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REVIEW ARTICLE

Cerebrospinal Fluid Biomarkers for Diagnosis of Alzheimer's Disease

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Abstract:

Alzheimer's disease is the most common cause of dementia among elderly people. The major pathological hallmarks of AD are the loss of neurons, occurrence of extracellular senile plaques as well as intracellular neurofibrillary tangles (NFT). Biochemical changes in the brain are reflected in the cerebrospinal fluid (CSF), and intense research efforts have been made to develop biomarkers for the central pathogenic processes in AD that can be used as diagnostic tools. Biomarkers are essential part of disease management as they are essential for diagnosis, monitoring the disease progression, detecting early onset of the disease, monitoring the effect of therapeutic intervention, and also avoiding false diagnosis of the disease. Unfortunately, none of the biomarkers presently available are able to accomplish the disease diagnosis single-handedly. Three CSF biomarkers, Aâ42, Total-tau (t-tau), and phosphorylated-tau (p-tau), have been found to have the highest diagnostic potential.

Keywords: *Dementia, Alzheimer's disease, CSF Biomarker, Tau protein, Amyloid beta.*

Introduction:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that causes dementia in approximately 10% of individuals older than 65 years [1]. It is estimated to afflict more than 27 million people worldwide[2]. AD accounts for at least 60% of all dementia diagnosed clinically. Alzheimer's disease is the most common cause of dementia among elderly people and will become a public health crisis within two to three decades if left untreated. Diagnosing AD and distinguishing it from other dementias depends primarily on clinical evaluation, and, ultimately, on investigator judgment[3]. There is also no definite clinical method to determine which patients with mild cognitive impairment (MCI) will develop AD. The major pathological hallmarks of AD are the loss of neurons, occurrence of extracellular senile plaques as well as intracellular neurofibrillary tangles (NFT) . Senile plaques are primarily composed of amyloid *â*-protein (A*â*), which is produced from the amyloid precursor protein (APP) by sequential proteolytic cleavages made by two proteolytic enzymes, *â*-secretase (*â*-site APP-cleaving enzyme; BACE) and *ã*-secretase (Figure 1)[2]. A definitive diagnosis of AD can only be made after death, when autopsy can reveal these senile plaques and neurofibrillary tangles in brain tissue. It is well known that the pathological processes in the brains of AD patients start more than a decade before the first symptoms are noticed. The temporal dynamics of biomarker levels in relation to changes in cognition have been described in a hypothetical model on the continuum of AD[4]. In line with this, the revised diagnostic guidelines identify three different stages of AD: preclinical AD, mild cognitive impairment (MCI) due to AD and AD with dementia. The main focus of this review is to provide insights on the various potential biomarkers with particular emphasis on CSF biomarkers characteristics of these three stages for AD diagnosis.

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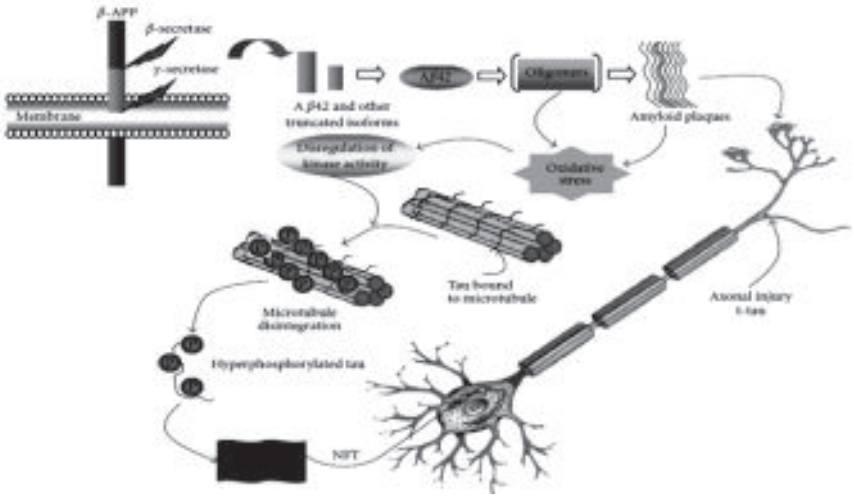

Fig.-1: *Pathological cascades and potential biomarkers of AD.*

Importance of diagnostic markers for AD and MCI:

The introduction of acetylcholine esterase (AChE) inhibitors as symptomatic treatment has highlighted the in the population of the availability of drug treatment has also made patients seek medical advice at an earlier stage of the disease, making the percentage of MCI cases at dementia clinics increase. This has increased the diagnostic challenge for physicians, because the characteristic clinical picture of AD with slowly progressive memory disturbances combined with parietal lobe symptoms has not yet developed in MCI cases. Accordingly, there is no clinical method to determine which MCI cases will progress to AD with dementia except for a very long clinical follow-up period. Thus, new diagnostic tools to aid the diagnosis of early AD and to identify incipient AD in MCI cases would be of great importance[5].

