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Review Article

Biomarkers and Their Implications in Alzheimer's Disease: A Literature Review

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder with a complex pathology that is not completely understood. Over time, AD reduces one's cortical and subcortical functioning. The incidence and prevalence of AD is projected to increase as the worldwide population continues to grow older. While advances in the field of neurology and medicine continue to improve, there are presently no novel therapeutic agents to prevent, halt, or cure patients suffering from AD. The utilization of biomarkers that aid the diagnostic algorithm, drug response monitoring and disease progression that add to further our understanding of the pathophysiology of neurodegenerative disease is vastly expanding. The significance of amyloid plaque deposition, tau pathology, and neurofibrillary tangle accumulation have been well-studied in the realm of neurodegenerative diseases for decades and are proposed biomarkers. However, it has been difficult to stratify physiological biomarkers of blood/plasma, cerebrospinal fluid, saliva/urine/hair/nail for diagnostic utility and overall understanding in the pathogenesis of neurodegeneration. We aim to review the most relevant, up-to-date biomarkers and their implications in AD.

Introduction

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease that commonly affects an aged population. AD is the most common cause of dementia worldwide, and accounts for over 60% of all confirmed cases.[1] The World Health Organization (WHO) claims that the total estimated cases of AD exceeds 6 million in the United States, and over 35 million globally.[2,3] The American population over 65 years of age is expected to increase from 58 million in 2021 to over 85 million by 2050.[4] According to the Alzheimer's Association, the percentage of individuals suffering from AD more than doubles from ages 65–54 to 75–54 (5.3%→13.8%), and more than 34% of people over the age of 85 years are living with AD.[3,4] With the growing global population, and advances in medicine allowing individuals to live longer than previous decades, the incidence and prevalence of AD is likely to increase if no curative measures are established.[3]

More than 95% of AD cases are sporadic in nature, with only 1–5% resulting from a genetic disposition.[5] The accumulation of amyloid plaques or amyloid-B (Aβ) peptides in the extracellular neural tissue and neurofibrillary tangles (NFT) composed of hyper-phosphorylated tau proteins within the intracellular tissue of the brain are the main pathological signs of AD.[2,6,7] However, several other factors have been described as significant contributors to the pathogenesis of AD. Prolonged activation of the brain's macrophages and other immune cells producing an inflammatory reaction have been shown to worsen amyloid and tau pathology.[8] Evidence has been found that the development of AD also cor-

Keywords: Biomarker(s); Alzheimer's disease; Neurodegeneration; Tau; Amyloid; Blood; CSF.

Abbreviations: Aβ, Amyloid beta; AD, Alzheimer's disease; ADM, Adrenomedullin; ANP, Atrial natriuretic peptide; APP, Amyloid Precursor Protein; BACE1, β-secretase 1; CSF, Cerebrospinal fluid; ELISA, Enzyme linked immunosorbent assay; ET1, Endothelin-1; Flt1, fms-related receptor tyrosine kinase; GAP43, Growth-associated protein-43; GFAP, Glial fibrillary acidic protein; hFABP, heart-type fatty acid-binding protein; ICAM1, Intercellular adhesion molecule 1; MAP, Microtubule associated protein; MCI, Mild cognitive impairment; MRI, Magnetic resonance imaging; NfL, Neurofilament-light chain; NFT, Neurofibrillary tangles; Ng, Neurogranin; NP/NPTX, Neuronal pentraxins; PET, Positron-emission tomography; PPAR1, Peroxisome proliferator-activated receptor; PSEN, Presenilin; p-tau, Phosphorylated tau; PUFA, Polyunsaturated fatty acid; S100B, S100 calcium-binding protein B; sIL-1R2, Soluble interleukin-1 receptor 2; SIMOA, Single-molecule arrays; SNAP25, Synaptosome–associated protein-25; Syt1, synaptotagmin-1; TDP, TAR-DNA binding protein; TREM2, Triggering receptor expressed on myeloid cells 2; VCAM1, Vascular cell adhesion protein 1; VILIP1, Visinin-like protein 1; YKL40, Chitinase-3-like protein 1.

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relates with increased oxidative stress from neural free radical production.[9] Mitochondrial dysfunction, calcium-mediated excitotoxicity, vascular injury, and immuno-dysregulation also appear to contribute to the development and/or exacerbation of AD.[2,10,11] These pathologies cause a neuronal cellular insult, resulting in impaired synaptic function, as well as an overall reduction in healthy functioning neural tissue.[12] The resulting chronic neurodegenerative changes produce cognitive, behavioral, and functional abnormalities that manifest as memory deterioration, confusion, and difficulty understanding visual and spatial relationships amongst many other clinical signs and symptoms.[2,11–13]

It has become an urgent priority within the medical community to identify biological markers and blood testing protocols to better understand the pathogenesis of AD in order to improve the efficiency of diagnosis, reduce associated costs, and prevent the occurrence of neurodegenerative diseases. Several methods have been previously deployed for aiding in the diagnostic algorithm of AD, including positron emission tomography (PET) scans to measure amyloid plaque deposits, lumbar punctures directed towards quantifying the degree of tau protein in the cerebrospinal fluid (CSF), and analyzing the amount of cortical atrophy via magnetic resonance imaging (MRI).[14,15] However, imaging to this degree is very costly and measuring protein levels within the CSF is an invasive procedure.[5,14,15]

Herein, we review the contributions made towards the relevance in blood testing and biomarker identification in the use for diagnosing AD. We discuss the current roles and propose future uses for biomarkers in improving diagnostic accuracy, cost efficiency, patient stratification, and monitoring disease staging and progression to novel treatments for AD. The focus of this review article is to improve our overall depth of comprehension of the role biomarkers play in understanding the pathogenesis and treatment of AD.

