosis. The pathologies underlying other types of dementia often involve very similar proteins to the ones used to diagnose AD, which complicates the ability to distinguish between them based solely on biomarker levels (Zou et al., 2020). Simply measuring protein concentrations is unlikely to provide sufficient information to differentiate effectively between various dementias.

Cost

The current landscape of AD research and the development of biomarkers, particularly blood-based biomarkers, is heavily skewed towards high-income countries (Rodda et al., 2025). To achieve equitable progress in the field, it's crucial to actively involve researchers and institutions from LMICs in the development, validation, and clinical application of BBMs. Key challenges remain, including the need to understand the real-world effectiveness, cost-efficiency, and acceptance of these tools in resource-limited environments. Financial constraints are a major barrier to the necessary research and validation in LMICs. Additionally, to effectively roll out BBMs in LMICs, significant investment is needed to build the necessary infrastructure and upskill the workforce. This could involve financial support from various sources, corporate engagement, and the use of innovative, practical approaches. For instance, using dried blood spots and task-shifting models (i.e. training community health workers to do tasks normally reserved for

specialists) where community health workers collect samples could make widespread use more feasible, especially in remote areas.

Generalisability

Not only are there technical and economic barriers, there exist obstacles in the generalisability of studies due to the lack of access to certain samples and communities of individuals. Most studies, especially plasma biomarker studies, to date have focused on genetically homogeneous populations in high-income countries. This highlights a critical need for large-scale international studies that include diverse populations with varying genetic backgrounds, comorbidities, and socioeconomic statuses. This is a significant issue because biomarker levels can vary among different racial and ethnic groups and are affected by certain long-term health conditions and medications (Rodda et al., 2025). A 2022 study showed that even though most participants expressed interest in biomarker research for AD, Black participants were comparatively more hesitant than White participants (Elicain et al., 2022). They were more concerned about the risks of participating and felt there were more barriers to undergoing brain scans. These differences remained even when accounting for their trust in researchers and their existing knowledge of AD. The study found that a lack of information was a major barrier for Black participants, while receiving information was a strong incentive. Black older adults expressed a desire for more details about AD, including its risks and prevention, as well as a better understanding of the general research process and specific biomarker procedures. This highlights the need for the scientific community to improve recruitment strategies for underrepresented groups with no prior research experience. The above could be done through improving information sharing and community awareness by increasing their presence in these communities and reducing the financial burden of participation.

Standardisation

A major hurdle for the widespread use of AD biomarkers, particularly blood-based ones, is a lack of standardization (Humpel & Hochstrasser, 2011). Currently, there are no uniform methods for sample collection, processing, or storage. This problem is compounded by the absence of established procedures to monitor long-term biomarker stability and the lack of certified reference standards. These inconsistencies introduce measurement variations, making it difficult to compare results between labs, establish reliable clinical thresholds, and ultimately use these biomarkers effectively in both research and patient care (Zeng et al., 2024).

Education of Staff

The widespread adoption of biomarkers, despite their accessibility, are significantly challenged by the need for more comprehensive education of primary care clinical staff and the development of clear, actionable guidelines for their use. Without this adequate training and clear protocols, there is a high risk of misinterpretation of results or the inappropriate use of these diagnostic tools (Schöll et al., 2024).

Overall, there still exist many translational hurdles, and it is only if these hurdles are overcome that biomarkers can achieve their full efficacy and potential. Reducing these hurdles also allows more people to access what could potentially be diagnostic tests for AD, bettering treatment plans via early detection.

Section 5: Ongoing efforts of bridging the divide

Early detection of AD could dramatically shift the way treatment is given to patients. Early detection allows for interventions during the prodromal or mild cognitive impairment stages, where therapeutic outcomes are more likely to be meaningful as early diagnosis is crucial in informing discussions, care and support, and treatment (Dubois et al., 2023). Moreover, biomarkers are critical not only for diagnosis, but also for other means like patient stratification in clinical trials and the development of potential disease-modifying therapies.

Hence, the limitations and translational challenges confronting AD biomarkers are being actively addressed. For example, significant progress is being made in developing more sensitive and specific assays for AD biomarkers. Ultra-sensitive platforms, like Single Molecule Array (SIMOA) technology, are enabling the reliable detection and quantification of biomarkers like p-tau and GFAP even if they are present at very low concentrations in biological fluids (Yuan et al., 2025). Other technologies include novel electrochemical sensing platforms like CRISPR/Cas12a-mediated electrochemical aptasensors, which are biosensors that use DNA/RNA molecules to detect proteins.

Recognising that no single biomarker is able to provide a complete picture of AD pathology and its progression, there is a growing emphasis on multimodal biomarker approaches and the integration of different data types (Gupta & Iftekhar, 2024). Combining imaging, fluid, cognitive and demographic data could offer the potential for enhanced diagnostic accuracy. AI and machine learning (ML) in particular are emerging as tools in this context.

Beyond refining extant markers, the field continues to pursue the discovery of novel biomarkers that can provide more specific and comprehensive insights into AD pathology.

Conclusion

While AD biomarkers are getting better at detecting neurobiological changes, their translation into widespread clinical use is still obstructed by challenges including limited accessibility. Georgakas et al. (2023) states that the ability to translate treatments to various wider populations are reliant on low cost, minimally invasive and more accessible diagnostic biomarkers in a general clinical setting. Bridging this gap is consequently essential to realising their full diagnostic and therapeutic potential. Another current limitation in our use of novel biomarkers is being able to distinguish between AD and other

neurodegenerative disorders, making the traditional biomarkers like A β and tau far more accurate and developed.

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EDITOR COMMENTS AND RECOMMENDATION:

Both reviewers agree that this review manuscript focused on biomarkers of Alzheimer's Disease is comprehensive, relevant, and timely with clear utilization of the most current literature. Reviewer critiques are primarily focused on improving the clarity of the manuscript as well as noting the absence of in-text citations where they are likely needed. Reviewer 1 has also provided a marked PDF of the manuscript with specific comments regarding stylistic suggestions as well as other minor corrections. Reviewer 2 is largely in agreement with this feedback, and also suggests a revision to the conclusion, perhaps in conjunction with an added Limitations section, to better capture the summary of the author's work. Given these critiques, the editor recommends accepting the manuscript following minor revisions.

REVIEWER 1

My recommendation for the review paper named "The Neurobiological Foundations and Translational Challenges of Current and New-Generation Alzheimer's Disease Biomarkers: A Review" is that it should be revised and resubmitted.

Overall, the author has conducted thorough research and produced a well-structured literature review. In most sections the flow is smooth, and the discussion of individual biomarkers is strong. The author demonstrates an ability to present both advantages and limitations of each biomarker, while also offering thoughtful perspectives that reflect a solid grasp of the scientific debates in this area. This critical evaluation strengthens the manuscript considerably and shows maturity in engaging with complex literature. The author also used loads of resources from 2020 onwards, which signify that they engaged with recent literature.

That said, there are several areas that need revision before resubmission. The most pressing issue is the consistent absence of citations across multiple paragraphs. While the author has clearly read widely, it is vital in academic writing to attribute all claims to their sources. This will likely take time to address, but it is non-negotiable for a piece of scientific scholarship. I have included comments throughout the PDF where the author can see where citations should be inserted.

In addition, I have used these comments in the PDF to suggest rephrasing certain sentences for clarity and making minor corrections throughout. Abbreviations also need more careful handling: currently, the author sometimes introduces the abbreviated form before the full name, or switches between them inconsistently. Best practice is to introduce the full name followed by the abbreviation in brackets upon first mention and then use only the abbreviation thereafter (except in headings, tables, or figure legends).

Regarding Table 1, I have recommended changes to improve clarity around the effectiveness of specific biomarkers. I also suggest that the legend be expanded to include full names of all abbreviations used, so that readers can access this information easily without needing to search back through the text.

To be clear, there are no fundamental issues that would justify rejecting this paper. Rather, the manuscript requires several minor revisions, particularly the insertion of citations, which will take time but are essential for academic rigour.

Overall, this is a significant, relevant, and original review that contributes meaningfully to current discussions in the field. Congratulations to the author for their achievement, and I encourage them to use this feedback to polish an already strong paper, even further. Doing so will elevate it to the standards expected of modern scientific writing and serve as a strong foundation for their future academic work.

REVIEWER 2

This is a very solid review of a) established biomarkers believed to play a role in the etiology of AD and b) novel biomarkers that are emerging as potential solutions. The great strength of the review is sections 4 and 5 which cover the difficult topic of how we effectively translate this science to effective clinical treatment. I feel the AD science is well covered. My comments below are intended to provide the author with advice on improving the clarity of the review for the reader. While I provide specific examples for the authors, they should give their review a thorough reading for these points throughout.

1. The introduction feels repetitive with the opening to section 1. Both sections start by summarizing AD in a broad sense. You do this in the Introduction, so it doesn't need repeating in Section 1. Even the Abstract feels like it repeats itself. For example, you don't need the "Hence ..." sentence in the middle because you just said this above.
2. The Conclusion kind of runs out of gas. Its final sentence notes a limitation when this should be the final summary statement. Take a little time to devote a sentence or two to each section of the paper. You could precede the Conclusions with a limitations section if you like. And then you need a sentence to summarize what you have done here.
3. Check for clarity throughout. Examples:
 - a. Page 7 paragraph 3, "between the levels of A β and that found in CSF" would be clearer if is said "levels of plasma A β "
 - b. Page 8 paragraph 2: Your sentence "This suggests that there is some degree of reliability of correlating A β levels with AD." Is not clear. Do you mean "This suggests that there is a question of reliability when using saliva samples to get an accurate measure of A β levels in studies of AD."
 - c. Page 3, paragraph 2: the entorhinal cortex is part of the hippocampal *formation*, together with the hippocampus proper, subiculum and dentate gyrus (so not inside hippocampus) – just say part of the hippocampal formation.
 - d. MRI is more a measure of blood oxygenation, rather than flow
 - e. Add the word "Markers" to your section 2 and 3 titles (after Established and Novel)
4. Check that acronyms for terms are provided on first usage. Examples:
 - a. NFTs are defined on page 3, but the full word is used on page 4
 - b. activities of daily living (ADL) is not defined on page 11, before using BADL
 - c. p-tau is introduced on page 6 but is defined on page 7. (This should probably occur on page 4 where tau is introduced).
5. Check that references are always provided when they should be. Examples:
 - a. on page 4, paragraph 2, there is a sentence that starts "Recent research has ..." with no reference that follows.
 - b. on page 6, paragraph 4, the sentence "However, there was no significant difference observed in the *total* CSF A β levels in AD patients and non-AD controls." has no reference.
6. Check that references are always provided when they should be. Examples:

- a. on page 4, paragraph 2, there is a sentence that starts “Recent research has ...” with no reference that follows.
- b. on page 6, paragraph 4, the sentence “However, there was no significant difference observed in the *total* CSF A β levels in AD patients and non-AD controls.” has no reference.

7. Check for typos. Examples:

- a. last sentence on page 6 ends without a period.
- b. Last word on page 7 should be “individuals”

8. Employ a thesaurus to improve word choices. Example:

- a. Page 21 paragraph 2 “way treatment is given to patients” could be “way treatment is provided to patients”
- b. Page 21 Conclusion “While AD biomarkers are getting better at detecting neurobiological changes, their” could be “While research developing biomarkers to reliably detect neurobiological changes in AD continues to progress, their”

Title: The Neurobiological Foundations and Translational Challenges of Current and New-Generation Alzheimer's Disease Biomarkers: A Review

Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative condition, and remains without a definitive cure despite decades of research. Biomarkers offer promising avenues for early diagnosis and disease monitoring, though their clinical adoption has proven to lag behind laboratory advances. This review synthesises recent literature on the neurobiological underpinnings of both well-known and novel AD biomarkers by drawing on 2015–2025, English, human-centered studies utilising assays, imaging, and assessments to highlight the mechanisms and limitations of current tools of diagnosis. This review also aims to analyse the key translational barriers involved in routine clinical diagnostics, and evaluate the clinical readiness and integration for molecular, imaging and functional biomarkers. Hence, this literature review investigates the neurobiological foundations and translational challenges of Alzheimer's disease biomarkers. Key findings include A β 42/40 being a strong biomarker for amyloid plaques, p-tau217 outperforming other plasma p-tau epitopes, and new technologies like second-gen tau PET being developed to reduce off-target issues. Other than certain technical limitations, AD biomarkers have strong neurobiological mechanistic evidence, but their translation is still impeded by practical and socioeconomic challenges, like barriers to access, standardisation and underrepresentation of communities in studies. Addressing and bridging these gaps would be essential for closing the bench-to-bedside divide in AD care, helping enable earlier intervention.

Keywords: Alzheimer's disease, biomarkers, neurobiology, translational neuroscience, diagnostics

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. It is characterised clinically by memory loss, impaired cognition, and behavioral changes. With prevalence rising sharply as populations age, AD poses mounting healthcare and socioeconomic challenges.

Despite being one of the most common forms of dementia and neurodegenerative disease, it remains an incurable illness. As of 2021, of the approximately 57 million people worldwide who have dementia, 60-70% have Alzheimer's (World Health Organisation, n.d.). This greatly affects their quality of life, and also places an enormous burden on caregivers and healthcare systems worldwide, costing economies US\$1.3 trillion globally, with 50% of these attributable to the care given by family members and close friends.

The pursuit of reliable biomarkers for AD has been driven by the need to detect the disease in its earlier preclinical stages, ideally before the onset of severe symptoms. If this is done, it could help facilitate the rapid development of an efficient treatment plan even

before the symptomatic clinical onset of the disease. Historically, the diagnosis of AD has relied on cognitive assessments and, ultimately, post-mortem histopathological confirmation. However, research in recent decades has witnessed rapid advancements in the identification of *in vivo* biomarkers, especially those reflecting amyloid-beta (A β) deposition, tau pathology, synaptic dysfunction and neuroinflammation, all of which are hallmarks of AD (Georgakas et al., 2023). Techniques such as positron emission tomography (PET), cerebrospinal fluid (CSF) assays, and blood-based biomarkers have shown increasing sensitivity and specificity in the past years. These are emphasised in research done on both animal (rat and primate) models and humans. Hansson et al. (2023), for example, states that blood-based biomarkers are an exceptionally promising avenue for revolutionizing the clinical practice of diagnostic and prognostic work-up of Alzheimer's disease. Anoop et al. (2010) explores the exciting recent advancements in CSF biomarkers for monitoring the early stages of AD before severe cognitive dysfunction is apparent. Márquez & Yassa (2019) also suggests a need for composite biomarkers in neuroimaging, as this can provide a deeper understanding of the pathophysiology of AD. Although the future of early diagnosis looks bright, it is undeniable that translational hurdles still persist, which have limited the availability of these diagnostics to certain communities due to factors like socioeconomic differences, standardisation, and education of clinical staff.

Methodology:

This literature review investigates how the neurobiological foundations of Alzheimer's disease biomarkers inform their utility, analysing the key translational challenges that hinder their integration into routine clinical practice.

The review is structured into four main sections. First, the molecular and cellular neurobiology underpinning key AD biomarkers will be discussed, with particular focus on the amyloid and tau hypotheses, neuroinflammation, mitochondrial dysfunction, and synaptic and neurotransmitter alterations. Second, it surveys the primary modalities used in biomarkers, their technical strength and mechanistic correlates. The third section examines translational challenges, including issues of socioeconomic accessibility, standardisation and technical limitations. Finally, the review evaluates the ongoing efforts in bridging the lab-to-clinic divide.

With a critical synthesis approach, this review analyses peer-reviewed literature across neuroscience, clinical neurology and translational medicine. It integrates findings from empirical studies, along with meta-analyses and systematic reviews to identify emerging consensus and unresolved tensions, such as the accuracies of specific biomarkers, within the field. A targeted narrative review of human and translational studies was conducted on Alzheimer's disease biomarkers published 2015 to 2025 in PubMed and Scopus, using terms combining Alzheimer's disease with biomarker, blood, CSF, MRI, PET, retina, saliva, urine, and volatile organic compounds. Inclusion prioritised peer-reviewed original research, along with systematic review and consensus statements. Seminal pre-2015 studies were

retained selectively to provide historical context. Non-peer-reviewed web sources were excluded except where clearly identified as illustrative, such as information about cost ranges.

Section 1: Molecular and cellular neurobiology and pathophysiology of AD

Alzheimer's disease is characterised by the slow, progressive neurodegeneration brought on by neuronal cell death and activity dysfunction, usually starting in the entorhinal cortex inside the hippocampus, an area crucial for various forms of explicit memory formation, particularly spatial memory (Karimani et al., 2024). The pathophysiological hallmarks of AD are accumulations of abnormal neuritic plaques and neurofibrillary tangles (NFTs). With these changes, there is also a loss of neurons, specifically cholinergic neurons in the basal forebrain and neocortex (Kumar et al., 2024). There are two main hypotheses that are popular within the scientific community, the first of which is the cholinergic hypothesis and the amyloid hypothesis. This literature review focuses on the amyloid hypothesis, as it is currently the most widely accepted pathophysiological mechanism for AD. However, it should be noted that this hypothesis has also faced challenges and criticisms, and it is believed that there are alternative or complementary mechanisms in the pathogenesis of AD (Nasb et al., 2024).

Amyloid beta

The peptide amyloid beta ($A\beta$) is derived from a protein, called the amyloid precursor protein (APP), through the abnormal activity of β - and γ -secretase enzymes responsible for breaking down proteins (Mucke & Selkoe, 2012). Typically, APP is cleaved by either α - or β -secretase and the smaller fragments formed are not neurotoxic. However, the cleavage by β - followed by γ -secretase results in peptides of 42 amino acids, known as $A\beta_{42}$, which is a long peptide more prone to aggregation than shorter species. $A\beta$ oligomers, which are smaller aggregates of these $A\beta$ peptides, are considered especially neurotoxic. Increase in $A\beta_{42}$ levels leads to the aggregation of amyloid, creating the commonly seen abnormal neuritic plaques. These are spherical microscopic lesions made of an extracellular $A\beta$ peptide surrounded by enlarged axonal endings, and are usually found within the cortical gray matter or around the meningeal and cerebral vessels of the brain. These deposits initially form in different areas, but grow and merge to comprise the larger more recognisable plaque structures, which then lead to neuronal toxicity. This is caused by $A\beta$ plaques damaging mitochondria as they accumulate inside neuronal cells, impairing its ability to generate ATP. Without sufficient energy, the neuron cannot maintain its normal function, leading to the initiation of apoptosis (programmed cell death). Furthermore, $A\beta$ oligomers can insert themselves into the cell membrane of neurons, forming pores that allow for the unregulated influx of calcium ions, disrupting the balance of calcium concentrations which is critical for signalling and cell survival. These high calcium levels within the cell can activate enzymes that degrade proteins and lipids, leading to the breakdown of the neuronal cytoskeleton, and further mitochondrial dysfunction.

Certain mutations, specifically those in the genes encoding APP and secretases (e.g. presenilin 1 and 2, shortened to PSEN1 and PSEN2) have been linked to cases of familial AD, lending support to the A β hypothesis. This is because these mutations increase the production of longer and more aggregation-prone isoforms of A β (Lanoiselée et al., 2017). Another gene is the apolipoprotein E (APOE) gene, which is the “strongest genetic risk factor for late-onset AD”, according to Kamatham et al. (2024), as it is associated with producing proteins that lead to higher levels of A β aggregation and reduced clearance.

Tau tangles

The aggregation of A β leads to a cascade of events. One of these events is the hyperphosphorylation of tau (the addition of too many phosphate groups which modifies the shape of tau) through the activation of kinases like GSK β , causing the protein that stabilises axonal microtubules vital for intracellular transport to become misfolded and aggregate within neurons (Kumar et al., 2024). With this, these tau molecules become prone to self-aggregation and form insoluble twisted paired helical filaments. Known as neurofibrillary tangles, they first appear in the hippocampus. Recent research has supported the idea that tau aggregates have prion-like properties, allowing their propagation throughout the brain by inducing the misfolding of normal tau in neighbouring neurons. This may explain why tau is often seen spreading throughout the cerebral cortex as AD progresses, contributing to cognitive decline in affected individuals.

Inflammation

Both A β peptides and NFTs can trigger inflammatory responses within the brain by activating microglia and astrocytes (Kamatham et al., 2024). These cells then become ramified and abnormally enlarged (hypertrophied) in NFT-rich brain regions. These immune responses are triggered to clear these harmful substances by the release of pro-inflammatory molecules. However, while this process is meant to protect the brain, chronic and dysregulated inflammation produces proinflammatory cytokines that lead to neuronal damage and exacerbate the progression of the disease. This sustained state of inflammation can exacerbate neuronal damage and further promote the spread of tau pathology.

Mitochondrial Dysfunction

A β peptides have also been shown to impair mitochondrial function by accumulating within the inter-mitochondrial membrane space and disrupting their normal function (Aran & Singh, 2023). This causes a reduction in ATP production and an increase in ROS generation, leading to oxidative stress and eventual damage to the organelle and its normal activity. This then triggers a positive feedback loop, as impaired mitochondrial function further promotes A β production and aggregation. In addition to this, abnormally phosphorylated and aggregated tau proteins have also been implicated in causing mitochondrial dysfunction by impairing mitochondrial transport along neuronal axons, which consequently leads to local energy deficits. It also disrupts fission and fusion processes crucial for maintaining a healthy mitochondrial network. This also triggers a

cycle, where mitochondrial dysfunction results in the formation of even more tau aggregates.

Synaptic and neurotransmitter alterations

Both protein aggregates of A β and tau prevent regular synaptic function by hindering the release of neurotransmitters (including acetylcholine, which plays an important role in memory and cognition), which reduces synaptic density and interferes with synaptic plasticity, particularly in cholinergic neurons (Kamatham et al., 2024). With this, there is a deficiency in signalling, contributing to the memory impairment and cognitive decline associated with AD. Glutamate neurotransmission is also affected, where it excessively activates glutamate receptors, contributing to excitotoxicity, which consequently leads to neuronal damage and cell death. As the membranes of these axonal cells are disrupted and the cells die, the intracellular contents, which contain essential structural components like neurofilaments (NFLs), leak out into the surrounding fluid.

Environmental Factors

Although novel, it has been posited that bacterial and viral infections may be implicated in the process of AD pathogenesis, as different types of microbes have been shown to stimulate A β aggregation and deposition. Therefore, there may be a pathogenic factor in the onset of AD as well (González-Sánchez et al., 2020).

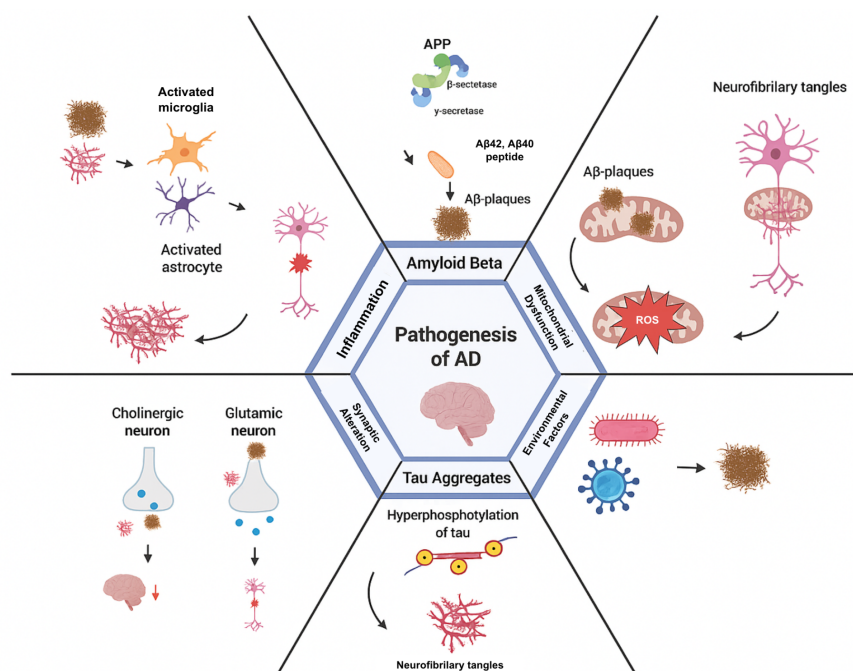


Figure 1. Diagram of the proposed forms of pathogenesis of AD

All the different pathologies of AD are largely thought to be dynamically interacting rather than isolated, suggesting that biomarkers that are able to capture multiple axes of degeneration may be most effective in diagnosing the disease.

Section 2: Types of Biomarkers and How They Work: Established

Biomarkers are defined as being naturally occurring molecules, genes, or characteristics by which a particular pathological or physiological process or disease can be identified. This literature review identifies three types of biomarkers typically employed in the diagnosis of AD: molecular, imaging and functional. Molecular biomarkers include proteins or other biological compounds found through the testing of substances like blood, CSF and other bodily fluids. Imaging biomarkers include scans like PET, MRI and CT, while functional biomarkers are measured through performance on cognitive or motor assessments and tasks. Usually, biomarkers are employed to determine the cause of mild cognitive impairment (MCI) in the initial stage of dementia. This literature review further investigates promising new biomarkers that have shown potential in predicting AD.

MOLECULAR

CSF

Cerebrospinal fluid is an ideal candidate for reflecting the molecular events of the brain, as it is in direct contact with the brain's extracellular space. Hence, experts agree that it is the best way to identify AD biomarkers that reflect its pathogenesis.

Because A β 42 is a large component of typical AD plaques, the amount of total A β in CSF is well-correlated with AD diagnosis. There is a positive correlation between lower CSF A β 42 levels and plaque burden, due to plaques acting as “amyloid sinks”, where the peptide is deposited and accumulates (Anoop et al., 2010). There has also been a correlation between a rise in A β 38 levels and a fall in A β 42, which supports the idea that the ratio A β 42:A β 38 may be more useful and precise in diagnosing AD. This reduced A β 42 has also been observed in other types of dementia, and many works and wider literature agrees that A β 42 is the most accurate CSF biomarker of AD in patients with MCI. However, there was no significant difference observed in the total CSF A β levels in AD patients and non-AD controls. Therefore, it seems that only A β 42 is a relatively accurate predictor of the disease.

Increased levels of tau and p-tau in CSF has been shown to be correlated with AD onset, with several studies suggesting that total tau (t-tau) concentration in AD patients' CSF is higher than the control (Papaliagkas et al., 2023). However, it is noted that this biomarker, although sensitive, is limited in its ability to differentiate AD from other forms of dementia, as an increase in t-tau is not a feature unique to Alzheimer's. Hence, p-tau is the more accurate biomarker, as it is more closely related to the pathophysiology of AD as compared to simply being a marker of axonal damage as t-tau is

Levels of GFAP (glial fibrillary acidic protein) tend to be increased in individuals with early A β pathology, as it is hypothesised to reflect the increased reactive astrocytes characteristic of AD (Marksteiner & Humpel, 2025). This can be measured reliably in the CSF. Plasma NfL levels also correlate well with the levels found in CSF, as it leaks from cells due to injury.

Another potential biomarker is neuronal threat proteins (NTPs). These are expressed in the brain during AD-related neurodegeneration, specifically abnormal AD7c-NTP. This has been found to be higher in postmortem CSF of AD individuals compared to controls (Kang & Wang, 2022).

Blood

As A β 42 and A β 40 peptides accumulate within the brain, plasma levels of individuals with AD are typically indicative of this. The plasma A β 42:A β 40 ratio is usually lower in A β 42-positive groups than in A β 42-negative groups, as has been demonstrated by different immunoassays and mass spectrometry-based assays. However, the performance of these different assays have been shown to vary substantially, with a recent comparison showing that certain mass spectrometry-based assays were far better at identifying A β pathology than more commonly used immunoassays. Regardless, these higher-performing A β 42:A β 40 assays still only exhibit modest correlations between the levels of A β and that found in CSF, likely due to the fact that A β in plasma is derived from peripheral sources, as posited by Hansson et al. (2023). Hence, it is agreed that plasma A β 42 levels alone cannot be considered an AD marker.

