Biomarkers for Alzheimer’s Disease: Imagination or Reality-View and Review!

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ABSTRACT
The Alzheimer’s disease (AD) is the most widespread chronic, insidious neurodegenerative disease causing dementia in elderly and leading to a massive burden on AD individuals, their families, and on social and health care systems. Its diagnosis is subjective, definite AD can only be diagnosed after pathological brain specimens are examined by either biopsy or autopsy, and it covers 50% - 70% of all dementia cases. It is estimated that, by 2050, the number of people aged 80 years or older will approach 370 million worldwide and that 50 percent of those aged 85 years or older will be afflicted with AD. Causes of the disease are multifactorial; where genetics and environmental risk factors work in harmony to cause the disease. Neuropathological features of AD depend on finding of extracellular deposits of β-amyloid peptides (Aβ) that lead to senile plaque formation and intracellular neurofibrillary tangles of hyperphosphorylated tau. However, increasing evidence has suggested that inflammation may play a critical role in AD pathogenesis as well. In the era of genome and sophisticated technology, AD early diagnosis still indecisive and valid biomarkers for AD to be used in routine clinical practice have met with dissatisfaction. Indeed, the relatively unchanged levels of plasma β-amyloid in AD, and a lack of analytical sensitivity for assays for the axonal damage marker have increased the effort to find an alternative ultra-sensitive assay for pathological markers in peripheral blood. We believe that early presymptomatological practical inexpensive strategies, for characterizing a potential surrogate marker in blood and CSF for AD is warranted, are of interest because they: 1) confirm diagnosis; 2) enable epidemiological screening; 3) identify distinct groups of patients (predictive testing); 4) monitor progression and response to treatment and aid in design/implementation of optimal therapeutic regimens; 5) further the study of the brain-behaviour relationship underlying neurodegeneration.

Keywords: Biomarkers; Alzheimer’s Disease; Amyloid-β; Tau; Cerebrospinal Fluid; Blood

1. Introduction
Alzheimer’s disease (AD), essentially, is multifactorial disease and a genetically heterogeneous disorder characterized by insidious chronic and progressive loss of neurons in discrete areas of the brain, causing debilitating symptoms and globally decreasing cognitive function such as dementia, loss of memory, loss of sensory or motor capability, decreased overall quality of life and well-being, and eventually, patient’s premature death. Not to mention the long sufferance and the heavy economical and emotional load placed on families and caregivers. No satisfactory cure is available yet; at best, existing treatments are symptomatic in nature and do not completely prevent or significantly impede the progression of the disease [1,2].

AD covers 50% - 70% of all dementia cases that affects millions of people globally. It is estimated that, by 2050, the number of people aged 80 years or older will approach 370 million worldwide and that 50 percent of those aged 85 years or older will be afflicted with AD [3,4].

However, rapid progress towards understanding the molecular underpinnings of AD is revolutionizing drug discovery for this condition. Furthermore, the development of models for this disorder is accelerating efforts to translate insights related to neurodegenerative mechanisms into disease-modifying therapies. Neither of Myriad approaches that had been undertaken to identify bio-
markers such as neurophysiological, imaging and cognitive testing in addition to newer sophisticated technologies for instance biochemical, proteomic, metabolomic and gene array profiling of tissue and biofluids from patients, and despite generous international effort, neither has proved satisfactory [5,6].

Hence, clear vision and an understanding of pathological progression of the illness can help to identify points of intervention and lead to promising therapeutic approaches. A fundamental approach for reducing the burden of AD is thus to slow or halt progression, and ultimately, to prevent the onset of the disease process. Strategies for neurorescue, neurorepair, or neuroprotection are being actively pursued by the basic, translational, and clinical research communities [7].

