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MODERN TECHNOLOGIES IN THE DIAGNOSIS OF ALZHEIMER'S DISEASE. A NARRATIVE REVIEW

Patrycja Piekarska[1] ✉, ***Adrianna Purwin[1]***, ***Zofia Wcisło[2]***, ***Karolina Lach[1]***, ***Grzegorz Szmit[2]***, ***Maria Chmielewska[3]***, ***Weronika Basak[2]***, ***Katarzyna Siwiec[1]***, ***Gabriela Krok[5]***, ***Wiktoria Modrzejewska[4]***

[1] *Medical University of Warsaw, Warsaw, Poland*
[2] *Military Institute of Medicine, Warsaw, Poland*
[3] *Mazovian Brodnowski Hospital, Warsaw, Poland*
[4] *National Medical Institute of the Ministry of Interior and Administration, Warsaw, Poland*
[5] *Henryk Klimontowicz Hospital in Gorlice, Gorlice, Poland*

✉ ***slodovnikowa@gmail.com***

ABSTRACT

Aim: The purpose of this review is to summarize and critically evaluate the current state of knowledge regarding modern diagnostic approaches to Alzheimer's (AD) disease with particular emphasis on emerging strategies and recent clinical developments, focusing on ophthalmic biomarkers, extracellular vesicles, magnetic resonance spectroscopy and blood biomarkers.

Methods: A narrative review based on full-text articles published in English. Sources include PubMed publications.

Results: Ophthalmic biomarkers, such as retinal nerve fiber layer thinning and retinal vascular changes, serve as promising accessible indicators of neurodegeneration. Extracellular vesicles, particularly neuron-enriched exosomes, contain a higher concentration of AD-related pathological proteins and exhibit altered exosomal microRNA expression, enhancing diagnostic precision. Magnetic resonance spectroscopy can reveal metabolic disturbances that precede structural brain changes. Blood-based biomarkers, especially phosphorylated tau isoforms, demonstrate high diagnostic accuracy comparable to cerebrospinal fluid and PET measures.

Conclusions: The integration of these diverse diagnostic modalities enhances specificity and reflects the complex pathophysiology of AD, allowing clinicians to obtain a more comprehensive understanding, which is crucial for early-stage diagnosis and timely implementation of potential therapies.

Keywords: Alzheimer Disease; diagnosis; magnetic resonance spectroscopy; MicroRNAs; biomarker

INTRODUCTION

Nowadays, the most common form of dementia is Alzheimer's disease (AD) [1]. Projections indicate that by 2050, the number of individuals affected by AD is expected to triple, increasing from 50 million to over 150 million [2]. European studies further confirm a steep age-related increase, from 0.6% (patients aged 65-69 years) to 22.2% (patients aged ≥90 years), emphasizing age as the key risk factor and the growing global burden [1]. Therefore, the disease is a major concern for a health system in the twenty-first century, especially its early diagnosis [3,4]. AD manifests as a progressive neurodegenerative disease. This disease is characterized by a prolonged asymptomatic phase during the preclinical stage [5]. It typically begins with mild memory loss in the early stage. As the disease progresses patients experience profound deterioration of cognitive abilities and executive functions [6].

The pathophysiology of AD usually includes neuronal loss, amyloid-beta (Aβ) aggregation, hyperphosphorylated tau-induced neurofibrillary tangles, and reduced acetylcholine levels [4].

In AD the pathological aggregation of Aβ and tau proteins not only constitutes a hallmark feature of the disorder but also elicits an immune system response. This inflammation complicates the identification of universal therapeutic methods because of its heterogeneous nature. Emerging evidence emphasizes the role of astrocytes, infiltrating immune cells and microglia, as being a part of immune perturbations, which plays a critical role in disease progression [6].

