Cerebrospinal Fluid Biomarkers for Understanding Multiple Aspects of Alzheimer's Disease Pathogenesis

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Abstract

Alzheimer's disease (AD) is a multifactorial age-related brain disease. Numerous pathological events run forth in the brain leading to AD. There is an initial long, dormant phase before the clinical symptoms become evident. There is a need to diagnose the disease at the preclinical stage since therapeutic interventions are most likely to be effective if initiated early. Undoubtedly, the core cerebrospinal fluid (CSF) biomarkers have a good diagnostic accuracy and have been used in clinical trials as end point measures. However, looking into the multifactorial nature of AD and the overlapping pathology with other forms of dementia, it is important to integrate the core CSF biomarkers with a broader panel of other biomarkers. These include proteins reflecting different aspects of pathology such as neurodegeneration, neuroinflammation and synaptic dysfunction. The combination of biomarkers would not only aid in preclinical diagnosis, but would also help in identifying early brain changes during the onset of disease. Successful treatment strategies can be devised by understanding the contribution of these markers in different aspects of disease pathogenesis.

Keywords: Biomarkers, Alzheimer's disease, Cerebrospinal fluid, Diagnosis, Pathogenesis

1 INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease whose pathology starts decades before the clinical symptoms appear (Price & Morris, 1999). The preclinical stage represents a dormant phase where neuropathological changes are accumulating but the person has normal cognition (Sperling *et al.*, 2011a). Numerous biochemical pathways have been described to explain the pathogenesis of AD. Starting with the identification of amyloid beta (Aβ) in 1985, as the main component of amyloid plaques (Masters *et al.*, 1985), our understanding of Aβ and amyloid precursor protein (APP) metabolism, and tau pathology (neurofibrillary tangles and neuropil threads) has improved with time. Thorough research has been carried out to understand other aspects of AD pathogenesis and thereafter, numerous hypotheses have been put forth. AD may therefore be considered a result of a number of pathological changes in the brain, such as amyloidosis, neurodegeneration, inflammation, synaptic dysfunction, disruption of neuronal signaling and neuronal membranes, oxidative stress and mitochondrial dysfunction (REF). These changes direct the trajectory of preclinical AD to AD dementia, and make AD a multifaceted disease.

Several AD drug trials have failed, probably because the treatments are initiated at an advanced stage where damage is too severe, and the drug is not able to demonstrate a clinical benefit because the brain is too compromised to benefit from a treatment (Morris & Selkoe, 2011; Sperling *et al.*, 2011b; Salloway *et al.*, 2014). It is imperative to initiate an early treatment, and ensure that the correct patient population is included in the clinical trials.

Therefore, there is an urgent need to diagnose AD and initiate treatment at the preclinical stage, so as to obtain a clinical benefit. The first step in devising successful treatment strategies is to identify biomarkers for accurate diagnosis of AD, and thereafter develop therapeutic strategies. It is essential to find an ideal biomarker that should also help in monitoring the mechanism of action and the biochemical effects of the treatment drug (Blennow, 2010). As per the regulatory bodies such as Food and Drug Administration (FDA) and European Medicine Agency (EMA), exploration and validation of a biomarker should be integrated with drug development in order to accelerate the journey towards development of an effective therapeutic intervention (Arneric *et al.*, 2016). Clinical trials that aim at evaluating the effectiveness of therapeutic strategies can come up with reliable results when the therapeutic effect of these agents is monitored using markers that reflect over the molecular changes of the disease. As far as AD is concerned the promising markers in this context are the cerebrospinal fluid (CSF) markers (Blennow, 2010). The CSF biomarkers are the potential candidates to facilitate early diagnosis of AD because the AD pathological hallmarks start decades before the appearance of cognitive symptoms (Ferreira *et al.*, 2014). The core CSF biomarkers (CSF Aβ-42, T-tau (Total tau) and P-tau (Phosphorylated tau)), have been extensively studied and validated in relation to AD pathology, conversion and progression. There is a further need to explore and evaluate additional CSF biomarkers, which can aid in early and accurate diagnosis of AD, as well as in monitoring the downstream effects of a therapeutic intervention. As seen from the high failure rate of AD drug trials, it is extremely essential to explore additional CSF biomarkers which reflect on individual pathologies, meet the regulatory qualification and can help to enrich the clinical trial populations.

2 THE CSF BIOMARKERS AS A PART OF AD DIAGNOSTIC CRITERIA

The biomarkers of AD have been divided into two main categories; the biomarkers of Aβ accumulation and the biomarkers of neuronal degeneration or injury (Jack *et al.*, 2011b). The biomarkers of Aβ accumulation include abnormal tracer retention on amyloid positron emission tomography (PET) imaging and CSF Aβ-42. The biomarkers of neuronal degeneration include CSF T-tau and P-tau, 18F-2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) and brain atrophy via magnetic resonance imaging (MRI). The brain imaging techniques have been successfully used as end points in clinical trials (Hampel *et al.*, 2010). However, the limited accessibility, lack of molecular specificity, exposure to radioactivity and cost factor involved in neuroimaging markers particularly Aβ imaging, restricts their use in routine analysis (Johnson *et al.*, 2012). Therefore, the CSF is being extensively studied worldwide, in AD biomarker research. The CSF is in direct contact with the brain and the biochemical changes occurring in the brain are reflected in it (Fishman, 1992). The CSF biomarkers have a causal relation to AD pathology and may provide an insight into the different aspects of AD pathogenesis. The core CSF biomarkers (decreased CSF Aβ-42 and elevated T-tau and P-tau), have shown a high specificity and sensitivity for AD diagnosis. CSF Aβ-42 correlates well with Aβ pathology (PMID: 29902934), whilst the correlation of the tau markers with pathology is less clear; recent data indicate that CSF T-tau and P-tau may be markers of a neuronal reaction to Aβ pathology, which with time will translate into full-blown pathology (neurodegeneration and tangle pathology) (ref: PMID: 29772204). In any case, these markers are quite specific for identifying an individual with preclinical AD (Dubois *et al.*, 2014).

In the recent years, with the advances in our understanding of AD pathophysiology, it has become evident that the relation between clinical symptoms and disease pathology varies and the cognitive impairment evolves gradually. As a result, in 2011 the National Institute on Aging (NIA) and the Alzheimer's Association (AA) revised the diagnostic and research criteria

for AD and included the CSF biomarkers in addition to the imaging markers (Jack *et al.*, 2011a). In 2014, the International Working Group (IWG) reanalysed the pathological and topographical biomarkers of AD. Diagnostic changes were proposed for typical, atypical, mixed and preclinical AD. According to this, the pathological markers such as decreased CSF Aβ-42 and elevated T-tau and P-tau were considered as specific makers of disease pathology (Dubois *et al.*, 2014).