2. Overview of Biomarkers Properties

Access to molecular and biochemical markers of AD would complement clinical approaches, and further the goals of early and accurate diagnosis. Hence, the importance of the biological biomarkers studies which are quantitative measurements that provide information about; intrinsic biological processes, a disease circumstances and risk of developing an illness (antecedent biomarkers), assist in diagnosing disease (diagnostic biomarkers), response to treatment (prognostic biomarkers), providing much-needed insight into preclinical and clinical data, all of these are still valid procedures for early detection of AD [8].

Detection the subject’s susceptibility to the disease prior to appearance of prodromal signs, and detection of neural dysfunction before irreversible cellular damage, will be tremendously valuable for developing; prevention and intervention strategies and early treatments. From here stems the truthfulness and reliability of biomarker to distinguish between normal and interested disease [9].

In fact, biomarkers are set of factors used to measure anatomic, physiologic, biochemical, pharmacological, or molecular parameters associated with the presence and severity of particular disease states or processes in humans and animals [10,11].

These characteristics are objectively measured and carefully evaluated as indicators of normal biologic or pathogenic processes or pharmacological responses to a therapeutic intervention. Actually, define biomarker panels comprehensively to quantify risk, assess prognosis, and determine response to therapy [12]. Years ago, biomarkers were primarily physiological indicators such as blood pressure or heart rate. More recently, biomarker is becoming an important tool in different disciplines such as in field of oncology, immunology, cardiovascular diseases and metabolic diseases [13]. Synonym for molecular biomarker, such as elevated prostate specific antigen (PSA) as a molecular biomarker for prostate cancer, or using enzyme assays as liver function tests [14].

Biomarkers also cover the use of molecular indicators of environmental exposure in epidemiologic studies such as human papilloma virus (HPV) or certain markers of tobacco exposure such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane (NNK) which is a nitrosamine present in tobacco that is a potent procarcinogen. It is activated by CYP2A6, and plays a role as a biomarker of exposure to cigarette smoke, produced upon the curing of tobacco [15,16]. Also, Genomic biomarkers have principal role in investigation diseases; such as Apolipoprotein Epsilon-4 allele (APOE-ε4) for AD, and HLA for looking for narcolepsy, CD19, Sialophorin, CD11 integrin cluster, and IL-4 receptor for Crohn’s disease. Other methods and assays are used like; Serum or Spinal fluid substance, Neuroimaging and Physiologic parameters [17-20].

2.1. Biomarkers and Alzheimer’s Disease (AD)

2.1.1. The Cerebrospinal Fluid (CSF) Biomarkers

Plthora of biomarkers has been evaluated for AD. According to the literature more than 25 potential biomarkers for AD had previously been identified and new ones are still under investigation [21].

The CSF is the direct target because of its straight contact with the extracellular space of the brain where many biochemical processes in the brain take place and are reflected in the CSF. Since AD pathology is restricted to the brain, CSF is an obvious source and justified biomarkers for AD. Indeed, early biomarker discovery efforts for AD is based on immunoassays to detect and measure pathophysiological molecules of AD, such as Cerebrospinal fluid-derived β-amyloid protein 1-42, total tau protein, and phosphorylated tau (181,199,231) protein [22].

Several of these immunoassays demonstrated effectiveness in improving AD diagnosis, and these molecules continue to be used as potential diagnostic biomarkers. Two categories of AD biomarker studies are available today in the literature: the first, aims to prove potential biomarkers that already exist, and the second, looks for clues novel AD biomarkers with an emphasis on research using Neuroimaging. This article will address the different aspects of AD biomarkers with an emphasis on the most significant and prevalent ones [23].

2.1.2. Amyloid Beta (Aβ)

Aβ is composed of a family of peptides produced by proteolytic cleavage of the type I transmembrane straddling glucoprotein amyloid precursor protein (APP). In fact, one of the extracellular pathological hallmarks lesions of AD is the accumulation of aggregated Aβ42 peptide in amyloid plaques. Aβ42 makes up less than 10% of total Aβ and it is the initial and major component
of amyloid plaque deposits in AD [24]. Increased Aβ42 production throughout life and failure of Aβ clearance mechanisms lead to accumulation of oligomerization of Aβ42 in limbic and association cortices [25] (Figure 1).