Definitive diagnosis requires postmortem brain tissue analysis or cerebrospinal fluid (CSF) biomarkers [4]. However, in living individuals it is possible to establish a probable diagnosis of AD by neuroimaging techniques combined with clinical criteria and screening studies involving multiple biomarkers [4,7-9]. For example, mesial temporal lobe atrophy detected by magnetic resonance imaging (MRI) has been utilized in clinical trials [3]. For clinical diagnosis physicians use standard diagnostic biomarkers for neurodegeneration include the levels of phosphorylated Tau (p-Tau), Aβ1-42/1-40, and total Tau (T-Tau) measured in cerebrospinal fluid (CSF), alongside positron emission tomography (PET) imaging. However, CSF collection requires invasive lumbar puncture, and PET scans are costly and time- consuming. Additionally, these methods exhibit limited specificity and sensitivity. Therefore, there is an urgent need for sensitive, accurate, and non-invasive biomarkers for the early diagnosis of AD [4,8].

In this context, the purpose of this literature review is to present and systematize existing and emerging strategies for the diagnosis of AD, together with an analysis of their mechanisms of action, clinical effectiveness, and directions for future scientific research.

METHODS

This literature review integrated reviews and articles found in PubMed and academic book sources. To maintain the reliability and contemporary relevance of the data included in this review, the literature search prioritized studies published from 2014 to 2025 in English and Polish. While the main focus of this paper is on emerging technologies, it also outlines the essential background of the disease, drawing on pioneering scientific studies and authoritative textbooks to provide the necessary context for interpreting these advancements.

RESULTS

OPHTHALMIC BIOMARKERS

The brain and ocular structures share a common embryological origin, with similar cellular development in related tissues. Damage to these structures can lead to accumulation of the proteins in an abnormal form [10,11].

The retina, as the only part of the central nervous system, extending beyond the skull, can potentially reflect brain changes, particularly in neurodegenerative diseases [10]. Unlike most structures of the central nervous system, the eye can be readily examined in vivo [12]. Recent research has shown a correlation between AD and degenerative alterations in retinal layers. Optical coherence tomography (OCT) has become a critical diagnostic tool in ophthalmology, allowing high-resolution imaging of the retina's structure, including the retina nerve fiber layer (RNFL) [10,12]. Studies using OCT have demonstrated that AD patients exhibit significant thinning of the RNFL. For example, a study involving 24 patients with mild cognitive impairment (MCI), 30 AD patients, and 24 age-matched healthy controls revealed substantial thinning of the RNFL, particularly in the inferior quadrant. Notably, AD patients also displayed thinning in the superior quadrant compared to controls. This heterogeneous reduction in RNFL thickness may be attributed to more severe degeneration of thicker nerve fibers compared to thinner sections. The peripapillary retinal nerve fiber layer (pRNFL) shows the most pronounced changes in AD, with this area being identified as the most sensitive to cognition related to this disease with positive correlation between the average thickness of pRNFL and cognitive deterioration [10,11]. Some studies demonstrated that pRNFL thinning occurred only in the superior region; others reported it in both superior and inferior regions, while still others observed it across all quadrants [12].

In a two-year longitudinal study using ultra-wide-band retinal imaging, drusen deposits were observed in the peripheral retina of AD patients, a feature not present in controls [10]. Drusen are abnormal extracellular deposits. These drusen are associated with retinal pigment epithelium (RPE) atrophy and photoreceptors degeneration, leading to measurable loss of visual function [13]. AD patients exhibited an increase in both the number of drusen and the affected retinal areas [10]. Some drusen contain Aβ, which reaches the eye via the optic nerve, transported along with cerebrospinal fluid [13,14]. Post-mortem analyses of individuals diagnosed with AD have revealed the presence of Aβ deposits in the retina, whereas retinal tissue from those without the disease exhibited little to no detectable accumulation, suggesting a potential link between retinal Aβ pathology and AD [15].

