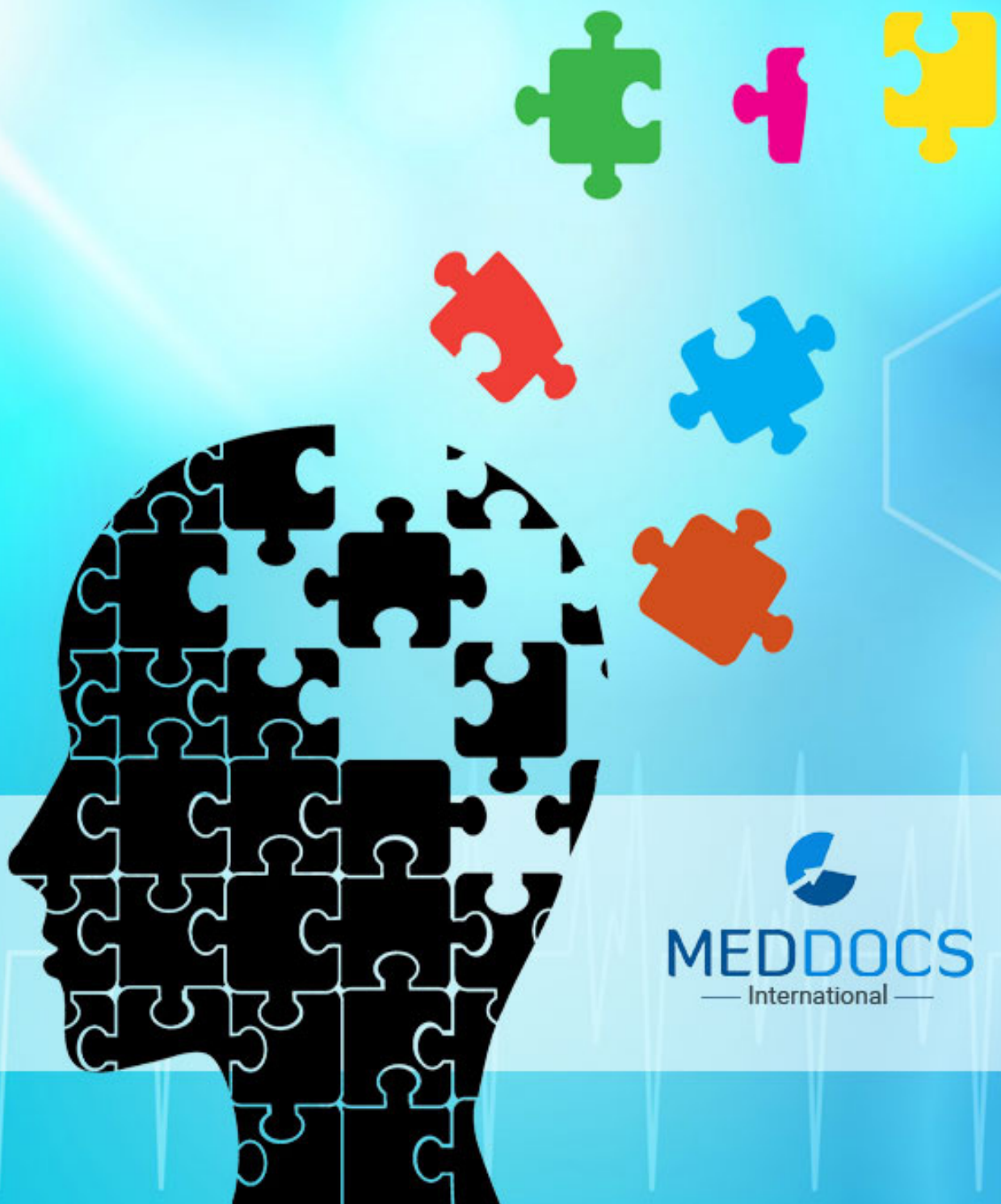


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Current and Novel Biomarkers for Alzheimer's disease