Pathogenesis and pathology

The pathogenesis and pathology of AD is important when discussing implicated biomarkers. Although the pathogenesis is not clear, the leading theory is that there is an accumulation of insoluble Aβ peptides.[16] Amyloid precursor protein (APP) is a transmembrane glycoprotein that is physiologically cleaved by alpha and gamma secretases to form two physiologic proteins, one soluble and one membrane bound. In the amyloid-producing process, APP is enzymatically cleaved by beta and gamma secretases, leading to the formation of various isoforms of Aβ peptides. These peptides aggregate to form fibrils and oligomers, ultimately leading to Aβ plaque formation. This eventually leads to inflammation and neuronal cell death, a process known as the amyloid cascade hypothesis.[16–18] Consequently, these neuritic plaques can be observed via microscopy of brain tissue from AD patients, along with neuronal synapse loss.[19–21]

Another protein involved in the suggested pathogenesis of AD is tau. Tau is an axonal, microtubule-associated protein (MAP) that regulates the assembly and function of microtubules, predominantly within neurons.[22,23] Physiologically, tau is highly soluble and undergoes phosphorylation to regulate its microtubule binding affinity. In AD, tau is hyper-phosphorylated, forming an insoluble protein that aggregates into toxic NFTs within neurons.[21,23] These NFTs are another characteristic pathological feature found in the brains of AD patients.[21] Furthermore, it is thought that tau has a role in mediating Aβ toxicity in AD, though this mechanism is unclear.[20]

Additionally, there are several genes implicated in the development of AD, one of the most notable being the e4 allele of the apolipoprotein E gene (APOE).[24] APOE plays a role in Aβ peptide clearance, and individuals with the e4 allele are at an increased risk of AD, most likely due to Aβ accumulation through unclear mechanisms.[25] In rare cases, genetic mutations of APP or presenilin (PSEN) genes can be inherited in an autosomal dominant pattern that results in an early onset AD. PSEN is a protein in the enzymatic gamma secretase complex that is responsible for pathological cleavage of APP in the development of AD.[24,26]

Biological markers (biomarkers)

A biomarker is a metric of a particular biological state that can be quantified or measured.[27] A biomarker may be used to evaluate normal physiological processes within the body, pathological processes, or a pharmacological response to medical intervention. Biomarkers have played valuable roles in the diagnostic algorithm of many diseases, as well as the assessment of disease progression and potential recurrence. What makes a biomarker such a useful tool for researchers and clinicians is the ability to detect a fundamental neuropathologic feature of AD and with a relatively high sensitivity and specificity (ability of a test to accurately identify individuals with versus without a disease).[28] There have been many biomarkers identified within the human body that serve as valuable tools in diagnosing and monitoring AD progression. Table 1 summaries the biomarkers that are covered in this review and demonstrates the relevant changes associated in AD. Below is a comprehensive review of the biomarkers that have been identified and used in the diagnosis and treatment of AD. An illustrative overview of the pathological mechanisms covered in this paper are presented in Figure 1.

Cerebrospinal fluid (CSF) biomarkers

The most widely studied CSF biomarkers related to neurodegenerative diseases are Aβ peptides, and in particular the Aβ42 protein, total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau181). These biomarkers exhibit greater than 95% sensitivity and 85% specificity in regards to diagnosing AD.[2] Low quantities of Aβ42 in the CSF are observed in AD individuals compared to controls,[29] while elevated levels of hyper-phosphorylated tau and t-tau have been identified in the CSF in AD patients.[2,30] Previous literature has shown that high t-tau and/or p-tau181 along with low Aβ42 in the CSF can be detected before patients with AD become symptomatic, and offer improved diagnostic accuracy of AD from other causes of dementia.[31] The ratio of t-tau/Aβ42 or p-tau181/Aβ42 within the CSF are reliable predictors in the progression of AD and in determining future cognitive impairment in individuals without a current neurological deficit over a 10-year follow-up period.[2,32,33]

Amyloid (Aβ) peptides

Aβ peptides are formed after being cleaved from amyloid precursor proteins. The Aβ peptides are then released into the CSF. This biological process allows for the level of Aβ peptides to be measured fairly easily. It has been well documented that a low level of Aβ42 peptides in the CSF and a high amyloid plaque concentration in the brain are highly suggestive of AD.[2,34,35] The pathogenesis of the diminished Aβ42 peptides in the CSF is a result of the aggregation of hydrophobic peptide-forming plaques.[34–47] A reduced level of Aβ42 has also been noted in patients with Lewy body demen-

Table 1. Comprehensive list of the biomarkers identified within the CSF and blood plasma, noting the relative changes in AD patients

Pathological mechanism	Biomarker	Elevated levels in CSF	Reduced levels in CSF	Elevated levels in plasma	Reduced levels in plasma
Amyloid	Aβ42		X		X
	Aβ40		X		X
	Aβ38		X		
Tau	p-tau	X		X	
	t-tau	X			
Amyloid precursor	BACE1	X			
Synapse	Ng	X			
	SNAP25	X			
	Syt1	X			
	GAP43	X			
	NPTX/NP	X			
Neuronal	NfL	X		X	
	VILIP1	X			
Vascular	VCAM1	X		X	
	ICAM1	X			
	Flt1	X			
	ANP			X	
	ADM			X	
	ET1				X
Inflammatory	IL6			X	
	IL15	X			
	IL18			X	
	sIL1R2			X	
	hFABP	X			
	TNFa	X			
	TREM2	X			
	YKL40	X			
	GFAP	X			
	S100B	X			
DNA binding	TDP43			X	
Metabolites	PUFA				X
	Bile acids			X	
	Tryptophan				X
Iron	Ferritin	X			

