

Review Article

From ambiguity to accuracy: A review of Alzheimer’s disease diagnostic errors and the need for non-invasive biomarkers

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ABSTRACT

Alzheimer’s disease (AD) is a progressive and irreversible form of dementia that is characterized by loss of memory and motor abilities, as well as psychiatric abnormalities. Accurate and early diagnosis of AD is crucial for both delaying the onset of the disease’s symptoms and prescribing a management plan. However, as many medical practitioners attest, precise diagnosis of AD is complex and difficult. Previously, AD diagnosis was based exclusively on clinical criteria; however, recent AD diagnosis guidelines consider the presence of biological markers. The current medicine is improving in novel non-invasive biomarker detection, neuroimaging techniques, and acquiring new knowledge of molecular pathways of AD. This review will provide a critical overview of AD diagnostic errors, current challenges, need for non-invasive biomarkers as well as how improvements in biomarkers and neuroimaging techniques can assist in early detection of AD.

Keywords: Aging, Alzheimer’s disease, Biomarkers, Dementia, Diagnostic errors

INTRODUCTION

Alzheimer’s disease (AD) is an intensive and irrevocable form of dementia that is exemplified by gradual memory loss, psychiatric abnormalities, motor difficulties, compromised glucose metabolism, spatial disorientation, aphasia, and cognitive decline.^[1] It has the largest prevalence, with nearly 60–80% of known dementia cases. AD prevalence increases from 10% >60 years of age to ~40% for individuals >80 years.^[2]

It is estimated that approximately 131 million individuals will be affected by AD worldwide by 2050, due mainly to an aging population. By 2050, humans aged 80 years and over will number 434 million, a 300% increase from the 2015 population of individuals aged 80 years and over. Furthermore, by 2030, 25% of the population in North and Latin America, Europe, and Oceania will be older and will increase to approximately 44% by 2050. Consequently, this exponential increase in aged individuals will place tremendous pressure on global healthcare systems to treat and support aged individuals with AD.^[2]

AD pathology is posited on three major biomarkers: tau protein (T-tau), phosphorylated tau (P-Tau), and amyloid beta (Aβ) peptide.^[1] The neuropathology of AD is

triggered by both genetic and environmental factors – a major reason why AD has been so difficult to identify and treat. AD contributing genetic factors involve multiple chromosomes (e.g. chromosomes 1, 14, 19, 21) which are not well understood.^[3] Both presenile and senile AD exist, which differ in genetic causation. The more common senile AD type involves the susceptible allele apolipoprotein E4. Homozygosity is a very high-risk factor in developing AD.^[3]

Although there is no cure for AD, patients are usually provided with pharmacological intervention and lifestyle advice. Medications such as cholinesterase inhibitors and memantine have improved life quality in AD patients without affecting the rate of decline. However, randomized control trials on the efficacy of donepezil in AD patients proved inconclusive.^[4] This mini-review will provide a critical overview of AD misdiagnosis, current challenges, need for non-invasive biomarkers as well as, how improvements in neuroimaging techniques can assist in early detection of AD.

DIAGNOSTIC AMBIGUITY WITH AD

Diagnosing AD is complicated for various reasons. First, early detection remains difficult, as clinical symptoms arise in the late stage of the disease. Furthermore, the similarity

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Received: 23 November 2024 Accepted: 03 January 2025 Epub ahead of print: 18 February 2025 Published: XXXXXX DOI: 10.25259/JNRP_431_2024

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of symptoms with other diseases makes disparity hard, and markers are often non-specific and also present in people without AD [Table 1]. Access to specialist testing, ethical problems, and financial considerations all hamper accurate and widespread diagnostics. Regardless of these issues, research is being undertaken to increase the precision and accessibility of AD detection and differentiation. It also seeks to discover new approaches to investigate and uncover AD in its initial phase.