3 THE NEED OF ADDITIONAL CSF BIOMARKERS

The research on core CSF biomarkers (CSF Aβ-42, T-tau and P-tau) began nearly two decades ago. Reduced CSF Aβ and elevated T-tau and P-tau were found in the CSF of AD patients. (Blennow *et al.*, 1995; Motter *et al.*, 1995). This created a pathway for further research to look over into the diagnostic potential of these biomarkers, which reflect upon brain amyloidosis and neurodegeneration. Today, these biomarkers are extensively used in diagnosis and clinical trials. These core CSF biomarkers, exhibit a typical AD signature (high concentration of T-tau, P-tau, and low Aβ-42)). They have a high enough diagnostic accuracy and reflect upon the neuropathological hallmarks of AD; the neurofibrillary tangles and amyloid plaques (Blennow & Hampel, 2003). Additional biomarkers are still needed to complement the core biomarkers for early diagnosis and prognosis of AD and get a better insight into the different pathogenic pathways associated with the AD.

The core CSF biomarkers are relatively stable in clinical AD and therefore, do not serve as good markers in studying disease progression (Perrin *et al.*, 2009; Zhou *et al.*, 2009). The CSF Aβ-42 is sharply reduced in in the preclinical phase of AD while the levels are found to be constant in the subsequent phases (Jack *et al.*, 2010). The altered levels of these core markers do not predict the rate of cognition decline as they do not correlate with the Mini Mental Status Examination (MMSE) (Kester *et al.*, 2009). In another multi-centre longitudinal study it was

found that, there was lack of association between the changes in CSF biomarkers and the rate of change or decline in cognition over a period of four years (Toledo *et al.*, 2013). Also, these markers do not perform well enough in differentiating AD from other forms of dementia due to partially coinciding pathologies (Blennow *et al.*, 2012). The therapeutic strategies that aim at reducing amyloid load have failed to show a clinical benefit in spite of clearing Aβ (Holmes *et al.*, 2008). The inter laboratory and inter assay variation in the measures of core CSF biomarkers necessitates the need of additional CSF biomarkers (Verwey *et al.*, 2009). Studies have shown that that the reduced levels of CSF Aβ negatively correlate with the brain amyloid load (Fagan *et al.*, 2006; Fagan *et al.*, 2009). However, this association does not match with the clinical diagnosis of AD. The discordance has been found mainly in the cognitively normal participants, which have reduced CSF Aβ but are amyloid negative as seen by PET. Therefore, CSF Aβ levels are altered in the preclinical stage (Fagan *et al.*, 2006; Leuzy *et al.*, 2015; Mattsson *et al.*, 2015; Palmqvist *et al.*, 2016). This has led to the contamination of cohort groups due to the inclusion of CSF Aβ positives in the control group. This necessitates the need for exploration and evaluation of additional or novel biomarkers that aid in accurate diagnosis, correlate with cognitive function, but also help in better understanding the disease progression and different aspects of AD pathology.

AD is a multifaceted disease, and AD dementia is a result of a number of pathological changes in the brain (Figure 1). Numerous proteins or other biomolecules play significant roles in these pathological pathways. A reduction or elevation of their levels in the CSF is associated with the pathological change. Elevation or reduction of their levels can directly highlight upon the extent of damage or can occur as a protective response against the damage. CSF biomarkers directing different AD associated pathologies can serve as targets for therapeutic agents aimed to combat the same, and a detailed understanding of disease pathogenesis at molecular level can help in designing new efficacious chemical entities for treatment. In context of a clinical

trial a biomarker can serve as a surrogate end point. The time consuming end points associated with the ongoing trails in AD can be reduced with the application of additional makers (Hsueh *et al.*, 2013; Counts *et al.*, 2016). An early diagnosis aided through CSF biomarkers would ensure cohort uniformity through recruitment of correct patient population. This would help in improving clinical trial design and interpretation (Fagan & Perrin, 2012). The clinical stages of AD are well defined and understood, but it is important to identify and understand the different pathophysiological stages of AD. CSF biomarkers associated with different pathologies would help in understanding and identifying these stages. In order to bring advancement in the field of AD biomarker and therapeutic research it is of utmost important that new biomarkers in relation to AD pathogenesis be explored in the CSF and their potential to diagnose AD at preclinical stage be evaluated in well-established cohorts.

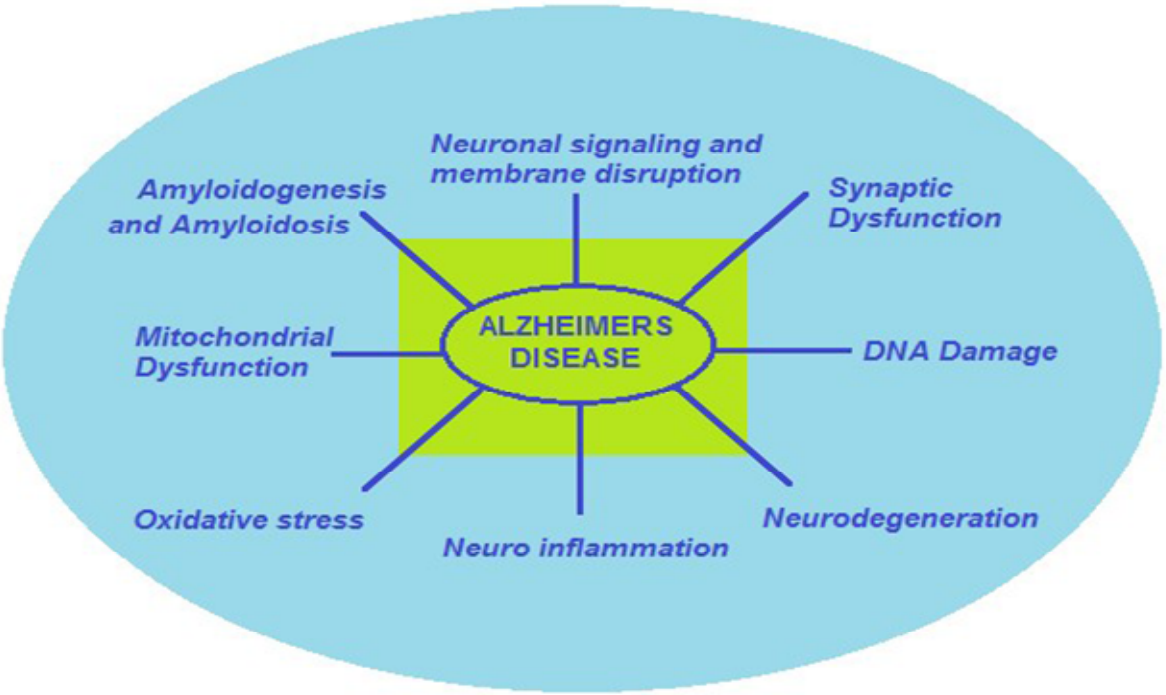

FIGURE 1: Alzheimer's disease: A multifaceted disease

This review highlights upon the various CSF biomarkers that reflect upon different aspects of multifaceted AD, and also highlights upon the different studies conducted on these

biomarkers in the past. Each biomarker helps to track different pathological events. An assessment of the levels of these markers in CSF might reveal an independent information or might unfold the association between individual pathologies. Altogether, the CSF measure of the biomarkers that relate to individual AD pathologies such as brain amyloidosis, neurodegeneration, synaptic dysfunction and neuroinflammation can help in better understanding the disease pathogenesis, accurate diagnosis and prognosis and thereby, help in devising effective treatment strategies (Figure 2).