Aβ, Amyloid beta; ADM, Adrenomedullin; ANP, Atrial natriuretic peptide; BACE1, β-secretase 1; ET1, Endothelin-1; Flt1, fms-related receptor tyrosine kinase; GAP43, Growth-associated protein-43; GFAP, Glial fibrillary acidic protein; hFABP, heart-type fatty acid-binding protein; ICAM1, Intercellular adhesion molecule 1; IL, Interleukin; NfL, Neurofilament-light chain; Ng, Neurogranin; NP/NPTX, Neuronal pentraxins; p-tau, Phosphorylated tau; PUFA, Polyunsaturated fatty acid; S100B, S100 calcium-binding protein B; sIL1R2, Soluble interleukin-1 receptor 2; SNAP25, Synaptosome–associated protein-25; Syt1, synaptotagmin-1; TDP, TAR-DNA binding protein; TNFa, Tumor necrosis factor alpha; TREM2, Triggering receptor expressed on myeloid cells 2; t-tau, Total tau; VCAM1, Vascular cell adhesion protein 1; VILIP1, Visinin-like protein 1; YKL40, Chitinase-3-like protein 1.

tia.[24,36] Enzyme-linked immunosorbent assay (ELISA) and mass spectrometry have been utilized for accurately measuring CSF levels of Aβ42 peptides.[34,38]

Measuring the CSF level of shortened Aβ peptides (Aβ38 and Aβ40) has proven to be of minimal reliability when diagnosing AD.[2,35] It has been identified that measuring Aβ peptide ratios may be advantageous over measuring the total Aβ42 peptide levels within the CSF. CSF measurements of Aβ42/Aβ40 and Aβ42/

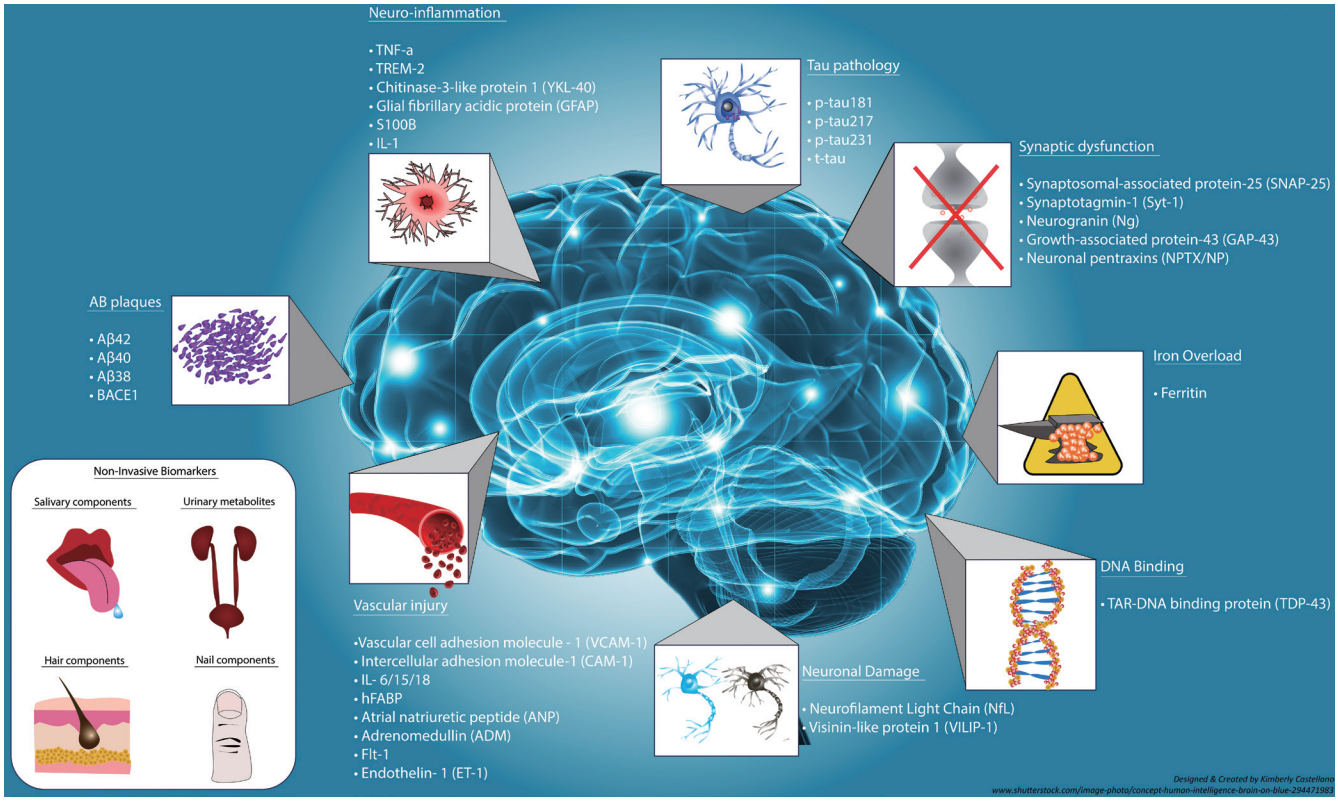

Fig. 1. Relevant physiological biomarkers and the proposed pathological mechanisms associated with AD. In this figure, the three-dimensional images reflect hypothetical relationships rather than direct causal links or specific cortical/subcortical locations of pathological mechanisms and neurodegeneration. This is not an all-inclusive list of pathophysiological mechanisms and/or implicated biomarkers in AD. Only the most relevant ones that are covered within this article are presented. Aβ, Amyloid beta; Flt1, fms-related receptor tyrosine kinase; hFABP, heart-type fatty acid-binding protein; IL, Interleukin; p-tau, Phosphorylated tau; S100B, S100 calcium-binding protein B; TNFa, Tumor necrosis factor alpha; TREM2, Triggering receptor expressed on myeloid cells 2; t-tau, Total tau.