Limitations of drug therapy

Correct diagnosis of AD remains challenging for many physicians. Accurate and early detection of AD is crucial for both delaying the onset of the disease's symptoms and prescribing a management plan. However, early detection and diagnosis of AD pose a considerable problem in many developed countries. The genetic and etiological complexity of AD represents obstacles to the precise diagnosis of AD. Even though AD diagnosis has improved in the past two decades, AD drug discovery has invariably been unsuccessful. To illustrate this, between 2002 and 2012, 244 drugs for AD were tested in 413 AD trials. Only the drug memantine had been successfully completed.^[5]

A new class of anti-amyloid monoclonal antibodies has been recently introduced for AD with two newly Food and Drug Administration-approved drugs aducanumab and lecanemab. These drugs share a common mechanism in targeting amyloid proteins and fibrils, however, aducanumab has failed in declining the progression of cognitive impairment and memory. While lecanemab lowered amyloid indicators in early AD resulting in a reasonably minor deterioration in measures of cognition and function than placebo at 18 months, it was, however, correlated with adverse events.^[6]

Another drug called sodium oligomannate, which has been approved in China has exhibited a decrease in A β and neuroinflammation. Nevertheless, the drug development landscape for AD has succumbed to failures since 2003 with less than a 2% success rate.

Symptoms of dementia can be difficult to differentiate between age-related behavioral changes and other common potentially curable conditions. An early diagnosis of AD can alleviate the burden on the geriatric population and their families. Unfortunately, many clinicians have difficulty diagnosing AD. Inaccurate diagnosis of AD prolongs the advancement of the pathological condition.

Researchers are continuing to explore and develop biomarkers to enhance diagnosis, considering the prevalence of misdiagnosis with AD due to flaws in the present diagnostic procedures, as well as the cost and availability of specific tests. Several types of brain scans, such as computed tomography CT, magnetic resonance imaging (MRI), and positron

emission tomography (PET), can assist clinicians detect AD or other dementias. However, on average, these tests are expensive and might not be accessible to poorer individuals.

Biomarkers in clinical examination for the diagnosis of AD

Previously, the disease diagnosis was exclusively dependent on clinical criteria; though, recent AD diagnosis guidelines consider the presence of biological markers. The National Institute on Aging and Alzheimer's Association "ATN" research paradigm can be implemented to make a neuropathologic diagnosis of AD with biomarkers. This concept recommends defining AD according to amyloid abnormalities ("A"), tau protein alterations ("T"), and an indication of neurodegeneration ("N"), regardless of the phenotypes or the non-existence of cognitive deficits. The ATN framework recommendations state that clinicians can confirm the existence of AD pathogenesis in the brain,^[1,6] but they cannot differentiate the clinicopathologic syndrome known as AD. However, there is still ongoing debate regarding the use of the ATN framework in practice.

Several procedures were used to screen for AD, such as cognitive assessments, neuroimaging, genetic testing, and blood and cerebrospinal fluid (CSF) analysis. Cognitive examinations evaluate memory function, whereas neuroimaging techniques such as MRI and PET could reveal structural and protein changes in the brain. CSF and blood analysis reveal markers linked to AD, whereas genetic evaluation identifies abnormalities in familial types of illness. Nevertheless, a clear diagnosis can be possible only with an autopsy.

Post-mortem prevails to be the "gold" standard for AD determination

To date, postmortem prevails to be the "gold standard" for accurately assessing the tau and amyloid markers of AD.^[2,4] This may be due to a lack of specificity of AD singular or combination features during macroscopic analysis. Although conducting live biopsy is common for identifying several diseases, biopsy in AD can be contraindicated due to elevated "risk/ratio."^[6]

Another difficulty in the current AD diagnostic methods relates to the heterogeneous features of this disease.^[7] In some cases, AD may follow other pathologies such as hippocampal sclerosis (HS) and cerebrovascular disease. These diseases may even continue after AD symptoms have leveled in patients ~90 years old.^[7,8]

A recent postmortem AD study found that some AD patients had multiple pathologies. Other studies have noted a >50% prevalence of AD subjects with mixed brain pathologies in community-dwelling establishments for dementia patients.

This, of course, challenges differential diagnosis since DSM-IV₂ criteria state that memory deficits are necessary for determining dementia.^[9,10] It is well known that several clinical disorders can mimic AD, which has invariably led to misdiagnosis. Both HS and dementia with Lewy bodies (especially at the prodromal stage) are often misdiagnosed as AD due to their similar clinical presentation with AD.^[11]

In general, many subjects in their 90s may have mild or undetected neuropathologies along with AD. However, these mixed pathologies may not be sufficiently significant due to their low levels (e.g. microinfarcts, mild amyloid angiopathy). Several studies have pointed out that between 12% and 23% of patients who had been diagnosed with AD had insufficient AD pathology at postmortem. A similar level of misdiagnosis in living subjects undergoing amyloid PET in a pharmaceutical industry drug trial has been recently noted. Reasons for this level of high misdiagnosis include lack of clinical neuroanatomical and etiological knowledge of AD progression, bias, failure to follow through with medical protocols, and institutional mismanagement that burdens AD patients to lengthy periods of potentially unsuitable treatment.^[12,13]