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Abstract

The rapid increase in the incidence of Alzheimer's disease (AD) in the approaching future prompts a need for an easy, efficient and precise diagnosis of the disease at its initial stages to halt or delay the disease progression. Conventional testing includes measuring amyloid beta and tau levels in the cerebrospinal fluid (CSF) aided by imaging techniques like positron emission tomography (PET) and magnetic resonance imaging (MRI). However, the cost factor and the invasiveness of the procedure curtails its utilization. Hence, there is a priority to investigate novel proteins, which are specific in predicting the development of AD, potentially enabling broader clinical assessments and efficient population screening. This chapter presents an overview of conventional and novel biomarkers under investigation to diagnose AD at the earliest and delay the memory deficits in addition to providing prognostic value.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly population worldwide. The number of cases of AD is currently assessed to be more than 5.4 million and is anticipated to quickly increase in the coming decades. AD is characterized by an irreversible, progressive neurodegeneration leading to memory loss, cognitive dysfunction and behavioral changes, which significantly interfere with social and occupational activities. The hallmarks of AD include extracellular senile plaques known as amyloid beta ($A\beta$) and intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein. Diagnostic criteria established by the National Institute on Aging (NIA) and the Alzheimer's Association (NIA-AA) for diagnosis of AD are mainly clinical based findings [1,2]. The current approach to diagnosis AD involves the patient's history, clinical examination, and diagnostic studies including imaging studies and various other biomarkers. However, the major concern in AD is to recognize the disease in its prodromal stage even prior to the appearance of cognitive deficits. Hence, there is an utmost need for improving the diagnosis of AD with objectives of earlier diagnosis and specificity in the diagnosis. As of late, investigation has started to center on developing modern tools, such as MRI, blood, lipid and CSF biomarkers that could

increase the specificity of the prodromal stage of AD. While this research can contribute to identifying biomarkers of AD, it can also permit identification of new therapeutic targets thereby preserving normal neuronal function or retarding the pathological changes. The need to use biomarkers more routinely will become necessary as disease-modifying treatments become available and an accurate subtype diagnosis is established, at an ideally pre-dementia stage. Therefore, it is of interest to focus on established and newly emerging central and peripheral biomarkers in AD.

Established biomarkers in AD:

According to the National Institutes of Health, "Biomarkers are evidence of any biological, pathogenic or pharmacogenomic response when administered to any therapeutic change"[3]. Biological markers are any kind of substances, structures or processes, which could be measured in/outside the body and may influence any changes in the body and probable prevalence of any disease in the body [4]. Biomarkers reflecting different types of pathophysiology in the brain can be used for clinical diagnosis, especially in the early stages of the disease, to predict progression, to monitor effects of novel drug candidates in clinical trials, and lastly in clinical research to deepen our under-



standing of the pathogenesis of the disease [5,6]. Biomarkers in AD are of great importance, since the cognitive symptoms often are diffuse and overlap with other disorders; the clinical progression is slow and variable even among patients with the same disease. For a possible potent biomarker for Alzheimer's disease, following criteria has been unanimously decided by researchers worldwide [7-10].

- Reflect aging of brain
- Describe pathophysiological processes in brain
- Any pharmacological change should be reflected
- Highly sensitive and specific
- Reproducible results over time changes
- Clear cut-off values with at least two-fold changes
- Easy collectible results and inexpensive tests

The major biomarkers used in AD research are divided into three categories based on the nature of the underlying pathophysiology as illustrated in Table 1:

Table 1: Conventional biomarkers in AD

Biomarkers of fibrillary A β deposition	1. Amyloid PET [11] 2. CSF A β 42[12]
Biomarkers of tau pathology	1. Elevated CSF phosphorylated tau (P-tau) 2. tau PET[13]
Biomarkers of neurodegeneration/ neuronal injury	1. CSF total tau (T-tau) 2. [18F]-fluorodeoxyglucose(FDG)-PET hypometabolism 3. Atrophy on structural MRI in regions characteristic of AD[14]

The above mentioned major AD biomarkers in Table-1 are divided into 3 classes and represent the A/T/N classification system. "A" refers to A β (PET or CSF A β), "T" referring to Tau and "N" referring to Neuronal injury [15]. Cerebrospinal fluid (CSF) tests for detecting amyloid deposits, tau pathology and neuronal injury are the established markers currently available and collectively increase the validity for diagnosis by giving results which are sensitive to >95% and specific to >85% [16-20]. Imaging biomarkers for AD include positron emission tomography (PET) of A β and tau to measure the amount of these protein deposits in the brain. Additionally, magnetic resonance imaging (MRI) is performed to measure brain volume and neuronal connectivity. These biomarkers are explained in detail in the following sections.