The detection that Aβ42 peptide forms the essential component of AD plaques and that is secreted by cells led to examinations of Aβ42 in the CSF. Previous studies showed a decrease in CSF-Aβ42 to about 40% - 50% of control levels has been found in AD in several papers [26].

It is not clear why Aβ42 is reduced in AD patients, but it is thought that its decrease reflects trapping of Aβ42 in the amyloid plaques in the brain. Indeed, studies suggest that decreased CSF Aβ42 correlates well with the levels of amyloid plaques in the AD brain as determined by amyloid imaging [27]. Decreased CSF Aβ42 appears to be an early marker for AD and might predict conversion to AD when combined with CSF tau measures. Considering the diagnostic sensitivity and specificity levels of Aβ42 in CSF-AD patients that ranged between 80% and 90%, may enhance its use as potential test in combinations with other tests [28-30].

2.1.3. Total Tau Protein

While tau has long been implicated in neurodegenerative conditions, its functions in the adult brain and the precise mechanisms by which it contributes to neuronal dysfunction and degeneration in these disorders remain to be elucidated.

Physiologically, tau proteins are an intracellular microtubule-associated protein acting as stabilizers microtubules in the cell cytoskeleton, and pathologically, tau proteins characterize the main component relating to intraneuronal changes in AD patients.

A flurry of recent studies has challenged major dogmas in this field, including the vision that filamentous tau aggregates are the most pernicious forms of tau, that failure of tau function plays a major role in the pathogenesis of tauopathies. Provocative discoveries suggest that tau regulates neuronal excitability and that it is required for Aβ and other neurotoxins to cause neuronal deficits, aberrant network activity and cognitive decline [31].

Microtubule-associated protein tau becomes abnormally hyperphosphorylated in AD and accumulates as tangles of paired helical filaments in neurons undergoing degeneration. Because of alternative splicing of T-mRNA, there are six isoforms ranging in size from 352 to 441 amino acids, with molecular weights of approximately 50 - 65 kDa [32].

In normal situations, tau is coordinated by phosphorylation. In abnormal conditions, tau becomes hyperphosphorylated (phospho-tau) and accumulates as paired helical filaments that aggregate into masses inside the neurons as neurofibrillary tangles (NFT), which represent one of the hallmarks of AD [33].

The logic for considering tau as biomarker is the presence of abnormal intraneuronal aggregates of phospho-tau observed in many tauopathies, including AD. Tau aggregates can be examined in the Brain and peripheral fluids. Biochemical and Immunohistochemical properties of Tau cumulative in brain permit postmortem categorization and differential diagnosis of tauopathies [34]. The first report on CSF T-T as a biomarker for AD was published in 1993. In that paper, an enzyme-linked immunosorbent assay (ELISA) with a polyclonal reporter antibody was used [35].

Total tau concentrations especially phosphorylated tau (181,199,231) proteins can be measured in the CSF as Aβ, and show a good correlation with the diagnosis of AD [36].

Previous studies have established 300% increase in the concentration of total-Tau in CSF Alzheimer’s patients 70 years and older versus control subjects younger than 50 years [ (>600 pg/mL vs <200 pg/mL), respectively] [21]. A strong correlation between age and t-tau in healthy individuals has been determined with a cut off value of >500 pg/mL (>70 years) versus 450 pg/mL (<70 years) [37]. CSF t-tau levels in AD patients have a sensitivity of 90% and specificity of 81% compared to healthy
Deficits as Potential Biomarkers for AD

Alzheimer’s disease (AD) per se has complex pathophysiology, involving multiple biochemical pathways. The loss of neuronal cells leads to deficits in various neurotransmitters. Changes in the cholinergic, serotonergic, glutamatergic noradrenergic, dopaminergic, GABAergic and somatostatinergic neurons and the potential involvement of inflammatory, oxidative and hormonal pathways were investigated to determine their roles in AD [44].