On the other hand, researchers have recently developed several high-sensitivity assays for the detection of different phosphorylated tau (p-tau) protein variants, or isoforms, including specific ones like p-tau181, p-tau231 and p-tau217 (Janelidze et al., 2025). When compared, it seems that assays quantifying plasma p-tau217 have been shown to be better at detecting AD pathology and predicting the development of AD dementia. However, it can be argued that p-tau231 is a good marker of AD in its early stages, as it starts increasing at very low A β levels. Hence, when combining plasma A β 42 and A β 40 with p-tau, early-stage amyloid positivity detection could be improved.

Similarly, GFAP can also be measured reliably in the blood as well as the CSF. Plasma NfL levels also correlate well with the levels found in CSF, although it seems this correlation is not as strong as that of p-tau217 (Garcia-Escobar et al., 2024). There is also little to no correlation between levels of NfL and A β 42 in the blood, which has led researchers to believe that NfL suggests neurodegeneration independently of the pathology of A β .

Janelidze et al. (2022)'s study has shown that the combination of plasma p-tau231 and A β 42:A β 40 could be used to detect very early stage AD, and it is supported by the fact that it was able to detect amyloid pathology in cognitively unimpaired individual.

Saliva

Saliva is not only an easily accessible body fluid, its secretion and composition might also be affected by various diseases (Surdu et al., 2025). It is posited that AD can influence these salivary parameters, as there seems to be a relationship between the brain and saliva. This occurs through six pathways communicating brain molecules with the saliva and vice versa. However, for both A β and tau, concentrations are considerably lower in non-plasma fluids.

From a review conducted by Nijakowski et al. (2024), it was seen that there was a statistically significant number of studies that have examined the saliva of AD patients and found elevated levels of A β . However, it was noted that in three studies, neither A β 40 or A β 42 were detected in AD patients. This suggests that there is some degree of reliability of correlating A β levels with AD.

Based on the meta-analysis performed by Nijakowski et al., it seems that the reliability of tau as a biomarker for AD is inconsistent and unreliable when considered alone. There is no consensus on whether t-tau, p-tau and their ratio are reliable indicators of AD. The studies examined by Nijakowski have reported conflicting results, and suggest that while tau may be part of the AD pathology, its biomarker use in saliva cannot be considered a standalone reliable biomarker.

Nijakowski et al. also explores lactoferrin (Lf), a protein with anti-inflammatory properties, and proposes that it may have neuroprotective effects in neurodegenerative diseases like AD. According to González-Sánchez et al. (2020), biomarkers that reflect the integrity of the immune system could be useful. Lf has previously been detected in senile plaques, neurofibrillary tangles and microglia in AD brains. Although it was particularly useful in differentiating AD and MCI patients from healthy individuals, it appears to be a promising, but not fully conclusive biomarker for AD. While several studies analysed by Nijakowski et al.'s paper showed a clear link between decreased salivary lactoferrin levels and AD, there are also conflicting findings that appear to warrant further investigation.

Urine

Both A β and tau are possible to detect in urine, because they can be eliminated from the organism by action of the kidneys (Armenta-Castra et al., 2024). These are detected through methods like ELISA and Western blot. Several studies show a correlation between the presence of these proteins in urine and AD, and it appears that A β oligomers are better indicators of AD progression, instead of the monomers A β 40 and A β 42. Both p-tau and tau were found to be significantly higher in AD patients compared to control individuals, but as was with saliva, these concentrations were much lower in urine than in plasma for both groups.

NTPs have been found in urine, as well as the CSF. Evidence for urinary AD7c-NTP is preliminary and replication in contemporary cohorts is limited, leaving clinical utility

uncertain. One such piece of evidence is the ELISA results from Ghanbari et al.'s 1998 study utilised ELISA to show that results in urine showed similar sensitivity and specificity to CSF, and that urinary AD7c-NTP was significantly higher than in the non-AD group.

IMAGING

Neuroimaging now allows for diagnosis of the AD pathophysiological process during life, even though neuropathology remains the definitive standard. Clinicians can begin to assess cases in vivo, with several modalities having been researched, including Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET).

Neurodegeneration leads to cerebral atrophy, which is most notable in the hippocampus and entorhinal cortex of patients with AD (Tabatabaei-Jafari et al., 2015). The level of atrophy in the medial temporal lobe is strongly correlated with scores on cognitive testing, and is therefore associated with disease progression. As this degeneration progresses to other cortical structures, lateral ventricles become enlarged in the later stages of the disease. In addition to this, limbic structures (such as the amygdala, cingulate gyrus, thalamus, and olfactory bulb tract) are impacted, and the atrophy leads to volumetric reductions in the frontal, parietal and temporal brain areas.

MRI

MRI is an imaging technique that is able to generate high-resolution images of water-containing tissue. This is done by applying a strong magnetic field and pulses of radio waves that manipulate electromagnetic fields and energy states of protons within the body. These protons emit energy, which are then converted to signals and images.

Structural MRIs are used to provide highly detailed images of anatomy as well as a strong gray/white matter contrast. These scans are able to display white matter hyperintensities that indicate demyelination and axonal loss (Meng et al., 2022). Compared to patients without AD, patients with AD have greater white matter hyperintensities in the frontal lobe that index small-vessel disease burden and help in differential diagnosis and staging, but are not necessarily specific to AD.

Diffusion Tensor Imaging (DTI) uses the displacement of water molecules to measure the integrity of the white matter tract. The direction of diffusion allows scientists to better understand tracts and specific fibre bundles that may be impacted by neurodegeneration (Esrael et al., 2021). The major measures of DTI are mean diffusivity (MD), which is the average rate of water molecule diffusivity, and fractional anisotropy (FA), which is the variability associated with diffusion. In AD brains, MD can be seen in all four areas of the brain (frontal, occipital, parietal and temporal), including the hippocampus. This is a clear difference from MCI, where these increases are not seen in the frontal and occipital regions. AD also presents as decreased FA localised to the cingulum, corpus callosum, uncinate fasciculus and superior lateral fasciculus, as well as throughout the temporal, occipital and frontal white matter. Patients with MCI have no irregularities of FA in

occipital and parietal areas, which sets MCI scans apart from AD. According to a study done by Magalhães et al. (2023), there was a significant correlation between white matter tracts and both p- and t-tau.

Functional MRI (fMRI) produces dynamic representations of brain activity through blood oxygen level-dependent (BOLD) signals (Chandra et al., 2018). This measures changes in blood volume and flow. When asked to perform memory tasks, AD patients showed little to no activation of hippocampal and other medial temporal structures as compared to non-AD individuals. Resting-state fMRIs of the AD brain also show abnormal coactivation of the medial temporal structures and the posterior cingulate cortex, which differs from those of normal brains.

PET

PET is an imaging modality that allows for the in vivo measurement of disease pathologies using radioligands to detect compounds of interest. In the case of AD, that would be neurofibrillary tangles and A β plaques.

FDG-PET uses 18-F-fluorodeoxyglucose (FDG), which allows researchers to estimate glucose metabolism in different brain areas and identify which ones may have abnormal metabolic activity (Bouter et al., 2019). From studies done, these scans show temporoparietal hypometabolism in patients with AD, which is characteristic of the disease. Furthermore, there was an observed decrease in metabolism in the posterior cingulate cortex and precuneus, which is thought to be related to the episodic memory deficits typical of AD.

Because of accumulated A β , there is known to be increased amyloid tracer binding in areas of the brain affected by the disease (more specifically, temporal parietal and frontal regions) (Villa et al., 2020). Tracers such as Pittsburgh Compound B (PiB) are able to bind to misfolded amyloid plaques, therefore helping to map distribution and quantify amyloid burden. Other tracers include florbetapir, 18-F-labeled flutemetamol and 18-F-labeled florbetaben.

Part of the underlying pathophysiology of AD is the neuroinflammatory cascades leading to an increase in microglial activation. Radiotracers that bind to TSPO (translocator protein-18 kDa) have been utilised as biomarkers of AD neuroinflammatory cascades (Georgakas et al., 2023). Since TSPO is a peripheral benzodiazepine receptor found on the membrane of the mitochondria, increased expression of this protein can be used as an estimate for increased inflammation, as the expression of this protein is upregulated by activated microglial cells. Studies done have found TSPO expression increased when compared to healthy controls.

Machine Learning and Artificial Intelligence

With the rise of better artificial intelligence algorithms, AI is being increasingly utilised in the diagnosis of AD (Kamatham et al., 2024). AI models are capable of analysing large, complex datasets containing neuroimaging data and biomarker profiles to identify patterns. MRI and PET generate vast amounts of data that can be analysed using AI to detect both structural and functional brain changes associated with AD. Biomarker data can also be incorporated in order to develop predictive models for risk assessment. This holds great potential for improving efficiency and accuracy, since AI models can therefore aid healthcare providers in making increasingly informed diagnostic decisions, facilitating the early detection of AD and the generation of personalised treatment plans based on individual profiles.

BEHAVIOURAL

Functional

The progressive loss of ability to perform activities of daily living unassisted is a primary characteristic of dementia (Chaves et al., 2011). These activities can be divided into basic (BADL) and instrumental (IADL). BADL includes the ability to carry out tasks necessary for personal hygiene, sphincter control and feeding. IADL, on the other hand, are more complex and are activities such as preparing meals, carrying out domestic chores, and managing finances and correspondence.

An example of an assessment used to evaluate changes in functional performance is through an assessment measure known as Functional Assessment Staging (FAST). This allows healthcare practitioners to specifically evaluate these changes throughout the entire course of AD (Sclan & Reisberg, 1992). Another such assessment measure is the Alzheimer's Disease Cooperative Study – Activities of Daily Living for Mild Cognitive Impairment, otherwise known as ADCS-MCI-ADL. This assesses the competence of AD patients in both basic and instrumental ADLs, and can be completed by a caregiver or administered by a clinician as a structured interview with a caregiver (Fish et al., 2011).

Cognitive

The initial and most common presenting symptom of AD is episodic short-term memory loss (Kumar et al., 2024). This includes difficulty retaining new information, and experiencing impairments in problem-solving, executive functioning, judgement, and organisational skills. Because of this, they may struggle with multitasking and abstract thinking, which affects their ability to carry out ADLs like driving, cooking, and activity planning. Following this, there are often language and visuospatial skill impairments, along with neuropsychiatric symptoms like changes in social behaviour.

A common example of a cognitive assessment is the Mini-Mental State Examination (MMSE) (Arevalo-Rodriguez et al., 2015). This is a common test for assessing changes in cognition, and evaluates functions such as orientation, language, memory and other functions. This is done through tasks that test reading, writing and short-term memory,

including tasks like subtraction, remembering a series of unconnected words or copying a diagram on paper.

The Alzheimer’s Disease Assessment Scale – Cognitive, or ADAS-Cog, is generally more thorough (Dementia Australia), and is considered the gold standard for assessing AD (Kueper et al., 2018). This includes 11 tasks, including both tests completed by the subject and assessments completed by the observer. When put together, these tasks assess memory, language, and praxis. Specific tasks, like the MMSE, include word recall, naming objects, commands, and word recognition among others.

As can be seen by this, there already exists a plethora of widely acknowledged and effective biomarkers, whether they be molecular, imaging or functional. A summary of the above information can be found in Table 1.

Table 1. Summary of Key Biomarkers and Diagnostic Modalities for Alzheimer’s Disease (AD)

Category	Subcategory	Biomarker / Method	Key Findings	Work Cited
Molecular	CSF	Aβ42	Lower levels correlate with plaque burden; most promising CSF biomarker for AD in MCI	Anoop et al., 2010
		Aβ42:Aβ38 ratio	May improve diagnostic precision	
		t-tau	Elevated in AD; sensitive for neurodegeneration	Papaliagkas et al., 2023
		p-tau	More closely related to AD pathology than t-tau	
		GFAP	Increased with early Aβ pathology; reflects reactive astrocytes	Marksteiner & Humpel, 2025
		NfL	Correlates with CSF levels; marker of axonal injury	
		AD7c-NTP	Higher in postmortem CSF of AD patients (still relatively new and emerging)	Kang & Wang, 2022
	Blood	Aβ42:Aβ40 ratio	Lower in Aβ-positive groups; better with mass spectrometry	Hansson et al., 2023
		p-tau217	Strong predictor of AD pathology and progression	Janelidze et al., 2025
		p-tau231	Early-stage AD marker (increases at low Aβ levels)	

Functional	Imaging	Saliva	p-tau181	Widely adopted, earlier generation with less specific indication of AD	
			GFAP	Detectable in blood; reflects astrocyte activation	Garcia-Escobar et al., 2024
			NfL	Detectable; less correlated with CSF than p-tau217	Janelidze et al., 2022
			Combined p-tau231 + Aβ42:Aβ40	Detects very early-stage AD	Hansson et al., 2023 Janelidze et al., 2025
			Aβ	Some studies show elevated levels in AD	Nijakowski et al., 2024
			t-tau, p-tau	Conflicting results; unreliable alone	
			Lactoferrin	Lower in AD; potential immune biomarker	González-Sánchez et al., 2020
		Urine	Aβ oligomers	Better indicators of progression than monomers	Armenta-Castro et al., 2024
			p-tau, tau	Higher in AD patients than controls	
			AD7c-NTP	Elevated; similar sensitivity/specificity to CSF	Ghanbari et al., 1998
	MRI	Structural MRI	Structural MRI	Hippocampal/entorhinal atrophy; white matter hyperintensities	Tabatabaei-Jafari et al., 2015 Meng et al., 2022
			DTI	Increased MD and reduced FA in specific tracts; correlates with tau	Esrael et al., 2021
			fMRI	Reduced hippocampal activation; altered resting-state connectivity	Chandra et al., 2018
		PET	FDG-PET	Temporoparietal hypometabolism; PCC/precuneus deficits	Bouter et al., 2019
			Amyloid PET	PiB and other tracers map amyloid burden	Villa et al., 2020
			TSPO PET	Detects neuroinflammation via microglial activation	Georgakas et al., 2023
Functional	Behavioural	AI/ML Integration	AI models	Detect structural/functional changes; integrate biomarkers	Kamatham et al., 2024
			BADL/IADL assessments (FAST, ADCS-MCI-A DL)	Measures decline in daily functioning	Chaves et al., 2011 Sclan & Reisberg, 1992 Fish et al., 2011

Section 3: Types of Biomarkers and How They Work: Novel

While traditional biomarkers have focused on the more established hallmarks of AD, such as A β and tau, recent research has expanded into a more diverse spectrum of novel biomarkers that may serve to capture alternative aspects of the disease's biology. These include new developments in both molecular and imaging biomarkers as technological advancements occur.

MOLECULAR

MicroRNA

MicroRNAs (miRNAs) are a type of short-length, non-coding, and single-stranded endogenous RNAs (also known as sncRNAs) found in a stable form in the blood, CSF, and various body fluids (Chimthanawala et al., 2023). By controlling the expression of genes and pathways, miRNAs by suppressing the translation of target mRNAs and facilitating their degradation, contributing to APP degradation and A β metabolism. AD patients had higher levels of miRNA-4722-5p and miRNA-615-3p, and combining these two AD-associated miRNAs provided a better diagnostic score than either miRNA alone. miRNA-20b-5p also downregulated APP levels, hence reducing the availability of A β . However, further research in this domain needs to be carried out, especially on three miRNAs that strongly correlated with the early stages of AD, namely miRNA-92a-3p, miRNA-486-5p, and miRNA-29a. Levels of miRNA-92a-3p and miRNA-486-5p were lower in AD patients compared to controls, but higher levels of miRNA-29a-3p were found in the plasma of AD and MCI patients. Plasma levels of miRNA-483-5p levels were higher in AD patients compared to healthy controls, and it was concluded that increased levels of miRNA-483-5p in plasma can reliably distinguish both MCI and AD groups from the healthy controls. The above all suggests that these miRNAs could be potential biomarkers in early AD diagnosis.

Long non-coding RNA

A second type of RNA that can be used is long non-coding RNAs (LncRNAs). These resemble mRNA (messenger RNA) molecules, but are not responsible for encoding proteins (Black et al., 2024). They typically contribute to the regulation of several processes by regulating chromatin remodelling, and providing alternative mRNA splicing. This affects gene expression, and has been shown to “directly or indirectly regulate the key features of AD including A β deposition, aberrant tau formation, oxidative stress, neuroinflammation, and neuronal death”. LncRNAs also interact with miRNAs, thereby

influencing AD-related neuropathology. The expression of numerous types of these molecules have been detected in plasma and CSF samples from AD patients, which indicates that lncRNAs could function as a likely biomarker in the diagnosis and progression of AD.

Apolipoprotein C3

A study done by Watanabe et al. (2020) suggests that Apolipoprotein C3 (ApoC3) may be a potential urinary biomarker for AD, although further analysis of this is warranted (Watanabe et al., 2020). ApoC3 is an abundant apolipoprotein mainly present in triglyceride-rich lipoproteins (chylomicrons and VLDL), as well as cholesterol particles. Watanabe et al. found that studies have reported people with AD had lower plasma levels of ApoC3 compared to healthy individuals, and that higher ApoC3 was associated with reduced grey matter volume in the brain. The paper also pointed to recent research suggesting that ApoC3 acts as a trigger for inflammation by activating the NLRP3 inflammasome. This is significant because the NLRP3 inflammasome is known to be activated in the brains of AD patients by amyloid beta. The result of Watanabe et al.'s experiment was that all samples revealed ApoC3 levels to be significantly higher in the AD group relative to the control group.

MCP-1

Monocyte Chemoattractant Protein 1 (MCP-1, also known as CCL2) is a protein that belongs to a family of signalling molecules called chemokines (Huang et al., 2023). They play key roles in the body's inflammatory response by attracting immune cells to sites of inflammation. As a biomarker, MCP-1 can be measured in both blood and urine. In AD, chronic inflammation in the brain (neuroinflammation) contributes to neuronal damage. It is thought that it is because of this that elevated levels of MCP-1 have been found in the blood and CSF of AD patients. However, this is not consistent across different studies, which is why Huang et al. hypothesised that MCP-1 levels impact individuals who are genetically at risk of developing AD.

Molecules Associated with Oxidative Stress

A β induces oxidative stress, the production of harmful reactive oxygen species (ROS), which is thought to be a major contributor to cellular damage in the brain (Gella & Durany, 2009). ROS are too unstable to measure directly, so measuring their byproducts is a method used to quantify oxidative stress. Several products, specifically 4-hydroxy-2,3-noneal (HNE), acrolein, F2-isoprostanes and malondialdehyde have been observed to exist in higher levels in AD brains. However, like many other biomarkers, oxidative stress is a general sign of cellular damage and is not necessarily specific to AD. It's a feature of many neurodegenerative disorders and is also affected by ageing and environmental factors.

Volatile Organic Compounds

Exhaled breath contains hundreds of different byproducts of metabolism, also known as volatile organic compounds (VOCs). In AD, metabolic changes in the brain could produce a unique “breathprint” (Zhang et al., 2025). Using sophisticated sensors or mass spectrometers, analysis of this breathprint can be carried out to identify patterns that correlate with this disease. However, this research is still in its early stages, and whether it can be used for AD diagnosis is unclear.

IMAGING

Retinal Imaging

In the last decade, there has been rapid progress in using retinal imaging to find markers of AD. Techniques like optical coherence tomography (OCT) are shown to be especially promising because of their non-invasive, affordable and accessible nature as compared to traditional brain imaging (Georgakas et al., 2023). Research has shown structural, vascular and neuropathological changes in the retinas of both mice and humans with AD. Structural markers, such as the thinning of the retinal nerve fibre layer (RNFL) are consistently seen in older adults with AD. This thinning may start early in disease progression, as it has been linked to levels of A β and tau in CSF, as well as to hippocampal volume in cognitively normal adults. This suggests its usefulness as a tool for early AD screening. Vascular changes are also being explored, as a 2013 study found that asymptomatic adults with high cerebral A β had less-branched retinal blood vessels. Another study found that a larger foveal avascular zone (FAZ), which is the area of the retina without blood vessels, was typically associated with higher cerebral A β in cognitively normal adults. This suggests that the FAZ area, along with retinal imaging in general, may be an effective marker for detecting preclinical AD. However, this research has yielded mixed results, with some finding strong correlations and others not.

CT

A CT scan uses a series of X-rays from different angles to generate cross-sectional images of the brain. While it cannot visualise amyloid plaques or tau tangles directly, it is able to detect brain atrophy, or shrinkage, of regions like the hippocampus (Pasi et al., 2011). However, it is only really supportive and non-specific with the typical patterns of AD. It may also prove more useful only for later-stage AD, as brain atrophy usually cannot be seen until patients begin to exhibit behavioural and cognitive changes. In addition to this, CT scans involve using ionizing radiation which, though at a low dose, carries a small risk, especially for repeated scans.

Tau PET

Tau PET molecular imaging is a relatively new technology. So far, there is a first-generation FDA-approved tracer, known as 18-F-flortaucipir, and has been shown to have high affinity for detecting the tau aggregates that are characteristic of AD (Petersen et al., 2022). However, off-target binding happens, where tracers bind to molecules other than tau, which produces unreliable results in a PET scan. This is why there are ongoing

efforts to develop second-generation tracers, which are being designed to have improved binding specificity.

Most non-CSF, non-blood matrices remain exploratory, but from current research, it's clear that many of them warrant significant potential to become alternative biomarkers. Some may even prove to be more advantageous than traditional biomarkers due to their cost-effectiveness and accessibility, as well as their specificity or accuracy.

Section 4: Translational challenges and barriers

Data standardization and comparability represent a critical challenge in both imaging data collection and biomarker fluid acquisition protocols that can vary considerably across different Alzheimer's Disease Research Centers (ADRCs) (Braudeau et al., 2025). This variability complicates the establishment of universal diagnostic thresholds and hinders the direct comparability and integration of findings from diverse research cohorts, making it difficult to generalize results and apply them uniformly in clinical practice. Consequently, even if a biomarker demonstrates exceptional scientific efficacy, its practical clinical utility is severely constrained if it cannot be widely deployed across diverse healthcare settings. This accessibility-utility gap highlights pressing health equity concerns in AD diagnosis, driving the urgent need for less invasive and more affordable alternatives, even if those alternatives present their own set of challenges.

IMAGING

High cost and limited accessibility are paramount challenges. Imaging costs can vary widely across settings, but illustrative ranges suggest substantial patient-level expense, with an MRI costing up to \$8,400, and a PET scan up to \$3,000 in some countries (Imaging Technology News, 2018). This substantial cost, coupled with the requirement for specialized equipment, trained personnel, and dedicated imaging facilities, restricts their availability, particularly in underserved regions and low-resource settings. This creates a significant barrier to widespread clinical use, contributing to health inequities in AD diagnosis.

Patient burden and contraindications also pose practical limitations (Decazes et al., 2021). Since PET scans involve a small dose of radiation, patients may be advised to avoid this scan if they are pregnant, and this radiation carries its own risks as well. Both MRI and PET scans require patients to remain still in enclosed spaces for extended periods, which can cause discomfort or claustrophobia. Furthermore, patients with certain medical devices or metal implants, such as pacemakers, may have contraindications for MRI due to the powerful magnetic fields involved.

PET

Cost and reimbursement represent major practical barriers to the widespread clinical adoption of amyloid PET (Lee et al., 2021). These scans are expensive, typically costing between \$3,000 and \$6,000. In the USA, third-party payers often do not reimburse for amyloid PET, citing a need for additional data demonstrating that the scan results directly impact clinical care and patient-based outcomes. This lack of coverage significantly restricts access for many patients.

Pittsburgh Compound B (PIB) utilises carbon-11, which has a very short half-life of 20 minutes, which limits its clinical applicability due to logistical challenges (Wolk et al., 2012). While more clinically viable F18-labeled tracers with a longer half-life of 110 minutes have since been developed, the cost and accessibility issues persist. Additionally, U.S. tracer labels and clinical practice typically use a binary (positive or negative) interpretation for amyloid PET results, which may oversimplify the continuous biological data and potentially obscure nuanced information about amyloid burden.

The designation of amyloid PET as a “gold standard” for detecting amyloid pathology is important to consider in context. While it excels at identifying the presence of amyloid plaques, a necessary component of AD, the presence of these plaques alone is not sufficient for a definitive AD diagnosis (Roberts et al., 2015). Amyloid plaques are found in other conditions and in a substantial proportion of cognitively normal individuals. Furthermore, the overall amyloid burden, as assessed by PET, does not consistently correlate strongly with cognitive deficits. This implies that a positive amyloid PET scan indicates the presence of amyloid pathology, but it does not definitively confirm symptomatic AD or establish amyloid as the sole cause of cognitive impairment.

MRI

In the very early stages of AD, an MRI scan of the brain may appear normal, making it less sensitive for preclinical detection compared to molecular imaging techniques (van Oostveen & de Lange, 2021). While it provides detailed structural changes, it cannot solely confirm the presence of AD pathology; comprehensive assessments combining MRI findings with clinical evaluations, cognitive testing, and patient history are essential for accurate diagnosis. Fundamentally, MRI primarily reflects neuronal loss and brain atrophy, which are consequences of amyloid and tau pathology, rather than directly visualizing these protein aggregates themselves, meaning it is less sensitive to preclinical pathology than molecular biomarkers. Emerging techniques like fMRI and DTI hold significant promise, but their widespread standardization and established clinical utility for routine AD diagnosis are still under development.

MOLECULAR

CSF

Despite how thoroughly characterised and well-established CSF markers for AD are, there are still significant limitations associated with these biomarkers, primarily revolving around the invasiveness of the lumbar puncture procedure used to procure the fluid

(Inekci et al., 2015). The discomfort and potential risks, although rare, including nerve damage, infection and herniation, are currently primary drivers for the ongoing search for less invasive diagnostic alternatives.

Blood

Compared to CSF, blood-based biomarkers are more appealing because they tend to be significantly less invasive in nature and more cost-effective extraction methods (Mielke et al., 2024). However, as aforementioned in this literature review, the challenges include lower biomarker concentrations in blood compared to CSF, and also less achievable accuracy due to complex matrix interference in blood. In addition to this, peripheral metabolism can significantly affect blood biomarker levels, making them less direct reflections of brain pathology.

Other Bodily Fluids

With research exploring the potential of other bodily fluids like saliva and urine, these fluids offer a theoretical advantage of maximal non-invasiveness, which makes them highly attractive for broader-scale screening (Kumari et al., 2023). However, this research is still in its nascent stages. Low concentrations of relevant analytes, high biological variability coupled with a significant lack of robust validation in studies limit their establishment for clinical use in AD diagnosis.

OVERALL

Specificity

Even with seemingly AD-specific biomarkers like tau and NfLs, there still exist difficulties in differential diagnosis. The pathologies underlying other types of dementia often involve very similar proteins to the ones used to diagnose AD, which complicates the ability to distinguish between them based solely on biomarker levels (Zou et al., 2020). Simply measuring protein concentrations is unlikely to provide sufficient information to differentiate effectively between various dementias.

Cost

The current landscape of AD research and the development of biomarkers, particularly blood-based biomarkers, is heavily skewed towards high-income countries (Rodda et al., 2025). To achieve equitable progress in the field, it's crucial to actively involve researchers and institutions from LMICs in the development, validation, and clinical application of BBMs. Key challenges remain, including the need to understand the real-world effectiveness, cost-efficiency, and acceptance of these tools in resource-limited environments. Financial constraints are a major barrier to the necessary research and validation in LMICs. Additionally, to effectively roll out BBMs in LMICs, significant investment is needed to build the necessary infrastructure and upskill the workforce. This could involve financial support from various sources, corporate engagement, and the use of innovative, practical approaches. For instance, using dried blood spots and task-shifting models (i.e. training community health workers to do tasks normally reserved for

specialists) where community health workers collect samples could make widespread use more feasible, especially in remote areas.