CSF biomarkers:

As discussed above, the three most important CSF biomarkers include the amyloid beta 42 isoform (A β 42), phosphorylated tau (P-tau) and total tau (T-tau) reflecting different aspects of disease pathogenesis [21]. In the course of disease, A β levels in the CSF decrease, whereas tau levels increase. This is due to P-tau and T-tau spilling into the CSF as the neurons are dying following which A β levels decrease in CSF as there is more A β being deposited within the brain tissue. These proteins are quantified routinely by using enzyme linked immunosorbent assay (ELISA)

which include an assay for T-tau that measures all tau isoforms irrespective of phosphorylation state [22], tau phosphorylated at threonine 181 (P-tau181) [23], the 42 amino acid forms of β -amyloid (A β -1-42) [24]. A marked increase in the levels of both T-tau and P-tau associated with decrease A β 42 in the CSF have been noted in various studies [25]. These tests experience inconsistencies that result from variation in reagent batches and in measurements between clinical laboratories; A β 42 is more susceptible than T-tau or P-tau to these limitations [26]. Standardization efforts to control these issues include the creation of a mass spectrometry-based Reference Measurement Procedures (RMP) for CSF A β 42 [27] and Certified Reference Materials (CRM) for the main AD CSF biomarkers [28]. In addition, precise measurements have been achieved by novel assays developed on fully automated laboratory equipment [29]. These developments will act as the foundation for authentic and consistent measurements of the AD CSF biomarker, enabling the introduction of systematic standards and a more practical use of the CSF diagnostic in the clinical evaluation of patients with cognitive dysfunction and potential AD.

CSF Biomarkers for early diagnosis:

The potential of the above-mentioned biomarkers in pre-AD or early phase of AD might offer benefits in terms of differentiating other disorders with similar manifestations in addition to introducing treatment with disease modifying agents, which are most effective in the early stage of AD before neuronal injury is severe. Thus, studies examining the potential of the AD CSF biomarkers for early diagnosis were warranted. The first study was published in 1999, by Andreasen and co-workers, which showed that patients with mild cognitive impairment (MCI) who progress to AD with dementia during clinical follow-up have the typical AD CSF biomarker profile (decreased CSF A β 42 together with increased T-tau and P-tau) [30]. Importantly, the CSF biomarkers were stable at follow-up when patients had reached the dementia stage, indicating that these biomarkers do not change during the clinical stages of the disease [31]. An extended clinical follow-up period is needed to ascertain which MCI will not progress to dementia, or even will improve so that they no longer have memory problems. The first study with such extended clinical follow-up was published in 2006 by Hansson and co-workers [32]. This study showed cognitively stable MCI patients do not have the AD biomarker profile, while progressive MCI patients (with prodromal AD) could be identified with 95% sensitivity and 92% specificity against elderly controls and 83% specificity against stable MCI cases [33]. This very high diagnostic accuracy for the core AD CSF biomarkers for prodromal AD was later verified in several large multi-center studies, including the DESCRIPA study in Europe [34], the American ADNI study [35] and the Swedish Brain Power study [36]. Taken together, these and several subsequent studies, support that the AD core CSF biomarker profile has diagnostic value to identify MCI prodromal AD cases in unselected MCI populations.

Emerging CSF biomarkers

There are lot of intensive research under way looking at the other CSF proteins and abnormalities as potential biomarkers. Other A β protein [37,38] levels and ratios (TAU/A β 42, A β 42/A β 40, A β 42/A β 38) also become abnormal with the signature of AD[39]. Neurofilament light chain (NfL) in plasma and CSF have been investigated as a reliable biomarker to monitor disease progression and treatment response in AD [40]. Possessing critical roles in axonal and dendritic branching and growth, NfL is a scaffolding protein found in the neuronal cytoskeleton.

NfL levels in the CSF are exacerbated by CNS axonal damage; in this way, NfL is a promising biomarker of axonal injury in various neurological diseases [41]. Recent studies have reported NfL in CSF to be elevated in AD and Parkinson's disease (PD) [42,43]. A post synaptic protein-neurogranin which is expressed exclusively in the dendritic spines seems to be a promising biomarker candidate. Neurogranin plays a vital role in learning and memory by maintaining synaptic plasticity and long-term potentiation through calmodulin pathway [44]. Decreased levels of neurogranin in AD brain tissue [45] and increase in CSF neurogranin in AD patients have been reported compared to controls [46]. Some of the newer biomarkers which are currently being investigated are visinin-like protein 1 [47], chitinase-3-like protein [48] and SNAP 25 which represent [49] different parts of the pathology that can be measured in the course of disease progression.