Among the various retinal alterations, the aggregation of amyloid beta and degeneration of retinal ganglion cells (RGCs) located in the inner retinal layers, particularly in the NFL and ganglion cell layer (GCL), can help differentiate AD- specific ocular pathology from other neurodegenerative diseases, such as glaucoma and age-related macular degeneration (AMD) [10]. Other researchers have shown that thinning of the GCL is a more sensitive indicator than RNFL thinning, allowing differentiation between patients with AD, Parkinson's disease, and healthy controls [16]. Furthermore, Aβ aggregation combined with RGC degeneration in the upper retinal layers has been shown to distinguish AD- related retinal changes from those seen in other conditions [10]. A promising direction for future investigation is the cerebral-to-ocular transport of Aβ, which may play a central role in the development of retinopathy in AD [14].

Currently, the ocular lens is also being investigated as a potential site for detecting pathological changes associated with AD. However, the available findings remain inconsistent [17]. Some studies have reported lens opacities and the presence of Aβ deposits in the supranuclear region of the lens in patients diagnosed with AD [16,17]. Other researchers employing Fluorescent Ligand Eye Scanning (FLES) observed that fluorescence signals originating from ocular lens were significantly associated with Aβ deposits measured via PET imaging [19]. These findings suggested that Aβ-induced cataract might serve as a potential non-invasive biomarker of AD; nevertheless, subsequent studies have not confirmed this hypothesis [17]. Another investigation concluded that neither the extent of lens opacification nor cataract severity provides a meaningful non-invasive measure of AD risk, as the observed associations with age were not statistically significant [18].

Although no clinical applications have yet been established for AD manifestations in other parts of the eye, it has been confirmed that these manifestations can also be detected in the pupil, choroid and optic nerve [13]. The study also revealed a significant increase in the venular width gradient, indicating that retinal vessels in AD patients show greater narrowing towards the periphery. This suggests that the peripheral retina may receive reduced blood supply and nutrients, providing insight into retinal degradation in AD. These vascular parameters show promise as potential biomarkers in AD, though further validation in large, longitudinal studies is needed [10].

While the available evidence is promising, ocular biomarkers have not yet been sufficiently validated for clinical implementation [20]. Despite the strong association between retinal degeneration and neurodegeneration in AD, the use of ophthalmic biomarkers remains challenging. Retinal degeneration is also present in other diseases, complicating the application of retinal imaging for AD diagnosis. For instance, AD and AMD share similar pathological mechanisms at the molecular and genetic levels, which can obscure the specificity of retinal imaging as a diagnostic tool for AD [10]. Nonetheless, it is increasingly proposed that ophthalmologists may assume a greater role in the diagnostic pathway of neurodegenerative diseases [20].

EXTRACELLULAR VESICLES

Extracellular vesicles (EVs) are nanoscale, membrane-enclosed particles that are secreted by various cell types, including neurons, astrocytes, microgia, oligodendrocytes, and endothelial cells. They are typically classified into microvesicles, apoptotic bodies, and exosomes according to their biogenesis pathways, molecular composition, dimensions and physiological functions [8]. They are capable of crossing the blood-brain barrier, which makes them accessible in studies of human body fluids [21].

Studies cited in the review show that neuron-enriched exosomes (NEEs) isolated from the plasma of AD patients contain significantly higher concentration of core AD pathological proteins, including Amyloid-β 42 (Aβ42), total Tau (T-tau) and phosphorylated Tau (P-T181-tau) compared to control groups. These findings suggest that exosomal biomarkers can reflect alterations accurately and are valuable for diagnosing AD [8].

MicroRNAs (miRNAs) are key components of exosomes that are transferred between cells [7]. They may influence mRNA translation and thereby modulate gene expression and influence the physiological activity of cells by interacting with the 3' UTR region of mRNAs [8,9]. Consequently, it may lead to either a decrease or an increase in the production of proteins [9].Owing to their wide distribution in multiple biological fluids- such as plasma, serum, cerebrospinal fluid, urine and saliva- exosomal miRNAs have attracted growing attention as potential biomarkers [8]. A further benefit is the exceptional stability of circulating miRNAs (as they are enclosed within EVs), which permits a