Generalisability

Not only are there technical and economic barriers, there exist obstacles in the generalisability of studies due to the lack of access to certain samples and communities of individuals. Most studies, especially plasma biomarker studies, to date have focused on genetically homogeneous populations in high-income countries. This highlights a critical need for large-scale international studies that include diverse populations with varying genetic backgrounds, comorbidities, and socioeconomic statuses. This is a significant issue because biomarker levels can vary among different racial and ethnic groups and are affected by certain long-term health conditions and medications (Rodda et al., 2025). A 2022 study showed that even though most participants expressed interest in biomarker research for AD, Black participants were comparatively more hesitant than White participants (Elicain et al., 2022). They were more concerned about the risks of participating and felt there were more barriers to undergoing brain scans. These differences remained even when accounting for their trust in researchers and their existing knowledge of AD. The study found that a lack of information was a major barrier for Black participants, while receiving information was a strong incentive. Black older adults expressed a desire for more details about AD, including its risks and prevention, as well as a better understanding of the general research process and specific biomarker procedures. This highlights the need for the scientific community to improve recruitment strategies for underrepresented groups with no prior research experience. The above could be done through improving information sharing and community awareness by increasing their presence in these communities and reducing the financial burden of participation.

Standardisation

A major hurdle for the widespread use of AD biomarkers, particularly blood-based ones, is a lack of standardization (Humpel & Hochstrasser, 2011). Currently, there are no uniform methods for sample collection, processing, or storage. This problem is compounded by the absence of established procedures to monitor long-term biomarker stability and the lack of certified reference standards. These inconsistencies introduce measurement variations, making it difficult to compare results between labs, establish reliable clinical thresholds, and ultimately use these biomarkers effectively in both research and patient care (Zeng et al., 2024).

Education of Staff

The widespread adoption of biomarkers, despite their accessibility, are significantly challenged by the need for more comprehensive education of primary care clinical staff and the development of clear, actionable guidelines for their use. Without this adequate training and clear protocols, there is a high risk of misinterpretation of results or the inappropriate use of these diagnostic tools (Schöll et al., 2024).

Overall, there still exist many translational hurdles, and it is only if these hurdles are overcome that biomarkers can achieve their full efficacy and potential. Reducing these hurdles also allows more people to access what could potentially be **diagnostic tests** for AD, bettering treatment plans via early detection.

Section 5: Ongoing efforts of bridging the divide

Early detection of AD could dramatically shift the way treatment is given to patients. Early detection allows for interventions during the prodromal or mild cognitive impairment stages, where therapeutic outcomes are more likely to be meaningful as early diagnosis is crucial in informing discussions, care and support, and treatment (Dubois et al., 2023). Moreover, biomarkers are critical not only for diagnosis, but also for other means **like** patient stratification in clinical trials and the development of potential disease-modifying therapies.

Hence, the limitations and translational challenges confronting AD biomarkers are being actively addressed. For example, significant progress is being made in developing more sensitive and specific assays for AD biomarkers. Ultra-sensitive platforms, **like** Single Molecule Array (SIMOA) technology, are enabling the reliable detection and quantification of biomarkers like p-tau and GFAP even if they are present at very low concentrations in biological fluids (Yuan et al., 2025). Other technologies include novel electrochemical sensing platforms like CRISPR/Cas12a-mediated electrochemical aptasensors, which are biosensors that use DNA/RNA molecules to **detect proteins**.

Recognising that no single biomarker is able to provide a complete picture of AD pathology and its progression, there is a growing emphasis on multimodal biomarker approaches and the integration of different data types (Gupta & Iftekhar, 2024). Combining imaging, fluid, cognitive and demographic data could offer the potential for enhanced diagnostic accuracy. AI and machine learning (ML) in particular are emerging as tools in this context.

Beyond refining extant markers, the field continues to pursue the discovery of novel biomarkers that can provide more specific and comprehensive insights into AD pathology.

Conclusion

While AD biomarkers are getting better at detecting neurobiological changes, their translation into widespread clinical use is still obstructed by challenges including **limited accessibility**. Georgakas et al. (2023) states that the ability to translate treatments to **various wider populations** are reliant on low cost, minimally invasive and more accessible diagnostic biomarkers in a general clinical setting. Bridging this gap is consequently essential to realising their full diagnostic and therapeutic potential. Another current limitation in our use of novel biomarkers is being able to distinguish between AD and other

neurodegenerative disorders, making the traditional biomarkers like A β and tau far more accurate and developed.

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Title: The Neurobiological Foundations and Translational Challenges of Current and New-Generation Alzheimer's Disease Biomarkers: A Review

Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative condition, and remains without a definitive cure despite decades of research. Biomarkers offer promising avenues for early diagnosis and disease monitoring, though their clinical adoption has proven to lag behind laboratory advances. This review synthesises recent literature on the neurobiological underpinnings of both well-known and novel AD biomarkers by drawing on 2015–2025, English, human-centered studies utilising assays, imaging, and assessments to highlight the mechanisms and limitations of current tools of diagnosis. This review also aims to analyse the key translational barriers involved in routine clinical diagnostics, and evaluate the clinical readiness and integration for molecular, imaging and functional biomarkers. Key findings include A β 42/40 being a strong biomarker for amyloid plaques, p-tau217 outperforming other plasma p-tau epitopes, and new technologies such as second-gen tau PET being developed to reduce off-target issues. Despite certain technical limitations, AD biomarkers have strong neurobiological mechanistic evidence, but their translation is still impeded by practical and socioeconomic challenges. These include barriers to access, standardisation and underrepresentation of communities in studies. Addressing and bridging these gaps would be essential for closing the bench-to-bedside divide in AD care, helping to enable earlier interventions.

Keywords: Alzheimer's disease, biomarkers, neurobiology, translational neuroscience, diagnostics

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. It is characterised clinically by memory loss, impaired cognition, and behavioral changes. With prevalence rising sharply as populations age, AD poses mounting healthcare and socioeconomic challenges.

Despite being one of the most common forms of dementia and neurodegenerative disease, it remains an incurable illness. As of 2021, out of the approximately 57 million people worldwide who have dementia, 60-70% have AD (World Health Organisation, n.d.). This greatly affects their quality of life, and also places an enormous burden on caregivers and healthcare systems worldwide, costing economies US\$1.3 trillion globally, with 50% of these attributable to the care given by family members and close friends, according to the World Health Organisation.

The pursuit of reliable biomarkers for AD has been driven by the need to detect the disease in its earlier preclinical stages, ideally before the onset of severe symptoms. If this is achieved, it could help facilitate the rapid development of an efficient treatment plan even before the symptomatic clinical onset of the disease. Historically, the diagnosis of AD

has relied on cognitive assessments and, ultimately, post-mortem histopathological confirmation. However, research in recent decades has witnessed rapid advancements in the identification of *in vivo* biomarkers, especially those reflecting amyloid-beta (A β) deposition, tau pathology, synaptic dysfunction and neuroinflammation, all of which are hallmarks of AD (Georgakas et al., 2023). Techniques such as positron emission tomography (PET), cerebrospinal fluid (CSF) assays, and blood-based biomarkers have shown increasing sensitivity and specificity in the past years. These are emphasised in research done on both animal (rat and primate) models and humans. Hansson et al. (2023), for example, states that blood-based biomarkers are an exceptionally promising avenue for revolutionizing the clinical practice of diagnostic and prognostic work-up of Alzheimer's disease. Anoop et al. (2010) explores the significant recent advancements in CSF biomarkers for monitoring the early stages of AD before severe cognitive dysfunction is apparent. Márquez & Yassa (2019) also suggests a need for composite biomarkers in neuroimaging, as this can provide a deeper understanding of the pathophysiology of AD. Although the future of early diagnosis looks bright, it is undeniable that translational hurdles still persist, which have limited the availability of these diagnostics to certain communities due to factors like socioeconomic differences, standardisation, and education of clinical staff. This literature review investigates how the neurobiological foundations of Alzheimer's disease biomarkers inform their utility, analysing the key translational challenges that hinder their integration into routine clinical practice.

Methodology:

The review is structured into five main sections. First, the molecular and cellular neurobiology underpinning key AD biomarkers will be discussed, with a particular focus on the amyloid and tau hypotheses, neuroinflammation, mitochondrial dysfunction, and synaptic and neurotransmitter alterations. Next, it surveys the primary modalities used in biomarkers, their technical strength and mechanistic correlates. The third section discusses more novel biomarkers, and the fourth examines translational challenges, including issues of socioeconomic accessibility, standardisation and technical limitations. Finally, the review evaluates the ongoing efforts in bridging the lab-to-clinic divide.

With a critical synthesis approach, this review analyses peer-reviewed literature across neuroscience, clinical neurology and translational medicine. It integrates findings from empirical studies, along with meta-analyses and systematic reviews to identify emerging consensus and unresolved tensions, such as the accuracies of specific biomarkers, within the field. A targeted narrative review of human and translational studies was conducted on Alzheimer's disease biomarkers published 2015 to 2025 in PubMed and Scopus, using terms combining *Alzheimer's disease* with *biomarker*, *blood*, *CSF*, *MRI*, *PET*, *retina*, *saliva*, *urine*, and *volatile organic compounds*. Inclusion prioritised peer-reviewed original research, along with systematic review and consensus statements. Seminal pre-2015 studies were retained selectively to provide historical context. Non-peer-reviewed web sources were excluded except where clearly identified as illustrative, such as information about cost ranges.

Section 1: Molecular and cellular neurobiology and pathophysiology of AD

AD is characterised by the slow, progressive neurodegeneration brought on by neuronal cell death and activity dysfunction. This usually starts in the entorhinal cortex (part of the hippocampal formation), an area crucial for various forms of explicit memory formation, particularly spatial memory (Karimani et al., 2024). The pathophysiological hallmarks of AD are accumulations of abnormal neuritic plaques and neurofibrillary tangles (NFTs). With these changes, there is also a loss of cholinergic neurons in the basal forebrain and neocortex (Kumar et al., 2024). There are two main hypotheses that are popular within the scientific community: the cholinergic hypothesis and the amyloid hypothesis. This literature review focuses on the amyloid hypothesis, as it is currently the most widely accepted pathophysiological mechanism for AD. However, it should be noted that this hypothesis has also faced challenges and criticisms, and it is believed that there are alternative or complementary mechanisms in the pathogenesis of AD (Nasb et al., 2024).

Amyloid beta

The peptide amyloid beta ($A\beta$) is derived from a protein called the amyloid precursor protein (APP), through the abnormal activity of β - and γ -secretase enzymes, which are responsible for breaking down proteins (Mucke & Selkoe, 2012). Typically, APP is cleaved by either α - or β -secretase and the smaller fragments formed are not neurotoxic. However, the cleavage by β - followed by γ -secretase results in a peptide of 42 amino acids, known as $A\beta_{42}$, which is a long peptide more prone to aggregation than shorter species. $A\beta$ oligomers, which are smaller aggregates of these $A\beta$ peptides, are considered especially neurotoxic. Increase in $A\beta_{42}$ levels leads to the aggregation of amyloid, creating the commonly seen abnormal neuritic plaques (Nhan et al. 2015). These are spherical microscopic lesions made of an extracellular $A\beta$ peptide surrounded by enlarged axonal endings, and are usually found within the cortical gray matter or around the meningeal and cerebral vessels of the brain. These deposits initially form in different areas, but grow and merge to comprise the larger more recognisable plaque structures, which then lead to neuronal toxicity (Kumar et al., 2024). This is caused by $A\beta$ plaques damaging mitochondria as they accumulate inside neuronal cells, impairing its ability to generate ATP. Without sufficient energy, the neuron cannot maintain its normal function, leading to the initiation of apoptosis (programmed cell death). Furthermore, $A\beta$ oligomers can insert themselves into the cell membrane of neurons, forming pores that allow for the unregulated influx of calcium ions, disrupting the balance of calcium concentrations which is critical for signalling and cell survival. High calcium levels within the cell can activate enzymes that degrade proteins and lipids, leading to the breakdown of the neuronal cytoskeleton, and further mitochondrial dysfunction (Fani et al., 2021).

Certain mutations, specifically those in the genes encoding APP and secretases (e.g. presenilin 1 and 2 (PSEN1 and PSEN2)) have been linked to cases of familial AD, supporting the $A\beta$ hypothesis. This is because these mutations increase the production of longer and more aggregation-prone isoforms of $A\beta$ (Lanoiselée et al., 2017). Another gene is the

apolipoprotein E (APOE) gene, which is the “strongest genetic risk factor for late-onset AD”, according to Kamatham et al. (2024), as it is associated with producing proteins that lead to higher levels of A β aggregation and simultaneously reduced clearance of the peptide.

Tau tangles

The aggregation of A β leads to a cascade of events. Tau is a protein that stabilises axonal microtubules which are vital for intracellular transport. One of these cascade events is the hyperphosphorylation of tau (the addition of too many phosphate groups which modifies its shape) through the activation of kinases like glycogen synthase kinase-3 β (GSK3 β), causing tau to become misfolded and aggregate within neurons (Kumar et al., 2024). With this, these tau molecules become prone to self-aggregation and form insoluble twisted paired helical filaments. Known as NFTs, they first appear in the hippocampus. Recent research has supported the idea that tau aggregates have prion-like properties, allowing their propagation throughout the brain by inducing the misfolding of normal tau in neighbouring neurons (Alyenbaawi et al., 2020). This may explain why tau is often seen spreading throughout the cerebral cortex as AD progresses, contributing to cognitive decline in affected individuals.

Inflammation

Both A β peptides and NFTs can trigger inflammatory responses within the brain by activating microglia and astrocytes. These cells then become ramified and abnormally enlarged (hypertrophied) in NFT-rich brain regions (Kamatham et al., 2024). The immune responses are triggered to clear these harmful substances through the release of pro-inflammatory molecules. However, while this process aims to protect the brain, chronic and dysregulated inflammation produces proinflammatory cytokines that lead to neuronal damage and exacerbate the progression of the disease (Adamu et al., 2024). This sustained state of inflammation can exacerbate neuronal damage and further promote the spread of tau pathology.

Mitochondrial Dysfunction

A β peptides have also been shown to impair mitochondrial function by accumulating within the inter-mitochondrial membrane space and disrupting their normal function (Aran & Singh, 2023). This causes a reduction in ATP production and an increase in ROS generation, leading to oxidative stress and eventual damage to the organelle and its normal activity. This then triggers a positive feedback loop, as impaired mitochondrial function further promotes A β production and aggregation. In addition to this, abnormally phosphorylated and aggregated tau proteins have also been implicated in causing mitochondrial dysfunction by impairing mitochondrial transport along neuronal axons, which consequently leads to local energy deficits (Reddy et al., 2012). It also disrupts fission and fusion processes, which are crucial for maintaining a healthy mitochondrial network (Zong et al., 2024). This also triggers a cycle, where mitochondrial dysfunction results in the formation of even more tau aggregates.

Synaptic and neurotransmitter alterations

Both protein aggregates of A β and tau prevent regular synaptic function by hindering the release of neurotransmitters (including acetylcholine, which plays an important role in memory and cognition), reducing synaptic density and interferes with synaptic plasticity, particularly in cholinergic neurons (Kamatham et al., 2024). Consequently, this causes signaling deficiencies, contributing to the memory impairment and cognitive decline associated with AD. Glutamate neurotransmission is also affected, as glutamate receptors are excessively activated, contributing to excitotoxicity, which then leads to neuronal damage and cell death. As the membranes of these axonal cells are disrupted and the cells die, the intracellular contents, which contain essential structural components including neurofilaments (NfLs), leak out into the surrounding fluid (Gafson et al., 2020). This release can activate an inflammatory response in the surrounding tissue, and the following inflammatory cascade can contribute to further neurodegeneration.

Environmental Factors

Although novel, it has been posited that bacterial and viral infections may be implicated in the process of AD pathogenesis, as different types of microbes have been shown to stimulate A β aggregation and deposition. Therefore, there may be a pathogenic factor in the onset of AD as well (González-Sánchez et al., 2020).

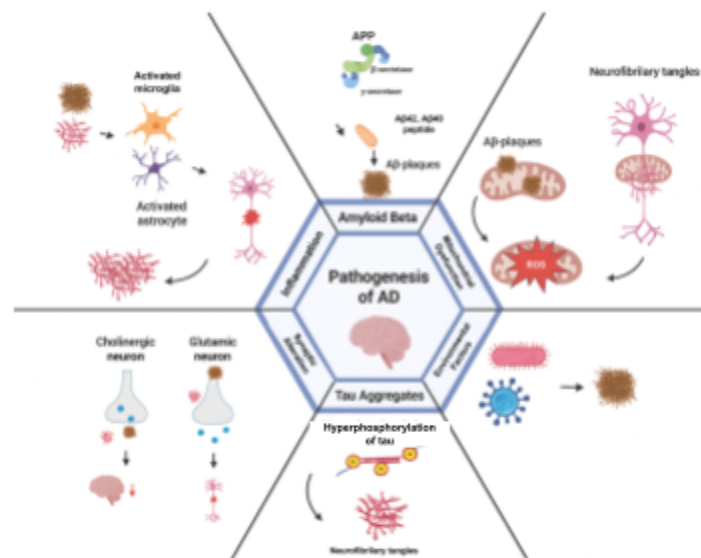


Figure 1. Diagram of the proposed forms of pathogenesis of AD

All the different pathologies of AD are largely thought to be dynamically interacting rather than isolated, as supported numerous studies, a few of which are Kamatham et al. (2024), Calabrò et al. (2020), and Kumar et al. (2024) suggesting that biomarkers that are able to capture multiple axes of degeneration may be most effective in diagnosing the disease.

Section 2: Types of Biomarkers and How They Work: Established Bioarkers

Biomarkers are defined as being naturally occurring molecules, genes, or characteristics by which a particular pathological or physiological process or disease can be identified. This literature review identifies three types of biomarkers typically employed in the diagnosis of AD: molecular, imaging and functional. Molecular biomarkers include proteins or other biological compounds found through the testing of substances like blood, cerebrospinal fluid (CSF) and other bodily fluids. Imaging biomarkers include scans like Positron Emission Tomography (PET), Magnetic Resonance Imagery (MRI) and Computed Tomography (CT), while functional biomarkers are measured through performance on cognitive or motor assessments and tasks. Usually, biomarkers are employed to determine the cause of mild cognitive impairment (MCI) in the initial stage of dementia. This literature review further investigates promising new biomarkers that have shown potential in predicting AD.

MOLECULAR

CSF

Cerebrospinal fluid is an ideal candidate for reflecting the molecular events of the brain, as it is in direct contact with the brain's extracellular space. Hence, it seems to be the general consensus that CSF has one of the highest potentials to identify AD biomarkers that reflect AD pathogenesis (Papaliagkas et al., 2023).

Because A β 42 is a large component of typical AD plaques, the amount of total A β in CSF is well-correlated with AD diagnosis. There is a positive correlation between lower CSF A β 42 levels and plaque burden, due to plaques acting as “amyloid sinks”, where the peptide is deposited and accumulates (Anoop et al., 2010). Anoop et al. also found a correlation between a rise in A β 38 levels and a fall in A β 42, which supports the idea that the ratio A β 42:A β 38 may be more useful and precise in diagnosing AD. Reduced A β 42 has also been observed in other types of dementia, and many works and wider literature agrees that A β 42 is the most accurate CSF biomarker of AD in patients with MCI (Sonnen et al., 2011). However, there was no significant difference observed in the *total* CSF A β levels in AD patients and non-AD controls in Anoop et al.'s study. Therefore, it seems that only A β 42 is a relatively accurate predictor of the disease.

Increased levels of tau and phosphorylated tau (p-tau) in CSF has been shown to be correlated with AD onset, with several studies suggesting that total tau (t-tau) concentration in AD patients' CSF is higher than the control (Papaliagkas et al., 2023). However, it is noted that this biomarker, although sensitive, is limited in its ability to differentiate AD from other forms of dementia, as an increase in t-tau is not a feature unique to AD. Hence, p-tau is the more accurate biomarker, because it is more closely related to the pathophysiology of AD. This is compared to t-tau which is simply a marker of axonal damage.

Levels of glial fibrillary acidic protein (GFAP) tend to be increased in individuals with early A β pathology, as it is hypothesised to reflect the increased reactive astrocytes, which are a characteristic of AD (Marksteiner & Humpel, 2025). This can be measured reliably in the CSF, as shown by Cicognola et al.'s 2021 study. Plasma NfL levels also correlate well with the levels of NfL found in CSF, as it leaks from cells due to injury (Gisslén et al., 2015).

Another potential biomarker is neuronal threat proteins (NTPs). These are expressed in the brain during AD-related neurodegeneration, with abnormal AD7c-NTP being an NTP that is especially expressed during this process. This has been found to be higher in the postmortem CSF of AD individuals compared to controls (Kang & Wang, 2022).

Blood

As A β 42 and A β 40 peptides accumulate within the brain, plasma levels of individuals with AD are typically indicative of this change. The plasma A β 42:A β 40 ratio is usually higher in A β 42-positive groups than in A β 42-negative groups, as has been demonstrated by different immunoassays and mass spectrometry-based assays (Doecke et al., 2020). However, different assays vary substantially, with a recent comparison showing that certain mass spectrometry-based assays were far better at identifying A β pathology than more commonly used immunoassays. Regardless, these higher-performing A β 42:A β 40 assays still only exhibit modest correlations between the levels of plasma A β and that found in CSF, likely due to the fact that A β in plasma is derived from peripheral sources, as posited by Hansson et al. (2023). Hence, it is agreed that plasma A β 42 levels alone cannot be considered an AD marker.

On the other hand, researchers have recently developed several high-sensitivity assays for the detection of different p-tau protein variants, or isoforms, including specific ones like p-tau181, p-tau231 and p-tau217 (Janelidze et al., 2025; Khalafi et al., 2024; Nabizadeh et al., 2022). When compared, it seems that assays quantifying plasma p-tau217 have been shown to be better at detecting AD pathology and predicting the development of AD dementia (Wang et al., 2025). However, it can be argued that p-tau231 is a good marker of AD in its early stages, as it starts increasing at very low A β levels (Ashton et al., 2022). Hence, when combining plasma A β 42 and A β 40 with p-tau, early-stage amyloid positivity detection could be improved.

Similarly, GFAP can also be measured reliably in the blood as well as in the CSF. Plasma NfL levels also correlate well with the levels found in CSF, although it seems that this correlation is not as strong as that of p-tau217 (Garcia-Escobar et al., 2024). This study has also shown little to no correlation between levels of NfL and A β 42 in the blood, which has led researchers to believe that NfL suggests neurodegeneration independently of the pathology of A β .

Janelidze et al. (2022)'s study has shown that the combination of plasma p-tau231 and A β 42:A β 40 could be used to detect very early stage AD, and it is supported by the fact that

it was able to detect amyloid pathology in cognitively unimpaired individual. This could prove useful as it would allow screening tests to be able to identify AD before the clinical onset of symptoms.

Saliva

Saliva is not only an easily accessible body fluid; its secretion and composition might also be affected by various diseases. It is posited that AD can influence salivary contents, as there seems to be a relationship between the brain and saliva. This occurs through six pathways connecting brain molecules with the saliva and vice versa (Surdu et al., 2025). However, for both A β and tau, concentrations are considerably lower in non-plasma fluids (Delaby et al., 2023).

A review by Nijakowski et al. (2024) showed that there was a statistically significant number of studies that have examined the saliva of AD patients and found elevated levels of A β . However, it was noted that in three studies, neither A β 40 or A β 42 were detected in AD patients. This suggests that there is a question of reliability when using saliva samples to get an accurate measure of A β levels in studies of AD, which is supported by similar findings from Sabbagh et al. (2018).

Based on the same review., it also appears that the reliability of tau as a biomarker for AD is inconsistent when considered alone. There is no consensus on whether t-tau, p-tau and their ratio are reliable indicators of AD. The studies examined by Nijakowski have reported conflicting results, and suggest that while tau may be part of the AD pathology, its biomarker use in saliva cannot be considered a standalone reliable biomarker.

Nijakowski et al. also explores lactoferrin (Lf), a protein with anti-inflammatory properties, and proposes that it may have neuroprotective effects in neurodegenerative diseases like AD. According to González-Sánchez et al. (2020), biomarkers that reflect the integrity of the immune system could be useful. Lf has previously been detected in senile plaques, NFTs and microglia in AD brains. Although it was particularly useful in differentiating AD and MCI patients from healthy individuals, it appears to be a promising, but not fully conclusive biomarker for AD (González-Sánchez et al., 2020). While several studies analysed by Nijakowski et al.'s paper showed a clear link between decreased salivary lactoferrin levels and AD, there are also conflicting findings that appear to warrant further investigation (Yang et al., 2021).

Urine

Both A β and tau are possible to detect in urine, because they can be eliminated from the organism by action of the kidneys (Armenta-Castra et al., 2024). These are detected through methods like enzyme-linked immunosorbent assay (ELISA) and Western blot. Several reviews like Kamatham et al. (2024) and Zhang et al. (2024) have shown a correlation between the presence of these proteins in urine and AD, and it appears that A β oligomers are better indicators of AD progression, instead of the monomers A β 40 and

A β 42. Both p-tau and tau were found to be significantly higher in AD patients compared to control individuals, but as was the case with saliva, these concentrations were much lower in urine than in plasma for both groups (Armenta-Castro et al., 2024).

NTPs have been found in urine, as in the CSF. Evidence for urinary AD7c-NTP is preliminary and replication in contemporary cohorts is limited, leaving clinical utility uncertain. One such piece of evidence is the ELISA results from Ma et al.'s 2016 study utilised ELISA to show that results in urine showed similar sensitivity and specificity to CSF, and that urinary AD7c-NTP was significantly higher than in the non-AD group.

IMAGING

Neuroimaging allows for diagnosis of the AD pathophysiological process during life, even though neuropathology remains the definitive standard. Clinicians can begin to assess cases in vivo, with several modalities having been researched, including MRI and PET (Dang et al., 2023).

Neurodegeneration leads to cerebral atrophy, which is most notable in the hippocampus and entorhinal cortex of patients with AD (Tabatabaei-Jafari et al., 2015). Woodworth et al (2022) found that the level of atrophy in the medial temporal lobe is strongly correlated with lower scores on cognitive testing, and is therefore associated with disease progression. As this degeneration progresses to other cortical structures, lateral ventricles become enlarged in the later stages of the disease. In addition to this, limbic structures (such as the amygdala, cingulate gyrus, thalamus, and olfactory bulb tract) are impacted, and the atrophy leads to volumetric reductions in the frontal, parietal and temporal brain areas (Woodworth et al., 2022).

MRI

MRI is an imaging technique that is able to generate high-resolution images of water-containing tissue. This is done by applying a strong magnetic field and pulses of radio waves that manipulate electromagnetic fields and energy states of protons within the body (Magnetic Resonance Imaging (MRI), n.d.). These protons emit energy, which are then converted to signals and images.

Structural MRIs are used to provide highly detailed images of anatomy as well as a strong gray/white matter contrast. These scans are able to display white matter hyperintensities that indicate demyelination and axonal loss (Meng et al., 2022). Compared to patients without AD, patients with AD have greater white matter hyperintensities in the frontal lobe that index small-vessel disease burden and help in differential diagnosis and staging, but are not necessarily specific to AD.