Blood based biomarkers:

Until date, there is no approved blood biomarker for AD. A lot of intense research is under way utilizing multiple-omics to investigate different metabolites and protein with microarrays to look for blood biomarkers for AD. They offer a unique advantage of being cost and time effective in addition to being easily accessible to the general population [50,51]. Serum A β has been investigated as a potential biomarker for AD. Some of the methods used are ELISA to measure A β 1-40/A β 1-42 ratio in patients with MCI [52] and electroluminescence to detect A β in the blood [53]. A recent meta-analysis showed a significant decrease in A β 1-42 ratio in plasma to predict AD [54]. Similarly, plasma A β 1-17 has been shown to be sensitive and specific biomarker of AD as the ratio of free to cell bound A β 1-17 of MCI and age matched control groups were significantly altered [55].

Plasma clusterin (ApoJ) has been proposed to be a blood-based biomarker for predicting future risk of dementia and stroke. Plasma clusterin levels are associated with increased risk of dementia especially among older adults (age>80) [56]. Exosome markers especially neuronal derived exosomes (NDEs) are investigated in predicting the progression from MCI to AD. Potential biomarkers present in cases of MCI that evolved into AD within 36 months include altered levels of P-tau in plasma NDE, A β 1-42, neurogranin (NRGN) and repressor element 1-silencing transcription factor. During the dementia stage of AD, an increase in the concentration of plasma total tau (T-tau) was observed; in comparison, the T-tau levels in the MCI stage were more ambiguous. The Mayo Clinic Study of Aging published that higher levels of T-tau were related to impaired memory recall and the decrease of cortical thickness in the AD-specific region [57] of cognitively normal individuals. A possible biomarker for AD that might provide a more accurate need for diagnosis is the NfL in blood. NfL concentrations in plasma and CSF are positively correlated. NfL levels in plasma, serum, and CSF in both dementia and MCI stages were observed to be increased. These two exosome biomarkers are promising candidates for diagnosis of AD; however, further studies must be completed before validation. Kallikrein 6 (KLK6) is a protease highly expressed in the CNS that is speculated to be a major factor in the process of aging. KLK6 was linked to proteolysis of extracellular proteins involved in various neurodegenerative diseases; furthermore, it can be identified as a potential biomarker for AD [58].

Biomarkers for oxidative stress

Oxidative stress is a major factor [59] that plays an important

role in the early stages of AD and is currently being investigated and explored for biomarkers in AD. Characteristically there is a pathophysiologic imbalance between oxidants and antioxidants [60]. The level of oxidative stress can be measured in plasma, serum, erythrocytes and leukocytes [61]. Increased reactive oxygen species (ROS) is seen in the affected regions of AD brain. Higher ROS levels lead to post-translational modification of proteins, toxic cell damage, fragmentation and aggregation of amyloid beta [62]. Oxidative damage due to ROS leads to alterations in the cellular membrane and protein structure causing DNA bases to be vulnerable to damage, impairing the functionality of membrane receptors, enzymes and fluidity of the cellular membrane. This sequentially leads to lipid formation [63]. Oxidative stress markers include elevated end products detection such as protein glutathionylation, Protein Carbonyl Content (PCC), free fatty acid releases, hydrogen peroxide, nitric oxide, DNA oxidation, 8-hydroxy-2-deoxyguanosine (8-OHdG), iso- & neuro-prostane, 4-Hydroxy 2 trans Nonenal (HNE), lipid peroxidation, Malondialdehyde (MDA), TBARS and advanced glycation [64-68]. Specifically, ROS combines with mitochondrial and nuclear DNA to produce 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker that can be measured in urine. This biomarker is used in the earliest stages of AD to monitor cellular dysfunction and is especially effective because the pathway of 8-OHdG is well established. Alternatively, lipid peroxidation produces isoprostanes, a biomarker that ropes a strong association between oxidative stress and AD [69-71]. Furthermore, isoprostanes were increased in urine, plasma and CSF of AD patients. Isoprostanes (IsoP) are formed by a free radical catalyzed mechanism from polyunsaturated fatty acids in lipid membranes prior to release by phospholipases. Additionally important biomarkers 8,12-isoPF(2alpha)-VI [72] and malondialdehyde (MDA) TBARS [73,74], were also established to be high in AD patients. Isoprostanes are an advantageous biomarker due to their chemical stability, ability to easily detect them in tissues and fluids, and the lack of effect on isoprostanes by lipids in diet. This biomarker for oxidative stress is typically measured in urine due to the stability of isoprostanes in urine and the lack of invasiveness of urine collection in patients. Research has proven the reliability of isoprostanes as oxidative stress biomarkers and found elevated levels in biological samples from patients with AD, including cerebrospinal fluid, urine, and plasma. Dynamic thiol-disulphide homeostasis has been used to assess oxidative stress as a biomarker of AD [75]. A study conducted to test the relationship between oxidative stress biomarkers in serum and cognitive function demonstrated that derivatives of reactive oxygen metabolites (d-ROM) were a crucial biomarker of oxidative stress. Higher values of d-ROMs correspond with higher levels of oxidative stress [76]. Superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase are antioxidant enzymes which may be induced or consumed by oxidative stress [77]. The changes in antioxidant enzymes activity can also be a specific marker for oxidative stress and the progression of disease. Another gene expressed by transcription factors that is prevalent in AD is Intracellular Adhesion Molecule I (ICAM-I). ICAM-I is regulated by TNF- β 1. VCAM-I and ICAM-I are both regulated by transcription factors that when treated with antioxidants, especially pyrroline dithiocarbamate, are reduced, therefore decreasing the VCAM-I and ICAM-I concentrations. Through this antioxidant reduction pathway, a correlation between antioxidant resistant transcription factors insinuates [78] that oxidative stress can be an important biomarker in the pathogenesis of AD.