Biomarkers:

The biomarkers are the entities whose concentration, presence, and activity are associated with disease. Biomarkers are essential part of disease treatments as they are essential for diagnosis, monitoring the disease progression, detecting early onset of the disease, monitoring the effect of therapeutic intervention, and also avoiding false diagnosis of the disease[6]. An ideal biomarker (1) should be highly specific, (2) should predict the course of illness accurately, and (3) should reflect the degree of response to treatment[7]. The biomarker research for AD has significantly advanced in recent years (Table-1). The neuroimaging techniques like CT, PET, PIB-PET, MRI assess the regional structure and function of the brain, as well as assist in identifying the biochemical profile of brain dysfunction[8-10].

The body fluids such as cerebrospinal fluid (CSF), plasma, and urine are considered as important sources for the AD biomarker development. Plasma biomarkers like α_{2-} Macroglobulin, Complement factor H, βâ 42 are noninvasive but less sensitive and specific tests for AD diagnosis[11-13]. But CSF is considered a better source for biomarker development as it is in direct contact with the extracellular space of the brain and can reflect biochemical changes that occur inside the brain. Thus far, three CSF biomarkers, Aβ42, total-tau (t-tau), and phosphorylated-tau (p-tau), have been found to have the highest diagnostic potential[10, 14]. Biomarkers of inflammation and oxidative stress and urine-based biomarkers are among the other sources that provide vital information on development and progression of AD. Unfortunately, none of the biomarkers presently available are able to accomplish the disease diagnosis single-handedly. Monitoring more than one biomarker at the same time is suggested to be suitable for detecting the disease progression.

Table-I

Some promising biomarkers in diagnosis of AD.

Category	Markers	Advantages	Limitations
Imaging	CT, PET, PIB-PET, MRI	(1)Noninvasive (2) Provides structural and functional details of brain immediately (3) Can reveal disease progression	(1) Expensive (2) Requires experienced personal (3) Sensitivity and specificity not satisfactory
Plasma	α_{2}- Macroglobulin, Cmplement factor H, Aβ 42	(1) Noninvasive (2) Samples are easily accessible	(1) Less correlation to AD (2) Less sensitive and specific for AD diagnosis
CSF	Aβ 42, t-tau,p-tau, p-tau/ Aβ 42, t-tau/ Aβ 42	(1) Can correlate AD directly (2) Highly sensitive and specific (3) Can detect AD progression	(1) Invasive , sample to be collected by LP (2) Irreproducible diagnosis due to sample storage and transportation

CSF Biomarker:

Biochemical changes in the brain are reflected in the cerebrospinal fluid (CSF), and intense research efforts have been made to develop biomarkers for the central pathogenic processes in AD that can be used as diagnostic tools. Early studies indicated that CSF biomarkers could be useful for defining a subgroup of patients with MCI at especially high risk of developing AD[15]. The best studied fluid proteins in AD have been CSF levels of Aβ42, the primary constituent of amyloid plaques, and tau protein, the primary component of neurofibrillary tangles. Levels of CSF Aβ42 are typically reduced in AD[16] reflecting its aggregation and deposition as amyloid in the brain , whereas levels of CSF tau and phosphorylated tau (p-tau) species are increased in AD[17]and are hypothesized to reflect the presence of neurofibrillary tangles, neurodegeneration, or both although not all studies support this conclusion[18]. Indeed, there is no proof that plaque formation is reason for Aβ42 concentration decrease, only correlation. A tau protein/ Aβ42 index, usually increased in AD patients, has high sensitivity, high specificity and also high negative predictive value in AD diagnosis.