Diffusion Tensor Imaging (DTI) uses the displacement of water molecules to measure the integrity of the white matter tract. The direction of diffusion allows scientists to better understand tracts and specific fibre bundles that may be impacted by neurodegeneration

(Esrael et al., 2021). The major measures of DTI are mean diffusivity (MD), which is the average rate of water molecule diffusivity, and fractional anisotropy (FA), which is the variability associated with diffusion. In AD brains, MD can be seen in all four areas of the brain (frontal, occipital, parietal and temporal), including the hippocampus. This is a clear difference from MCI, where these increases are not seen in the frontal and occipital regions. AD also presents as decreased FA localised to the cingulum, corpus callosum, uncinate fasciculus and superior lateral fasciculus, as well as throughout the temporal, occipital and frontal white matter (Chandra et al., 2018). Patients with MCI have no irregularities of FA in occipital and parietal areas, which sets MCI scans apart from AD, according to Chandra et al. (2018). According to a study done by Magalhães et al. (2023), there was a significant correlation between white matter tracts and both p- and t-tau.

Functional MRI (fMRI) produces dynamic representations of brain activity through blood oxygen level-dependent (BOLD) signals (Chandra et al., 2018). When asked to perform memory tasks, AD patients showed little to no activation of hippocampal and other medial temporal structures as compared to non-AD individuals (Casagrande et al., 2023). Resting-state fMRIs of the AD brain also show abnormal coactivation of the medial temporal structures and the posterior cingulate cortex, which differs from those of normal brains (Aramadaka et al., 2023).

PET

PET is an imaging modality that allows for the in vivo measurement of disease pathologies using radioligands to detect compounds of interest. In the case of AD, that would be NFTs and A β plaques.

FDG-PET uses 18-F-fluorodeoxyglucose (FDG), which allows researchers to estimate glucose metabolism in different brain areas and identify which ones may have abnormal metabolic activity (Bouter et al., 2019). From studies done, such as Womack et al. (2012) and Schilling et al. (2016), these scans show temporoparietal hypometabolism in patients with AD, which is characteristic of the disease. Furthermore, there was an observed decrease in metabolism in the posterior cingulate cortex and precuneus, which is thought to be related to the episodic memory deficits typical of AD (Villa et al., 2020).

Because of accumulated A β , there is an increased level of amyloid tracer binding in areas of the brain affected by the disease (more specifically, temporal, parietal and frontal regions), according to Villa et al. (2020). Tracers such as Pittsburgh Compound B (PiB) are able to bind to misfolded amyloid plaques, therefore helping to map distribution and quantify amyloid burden. Other tracers include florbetapir (Matsuda et al., 2022), 18-F-labeled flutemetamol (Park et al., 2025) and 18-F-labeled florbetaben (Son et al., 2018).

Part of the underlying pathophysiology of AD includes the neuroinflammatory cascades leading to an increase in microglial activation. Radiotracers that bind to translocator protein-18 kDa (TSPO) have been utilised as biomarkers of AD neuroinflammatory

cascades (Georgakas et al., 2023). Since TSPO is a peripheral benzodiazepine receptor found on the membrane of the mitochondria, increased expression of this protein can be used as an estimate for increased inflammation, as the expression of this protein is upregulated by activated microglial cells. Studies done have found increased TSPO expression levels in individuals with MCI and both prodromal and severe AD stages when compared to healthy controls (Nutma et al., 2021; Zhang et al., 2021).

Machine Learning and Artificial Intelligence

With the rise of better artificial intelligence (AI) algorithms, AI is being increasingly utilised in the diagnosis of AD (Kamatham et al., 2024). AI models are capable of analysing large, complex datasets containing neuroimaging data and biomarker profiles to identify patterns. MRI and PET generate vast amounts of data that can be analysed using AI to detect both structural and functional brain changes associated with AD. Biomarker data can also be incorporated in order to develop predictive models for risk assessment. This holds great potential for improving efficiency and accuracy, since AI models can therefore aid healthcare providers in making increasingly informed diagnostic decisions, facilitating the early detection of AD and the generation of personalised treatment plans based on individual profiles (Chong et al., 2025).

BEHAVIOURAL

Functional

The progressive loss of ability to perform activities of daily living (ADL) unassisted is a primary characteristic of dementia (Chaves et al., 2011). These activities can be divided into basic (BADL) and instrumental (IADL). BADL includes the ability to carry out tasks necessary for personal hygiene, sphincter control and feeding. IADL, on the other hand, are more complex and are activities such as preparing meals, carrying out domestic chores, and managing finances and correspondence.

An example of an assessment used to evaluate changes in functional performance is through an assessment measure known as Functional Assessment Staging (FAST). This allows healthcare practitioners to specifically evaluate these changes throughout the entire course of AD (Sclan & Reisberg, 1992). Another such assessment measure is the Alzheimer's Disease Cooperative Study – Activities of Daily Living for Mild Cognitive Impairment, otherwise known as ADCS-MCI-ADL. This assesses the competence of AD patients in both basic and instrumental ADLs, and can be completed by a caregiver or administered by a clinician as a structured interview with a caregiver (Fish et al., 2011).

Cognitive

The initial and most common presenting symptom of AD is episodic short-term memory loss (Kumar et al., 2024). This includes difficulty retaining new information, and experiencing impairments in problem-solving, executive functioning, judgement, and organisational skills. Because of this, individuals with AD may struggle with multitasking and abstract thinking, which affects their ability to carry out ADLs such as driving,

cooking, and activity planning. Following this, there are often language and visuospatial skill impairments, along with neuropsychiatric symptoms like changes in social behaviour (Kumar et al., 2024).

A common example of a cognitive assessment is the Mini-Mental State Examination (MMSE) (Arevalo-Rodriguez et al., 2015). This is a common test for assessing changes in cognition, and evaluates functions such as orientation, language, memory and other functions. This is done through tasks that test reading, writing and short-term memory, including tasks like subtraction, remembering a series of unconnected words or copying a diagram on paper.

The Alzheimer’s Disease Assessment Scale - Cognitive, or ADAS-Cog, is generally more thorough, and is considered the gold standard for assessing AD (Dementia Australia, n.d.; Kueper et al., 2018). This includes 11 tasks, including both tests completed by the subject and assessments completed by the observer. When put together, these tasks assess memory, language, and praxis. Specific tasks, like the MMSE, include word recall, naming objects, commands, and word recognition among others.

As can be seen by this, there already exists a plethora of widely acknowledged and effective biomarkers, whether they be molecular, imaging or functional. A summary of the above information can be found in Table 1.

Table 1. Summary of Key Biomarkers and Diagnostic Modalities for Alzheimer’s Disease (AD)

Category	Subcategory	Biomarker / Method	Key Findings	Work Cited
Molecular	CSF	Aβ42	Lower levels correlate with plaque burden; most promising CSF biomarker for AD in MCI (Kumar et al., 2024)	Anoop et al., 2010
		Aβ42:Aβ38 ratio	May improve diagnostic precision compared to Aβ42 alone (Doecke et al., 2020)	Doecke et al., 2020
		t-tau	Elevated in AD; sensitive for neurodegeneration	Papaliagkas et al., 2023
		p-tau	More closely related to AD pathology than t-tau	Janelidze et al., 2025; Ashton et al., 2022
		GFAP	Increased with early Aβ pathology; reflects reactive astrocytes	Marksteiner & Humpel, 2025
		NfL	Elevated; marker of axonal injury and neurodegeneration	Garcia-Escobar et al., 2024

		AD7c-NTP	Higher in postmortem CSF of AD patients (emerging biomarker)	Kang & Wang, 2022
	Blood	A β 42:A β 40 ratio	Lower in A β -positive groups; better with mass spectrometry	Hansson et al., 2023
		p-tau217	Strong predictor of AD pathology and progression	Khalafi et al., 2024
		p-tau231	Early-stage AD marker (increases at low A β levels)	Nabizadeh et al., 2022
		p-tau181	Widely adopted, earlier generation with lower AD specificity	Janelidze et al., 2025
		GFAP	Detectable in blood plasma; reflects astrocyte activation	Garcia-Escobar et al., 2024
		NfL	Detectable in blood plasma; less correlated with CSF than p-tau217	Janelidze et al., 2022
		Combined p-tau231 + A β 42:A β 40	Detects very early-stage AD with improved accuracy	Hansson et al., 2023 Janelidze et al., 2025
	Saliva	A β	Some studies show elevated levels in AD	Sabbagh et al., 2018
		t-tau, p-tau	Conflicting results; unreliable as standalone biomarkers	Nijakowski et al., 2024
		Lactoferrin	Lower in AD; potential immune-related biomarkers	González-Sánchez et al., 2020
	Urine	A β oligomers	Better indicators of disease progression than monomers	Armenta-Castro et al., 2024
		p-tau, tau	Higher in AD patients than controls	Armenta-Castro et al., 2024
		AD7c-NTP	Elevated; similar sensitivity/specificity to CSF assays	Ghanbari et al., 1998
Imaging	MRI	Structural MRI	Hippocampal and entorhinal atrophy; white matter hyperintensities	Tabatabaei-Jafari et al., 2015 Meng et al., 2022
		DTI	Increased MD and reduced FA in specific tracts; correlates with tau pathology	Esrael et al., 2021
		fMRI	Reduced hippocampal activation; altered resting-state connectivity	Chandra et al., 2018
	PET	FDG-PET	Temporoparietal hypometabolism; PCC/precuneus deficits	Bouter et al., 2019

		Amyloid PET	PiB and other tracers map amyloid burden in vivo	Villa et al., 2020
		TSPO PET	Detects neuroinflammation via microglial activation	Georgakas et al., 2023
	AI/ML Integration	AI models	Detect structural/functional changes; integrate biomarkers for improved prediction	Kamatham et al., 2024
Functional	Behavioural	BADL/IADL assessments (FAST, ADCS-MCI-A DL)	Measures decline in daily functioning	Chaves et al., 2011 Sclan & Reisberg, 1992 Fish et al., 2011
	Cognitive	MMSE/ADAS-Cog	Screen orientation, memory, language; widely used cognitive assessments	Kumar et al., 2024 Arevalo-Rodriguez et al., 2015 Kueper et al., 2018

Abbreviations:

A β = amyloid-beta; APP = amyloid precursor protein; APOE = apolipoprotein E; ApoC3 = apolipoprotein C3; AT(N) = amyloid, tau, neurodegeneration framework; BBM = blood-based biomarker; CSF = cerebrospinal fluid; DTI = diffusion tensor imaging; FA = fractional anisotropy; FDG-PET = fluorodeoxyglucose positron emission tomography; fMRI = functional magnetic resonance imaging; GFAP = glial fibrillary acidic protein; IADL = instrumental activities of daily living; Lf = lactoferrin; LncRNA = long non-coding RNA; MCI = mild cognitive impairment; MD = mean diffusivity; miRNA = microRNA; MRI = magnetic resonance imaging; MCP-1 / CCL2 = monocyte chemoattractant protein-1 (chemokine ligand 2); NfL = neurofilament light chain; NFTs = neurofibrillary tangles; NTPs = neuronal thread proteins; OCT = optical coherence tomography; PET = positron emission tomography; p-tau = phosphorylated tau; RNFL = retinal nerve fiber layer; ROS = reactive oxygen species; SIMOA = single molecule array; SUVR = standard uptake value ratio; TSPO = translocator protein (18 kDa); VOC = volatile organic compound.

Section 3: Types of Biomarkers and How They Work: Novel Bioarkers

While traditional biomarkers have focused on the more established hallmarks of AD, such as A β and tau, recent research has expanded into a more diverse spectrum of novel biomarkers that may serve to capture alternative aspects of the disease's pathology. These include new developments in both molecular and imaging biomarkers as technological advancements occur.

MOLECULAR

MicroRNA

Micro ribonucleic acids (miRNAs) are a type of short-length, non-coding, and single-stranded endogenous RNAs (also known as sncRNAs) found in a stable form in the blood, CSF, and various body fluids (Chimthanawala et al., 2023). By controlling the expression of genes and pathways, miRNAs act through suppressing the translation of target messenger RNAs (mRNAs) and facilitating their degradation, contributing to APP

degradation and A β metabolism. AD patients had higher levels of miRNA-4722-5p and miRNA-615-3p, and combining these two AD-associated miRNAs provided a better diagnostic score than either miRNA alone (Liu et al., 2022). miRNA-20b-5p also downregulated APP levels, hence reducing the availability of A β (Wang et al., 2022). However, further research in this domain needs to be carried out, especially on three miRNAs that strongly correlated with the early stages of AD, namely miRNA-92a-3p, miRNA-486-5p, and miRNA-29a. Levels of miRNA-92a-3p and miRNA-486-5p were lower in AD patients compared to controls, but higher levels of miRNA-29a-3p were found in the plasma of AD and MCI patients (Peña-Bautista et al., 2022). The same source also found that plasma levels of miRNA-483-5p levels were higher in AD patients compared to healthy controls, and it was concluded that increased levels of miRNA-483-5p in plasma can reliably distinguish both MCI and AD groups from the healthy controls. The above findings suggest that these miRNAs could be potential biomarkers in early AD diagnosis.

Long non-coding RNA

A second type of RNA that can be used is long non-coding RNAs (LncRNAs). These resemble mRNA molecules, but are not responsible for encoding proteins (Black et al., 2024). They typically contribute to the regulation of several processes by controlling chromatin remodelling, and providing alternative mRNA splicing. Black et al. (2024) has found that this affects gene expression, and has been shown to “directly or indirectly regulate the key features of AD including A β deposition, aberrant tau formation, oxidative stress, neuroinflammation, and neuronal death”. LncRNAs also interact with miRNAs, therefore influencing AD-related neuropathology. The expression of numerous types of these molecules has been detected in plasma and CSF samples from AD patients, which indicates that LncRNAs could function as a likely biomarker in the diagnosis and progression of AD (Black et al., 2024). Specific candidate LncRNAs include BC200, NEAT1, BACE1-AS, and 51A, which were both elevated in AD patients, as found in a review by Wu et al. (2024).

Apolipoprotein C3

A study done by Watanabe et al. (2020) suggests that Apolipoprotein C3 (ApoC3) may be a potential urinary biomarker for AD, although further analysis of this is warranted (Watanabe et al., 2020). ApoC3 is an abundant apolipoprotein mainly present in triglyceride-rich lipoproteins (chylomicrons and very-low-density lipoprotein (VLDL)), as well as cholesterol particles. Watanabe et al. showed that studies have reported that people with AD had lower plasma levels of ApoC3 compared to healthy individuals, and that higher ApoC3 was associated with reduced grey matter volume in the brain. The paper also pointed to recent research suggesting that ApoC3 acts as a trigger for inflammation by activating the NLRP3 inflammasome (Watanabe et al., 2020). This is significant because the NLRP3 inflammasome is known to be activated in the brains of AD patients by amyloid beta. The result of Watanabe et al.'s experiment was that all samples revealed ApoC3 levels to be significantly higher in the AD group relative to the control group.

MCP-1

Monocyte Chemoattractant Protein 1 (MCP-1, also known as CCL2) is a protein that belongs to a family of signalling molecules called chemokines (Huang et al., 2023). They play key roles in the body's inflammatory response by attracting immune cells to sites of inflammation. As a biomarker, MCP-1 can be measured in both blood and urine. In AD, neuroinflammation contributes to neuronal damage. It is thought that it is because of this that elevated levels of MCP-1 have been found in the blood and CSF of AD patients. However, this is found to varying extents across different studies (Fenoglio et al., 2004; Galimberti et al., 2006), which is why Huang et al. hypothesised that MCP-1 levels impact individuals who are genetically at risk of developing AD.

Molecules Associated with Oxidative Stress

A β induces oxidative stress, the production of harmful reactive oxygen species (ROS), which is thought to be a major contributor to cellular damage in the brain (Gella & Durany, 2009). ROS are too unstable to measure directly, so measuring their byproducts is a method used to quantify oxidative stress. Several products, specifically 4-hydroxy-2,3-noneal (HNE), acrolein, F2-isoprostanes and malondialdehyde have been observed to exist in higher levels in AD brains (Singh et al., 201). However, like many other biomarkers, oxidative stress is a general sign of cellular damage and is not necessarily specific to AD. It is a feature of many neurodegenerative disorders and is also affected by ageing and environmental factors (Dash et al., 2025).

Volatile Organic Compounds

Exhaled breath contains hundreds of different byproducts of metabolism, also known as volatile organic compounds (VOCs). In AD, metabolic changes in the brain could produce a unique "breathprint" (Zhang et al., 2025). Using sophisticated sensors or mass spectrometers, analysis of this breathprint can be carried out to identify patterns that correlate with this disease. Zhang et al. (2025) identifies six key candidate VOCs of interest, including ethanol, isopropanol, chloroethane, pyrrole, 1-butanol and benzene. However, this research is still in its early stages, and whether it can be used for AD diagnosis is unclear.

IMAGING

Retinal Imaging

The past decade has seen rapid progress in using retinal imaging to find markers of AD. Techniques like optical coherence tomography (OCT) are shown to be especially promising because of their non-invasive, affordable and accessible nature when compared to traditional brain imaging (Georgakas et al., 2023). Research has shown structural, vascular and neuropathological changes in the retinas of both mice and humans with AD. Structural markers, such as the thinning of the retinal nerve fibre layer (RNFL) are consistently seen in older adults with AD (Sánchez-Puebla et al., 2024). This thinning may start early in disease progression, as it has been linked to levels of A β and tau in CSF, as well as to

hippocampal volume in cognitively normal adults (Chen et al., 2023). This suggests its usefulness as a tool for early AD screening. Vascular changes are also being explored, as a 2013 study by Frost et al. found that asymptomatic adults with high cerebral A β had less-branched retinal blood vessels. Vagiakis et al.'s 2024 study found that a larger foveal avascular zone (FAZ), which is the area of the retina without blood vessels, was typically associated with higher cerebral A β in cognitively normal adults. This suggests that the FAZ area, along with retinal imaging in general, may be an effective marker for detecting preclinical AD (Vagiakis et al., 2024). However, Ashraf et al. (2023) has found weak evidence, suggesting that research in this area is currently yielding mixed results, with some finding strong correlations and others not.

CT

A CT scan uses a series of X-rays from different angles to generate cross-sectional images of the brain. While it cannot visualise amyloid plaques or tau tangles directly, it is able to detect brain atrophy of regions such as the hippocampus (Pasi et al., 2011). However, it is only really supportive when a patient presents with the typical patterns of AD (i.e. it is not specific to AD and may also appear in other conditions). It may also prove more useful only for later-stage AD, as brain atrophy usually cannot be detected until patients begin to exhibit behavioural and cognitive changes. In addition to this, CT scans involve using ionizing radiation which, though at a low dose, carries a small risk, especially for people undergoing repeated scans.

Tau PET

Tau PET molecular imaging is a relatively new technology. So far, there is a first-generation FDA-approved tracer, known as 18-F-flortaucipir, and has been shown to have a high affinity for detecting the tau aggregates that are characteristic of AD (Petersen et al., 2022). However, off-target binding is an obstacle in this procedure, where tracers bind to molecules other than tau, producing unreliable results in a PET scan. This is why there are ongoing efforts to develop second-generation tracers, which are being designed to have improved binding specificity (Bischof et al., 2021). These second-generation tracers include 18F-RO-948, 18F-MK-6240 and 8F-PI-2620, to name a few included in Bischof et al. (2021)'s review.

Most non-CSF, non-blood matrices remain exploratory, but from current research, it is clear that many of them warrant significant potential to become alternative biomarkers. Some may even prove to be more advantageous than traditional biomarkers due to their cost-effectiveness and accessibility, as well as their specificity and accuracy.

Section 4: Translational challenges and barriers

Data standardization and comparability represent a critical challenge in both imaging data collection and biomarker fluid acquisition protocols that can vary considerably across different Alzheimer's Disease Research Centers (ADRCs) (Braudeau et al., 2025). This

variability complicates the establishment of universal diagnostic thresholds and hinders the direct comparability and integration of findings from diverse research cohorts, making it difficult to generalize results and apply them uniformly in clinical practice.

Consequently, even if a biomarker demonstrates exceptional scientific efficacy, its practical clinical utility is severely constrained if it cannot be widely deployed across diverse healthcare settings. This accessibility-utility gap highlights pressing health equity concerns in AD diagnosis, driving the urgent need for less invasive and more affordable alternatives, even if those alternatives present their own set of challenges.

IMAGING

High cost and limited accessibility are paramount challenges. Imaging costs can vary widely across settings, but illustrative ranges suggest substantial patient-level expense, with an MRI costing up to \$8,400, and a PET scan up to \$3,000 in some countries (Imaging Technology News, 2018). This substantial cost, coupled with the requirement for specialized equipment, trained personnel, and dedicated imaging facilities, restricts their availability, particularly in underserved regions and low-resource settings. This creates a significant barrier to widespread clinical use, contributing to health inequities in AD diagnosis.

Patient burden and contraindications also pose practical limitations (Decazes et al., 2021). Since PET scans involve a small dose of radiation, patients may be advised to avoid this scan if they are pregnant. This radiation also carries the small, but increased, lifetime risk of developing cancer. Both MRI and PET scans require patients to remain still in enclosed spaces for extended periods, which can cause discomfort or claustrophobia (Munn et al., 2015). Furthermore, patients with certain medical devices or metal implants, such as pacemakers, may have contraindications for MRI due to the powerful magnetic fields involved (Ghadimi et al., 2023).

PET

Cost and reimbursement represent major practical barriers to the widespread clinical adoption of amyloid PET (Lee et al., 2021). These scans are expensive, typically costing between \$3,000 and \$6,000. In the USA, third-party payers often do not reimburse for amyloid PET, citing a need for additional data demonstrating that the scan results directly influence clinical care and patient-based outcomes. This lack of coverage significantly restricts access for many patients.

Pittsburgh Compound B (PIB) utilises carbon-11, which has a very short half-life of 20 minutes, naturally limiting its clinical applicability due to logistical challenges (Wolk et al., 2012). While more clinically viable F18-labeled tracers with a longer half-life of 110 minutes have since been developed, the cost and accessibility issues persist (Trotter et al., 2023). Additionally, U.S. tracer labels and clinical practice typically use a binary (positive or negative) interpretation for amyloid PET results, which may oversimplify the continuous

biological data and potentially obscure nuanced information about amyloid burden (Suppiah et al., 2019).

The designation of amyloid PET as a “gold standard” for detecting amyloid pathology is important to consider in context. While it excels at identifying the presence of amyloid plaques, a necessary component of AD, the presence of these plaques alone is not sufficient for a definitive AD diagnosis (Roberts et al., 2015). Amyloid plaques are found in other conditions, and also in a substantial proportion of cognitively normal individuals. Furthermore, the overall amyloid burden, as assessed by PET, does not consistently correlate strongly with cognitive deficits (Villemagne & Rowe 2011). This implies that a positive amyloid PET scan indicates the presence of amyloid pathology, but it does not definitively confirm symptomatic AD or establish amyloid as the sole cause of cognitive impairment.

MRI

In the very early stages of AD, an MRI scan of the brain may appear normal, making it less sensitive for preclinical detection compared to molecular imaging techniques (van Oostveen & de Lange, 2021). While it provides detailed structural changes, it cannot solely confirm the presence of AD pathology; comprehensive assessments combining MRI findings with clinical evaluations, cognitive testing, and patient history are essential for accurate diagnosis. Fundamentally, MRI primarily reflects neuronal loss and brain atrophy, which are consequences of amyloid and tau pathology, rather than directly visualizing these protein aggregates themselves, meaning it is less sensitive to preclinical pathology than molecular biomarkers. Emerging techniques like fMRI and DTI hold significant promise, but their widespread standardization and established clinical utility for routine AD diagnosis are still under development. (Chandrasekar et al., 2025)

MOLECULAR

CSF

Despite how thoroughly characterised and well-established CSF markers for AD are, there are still significant limitations associated with these biomarkers, primarily revolving around the invasiveness of the lumbar puncture procedure used to procure the fluid (Inekci et al., 2015). The discomfort and potential risks, although rare, including nerve damage, infection and herniation, are currently primary drivers for the ongoing search for less invasive diagnostic alternatives.

Blood

Compared to CSF, blood-based biomarkers are more appealing because they tend to be significantly less invasive in nature and more cost-effective extraction methods (Mielke et al., 2024). However, as aforementioned in this literature review, the challenges include lower biomarker concentrations in blood compared to CSF, and also less achievable accuracy due to complex matrix interference in blood. In addition to this, peripheral

metabolism can significantly affect blood biomarker levels, making them less direct reflections of brain pathology (Janigro et al., 2021).

Emerging Peripheral Biofluids

With research exploring the potential of other bodily fluids like saliva and urine, these can offer a theoretical advantage of maximal non-invasiveness, which makes them highly attractive for broader-scale screening (Kumari et al., 2023). However, this research is still in its nascent stages. Low concentrations of relevant analytes and high biological variability coupled with a significant lack of robust validation in studies limit their establishment for clinical use in AD diagnosis.

OVERALL

Specificity

Even with seemingly AD-specific biomarkers like tau and NfLs, there still exist difficulties in differential diagnoses. The pathologies underlying other types of dementia often involve very similar proteins to the ones used to diagnose AD, which complicates the ability to distinguish between them based solely on biomarker levels (Zou et al., 2020). Simply measuring protein concentrations is unlikely to provide sufficient information to differentiate effectively between various dementias.

Cost

The current landscape of AD research and the development of biomarkers, particularly blood-based biomarkers, is heavily skewed towards high-income countries (Rodda et al., 2025). To achieve equitable progress in the field, it is crucial to actively involve researchers and institutions from low- or middle-income countries (LMICs) in the development, validation, and clinical application of biomarkers. Key challenges remain, including the need to understand the real-world effectiveness, cost-efficiency, and acceptance of these tools in resource-limited environments. Financial constraints are a major barrier to the necessary research and validation in LMICs (Dakhil et al., 2024). Additionally, to effectively roll out biomarkers in LMICs, significant investment is needed to build the necessary infrastructure and upskill the workforce. This could involve financial support from various sources, corporate engagement, and the use of innovative, practical approaches. For instance, using dried blood spots and task-shifting models (i.e. training community health workers to do tasks normally reserved for specialists) where community health workers collect samples could make widespread use more feasible, especially in remote areas (Babulal et al., 2025; Tarawneh, 2020).

Generalisability

Not only are there technical and economic barriers, there exist obstacles in the generalisability of studies due to the lack of access to certain samples and communities of individuals. Most studies, especially plasma biomarker studies, to date have focused on genetically homogeneous populations in high-income countries. This highlights a critical need for large-scale international studies that include diverse populations with varying

genetic backgrounds, comorbidities, and socioeconomic statuses. This is a significant issue because biomarker levels can vary among different racial and ethnic groups and are affected by certain long-term health conditions and medications (Rodda et al., 2025). A 2022 study showed that even though most participants expressed interest in biomarker research for AD, Black participants were comparatively more hesitant than White participants (Elicain et al., 2022). They were more concerned about the risks of participating and felt there were more barriers to undergoing brain scans. These differences remained even when accounting for their trust in researchers and their existing knowledge of AD. The study found that a lack of information was a major barrier for Black participants, while receiving information was a strong incentive. Black older adults expressed a desire for more details about AD, including its risks and prevention, as well as a better understanding of the general research process and specific biomarker procedures. This emphasises that the scientific community has to improve recruitment strategies for underrepresented groups with no prior research experience. The above could be done through refined information sharing and awareness approaches. These could be achieved through increasing the presence of the scientific community in these groups and reducing the financial burden of participation.

Standardisation

A major hurdle for the widespread use of AD biomarkers, and particularly blood-based ones, is a lack of standardization (Humpel & Hochstrasser, 2011). Currently, there are no uniform methods for sample collection, processing, or storage. This problem is compounded by the absence of established procedures to monitor long-term biomarker stability and the lack of certified reference standards. These inconsistencies introduce measurement variations, making it difficult to compare results between labs, establish reliable clinical thresholds, and ultimately use these biomarkers effectively in both research and patient care (Zeng et al., 2024).