Consequently, these pathways were carefully planned as potential targets for AD early detection, treatment and prevention strategies. In this manuscript we will spotlight on some important neurotransmitters involved in pathology of this devastating disease. Indeed, in the brain the cholinergic pathway represented by acetylcholine (Ach) which is a critical neurotransmitter responsible for information processing, memory; learning and attention. Decreasing levels of Ach synthesis are significantly correlated with increasing severity of dementia in patients with AD [45]. Cortical deficiencies Ach contribute to a loss of cholinergic neurons in the basal forebrain, reduced acetylcholine (Ach) levels, and a decrease in the synthesis of choline acetyltransferase, the enzyme that catalyzes the synthesis of Ach from choline and acetyl coenzyme-A by 35% to 50% in AD comparing to healthy subjects. Treatments such as, Acetylcholinesterase inhibitors (Donepezil Hydrochloride-Aricept), Tacrine which is a centrally acting anticholinesterase and indirectly acting as cholinergic agonist (parasympathomimetic), the reversible cholinesterase inhibitor (rivastigmine tartrate-Exelon) and Ach receptor agonists, including nicotine, have been used to treat AD. The slight success of these medications suggests that, in addition to Ach deficiency, other deficit neurotransmitters may play a role and may involved together with Ach reduction to cause the profound deteriorations in the cognitive impairment.

The glutamatergic pathway involvement in AD comes from its responsibility on different input and output pathways responsible for thinking and remembering, and is driven from the connection between the hippocampus...
and neocortex [46].

All such pathways activity depends on signaling mediated by the neurotransmitter glutamate. In intact brain glutamatergic cycles start in the hippocampus mitochondria neural cells, where the conversion of glutamine to glutamate takes place by the enzyme glutaminase and end on the packaging of the glutamate molecule into vesicles, glutamate released from vesicles into the synaptic cleft leading to increase free glutamate [47].

The interaction between glutamatergic receptor and postsynaptic neurons provokes transmission of neural signals, followed by quick synaptic glutamate levels restoration to normal concentrations, however, through the rapid uptake of unbound glutamate molecules by nearby glial cells, which subsequently convert these glutamate molecules to glutamine [48]. In AD patients’ interruption of gulamatergic cycle at the point of glial cell reuptake of free glutamine from the synapse is noticeable, and was confirmed by many studies who demonstrate decrease levels of glutamate reuptake in the cortex in addition to the reduction uptake by vesicular glutamate transported [49]. As a result of the diminution in vesicular glutamate uptake, fewer glutamate will be stored in each vesicle, then neurons are left with low neurotransmitter molecules to release into the synaptic cleft in times of neuronal activity, consequently, the neural impulse will attenuated, leading to inadequate neurotransmission [50].

According to this hypothesis, it seems that low clearance of free glutamate from the synapse leads to high accumulation and high concentrations under resting conditions leading to abnormalities on neuronal signaling and neural death. In fact, the glutamatergic hypothesis links cognitive decline in patients with Alzheimer’s to neuronal damage resulting from over activation of N-methyl-d-aspartate (NMDA) receptors by glutamate [51].

The sustained low-level activation of NMDA receptors, which are pivotal in learning and memory, may result from deficiencies in glutamate reuptake by astroglial cells in the synaptic cleft [51].

In 2003, the FDA approved a glutamate antagonist memantine (Namenda) for the treatment of moderate to severe AD [52].

The medication helps block the activity of the neurotransmitter glutamate by binding to N-methyl-D-aspartate (NMDA) receptors on the surface of brain cells. If glutamate levels are too low, cognitive problems develop. If levels are too high, glutamate over stimulates nerve cells, leading to cell death [53].