longer time between sample collection and subsequent analysis [9,22,23]. In the course of certain diseases, dysregulation of specific miRNAs or miRNAs panels has been observed [23]. In a cohort of 436 subjects (208 probable Alzheimer's disease patients and 228 controls), the expression of exosomal miR-135a, miR-193b, and miR-384 was analyzed in serum samples. The study revealed a marked elevation of miR-135a levels in AD patients compared with healthy controls. Meanwhile, exosomal miR-384 expression was lower than in dementia of the Alzheimer's type (DAT). Importantly, the combined evaluation of miR-193b, miR-384, and miR-135a expression appeared to enhance the diagnostic precision for detecting early-stage AD [8]. In separate study examining patients with AD, increased expression of several miRNAs was reported, including miR-106a-5p, miR-16-5p, miR-223-3p, miR-25-3p, miR-30b-5p, miR-92a-3p and miR-451a [18]. The inconsistencies reported across studies are likely attributable to methodological heterogeneity in EV isolation and miRNA profiling approaches, as well as differences in age, sex, genetic background- together with technical disparities and limited comparability arising from variation in the stage of AD among individual patients [23,24].

Microparticles (MPs), which are also a subclass of EVs, contribute to both hemostatic and neuroinflammatory mechanisms [8]. Their elevated levels may be caused by central nervous system disorders, including neurodegenerative diseases [25]. Altered plasma concentrations of MPs- particularly those derived from neurons, leukocytes, endothelial cells, and platelets, as well as MPs expressing tissue factors- have been observed in AD patients. These abnormalities suggest that MPs may serve as indicators of vascular and inflammatory dysregulation associated with AD pathology [8]. Another study emphasized the potential utility of endothelial-derived MPs (EMPs) in assessing AD stage, reporting that increased EMPs levels were associated with impaired cognitive function [26].

Together, EVs are emerging as highly promising, minimally invasive tools for the diagnosis and monitoring of neurodegenerative disorders. The precise mechanisms by which exosomes influence the pathophysiological processes of AD remain to be fully clarified, not to mention their small size, biological compatibility, and unique ability to traverse the blood-brain barrier make them particularly advantageous for studying the central nervous system and other diseases [8]. Interestingly, several studies have demonstrated that miRNAs are involved in Aβ clearance, making them a promising avenue for research into AD treatment [9].

MAGNETIC RESONANCE SPECTROSCOPY

In patients with cognitive impairment, magnetic resonance imaging is routinely performed. However, it is not used to confirm AD, but rather to exclude alternative causes of patients' symptoms [5]. Standard MRI examinations are characterized by limited specificity and sensitivity of the obtained data in the context of AD diagnosis; moreover, they do not reveal much in terms of biochemical or physiological changes. Efforts have been made to implement Magnetic Resonance Spectroscopy Imaging Technology (MRS) in the diagnosis of AD as a non-invasive method [27,28]. Thanks to the combination of the principle of magnetic resonance and the phenomenon of the chemical shift in MRS, this imaging technique is distinguished by high resolution, imaging without the use of contrast agents, absence of bone artifacts, and lack of complications from ionizing radiation. In MRS studies, it is possible to distinguish chemical compounds based on spectral peaks, which results from differences in their chemical shifts [27]. Furthermore, MRS allows for the assessment of their concentrations [29]. In the human brain, there are five resonance spectrum peaks: N-acetyl aspartate (NAA), choline complex (Cho), myoinositol (MI), creatine (Cr), and glutamate [26]. NAA reflects the status of neurons and their synapses [28]. In a healthy brain, the concentration of NAA is approximately 12mM, and a decrease in this concentration may indicate damage to or loss of neurons in the brain. Cho is associated with cognitive functions, neurotransmitter metabolism, and ion transport, as it is a component of the cell membrane or may act as a neurotransmitter, being a precursor to acetylcholine and phosphatidylcholine. Meanwhile, MI, since it is present only in glial cells, increases in states of glial damage [27]. The neuroprotective effects of Cr have been demonstrated in studies conducted on rodents [29]. As Cr is a phosphate substance that transforms under the influence of ATP/ADP, states of declining metabolism cause its concentration to rise [22]. In turn, during hypoxia and ischemia of brain tissue, the concentration of glutamine, as an excitatory amino acid, increases [27].