Some researchers mention a lack of correspondence between clinical AD diagnosis and neuropathological changes, highlighting the need for physicians to focus on neuropathological testing in clinical practice. Furthermore, older individuals have a higher risk of being misdiagnosed with AD as they generally have more etiologies than younger individuals. Moreover, there is a correlation between increasing pathologic detection rates with higher dementia severity.^[14]

Other diagnostic issues

Some researchers mention a lack of correspondence between clinical AD diagnosis and neuropathological changes, highlighting the need for physicians to focus on neuropathological testing in clinical practice.^[15] Furthermore, older individuals have a higher risk of being misdiagnosed with AD as they generally have more etiologies than younger individuals.^[14] Moreover, there is a correlation between increasing pathologic detection rates with higher dementia severity.

The apparent AD diagnostic ambiguity has not only increased new ways of thinking about AD but also has sometimes spurred some physicians to confabulate research discoveries. CSF is found to be a potential method for diagnosing AD. Having found tau and amyloid in the CSF, AD in 302 subjects and 114 normal subjects (at a false positive rate of 36% and specificity of 64%), many physicians believed that the CSF test was the awaited breakthrough for accurately identifying AD.^[16] The alleged discovery was a misnomer. This episode serves as a caveat for physicians who may not be aware of the neuropathological and life course complexities of AD.

CURRENT AND FUTURE APPLICATIONS FOR IMPROVING AD DIAGNOSIS

Neuroimaging

Improvements in neuroimaging techniques will in the next few years provide new ways for early diagnosis of AD. AD sequelae are identifiable in neuroimaging in later stages but are not easily detectable in the early onset of the disease. While X-ray has diagnostic value in identifying bone tissue abnormalities, it fails to assess CNS anomalies. Similarly, CT has limited diagnostic scope, as it lacks the capacity to identify mild cognitive impairment (MCI) and AD.^[16-18]

Furthermore, head MRI is poor in assessing biochemical changes associated with AD. PET is a significant diagnostic neuroimaging technique that is currently able to diagnose A β deposition in AD patients.^[16] Unfortunately, the stage of such diagnosis usually means that AD has progressed. However, Gordon *et al.*^[18] identified A β deposition, cortical thinning, and reduced glucose metabolism in autosomal dominant patients, as well as, speculating that such glucose metabolism had commenced approximately 19 years prior to AD onset.

Recently, Segovia *et al.*^[19] used intermediate and late fusion positron emission tomography (PET) methods on 43 cognitively impaired subjects and found good specificity and sensitivity. Other studies have highlighted that data obtained after the use of injected radiotracer correlated with 18F fluorodeoxyglucose (the most common substance used in PET neuroimaging). Nakamura *et al.*^[20] identified that mass spectrometry and immunoprecipitation for determining high-performance plasma A β biomarkers showed improved synchronization when diagnosing AD than A β -PET imaging looking for A β deposits.

Like PET, MRI is a widely used set of neuroimaging techniques for diagnosing AD and is known for accurately observing brain structures without using contrastive agents.^[21] MRI-based techniques (functional MRI, magnetic resonance spectroscopy, magnetic resonance-diffusion tensor imaging, magnetic resonance-perfusion-weighted imaging, magnetic resonance susceptibility-weighted imaging) are now being developed to improve in discriminating between AD and MCI subjects. MRI techniques are also being used in medical research and AD diagnosis. The range of these techniques is beyond the scope of this analysis. Chen *et al.*^[21] have used a deformation-based learning approach using high-resolution MRI to assess and compute morphological variation between these two groups. This technique predicted AD with 96.5% accuracy and 91.74% accuracy for MCI in elderly patients, respectively.^[22] Moreover, it has been shown that MRI neuroimaging can identify early-onset AD, especially in relation to hippocampal volume loss.