Aβ38 ratios were shown to better differentiate AD from dementia of a non-AD cause. The ratios are more closely associated with overall amyloid plaque deposition on PET scans and may be a superior target measure for newly implemented clinical trials of amyloid-based treatments than of CSF Aβ42 alone.[2,39,40] A limiting factor of measuring only Aβ42 is the potentially confounding effect that results from differences in CSF subtleties or the variable rate of amyloid production from person to person. Importantly, this limitation is corrected when using the CSF Aβ42/Aβ40 ratio instead.[2,41] When CSF Aβ42 is measured in conjunction with CSF Aβ40, it provides a useful measure for target engagement of β-secretase (BACE1) modulators to inhibit Aβ peptide production and deposition.[42] There have also been promising results in tracking the physiological response to treatment with γ-secretase inhibitors when low CSF Aβ42 and Aβ40 levels are found with increased amounts of shortened fragments of CSF Aβ37 and Aβ38.[43]

Phosphorylated Tau (p-tau)

NFTs are composed of aggregates of abnormally hyper-phosphorylated p-tau.[2,34,44] Excessive amounts of p-tau in the CSF have been widely documented in AD patients, and are associated with an increased rate of disease progression.[34,45,46] The hyper-phosphorylation of these proteins results in the dysfunction of axonal transport in the brain.[47] ELISA offers an effective way to measure p-tau by recognizing specific epitopes.[45] Tau proteins phosphorylated at threonine 181 (p-tau181) have been the most thoroughly studied form of tau in conjunction with neurodegenerative disease.[2,34,44,45] However, recent literature that has studied p-tau231 and p-tau199 levels in the CSF confirmed a similar specificity to p-tau181 in differentiating AD from healthy controls.[48] Furthermore, p-tau231 demonstrated high sensitivity and specificity as a reliable biomarker for differentiating AD from non-AD dementias.[48]

A study completed in 2020, found elevated levels of tau phosphorylated at threonine 217 (p-tau217) in the CSF in patients with AD and proved to more accurately differentiate AD from non-AD dementias than CSF p-tau181.[49] In the same study, a higher level of p-tau217 in the CSF demonstrated a closer correlation with the measured amount of cortical amyloid present on PET scans and in the CSF compared to that of p-tau181.[49] Lastly, baseline and longitudinal measurements of CSF p-tau217 correlated with cortical tau deposition to a better extent than CSF p-tau181 when measured by the PET tau tracer [18F] flortaucipir.[49] Several other p-tau proteins have also been studied (235, 396, and 404) that may be of value as potential biomarkers after further research is performed to identify neurodegenerative disease states in the future.[50]

Total Tau (t-tau)

T-tau is utilized as an indicator for overall neurodegeneration.[51]

In healthy individuals, CSF t-tau increases with age: <300 pg/ml (21–10 yrs), <450 pg/ml (51–10 yrs), and <500 pg/ml (>70 yrs).[52] CSF t-tau has also been found to be significantly elevated in AD patients when compared to age-matched controls (>600 pg/ml in AD patients >70 yrs).[52,53] The t-tau level in the CSF is also potentiated as a prognostic marker for the conversion of mild cognitive impairment (MCI) to AD.[52] High CSF t-tau levels have been found in over 90 % of patients diagnosed with MCI that progressed to AD.[54] Interestingly, patients with stable MCI did not go on to develop AD.[54] CSF levels of t-tau can be accurately measured by ELISA.[55]

Beta site-amyloid precursor protein (APP)-cleaving enzyme 1 (BACE1)

BACE1 is a major β-secretase involved in plaque formation in the brain.[34] BACE1 expression is influenced by an inflammatory state, and in AD, the upregulation of neuritic cytokines reduces the peroxisome proliferator-activated receptors (PPAR1) which acts as an inhibitor of BACE1.[56] CSF levels of BACE1 have been previously measured by Western blot and ELISA in both MCI and AD patients, as well as healthy controls.[57] Along these lines, a significantly increased level of CSF BACE1 was found in MCI subjects versus AD and healthy controls.[57] The high BACE1 level in the CSF of MCI patients may reflect an overproduction of BACE1 by stressed neurons and/or glial cells in MCI, which then decreases while cells die during the progression to AD.[57,58] Nevertheless, further analysis is needed before BACE1 can be considered a reliable CSF biomarker for AD.

Synaptic biomarkers

A synapse or neuronal junction is the site of transmission of electric impulses between neurons (nerve cells) or between a neuron and a gland or muscle cell.[59] A synapse functions by storing neurotransmitters in presynaptic vesicles that are then released into the inter-neural space or synaptic cleft. This process allows communication via postsynaptic receptors with an adjacent cell or neuron after a cascade of electric stimuli traverses the nerve.[60] Significant loss of synaptic volume and degeneration within the grey matter of the brain are hallmarks of the early stages AD and produce cerebral impairment.[61] Numerous CSF biomarkers have been studied in regard to synaptic dysfunction in AD patients that may be useful for further advancing our understanding of the pathogenesis and treatment of AD.

The most promising biomarkers associated with synaptic dysfunction in AD patients are the postsynaptic protein neurogranin (Ng) and the presynaptic proteins synaptosome–associated protein-25 (SNAP25) and synaptotagmin-1 (Syt1).[2] The overall neuronal specificity and abundant expression of Ng, SNAP25, and Syt1 allow these biomarkers to accurately reflect the degree of neuronal and synaptic injury. This is because the CSF level of these biomarkers correlate with damage to neuronal and synaptic structures, as well as with the release of neuronal or synaptic components into the extracellular compartment as neurodegeneration progresses.[2,62,63] Cortical and hippocampal synaptic density is reduced by nearly 50 % in patients suffering from AD.[64] This significant loss in brain volume is attributed to global neuronal loss and a reduction in synaptic density.[64,65]