Additionally, in the early stages of Alzheimer's disease, abnormalities in the structure of brain tissue may not be present, with only disturbances in the function of the nervous system appearing. At the very early stage of AD development, when no structural brain abnormalities are detectable, MRS is able to reveal irregularities in the spectrum. For example, studies have shown a decrease in NAA levels in the hippocampus, the anterior and posterior cingulate gyrus, as well as in the neocortex in patients with AD compared to the control group [27]. Reduced Cr levels have been observed in both MCI and AD compared to healthy controls [29]. A reduced NAA/Cr ratio has also been demonstrated, along with an increased MI/Cr ratio. It has been proposed that an MI/Cr ratio of 0,7 corresponds to a probable diagnosis of AD, and a decreasing NAA/Cr ratio indicates the rate of hippocampal neuronal cell loss, which may be a marker of progression from mild cognitive impairment to AD [27]. In patients with AD, poorer performance in cognitive assessments correlated with lower of NAA or the NAA/Cr ratio [30]. Significant elevation of MI concentration has also been demonstrated in areas such as the hippocampus, occipital lobe and other areas in patients with AD. A significant improvement in quality and the ability to quantitatively determine substances not

detectable with low magnetic fields can be observed with the recently introduced high magnetic fields, for example, glutamic acid or taurine. It is expected that spectroscopy using high magnetic fields will reveal the metabolic profile of AD and thus show a very wide range of neuronal metabolites. However, this still requires further scientific research [27].

BLOOD BIOMARKERS

The principal limitation of standard AD biomarkers obtained from cerebrospinal fluid lies in the invasive nature of their acquisition, restricted availability and substantial costs associated with these assessments [31,32]. Blood-based biomarkers (BBM) appear highly promising due to their minimally invasive nature, low cost, and significantly reduced time requirements [33]. The characteristics of an ideal biomarker are presented in Table 1.

Table 1. Key characteristics of an ideal biomarker for Alzheimer's disease, adapted from [7].

Relationship to disease pathology	The biomarker should reflect the underlying biological mechanisms and pathological processes associated with Alzheimer's disease	Biomarkers such as Aβ and phosphorylated tau are closely linked to AD pathophysiology and disease progression
High diagnostic accuracy	It should demonstrate strong sensitivity and specificity, enabling reliable discrimination between Alzheimer's disease and other types of dementia	Phosphorylated tau isoforms (e.g., p-tau217) show high diagnostic accuracy comparable to CSF and PET measures
Capability for early identification	The biomarker should allow detection of the disease at an early stage, providing valuable diagnostic and prognostic insights and supporting timely therapeutic decisions	Changes in Aβ metabolism and tau phosphorylation occur years before clinical symptoms, supporting early detection potential
Predictive value	The marker should possess a high positive predictive value, indicating a strong association with disease presence or progression	Elevated tau and altered Aβ ratios are associated with increased risk of disease progression
Accessibility and practicality	Ideally, the biomarker should be measurable in biological fluids (e.g., blood, cerebrospinal fluid, or peripheral tissues) using minimally invasive and cost-effective methods	Blood-based biomarkers are increasingly recognized as practical alternatives to CSF and PET due to lower cost and easier accessibility

Such BBM as tau protein, Aβ40 and Aβ42 peptides, neurofilament (NfL) can be assessed [33].