Development of non-invasive biomarkers

Retinal biomarkers

Recent advancements in imaging technologies have unveiled the potential of retinal biomarkers, which could offer non-invasive alternatives to traditional methods for diagnosing and observing the progression of AD. Research indicates that alterations in the retina, such as changes in the retinal nerve fiber layer and abnormalities in the blood-retinal barrier, may correlate with the biomarkers of AD-like A β plaques and tau tangles.^[23]

These findings suggest that retinal imaging could serve as a valuable adjunct to CSF analysis and neuroimaging techniques, potentially facilitating earlier intervention strategies and improving overall patient outcomes. Furthermore, the accessible nature of the retina and the ease of conducting retinal examinations make this approach a compelling option for clinicians, mostly in resource-limited settings where the approach to more specialized diagnostic tools may be limited. Retinal alterations are evident in the initial stages of AD as identified by the post-mortem studies. Clinical indications of AD include enlarged pupil size,^[24] reduced pupillary light sensitivity,^[25] and A β 40 levels in the aqueous humor analogous to CSF fluid.^[26] In post-mortem eyes of AD subjects, A β deposits were not found in the lens.^[27] AD has been correlated with greater cupping of the optic nerve, although a pre-clinical study utilizing A β injection revealed minimal axonal damage.^[28] Furthermore, decreased pTau and A β levels were observed in the vitreous humor and associated with reduced cognitive activity.^[28]

Besides physical symptoms, visual and motor deficits were described in AD patients. These problems comprise anomalous hypometric saccades, decreased visual acuity, diminished contrast sensitivity, stereopsis, poor hand-eye coordination, improper eye fixation, and difficulties in distinguishing objects. These variations indicate prognostic and progressive evidence of neurodegenerative processes and are prospective ocular biomarkers of AD.

Optical coherence tomography (OCT) allows to obtain three-dimensional pictures of the retina and helps to study the retinal and macular thinning along with vascular changes.

Research has shown decreased macular volume and thickness in non-symptomatic subjects having more genetic risk^[29] and decreased central macular thickness in AD patients in comparison with normal subjects.^[30] Moreover, the thinning of macular and peripapillary retinal nerve fiber layers was reported in various studies in AD in studies that utilized OCT.^[31] This indicates that OCT can be a non-invasive retinal imaging diagnostic tool to detect early AD. OCT angiography is another tool that helps to detect retinal microvascular changes in evaluating the progress of AD. Similarly, hyperspectral imaging and fundus photography can be useful tools in detecting A β and also vascular changes in the retina.^[32]

In comparison to established clinical brain diagnostic approaches, retinal analysis like OCT and hyperspectral imaging have significant benefits in diagnosing AD. Retinal analyses are non-invasive, inexpensive, time-saving, and well-tolerated by patients and have become useful tools for early identification. They could identify minor retinal variations in AD earlier the symptom onset.

Salivary biomarkers

Saliva is an accessible bodily fluid with recurring variations in composition in various pathological situations such as gastrointestinal, cardiovascular, and neurological disorders.^[33] AD may also have an influence on both qualitative and quantitative salivary characteristics. Furthermore, there appears to be a link between the brain and saliva that happens through six distinct channels that communicate brain chemicals with saliva and vice versa. Possible routes for the oral-brain axis include the cranial nerves, the gut-brain axis through the vagal route, the lymphatic system, the peripheral circulation, and the sublingual intranasal routes.

Numerous studies indicated that salivary A β 42 levels are elevated in AD.^[34]

In a clinical study involving 160 age-matched controls, 53 AD subjects employed ultrasensitive single molecule microarray assay to estimate tau levels in saliva. Interestingly, in 95% of subjects, t-tau was four-fold higher than in plasma; however, they did not find any association of tau with the cognitive scores.^[34] Apart from A β and tau, lactoferrin, an antimicrobial peptide with A β binding capacity is turned out to be abundant in human saliva. In AD patients, unstimulated lactoferrin was found to be positively linked with the Mini-Mental State Examination (MMSE) score, which could make early diagnosis of AD possible.^[35] Furthermore, acetylcholinesterase (AChE) enzyme activity can also be confirmed in saliva, as the AChE inhibitors are the major drugs currently being prescribed for AD. Biological markers linked to oxidative stress, inflammation, and redox balance such as salivary levels of glial fibrillary acidic protein, interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF- α), cyclooxygenase-2, caspase-8, haptoglobin, catalase, glutathione, and other markers were analyzed, and these biomarkers were found to be altered in the AD subjects.