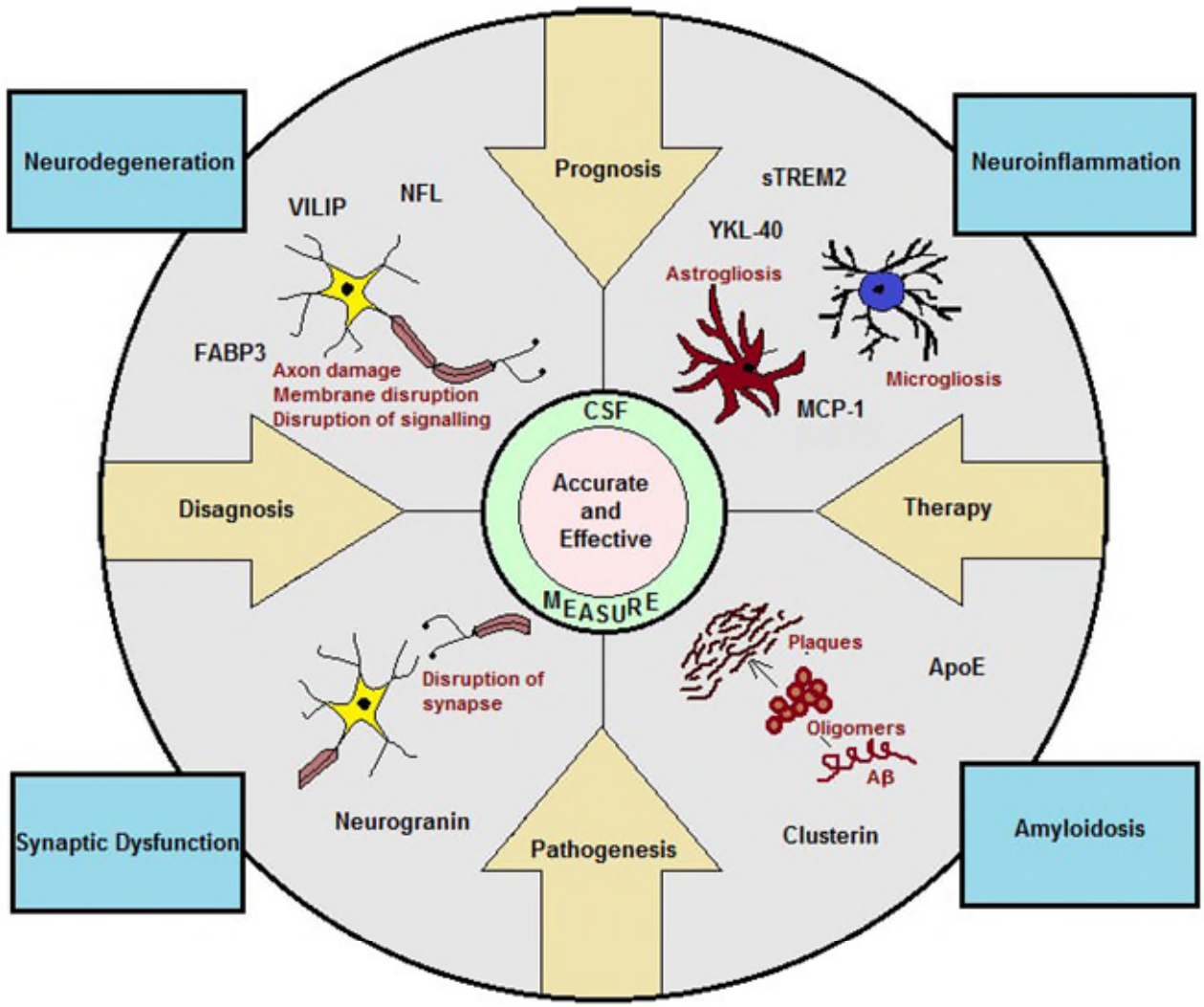

FIGURE 2: CSF biomarkers for AD diagnosis and understanding different aspects of pathology.

4 THE BIOMARKERS OF AMYLOIDOGENESIS AND BRAIN AMYLOIDOSIS

4.1 APOLIPOPROTEIN E (APOE)

4.1.1 ROLE IN AD PATHOGENESIS

ApoE is a glycoprotein, which is highly expressed in the liver and the Central Nervous System (CNS) (Mahley, 1988). In the CNS it is mainly expressed by astrocytes and to some extent by the microglia (Pitas *et al.,* 1987; Stone *et al.,* 1997). It is a constituent of lipoproteins, and in the CNS it is mainly confined to the HDL (high density lipoproteins) (DeMattos *et al.,* 2001). In the brain, apoE plays a vital role in regulating cholesterol metabolism and transport (Leduc *et al.,* 2010). ApoE plays a significant role in AD pathogenesis by affecting amyloid and tau pathology, and the isoforms have a differential role in pathogenesis (Figure 3). In response to neuronal injury, the expression of apoE is upregulated (Poirier *et al.,* 1991). The three isoforms of apoE (E2, E3, E4), differentially affect cholesterol transport, metabolism and synaptic plasticity, repair and neurite growth. The E4 isoform, is least effective in regulating cholesterol transport, efflux and synaptic plasticity (Michikawa *et al.,* 2000; Gong *et al.,* 2002).

ApoE mediates the cellular uptake and degradation of Aβ . Lipidated apoE binds to Aβ, and thereby influences plaque formation and Aβ transport (Tokuda *et al.,* 2000). The cellular uptake of Aβ is facilitated via apoE, through the endocytosis Aβ lipoprotein complexes. Due to the differential affinity of different isoforms with Aβ, the endocytosis is isoform dependent (Holtzman *et al.,* 2012). ApoE also modulates the accumulation and clearance of Aβ, in an isoform dependent manner. The E4 isoform modulates least clearance of Aβ, and also promotes a greater fibrilization of Aβ (Ma *et al.,* 1994; Castellano *et al.,* 2011). Neurodegeneration in AD is also influenced by apoE. ApoE affects neuroinflammation, and tau-mediated neurodegeneration. ApoE4 exacerbates neuronal death and modulates microglial activation (Shi *et al.,* 2017), and overexpression of apoE4 results in tau hyperphosphorylation (Tesseur *et*

al., 2000). Recent data also suggest intriguing interactions between apoE isoforms and the activation state of disease-associated microglia, which may be part of the disease-promoting effect of apoE4 (PMID: 30140051).