Building on the assumption that amyloidogenesis constitutes a pivotal mechanism in the development of AD, numerous efforts have focused on detecting peripheral blood indicators of amyloid pathology [31,34]. The Aβ42 isoform- the primary constituent of senile plaques-aries from the proteolytic processing of amyloid precursor protein and can induce DNA damage through mechanisms involving oxidative stress. Circulating Aβ is derived from both the central nervous system and peripheral tissues [31]. The structure of Aβ is unstable [35]. A wide range of biochemical, methodological, clinical, demographic and genetic variables has been shown to influence Aβ concentrations. For instance, owing to its hydrophobic properties, Aβ in the bloodstream tends to adhere to plasma proteins and even to tube surfaces, which may obscure epitopes and interfere with analytical procedures. Only a modest correlation exists between Aβ levels measured in blood and those found in CSF [31]. Nevertheless, several studies report that, within the AD continuum, shifts in the Aβ42/Aβ40 ratio in both plasma and CSF manifest before amyloid positivity is detectable via PET imaging, indicating early pathological processes [35,36]. In a separate study, serum analysis revealed elevated Aβ43 concentrations and reduced Aβ42 levels in patients with AD relative to controls, indicating that the Aβ42/Aβ43 ratio may have potential diagnostic value [35]. In older adults, increased plasma Aβ42, decreased Aβ40, and a lower Aβ42/Aβ40 ratio may reflect a transition from normal cognition toward MCI or AD [31]. However, commonly used medications in older adults may influence plasma Aβ levels [36]. However, due to the substantial influence of multiple confounding factors, the inconsistent reproducibility of findings, and ongoing debate surrounding these results, the role of Aβ as a dependable plasma biomarker cannot currently be clearly defined [31].

Concentration of NfL reflects injury to spinal cord structures, the brain axons and axonal degeneration. Plasma NfL concentration serves as an index of neurodegeneration (similarly to the NfL levels in cerebrospinal fluid) and exhibits a strong correlation with its cerebrospinal fluid concentration [37].Other studies have reported equivalent diagnostic efficacy of NfL in both plasma and CSF [38]. NfL levels are not specific to AD, elevated levels can also be detected in pathological conditions [31]. Nevertheless, NfL can be regarded as an indicator of the intensity of ongoing neurodegeneration [37]. Therefore, the use of NfL as a marker of neurodegeneration and as a tool for identifying individuals susceptible to cognitive impairment and brain atrophy can be considered [33].

Although elevations in plasma NfL can be observed in individuals with MCI or dementia attributable to AD, these markers provide only limited added value for reliably identifying AD pathology when used alongside high-performance plasma assays for p-tau [37]. On the contrary, multiple phosphorylated tau isoforms- such as p-tau181, p-tau217 and p-tau231- are markedly elevated in the plasma of patients with MCI or AD-related dementia, enabling differentiation of AD from other neurodegenerative conditions with a high degree of diagnostic precision, often comparable to PET imaging and CSF- based AD biomarkers [37, 39]. Among these variants, p-tau217 demonstrates the most pronounced relative increase in symptomatic AD, typically rising by approximately 300-700% in comparison with both cognitively unimpaired individuals and patients with alternative neurodegenerative disorders [37,38]. In particular, elevated concentrations of p-tau217 may be associated with an increased likelihood that individuals diagnosed with MCI will subsequently develop AD over a period of approximately four years [40]. As a result, the diagnostic performance of p-tau217 shows reduced vulnerability to test-retest inconsistencies relative to many other blood-based biomarkers, and comorbid conditions- such as renal impairment- exert only minimal influence on its plasma concentration [37]. Collectively, these attributes render plasma p-tau217 a highly reliable biomarker for identifying AD in clinical settings among patients presenting with MCI or dementia, and potentially even in individuals without cognitive symptoms [37,39, 40]. Its potential role as a screening test has also been discussed [39].

Plasma clusterin levels have also been investigated as a potential biomarker for AD. However, they have been shown not to be associated with the prevalence of AD, but rather with the severity of its progression [41].

Currently, the diagnosis of AD based on biomarker results is presented in the scientific literature as a powerful and excellent clinical method [42,43]. However, it is important to foster discussion between practicing clinicians and scientific researchers, as a discrepancy exists between expectations and everyday clinical practice. Therefore, a study was conducted to explore the opinions of Dutch physicians. Among the views expressed was the argument that the effects of an early diagnosis of AD on the subsequent course and organization of patient care remain insufficiently established [42].