MicroRNAs are tiny, non-coding RNAs that influence gene expression. It examined^[36] miRNA-485-3p levels in salivary exosome-enriched extracellular vesicles (EE-EV) from 27 AD and 13 normal subjects. The study found that miRNA-485-3p concentrations in salivary EE-EV from AD patients were considerably higher than in the healthy controls. The receiver operating characteristic (ROC) scrutiny for discriminating AD and healthy persons revealed that this biomarker performed well: Significant relationships were found among miRNA-485-3p levels in salivary EE-EV and MMSE or A β

PET findings, with a greater correlation with the latter. In AD patients, unstimulated lactoferrin was found positively linked with the MMSE score.

Urinary biomarkers

Increasing data indicates that inflammatory reactions elicit a significant feature in the etiology of AD. Amyloid plaques and neurofibrillary tangles (NFTs) bind to pattern recognition receptors on microglia and astrocytes, inducing an immune response and triggering the discharge of inflammatory mediators that increase the disease progression.^[10,37] In a cross-sectional study by Saiyed, *et al.* 2023, conducted in 25 AD patients they identified urinary cytokines, which are the hallmarks of inflammatory response. The study established a significant association between AD and urinary markers such as IL-6 Cytokine Family Signal Transducer, Matrix metalloproteinase 2, TNF Receptor Superfamily Member 8, and IL-19.^[38]

Neuronal thread proteins (NTPs) are phosphoproteins found in brain and neuroectodermal tumor cells during proliferation, differentiation, brain development, and AD neurodegeneration.

AD7c-NTP is commonly articulated in neurons and found to be amplified in AD. Due to its immunoreactivity, it is easily detected in senile plaques and neurofibrillary tangles. Apart from CSF, AD7c-NTP exhibited sensitivity and specificity in urine samples of AD patients.^[39]

Nanotechnological-based systems as potential AD biomarkers

Due to the protracted pre-clinical period of AD, various nanotechnological methods are currently being developed based on nanoparticulate systems which have the potential to provide precise and early identification of AD biomarkers. Nanoparticles (NPs) offer state-of-the-art specific and high-loading drug delivery due to their unique characteristics, high stability post administration *in vivo*, and possess high surface-to-volume ratio for optimal absorption.^[40]

Synthetic-based NPs include gold NPs (AuNP), nanoliposomes, carbon nanotubes, peptides, and polymeric NPs. NPs consist of two parts: The core/central material and the surface modifier that inform transformations of the physiochemical properties of the former. The current diagnostic techniques for AD are invasive, time consuming (neuropsychological assessment), and expensive (e.g. neuroimaging), thus having limited AD diagnostic capacities. These limitations are well known to clinicians and biomedical technologists.^[40]

Although in the past 20 years, nano-based therapeutics have been used; for drug delivery, these failed to demonstrate the potential of nanoparticulates. Park *et al.*,^[41] state two reasons for this: Drug delivery was administered at a very late stage for

it to be beneficial; second, due to scarce experimental evidence showing the efficacy of nano-based drug delivery systems.

One method that is currently being developed is based on an AD diagnosis kit using fluorescent NPs which will detect antigens and miRNAs.^[41] Although holding incredible potential for superior AD treatment, NPs have the ability to interfere with amyloid protein structures which have yet to be understood due to the diverse characteristics of oligomers. Consequently, research needs to be conducted to assess both *in vitro* and *in vivo* interactions of NPs and nanocomposites in hindering amyloidosis. For example, research shows that synthetic and phenolic molecules can inhibit amyloid accumulation. Organic flavonoids such as curcumin and resveratrol, potent radical scavengers at the cellular membrane level, may have limited *in vivo* applicability due to their low dissolubility and molecular indiscrimination.^[42,43]

The use of inorganic metals as nano-therapeutic agents has received considerable medical attention, especially in the context of promising cancer treatment and drug delivery. Proposed metals include cerium, molybdenum gold, and iron. At a molecular level, these metals possess impressive drug-loading capacity and precision site delivery.^[44]

Gold is a promising candidate due to its amyloid cysteine binding ability during fibrilization probing and inducing anti-amyloidosis. Furthermore, AuNP has excellent biocompatibility, minimal toxicity, and blood-brain barrier (BBB) penetration. Thus, AuNP ability to cross the BBB allows it to bypass problems of small-molecule anti-AD drugs.