Education of Staff

The widespread adoption of biomarkers, despite their accessibility, is significantly challenged by the need for more comprehensive education of primary care clinical staff and the development of clear, actionable guidelines for their use. Without this adequate training and clear protocols, there is a high risk of misinterpretation of results or the inappropriate use of these diagnostic tools (Schöll et al., 2024).

Overall, there still exist many translational hurdles, and it is only if these hurdles are overcome that biomarkers can achieve their full efficacy and potential. Reducing these hurdles also allows more people to access what could potentially be advanced diagnostic tests for AD, bettering treatment plans via early detection.

Section 5: Ongoing efforts of bridging the divide

Early detection of AD could dramatically shift the way treatment is provided to patients. Early detection allows for interventions during the prodromal or mild cognitive

impairment stages, where therapeutic outcomes are more likely to be meaningful as early diagnosis is crucial in informing discussions, care and support, and treatment (Dubois et al., 2023). Moreover, biomarkers are critical not only for diagnosis, but also for other means including patient stratification in clinical trials and the development of potential disease-modifying therapies.

Hence, the limitations and translational challenges confronting AD biomarkers are being actively addressed. For example, significant progress is being made in developing more sensitive and specific assays for AD biomarkers. Ultra-sensitive platforms, such as Single Molecule Array (SIMOA) technology, are enabling the reliable detection and quantification of biomarkers like p-tau and GFAP even if they are present at very low concentrations in biological fluids (Yuan et al., 2025). Other technologies include novel electrochemical sensing platforms like CRISPR/Cas12a-mediated electrochemical aptasensors, which are biosensors that use DNA/RNA molecules to detect proteins (Yuan et al., 2025).

Recognising that no single biomarker is able to provide a complete picture of AD pathology and its progression, there is a growing emphasis on multimodal biomarker approaches and the integration of different data types (Gupta & Iftekhhar, 2024). Combining imaging, fluid, cognitive and demographic data could offer the potential for enhanced diagnostic accuracy. AI and machine learning (ML) in particular are emerging as tools in this context.

Beyond refining extant markers, the field continues to pursue the discovery of novel biomarkers that can provide more specific and comprehensive insights into AD pathology.

Conclusion

Our ability to discover, develop and validate stronger biomarkers is improving, with research on AD biomarkers having progressed considerably over the past decade. Novel molecular, fluid and imaging markers show growing promise for earlier and more accurate detection. Yet, the translation of these biomarkers into widespread clinical use is still obstructed by challenges. Accessibility is limited by high costs and the invasive nature of some procedures, as supported by Georgakas et al. (2023)'s paper stating that the ability to translate treatments to diverse populations is reliant on low cost, minimally invasive and more accessible diagnostic biomarkers in a general clinical setting. Specificity also continues to be a central challenge, as biomarkers often overlap with other neurodegenerative conditions. These obstacles hinder the integration of biomarkers into routine clinical practice, despite their strong scientific potential. Bridging this gap is consequently essential to realising the full diagnostic potential of biomarkers. Achieving low-cost, minimally invasive and widely validated biomarkers tested across diverse populations will not only democratise access but also improve diagnostic accuracy. Ultimately, translation of these scientific advances into clinical use can reshape AD care, enabling earlier detection and therefore earlier and more effective interventions.

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EDITOR COMMENTS AND RECOMMENDATION:

Both reviewers agree that this review manuscript focused on biomarkers of Alzheimer's Disease is comprehensive, relevant, and timely with clear utilization of the most current literature. Reviewer critiques are primarily focused on improving the clarity of the manuscript as well as noting the absence of in-text citations where they are likely needed. Reviewer 1 has also provided a marked PDF of the manuscript with specific comments regarding stylistic suggestions as well as other minor corrections. Reviewer 2 is largely in agreement with this feedback, and also suggests a revision to the conclusion, perhaps in conjunction with an added Limitations section, to better capture the summary of the author's work. Given these critiques, the editor recommends accepting the manuscript following minor revisions.

REVIEWER 1

My recommendation for the review paper named "The Neurobiological Foundations and Translational Challenges of Current and New-Generation Alzheimer's Disease Biomarkers: A Review" is that it should be revised and resubmitted.

Overall, the author has conducted thorough research and produced a well-structured literature review. In most sections the flow is smooth, and the discussion of individual biomarkers is strong. The author demonstrates an ability to present both advantages and limitations of each biomarker, while also offering thoughtful perspectives that reflect a solid grasp of the scientific debates in this area. This critical evaluation strengthens the manuscript considerably and shows maturity in engaging with complex literature. The author also used loads of resources from 2020 onwards, which signify that they engaged with recent literature.

That said, there are several areas that need revision before resubmission. The most pressing issue is the consistent absence of citations across multiple paragraphs. While the author has clearly read widely, it is vital in academic writing to attribute all claims to their sources. This will likely take time to address, but it is non-negotiable for a piece of scientific scholarship. I have included comments throughout the PDF where the author can see where citations should be inserted.

In addition, I have used these comments in the PDF to suggest rephrasing certain sentences for clarity and making minor corrections throughout. Abbreviations also need more careful handling: currently, the author sometimes introduces the abbreviated form before the full name, or switches between them inconsistently. Best practice is to introduce the full name followed by the abbreviation in brackets upon first mention and then use only the abbreviation thereafter (except in headings, tables, or figure legends).

Regarding Table 1, I have recommended changes to improve clarity around the effectiveness of specific biomarkers. I also suggest that the legend be expanded to include full names of all abbreviations used, so that readers can access this information easily without needing to search back through the text.

To be clear, there are no fundamental issues that would justify rejecting this paper. Rather, the manuscript requires several minor revisions, particularly the insertion of citations, which will take time but are essential for academic rigour.

Overall, this is a significant, relevant, and original review that contributes meaningfully to current discussions in the field. Congratulations to the author for their achievement, and I encourage them to use this feedback to polish an already strong paper, even further. Doing so will elevate it to the standards expected of modern scientific writing and serve as a strong foundation for their future academic work.

REVIEWER 2

This is a very solid review of a) established biomarkers believed to play a role in the etiology of AD and b) novel biomarkers that are emerging as potential solutions. The great strength of the review is sections 4 and 5 which cover the difficult topic of how we effectively translate this science to effective clinical treatment. I feel the AD science is well covered. My comments below are intended to provide the author with advice on improving the clarity of the review for the reader. While I provide specific examples for the authors, they should give their review a thorough reading for these points throughout.

1. The introduction feels repetitive with the opening to section 1. Both sections start by summarizing AD in a broad sense. You do this in the Introduction, so it doesn't need repeating in Section 1. Even the Abstract feels like it repeats itself. For example, you don't need the "Hence ..." sentence in the middle because you just said this above.

This sentence has been deleted also in accordance with Reviewer 1's feedback.

2. The Conclusion kind of runs out of gas. Its final sentence notes a limitation when this should be the final summary statement. Take a little time to devote a sentence or two to each section of the paper. You could precede the Conclusions with a limitations section if you like. And then you need a sentence to summarize what you have done here.

The conclusion has been reworded and restructured in order to provide a clearer synthesis of the paper's contents as recommended.

3. Check for clarity throughout. Examples:

- a. Page 7 paragraph 3, "between the levels of A β and that found in CSF" would be clearer if is said "levels of plasma A β "
Fixed.
- b. Page 8 paragraph 2: Your sentence "This suggests that there is some degree of reliability of correlating A β levels with AD." Is not clear. Do you mean "This suggests that there is a question of reliability when using saliva samples to get an accurate measure of A β levels in studies of AD."
Fixed.
- c. Page 3, paragraph 2: the entorhinal cortex is part of the hippocampal *formation*, together with the hippocampus proper, subiculum and dentate gyrus (so not inside hippocampus) – just say part of the hippocampal formation.
Fixed.
- d. MRI is more a measure of blood oxygenation, rather than flow
Fixed.
- e. Add the word "Markers" to your section 2 and 3 titles (after Established and Novel)
Fixed.

4. Check that acronyms for terms are provided on first usage. Examples:

- a. NFTs are defined on page 3, but the full word is used on page 4
Fixed.
- b. activities of daily living (ADL) is not defined on page 11, before using BADL
Fixed.

- c. p-tau is introduced on page 6 but is defined on page 7. (This should probably occur on page 4 where tau is introduced).

Fixed.

5. Check that references are always provided when they should be. Examples:

- a. on page 4, paragraph 2, there is a sentence that starts “Recent research has ...” with no reference that follows.
- b. on page 6, paragraph 4, the sentence “However, there was no significant difference observed in the *total* CSF A β levels in AD patients and non-AD controls.” has no reference.

Fixed.

Fixed.

6. Check for typos. Examples:

- a. last sentence on page 6 ends without a period.
- b. Last word on page 7 should be “individuals”

Fixed.

Fixed.

7. Employ a thesaurus to improve word choices. Example:

- a. Page 21 paragraph 2 “way treatment is given to patients” could be “way treatment is provided to patients”
- b. Page 21 Conclusion “While AD biomarkers are getting better at detecting neurobiological changes, their” could be “While research developing biomarkers to reliably detect neurobiological changes in AD continues to progress, their”

Fixed.

Fixed.

Title: The Neurobiological Foundations and Translational Challenges of Current and New-Generation Alzheimer's Disease Biomarkers: A Review

Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative condition, and remains without a definitive cure despite decades of research. Biomarkers offer promising avenues for early diagnosis and disease monitoring, though their clinical adoption has proven to lag behind laboratory advances. This review synthesises recent literature on the neurobiological underpinnings of both well-known and novel AD biomarkers by drawing on 2015–2025, English, human-centered studies utilising assays, imaging, and assessments to highlight the mechanisms and limitations of current tools of diagnosis. This review also aims to analyse the key translational barriers involved in routine clinical diagnostics, and evaluate the clinical readiness and integration for molecular, imaging and functional

ence, this literature review investigates the neurobiological foundations and translational challenges of current and new-generation Alzheimer's disease biomarkers.

being a strong biomarker for amyloid plaques, p-tau217 outperforming other plasma p-tau epitopes, and new technologies like second-gen tau PET being developed to reduce off-target issues. However, certain technical limitations, AD biomarkers have strong neurobiological mechanistic evidence, but their translation is still impeded by practical and socioeconomic challenges like barriers to access, standardisation and underrepresentation of communities in studies. Addressing and bridging these gaps would

for closing the bench-to-bedside divide in AD care, helping

Keywords: Alzheimer's disease, biomarkers, neurobiology, translational neuroscience, diagnostics

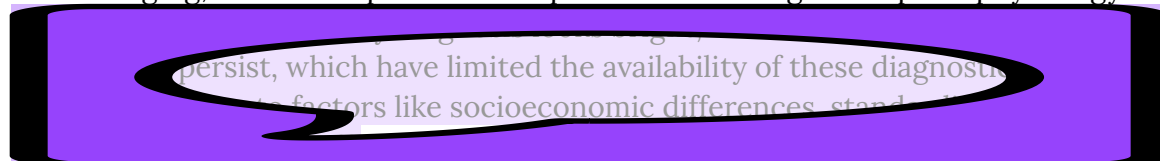
Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. It is characterised clinically by memory loss, impaired cognition, and behavioral changes. With prevalence rising sharply as populations age, AD poses mounting healthcare and socioeconomic challenges.

Despite being one of the most common forms of dementia and neurodegenerative disease, it remains an incurable illness. As of 2021, approximately 57 million people worldwide who have dementia, 60-70% have Alzheimer's (World Health Organisation, n.d.). This greatly affects their quality of life, and also places an enormous burden on caregivers and healthcare systems worldwide, costing economies US\$1.3 trillion globally, with 50% of these attributable to the care given by family members and close friends.

The pursuit of reliable biomarkers for AD has been driven by the need to detect the disease in its earlier preclinical stages, ideally before the onset of severe symptoms. If this is possible, it could help facilitate the rapid development of an efficient treatment plan even

before the symptomatic clinical onset of the disease. Historically, the diagnosis of AD has relied on cognitive assessments and, ultimately, post-mortem histopathological confirmation. However, research in recent decades has witnessed rapid advancements in the identification of *in vivo* biomarkers, especially those reflecting amyloid-beta (A β) deposition, tau pathology, synaptic dysfunction and neuroinflammation, all of which are hallmarks of AD (Georgakas et al., 2023). Techniques such as positron emission tomography (PET), cerebrospinal fluid (CSF) assays, and blood-based biomarkers have shown increasing sensitivity and specificity in the past years. These are emphasised in research done on both animal (rat and primate) models and humans. Hansson et al. (2023), for example, states that blood-based biomarkers are an exceptionally promising avenue for revolutionizing the clinical practice of diagnostic and prognostic work-up of Alzheimer's disease. Anoop et al. (2010) explores the **emerging recent advances** in CSF biomarkers for monitoring the early stages of AD before severe cognitive dysfunction is apparent. Márquez & Yassa (2019) also suggests a need for composite biomarkers in neuroimaging, as this can provide a deeper understanding of the pathophysiology of AD.



Methodology:

This literature review investigates how the neurobiological foundations of Alzheimer's disease biomarkers inform their utility, analysing the key translational challenges that hinder their integration into routine clinical practice.

This review is structured into four main sections. First, the molecular and cellular neurobiology underpinning key AD biomarkers will be discussed, **each part** focus on the amyloid and tau hypotheses, neuroinflammation, mitochondrial dysfunction, and synaptic and neurotransmitter alterations. **scope** it surveys the primary modalities used in biomarkers, their technical strength and mechanistic correlates. The third section examines translational challenges, including issues of socioeconomic accessibility, standardisation and technical limitations. Finally, the review evaluates the ongoing efforts in bridging the lab-to-clinic divide.

With a critical synthesis approach, this review analyses peer-reviewed literature across neuroscience, clinical neurology and translational medicine. It integrates findings from empirical studies, along with meta-analyses and systematic reviews to identify emerging consensus and unresolved tensions, such as the accuracies of specific biomarkers, within the field. A targeted narrative review of human and translational studies was conducted on Alzheimer's disease biomarkers published 2015 to 2025 in PubMed and Scopus, using terms combining Alzheimer's disease with *biomarker*, *blood*, *CSF*, *MRI*, *PET*, *retina*, *saliva*, *urine*, and *volatile organic compounds*. Inclusion prioritised peer-reviewed original research, along with systematic review and consensus statements. Seminal pre-2015 studies were

retained selectively to provide historical context. Non-peer-reviewed web sources were excluded except where clearly identified as illustrative, such as information about cost ranges.

Section 1: Molecular and cellular neurobiology and pathophysiology of AD

Alzheimer's disease is characterised by the slow, progressive neurodegeneration brought on by neuronal cell death and activity dysfunction starting in the entorhinal cortex inside the hippocampus, an area crucial for various forms of explicit memory formation, particularly spatial memory (Karimani et al., 2024). The pathophysiological hallmarks of AD are accumulations of abnormal neuritic plaques and neurofibrillary tangles (NFTs). With these changes, there is also a loss of cholinergic neurons in the basal forebrain and neocortex (Kumar et al., 2024). There are two main hypotheses that are popular within the scientific community: the cholinergic hypothesis and the amyloid hypothesis. This literature review focuses on the amyloid hypothesis, as it is currently the most widely accepted pathophysiological mechanism for AD. However, it should be noted that this hypothesis has also faced challenges and criticisms, and it is believed that there are alternative or complementary mechanisms in the pathogenesis of AD (Nasb et al., 2024).

Amyloid beta

The peptide amyloid beta ($A\beta$) is derived from a protein called the amyloid precursor protein (APP), through the abnormal activity of β - and γ -secretase for breaking down proteins (Mucke & Selkoe, 2012). Typically, APP is cleaved by either α - or β -secretase and the smaller fragments formed are not neurotoxic. However, the cleavage by β - followed by γ -secretase results in a peptide of 42 amino acids, known as $A\beta_{42}$, which is a long peptide more prone to aggregation than shorter species. $A\beta$ oligomers, which are smaller aggregates of these $A\beta$ peptides, are considered especially neurotoxic. Increase in $A\beta_{42}$ levels leads to the aggregation of amyloid, creating the commonly seen abnormal neuritic plaques. These are spherical microscopic lesions made of an extracellular $A\beta$ peptide surrounded by enlarged axonal endings, and are usually found within the cortical gray matter or around the meningeal and cerebral vessels of the brain. These deposits initially form in different areas, but grow and merge to comprise the larger more recognisable plaque structures, which then lead to neuronal toxicity. This is caused by $A\beta$ plaques damaging mitochondria as they accumulate inside neuronal cells, impairing its ability to generate ATP. Without sufficient energy, the neuron cannot maintain its normal function, leading to the initiation of apoptosis (programmed cell death). Furthermore, $A\beta$ oligomers can insert themselves into the cell membrane of neurons, forming pores that allow for the unregulated influx of calcium ions, disrupting the balance of calcium concentrations which is critical for signalling and cell survival. High calcium levels within the cell can activate enzymes that degrade proteins and lipids, leading to the breakdown of the neuronal cytoskeleton, and further mitochondrial dysfunction.

Certain mutations, specifically those in the genes encoding APP and secretases (e.g. presenilin 1 and 2, ) have been linked to cases of familial AD,  A β hypothesis. This is because these mutations increase the production of longer and more aggregation-prone isoforms of A β (Lanoiselée et al., 2017). Another gene is the apolipoprotein E (APOE) gene, which is the “strongest genetic risk factor for late-onset AD”, according to Kamatham et al. (2024), as it is associated with producing proteins that lead to higher levels of A β aggregation and .

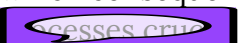
tangles

The aggregation of A β leads to a cascade of events. One of these events is the hyperphosphorylation of  (the addition of too many phosphate groups which modifies the shape of tau) through the activation of kinases like GSK β , causing the protein that stabilises axonal  intracellular transport to become misfolded and aggregate within neurons (Kumar et al., 2024). With this, these tau molecules become prone to self-aggregation and form insoluble twisted paired helical filaments. Known as neurofibrillary tangles, they first appear in the hippocampus. Recent research has supported the idea that tau aggregates have prion-like properties, allowing their propagation throughout the brain by inducing the misfolding of normal tau in . This may explain why tau is often seen spreading throughout the cerebral cortex as AD progresses, contributing to cognitive decline in affected individuals.

Inflammation

Both A β peptides and NFTs can trigger inflammatory responses within the brain by activating microglia and astrocytes (Kamatham et al., 2024). These cells then become ramified and abnormally enlarged (hypertrophied) in NFT-rich brain regions.  immune responses are triggered to clear these harmful substances  the release of pro-inflammatory molecules. However, while this process  to protect the brain, chronic and dysregulated inflammation produces proinflammatory cytokines that lead to neuronal damage and exacerbate the progression of the disease. This sustained state of inflammation can exacerbate neuronal damage and further promote the spread of tau .

Mitochondrial Dysfunction

A β peptides have also been shown to impair mitochondrial function by accumulating within the inter-mitochondrial membrane space and disrupting their normal function (Aran & Singh, 2023). This causes a reduction in ATP production and an increase in ROS generation, leading to oxidative stress and eventual damage to the organelle and its normal activity. This then triggers a positive feedback loop, as impaired mitochondrial function further promotes A β production and aggregation. In addition to this, abnormally phosphorylated and aggregated tau proteins have also been implicated in causing mitochondrial dysfunction by impairing mitochondrial transport along neuronal axons, which consequently leads to local energy deficits. It also disrupts fission and fusion  for maintaining a healthy mitochondrial network. This also triggers a

mitochondrial dysfunction results in the formation of

Synaptic and neurotransmitter alterations

Both protein aggregates of A β and tau prevent regular synaptic function by hindering the release of neurotransmitters (including acetylcholine, which plays an important role in memory and cognition), which reduces synaptic density and interferes with synaptic

transmission, particularly in cholinergic neurons (Kamatham et al., 2024). This contributes to the cognitive decline seen in AD.

In AD, glutamate neurotransmission is also affected, where excess glutamate in the synaptic cleft contributes to excitotoxicity, leading to neuronal damage and cell death.

As the membranes of these axonal cells are disrupted and the cells die, the intracellular contents, which contain essential structural components like neurofilaments (NFLs), leak out into the surrounding tissue.

Environmental Factors

Although novel, it has been posited that bacterial and viral infections may be implicated in the process of AD pathogenesis, as different types of microbes have been shown to stimulate A β aggregation and deposition. Therefore, there may be a pathogenic factor in the onset of AD as well (González-Sánchez et al., 2020).

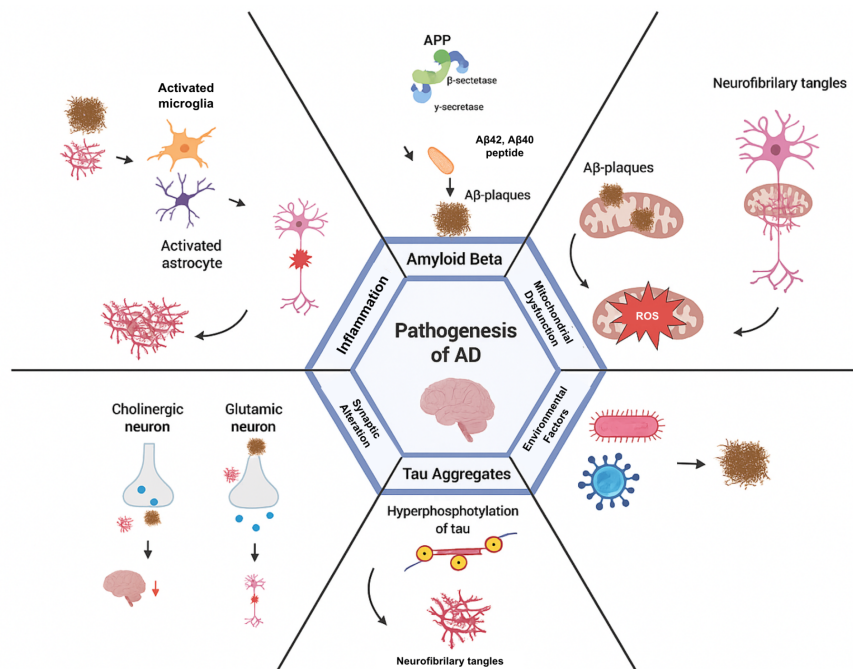


Figure 1. Diagram of the proposed forms of pathogenesis of AD

All the different pathologies of AD are largely thought to be dynamically interacting rather than isolated, suggesting that biomarkers that are able to capture multiple axes of degeneration may be most effective in **diagnosing the disease**.

Section 2: Types of Biomarkers and How They Work: Established

Biomarkers are defined as being naturally occurring molecules, genes, or characteristics by which a particular pathological or physiological process or disease can be identified. This literature review identifies three types of biomarkers typically employed in the diagnosis of AD: molecular, imaging and functional. Molecular biomarkers include proteins or other biological compounds found through the testing of substances like blood, **CSF** and other bodily fluids. Imaging biomarkers include scans like **CT, MRI and PET**, while functional biomarkers are measured through performance on cognitive or motor assessments and tasks. Usually, biomarkers are employed to determine the cause of mild cognitive impairment (MCI) in the initial stage of dementia. This literature review further investigates promising new biomarkers that have shown potential in predicting AD.

MOLECULAR

CSF

Cerebrospinal fluid is an ideal candidate for reflecting the molecular events of the brain, as it is in direct contact with the brain's extracellular space. Hence, experts agree that it is the best way to identify AD biomarkers that reflect **neurodegenerative pathogenesis**.

Because A β 42 is a large component of typical AD plaques, the amount of total A β in CSF is well-correlated with AD diagnosis. There is a positive correlation between lower CSF A β 42 levels and plaque burden, due to plaques acting as “amyloid sinks”, where the peptide is deposited and accumulates (Anoop et al., 2010). There has also been a correlation between a rise in A β 38 levels and a fall in A β 42, which supports the idea that the ratio A β 42:A β 38 may be more useful and precise in diagnosing AD. **Reduced A β 42** also been observed in other types of dementia, and many works and wider literature agrees that A β 42 is the most accurate CSF biomarker of AD in **patients with AD**. However, there was no significant difference observed in the total CSF A β levels in AD patients and non-AD **control**. Therefore, it seems that only A β 42 is a relatively accurate predictor of the disease.

Increased levels of tau and **t-tau** in CSF has been shown to be correlated with AD onset, with several studies suggesting that total tau (t-tau) concentration in AD patients' CSF is higher than the control (Papaliagkas et al., 2023). However, it is noted that this biomarker, although sensitive, is limited in its ability to differentiate AD from other forms of

Increased levels of tau is not a feature unique to Alzheimer's disease. It is a biomarker, as it is more closely related to the pathophysiology of the disease.

Levels of β -amyloid fibrillary acidic protein tend to be increased in individuals with early AD, as it is hypothesised to reflect the increased reaction of the immune system to the amyloid plaques. Plasma β -amyloid levels also correlate well with the levels found in CSF, as it leaks from cells due to BBB dysfunction.

Another potential biomarker is neuronal thread proteins (NTPs). These are expressed in the brain during AD-related neurodegeneration, and are found to be higher in the CSF of AD individuals compared to controls (Kang & Wang, 2022).

Blood

As A β 42 and A β 40 peptides accumulate within the brain, plasma levels of individuals with AD are typically indicative of the disease. The plasma A β 42:A β 40 ratio is usually lower in A β 42-positive groups than in A β 42-negative groups, as has been demonstrated by different immunoassays and mass spectrometry-based assays. However, the performance of these assays has been shown to be substantially lower than that of CSF assays, with a recent comparison showing that certain mass spectrometry-based assays were far better at identifying A β pathology than more commonly used immunoassays. Regardless, these higher-performing A β 42:A β 40 assays still only exhibit modest correlations between the levels of A β and that found in CSF, likely due to the fact that A β in plasma is derived from peripheral sources, as posited by Hansson et al. (2023). Hence, it is agreed that plasma A β 42 levels alone cannot be considered an AD marker.

On the other hand, researchers have recently developed several high-sensitivity assays for the detection of different phosphorylated tau protein variants, or isoforms, including specific ones like p-tau181, p-tau231 and p-tau217 (Janelidze et al., 2025). When compared, it seems that assays quantifying plasma p-tau217 have been shown to be better at detecting AD pathology and predicting the progression of AD. However, it can be argued that p-tau231 is a good marker of AD in its early stages, as it starts increasing at low A β levels. Hence, when combining plasma A β 42 and A β 40 with p-tau, early-stage amyloid positivity detection could be improved.

Similarly, GFAP can also be measured reliably in the blood as well as in CSF. Plasma NFL levels also correlate well with the levels found in CSF, although its correlation is not as strong as that of p-tau217 (Garcia-Escobar et al., 2024). There is also little to no correlation between levels of NFL and A β 42 in the blood, which has led researchers to believe that NFL suggests neurodegeneration independently of the amyloid pathology.

Janelidze et al. (2022)'s study has shown that the combination of plasma p-tau231 and A β 42:A β 40 could be used to detect very early stage AD, and it is supported by the fact that it was able to detect amyloid pathology in cognitively unimpaired individuals.

Saliva

Saliva is not only an easily accessible body **fluid**, its secretion and composition might also be affected by various diseases (Surdu et al., 2025). It is posited that AD can influence **salivary parameters**, as there seems to be a relationship between the brain and saliva. This occurs through six pathways of **communication** between brain molecules with the saliva and **vice versa**. However, for both A β and tau, concentrations are considerably lower in **saliva than in plasma**.

A meta-analysis conducted by Nijakowski et al. (2024) was seen was a statistically significant number of studies that have examined the saliva of AD patients and found elevated levels of A β . However, it was noted that in three studies, neither A β 40 or A β 42 were detected in AD patients. This suggests that there is some degree of reliability **in** correlating A β levels with AD.