CSF Amyloid-β

Amyloid-β (Aβ) is a secreted peptide of unknown physiological function that is cleaved from the amyloid precursor protein (APP) by the sequential activities of β-secretase and β-secretase enzymes. The majority of Aβ is produced in the brain and secreted into the brain extracellular space. Some fraction of CNS-produced Aâ diffuses into the CSF, appearing in modest concentrations (~10–15 ng/ml). Aβ occurs in multiple forms, including those ranging from 37 to 43 amino acids in length (Fig.2). Among these, Aâ40 is the most abundant species, but Aβ42 seems to be essential for initiating amyloid-â aggregation and is considered central to the amyloid cascade hypothesis of AD[19]. Of these two species, Aâ42 has emerged as a useful biomarker for AD.

CSF Tau

Tau is a cytosolic protein predominantly expressed in neurons, wherein its primary function seems to be regulation of microtubule stability within the axon. The tau proteins are the product of alternative splicing from a single gene that in humans is designated *MAPT* (microtubule-associated protein tau) and is located on chromosome 17[20]. They were discovered in 1975 in Marc Kirschner's laboratory at Princeton University[21]. This function is regulated by several different post-translational modifications, principally phosphorylation of numerous serine and threonine residues. In AD, hyperphosphorylated tau often fills the dystrophic neurites of neuritic plaques, and is the principal component of the paired helical

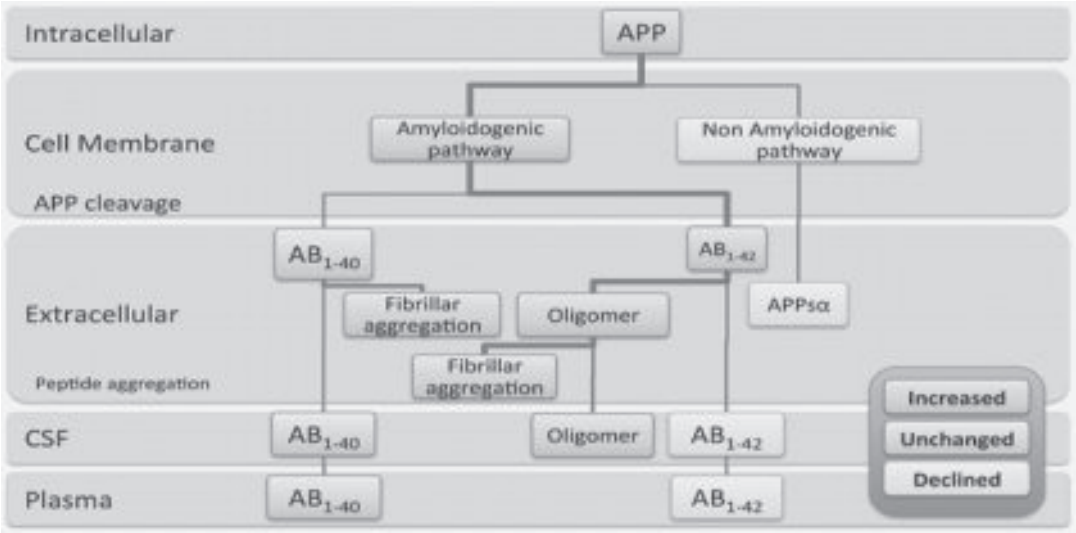

Fig.-2: *Amyloid precursor protein metabolism and biomarker of amyloid pathology.*

filaments that constitute NFTs that are present in neuronal cell bodies. The precise forms of tau that appear in the CSF, and the mechanism or mechanisms by which they get there, are not entirely understood, but recent studies demonstrate that virtually all domains of the protein are represented, and it is widely assumed (but not proven) that the major sources of increases in tau and phosphorylated tau (Fig. 3) in the CSF in AD are either due to synaptic/neuronal injury, cell death, or possibly neurofibrillary tangles.

Total (T)-Tau

Tau is the major protein component of intra-neuronal NFT and is elevated in the CSF in most patients with AD. In addition to the presence of tau in neurofibrillary tangles, it has been shown that tau levels in CSF can increase rapidly as a result of neuronal injury, and therefore, may indicate the severity of the underlying neurodegeneration. Over 50 studies have demonstrated an increase in the concentration of total tau (t-tau) by approximately 2–3 fold in AD compared with non-demented elderly subjects .Elevation of CSF tau differentiates AD from non-demented, age-matched elderly with a sensitivity and specificity of ~90%[22]. As mentioned previously, tau elevation seems to occur at the early symptomatic stages of disease (MCI/ very mild dementia) and in some cognitively normal individuals, where its levels correlate with the amount of amyloid