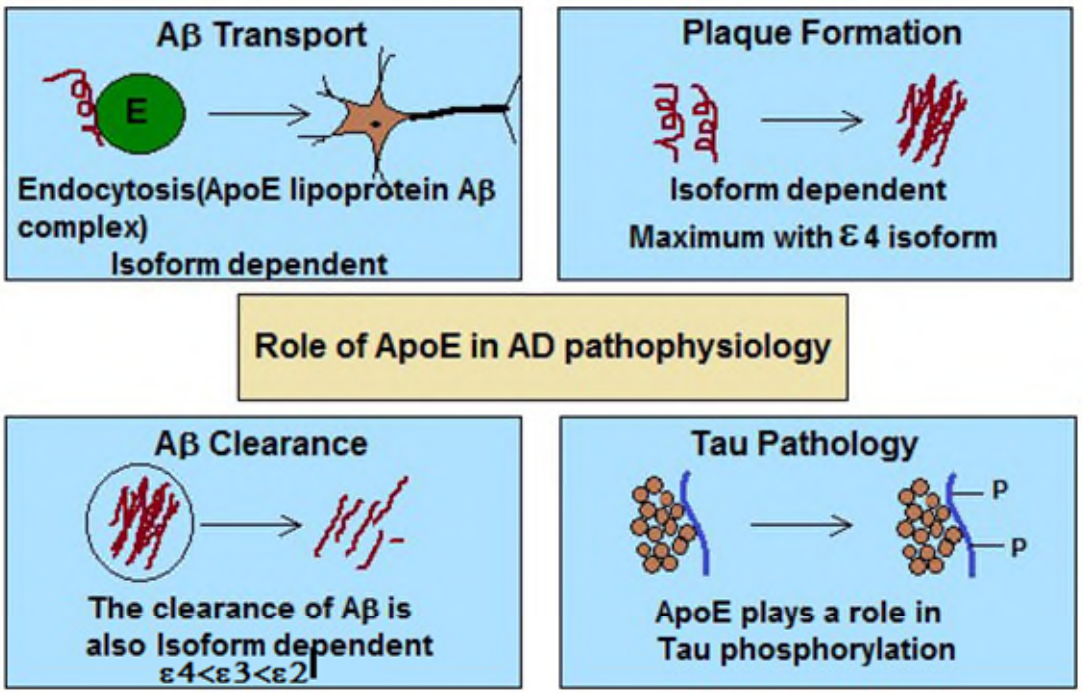

FIGURE 3: Role of apoE in the pathophysiology of AD. AD: Alzheimer's disease; ApoE: Apolipoprotein E; Aβ: Amyloid beta

4.1.2 CSF BIOMARKER STUDIES PERTAINING TO APOE

ApoE is a major apolipoprotein found in the CSF (Roheim *et al.*, 1979). Numerous studies have evaluated the levels of apoE in the CSF, so as to establish it as a potential marker (Table 1). To evaluate the CSF levels researches have used methods such as enzyme linked immuno sorbent assay (ELISA), mass spectrometry, multiplex assays and flow cytometry. Studies on CSF levels of apoE in AD show inconstant results, with either decreased (REF) or elevated (Lindh *et al.*, 1997; Merched *et al.*, 1997; Fukuyama *et al.*, 2000) levels as compared to controls. As per some studies *APOE* genotype may influence CSF ApoE levels. Higher CSF levels of apoE have been reported in individuals having *APOE* ε4 alleles (Hesse *et al.*, 2000).

Strong positive correlations have been found between CSF apoE levels and CSF tau in AD patients as compared to controls (Lindh *et al.*, 1997). The correlation between CSF apoE levels and CSF tau are also *APOE* genotype dependent (Martinez-Morillo *et al.*, 2014). The correlation between the two markers suggest that altered apoE levels in CSF could be attributed to the neurodegeneration in AD or vice versa. ApoE binds to protein tau in an isoform dependent manner (Strittmatter *et al.*, 1994). The association of apoE CSF levels with genotype, and genotype dependent correlation between ApoE and CSF Tau, suggest that neurodegeneration is isoform influenced.

Thus, quantification of apoE levels can highlight upon state of amyloid and tau pathology in AD brains. Owing to the a significant role of apoE in AD pathogenesis, further studies should be conducted in well-established cohorts in order to establish apoE as a potential CSF diagnostic and theragnostic biomarker. There have been inconsistencies with regard to CSF apoE levels. However, these inconsistencies could be attributed to a number of factors such as sample variability, variability in method or technique of analysis or unequal gender distribution in study groups.

TABLE 1 Studies conducted to evaluate the role of apoE as a potential CSF biomarker

Study	***Study groups***	***CSF levels in AD/study groups***	***Association with APOE genotype/core markers***	***Analysis method***
(Rezeli et al., 2015)	AD and non-AD	No significant difference between study groups	ApoE levels in CSF were not associated with APOE genotype; CSF levels positively correlated with P-tau in ε4 non carriers	Mass spectrometry
(Richens et al., 2014)	AD and controls	No significant difference between study groups		Luminex Multiplex assay

(Toledo et al., 2014)	Normal controls, MCI and AD	CSF apoE levels were associated with cognitive decline and atrophy rate	Results were only significant in the group without the ε4 allele	Luminex multiplex assay
(Perrin et al., 2011)	Cognitively normal (Clinical dementia rating (CDR) 0) and mild AD or probable AD (CDR 1)	Did not differ significantly between the two groups		ELISA
(Zhang et al., 2008)	AD, Parkinson's disease (PD), controls	Reduced in AD as compared to controls, but did not differ from PD		Multiplex assay
(Hesse et al., 2000)	AD patients and normal controls	Reduced in AD as compared to the controls	Individuals having ε4 allele had higher apoE levels	ELISA
(Fukuyama et al., 2000)	AD patients (early onset Alzheimer's disease (EOAD) and late onset Alzheimer's disease (LOAD)) and normal controls	ApoE levels were reduced with age in normal controls and increased in AD (more distinctly in EOAD). The elevated levels were positively correlated with decline in cognition		ELISA
(Lindh et al., 1997)	AD, MCI, other dementia disorders (ODD) and age matched healthy controls	Elevated in AD, MCI and ODD as compared to healthy controls and significantly increased in AD at follow up	CSF levels significantly correlated with CSF tau in AD	ELISA
(Merched et al., 1997)	AD, controls and those suffering from other neurological disorders The CSF apoE levels were	Significantly elevated in patients with LOAD as compared to controls and other neurological disorders		ELISA

4.2 CLUSTERIN

4.2.1. ROLE IN AD PATHOGENESIS

Clusterin also called apolipoprotein J, is a stress induced chaperone glycoprotein which can stabilize stressed protein structures. It does so by binding to the hydrophobic surfaces of the partially unfolded proteins (Humphreys *et al.*, 1999; Nuutinen *et al.*, 2009). In the brain, it is highly expressed by astrocytes (De Silva *et al.*, 1990). It plays varied roles in AD pathology.

Clusterin affects amyloid pathology in multiple ways. It interacts with the Aβ peptides to form complexes. The antibodies specific to clusterin strongly stain the amyloid deposits in AD brain (McGeer *et al.*, 1992). This interaction keeps Aβ solubilized and prevents its fibrillation, and also regulate its transport across the BBB (Matsubara *et al.*, 1996; Calero *et al.*, 2000; Bell *et al.*, 2007). The levels are significantly increased in frontal cortex and in the hippocampus in AD (Lidström *et al.*, 1998). The elevated levels are localised to the regions abundant in Aβ (Miners *et al.*, 2016). This could be attributed as a protective response to combat the excessive Aβ deposition within the brain tissue. It likely suppresses Aβ deposition in conjunction with ApoE. This is evident by the elevation of Aβ in the CSF and brain interstitial fluid of both *APOE* and *CLU* knockout mice (DeMattos *et al.*, 2004).