Ng is a calmodulin-binding neuronal protein that is largely found in postsynaptic membranes of the hippocampus and basal forebrain.[66] This protein plays a key role in the brain's adaptability to learning and memory function, as well as long-term potentiation (LTP).[2,62] Prior literature has discovered that baseline Ng levels in the CSF strongly correlate with neurodegeneration and cortical atrophy in AD patients.[2,62,67] Research has shown that measuring CSF Ng levels can serve as a reliable marker for future impairment to a similar degree as CSF t-tau and Aβ42 in patients who are presently cognitively normal.[2,62] Additionally, the abundance of CSF Ng may provide another way for researchers to differentiate AD from other neurodegenerative disorders with high reliability.[68]

The pre-synaptic protein SNAP25 is essential for exocytosis of synaptic vesicles via vesicle docking, neurotransmitter release, and neurite outgrowth.[2,34,69] Elevated levels of fragmented SNAP25 have been found in the CSF of AD patients when compared to healthy controls.[70] Also, the amount of CSF SNAP25 may be associated with cortical atrophy and the overall risk of cognitive deterioration over time.[70,71] Two distinct variants of SNAP25 have been isolated; SNAP25a and SNAP25b.[34,69] Further investigation is needed before either isoform can be credited as a reliable marker for AD and neurodegeneration.[72]

Another important pre-synaptic biomarker found in the CSF of AD patients is Syt1. This protein acts as a calcium sensor to allow neurotransmitter release into the synaptic cleft.[73] Increased amounts of Syt1 levels were identified in the CSF in the early stages of AD and MCI compared to healthy controls when using mass spectrometry.[2,74] A similar study concerning CSF biomarkers found low reliability in differentiating MCI due to AD and AD dementia when CSF Syt1 was compared to CSF levels of Ng and SNAP25a.[75] The use of Syt1 needs more validation through future studies before being considered a reliable CSF biomarker for neurodegenerative diseases.

A lesser-studied CSF biomarker in AD is growth-associated protein-43 (GAP43, neuromodulin). GAP43 is a crucial component of the neuronal axon and presynaptic terminal, and is primarily responsible for growth or "synaptic plasticity".[76] This protein also functions in pre-synaptic vesicle recycling through communication with synaptophysin and SNAP25.[2,77] A strong association has been found with increased GAP43 levels in the CSF with amyloid and p-tau/t-tau in AD patients, as well as most other neurodegenerative conditions.[2] This biomarker may have strong potential for use in the diagnostic algorithm of neurodegenerative disease.[2,78]

Pre-synaptic glycoproteins, often referred to as neuronal pentraxins (NPTX, NP) are involved in excitatory synapse formation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and are responsible for apoptotic neuronal death.[79] Aβ amyloid oligomers induce the pre-synaptic release of NP1, which is partially responsible for the synaptic and mitochondrial insult seen in the amyloid pathology of neurodegeneration.[80] Previous studies have correlated higher CSF levels of the pentraxin receptor, with increased severity of dementia in patients suffering from early-onset AD symptoms.[81] Current information available regarding the usefulness of neuronal pentraxins as CSF biomarkers of synaptic injury is inadequate, and further research is necessary.

Neuronal biomarkers

A neuron is a highly specialized cell within the nervous system that is composed of several unique structures that include dendrites, a cell body and an axon. Few neuron-specific biomarkers have been studied in depth, however, the most relevant neuronal biomarkers found in the CSF include neurofilament-light chain (NfL) and visinin-like protein 1 (VILIP1).[82]

NfL is a measurable component of the CSF when using immu-

noassay.[83] This marker is largely found within neuron axons and can be used to evaluate axonal damage in many neurological disorders.[82] Elevated levels of CSF NfL may offer another reliable biomarker for grading AD severity and progression.[2,82] Research has revealed elevated levels of NfL in the CSF during the early stages of symptom onset in AD.[82] Furthermore, rising levels of CSF NfL have been associated with the degree of cortical atrophy, cognitive impairment, and overall death rate of AD.[82]

VILIP1 is a protein that is expressed in abundance within the cerebellum and functions as a calcium-sensing receptor that is responsible for controlling intracellular signaling pathways through that regulation of adenylyl cyclase.[84] Previous literature has found a strong correlation between the level of VILIP1 in the CSF and overall cortical atrophy, as well as with amyloid/t-tau levels in patients displaying varying degrees of AD progression.[32,85] Similar to other CSF biomarkers, VILIP1 levels were increased in patients with MCI as a result of AD, in addition to individuals with AD dementia, when compared to healthy controls.[32] The ratio of CSF VILIP1/Aβ42 may serve as a better marker for determining whole-brain or regional cortical atrophy and cognitive deterioration when evaluated against some of the most widely studied CSF biomarkers that include t-tau, p-tau181, Aβ42 and t-tau/Aβ42 or p-tau181/Aβ42.[85] CSF VILIP1 has been determined to be a promising marker for not only neuronal injury in AD, but also as a reliable marker of future impairment in patients who are intellectually normal.[85,86]

Vascular markers of the CSF

Vascular injury and insult have provided some promising potential CSF biomarkers regarding neurodegenerative disease. Some of the previously identified vascular CSF biomarkers include vascular cell adhesion protein 1 (VCAM1), which mediates the binding of immune cells when endothelial damage occurs inside of blood vessels,[87] intercellular adhesion molecule 1 (ICAM1), a protein present on the surface of leukocytes that is responsible for cellular-vessel wall adhesion,[88] interleukin-15 (IL15), a pro-inflammatory cytokine,[89] and fms-related receptor tyrosine kinase (Flt1), a transmembrane domain responsible for angiogenic growth factor binding.[90] A 2018 study demonstrated increased CSF level of VCAM1, ICAM1, IL15 and Flt1 in patients diagnosed with AD in symptomatic and pre-symptomatic states when compared to healthy controls.[91] Adhesion proteins (ICAM1 and VCAM1) were also found to be strongly associated with future cognitive decline.[91]

A cytoplasmic cardiac protein called heart-type fatty acid-binding protein (hFABP), which is released during periods of myocardial ischemia,[92] has been isolated in the CSF at elevated levels in patients with AD and vascular dementia.[93] An increased level of hFABP in the CSF is also correlated with lower CSF Aβ42,[94] as well as cortical deterioration in patients who displayed amyloid plaque accumulation.[95] This may therefore be a potentially useful marker in distinguishing characteristics between vascular dementia, AD and other forms of neurodegeneration.