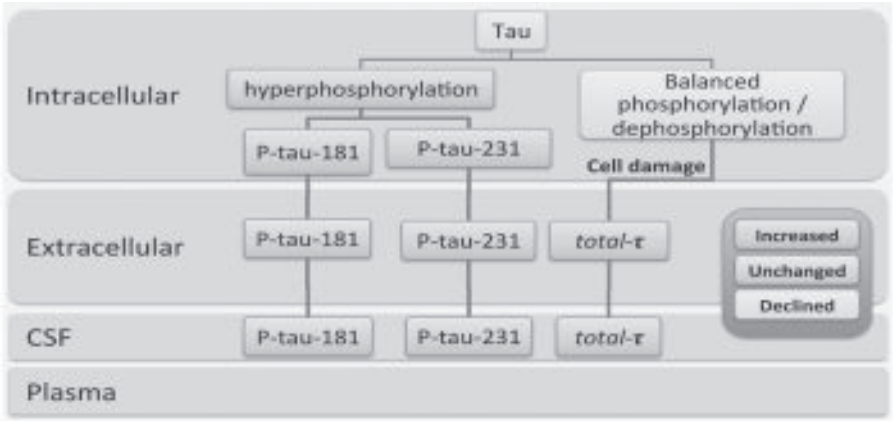

Fig.-3: *Biomarkers of tau pathology*

deposition and together with Aβ42 predict cognitive decline (see below). Cognitively normal individuals with evidence of amyloid deposition and increased tau are likely to have preclinical AD (see below). However, it is important to consider that tau elevation can be seen in other neurodegenerative diseases, potentially limiting the utility of tau alone in the differential diagnosis of AD .Tau, as a marker of neuronal injury, can be transiently increased after any acute brain injury (such as stroke or trauma)[23]. Moreover, tau levels seem to remain relatively stable throughout the clinically symptomatic period of AD and do not correlate well with dementia severity. Age might affect the CSF levels of tau; however, studies have been conflicting regarding the direction and significance of such an effect.

Phosphorylated (P)-tau

Abnormal tau phosphorylation is present in neurofibrillary tangles and has been investigated as a marker of AD pathology. As many as 30 different phosphorylation sites of p-tau have been identified[24], and ELISAs (enzyme-linked immunoassays) have been developed for at least 5 of them. Studies examining the utility of different forms of p-tau in the early diagnosis of AD, and in the differentiation from other causes of dementia, have consistently shown that p-tau 181[25], p-tau 231-235, or p-tau 396-404 offer at least equivalent diagnostic utility for AD as compared to total tau. Studies comparing the diagnostic performance of different phosphorylation sites (p-181, p-199, and p-231) suggest that all three assays are equally effective in differentiating AD from non-demented controls. P-tau 231 may provide diagnostic specificity for AD and may improve the differentiation between AD and FTD, while there is some evidence that p-tau 181 improves the differentiation between AD and DLB. P-tau 396-404, and the ratio of p-tau 396-404/t-tau, but not tau alone, has been shown in one study to differentiate AD from vascular dementia. In contrast to t-tau, p-tau does not appear to be increased secondary to acute brain injury, further adding to its diagnostic specificity.

Combination of Aâ42 and tau

Based on current data, the use of CSF Aβ42 alone but especially together with t-tau or ptau181 is very useful in both diagnosis and prognosis of individuals with MCI/very mild dementia and also in predicting progression from cognitive normalcy to MCI/very mild dementia. This is likely due to the fact that the levels of the markers together can identify two aspects of AD pathology, plaques (Aβ42) and tauopathy / neurodegeneration (tau).

Use of AD Biomarkers in CSF in Clinical Practice

The diagnostic performance of AD biomarkers has been evaluated in clinical practice in two studies[26, 27]. In these studies, the CSF markers have been evaluated on prospective patient samples from clinical practice, and ELISA assays have been run each week in clinical neurochemical routine. The diagnostic performance of CSF T-tau[26] and the combination of CSF T-tau and Aβ42[27] has been similar to that found in other studies, with a high ability to differentiate AD from normal aging, depression, and PD, but lower specificity against other dementias like VAD and LBD. A summary of the diagnostic performance of T-tau, P-tau, and Aβ42 is given in Table 2. Details on the performance of these CSF markers in the separation between AD and nondemented aged individuals have been published previously.