It acts as a neuroprotectant by combating oxidative stress and apoptosis (Viard *et al.*, 1999). It prevents the mitochondrial transfer of activated Bcl-2 associated X (Bax) protein, a member of Bcl-2 protein family, which is known to accelerate apoptosis. Clusterin is also involved in double stranded DNA break repair (Wolter *et al.*, 1997; Zhang *et al.*, 2005; Shannan *et al.*, 2006). Clusterin also influences inflammation and immune response. The expression of clusterin by astrocytes is increased on treatment with Interleukin 2 (Zwain *et al.*, 1994). It inhibits the membrane binding of membrane attack complex and regulates the nuclear factor kappa light chain enhancer of activated B cell (NF-κB) pathway (Kirszbaum *et al.*, 1992; Wu *et al.*, 2012). Therefore, clusterin plays varied roles in AD pathology and serves as neuroprotectant by combating apoptosis, regulating inflammation and immune response and

preventing aggregation of Aβ (Figure 4). It can certainly serve a potential stage and state AD biomarker.

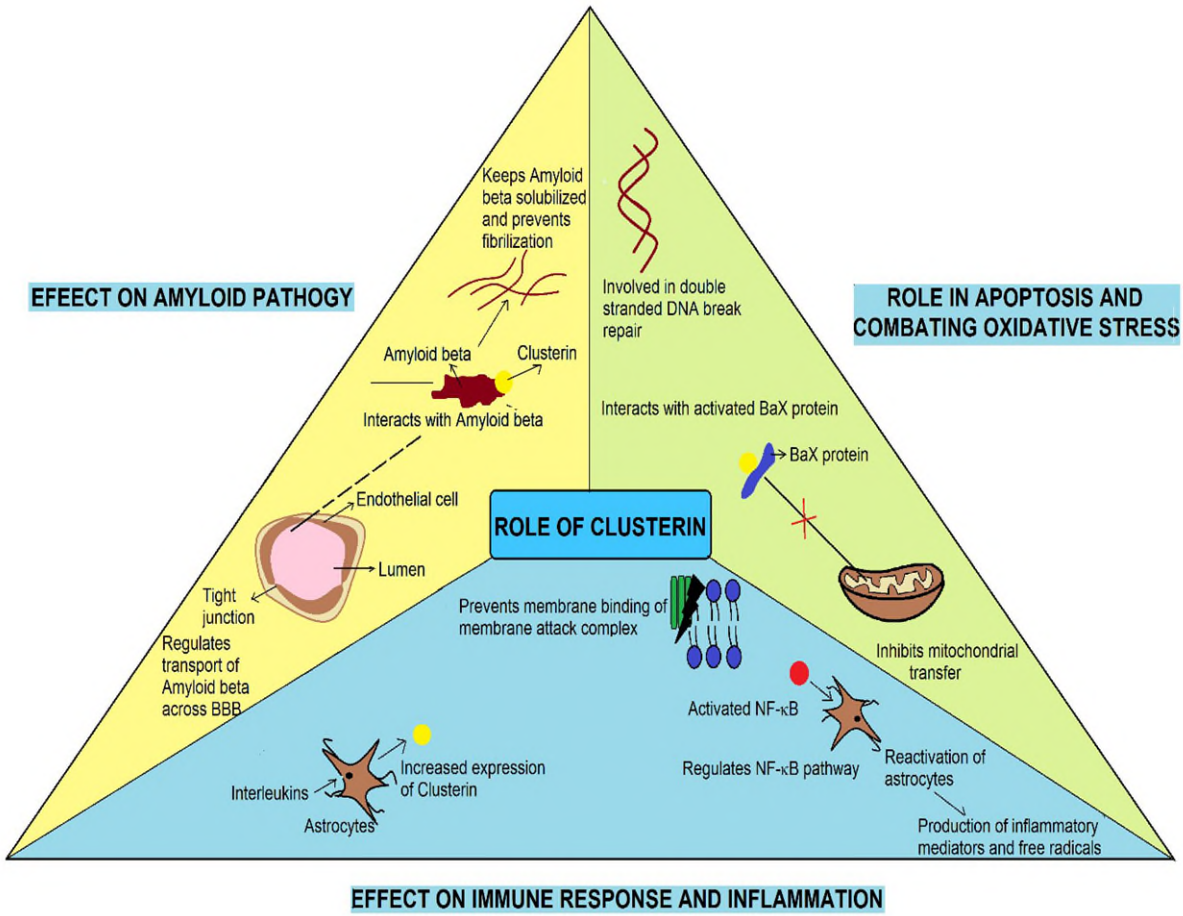

FIGURE 4: Varied roles of clusterin in AD pathology. Bax protein: Bcl-2 associated X protein; BBB: Blood brain barrier, DNA: Deoxyribonucleic acid; NF-κB: Nuclear factor kappa light chain enhancer of activated B cells.

4.2.2 CSF BIOMARKER STUDIES PERTAINING TO CLUSTERIN

Numerous studies have evaluated the diagnostic potential of clusterin in CSF by using different methods such as ELISA, mass spectrometry and multiplex assays. Most of the studies have reported that clusterin is significantly increased in CSF of AD patients (Table 2). The increased levels of clusterin could be attributed as a defence against neurodegeneration. CSF

clusterin levels correlate well with the core CSF biomarkers (T-tau and P-tau and Aβ-42), and are also significantly associated with CSF tau/Aβ ratio (Nilselid *et al.*, 2006; Jongbloed *et al.*, 2014; Deming *et al.*, 2016). CSF clusterin levels were found to be associated with the entorhinal cortex atrophy rate among CSF Aβ-42 positive individuals. (Desikan *et al.*, 2014). These correlations very likely suggest that CSF clusterin levels are elevated in relation to the pathological changes in the brain. Elevation in CSF levels of clusterin and the correlation with core biomarkers suggest that, elevated levels of clusterin could be attributed as a protective response to the amyloidosis and increased neurodegeneration in the AD brain. Looking at the role of clusterin in AD pathogenesis, a further exploration of its role as an AD biomarker is needed in the CSF.

TABLE 2 Studies conducted to evaluate the role of clusterin as a potential CSF biomarker

Study	***Study groups***	***CSF levels in AD/study groups***	***Association with APOE genotype/core markers***	***Analysis method***
(Deming et al., 2016)	AD and controls	Clusterin CSF levels were significantly elevated in AD	CSF levels were not associated with *APOE* genotype	Multiplex immunoassay
(Prikrylova Vranova et al., 2016)	PD, Parkinson's disease dementia (PDD), dementia with Lewy bodies (DLB), AD, progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and control group	No significant difference between AD and other diagnostic groups		ELISA
(Jongbloed et al., 2014)	Subjective memory complainers (SMC) and volunteers	The clusterin CSF levels were reduced in SMC. The difference in the CSF levels between study groups was more clear with the CSF clusterin/plasma ratio	Significant positive correlation with CSF T-tau and P-tau	ELISA

(Sihlbom et al., 2008)	AD and controls	The clusterin CSF levels were elevated in AD		Mass spectrometry
(Nilselid et al., 2006)	AD and controls	The clusterin CSF levels (native and deglycosylated) were elevated in AD	Both native and deglycosylated clusterin levels positively corrected with CSF tau and Aβ-42	ELISA
(Finehout et al., 2007)	AD and controls	The clusterin CSF levels were elevated in AD		Mass spectrometry

Please also add this paper:

Lidström AM, Hesse C, Rosengren L, Davidsson P, Blennow K. Normal levels of clusterin in cerebrospinal fluid in Alzheimer's disease, and no change after acute ischemic stroke. J Alzhemier Disease 2001;3:435-442.