Serotonin (5-hydroxytryptamine or 5-HT) is synthesized from the amino acid tryptophan and considered a key neurotransmitter in the central and peripheral nervous systems [54].

Its pathways spread extensively throughout the brainstem, the cerebral cortex and the spinal cord, forming the principal serotonin centers in the caudal and rostral raphe nuclei [55].

It was observed that transmission of serotonin descending from the rostral raphe nuclei to the temporal lobe is decreased in AD patients, and there is an extensive degeneration of serotonergic neurons, with corresponding losses of the serotonin (5HT) transporter (5HTT), which is responsible for the reuptake of 5HT from the synaptic cleft. Studies indicates that allelic variation of the 5HTT gene promoter (5HTT gene-linked polymorphic region, 5HTTLPR) determines high or low 5HT uptake in normal human brain [56].

Because of the extensive serotonergic denervation that has been observed in the AD brain and the important role played by serotonin (5-HT) in executive functions control, this neurotransmitter system has become a focus of concerted research efforts to identify new treatments for AD [56,57].

3. Summary

Ones of the major novelties in the field of medical science are represented by biomarkers. Biomarkers are objectively and impartially measured and evaluated as an indicator of physiological processes, pathological processes or pharmacological responses to the applied therapy [58,59].

The scientific rationale and regulatory acceptance for biomarkers in medicine and in drug development has become more fully recognized, and biomarkers in medicine provide the platform for all players in this increasingly crucial area to converse and join forces to find confident biomarkers. Because of the complexity and pathological ramification of the disease, and after careful consideration to almost all medical literature who investigated AD biomarkers, we are convinced that joint action and cross-institutional standards must be employed in order to validate a novel biomarker.

Planned synergistic collaborations between academic institutions, pharmaceutical companies and supporting organizations for the establishment of standards and networks for the identification and qualification of biological marker candidates are urgently warranted.

Indeed, Interruption of the cascade of events that underling AD pathology by novel therapeutic strategies that guide to even small delays, or even reject the onset and progression of the AD would significantly decrease the global burden of the disease, and a blessing advancement in deceasing suffrecence of the patients.

Presently, Aβ1-42, t-tau and p-tau in CSF are considered the most consistent, sensitive and specific biomarkers for AD; and recently these biomarkers were found to be useful in differential dementia diagnosis. Patients with progressive supranuclear palsy had normal CSF biomarker values in 90%. Patients with Creutzfeldt-
Jakob disease demonstrated an extremely high CSF t-tau at a relatively normal CSF p-tau. CSF AD biomarker profile was seen in 47% of patients with dementia with Lewy bodies (DLB), 38% in corticobasal degeneration (CBD), and almost 30% in frontotemporal lobar degeneration (FTLD) and vascular dementia (VaD). Concordance between clinical and neuropathologic diagnosis was 85% and CSF markers reflected neuropathology in 94% [60]. However, CSF collection, despite its importance, is an invasive procedure and many researchers steer clear of it, and sensitive assays to detect pathological molecules in CSF are still unavailable [61-64].

Therefore, more attention was focus toward blood-based biomarkers; namely many biomarkers in blood depending on pathophysiological processes have been identified, such as amyloid plaque formation (Aβ), Aβ autoantibodies, platelet amyloid precursor protein (APP) isoforms, inflammation (cytokines), oxidative stress (vitamin E, isoprostanes), lipid metabolism (Apolipoprotein Epsilon-4 allele (ApoE-4), 24S-hydroxycholesterol), 3-nitrotyrosin, α-1-antichymotrypsin, C-reactive protein, Clq complementary systems, and vascular disease (homocysteine, lipoprotein) [65-68].