Cytokines and Inflammatory mediators

Neuro-inflammation is recognized as a fundamental component in the pathological process observed in AD. For over forty years, literature has indicated protective effects against AD when patients take anti-inflammatory agents for various other unrelated diseases.[8,96] Research hypothesizes the overwhelmingly increased deposition of Aβ plaques and a prolonged inflammatory response in an attempt to combat this pathology. The results indicate a sustained activation of microglia in a feed-forward loop that causes inevitable progression of the disease.[97,98]

Tumor necrosis factor-alpha (TNFa) is a small signaling protein (cytokine) released from microglial cells and astrocytes in the brain in response to an inflammatory reaction.[99] CSF levels of TNFa, as well as TNFa converting enzyme (TACE), have been found to be higher in AD patients when compared to healthy controls.[2,100,101] Another well-studied inflammatory biomarker is triggering receptor expressed on myeloid cells 2 (TREM2). Research has described missense mutations of TREM2, which induces phagocytosis of amyloid plaques, as a significant risk for the development of AD and other neurodegenerative diseases.[102] There have been similar reports from animal studies and human models that have identified elevated CSF levels of TREM2 in pre-symptomatic stages of AD patients.[2,8] Recent literature also suggests that CSF TREM2 levels may be directly associated with the degree of tau and/or amyloid pathology present.[2,8] An astrocytic pro-inflammatory biomarker called chitinase-3-like protein 1 (YKL40) has been studied to assess the diagnostic accuracy in the CSF of AD patients.[103,104] In a study focusing on CSF YKL40 levels, researchers were able to positively discriminate AD from cognitively normal controls and patients with frontotemporal dementia (FTD).[104] Furthermore, this same study found that the CSF level of YKL40 appropriately identified tau-positive individuals and AD pathophysiology-positive individuals from healthy controls and FTD patients.[104] Increased levels of CSF YKL40 have shown a positive correlation with cortical thinning in AD patients who displayed the APOE4 mutation.[105] CSF YKL40 has been positively associated with tau protein during the asymptomatic and preclinical stages of AD.[106] YKL40 may also serve as a reliable biomarker for future cognitive decline.[106] The utilization of CSF YKL40 as a biomarker in neurodegenerative diseases adds to the growing array of markers used for understanding and treating these diseases.

Glial fibrillary acidic protein (GFAP) is an intermediate filament expressed in astrocytes and ependymal cells throughout the central nervous system.[107] Increased CSF levels of GFAP have been identified in several neurodegenerative diseases, including AD, FTD and Lewy body dementia.[108] Another potentially useful biomarker is S100 calcium-binding protein B (S100B). This protein is exclusively expressed in astrocytes and has several theorized functions, such as neurite expansion and growth.[109] Elevated CSF S100B levels may offer diagnostic value during the initial stages of AD, especially when evaluating short-term memory recall.[110]

Blood and Plasma Biomarkers

Amyloid (Aβ) peptides

Detecting Aβ in plasma and accurately using it as a biomarker for amyloid pathology has been difficult using ELISA.[111] Not only are plasma Aβ levels much lower than in the CSF, but there is also a poor correlation between CSF and plasma levels of Aβ alone.[111,112] For this reason, and due to the contribution of Aβ from other peripheral sources, there has been inconsistency in plasma measurements of Aβ in different laboratories using ELISA.[111,113] This aside, one study showed that decreasing levels of Aβ42, an Aβ peptide with 42 residues that contributes to plaque formation, and decreasing ratios of Aβ42/Aβ40 in serial measurements using ELISA were associated with cognitive decline and the development of AD.[114,115]

The past decade has exhibited improvements in accurate measurements of Aβ using immunoaffinity-based assays, including single-molecule arrays (SIMOA) and mass spectrometry.[116] Specifically, studies using these methods to measure Aβ42/Aβ40 or Aβ40/Aβ42 ratios in plasma have shown a correlation with AD pathology, suggesting that it can serve as a prognostic indicator.[117–119] Plasma levels of Aβ42/Aβ40 were additionally able to predict amyloid pathology in patients without cognitive decline who were at risk for AD.[118,119] Recent studies have used immunoprecipitation and mass spectrometry to test for plasma biomarkers in both cognitively normal and abnormal patients. These works showed that there is a strong correlation between both APP/Aβ42 and Aβ42/40 plasma levels with Aβ-PET scan burden and CSF levels of Aβ42, which are more established markers of amyloidosis seen in AD.[2,117,118] The use of SIMOA to detect the plasma level of Aβ42/Aβ40 also showed a positive correlation with abnormal CSF level of Aβ42.[120] In terms of prognosis, a lower plasma level of Aβ42/Aβ40 has been shown to be associated with a more rapid decline in cognitive function in patients with subjective cognitive decline.[119]

Another Aβ related plasma biomarker that has shown prognostic value in AD is the detection of Aβ mis-folding using immune-infrared-sensor technology.[121] In one cohort study, the presence of Aβ mis-folding and Aβ42/Aβ40 plasma levels were tracked in patients with only subjective cognitive decline. The presence of mis-folding and positive Aβ ratios were strongly correlated with the progression to MCI and dementia due to AD.[121]