The meta-analysis performed by Nijakowski et al. (2024) found it seems that the reliability of tau as a biomarker for AD is inconsistent **and unreliable** when considered alone. There is no consensus on whether t-tau, p-tau and their ratio are reliable indicators of AD. The studies examined by Nijakowski have reported conflicting results, and suggest that while tau may be part of the AD pathology, its biomarker use in saliva cannot be considered a standalone reliable biomarker.

Nijakowski et al. also explores lactoferrin (Lf), a protein with anti-inflammatory properties, and proposes that it may have neuroprotective effects in neurodegenerative diseases like AD. According to González-Sánchez et al. (2020), biomarkers that reflect the integrity of the immune system could be useful. Lf has previously been detected in senile plaques, neurofibrillary tangles and microglia in **the brain**. Although it was particularly useful in differentiating AD and MCI patients from healthy individuals, it appears to be a promising, but not fully conclusive biomarker for AD. While several studies analysed by Nijakowski et al.'s paper showed a clear link between decreased salivary lactoferrin levels and AD, there are also conflicting findings that appear to **warrant further investigation**.

Urine

Both A β and tau are possible to detect in urine, because they can be eliminated from the organism by action of the kidneys (Armenta-Castra et al., 2024). These are detected through methods like **ELISA** and Western blot. Several studies show a correlation between the presence of these proteins in urine and AD, and it appears that A β oligomers are better indicators of AD progression, instead of the monomers **A β 40 and A β 42**. Both p-tau and tau were found to be significantly higher in AD patients compared to control individuals, but **when compared** with saliva, these concentrations were much lower in urine than in plasma for both **proteins**.

NTPs have been found in urine, as well **as in** CSF. Evidence for urinary AD7c-NTP is preliminary and replication in contemporary cohorts is limited, leaving clinical utility

uncertain. One such piece of evidence is the ELISA results from Ghanbari et al.'s 1998 study utilised ELISA to show that results in urine showed similar sensitivity and specificity to CSF, and that urinary AD7c-NTP was significantly higher than in the **AD group**.

IMAGING

Neuroimaging **allows** for diagnosis of the AD pathophysiological process during life, even though neuropathology remains the definitive standard. Clinicians can begin to

in vivo, with several modalities having been researched, including Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET).

Neurodegeneration leads to cerebral atrophy, which is most notable in the hippocampus and entorhinal cortex of patients with AD (Tabatabaei-Jafari et al., 2015). The level of atrophy in the medial temporal lobe is strongly correlated **with scores** on cognitive testing, and is therefore associated with disease progression. As this degeneration progresses to other cortical structures, lateral ventricles become enlarged in the later stages of the disease. In addition to this, limbic structures (such as the amygdala, cingulate gyrus, thalamus, and olfactory bulb tract) are impacted, and the atrophy leads to volumetric reductions in the frontal, parietal and **temporal brain areas**.

MRI

MRI is an imaging technique that is able to generate high-resolution images of water-containing tissue. This is done by applying a strong magnetic field and pulses of radio waves that manipulate electromagnetic fields and energy states of protons within the body. These protons emit energy, which are then converted to **signals and images**.

Structural MRIs are used to provide highly detailed images of anatomy as well as a strong gray/white matter contrast. These scans are able to display white matter hyperintensities that indicate demyelination and axonal loss (Meng et al., 2022). Compared to patients without AD, patients with AD have greater white matter hyperintensities in the frontal lobe that index small-vessel disease burden and help in differential diagnosis and staging, but are not necessarily specific to AD.

Diffusion Tensor Imaging (DTI) uses the displacement of water molecules to measure the integrity of the white matter tract. The direction of diffusion allows scientists to better understand tracts and specific fibre bundles that may be impacted by neurodegeneration (Esrael et al., 2021). The major measures of DTI are mean diffusivity (MD), which is the average rate of water molecule diffusivity, and fractional anisotropy (FA), which is the variability associated with diffusion. In AD brains, MD can be seen in all four areas of the brain (frontal, occipital, parietal and temporal), including the hippocampus. This is a clear

in MCI, where these increases are not seen in the frontal, parietal and temporal regions, as well as decreased FA in the frontal, parietal and temporal regions.

uncinate fasciculus and superior lateral fasciculus, as well as throughout the temporal, occipital and frontal white matter. Patients with MCI have no irregularities of FA in

occipital and parietal areas, [redacted] According to a study done by Magalhães et al. (2023), there was a significant correlation between white matter tracts and both p- and t-tau.

Functional MRI (fMRI) produces dynamic representations of brain activity through blood oxygen level-dependent (BOLD) signals (Chandra et al., 2018). This measures changes in blood volume and flow. When asked to perform memory tasks, AD patients showed little to no activation of hippocampal and other medial temporal structures as compared to non-AD individuals. Resting-state fMRIs of the AD brain also show abnormal coactivation of the medial temporal structures and the posterior cingulate cortex, which differs from [redacted]

PET

PET is an imaging modality that allows for the in vivo measurement of disease pathologies using radioligands to detect compounds of interest. In the case of AD, that would be [redacted] and A β plaques.

FDG-PET uses 18-F-fluorodeoxyglucose (FDG), which allows researchers to estimate glucose metabolism in different brain areas and identify which ones may have abnormal metabolic activity (Bouter et al., 2019). From studies done, these scans show temporoparietal hypometabolism in patients with AD, which is characteristic of the [redacted] Furthermore, there was an observed decrease in metabolism in the posterior cingulate cortex and precuneus, which is thought to be related to the episodic memory [redacted]

Because of accumulated A β , [redacted] in areas of the brain affected by the disease (more specifically, [redacted] and frontal regions) (Villa et al., 2020). Tracers such as Pittsburgh Compound B (PiB) are able to bind to misfolded amyloid plaques, therefore helping to map distribution and quantify amyloid [redacted]

Part of the underlying pathophysiology of AD [redacted] the neuroinflammatory cascades leading [redacted] in microglial activation. Radiotracers that bind to TSPO [redacted] can be used as a marker of inflammation (Georgakas et al., 2023). Since TSPO is a peripheral benzodiazepine receptor found on the membrane of the mitochondria, increased expression of this protein can be used as an estimate for increased inflammation, as the expression of this protein is upregulated by [redacted]

With the rise of better **artificial intelligence** algorithms, AI is being increasingly utilised in the diagnosis of AD (Kamatham et al., 2024). AI models are capable of analysing large, complex datasets containing neuroimaging data and biomarker profiles to identify patterns. MRI and PET generate vast amounts of data that can be analysed using AI to detect both structural and functional brain changes associated with AD. Biomarker data can also be incorporated in order to develop predictive models for risk assessment. This holds great potential for improving efficiency and accuracy, since AI models can therefore aid healthcare providers in making increasingly informed diagnostic decisions, facilitating **the early detection of AD and the generation of personalised treatment plans**.

BEHAVIOURAL

Functional

The progressive loss of ability to perform **activities of daily living** unassisted is a primary characteristic of dementia (Chaves et al., 2011). These activities can be divided into basic (BADL) and instrumental (IADL). BADL includes the ability to carry out tasks necessary for personal hygiene, sphincter control and feeding. IADL, on the other hand, are more complex and are activities such as preparing meals, carrying out domestic chores, and managing finances and correspondence.

An example of an assessment used to evaluate changes in functional performance is through an assessment measure known as Functional Assessment Staging (FAST). This allows healthcare practitioners to specifically evaluate these changes **throughout the** entire course of AD (Sclan & Reisberg, 1992). Another such assessment measure is the Alzheimer's Disease Cooperative Study – Activities of Daily Living for Mild Cognitive Impairment, otherwise known as ADCS-MCI-ADL. This assesses the competence of AD patients in both basic and instrumental ADLs, and can be completed by a caregiver or administered by a clinician as a structured interview with a caregiver (Fish et al., 2011).

Cognitive

The initial and most common presenting symptom of AD is episodic short-term memory loss (Kumar et al., 2024). This includes difficulty retaining new information, and experiencing impairments in problem-solving, executive functioning, judgement, and organisational skills. Because of this, **people** may struggle with multitasking and abstract thinking, which affects their ability to carry out ADLs **like** driving, cooking, and activity planning. Following this, there are often language and visuospatial skill impairments, along with neuropsychiatric symptoms like changes in **social behaviour**.

A common example of a cognitive assessment is the Mini-Mental State Examination (MMSE) (Arevalo-Rodriguez et al., 2015). This is a common test for assessing changes in cognition, and evaluates functions such as orientation, language, memory and other functions. This is done through tasks that test reading, writing and short-term memory,

including tasks like subtraction, remembering a series of unconnected words or copying a diagram on paper.

The Alzheimer’s Disease Assessment Scale - Cognitive, or ADAS-Cog, is generally more thorough than the MMSE and is considered the gold standard for assessing AD (Kueper et al., 2018). This includes 11 tasks, including both tests completed by the subject and assessments completed by the observer. When put together, these tasks assess memory, language, and praxis. Specific tasks, like the MMSE, include word recall, naming objects, commands, and word recognition among others.

As can be seen by this, there already exists a plethora of widely acknowledged and effective biomarkers, whether they be molecular, imaging or functional. A summary of the above information can be found in Table 1.

Summary of Key Biomarkers and Diagnostic Modalities for Alzheimer’s Disease

Category	Subcategory	Biomarker / Method	Key Findings	Work Cited
Molecular	CSF	Aβ42	Lower levels correlate with plaque burden; most promising CSF biomarker for AD in MCI	Anoop et al., 2010
		Aβ42:Aβ38 ratio	May improve diagnostic accuracy	
		t-tau	Elevated in AD; sensitive for neurodegeneration	Papaliagkas et al., 2023
		p-tau	More closely related to AD pathology than t-tau	
		GFAP	Increased with early Aβ pathology; reflects reactive astrocytes	Marksteiner & Humpel, 2025
		NfL	Correlates with CSF Aβ; marker of neurodegeneration	
		AD7c-NTP	Higher in postmortem CSF of AD patients (still relatively new)	Kang & Wang, 2022
	Blood	Aβ42:Aβ40 ratio	Lower in Aβ-positive groups; better with mass spectrometry	Hansson et al., 2023
		p-tau217	Strong predictor of AD pathology and progression	Janelidze et al., 2025
		p-tau231	Early-stage AD marker (increases at low Aβ levels)	

		p-tau181	Widely adopted, earlier generation with specific indication	
		GFAP	Detectable in 100% ; reflects astrocyte activation	Garcia-Escobar et al., 2024
		NfL	Detectable ; less correlated with CSF than p-tau217	Janelidze et al., 2022
		Combined p-tau231 + Aβ42:Aβ40	detects very early stages	Hansson et al., 2023 Janelidze et al., 2025
	Saliva	Aβ	Some studies show elevated levels in AD	Nijakowski et al., 2024
		t-tau, p-tau	Conflicting results; reliable	
		Lactoferrin	Lower in AD; potential marker	González-Sánchez et al., 2020
	Urine	Aβ oligomers	Better indicators of progression than monomers	Armenta-Castro et al., 2024
		p-tau, tau	Higher in AD patients than controls	
		AD7c-NTP	Elevated; similar sensitivity/specificity CSF	Ghanbari et al., 1998
Imaging	MRI	Structural MRI	hippocampal/entorhinal atrophy; white matter hyperintensities	Tabatabaei-Jafari et al., 2015 Meng et al., 2022
		DTI	Increased MD and reduced FA in specific tracts; correlates with	Esrael et al., 2021
		fMRI	Reduced hippocampal activation; altered resting-state connectivity	Chandra et al., 2018
	PET	FDG-PET	Temporoparietal hypometabolism; PCC/precuneus deficits	Bouter et al., 2019
		Amyloid PET	PiB and other tracers map amyloid load	Villa et al., 2020
		TSPO PET	Detects neuroinflammation via microglial activation	Georgakas et al., 2023
	AI/ML Integration	AI models	Detect structural/functional changes; create biomarkers	Kamatham et al., 2024
Functional	Behavioural	BADL/IADL assessments (FAST, ADCS-MCI-A DL)	Measures decline in daily functioning	Chaves et al., 2011 Sclan & Reisberg, 1992 Fish et al., 2011

Section 3: Types of Biomarkers and How They Work: Novel

While traditional biomarkers have focused on the more established hallmarks of AD, such as A β and tau, recent research has expanded into a more diverse spectrum of novel biomarkers that may serve to capture alternative aspects of the disease's **biology**. These include new developments in both molecular and imaging biomarkers as technological advancements occur.

MOLECULAR

MicroRNA

MicroRNAs (miRNAs) are a type of short-length, non-coding, and single-stranded endogenous RNAs (also known as sncRNAs) found in a stable form in the blood, CSF, and various body fluids (Chimthanawala et al., 2023). By controlling the expression of genes and pathways, **miRNAs** **by suppressing** the translation of target **RNA** and facilitating their degradation, contributing to APP degradation and A β metabolism. AD patients had higher levels of miRNA-4722-5p and miRNA-615-3p, and combining these two AD-associated miRNAs provided a better diagnostic score than either **miRNA alone**. miRNA-20b-5p also downregulated APP levels, hence reducing the **stability of**. However, further research in this domain needs to be carried out, especially on three miRNAs that strongly correlated with the early stages of AD, namely miRNA-92a-3p, miRNA-486-5p, and miRNA-29a. Levels of miRNA-92a-3p and miRNA-486-5p were lower in AD patients compared to controls, but higher levels of miRNA-29a-3p were found in the plasma of AD and MCI patients. Plasma levels of miRNA-483-5p levels were higher in AD patients compared to healthy controls, and it was concluded that increased levels of miRNA-483-5p in plasma can reliably distinguish both MCI and AD groups from the healthy controls. The above **suggests** that these miRNAs could be potential biomarkers in early **diagnosis**.

Long non-coding RNA

A second type of RNA that can be used is long non-coding RNAs (LncRNAs). These resemble mRNA (**messenger RNA**) molecules, but are not responsible for encoding proteins (Black et al., 2024). They typically contribute to the regulation of several processes by **regulating** chromatin remodelling, and providing alternative mRNA splicing. This affects gene expression, and has been shown to “directly or indirectly regulate the key features of AD including A β deposition, aberrant tau formation, oxidative stress, neuroinflammation, and **neuronal death**”. LncRNAs also interact with miRNAs, **thereby**

influencing AD-related neuropathology. The expression of numerous types of these molecules have been detected in plasma and CSF samples from AD patients, which indicates that lncRNAs could function as a likely biomarker in the diagnosis and

Apolipoprotein C3

A study done by Watanabe et al. (2020) suggests that Apolipoprotein C3 (ApoC3) may be a potential urinary biomarker for AD, although further analysis of this is warranted (Watanabe et al., 2020). ApoC3 is an abundant apolipoprotein mainly present in triglyceride-rich lipoproteins (chylomicrons and VLDL), as well as cholesterol particles. Watanabe et al. found that studies have reported people with AD had lower plasma levels of ApoC3 compared to healthy individuals, and that higher ApoC3 was associated with reduced grey matter volume in the brain. The paper also pointed to recent research suggesting that ApoC3 acts as a trigger for inflammation by activating the NLRP3 inflammasome. This is significant because the NLRP3 inflammasome is known to be activated in the brains of AD patients by amyloid beta. The result of Watanabe et al.'s experiment was that all samples revealed ApoC3 levels to be significantly higher in the AD group relative to the control group.

MCP-1

Monocyte Chemoattractant Protein 1 (MCP-1, also known as CCL2) is a protein that belongs to a family of signalling molecules called chemokines (Huang et al., 2023). They play key roles in the body's inflammatory response by attracting immune cells to sites of inflammation. As a biomarker, MCP-1 can be measured in both blood and urine. In AD, neuroinflammation in the brain (neuroinflammation) contributes to neuronal damage. It is thought that it is because of this that elevated levels of MCP-1 have been found in the blood and CSF of AD patients. However, this is not consistent across different studies, which is why Huang et al. hypothesised that MCP-1 levels impact individuals who are genetically at risk of developing AD.

Molecules Associated with Oxidative Stress

A β induces oxidative stress, the production of harmful reactive oxygen species (ROS), which is thought to be a major contributor to cellular damage in the brain (Gella & Durany, 2009). ROS are too unstable to measure directly, so measuring their byproducts is a method used to quantify oxidative stress. Several products, specifically 4-hydroxy-2,3-noneal (HNE), acrolein, F2-isoprostanes and malondialdehyde have been observed to exist in higher levels in AD brains. However, like many other biomarkers, oxidative stress is a general sign of cellular damage and is not necessarily specific to AD. It is a feature of many neurodegenerative disorders and is also affected by ageing and

Volatile Organic Compounds

Exhaled breath contains hundreds of different byproducts of metabolism, also known as volatile organic compounds (VOCs). In AD, metabolic changes in the brain could produce a unique “breathprint” (Zhang et al., 2025). Using sophisticated sensors or mass spectrometers, analysis of this breathprint can be carried out to identify patterns that

are associated with this disease. However, this research is still in its early stages, and this is unclear.

IMAGING

Retinal Imaging

In the last decade, there has been rapid growth in using retinal imaging to find markers of AD. Techniques like optical coherence tomography (OCT) are shown to be especially promising because of their non-invasive, affordable and accessible nature compared to traditional brain imaging (Georgakas et al., 2023). Research has shown structural, vascular and neuropathological changes in the retinas of both mice and humans with AD. Structural markers, such as the thinning of the retinal nerve fibre layer (RNFL) are consistently seen in older adults with AD. This thinning may start early in disease progression, as it has been linked to levels of A β and tau in CSF, as well as to hippocampal volume in cognitively normal adults. This suggests its usefulness as a tool for early AD screening. Vascular changes are also being explored, as a 2013 study found that asymptomatic adults with high cerebral A β had less-branched retinal blood vessels. Another study found that a larger foveal avascular zone (FAZ), which is the area of the retina without blood vessels, was typically associated with higher cerebral A β in cognitively normal adults. This suggests that the FAZ area, along with retinal imaging in general, may be an effective marker for detecting AD. However, this research has yielded mixed results, with some finding strong correlations and others not.

CT

A CT scan uses a series of X-rays from different angles to generate cross-sectional images of the brain. While it cannot visualise amyloid plaques or tau tangles directly, it is able to detect brain atrophy, or shrinkage, of regions like the hippocampus (Pasi et al., 2011). However, it is only really supportive and non-specific of AD. It may also prove more useful only for later-stage AD, as brain atrophy usually cannot be seen until patients begin to exhibit behavioural and cognitive changes. In addition to this, CT scans involve using ionizing radiation which, though at a low dose, carries a small risk, especially if repeated scans are used.

Tau PET

Tau PET molecular imaging is a relatively new technology. So far, there is a first-generation FDA-approved tracer, known as 18-F-flortaucipir, and has been shown to have high affinity for detecting the tau aggregates that are characteristic of AD (Petersen et al., 2022). However, off-target binding, where tracers bind to molecules other than tau, can produce unreliable results in a PET scan. This is why there are ongoing



Most non-CSF, non-blood matrices remain exploratory, but from current research,  clear that many of them warrant significant potential to become alternative biomarkers. Some may even prove to be more advantageous than traditional biomarkers due to their cost-effectiveness and accessibility, as well as their specificity  accuracy.

Section 4: Translational challenges and barriers

Data standardization and comparability represent a critical challenge in both imaging data collection and biomarker fluid acquisition protocols that can vary considerably across different Alzheimer's Disease Research Centers (ADRCs) (Braudeau et al., 2025). This variability complicates the establishment of universal diagnostic thresholds and hinders the direct comparability and integration of findings from diverse research cohorts, making it difficult to generalize results and apply them uniformly in clinical practice. Consequently, even if a biomarker demonstrates exceptional scientific efficacy, its practical clinical utility is severely constrained if it cannot be widely deployed across diverse healthcare settings. This accessibility-utility gap highlights pressing health equity concerns in AD diagnosis, driving the urgent need for less invasive and more affordable alternatives, even if those alternatives present their own set of challenges.

IMAGING

High cost and limited accessibility are paramount challenges. Imaging costs can vary widely across settings, but illustrative ranges suggest substantial patient-level expense, with an MRI costing up to \$8,400, and a PET scan up to \$3,000 in some countries (Imaging Technology News, 2018). This substantial cost, coupled with the requirement for specialized equipment, trained personnel, and dedicated imaging facilities, restricts their availability, particularly in underserved regions and low-resource settings. This creates a significant barrier to widespread clinical use, contributing to health inequities in AD diagnosis.

Patient burden and contraindications also pose practical limitations (Decazes et al., 2021). Since PET scans involve a small dose of radiation, patients may be advised to avoid this scan if they are pregnant, and this radiation carries its own risks as . Both MRI and PET scans require patients to remain still in enclosed spaces for extended periods, which can cause discomfort or claustrophobia. Furthermore, patients with certain medical devices or metal implants, such as pacemakers, may have contraindications for MRI due to the powerful magnetic fields invol.

PET

Cost and reimbursement represent major practical barriers to the widespread clinical adoption of amyloid PET (Lee et al., 2021). These scans are expensive, typically costing between \$3,000 and \$6,000. In the USA, third-party payers often do not reimburse for amyloid PET, citing a need for additional data demonstrating that the scan results directly impact clinical care and patient-based outcomes. This lack of coverage significantly restricts access for many patients.

Pittsburgh Compound B (PIB) utilises carbon-11, which has a very short half-life of 20 minutes, limiting its clinical applicability due to logistical challenges (Wolk et al., 2012). While more clinically viable F18-labeled tracers with a longer half-life of 110 minutes have since been developed, the cost and accessibility issues persist. Additionally, U.S. tracer labels and clinical practice typically use a binary (positive or negative) interpretation for amyloid PET results, which may oversimplify the continuous biological data and potentially obscure nuanced information about amyloid burden.

The designation of amyloid PET as a “gold standard” for detecting amyloid pathology is important to consider in context. While it excels at identifying the presence of amyloid plaques, a necessary component of AD, the presence of these plaques alone is not sufficient for a definitive AD diagnosis (Roberts et al., 2015). Amyloid plaques are found in some conditions and a substantial proportion of cognitively normal individuals. Furthermore, the overall amyloid burden, as assessed by PET, does not consistently correlate strongly with cognitive decline. This implies that a positive amyloid PET scan indicates the presence of amyloid pathology, but it does not definitively confirm symptomatic AD or establish amyloid as the sole cause of cognitive impairment.

MRI

In the very early stages of AD, an MRI scan of the brain may appear normal, making it less sensitive for preclinical detection compared to molecular imaging techniques (van Oostveen & de Lange, 2021). While it provides detailed structural changes, it cannot solely confirm the presence of AD pathology; comprehensive assessments combining MRI findings with clinical evaluations, cognitive testing, and patient history are essential for accurate diagnosis. Fundamentally, MRI primarily reflects neuronal loss and brain atrophy, which are consequences of amyloid and tau pathology, rather than directly visualizing these protein aggregates themselves, meaning it is less sensitive to preclinical pathology than molecular biomarkers. Emerging techniques like fMRI and DTI hold significant promise, but their widespread standardization and established clinical utility for routine AD diagnosis are still under development.

MOLECULAR

CSF

Despite how thoroughly characterised and well-established CSF markers for AD are, there are still significant limitations associated with these biomarkers, primarily revolving around the invasiveness of the lumbar puncture procedure used to procure the fluid

(Inekci et al., 2015). The discomfort and potential risks, although rare, including nerve damage, infection and herniation, are currently primary drivers for the ongoing search for less invasive diagnostic alternatives.

Blood

Compared to CSF, blood-based biomarkers are more appealing because they tend to be significantly less invasive in nature and more cost-effective extraction methods (Mielke et al., 2024). However, as aforementioned in this literature review, the challenges include lower biomarker concentrations in blood compared to CSF, and also less achievable accuracy due to complex matrix interference in blood. In addition to this, peripheral metabolism can significantly affect blood biomarker levels, making them less direct reflections of brain pathology.

Other Bodily Fluids

With research exploring the potential of other bodily fluids like saliva and urine, these fluids offer a theoretical advantage of maximal non-invasiveness, which makes them highly attractive for broader-scale screening (Kumari et al., 2023). However, this research is still in its nascent stages. Low concentrations of relevant analytes, high biological variability coupled with a significant lack of robust validation in studies limit their establishment for clinical use in AD diagnosis.

OVERALL

Specificity

Even with seemingly AD-specific biomarkers like tau and NfLs, there still exist difficulties in differential diagnosis. The pathologies underlying other types of dementia often involve very similar proteins to the ones used to diagnose AD, which complicates the ability to distinguish between them based solely on biomarker levels (Zou et al., 2020). Simply measuring protein concentrations is unlikely to provide sufficient information to differentiate effectively between various dementias.

Cost

The current landscape of AD research and the development of biomarkers, particularly blood-based biomarkers, is heavily skewed towards high-income countries (Rodda et al., 2025). To achieve equitable progress in the field, it is crucial to actively involve researchers and institutions from LMICs in the development, validation, and clinical application of BBMs. Key challenges remain, including the need to understand the real-world effectiveness, cost-efficiency, and acceptance of these tools in resource-limited environments. Financial constraints are a major barrier to the necessary research and validation in LMICs. Additionally, to effectively roll out BBMs in LMICs, significant investment is needed to build the necessary infrastructure and upskill the workforce. This could involve financial support from various sources, corporate engagement, and the use of innovative, practical approaches. For instance, using dried blood spots and task-shifting models (i.e. training community health workers to do tasks normally reserved for

specialists) where community health workers collect samples could make widespread use more feasible, especially 

Generalisability

Not only are there technical and economic barriers, there exist obstacles in the generalisability of studies due to the lack of access to certain samples and communities of individuals. Most studies, especially plasma biomarker studies, to date have focused on genetically homogeneous populations in high-income countries. This highlights a critical need for large-scale international studies that include diverse populations with varying genetic backgrounds, comorbidities, and socioeconomic statuses. This is a significant issue because biomarker levels can vary among different racial and ethnic groups and are affected by certain long-term health conditions and medications (Rodda et al., 2025). A 2022 study showed that even though most participants expressed interest in biomarker research for AD, Black participants were comparatively more hesitant than White participants (Elicain et al., 2022). They were more concerned about the risks of participating and felt there were more barriers to undergoing brain scans. These differences remained even when accounting for their trust in researchers and their existing knowledge of AD. The study found that a lack of information was a major barrier for Black participants, while receiving information was a strong incentive. Black older adults expressed a desire for more details about AD, including its risks and prevention, as well as a better understanding of the general research process and specific biomarker procedures. This  for the scientific community to improve recruitment strategies for underrepresented groups with no prior research experience. The above could be done through  by increasing their presence in these communities and reducing the financial burden of participation.

Standardisation

A major hurdle for the widespread use of AD  blood-based ones, is a lack of standardization (Humpel & Hochstrasser, 2011). Currently, there are no uniform methods for sample collection, processing, or storage. This problem is compounded by the absence of established procedures to monitor long-term biomarker stability and the lack of certified reference standards. These inconsistencies introduce measurement variations, making it difficult to compare results between labs, establish reliable clinical thresholds, and ultimately use these biomarkers effectively in both research and patient care (Zeng et al., 2024).

Education of Staff

The widespread adoption of biomarkers, despite their accessibility,  significantly challenged by the need for more comprehensive education of primary care clinical staff and the development of clear, actionable guidelines for their use. Without this adequate training and clear protocols, there is a high risk of misinterpretation of results or the inappropriate use of these diagnostic tools (Schöll et al., 2024).

Overall, there still exist many translational hurdles, and it is only if these hurdles are overcome that biomarkers can achieve their full efficacy and potential. Reducing these hurdles also allows more people to access what could potentially be a diagnostic tool for AD, bettering treatment plans via early detection.

Section 5: Ongoing efforts of bridging the divide

Early detection of AD could dramatically shift the way treatment is given to patients. Early detection allows for interventions during the prodromal or mild cognitive impairment stages, where therapeutic outcomes are more likely to be meaningful as early diagnosis is crucial in informing discussions, care and support, and treatment (Dubois et al., 2023). Moreover, biomarkers are critical not only for diagnosis, but also for other means of patient stratification in clinical trials and the development of potential disease-modifying therapies.