Previous studies found that plasma concentrations of Aβ do not correlate with those in CSF, and no consistent change in plasma Aβ over time in AD patients, and no cross-sectional Aβ differences between AD patients and controls was found that would allow plasma Aβ concentrations to be used as a diagnostic measure [68]. James D. Doecke et al., (2012) identify panel of plasma biomarkers that distinguish individuals with AD from cognitively healthy control with high sensitivity (85%) and specificity (93%) cross-validated accuracy measures, the revealed biomarkers panel that included markers significantly increased (cortisol, pancreatic polypeptide, insulin like growth factor binding protein 2, β3 microglobulin, vascular cell adhesion molecule 1, carcinoembryonic antigen, matrix metalloprotein 2, CD40, macrophage inflammatory protein 1α, superoxide dismutase, and homocysteine) and decreased (apolipoprotein E, epidermal growth factor receptor, hemoglobin, calcium, zinc, interleukin 17, and albumin) in AD [69]. Indeed [69], Plasma biomarker results confirm cerebrospinal fluid studies reporting increased levels of pancreatic polypeptide and N-terminal protein B-type brain natriuretic peptide in patients with AD and mild cognitive impairment. Incorporation of plasma biomarkers yielded high sensitivity with improved specificity, supporting their usefulness as a screening tool. The ApoE genotype was associated with a unique biochemical profile irrespective of diagnosis, highlighting the importance of genotype on blood protein profiles [70].

J. B. Toledo et al. (2013) mentioned that different inflammatory and metabolic pathways have been associated with AD. The authors recruited 818 subjects and studied multi-analyte panels using a large number of molecules that point to the affected pathways and also studied the relationship between a panel of plasma biomarkers and presence of AD-like brain atrophy patterns using MRI. Their results indicate that stress and insulin responses and cytokines associated with recruitment of inflammatory cells in Mild Cognitive Impairment-AD are associated with its characteristic AD-like brain atrophy pattern and correlate with clinical changes or CSF biomarkers [71].

Thus, a neuropathological diagnosis which leads to synchronization and correlation between CSF biomarkers and blood biomarkers levels may relatively reflect the pathological processes in the brain and increase the sensitivity, specificity and validity for putative biomarkers [72,73].

The most promising approach to diagnose AD is the combination of numerous biomarkers, and isolated tests should not be used alone. Unifying multiplex methodological techniques in one assay; using standardized tests for measuring peripheral proteins to avoid contradictory results; and combining panels of existing biomarkers pattern who reflect the whole spectrum of abnormal proteins deposited in the brain or surveying the range of proteins in plasma (proteomics) will show promise for discovering biomarker profiles that are characteristic of AD, yet distinct from non-demented patients or patients with other forms of dementia. Furthermore, these proteins might serve as interesting biomarker candidates for multiple biomarkers strategies [74].

We believe that we should consider the centenarian’s hypothesis, where 90% of all of the centenarians were still independently functioning at the average age of 93 years by searching for their protective genes and genetic markers. Recent studies found 281 genetic markers that are 61% accurate in predicting who 100 years old is. These markers point to at least 130 genes, many of which have been shown to play roles in chronic diseases including the basic biological mechanisms of aging. Therefore, centenarian’s tremendous survival advantage may in great part due to the existence of longevity associated genetic variants that counteract or restrain the onset and the progression or even escape age-related diseases like AD. Our driving hypothesis is that we can examine these genes and signature markers among AD patients to verify if they possess the protective genes or markers, and to use this model as a diagnostic test [75-77].

Finally, we believe that prior to search an accurate biomarker, we must be confident of the actual diagnostic guidelines for AD. Reevaluation of current criteria, changes in the mentality and the way that many clinicians diagnose AD are mandatory, because diagnosis of AD is still largely based on exclusion criteria of secon-
mary causes and other forms of dementia.

Despite the frustrations, enormous efforts have been made and others energetic efforts are ongoing to find adequate biomarkers characterized by high sensitivity and specificity for this complex neurodegenerative disorder. The future is full of surprises and simple answer to complex disease will be sooner.

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