Biomarkers for synaptic dysfunction and degeneration:

Various studies have discussed that loss of synapse occurs early in Alzheimer's disease (AD) and is more intensely interrelated with cognitive decline. Therefore, quantifiable biomarker measures loss of synapse or synaptic degeneration, dysfunction which maybe predominantly useful for recognizing the neuronal pathology at an early stage of AD and for predicting forthcoming cognitive impairment [79,80]. Interestingly, there is no established or authenticated biomarker test for synaptic dysfunction or degeneration which is a crucial feature of AD pathophysiology. Disruption of synaptic transmission results in neuronal dysfunction and degeneration associated with patho-anatomical mechanisms at the synapse. Novel biomarker assays have been developed to measure the synapse-related proteins, monitor synaptic and dendritic function. Synaptotagmin is a pre-synaptic calcium sensor vesicle protein, novel biomarker for Alzheimer's disease. It facilitates neurotransmitter release from the synaptic vesicle by exocytosis and also functions as an essential vesicle cargo molecule [81] in hippocampal neurons. Various studies have shown that decrease in synaptotagmin-1 is seen in patients of Alzheimer's disease [82]. Therefore, synaptotagmin-1 is considered as a potential CSF biomarker in dementia due to Alzheimer's disease [82]. Synaptosomal-associated protein 25 (SNAP-25) is an essential component of the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex which mediate synaptic communication by initiating fusion of synaptic vesicles [83]. Negative correlation between SNAP-25 and cognitive decline is observed in a study done by Brinkmalm et. al., [84] indicating that this is a promising novel cerebrospinal fluid biomarker for synapse degeneration in Alzheimer's disease. Neurogranin (NGRN) is a post-synaptic dendritic protein expressed in the cortex and hippocampus of the excitatory neurons [37,38], that binds to calmodulin in the [85] absence of calcium and plays an important role in synaptic plasticity and long-term potentiation processes essential for learning [39,40]. A study done by Kester et. al., confirmed that CSF levels of NGRN are increased in patients with AD [86] [45,46] [49]. Therefore, measurement of neurogranin in CSF may be a promising biomarker reflecting dendritic instability, degeneration and pathophysiological process and so it serves as the direct link to clinical symptoms in AD. Neurosecretory protein VGF (VGF), chromogranin A (CHGA), secretogranin 2 (SCG2), and granins are various proteins involved in axonal or synaptic vesicle transport, synapse formation, plasticity, and stability [12]. Cystatin C (CysC) is a protease involved in A β degradation [87,88]; β 2-microglobulin (β 2M), lysozyme C (LysC) are proteins involved in the innate immune system [89,90]; neurexins (NRXNs) NRXN-1, NRXN-2, and NRXN-3, neuronal pentraxin 1 (NPTX1), neurofascin (NFASC) and neurocan core protein (NCANP). All the mentioned proteins are also involved in synapse formation and stabilization [91]. Several of these proteins, including VGF, CHGA, SCG2, CysC and β 2M have been suggested in previous studies to be involved in AD pathology. A recent study in mice suggested that NRXN-2 interacts with A β oligomers, resulting in loss of synapses, whereas blocking of this interaction prevented A β -induced memory impairment. NPTX1 is expressed in excitatory neurons, where it is involved in the function of AMPA receptors and GluA1 at [92] the synapse. NPTX1 negatively regulates mitochondrial function, transport and caspase activation through the intrinsic program of apoptosis pathway and negatively regulates excitatory synapse density. NPTX 1 has been found to be increased *in vitro* and be present in dystrophic neurites and around A β plaques in AD brains