5 BIOMARKERS OF NEUROINFLAMMATION

5.1 YKL-40/CHITINASE-3-LIKE PROTEIN 1 (CHI3L1)

5.1.1 ROLE IN AD PATHOGENESIS

YKL-40 is a glycoprotein belonging to the family of 18 glycosyl hydrolases. It is also called human cartilage glycoprotein-39 (HC gp-39) or chitinase-3-like-1 protein (CHI3L1). It binds with chitin but does not have a chitinase activity (Kazakova & Sarafian, 2009). It is secreted by the chondrocytes, synovial cells, vascular smooth muscle cells, macrophages and neutrophils (Volck *et al.*, 1998; Johansen, 2006). It is named based on the first three terminal amino acids: tyrosine (Y), lysine (K), leucine (L) (Johansen *et al.*, 1992; Johansen, 2006). YKL-40 plays a key role in inflammation, therefore influences AD pathology. In response to neuroinflammation the expression of YKL-40 is increased and is localized to astrocytes in the region of inflammation (Bonneh-Barkay *et al.*, 2010). It is expressed by the microglia and the

expression of YKL-40 mRNA is increased in AD (Colton *et al.*, 2006). Microglia and astrocytes are associated with senile plaques in AD and play a key role in immune response in the brain (Rubio-Perez & Morillas-Ruiz, 2012). The microglia are activated in response to neurodegeneration. The plaque associated activated microglia are large and mostly phagocytic (Sheng *et al.*, 1997). They constantly scavenge the plaques, damaged neurons, infectious agents and promote inflammation in damaged tissue (Lee & Landreth, 2010; Neniskyte *et al.*, 2011). Aβ, either alone or together with inflammatory mediators, sets up an activation cycle to activate the microglia and thereby, generate an immune response in the brain (Mrak & Griffin, 2005). Microglial activation thereby plays an important role in AD (Janelidze *et al.*, 2016). Therefore, microglial expressed protein YKL-40 is a potential marker of neuroinflammation, and plays a significant role in AD pathogenesis.

5.1.2 CSF BIOMARKER STUDIES PERTAINING TO YKL-40

The CSF levels of YKL-40 are elevated in AD. Through numerous studies it has been have found that increased levels of YKL-40 in CSF have prognostic and diagnostic utility as a biomarker for AD. YKL-40 aids in preclinical AD diagnosis and discriminating cognitively normal individuals from mild cognitive impairment (MCI) or AD patients (Table 3). The role of YKL-40 is also seen in differential diagnosis of dementia (Wennström *et al.*, 2015). The levels of YKL-40 have been found to significantly correlate with MMSE scores (Antonell *et al.*, 2014). Studies suggest YKL-40 is elevated early in the AD continuum and can serve as a valuable neuroinflammatory marker to detect early pathological changes and can even be used to study disease progression. The association of CSF YKL-40 with CSF T-tau and P-tau (Table 3), indicates that YKL-40 can help in tracking the neuroinflammation associated to neurodegeneration. Being a potential diagnostic and prognostic marker it can serve as a target to combat AD associated neuroinflammation. YKL-40 levels are consistently increased with

age. This suggests that neuroinflammation occurs normally with aging. However, the still higher increase in ε4 carriers suggest that neuroinflammation is exacerbated with amyloidosis and neurodegeneration (Sutphen *et al.*, 2015). On the contrary, a recent study also indicates that inflammation could be driven by amyloidosis but, independent of the *APOE* ε4 status. In this study the CSF levels were elevated in Aβ positive individuals, who were *APOE* ε4 non carriers (Hoglund *et al.*, 2017). Therefore, YKL-40 can be used a potential marker to stage the neuroinflammation associated with AD.

TABLE 3 Studies conducted to evaluate the role of YKL-40 as a potential CSF biomarker

Study	***Study groups***	***CSF levels in AD/study groups***	***Association with core biomarkers/APOE genotype***	***Analysis method***
(Gispert et al., 2017)	Controls, preclinical AD, MCI due to AD, mild AD dementia	Elevated in preclinical AD, AD and MCI	Increased in *APOE* ε4 carriers within a study group; positively associated with P-tau	ELISA
(Hoglund et al., 2017)	Healthy older individuals (divided into Aβ positive and negative)	No difference between two groups	Significantly elevated in *APOE* ε4 non carriers who were Aβ positive	ELISA
(Gispert et al., 2016a)	Normal controls, preclinical AD (CSF Aβ < 500 pg/mL), MCI due to AD, mild AD dementia	Significantly increased in MCI due to AD compared to controls	No association with Aβ-42; positive linear association with P-tau	ELISA
(Janelidze et al., 2016)	Healthy controls, Stable mild cognitive impairment (sMCI), MCI who later developed AD (MCI-AD), AD dementia, PDD, DLB, Vascular dementia (VaD), and Frontotemporal	Significantly increased compared to healthy controls and sMCI. Elevated as compared with DLB, PDD but not with VaD or FTD.	Positively correlated with Aβ-42 in AD patients and with tau in all diagnostic groups	ELISA

	dementia (FTD)			
(Wennström et al., 2015)	AD patients with mild to moderate dementia, PD, DLB and non-demented controls	Elevated compared to non-demented controls, DLB and PD patients	Not associated with CSF P-tau T-tau or Aβ–42	ELISA
(Hellwig et al., 2015)	AD dementia, MCI due to AD, MCI not due to AD, non-AD dementia	Significantly elevated in AD compared to MCI not due to AD or non-AD dementia. Also elevated in MCI-AD	Significant correlation with T-tau and P-tau in the non-AD group; no association CSF with Aβ	ELISA
(Kester et al., 2015b)	Cognitively normal, MCI, AD	Elevated in MCI and AD Baseline levels in MCI predicted progression to AD. Longitudinally increased in AD and MCI		ELISA
(Alcolea et al., 2015)	Cognitively normal controls and amnestic MCI	Elevated significantly in amnesic MCI as compared to controls	Strong correlation with T-tau and P-tau; no correlation between YKL-40 and Aβ-42	ELISA
(Sutphen et al., 2015)	Cognitively normal middle aged individuals (CDR 0), classified as early, mid and late	Significantly elevated in mid and late middle aged individuals	Biomarker changes more evident in ε4 carriers	ELISA

5.2 MONOCYTE CHEMO ATTRACTANT PROTEIN 1 (MCP-1)

5.2.1 ROLE IN AD PATHOGENESIS

Chemokines are low molecular weight cytokines. They are secondary inflammatory mediators induced by the primary mediators such as interleukin-1. These act as chemo-attractants and direct leucocytes to the site of inflammation. They express their action through guanine nucleotide associated proteins (G protein) coupled receptors. There are approximately 50 cytokines which are classified into four families; CC cytokines (have two adjacent cysteine residues at the N terminal), CXC cytokines (have two terminal cysteine residues separated by one amino acid), C cytokines (have two cysteine residues in total, one at N terminal and other at the downstream) and CX_3C cytokines (have two cysteine residues separated by three amino acids at the N terminal) (Graves & Jiang, 1995; Charo & Ransohoff 2006). Inflammation plays a significant role in AD pathogenesis. The cytokines and chemokines, being inflammatory mediators are involved in AD pathogenesis. They are released by the astrocytes, which play a role in Aβ generation and degeneration (Kato *et al.*, 1998). The astrocytes proliferate in response to neurodegeneration and increase the deposition of toxic Aβ (Blasko *et al.*, 2004). Aβ itself increases the expression of chemokines and cytokines by astrocytes, by reactivating them. There is a continuous cycle of activation and reactivation of astrocytes leading to inflammation and neuronal injury. (Forloni *et al.*, 1997; Wyss-Coray *et al.*, 2003). Therefore, the chemokines being mediators of inflammation, play a significant role in AD pathogenesis.