However, the use of blood-derived biomarkers is constrained by the complex nature of blood itself- a readily accessible yet highly intricate biological matrix whose composition may be influenced by numerous factors. These influences may lead to variability in the concentrations of specific biomarkers and may affect both the sensitivity and specificity of the diagnostic assays [31]. Moreover, AD is a slowly progressive disorder, and the extent of blood-brain barrier integrity loss remains insufficiently understood. Nevertheless, disruption of this barrier has been documented, enabling the exchange of molecules between cerebrospinal fluid and blood [44].

CONCLUSION

Modern diagnostic innovations offer less invasive approaches for detecting Alzheimer’s disease. The comparison of the aforementioned methods compared to CSF and PET are presented in Table 2. Ophthalmic biomarkers and retinal structural changes provide accessible indicators of neurodegeneration that mirror cerebral pathology. Extracellular vesicles, especially neuron-derived exosomes, deliver valuable peripheral insights into molecular alterations associated with AD. Magnetic resonance spectroscopy enables early detection of metabolic disturbances that precede structural brain abnormalities. Among blood biomarkers, phosphorylated tau isoforms- particularly p-tau217- demonstrate high diagnostic accuracy comparable to CSF and PET measures. In contrast, other approaches- including ophthalmic biomarkers, extracellular vesicles, and magnetic resonance spectroscopy- remain at earlier stages of validation. Evidence supporting these methods is largely derived from small, heterogeneous, and often exploratory studies, which limits their reproducibility and generalizability. Furthermore, inconsistencies in results, lack of standardized methodologies, and absence of clearly defined diagnostic thresholds reduce their immediate clinical applicability.

Table 2. The comparison of the diagnosis methods of AD.

Method	Biological basis	Key advantages	Limitations	Level of evidence

Blood-based biomarkers	Reflect amyloid and tau pathology, neurodegeneration	Minimally invasive, relatively low cost, high diagnostic accuracy (especially p-tau217), scalable	Influenced by comorbidities and peripheral factors; variability in assays; Aβ instability	High
Extracellular vesicles	Transport AD-related proteins and genetic material across BBB	Reflect CNS processes; high molecular specificity; stable miRNAs	Lack of standardization (isolation, analysis); inconsistent results; technical complexity	Moderate–low
Ophthalmic biomarkers	Retina as extension of CNS; reflects neurodegeneration and Aβ deposition	Non-invasive, accessible, low cost, repeatable	Low specificity (overlap with glaucoma, AMD); inconsistent findings; limited validation	Low–moderate
Magnetic Resonance Spectroscopy	Detects metabolic changes (NAA, MI, Cr, glutamate)	Identifies early biochemical changes before structural damage; non-invasive	Limited availability; lack of standardized thresholds; complex interpretation	Moderate
CSF biomarkers (reference standard)	Direct measurement of Aβ, T-tau, p-tau	High diagnostic accuracy; well-established	Invasive (lumbar puncture); limited accessibility	High
PET imaging	Visualization of amyloid and tau deposition	High specificity; gold standard imaging	Expensive; limited availability; radiation exposure	High

Early-stage diagnosis is of paramount importance, as it enables the identification of AD before irreversible neuronal damage occurs and may increase the likelihood of successful implementation of future disease-modifying therapies. The integration of multiple complementary diagnostic modalities enhances specificity and reflects the complex pathophysiology of AD. Continuous development of new methods remains essential, as no single biomarker captures the full spectrum of neurobiological changes underlying the disease. Overall, the current level of evidence supports the use of blood-based biomarkers as the most clinically advanced tools, whereas other techniques should still be considered investigational. Ongoing exploration of innovative diagnostic tools improves detection and provides knowledge crucial for designing more effective therapeutic strategies. Together these advancements hold significant promise for improving diagnosis. Studies that have led to the development of new imaging techniques and biomarkers now enable the detection of AD even before the onset of symptoms [45].