which has shown to modulate synaptic transmission. Interestingly, expression of NPTX1 is disrupted in AD models. Therefore, NPTX1 may represent a potential plasma biomarker for excitatory synaptic dysfunction AD. It is clear that reliable synaptic biomarkers may eventually be useful for early disease diagnosis, predicting and monitoring cognitive decline during disease progression and to monitor synaptic and dendritic function directly in AD patients and cognitively normal elderly. This monitoring will be a very valuable addition to the AD diagnostic biomarker toolbox, but also in clinical trials to monitor pharmacodynamics effects of novel drug candidates on synaptic dysfunction and degeneration.

Biomarkers for neuroinflammation In AD:

Neuroinflammation is considered as a chief mechanism responsible for the progression of AD. Neuroinflammation involving astrocytes, microglia, and secreted compounds like reactive oxygen species, cytokines, and chemokines are some of the major biological processes assessed when diagnosing AD. When inflammation is triggered, pro-inflammatory cytokines are predominantly secreted. These pro-inflammatory mediators are dysregulated, can cause neuronal death, leading to cognitive decline [93]. One of the most potent pro-inflammatory cytokines is the tumor necrosis factor (TNF)- α and can be used as an inflammatory biomarker for AD. One study tested the levels of (TNF)- α in AD, mild-infarct dementia (MID) and non-demented elderly. TNF- α was the only outlier for AD, making it an appropriate biomarker for disguising AD from MID. Not only has TNF- α been correlated to AD, but also other pro-inflammatory cytokine interleukin-IL- β . IL- β is activated by caspase 1, due to mitochondrial dysfunction, a common pathophysiological feature in AD neuropathology. Along with TNF- α and IL- β , there are several other biomarkers associated with AD. The other biomarkers are IL- 2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, Interferon (IFN)- γ , Transforming Growth Factor (TGF)- β , and acute phase Reactant Protein c (CRP).

Sulfatides (ST) control cell growth, protein trafficking, signal transduction, cell adhesion, neuronal plasticity, signal transduction and cell morphogenesis. The depletion of sulfatides in both grey matter and white matter of AD patients, results decreased hippocampal volume and cognitive decline. Therefore, ST can be used as an early diagnostic marker of AD. In contrast, ceramides (Cer) levels increase in AD patient. Ceramides are ST degradation products which are often increased in correlation with AD. This increase of Cer can cause up-regulation of cytokines, an increase in ROS, mitochondrial dysfunction, and apoptosis. Plasmalogens are glycerophospholipids, major component of neuronal membranes whose deficiency affect synaptic function and structure, leading to cholinergic system dysfunction in AD. Plasmalogen inhibits the processing of amyloid precursor protein metabolism through γ -secretase activity, thereby resulting in formation of A β peptides. The vascular cell adhesion molecule-I (VCAM-I) expression is an early marker for microvascular injuries in AD. VCAM-I can be coupled with oxidative stress and is activated by posttranscriptional regulatory factors, IL- β . This leads to higher levels of VCAM-1 and cognitive decline.