Tau

Detecting tau in plasma using immunoassay techniques and using it as a biomarker for AD pathology has shown promise in numerous studies.[122–125] Several cohorts showed that an increased level of p-tau181 in plasma, as measured by SIMOA and solid-phase enzyme immunoassay, is highly accurate in confirming AD pathology and in differentiating it from other non-AD pathologies in dementia patients.[122,123] Furthermore, a high plasma p-tau181 level more accurately predicted AD neuropathology compared to clinical diagnosis up to 8 years prior to postmortem examination of brain tissue.[122] An association also exists between a high plasma p-tau181 level and the development of AD dementia in unimpaired patients and those with MCI. This correlates with CSF p-tau181 and is predictive of positive PET scans.[124] Nevertheless, the plasma measurement of p-tau217 shows even more promise. Elevated plasma levels of p-tau217 discriminated AD dementia from other non-AD pathologies with greater accuracy than plasma p-tau181, plasma NfL and the detection of brain atrophy on MRI.[125]

Neurofilament Light Chain (NfL)

NfLs are components of axons that are another potential biomarker of use in AD.[82,126,127] High plasma NfL levels, measured using SIMOA, have been associated with the diagnoses of MCI and AD with Aβ pathology, and are correlated with brain atrophy on neuroimaging scans associated with AD.[126,127] Additionally, plasma NfL has been predictive of the rate of MRI brain atrophy in AD patients when using the Mini-Mental State Examination and Logical Memory Test for assessment.[127] In that same study, serial NfL measurements showed a high rate of change in AD patients when changing from a pre-symptomatic to symptomatic alteration, demonstrating utility in predicting the progression of AD.[127]

Inflammatory markers

Due to the inflammation involved in the pathogenesis of AD, there are numerous changes in the level of peripheral inflammatory markers, especially IL1-related cytokines and receptors.[128,129] Using ELISA, one study found an increase in serum sIL1R2 (soluble interleukin-1 receptor 2) and free IL18 in MCI, which also disappeared in AD.[128] They also found an increase in IL1Ra (interleukin-1 receptor antagonist), sIL1R1, sIL1R4, and IL18BP (interleukin-18 binding protein) in patients with AD but not with MCI, which may aid in showing the progression from MCI to AD.[128] Another peripheral cytokine of possible importance is interleukin-6 (IL6) which was found to be elevated in AD patients compared with healthy controls and showed an inverse correlation with Mini-Mental Status Examination Scores.[129]

Additionally, there are reported changes in peripheral T-cell presence and receptor expression in AD patients.[130–132] One study found an "immune signature" consisting of an increase in CD8+T effector memory CD45RA+ (TEMRA) cells, which additionally showed a negative correlation with cognition.[130] It has also been found that there are lower levels of CD45RA on CD4+T cells in AD patients compared to patients with other forms of dementia. In addition, the sensitivity and predictive value increased for classifying AD when combining CD45RA with the APOE genotype.[131]

Vascular markers

Vascular and microvascular dysregulation has been suggested as a causal role for AD and may precede neurodegeneration with up to 30 % of AD cases presenting with cerebrovascular pathology.[93,133,134] One study showed elevated levels of soluble E-selectins and VCAM1 in patients with AD compared to healthy controls.[135] Additionally, altered levels of the endothelin regulator and vasodilators endothelin-1 (ET1), atrial natriuretic peptide (ANP), and adrenomedullin (ADM) have been found in AD patients using special immunoassays to detect their precursors.[133,136] These assays measured C-terminal endothelin-1 precursor fragment (CT-proET1), mid-regional pro-adrenomedullin (MR-proADM), and mid-regional pro-atrial natriuretic peptide (MR-proANP) in the plasma since their final products have short half-lives.[133,136] In patients with AD, there were increased blood levels of MR-proADM and MR-proANP with decreased levels of CT-proET1. Additionally, both the sensitivity and specificity were increased when measuring the MR-proANP/CT-proET1 ratio.[136] Furthermore, increased plasma levels of MR-proANP and MR-proADM showed predictive value for progression from MCI to AD.[137] It is important to note that these markers might represent systemic microvascular or inflammatory changes, and may warrant further investigation for their clinical utility in diagnosing and predicting AD.[133]

TAR-DNA binding protein (TDP43)

TDP43 is an RNA and DNA binding protein that is involved in regulating splicing and transcriptional repression, and is a major component of cytoplasmic inclusions within neurons in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD).[138] It has recently been shown that 25–50 % of AD cases present with TDP43 pathological changes, which may be a factor in the development of AD, especially in more severe cases.[139–142] Increased plasma levels of TDP43 and TDP43 variants have been

detected in both AD and MCI before conversion to AD.[141,143] Due to increased levels of TDP43 in other neurodegenerative disorders, its clinical utility may be limited in distinguishing between various disorders, but it could still be used to narrow-down potential diagnoses.[141]

Metabolites

Dysregulation of several metabolic pathways with changes in plasma metabolites has been associated with and may contribute to AD pathology and impairment.[5] Cholesterol metabolism, fatty acid (FA) metabolism, bile acid synthesis, and amino acid metabolism may be the most associated with AD. Therefore, changes in plasma FAs, bile acids, and amino acids are some of the most apparent alterations reported in previous studies.[5,144–146]

The reduction in the level of polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (DHA), has been associated with cognitive impairment due to AD and may be due to impaired FA metabolism in the liver.[5,147,148] One study showed that cognitive performance improved with dietary supplementation of DHA in AD patients, which could have been due to neuroprotective properties of PUFAs.[148,149] To further implicated liver dysfunction, the level of the bile acids cholic acid, chenodeoxycholic acid, and allocholic acid all increased with disease severity in AD.[5] The role of bile acids as biomarkers in AD is further propounded by evidence of association between the traditional AD biomarkers, Aβ and tau, and bile acid profiles.[5,150]

Regarding alterations in FA metabolism, studies have shown declining acyl-carnitines across subjects from healthy individuals to those with MCI and AD, with significantly reduced levels of medium- and long-chain acyl-carnitines in those with AD.[5,151] Impaired energy metabolism is further indicated by one study that analyzed RNA transcripts to find decreased beta-oxidation, mitochondrial transport, and carnitine shuttle activity in patients with AD.[152]