Recently, the role of AuNP in detracting A β amyloid fibrils and oligomers was demonstrated through an AuNP surface plasmon resonance absorption band. This AuNP-derived method may lead to better A β 40 amyloid oligomers/fibril detection, leading to effective probing.^[43]

In a rat model, AuNP acted as an anti-inflammatory agent and in preventing cognitive decline due to oxidative stress. AuNP inhibitory agent of A β has been further enhanced by conjugating peptide inhibitors, VVIA and LPFFD, highlighting the potent AuNP/peptide hybridization.^[44] Another AuNP-based method relies on isolating antigens involving oligonucleotides through a "sandwich process," which then undergoes magnetic separation where the antigens are purified. This sequestration process in solution results in numerous DNA improves identification of A β -derived diffusible ligands by a magnitude of 6 orders.

Other promising diagnostic nanotechnologies are carbon dots due to their biocompatibility and high photostability, photoluminescent aspects, and applicability are highly suitable for BBB crossing drug delivery and bioimaging; nanoliposomes incorporating either cardiolipins or phosphatidic acid for the targeting of amyloid beta peptide nanobiosensors, along with biochemical agents capable of single conversion for the assessment of biomarkers in Alzheimer's disease.^[40,45]

Table 1: Various neurological diseases/deficiencies which have similar symptomatology with Alzheimer's disease (AD).

Clinical features of AD	Disorders with similar symptomatology with AD
Cortical dysfunction and impairment, poor memory performance, psychiatric symptoms, dysexecutive syndrome	B12 deficiency ^[49]
Neurocognitive, deficits, irritability, depression	Neurosyphilis ^[46]
RMN or TAC positive for vascular antecedents focal deficits, less striking memory deficit	Vascular dementia ^[47]
Cognitive deficits, reduction in speech fluency and word retrieval, mood changes	Lyme disease ^[48]
Systematized delusions, hallucinations, fluctuation cognition, progressive cognitive decline	Lewy's dementia ^[49]
Cognitive disorder, ataxia, psychiatric problems	Niemann Pick type C ^[50]

Strengths of the study

Utilization of non-invasive retinal, urinary, and salivary biomarkers is cost-effective and can serve in precise detection of AD.

Nanotechnological-based markers and advanced neuroimaging based on brain mapping could assist in avoiding diagnostic errors in AD.

Research and clinical investigations indicate a promising application of these biomarkers in the early detection of the disease.

Limitations and challenges

AD has a unique pathological heterogeneity; this complexity consists of various co-factors, hence making precise diagnosis difficult.

Availability or access to medical equipment/expertise could be a challenge in undeveloped countries.

Future directions

The exponential increase in AD demands the development of more reliable diagnostic techniques that will be beneficial for both physicians and patients. NPs may even be able to detect nascent AD biomarkers in younger individuals that will assist in providing treatments for preventing the onset of AD. More research is needed to establish new knowledge of molecular pathways of AD for precision diagnosis. In the future, early diagnosis of AD can be accomplished using a combination of non-invasive biomarkers, nanoparticulate systems, and advanced neuroimaging.

CONCLUSION

Due to the high rate of AD diagnostic errors in the recent past and present, physicians have been placed in a tenuous situation between having to correctly identify AD from other neurological diseases and then providing a realistic management plan for patients. To maximize the clinical

effectiveness of the very few number of available drugs for AD, the diagnosis should be accurate. Although pre-clinical and clinical trials for AD have shown that advanced neuroimaging and nanotechnological markers can be significant in the precise diagnosis of the disease, they are invasive and non-patient complaints. Research has identified that non-invasive biological markers such as retinal, urinary, and salivary markers as mentioned above can offer an accurate and detailed diagnosis of AD. The current medicine recognizes that AD has genetic and environmental components which may commence decades before AD symptoms manifest. Consequently, we endorse that medical understandings of AD move toward pathologically driven diagnosis instead of relying on symptoms-based diagnosis.

Ethical approval: Institutional Review Board approval is not required.

Declaration of patient consent: Patient's consent is not required, as there are no patients in this study.

Financial support and sponsorship: Nil.

Conflicts of interest: There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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How to cite this article: Rapaka D, Saniotis A, Mohammadi K, Galassi FM, Kumar PO, Bitra VR. From ambiguity to accuracy: A review of Alzheimer's disease diagnostic errors and the need for non-invasive biomarkers. *J Neurosci Rural Pract.* doi: 10.25259/JNRP_431_2024