DISCUSSION

Among the diagnostic methods outlined above, blood-based biomarkers- particularly phosphorylated tau isoforms (p-tau181, p-tau217, p-tau231) demonstrate the strongest diagnostic performance and the highest level of evidence. Their reported diagnostic accuracy approaches that of established methods such as cerebrospinal fluid (CSF) biomarkers and positron emission tomography (PET), especially in differentiating AD from other neurodegenerative disorders. In contrast, plasma Aβ measurements remain less reliable due to biological instability and susceptibility to multiple confounding factors affecting circulating concentrations.

Extracellular vesicles, especially neuron-derived exosomes, provide a promising tool for detecting AD-related proteins

(Aβ42, T-tau, p-tau) and dysregulated microRNAs. However, their clinical applicability is limited by methodological heterogeneity, particularly in EV isolation techniques and miRNA profiling, which contributes to inconsistent findings across studies. Similarly, ophthalmic biomarkers, such as retinal nerve fiber layer (RNFL) thinning and retinal vascular alterations, offer a non-invasive window into neurodegeneration. Nevertheless, their diagnostic specificity remains insufficient, as similar changes are observed in other conditions, including glaucoma and age-related macular degeneration.

Magnetic resonance spectroscopy enables detection of early metabolic disturbances, such as reduced N-acetylaspartate (NAA) and altered NAA/Cr and MI/Cr ratios, which may precede structural brain changes. Despite this advantage, MRS is not widely implemented in clinical practice due to limited accessibility, lack of standardized diagnostic thresholds, and variability in interpretation.

Several limitations affect the overall reliability of the discussed methods. Many studies are based on relatively small cohorts and differ in terms of patient selection, disease stage, and analytical protocols, which limits comparability and reproducibility. Blood-based biomarkers may be influenced by comorbidities and systemic factors, while EV-based and ophthalmic approaches lack standardized methodologies. Additionally, inconsistencies in the literature- such as conflicting data on lens biomarkers and miRNA expression profiles- highlight the need for harmonization of research protocols.

From a clinical perspective, no single biomarker currently captures the full complexity of AD pathology. Therefore, a multimodal approach integrating fluid biomarkers, imaging techniques, and clinical assessment appears to be the most rational strategy. However, translation into routine clinical practice requires further large-scale, longitudinal studies, as well as cost-effectiveness analyses and the development of standardized diagnostic algorithms.

CONCLUSIONS

By integrating the emerging evidence from ophthalmic imaging and metabolic screening, we can bridge the gap between initial clinical suspicion and definitive molecular confirmation of Alzheimer’s disease.

1. The diagnosis of Alzheimer's is shifting from invasive gold standards to accessible non-invasive "liquid biopsies" and retinal imaging that offer comparable accuracy for early detection.
2. Advancements in technology now allow for the identification of metabolic and molecular changes, such as p-tau isoforms and neuronal exosomes, before the onset of permanent structural brain damage.
3. The integration of these diverse diagnostic modalities enhances specificity and reflects the complex pathophysiology of the disease, allowing clinicians to obtain a more comprehensive understanding crucial for early-stage diagnosis and timely treatment.

DISCLOSURE

AUTHORS CONTRIBUTIONS:

Concept and design of the study: Patrycja Piekarska, Adrianna Purwin;

Literature review: Zofia Wcisło, Karolina Lach;

Resources: Grzegorz Szmit, Maria Chmielewska;

Data curation: Weronika Basak, Katarzyna Siwiec;

Writing, draft preparation: Patrycja Piekarska;

Review and editing: Gabriela Krok;

Supervision – Patrycja Piekarska;

Project administration: Wiktoria Modrzejewska;

Critical review and approval of the final version: Patrycja Piekarska, Adrianna Purwin, Zofia Wcisło, Karolina Lach, Grzegorz Szmit, Maria Chmielewska, Weronika Basak, Katarzyna Siwiec, Gabriela Krok, Wiktoria Modrzejewska

USE OF AI

Data presented in this study are available upon request from the author correspondence.

Artificial intelligence tools were used solely to assist with vocabulary refinement and

language editing. All conceptual decisions, including study design, data interpretation, and final approval of the manuscript, were made independently by the authors to ensure the integrity and originality of the work.

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