The diagnostic performance of the CSF markers seems to be highest in the differentiation between AD and several important differential diagnoses, including normal aging, depression, alcohol dementia, and PD (Table 2). Another useful clinical application is the identification of CJD in cases with rapidly progressive dementia, in which the combination of very marked increased CSF T-tau and normal or mildly increased P-tau has high diagnostic value. Lastly, these CSF markers may be useful in identifying mixed AD/VAD dementia.

Candidate biomarkers

The search for new AD biomarkers may be facilitated by the core CSF AD biomarkers. By including patients with signs of an AD pathological process and ensuring that control subjects lack this profile, future biomarker studies may be more successful.

BACE1

For Aβ to be produced, the amyloid precursor protein (APP) is cut by two different enzymes, â-secretase

Table-II

Summary of the Specificity of CSF Markers for Alzheimer's Disease

Disorder	Total Tau	Phospho-Tau	Aβ42
Alzheimer's disease	Moderate to marked increase	Moderate to marked increase	Moderate to marked decrease
Normal aging	Normal	Normal	Normal
Depression	Normal	Normal	Normal
Alcohol dementia	Normal	Normal	Normal
Parkinson's disease	Normal	Normal	Normal
Creutzfeldt-Jakob disease	Very marked increase	Normal, but some cases with mild increase	Normal to marked decrease
Frontotemporal dementia	Normal to mild increase	Normal	Mild to moderate decrease
Lewy body dementia	Normal to mild increase	Normal to mild increase	Moderate decrease
Vascular dementia	Inconsistent data, some studies with normal and some with increased levels	Normal	Mild to moderate decrease
Acute stroke	Mild to very marked increase, depending on the infarct size	Normal	Normal
Non-acute CVD without dementia	Normal	N.E.	Normal

CVD = cerebrovascular disease; N.E. = not examined.

and â-secretase. The major â-secretase in the brain is called β-site APP cleaving enzyme-1 (BACE1). Several studies have investigated the levels of CSF BACE1 activity in patients with MCI and AD, but the results are not univocal.

Ubiquitin

Ubiquitin is a small (8.7 kDa) protein involved in the ATP-dependent degradation of proteins, in which it is covalently conjugated to lysine residues of target proteins, for which it serves as a signal for degradation of the protein by proteases.

NF proteins

NF proteins are structural components in the neuronal axons that are important for axonal structure and transport. Neurofilaments are composed of three subunits based on the molecular weight, termed high (NF-H), medium (NF-M), and light (NF-L).

GAP43 (neuromodulin)

GAP43, or neuromodulin, is a protein localized in the presynaptic terminals and axons of cortical neurons. In AD brain, GAP43 is found in the dystrophic neuritis in plaques. GAP43 protein levels are decreased in the frontal cortex and the hippocampus in AD.

NTP and AD7c protein

NTP was identified a brain protein cross-reacting with antibodies against pancreatic thread protein (PTP). Both brain NTP immunoreactivity and mRNA levels are increased in AD.

Limitations:

Although CSF biomarkers have proved to be highly informative, sensitive, and specific for detection of clinical AD and early stage of AD, their regular use in clinic is still limited. One of the major reasons against the vast applicability of CSF in AD diagnosis is lumbar puncture, an invasive method to collect the CSF sample. However, the technique of lumbar puncture has considerably improved and, as a consequence, the incidence of headache after lumbar puncture in elderly patients is 2% or less. Other issues including inconsistency of data analysis of CSF sample due to sample collection, transportation, storage, and high expense of the test might limit the use of CSF for routine diagnosis. The major and inherent limitation of fluid biomarkers is the lack of anatomical precision in the measurements. Another limitation is the lack of assay standardization. Different assays for, e.g., Aâ42 correlate but give different absolute concentrations of the protein. This prevents from the use of global reference limits and diagnostic cut-points.

Conclusion:

The potential uses for AD biomarkers are many. Besides from diagnostics, CSF biomarkers may be utilized for prognosis, assessing disease

progression, developing treatments, monitoring treatment effects and studying disease mechanisms. Intense research has led to the development of CSF biomarkers reflecting different aspects of AD pathogenesis. Currently, validated and reliable biomarkers exist for amyloid pathology and axonal degeneration. Measuring CSF Aβ42, t-tau and p-tau alone or in combination may be especially useful for the selection of pre-symptomatic individuals with known MCI/ pre-clinical AD pathology and for enrollment in prevention trials of disease modifying therapies. Apart from the core biomarkers, there are several candidate CSF and blood biomarkers in the pipeline, but they need verification in further studies.

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