Hence, the limitations and translational challenges confronting AD biomarkers are being actively addressed. For example, significant progress is being made in developing more sensitive and specific assays for AD biomarkers. Ultra-sensitive platforms, such as Single Molecule Array (SIMOA) technology, are enabling the reliable detection and quantification of biomarkers like p-tau and GFAP even if they are present at very low concentrations in biological fluids (Yuan et al., 2025). Other technologies include novel electrochemical sensing platforms like CRISPR/Cas12a-mediated electrochemical aptasensors, which are biosensors that use DNA/RNA molecules to detect protein targets.

Recognising that no single biomarker is able to provide a complete picture of AD pathology and its progression, there is a growing emphasis on multimodal biomarker approaches and the integration of different data types (Gupta & Iftekhar, 2024). Combining imaging, fluid, cognitive and demographic data could offer the potential for enhanced diagnostic accuracy. AI and machine learning (ML) in particular are emerging as tools in this context.

Beyond refining extant markers, the field continues to pursue the discovery of novel biomarkers that can provide more specific and comprehensive insights into AD pathology.

Conclusion

While we are getting better at detecting neurobiology, the path to widespread clinical use is still obstructed by challenges including the need for wider population-based studies. Bridging this gap is consequently essential to realising their full diagnostic and therapeutic potential. Another current limitation in our use of novel biomarkers is being able to distinguish between AD and other

neurodegenerative disorders, making the traditional biomarkers like A β and tau far more



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Beautiful job reworking the Introduction and the Conclusion. I have some suggestions below that might help with some of the wording in these sections.

Intro:

Page 2, Para 1

Change: which have limited the availability of these diagnostics to certain communities
To: thereby limiting the availability of proven diagnostics to diverse communities

(you can decide if the word “diverse” is correct in this context. “underserved” is another possibility)

Conclusions:

We generally don't have a reference in a Conclusions section because ideally the info should be in the paper. Since that is not the case for this exact information, and it might be awkward to force it up there, I am ok with it here, but suggest you always cite a paper without referring to it per se (possessive).

Page 22, Para 5

Change: Accessibility is limited by high costs and the invasive nature of some procedures, as supported by Georgakas et al. (2023)'s paper stating that the ability to translate treatments to diverse populations is reliant on low cost, minimally invasive and more accessible diagnostic biomarkers in a general clinical setting.

To: Accessibility will depend on our ability to translate treatments to diverse populations by being reliant on accessible, low cost, and minimally invasive diagnostic biomarkers available to a general clinical setting (Georgakas et al., 2023).

Change: Achieving low-cost, minimally invasive and widely validated biomarkers tested across diverse populations will not only democratise access but also improve diagnostic accuracy.

To: Achieving the above stated goals will not only democratise access but also improve diagnostic accuracy.

(the former was repetitive with above)

Title: The Neurobiological Foundations and Translational Challenges of Current and New-Generation Alzheimer's Disease Biomarkers: A Review

Abstract:

Alzheimer's disease (AD) is a progressive neurodegenerative condition, and remains without a definitive cure despite decades of research. Biomarkers offer promising avenues for early diagnosis and disease monitoring, though their clinical adoption has proven to lag behind laboratory advances. This review synthesises recent literature on the neurobiological underpinnings of both well-known and novel AD biomarkers by drawing on 2015–2025, English, human-centered studies utilizing assays, imaging, and assessments to highlight the mechanisms and limitations of current tools of diagnosis. This review also aims to analyse the key translational barriers involved in routine clinical diagnostics, and evaluate the clinical readiness and integration for molecular, imaging and functional biomarkers. Key findings include A β 42/40 being a strong biomarker for amyloid plaques, p-tau217 outperforming other plasma p-tau epitopes, and new technologies such as second-gen tau PET being developed to reduce off-target issues. Despite certain technical limitations, AD biomarkers have strong neurobiological mechanistic evidence, but their translation is still impeded by practical and socioeconomic challenges. These include barriers to access, standardization and underrepresentation of communities in studies. Addressing and bridging these gaps would be essential for closing the bench-to-bedside divide in AD care, helping to enable earlier interventions.

Keywords: Alzheimer's disease, biomarkers, neurobiology, translational neuroscience, diagnostics

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. It is characterized clinically by memory loss, impaired cognition, and behavioral changes. With prevalence rising sharply as populations age, AD poses mounting healthcare and socioeconomic challenges.

Despite being one of the most common forms of dementia and neurodegenerative disease, it remains an incurable illness. As of 2021, out of the approximately 57 million people worldwide who have dementia, 60–70% have AD (World Health Organization, n.d.). This greatly affects their quality of life, and also places an enormous burden on caregivers and healthcare systems worldwide, costing economies US\$1.3 trillion globally, with 50% of these attributable to the care given by family members and close friends, according to the World Health Organization.

The pursuit of reliable biomarkers for AD has been driven by the need to detect the disease in its earlier preclinical stages, ideally before the onset of severe symptoms. If this is achieved, it could help facilitate the rapid development of an efficient treatment plan even before the symptomatic clinical onset of the disease. Historically, the diagnosis of AD has relied on cognitive assessments and, ultimately, post-mortem histopathological confirmation. However, research in recent decades has witnessed rapid advancements in the identification of *in vivo* biomarkers, especially those reflecting amyloid-beta ($A\beta$) deposition, tau pathology, synaptic dysfunction and neuroinflammation, all of which are hallmarks of AD (Georgakas et al., 2023). Techniques such as positron emission tomography (PET), cerebrospinal fluid (CSF) assays, and blood-based biomarkers have shown increasing sensitivity and specificity in the past years. These are emphasized in research done on both animal (rat and primate) models and humans. Hansson et al. (2023), for example, states that blood-based biomarkers are an exceptionally promising avenue for revolutionizing the clinical practice of diagnostic and prognostic work-up of AD. Anoop et al. (2010) explores the significant recent advancements in CSF biomarkers for monitoring the early stages of AD before severe cognitive dysfunction is apparent. Márquez & Yassa (2019) also suggests a need for composite biomarkers in neuroimaging, as this can provide a deeper understanding of the pathophysiology of AD. Although the future of early diagnosis looks bright, it is undeniable that translational hurdles still persist, thereby limiting the availability of proven diagnostics to underserved communities due to factors like socioeconomic differences, standardization, and education of clinical staff. This literature review investigates how the neurobiological foundations of Alzheimer's disease biomarkers inform their utility, analysing the key translational challenges that hinder their integration into routine clinical practice.

Methodology:

The review is structured into five main sections. First, the molecular and cellular neurobiology underpinning key AD biomarkers will be discussed, with a particular focus on the amyloid and tau hypotheses, neuroinflammation, mitochondrial dysfunction, and synaptic and neurotransmitter alterations. Next, it surveys the primary modalities used in biomarkers, their technical strength and mechanistic correlates. The third section discusses more novel biomarkers, and the fourth examines translational challenges, including issues of socioeconomic accessibility, standardization and technical limitations. Finally, the review evaluates the ongoing efforts in bridging the lab-to-clinic divide.

With a critical synthesis approach, this review analyses peer-reviewed literature across neuroscience, clinical neurology and translational medicine. It integrates findings from empirical studies, along with meta-analyses and systematic reviews to identify emerging consensus and unresolved tensions, such as the accuracies of specific biomarkers, within the field. A targeted narrative review of human and translational studies was conducted on

D biomarkers published 2015 to 2025 in PubMed and Scopus, using terms combining *Alzheimer's disease* with *biomarker*, *blood*, *CSF*, *MRI*, *PET*, *retina*, *saliva*, *urine*, and *volatile organic compounds*. Inclusion prioritized peer-reviewed original research, along with systematic review and consensus statements. Seminal pre-2015 studies were retained selectively to provide historical context. Non-peer-reviewed web sources were excluded except where clearly identified as illustrative, such as information about cost ranges.

Section 1: Molecular and cellular neurobiology and pathophysiology of AD

AD is characterized by the slow, progressive neurodegeneration brought on by neuronal cell death and activity dysfunction. This usually starts in the entorhinal cortex (part of the hippocampal formation), an area crucial for various forms of explicit memory formation, particularly spatial memory (Karimani et al., 2024). The pathophysiological hallmarks of AD are accumulations of abnormal neuritic plaques and neurofibrillary tangles (NFTs). With these changes, there is also a loss of cholinergic neurons in the basal forebrain and neocortex (Kumar et al., 2024). There are two main hypotheses that are popular within the scientific community: the cholinergic hypothesis and the amyloid hypothesis. This literature review focuses on the amyloid hypothesis, as it is currently the most widely accepted pathophysiological mechanism for AD. However, it should be noted that this hypothesis has also faced challenges and criticisms, and it is believed that there are alternative or complementary mechanisms in the pathogenesis of AD (Nasb et al., 2024).

Amyloid beta

The peptide amyloid beta ($A\beta$) is derived from a protein called the amyloid precursor protein (APP), through the abnormal activity of β - and γ -secretase enzymes, which are responsible for breaking down proteins (Mucke & Selkoe, 2012). Typically, APP is cleaved by either α - or β -secretase and the smaller fragments formed are not neurotoxic. However, the cleavage by β - followed by γ -secretase results in a peptide of 42 amino acids, known as $A\beta_{42}$, which is a long peptide more prone to aggregation than shorter species. $A\beta$ oligomers, which are smaller aggregates of these $A\beta$ peptides, are considered especially neurotoxic. Increase in $A\beta_{42}$ levels leads to the aggregation of amyloid, creating the commonly seen abnormal neuritic plaques (Nhan et al. 2015). These are spherical microscopic lesions made of an extracellular $A\beta$ peptide surrounded by enlarged axonal endings, and are usually found within the cortical gray matter or around the meningeal and cerebral vessels of the brain. These deposits initially form in different areas, but grow and merge to comprise the larger more recognisable plaque structures, which then lead to neuronal toxicity (Kumar et al., 2024). This is caused by $A\beta$ plaques damaging mitochondria as they accumulate inside neuronal cells, impairing its ability to generate ATP. Without sufficient energy, the neuron cannot maintain its normal function, leading to the initiation of apoptosis (programmed cell death). Furthermore, $A\beta$ oligomers can insert themselves into the cell membrane of neurons, forming pores that allow for the unregulated influx of calcium ions, disrupting the balance of

calcium concentrations which is critical for signalling and cell survival. High calcium levels within the cell can activate enzymes that degrade proteins and lipids, leading to the breakdown of the neuronal cytoskeleton, and further mitochondrial dysfunction (Fani et al., 2021).

Certain mutations, specifically those in the genes encoding APP and secretases (e.g. presenilin 1 and 2 (PSEN1 and PSEN2)) have been linked to cases of familial AD, supporting the A β hypothesis. This is because these mutations increase the production of longer and more aggregation-prone isoforms of A β (Lanoiselée et al., 2017). Another gene is the apolipoprotein E (APOE) gene, which is the “strongest genetic risk factor for late-onset AD”, according to Kamatham et al. (2024), as it is associated with producing proteins that lead to higher levels of A β aggregation and simultaneously reduced clearance of the peptide.

Tau tangles

The aggregation of A β leads to a cascade of events. Tau is a protein that stabilises axonal microtubules which are vital for intracellular transport. One of these cascade events is the hyperphosphorylation of tau (the addition of too many phosphate groups which modifies its shape) through the activation of kinases like glycogen synthase kinase-3 β (GSK3 β), causing tau to become misfolded and aggregate within neurons (Kumar et al., 2024). With this, these tau molecules become prone to self-aggregation and form insoluble twisted paired helical filaments. Known as NFTs, they first appear in the hippocampus. Recent research has supported the idea that tau aggregates have prion-like properties, allowing their propagation throughout the brain by inducing the misfolding of normal tau in neighbouring neurons (Alyenbaawi et al., 2020). This may explain why tau is often seen spreading throughout the cerebral cortex as AD progresses, contributing to cognitive decline in affected individuals.

Inflammation

Both A β peptides and NFTs can trigger inflammatory responses within the brain by activating microglia and astrocytes. These cells then become ramified and abnormally enlarged (hypertrophied) in NFT-rich brain regions (Kamatham et al., 2024). The immune responses are triggered to clear these harmful substances through the release of pro-inflammatory molecules. However, while this process aims to protect the brain, chronic and dysregulated inflammation produces proinflammatory cytokines that lead to neuronal damage and exacerbate the progression of the disease (Adamu et al., 2024). This sustained state of inflammation can exacerbate neuronal damage and further promote the spread of tau pathology.

Mitochondrial Dysfunction

A β peptides have also been shown to impair mitochondrial function by accumulating within the inter-mitochondrial membrane space and disrupting their normal function (Aran & Singh, 2023).

This causes a reduction in ATP production and an increase in ROS generation, leading to oxidative stress and eventual damage to the organelle and its normal activity. This then triggers a positive feedback loop, as impaired mitochondrial function further promotes A β production and aggregation. In addition to this, abnormally phosphorylated and aggregated tau proteins have also been implicated in causing mitochondrial dysfunction by impairing mitochondrial transport along neuronal axons, which consequently leads to local energy deficits (Reddy et al., 2012). It also disrupts fission and fusion processes, which are crucial for maintaining a healthy mitochondrial network (Zong et al., 2024). This also triggers a cycle, where mitochondrial dysfunction results in the formation of even more tau aggregates.

Synaptic and neurotransmitter alterations

Both protein aggregates of A β and tau prevent regular synaptic function by hindering the release of neurotransmitters (including acetylcholine, which plays an important role in memory and cognition), reducing synaptic density and interferes with synaptic plasticity, particularly in cholinergic neurons (Kamatham et al., 2024). Consequently, this causes signaling deficiencies, contributing to the memory impairment and cognitive decline associated with AD. Glutamate neurotransmission is also affected, as glutamate receptors are excessively activated, contributing to excitotoxicity, which then leads to neuronal damage and cell death. As the membranes of these axonal cells are disrupted and the cells die, the intracellular contents, which contain essential structural components including neurofilaments (NfLs), leak out into the surrounding fluid (Gafson et al., 2020). This release can activate an inflammatory response in the surrounding tissue, and the following inflammatory cascade can contribute to further neurodegeneration.

Environmental Factors

Although novel, it has been posited that bacterial and viral infections may be implicated in the process of AD pathogenesis, as different types of microbes have been shown to stimulate A β aggregation and deposition. Therefore, there may be a pathogenic factor in the onset of AD as well (Gonzalez-Sanchez et al., 2020).

Figure 1. Diagram of the proposed forms of pathogenesis of AD

All the different pathologies of AD are largely thought to be dynamically interacting rather than isolated, as supported numerous studies, a few of which are Kamatham et al. (2024), Calabrò et al. (2020), and Kumar et al. (2024) suggesting that biomarkers that are able to capture multiple axes of degeneration may be most effective in diagnosing the disease.

Section 2: Types of Biomarkers and How They Work: Established Biomarkers

Biomarkers are defined as being naturally occurring molecules, genes, or characteristics by which a particular pathological or physiological process or disease can be identified. This literature review identifies three types of biomarkers typically employed in the diagnosis of AD: molecular, imaging and functional. Molecular biomarkers include proteins or other biological compounds found through the testing of substances like blood, cerebrospinal fluid (CSF) and other bodily fluids. Imaging biomarkers include scans like Positron Emission Tomography (PET), Magnetic Resonance Imagery (MRI) and Computed Tomography (CT), while functional biomarkers are measured through performance on cognitive or motor assessments and tasks. Usually, biomarkers are employed to determine the cause of mild cognitive impairment (MCI) in the initial stage of dementia. This literature review further investigates promising new biomarkers that have shown potential in predicting AD.

MOLECULAR

CSF

Cerebrospinal fluid is an ideal candidate for reflecting the molecular events of the brain, as it is in direct contact with the brain's extracellular space. Hence, it seems to be the general consensus that CSF has one of the highest potentials to identify AD biomarkers that reflect AD pathogenesis (Papaliagkas et al., 2023).

Because A β 42 is a large component of typical AD plaques, the amount of total A β in CSF is well-correlated with AD diagnosis. There is a positive correlation between lower CSF A β 42 levels and plaque burden, due to plaques acting as “amyloid sinks”, where the peptide is deposited and accumulates (Anoop et al., 2010). Anoop et al. also found a correlation between a rise in A β 38 levels and a fall in A β 42, which supports the idea that the ratio A β 42:A β 38 may be more useful and precise in diagnosing AD. Reduced A β 42 has also been observed in other types of dementia, and many works and wider literature agrees that A β 42 is the most accurate CSF biomarker of AD in patients with MCI (Sonnen et al., 2011). However, there was no significant difference observed in the *total* CSF A β levels in AD patients and non-AD controls in Anoop et al.'s study. Therefore, it seems that only A β 42 is a relatively accurate predictor of the disease.

Increased levels of tau and phosphorylated tau (p-tau) in CSF has been shown to be correlated with AD onset, with several studies suggesting that total tau (t-tau) concentration in AD patients' CSF is higher than the control (Papaliagkas et al., 2023). However, it is noted that this biomarker, although sensitive, is limited in its ability to differentiate AD from other forms of dementia, as an increase in t-tau is not a feature unique to AD. Hence, p-tau is the more accurate biomarker, because it is more closely

related to the pathophysiology of AD. This is compared to t-tau which is simply a marker of axonal damage.

Levels of glial fibrillary acidic protein (GFAP) tend to be increased in individuals with early A β pathology, as it is hypothesized to reflect the increased reactive astrocytes, which are a characteristic of AD (Marksteiner & Humpel, 2025). This can be measured reliably in the CSF, as shown by Cicognola et al.'s 2021 study. Plasma NfL levels also correlate well with the levels of NfL found in CSF, as it leaks from cells due to injury (Gisslén et al., 2015).

Another potential biomarker is neuronal threat proteins (NTPs). These are expressed in the brain during AD-related neurodegeneration, with abnormal AD7c-NTP being an NTP that is especially expressed during this process. This has been found to be higher in the postmortem CSF of AD individuals compared to controls (Kang & Wang, 2022).

Blood

As A β 42 and A β 40 peptides accumulate within the brain, plasma levels of individuals with AD are typically indicative of this change. The plasma A β 42:A β 40 ratio is usually higher in A β 42-positive groups than in A β 42-negative groups, as has been demonstrated by different immunoassays and mass spectrometry-based assays (Doecke et al., 2020). However, different assays vary substantially, with a recent comparison showing that certain mass spectrometry-based assays were far better at identifying A β pathology than more commonly used immunoassays. Regardless, these higher-performing A β 42:A β 40 assays still only exhibit modest correlations between the levels of plasma A β and that found in CSF, likely due to the fact that A β in plasma is derived from peripheral sources, as posited by Hansson et al. (2023). Hence, it is agreed that plasma A β 42 levels alone cannot be considered an AD marker.

On the other hand, researchers have recently developed several high-sensitivity assays for the detection of different p-tau protein variants, or isoforms, including specific ones like p-tau181, p-tau231 and p-tau217 (Janelidze et al., 2025; Khalafi et al., 2024; Nabizadeh et al., 2022). When compared, it seems that assays quantifying plasma p-tau217 have been shown to be better at detecting AD pathology and predicting the development of AD dementia (Wang et al., 2025). However, it can be argued that p-tau231 is a good marker of AD in its early stages, as it starts increasing at very low A β levels (Ashton et al., 2022). Hence, when combining plasma A β 42 and A β 40 with p-tau, early-stage amyloid positivity detection could be improved.

Similarly, GFAP can also be measured reliably in the blood as well as in the CSF. Plasma NfL levels also correlate well with the levels found in CSF, although it seems that this correlation is not as strong as that of p-tau217 (Garcia-Escobar et al., 2024). This study has

also shown little to no correlation between levels of NfL and A β 42 in the blood, which has led researchers to believe that NfL suggests neurodegeneration independently of the pathology of A β .

Janelidze et al. (2022)'s study has shown that the combination of plasma p-tau231 and A β 42:A β 40 could be used to detect very early stage AD, and it is supported by the fact that it was able to detect amyloid pathology in cognitively unimpaired individual. This could prove useful as it would allow screening tests to be able to identify AD before the clinical onset of symptoms.

Saliva

Saliva is not only an easily accessible body fluid; its secretion and composition might also be affected by various diseases. It is posited that AD can influence salivary contents, as there seems to be a relationship between the brain and saliva. This occurs through six pathways connecting brain molecules with the saliva and vice versa (Surdu et al., 2025). However, for both A β and tau, concentrations are considerably lower in non-plasma fluids (Delaby et al., 2023).

A review by Nijakowski et al. (2024) showed that there was a statistically significant number of studies that have examined the saliva of AD patients and found elevated levels of A β . However, it was noted that in three studies, neither A β 40 or A β 42 were detected in AD patients. This suggests that there is a question of reliability when using saliva samples to get an accurate measure of A β levels in studies of AD, which is supported by similar findings from Sabbagh et al. (2018).

Based on the same review., it also appears that the reliability of tau as a biomarker for AD is inconsistent when considered alone. There is no consensus on whether t-tau, p-tau and their ratio are reliable indicators of AD. The studies examined by Nijakowski have reported conflicting results, and suggest that while tau may be part of the AD pathology, its biomarker use in saliva cannot be considered a standalone reliable biomarker.

Nijakowski et al. also explores lactoferrin (Lf), a protein with anti-inflammatory properties, and proposes that it may have neuroprotective effects in neurodegenerative diseases like AD. According to Gonzalez-Sanchez et al. (2020), biomarkers that reflect the integrity of the immune system could be useful. Lf has previously been detected in senile plaques, NFTs and microglia in AD brains. Although it was particularly useful in differentiating AD and MCI patients from healthy individuals, it appears to be a promising, but not fully conclusive biomarker for AD (Gonzalez-Sanchez et al., 2020). While several studies analysed by Nijakowski et al.'s paper showed a clear link between decreased salivary lactoferrin levels and AD, there are also conflicting findings that appear to warrant further investigation (Yang et al., 2021).

Urine

Both A β and tau are possible to detect in urine, because they can be eliminated from the organism by action of the kidneys (Armenta-Castra et al., 2024). These are detected through methods like enzyme-linked immunosorbent assay (ELISA) and Western blot. Several reviews like Kamatham et al. (2024) and Zhang et al. (2024) have shown a correlation between the presence of these proteins in urine and AD, and it appears that A β oligomers are better indicators of AD progression, instead of the monomers A β 40 and A β 42. Both p-tau and tau were found to be significantly higher in AD patients compared to control individuals, but as was the case with saliva, these concentrations were much lower in urine than in plasma for both groups (Armenta-Castro et al., 2024).

NTPs have been found in urine, as in the CSF. Evidence for urinary AD7c-NTP is preliminary and replication in contemporary cohorts is limited, leaving clinical utility uncertain. One such piece of evidence is the ELISA results from Ma et al.'s 2016 study utilized ELISA to show that results in urine showed similar sensitivity and specificity to CSF, and that urinary AD7c-NTP was significantly higher than in the non-AD group.

IMAGING

Neuroimaging allows for diagnosis of the AD pathophysiological process during life, even though neuropathology remains the definitive standard. Clinicians can begin to assess cases in vivo, with several modalities having been researched, including MRI and PET (Dang et al., 2023).

Neurodegeneration leads to cerebral atrophy, which is most notable in the hippocampus and entorhinal cortex of patients with AD (Tabatabaei-Jafari et al., 2015). Woodworth et al (2022) found that the level of atrophy in the medial temporal lobe is strongly correlated with lower scores on cognitive testing, and is therefore associated with disease progression. As this degeneration progresses to other cortical structures, lateral ventricles become enlarged in the later stages of the disease. In addition to this, limbic structures (such as the amygdala, cingulate gyrus, thalamus, and olfactory bulb tract) are impacted, and the atrophy leads to volumetric reductions in the frontal, parietal and temporal brain areas (Woodworth et al., 2022).

MRI

MRI is an imaging technique that is able to generate high-resolution images of water-containing tissue. This is done by applying a strong magnetic field and pulses of radio waves that manipulate electromagnetic fields and energy states of protons within the body (Magnetic Resonance Imaging (MRI), n.d.). These protons emit energy, which are then converted to signals and images.

Structural MRIs are used to provide highly detailed images of anatomy as well as a strong gray/white matter contrast. These scans are able to display white matter hyperintensities that indicate demyelination and axonal loss (Meng et al., 2022). Compared to patients without AD, patients with AD have greater white matter hyperintensities in the frontal lobe that index small-vessel disease burden and help in differential diagnosis and staging, but are not necessarily specific to AD.

Diffusion Tensor Imaging (DTI) uses the displacement of water molecules to measure the integrity of the white matter tract. The direction of diffusion allows scientists to better understand tracts and specific fibre bundles that may be impacted by neurodegeneration (Esrael et al., 2021). The major measures of DTI are mean diffusivity (MD), which is the average rate of water molecule diffusivity, and fractional anisotropy (FA), which is the variability associated with diffusion. In AD brains, MD can be seen in all four areas of the brain (frontal, occipital, parietal and temporal), including the hippocampus. This is a clear difference from MCI, where these increases are not seen in the frontal and occipital regions. AD also presents as decreased FA localized to the cingulum, corpus callosum, uncinate fasciculus and superior lateral fasciculus, as well as throughout the temporal, occipital and frontal white matter (Chandra et al., 2018). Patients with MCI have no irregularities of FA in occipital and parietal areas, which sets MCI scans apart from AD, according to Chandra et al. (2018). According to a study done by Magalhães et al. (2023), there was a significant correlation between white matter tracts and both p- and t-tau.

Functional MRI (fMRI) produces dynamic representations of brain activity through blood oxygen level-dependent (BOLD) signals (Chandra et al., 2018). When asked to perform memory tasks, AD patients showed little to no activation of hippocampal and other medial temporal structures as compared to non-AD individuals (Casagrande et al., 2023). Resting-state fMRIs of the AD brain also show abnormal coactivation of the medial temporal structures and the posterior cingulate cortex, which differs from those of normal brains (Aramadaka et al., 2023).

PET

PET is an imaging modality that allows for the in vivo measurement of disease pathologies using radioligands to detect compounds of interest. In the case of AD, that would be NFTs and A β plaques.

FDG-PET uses 18-F-fluorodeoxyglucose (FDG), which allows researchers to estimate glucose metabolism in different brain areas and identify which ones may have abnormal metabolic activity (Bouter et al., 2019). From studies done, such as Womack et al. (2012) and Schilling et al. (2016), these scans show temporoparietal hypometabolism in patients

with AD, which is characteristic of the disease. Furthermore, there was an observed decrease in metabolism in the posterior cingulate cortex and precuneus, which is thought to be related to the episodic memory deficits typical of AD (Villa et al., 2020).

Because of accumulated A β , there is an increased level of amyloid tracer binding in areas of the brain affected by the disease (more specifically, temporal, parietal and frontal regions), according to Villa et al. (2020). Tracers such as Pittsburgh Compound B (PiB) are able to bind to misfolded amyloid plaques, therefore helping to map distribution and quantify amyloid burden. Other tracers include florbetapir (Matsuda et al., 2022), 18-F-labeled flutemetamol (Park et al., 2025) and 18-F-labeled florbetaben (Son et al., 2018).

Part of the underlying pathophysiology of AD includes the neuroinflammatory cascades leading to an increase in microglial activation. Radiotracers that bind to translocator protein-18 kDa (TSPO) have been utilized as biomarkers of AD neuroinflammatory cascades (Georgakas et al., 2023). Since TSPO is a peripheral benzodiazepine receptor found on the membrane of the mitochondria, increased expression of this protein can be used as an estimate for increased inflammation, as the expression of this protein is upregulated by activated microglial cells. Studies done have found increased TSPO expression levels in individuals with MCI and both prodromal and severe AD stages when compared to healthy controls (Nutma et al., 2021; Zhang et al., 2021).