5.2.2 CSF BIOMARKER STUDIES PERTAINING TO MCP-1

Many studies have demonstrated the role of CC chemokine, MCP-1 or CCL2 in AD diagnosis. Studies have reported elevated CSF levels of MCP-1 in AD (Table 4). MCP-1 levels in CSF are positively correlated with the decrease in MMSE scores and higher baseline levels predict a faster rate of cognitive decline in AD (Blasko *et al.*, 2006; Galimberti *et al.*, 2006; Westin *et al.*, 2012). Therefore, MCP-1 could serve as a marker of cognitive decline along the

AD continuum. MCP-1 plays an important role in AD associated neuroinflammation and can serve a potential biomarker to track the same.

TABLE 4 Studies conducted to evaluate the role of MCP-1 as a potential CSF biomarker

Study	***Study groups***	**CSF *levels in AD***	***Association with core biomarkers/APOE genotype***	***Analysis method***
(Wennström et al., 2015)	AD patients with mild to moderate dementia, PD, DLB and non-demented controls	Elevated compared to non-demented controls; difference not detectable on age correction		Electro chemiluminescence Immunoassay (Meso scale discovery, (MSD))
(Rosén et al., 2014)	AD and controls	No significant difference between AD and controls		MSD assay
(Westin et al., 2012)	Controls and MCI	Elevated in sMCI and even in MCI to AD converters; higher baseline values predict rate of future cognitive decline		Electro chemiluminescence Immunoassay (MSD)
(Correa et al., 2011)	AD and healthy controls	Significantly elevated in AD	Positively correlated with CSF P-tau and Aβ	ELISA
(Choi et al., 2008)	AD, PD, healthy controls	Elevated in AD and PD but not significantly as compared to controls		Multiplex immunoassay (Luminex xMAP technology)
(Galimberti et al., 2006)	Amnestic MCI, AD, subjects with Non inflammatory affections of the nervous system.	Significantly elevated in in MCI and AD compared to controls		ELISA
(Blasko et al., 2006)	AD, FTD, alcohol dementia, major	Significantly increased in AD compared to	Positive correlation with T-tau	ELISA

depression and control patients	controls (no difference in case of older controls	but not with Aβ and P-tau

6 BIOMARKER OF SYNAPTIC DYSFUNCTION

6.1 NEUROGRANIN

6.1.1 ROLE IN AD PATHOGENESIS

Neurogranin is a calmodulin-binding, postsynaptic protein found in the dendrites (Gerendasy & Sutcliffe, 1997). It plays an important role in memory potentiation. It binds with calmodulin and releases the same when intracellular concentration of calcium increases. The released calmodulin binds with the calcium ions and activates a signal transduction pathway (Hayashi, 2009). Synaptic dysfunction is linked to decline in cognition and occurs prior to neuronal degeneration (DeKosky & Scheff, 1990; Bertoni-Freddari *et al.*, 1996). The brain levels of synaptic proteins including neurogranin are reduced in AD at an early stage. The synaptic dysfunction in terms of reduction in synapses is also seen in MCI which is higher in mild AD. Thus, synaptic dysfunction occurs early in AD and indicates disease progression (Masliah *et al.*, 1994; Masliah *et al.*, 2001; Reddy *et al.*, 2005; Scheff *et al.*, 2007; Fyfe, 2015). Neurogranin regulates the calcium-dependent postsynaptic signalling triggered by calmodulin (Kubota *et al.*, 2007). Reduced brain levels of neurogranin can cause a dysregulation of post synaptic signalling. The expression of neurogranin is reduced in hippocampal and retrosplenial regions of brain in aged mice (Mons *et al.*, 2001). Therefore, a reduction of synaptic proteins such as neurogranin in the brain relates to synaptic dysfunction and the CSF levels of such proteins can be used for disease diagnosis and monitor the progression.

6.1.2 CSF BIOMARKER STUDIES PERTAINING TO NEUROGRANIN

In the past few years, a number of researchers have evaluated the diagnostic and prognostic potential of the biomarker neurogranin. A number of assay methods have been developed to quantify neurogranin in the CSF and have reported elevated neurogranin levels are elevated in AD (Table 5). In a study conducted on various synaptic proteins including neurogranin in post-mortem brain samples, it was found that synaptic proteins discriminated dementia cases from controls with over 90% sensitivity and specificity (Bereczki *et al.*, 2016). The CSF neurogranin levels correlate with brain atrophy and amyloid load and also help in predicting decline in cognition. The CSF levels differ significantly between stable MCI (sMCI) and MCI to AD converters and between sMCI and AD (Kester *et al.*, 2015a; Kvartsberg *et al.*, 2015; Portelius *et al.*, 2015; Tarawneh *et al.*, 2016). Increased CSF levels of neurogranin are specific to AD and not seen in other neurodegenerative diseases (Wellington *et al.*, 2016). Therefore, it is a promising biomarker for early AD diagnosis, predicting progression and distinguishing AD from other forms of dementia. It can act as a theragnostic marker, which can help in monitoring biochemical effects of drugs used to improve synaptic function. Since, synaptic dysfunction is associated to cognitive decline, neurogranin can help in staging the rate of cognitive decline along the AD continuum. However, large longitudinal studies are needed to further validate the role of neurogranin in AD diagnosis and prognosis.

TABLE 5 Studies conducted to evaluate the role of neurogranin as a potential CSF biomarker

Study	***Study groups***	***CSF levels in AD***	***Association with core biomarkers/APOE genotype***	***Analysis method***
(Hoglund et al., 2017)	Healthy older individuals (divided into Aβ positive and negative)	Significantly elevated in Aβ positive group	Significantly elevated in *APOE* ε4 non carriers who were Aβ positive	MSD assay