A study on CD4⁺ T cells and AD was performed, showing that an increase in CD4⁺ T cells, increased cognitive function and decreased A β plaques. For this reason, CD4⁺ T cells can also be a biomarker in the diagnoses of AD. In many patients, there will be an increased level of CD4⁺ T cell apoptosis, which allows the A β accumulation and neurodegeneration. Furthermore, there

have been studies showing an increased level of B-cell lymphoma 2 (Bcl-2), a regulatory protein that either upregulates or downregulates apoptosis. The increase of Bcl-2 and the increase of CD4⁺ T cell apoptosis suggest that the Bcl-2 is working to upregulate apoptosis of CD4⁺ T cells, causing cognitive decline. For this, Bcl-2 levels can also be used as an important biomarker for AD. Triggering receptor expressed on myeloid cells 2 (TREM2), belongs to the immunoglobulin super family of receptors that is primarily expressed in osteoclasts and microglia. This regulates phagocytosis and anti-inflammatory activity. Rare mutation of R47H in TREM2 affects the phagocytic activity of microglia and consequently contributes to accumulation of A β . Another biomarker that has been recently identified is Ecto-nucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2), commonly named autotaxin (ATX). Amongst AD patients, an increase in ATX is observed with increased insulin resistance and neurofibrillary tangles. ATX is measured in the CSF and serum through enzymatic photometric methods. In addition, ATX is involved in the regulation of glucose metabolism and the formation of lysophosphatidic acid (LPA) from the precursor molecule lysophosphatidylcholine (LPC). LPA is a signaling molecule and lipid-based growth factor which is involved in the regulation of neurotransmitter release, the pro-inflammatory response, cell activation, survival, and migration via G protein-coupled receptors. LPA can be measured by colorimetric assays or mass spectrometry. Further investigation into the biochemical cascade of autotaxin and its related metabolites is necessary to understand their primary function in AD. Overall, these inflammatory markers offer a new avenue for biomarker exploration and many research studies have begun to identify potential ones as mentioned above.

Circulatory miRNA as biomarkers in AD:

Circulatory miRNAs are a more recently proposed biomarker for AD. This is due to their ability to bind to and suppress or degrade messenger transcripts. miRNAs are a key factor in gene regulation. Several thousand types of miRNAs have been discovered thus far and many of those types are specific to certain tissue and cell types. Extracellular miRNAs can be found in serum and plasma. Extracellular and intracellular miRNAs can be used as biomarkers of neurodegenerative diseases. A study was undertaken to measure the circulatory miRNAs in plasma samples collected from patients with neurodegenerative diseases including AD, PD, and Amyotrophic lateral sclerosis (ALS). The study evaluated the differentiation of neurodegenerative diseases from each other and from a control group based on pairs of miRNAs. The results of the study show that circulatory miRNAs have the potential to be used as biomarkers for specific neurodegenerative diseases. Enrichment of miRNAs in synapses and specific cell types is a valuable addition to the set of biomarkers available for AD due to the low cost and lack of invasiveness in relation to other biomarkers. Microarray and RNA sequencing are techniques used to find miRNAs in bio fluid. Recent research has identified miR-9, miR-125b, miR-146a, miR181c, let-7g-5p, and miR191-5p as six miRNAs which are possible biomarkers of AD. Certain studies of miRNA as an AD biomarker have focused on various stages of AD progression and pathologies, but further detailed studies are needed to focus on miRNAs as AD progresses and establish circulatory miRNAs as peripheral biomarkers for AD dementia. The RNA which originates in diseased tissue from the central nervous system, such as neurons, can move from the CNS to the peripheral nervous system and is therefore, able to be tested. Micro vesicles and binding proteins transport RNA from CNS cells to the PNS without the RNA

being degraded. Circulatory miRNA that has made its way to the periphery from the CNS can be a biomarker for neurodegenerative disease and give indication of cellular changes related to disease. Research to connect information from RNA in the peripheral nervous system to the neurodegenerative standing of patients with AD assesses miRNA in serum and cerebrospinal fluid. Next generation small RNA sequencing (NGS) has been used to compare the miRNA of AD patients, Parkinson's patients, and a control group of neurologically normal individuals. NGS can detect and measure amounts of various types of miRNA in serum and cerebrospinal fluid. Differences were observed in miRNA expression and related to plaques, tangles, and dementia. There was a connection found between these differentially expressed miRNAs and previous research on miRNAs deregulated in the CNS of patients with neurodegenerative disease. Some proteins, mRNA and miRNAs are transported in vesicles known as exosomes [35]. In consideration of miRNAs as a biomarker for neurodegenerative diseases, research into an exosome-mediated miRNA signature could be beneficial. There are some exosomal miRNAs which could be a risk factor for AD and some of these miRNAs even contribute to cognitive dysfunction [6]. Circulating exosomes are distinctive enough that they can be used in tandem with information about association between particular miRNAs and exosomes to increase efficacy of miRNA as a biomarker for AD [94]. Deviant miRNA expression studied in transgenic mice models has been shown to increase formation of A β plaques [95,96]. Changes in miRNA expression might also contribute to nonstandard degradation of tau [97].