Machine Learning and Artificial Intelligence

With the rise of better artificial intelligence (AI) algorithms, AI is being increasingly utilized in the diagnosis of AD (Kamatham et al., 2024). AI models are capable of analysing large, complex datasets containing neuroimaging data and biomarker profiles to identify patterns. MRI and PET generate vast amounts of data that can be analysed using AI to detect both structural and functional brain changes associated with AD. Biomarker data can also be incorporated in order to develop predictive models for risk assessment. This holds great potential for improving efficiency and accuracy, since AI models can therefore aid healthcare providers in making increasingly informed diagnostic decisions, facilitating the early detection of AD and the generation of personalized treatment plans based on individual profiles (Chong et al., 2025).

behaviorAL

Functional

The progressive loss of ability to perform activities of daily living (ADL) unassisted is a primary characteristic of dementia (Chaves et al., 2011). These activities can be divided into basic (BADL) and instrumental (IADL). BADL includes the ability to carry out tasks necessary for personal hygiene, sphincter control and feeding. IADL, on the other hand,

are more complex and are activities such as preparing meals, carrying out domestic chores, and managing finances and correspondence.

An example of an assessment used to evaluate changes in functional performance is through an assessment measure known as Functional Assessment Staging (FAST). This allows healthcare practitioners to specifically evaluate these changes throughout the entire course of AD (Sclan & Reisberg, 1992). Another such assessment measure is the Alzheimer's Disease Cooperative Study – Activities of Daily Living for Mild Cognitive Impairment, otherwise known as ADCS-MCI-ADL. This assesses the competence of AD patients in both basic and instrumental ADLs, and can be completed by a caregiver or administered by a clinician as a structured interview with a caregiver (Fish et al., 2011).

Cognitive

The initial and most common presenting symptom of AD is episodic short-term memory loss (Kumar et al., 2024). This includes difficulty retaining new information, and experiencing impairments in problem-solving, executive functioning, judgement, and organizational skills. Because of this, individuals with AD may struggle with multitasking and abstract thinking, which affects their ability to carry out ADLs such as driving, cooking, and activity planning. Following this, there are often language and visuospatial skill impairments, along with neuropsychiatric symptoms like changes in social behavior (Kumar et al., 2024).

A common example of a cognitive assessment is the Mini-Mental State Examination (MMSE) (Arevalo-Rodriguez et al., 2015). This is a common test for assessing changes in cognition, and evaluates functions such as orientation, language, memory and other functions. This is done through tasks that test reading, writing and short-term memory, including tasks like subtraction, remembering a series of unconnected words or copying a diagram on paper.

The Alzheimer's Disease Assessment Scale - Cognitive, or ADAS-Cog, is generally more thorough, and is considered the gold standard for assessing AD (Dementia Australia, n.d.; Kueper et al., 2018). This includes 11 tasks, including both tests completed by the subject and assessments completed by the observer. When put together, these tasks assess memory, language, and praxis. Specific tasks, like the MMSE, include word recall, naming objects, commands, and word recognition among others.

As can be seen by this, there already exists a plethora of widely acknowledged and effective biomarkers, whether they be molecular, imaging or functional. A summary of the above information can be found in Table 1.

Table 1. Summary of Key Biomarkers and Diagnostic Modalities for AD

Category	Subcategory	Biomarker / Method	Key Findings	Work Cited
Molecular	CSF	A β 42	Lower levels correlate with plaque burden; most promising CSF biomarker for AD in MCI (Kumar et al., 2024)	Anoop et al., 2010
		A β 42:A β 38 ratio	May improve diagnostic precision compared to A β 42 alone (Doecke et al., 2020)	Doecke et al., 2020
		t-tau	Elevated in AD; sensitive for neurodegeneration	Papaliagkas et al., 2023
		p-tau	More closely related to AD pathology than t-tau	Janelidze et al., 2025; Ashton et al., 2022
		GFAP	Increased with early A β pathology; reflects reactive astrocytes	Marksteiner & Humpel, 2025
		NfL	Elevated; marker of axonal injury and neurodegeneration	Garcia-Escobar et al., 2024
		AD7c-NTP	Higher in postmortem CSF of AD patients (emerging biomarker)	Kang & Wang, 2022
	Blood	A β 42:A β 40 ratio	Lower in A β -positive groups; better with mass spectrometry	Hansson et al., 2023
		p-tau217	Strong predictor of AD pathology and progression	Khalafi et al., 2024
		p-tau231	Early-stage AD marker (increases at low A β levels)	Nabizadeh et al., 2022
		p-tau181	Widely adopted, earlier generation with lower AD specificity	Janelidze et al., 2025
		GFAP	Detectable in blood plasma; reflects astrocyte activation	Garcia-Escobar et al., 2024
		NfL	Detectable in blood plasma; less correlated with CSF than p-tau217	Janelidze et al., 2022
		Combined p-tau231 + A β 42:A β 40	Detects very early-stage AD with improved accuracy	Hansson et al., 2023 Janelidze et al., 2025
	Saliva	A β	Some studies show elevated levels in AD	Sabbagh et al., 2018

		t-tau, p-tau	Conflicting results; unreliable as standalone biomarkers	Nijakowski et al., 2024
		Lactoferrin	Lower in AD; potential immune-related biomarkers	Gonzalez-Sanchez et al., 2020
	Urine	A β oligomers	Better indicators of disease progression than monomers	Armenta-Castro et al., 2024
		p-tau, tau	Higher in AD patients than controls	Armenta-Castro et al., 2024
		AD7c-NTP	Elevated; similar sensitivity/specificity to CSF assays	Ghanbari et al., 1998
Imaging	MRI	Structural MRI	Hippocampal and entorhinal atrophy; white matter hyperintensities	Tabatabaei-Jafari et al., 2015 Meng et al., 2022
		DTI	Increased MD and reduced FA in specific tracts; correlates with tau pathology	Esrael et al., 2021
		fMRI	Reduced hippocampal activation; altered resting-state connectivity	Chandra et al., 2018
	PET	FDG-PET	Temporoparietal hypometabolism; PCC/precuneus deficits	Bouter et al., 2019
		Amyloid PET	PiB and other tracers map amyloid burden in vivo	Villa et al., 2020
		TSPO PET	Detects neuroinflammation via microglial activation	Georgakas et al., 2023
	AI/ML Integration	AI models	Detect structural/functional changes; integrate biomarkers for improved prediction	Kamatham et al., 2024
Functional	Behavioral	BADL/IADL assessments (FAST, ADCS-MCI-A DL)	Measures decline in daily functioning	Chaves et al., 2011 Sclan & Reisberg, 1992 Fish et al., 2011
	Cognitive	MMSE/ADAS-Cog	Screen orientation, memory, language; widely used cognitive assessments	Kumar et al., 2024 Arevalo-Rodriguez et al., 2015 Kueper et al., 2018

Abbreviations:

A β = amyloid-beta; APP = amyloid precursor protein; APOE = apolipoprotein E; ApoC3 = apolipoprotein C3; AT(N) = amyloid, tau, neurodegeneration framework; BBM = blood-based biomarker; CSF = cerebrospinal fluid; DTI = diffusion tensor imaging; FA = fractional anisotropy; FDG-PET = fluorodeoxyglucose positron emission tomography; fMRI = functional magnetic resonance

imaging; GFAP = glial fibrillary acidic protein; IADL = instrumental activities of daily living; Lf = lactoferrin; LncRNA = long non-coding RNA; MCI = mild cognitive impairment; MD = mean diffusivity; miRNA = microRNA; MRI = magnetic resonance imaging; MCP-1 / CCL2 = monocyte chemoattractant protein-1 (chemokine ligand 2); NfL = neurofilament light chain; NFTs = neurofibrillary tangles; NTPs = neuronal thread proteins; OCT = optical coherence tomography; PET = positron emission tomography; p-tau = phosphorylated tau; RNFL = retinal nerve fiber layer; ROS = reactive oxygen species; SIMOA = single molecule array; SUVR = standard uptake value ratio; TSPO = translocator protein (18 kDa); VOC = volatile organic compound.

Section 3: Types of Biomarkers and How They Work: Novel Biomarkers

While traditional biomarkers have focused on the more established hallmarks of AD, such as A β and tau, recent research has expanded into a more diverse spectrum of novel biomarkers that may serve to capture alternative aspects of the disease's pathology. These include new developments in both molecular and imaging biomarkers as technological advancements occur.

MOLECULAR

MicroRNA

Micro ribonucleic acids (miRNAs) are a type of short-length, non-coding, and single-stranded endogenous RNAs (also known as sncRNAs) found in a stable form in the blood, CSF, and various body fluids (Chimthanawala et al., 2023). By controlling the expression of genes and pathways, miRNAs act through suppressing the translation of target messenger RNAs (mRNAs) and facilitating their degradation, contributing to APP degradation and A β metabolism. AD patients had higher levels of miRNA-4722-5p and miRNA-615-3p, and combining these two AD-associated miRNAs provided a better diagnostic score than either miRNA alone (Liu et al., 2022). miRNA-20b-5p also downregulated APP levels, hence reducing the availability of A β (Wang et al., 2022). However, further research in this domain needs to be carried out, especially on three miRNAs that strongly correlated with the early stages of AD, namely miRNA-92a-3p, miRNA-486-5p, and miRNA-29a. Levels of miRNA-92a-3p and miRNA-486-5p were lower in AD patients compared to controls, but higher levels of miRNA-29a-3p were found in the plasma of AD and MCI patients (Peña-Bautista et al., 2022). The same source also found that plasma levels of miRNA-483-5p levels were higher in AD patients compared to healthy controls, and it was concluded that increased levels of miRNA-483-5p in plasma can reliably distinguish both MCI and AD groups from the healthy controls. The above findings suggest that these miRNAs could be potential biomarkers in early AD diagnosis.

Long non-coding RNA

A second type of RNA that can be used is long non-coding RNAs (LncRNAs). These resemble mRNA molecules, but are not responsible for encoding proteins (Black et al., 2024). They typically contribute to the regulation of several processes by controlling chromatin

remodelling, and providing alternative mRNA splicing. Black et al. (2024) has found that this affects gene expression, and has been shown to “directly or indirectly regulate the key features of AD including A β deposition, aberrant tau formation, oxidative stress, neuroinflammation, and neuronal death”. LncRNAs also interact with miRNAs, therefore influencing AD-related neuropathology. The expression of numerous types of these molecules has been detected in plasma and CSF samples from AD patients, which indicates that lncRNAs could function as a likely biomarker in the diagnosis and progression of AD (Black et al., 2024). Specific candidate lncRNAs include BC200, NEAT1, BACE1-AS, and 51A, which were both elevated in AD patients, as found in a review by Wu et al. (2024).

Apolipoprotein C3

A study done by Watanabe et al. (2020) suggests that Apolipoprotein C3 (ApoC3) may be a potential urinary biomarker for AD, although further analysis of this is warranted (Watanabe et al., 2020). ApoC3 is an abundant apolipoprotein mainly present in triglyceride-rich lipoproteins (chylomicrons and very-low-density lipoprotein (VLDL)), as well as cholesterol particles. Watanabe et al. showed that studies have reported that people with AD had lower plasma levels of ApoC3 compared to healthy individuals, and that higher ApoC3 was associated with reduced grey matter volume in the brain. The paper also pointed to recent research suggesting that ApoC3 acts as a trigger for inflammation by activating the NLRP3 inflammasome (Watanabe et al., 2020). This is significant because the NLRP3 inflammasome is known to be activated in the brains of AD patients by amyloid beta. The result of Watanabe et al.'s experiment was that all samples revealed ApoC3 levels to be significantly higher in the AD group relative to the control group.

MCP-1

Monocyte Chemoattractant Protein 1 (MCP-1, also known as CCL2) is a protein that belongs to a family of signalling molecules called chemokines (Huang et al., 2023). They play key roles in the body's inflammatory response by attracting immune cells to sites of inflammation. As a biomarker, MCP-1 can be measured in both blood and urine. In AD, neuroinflammation contributes to neuronal damage. It is thought that it is because of this that elevated levels of MCP-1 have been found in the blood and CSF of AD patients. However, this is found to varying extents across different studies (Fenoglio et al., 2004; Galimberti et al., 2006), which is why Huang et al. hypothesized that MCP-1 levels impact individuals who are genetically at risk of developing AD.

Molecules Associated with Oxidative Stress

A β induces oxidative stress, the production of harmful reactive oxygen species (ROS), which is thought to be a major contributor to cellular damage in the brain (Gella & Durany, 2009). ROS

are too unstable to measure directly, so measuring their byproducts is a method used to quantify oxidative stress. Several products, specifically 4-hydroxy-2,3-nonenal (HNE), acrolein, F2-isoprostanes and malondialdehyde have been observed to exist in higher levels in AD brains (Singh et al., 201). However, like many other biomarkers, oxidative stress is a general sign of cellular damage and is not necessarily specific to AD. It is a feature of many neurodegenerative disorders and is also affected by ageing and environmental factors (Dash et al., 2025).

Volatile Organic Compounds

Exhaled breath contains hundreds of different byproducts of metabolism, also known as volatile organic compounds (VOCs). In AD, metabolic changes in the brain could produce a unique “breathprint” (Zhang et al., 2025). Using sophisticated sensors or mass spectrometers, analysis of this breathprint can be carried out to identify patterns that correlate with this disease. Zhang et al. (2025) identifies six key candidate VOCs of interest, including ethanol, isopropanol, chloroethane, pyrrole, 1-butanol and benzene. However, this research is still in its early stages, and whether it can be used for AD diagnosis is unclear.

IMAGING

Retinal Imaging

The past decade has seen rapid progress in using retinal imaging to find markers of AD. Techniques like optical coherence tomography (OCT) are shown to be especially promising because of their non-invasive, affordable and accessible nature when compared to traditional brain imaging (Georgakas et al., 2023). Research has shown structural, vascular and neuropathological changes in the retinas of both mice and humans with AD. Structural markers, such as the thinning of the retinal nerve fibre layer (RNFL) are consistently seen in older adults with AD (Sánchez-Puebla et al., 2024). This thinning may start early in disease progression, as it has been linked to levels of A β and tau in CSF, as well as to hippocampal volume in cognitively normal adults (Chen et al., 2023). This suggests its usefulness as a tool for early AD screening. Vascular changes are also being explored, as a 2013 study by Frost et al. found that asymptomatic adults with high cerebral A β had less-branched retinal blood vessels. Vagiakis et al.'s 2024 study found that a larger foveal avascular zone (FAZ), which is the area of the retina without blood vessels, was typically associated with higher cerebral A β in cognitively normal adults. This suggests that the FAZ area, along with retinal imaging in general, may be an effective marker for detecting preclinical AD (Vagiakis et al., 2024). However, Ashraf et al. (2023) has found weak evidence, suggesting that research in this area is currently yielding mixed results, with some finding strong correlations and others not.

CT

A CT scan uses a series of X-rays from different angles to generate cross-sectional images of the brain. While it cannot visualise amyloid plaques or tau tangles directly, it is able to detect brain atrophy of regions such as the hippocampus (Pasi et al., 2011). However, it is only really supportive when a patient presents with the typical patterns of AD (i.e. it is not specific to AD and may also appear in other conditions). It may also prove more useful only for later-stage AD, as brain atrophy usually cannot be detected until patients begin to exhibit behavioral and cognitive changes. In addition to this, CT scans involve using ionizing radiation which, though at a low dose, carries a small risk, especially for people undergoing repeated scans.

Tau PET

Tau PET molecular imaging is a relatively new technology. So far, there is a first-generation FDA-approved tracer, known as 18-F-flortaucipir, and has been shown to have a high affinity for detecting the tau aggregates that are characteristic of AD (Petersen et al., 2022). However, off-target binding is an obstacle in this procedure, where tracers bind to molecules other than tau, producing unreliable results in a PET scan. This is why there are ongoing efforts to develop second-generation tracers, which are being designed to have improved binding specificity (Bischof et al., 2021). These second-generation tracers include 18F-RO-948, 18F-MK-6240 and 8F-PI-2620, to name a few included in Bischof et al. (2021)'s review.

Most non-CSF, non-blood matrices remain exploratory, but from current research, it is clear that many of them warrant significant potential to become alternative biomarkers. Some may even prove to be more advantageous than traditional biomarkers due to their cost-effectiveness and accessibility, as well as their specificity and accuracy.

Section 4: Translational challenges and barriers

Data standardization and comparability represent a critical challenge in both imaging data collection and biomarker fluid acquisition protocols that can vary considerably across different Alzheimer's Disease Research Centers (ADRCs) (Braudeau et al., 2025). This variability complicates the establishment of universal diagnostic thresholds and hinders the direct comparability and integration of findings from diverse research cohorts, making it difficult to generalize results and apply them uniformly in clinical practice.

Consequently, even if a biomarker demonstrates exceptional scientific efficacy, its practical clinical utility is severely constrained if it cannot be widely deployed across diverse healthcare settings. This accessibility-utility gap highlights pressing health equity concerns in AD diagnosis, driving the urgent need for less invasive and more affordable alternatives, even if those alternatives present their own set of challenges.

IMAGING

High cost and limited accessibility are paramount challenges. Imaging costs can vary widely across settings, but illustrative ranges suggest substantial patient-level expense, with an MRI costing up to \$8,400, and a PET scan up to \$3,000 in some countries (Imaging Technology News, 2018). This substantial cost, coupled with the requirement for specialized equipment, trained personnel, and dedicated imaging facilities, restricts their availability, particularly in underserved regions and low-resource settings. This creates a significant barrier to widespread clinical use, contributing to health inequities in AD diagnosis.

Patient burden and contraindications also pose practical limitations (Decazes et al., 2021). Since PET scans involve a small dose of radiation, patients may be advised to avoid this scan if they are pregnant. This radiation also carries the small, but increased, lifetime risk of developing cancer. Both MRI and PET scans require patients to remain still in enclosed spaces for extended periods, which can cause discomfort or claustrophobia (Munn et al., 2015). Furthermore, patients with certain medical devices or metal implants, such as pacemakers, may have contraindications for MRI due to the powerful magnetic fields involved (Ghadimi et al., 2023).

PET

Cost and reimbursement represent major practical barriers to the widespread clinical adoption of amyloid PET (Lee et al., 2021). These scans are expensive, typically costing between \$3,000 and \$6,000. In the USA, third-party payers often do not reimburse for amyloid PET, citing a need for additional data demonstrating that the scan results directly influence clinical care and patient-based outcomes. This lack of coverage significantly restricts access for many patients.

Pittsburgh Compound B (PIB) utilises carbon-11, which has a very short half-life of 20 minutes, naturally limiting its clinical applicability due to logistical challenges (Wolk et al., 2012). While more clinically viable F18-labeled tracers with a longer half-life of 110 minutes have since been developed, the cost and accessibility issues persist (Trotter et al., 2023). Additionally, U.S. tracer labels and clinical practice typically use a binary (positive or negative) interpretation for amyloid PET results, which may oversimplify the continuous biological data and potentially obscure nuanced information about amyloid burden (Suppiah et al., 2019).

The designation of amyloid PET as a “gold standard” for detecting amyloid pathology is important to consider in context. While it excels at identifying the presence of amyloid plaques, a necessary component of AD, the presence of these plaques alone is not

sufficient for a definitive AD diagnosis (Roberts et al., 2015). Amyloid plaques are found in other conditions, and also in a substantial proportion of cognitively normal individuals. Furthermore, the overall amyloid burden, as assessed by PET, does not consistently correlate strongly with cognitive deficits (Villemagne & Rowe 2011). This implies that a positive amyloid PET scan indicates the presence of amyloid pathology, but it does not definitively confirm symptomatic AD or establish amyloid as the sole cause of cognitive impairment.

MRI

In the very early stages of AD, an MRI scan of the brain may appear normal, making it less sensitive for preclinical detection compared to molecular imaging techniques (van Oostveen & de Lange, 2021). While it provides detailed structural changes, it cannot solely confirm the presence of AD pathology; comprehensive assessments combining MRI findings with clinical evaluations, cognitive testing, and patient history are essential for accurate diagnosis. Fundamentally, MRI primarily reflects neuronal loss and brain atrophy, which are consequences of amyloid and tau pathology, rather than directly visualizing these protein aggregates themselves, meaning it is less sensitive to preclinical pathology than molecular biomarkers. Emerging techniques like fMRI and DTI hold significant promise, but their widespread standardization and established clinical utility for routine AD diagnosis are still under development. (Chandrasekar et al., 2025)

MOLECULAR

CSF

Despite how thoroughly characterized and well-established CSF markers for AD are, there are still significant limitations associated with these biomarkers, primarily revolving around the invasiveness of the lumbar puncture procedure used to procure the fluid (Inekci et al., 2015). The discomfort and potential risks, although rare, including nerve damage, infection and herniation, are currently primary drivers for the ongoing search for less invasive diagnostic alternatives.

Blood

Compared to CSF, blood-based biomarkers are more appealing because they tend to be significantly less invasive in nature and more cost-effective extraction methods (Mielke et al., 2024). However, as aforementioned in this literature review, the challenges include lower biomarker concentrations in blood compared to CSF, and also less achievable accuracy due to complex matrix interference in blood. In addition to this, peripheral metabolism can significantly affect blood biomarker levels, making them less direct reflections of brain pathology (Janigro et al., 2021).

Emerging Peripheral Biofluids

With research exploring the potential of other bodily fluids like saliva and urine, these can offer a theoretical advantage of maximal non-invasiveness, which makes them highly attractive for broader-scale screening (Kumari et al., 2023). However, this research is still in its nascent stages. Low concentrations of relevant analytes and high biological variability coupled with a significant lack of robust validation in studies limit their establishment for clinical use in AD diagnosis.

OVERALL

Specificity

Even with seemingly AD-specific biomarkers like tau and NfLs, there still exist difficulties in differential diagnoses. The pathologies underlying other types of dementia often involve very similar proteins to the ones used to diagnose AD, which complicates the ability to distinguish between them based solely on biomarker levels (Zou et al., 2020). Simply measuring protein concentrations is unlikely to provide sufficient information to differentiate effectively between various dementias.

Cost

The current landscape of AD research and the development of biomarkers, particularly blood-based biomarkers, is heavily skewed towards high-income countries (Rodda et al., 2025). To achieve equitable progress in the field, it is crucial to actively involve researchers and institutions from low- or middle-income countries (LMICs) in the development, validation, and clinical application of biomarkers. Key challenges remain, including the need to understand the real-world effectiveness, cost-efficiency, and acceptance of these tools in resource-limited environments. Financial constraints are a major barrier to the necessary research and validation in LMICs (Dakhil et al., 2024). Additionally, to effectively roll out biomarkers in LMICs, significant investment is needed to build the necessary infrastructure and upskill the workforce. This could involve financial support from various sources, corporate engagement, and the use of innovative, practical approaches. For instance, using dried blood spots and task-shifting models (i.e. training community health workers to do tasks normally reserved for specialists) where community health workers collect samples could make widespread use more feasible, especially in remote areas (Babulal et al., 2025; Tarawneh, 2020).

Generalizability

Not only are there technical and economic barriers, there exist obstacles in the generalizability of studies due to the lack of access to certain samples and communities of individuals. Most studies, especially plasma biomarker studies, to date have focused on genetically homogeneous populations in high-income countries. This highlights a critical need for large-scale international studies that include diverse populations with varying genetic backgrounds, comorbidities, and socioeconomic statuses. This is a significant

issue because biomarker levels can vary among different racial and ethnic groups and are affected by certain long-term health conditions and medications (Rodda et al., 2025). A 2022 study showed that even though most participants expressed interest in biomarker research for AD, Black participants were comparatively more hesitant than White participants (Elicain et al., 2022). They were more concerned about the risks of participating and felt there were more barriers to undergoing brain scans. These differences remained even when accounting for their trust in researchers and their existing knowledge of AD. The study found that a lack of information was a major barrier for Black participants, while receiving information was a strong incentive. Black older adults expressed a desire for more details about AD, including its risks and prevention, as well as a better understanding of the general research process and specific biomarker procedures. This emphasises that the scientific community has to improve recruitment strategies for underrepresented groups with no prior research experience. The above could be done through refined information sharing and awareness approaches. These could be achieved through increasing the presence of the scientific community in these groups and reducing the financial burden of participation.

Standardization

A major hurdle for the widespread use of AD biomarkers, and particularly blood-based ones, is a lack of standardization (Humpel & Hochstrasser, 2011). Currently, there are no uniform methods for sample collection, processing, or storage. This problem is compounded by the absence of established procedures to monitor long-term biomarker stability and the lack of certified reference standards. These inconsistencies introduce measurement variations, making it difficult to compare results between labs, establish reliable clinical thresholds, and ultimately use these biomarkers effectively in both research and patient care (Zeng et al., 2024).

Education of Staff

The widespread adoption of biomarkers, despite their accessibility, is significantly challenged by the need for more comprehensive education of primary care clinical staff and the development of clear, actionable guidelines for their use. Without this adequate training and clear protocols, there is a high risk of misinterpretation of results or the inappropriate use of these diagnostic tools (Schöll et al., 2024).

Overall, there still exist many translational hurdles, and it is only if these hurdles are overcome that biomarkers can achieve their full efficacy and potential. Reducing these hurdles also allows more people to access what could potentially be advanced diagnostic tests for AD, bettering treatment plans via early detection.

Section 5: Ongoing efforts of bridging the divide

Early detection of AD could dramatically shift the way treatment is provided to patients. Early detection allows for interventions during the prodromal or mild cognitive impairment stages, where therapeutic outcomes are more likely to be meaningful as early diagnosis is crucial in informing discussions, care and support, and treatment (Dubois et al., 2023). Moreover, biomarkers are critical not only for diagnosis, but also for other means including patient stratification in clinical trials and the development of potential disease-modifying therapies.

Hence, the limitations and translational challenges confronting AD biomarkers are being actively addressed. For example, significant progress is being made in developing more sensitive and specific assays for AD biomarkers. Ultra-sensitive platforms, such as Single Molecule Array (SIMOA) technology, are enabling the reliable detection and quantification of biomarkers like p-tau and GFAP even if they are present at very low concentrations in biological fluids (Yuan et al., 2025). Other technologies include novel electrochemical sensing platforms like CRISPR/Cas12a-mediated electrochemical aptasensors, which are biosensors that use DNA/RNA molecules to detect proteins (Yuan et al., 2025).

Recognizing that no single biomarker is able to provide a complete picture of AD pathology and its progression, there is a growing emphasis on multimodal biomarker approaches and the integration of different data types (Gupta & Iftekhar, 2024). Combining imaging, fluid, cognitive and demographic data could offer the potential for enhanced diagnostic accuracy. AI and machine learning (ML) in particular are emerging as tools in this context.

Beyond refining extant markers, the field continues to pursue the discovery of novel biomarkers that can provide more specific and comprehensive insights into AD pathology.

Conclusion

Our ability to discover, develop and validate stronger biomarkers is improving, with research on AD biomarkers having progressed considerably over the past decade. Novel molecular, fluid and imaging markers show growing promise for earlier and more accurate detection. Yet, the translation of these biomarkers into widespread clinical use is still obstructed by challenges. Accessibility will depend on our ability to translate treatments to diverse populations by being reliant on accessible, low cost, and minimally invasive diagnostic biomarkers available to a general clinical setting (Georgakas et al., 2023). Specificity also continues to be a central challenge, as biomarkers often overlap with other neurodegenerative conditions. These obstacles hinder the integration of biomarkers into routine clinical practice, despite their strong scientific potential. Bridging this gap is consequently essential to realizing the full diagnostic potential of biomarkers. Achieving the above stated goals will not only democratise access but also improve diagnostic

accuracy. Ultimately, translation of these scientific advances into clinical use can reshape AD care, enabling earlier detection and therefore earlier and more effective interventions.

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