Amino acid metabolism may also play a role in the use of metabolic profiles as biomarkers of AD, as reduced levels of tryptophan have been found in AD subjects, along with its derivatives of serotonin and indole-3-lactic acid.[5] The decrease in tryptophan and indole-3-lactic acid levels were further associated with disease severity of AD, which may serve as a biomarker for AD disease progression.[5] This follows our physiologic understanding of tryptophan's role as a precursor for neurotransmitters and their role in neuronal activity.[153]

Noninvasive biomarkers

Saliva is an extracellular fluid that functions primarily to aid in the digestion of food and maintain appropriate oral hygiene.[154] It is composed largely of water, and a very minuscule amount of electrolytes, mucus, antibacterial compounds and various enzymes.[154] Salivary testing offers an excellent alternative to expensive laboratory blood tests and invasive CSF measures via lumbar puncture. The most relevant finding has been the elevated salivary cortisol level in AD patients compared to healthy controls.[155] In the same study, the level of evening cortisol was lower in AD patients than in control subjects.[155] Despite these results, more research is needed before any salivary components can be reliably used as biomarkers in AD and neurodegeneration.

Another potential low-cost biomarker that can be easily collected and stored is a hair sample. Hair is a protein filament composed of keratin that grows from the dermis of the skin.[156] What makes hair follicles a potential biomarker for neurodegenerative disease is the fact that elemental components in its structure can be maintained for extended periods of time.[2,157] Several elemental metals have been shown to be elevated in AD patients' hair samples, including Br, K, Na and Zn. By contrast, Al, Ca, Co, Cu, Fe, Hg and Pb levels were reduced in the same hair samples.[2,157]

Nails are a keratinous plate at the fingertips and toe-tips, and similar to hair, are able to store elemental components for an extended amount of time.[158] Zinc is an abundant element within the brain and may play a role in several pathways relevant to the pathogenesis of AD, most importantly the processing of APP and aggregation of Aβ.[159] The level of zinc and numerous other metal chelators were shown to be decreased in nail samples of patients with AD.[2,159] However, there is no reliable literature that has consistently linked the use of elemental findings from nail samples as biomarkers in AD, and therefore further analysis is indicated.

The urinary tract may offer the most promise in identifying biomarkers that may distinguish neurodegeneration. Testing urine samples from AD patients is thought to recognize markers or patterns of free radical damage, or oxidative stress that may point to a pathological process of AD.[2] 8-hydroxy-2-deoxy-guanosine (8OHdG) is a major product of DNA oxidative damage[160] and serves as a widely studied biomarker. Previous literature has found elevated 8OHdG levels in the urine by more than ten-fold in AD patients when compared to cognitively normal controls.[161] Isoprostanes and neuroprostanes are prostaglandin-like compounds formed from free radical-catalyzed peroxidation of fatty acids,[162] and are excreted in the urine and may be reliable biomarkers for AD. The level of isoprostanes was shown to be elevated in patients with MCI compared to healthy controls and elevated to a higher extent in AD compared to MCI.[2] Lastly, urinary levels of amino acids are also theorized to be potential biomarkers of AD.[2,163] Elevated levels of glycine, histidine, 3-methyl histidine and carnosine were isolated in urinary samples of AD patients.[2,164] While these results demonstrate the increasing use of urine components as biomarkers, the reliability of these components needs further development.

Iron overload

Iron plays several important roles within the brain to maintain homeostatic function. Iron is responsible for neuronal oxygen transportation, DNA and myelin synthesis and appropriate mitochondrial functioning.[165,166] However, iron overload may be detrimental to neuronal health. Previous research has found an increased amount of iron deposits within the brain of patients with AD[167] and MCI.[168] Interestingly, iron facilitated the aggregation of Aβ plaques and p-tau by influencing the function of APP.[169] The utilization of brain imaging (MRI and PET scans) confirmed increased levels of iron in the brain in patients with elevated Aβ deposits, suggesting that iron may accelerate the aggregation of amyloid pathology in this population.[170] Research also points in favor of ferritin as a potential biomarker of AD.[165,169] Elevated ferritin levels in the CSF have been previously documented in APOE-e4 carriers and reflect a faster cognitive decline in MCI patients as they progress to AD.[171] However, plasma levels of ferritin have not demonstrated a strong correlation with CSF findings in AD and MCI patients.[171] While data is limited on ferritin as a biomarker, it may be more useful as a prognostic marker in the CSF when evaluating AD.

Prospect

Medical advancements and drug therapy trials targeting neurodegeneration have largely failed to provide any significant advancement in the detection, treatment, and prevention of neurodegeneration.[165,172,173] AD treatment is trending in the direction of a precision-based model to individualize diagnostic algorithms and treatment plans. By incorporating the accuracy and reliability of more physiological biomarkers, we may be able to better understand the patient population at higher risk for neurodegeneration and slow disease progression. Individualized biomarkers in AD and other neurodegenerative diseases may provide a path towards prevention and potential curative measures.

Conclusions

Biomarkers have played a crucial role in improving the diagnostic efficiency, cost analysis, and overall enhancement of our understanding of the pathophysiology in neurodegeneration. Biomarker inclusion has remained an overwhelming target in AD research, with the most reliable and widely studied ones comprising amyloid and tau pathology. More data is needed to standardize and stratify biomarkers indicating vascular pathology, neuro-inflammatory response, and reliable noninvasive markers outside of the blood and CSF.

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Conflict of interest

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Author contributions

Study concept and design (JK), acquisition of data (VM, JK), manuscript writing (VM, JK), critical revision of the manuscript for important intellectual content (VM, JK), analysis and interpretation of data (VM, JK).

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