Neuroimaging novel approaches:

Functional and molecular neuroimaging provide insights into brain structure and physiology. This novel technique can detect specific proteins and protein aggregates associated with AD. In AD, structural imaging data from computerized tomography (CT) and magnetic resonance imaging (MRI) show a progressive cerebral atrophy as a probable reflection of dendritic and neuronal loss, a neurodegenerative hallmark of the disease. Another type of information can be acquired using radioligands to detect molecules of interest using positron emission tomography (PET) imaging. In AD, the most applied imaging modalities so far are the ones measuring brain metabolism [18F] fluorodeoxyglucose (18F-FDG) and amyloid load 18F-florbetapir and [11C] Pittsburg Compound B (11C-PIB). Imaging tracers targeting microglial activation, synaptic density, gene expression and phosphorylated tau are under development, and hopefully they will improve our knowledge about the mechanisms underlying AD pathology. Regarding AD, one of the most eagerly anticipated PET modality is the tau imaging. However, developing a tau tracer has been challenging as there are several isoforms of the protein in the brain and because of the spatial characteristics of tau aggregates. Several radioligands have presented high "off-target" retention that hinders their use for research or clinical purposes. Despite that, the tracers currently being tested have shown reasonable uptake patterns akin to those described in Braak staging (Braak & Braak, 1991) and a significant difference in tracer accumulation is detectable between AD, MCI and normal subjects (Saint-Aubert et al., 2017). This reassures the potential of establishing tau radioligands to track disease progression. Two new radioligands are being tested [11C] Martinostat and [11C] UCB-J, for indexing gene expression and synaptic density, respectively. [11C] Martinostat is a PET tracer which binds to histone deacetylases (HDACs), which is known to silence expression of genes associated with neuroplasticity [98,99]. This PET compound is currently being tested in AD pa-

tients, who are expected to present fewer uptakes as compared to cognitively preserved subjects. If this holds true, this tracer could become a biomarker of gene expression, determining genetic and/or epigenetic signatures in such complex scenario [100]. This subsequently can help pharmaceutical companies to identify the best individuals to undergo clinical trials of HDAC inhibitors-which have been proposed as treatment for degenerative diseases. SV2A may be a good proxy of synaptic activity. Cognitive impairment has already been associated with synaptic loss in AD but being able to quantify this in clinical trial settings would probably boost the understanding of disease onset and progression.

Ocular biomarkers In AD:

The eye offers itself as a transparent medium to cerebral pathology and has in this way potentiated the advancement of ocular biomarkers for AD. AD patients have ocular manifestations such as anomalies of color vision, retinal changes such as cupping of optic nerve head and nerve fiber layer thinning, impaired contrast sensitivity, abnormal ocular movements and cataractous lens. The eye being a transparent structure can be visualized non-invasively, at cellular level, approving inexpensive testing of biomarkers in a clinical setting. Changes in retinal nerve fiber layer thickness, measures of decreased retinal ganglion cell function and loss of nerve fiber layer as quantified by optical coherence tomography (OCT) have been widely observed in AD patients [101,102]. However, there is an inadequate evidence to support the use of retinal vascular biomarkers of AD. Decreased choroidal thickness as noted by OCT is also seen in AD patients. A β accumulation in lens and supranuclear cataracts was observed in Down's syndrome patients with APP gene and early onset AD. Fluorescence lifetime imaging and A β PET imaging are done to notice lenticular changes. Accordingly, the identification of ocular manifestations of AD may be proven useful for diagnosis and monitoring of progression [103,104,105].

Conclusion

It is extremely challenging to determine the appropriate biomarkers for neurodegenerative diseases because tissues affected by neurodegenerative diseases are generally difficult to access. However, the conceivable benefits of biomarkers in clinical practice include outcome prediction, supplementary and influence therapeutic regimens. Therefore, successful translation of the assessment of novel and valid biomarkers in clinical setting for neurodegenerative disorders like AD offers the promise of not only improving outcome prediction but also a more scientific basis for therapeutic options.

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