(Tarawneh et al., 2016)	Cognitively normal controls (CDR 0) and AD (CDR 0.5, 1, 2), non-AD dementias	Significantly elevated in participants with CDR ≥ 0.5 compared to CDR 0 and non-AD dementias	Positively correlated with CSF T-tau and P-tau	Two-site immunoassay (Erenna Singulex)
(Mattsson et al., 2016)	AD, MCI and controls	Significantly predicted AD vs. controls; elevated in AD	CSF Neurogranin levels were associated with Aβ positivity in all groups; correlated strongly with T-tau	Electro chemiluminescence Immunoassay (MSD)
(Sanfilippo et al., 2016)	Healthy controls, MCI, Prodromal AD, AD, major depressive disorder (MDD)	Significantly elevated in AD, prodromal AD vs. controls and MDD; also in Significantly in prodromal AD vs. MCI	Correlated positively with CSF T-tau and P-tau	ELISA
(De Vos et al., 2016)	Healthy controls , MCI due to AD, AD	Significantly elevated in MCI vs. controls but not in AD; no significant differences between MCI and AD	Correlated with T-tau in MCI and AD but not with Aβ-42	ELISA
(Kester et al., 2015a)	Cognitively normal, MCI and AD	Significantly elevated in AD compared to controls; no difference between MCI and AD, MCI and cognitively normal	Strong positive correction with CSF T-tau and P-tau	Sandwich immunoassay (Erenna Singulex)
(Kvartsberg et al., 2015)	Three cohorts consisting of AD, MCI and controls	Significantly elevated in AD vs. controls (using both methods); Significantly elevated in MCI-AD vs. sMCI, AD vs. sMCI	Strong positive correction with CSF T-tau and P-tau in AD and controls	Mass spectrometry and ELISA
(Hellwig et al., 2015)	AD dementia, MCI due to AD, MCI not	Significantly increased in AD, MCI due to AD	Moderate correlation with CSF T-tau and P-	MSD assay

	due to AD, non-AD dementia	compared to MCI not due to AD	tau but not with Aβ-42	
(Portelius et al., 2015)	Cognitively normal, MCI and AD	Significantly elevated in progressive MCI, AD vs. controls	Negatively correlated with CSF Ab-42 in sMCI, progressive MCI and AD; strong positive correlation with CSF T-tau and P-tau in all study groups	Electro chemilumin escence Immunoass ay (MSD)

Please add this paper here:

Thorsell A, Bjerke M, Gobom J, Brunhage E, Vanmechelen E, Andreasen N, Hansson O, Minthon L, Zetterberg H, Blennow K. Neurogranin in cerebrospinal fluid as a marker of synaptic degeneration in Alzheimer's disease. Brain Res 2010;1362:13-22.

7 BIOMARKER OF ALTERED MICROGLIAL ACTIVITY

7.1 SOLUBLE ECTODOMAIN OF TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS (sTREM2)

7.1.1 ROLE IN AD PATHOGENESIS

Ectodomain of triggering receptor expressed on myeloid cells (TREM2) is a transmembrane glycoprotein immune receptor expressed in a number of cells such as dendritic cells, osteoclasts, tissue macrophages and the microglia. It contains an ectodomain with three N-glycosylation residues, a transmembrane sequence and a short intracellular tail. Its functions are mediated via DNAX activating protein of 12 kDa (DAP12) signaling (Bouchon *et al.*, 2000; Colonna, 2003). In the brain, it is expressed on the microglial cells and regulates microglial mediated phagocytosis and clearance of apoptotic neurons (Schmid *et al.*, 2002; Hsieh *et al.*,

2009). It plays an important role in regulating immune responses in the brain and the production of inflammatory cytokines (Takahashi *et al.*, 2005; Hamerman *et al.*, 2006). Therefore, the role of TREM2 has been widely studied in AD in relation to down regulating inflammation and clearing out apoptotic neurons via microgliosis. TREM2 is up regulated in mice with mutant APP and amyloid deposition (Frank *et al.*, 2008). The mutations associated with the *TREM2* gene are associated with an increased risk for AD. Rare variants of the gene increase the risk of developing AD by twofold (Jonsson *et al.*, 2013; Jin *et al.*, 2014). It has also been found that mutations in *TREM2* reduce Aβ clearance (Kleinberger *et al.*, 2014). TREM2 undergoes regulated membrane proteolytic processing by ADAM 10 (A disintegrin and metalloproteinase domain containing protein 10) and γ secretase, and releases the soluble ectodomain sTREM2 into the extracellular space (Colonna & Wang, 2016). The sTREM2 is detectable in the CSF and the levels have been quantified in different neurological disorders such as AD, fronto temporal dementia (FTD) and mutiple sclerosis (Piccio *et al.*, 2008; Piccio *et al.*, 2016). Since, TREM2 regulates microgliosis, the soluble fragment of the protein, sTREM2, could play a role in regulating TREM2 mediated microgliosis. The exact biological role of the soluble fragment is unclear. However, using in vitro and in vivo models, Zhong, et.al. have shown that sTREM2 promotes microglial survival and induces production of inflammatory cytokines (Zhong *et al.*, 2017). Therefore, sTREM2 likely plays a role in microgliosis, but further studies are needed to affirmatively elucidate the exact role of sTREM2.

7.1.2 CSF BIOMARKER STUDIES PERTAINING TO STREM2

Numerous studies have revealed that CSF levels of sTREM2 are altered in AD (Table 6). The levels are elevated in dominantly inherited AD cases years before the onset of symptoms (Suarez-Calvet *et al.*, 2016a). This signifies that microgliosis occurs prior to the onset of symptoms and later to brain amyloidosis. The Nasu-Hakola disease (NHD) *TREM2*

mutation carriers have lower CSF levels of sTREM2 (Piccio *et al.*, 2016). This signifies the altered protein production in mutation carriers. Studied have found the CSF levels of TREM2 are increased in AD at early stage and correlate well with the markers of neurodegeneration and tau pathology. Therefore, microgliosis is most likely an early event that occurs along the AD and occurs in response to neurodegeneration. The CSF levels are lesser in AD as compared to MCI who later developed AD (MCI-AD).This signifies that microgliosis increases from the preclinical AD to MCI-AD and there after reduces in AD, probably due to reduction in immune response (Schindler & Holtzman, 2016). Higher CSF levels in MCI patients are associated to increased gray matter volume. This reflects upon the protective response of microglia in response to neurodegeneration (Gispert *et al.*, 2016b). The role of TREM2 in regulating brain immune response, microgliosis and inflammation needs to be further explored. The CSF levels of sTREM2 can help in tracking the altered microgliosis along the disease trajectory and can serve as a potential stage biomarker for identifying early stages of AD and as theragnostic marker to monitor therapeutic effects of drugs administered at an early stage.

TABLE 6 Studies conducted to evaluate the role of sTREM2 as a potential CSF biomarker

Study	***Study groups***	***CSF levels in AD***	***Association with core biomarkers/APOE genotype***	***Analysis method***
(Gispert et al., 2017)	Control; Preclinical AD; MCI due to AD; Mild AD dementia	Elevated in AD and MCI however no significant difference upon age correction	Positively associated with CSF P-tau not significant difference between *APOE* ε4 carriers and non-carriers in any study group	ELISA
(Heslegrave et al., 2016)	AD and cognitively normal controls	Significantly elevated in AD	Significant positive correlation with CSF T-tau and P-tau but not with CSF Aβ-42	Mass spectrometry

(Suarez-Calvet et al., 2016b)	Controls, Preclinical AD, MCI due to AD and AD dementia	Significantly elevated in MCI-AD compared to controls and AD dementia	Positively correlated with CSF T-tau and P-tau (stronger in preclinical AD, MCI due to AD and AD); not affected by APOE ε4 status	ELISA (MSD platform)
(Henjum et al., 2016)	Controls, MCI and AD	No statistical difference among the diagnostic groups	Positively correlated with CSF Aβ-42, T-tau and P-tau among controls	ELISA
(Piccio et al., 2016)	Cognitive normal, AD, FTD and *TREM2* risk variant carriers	Significantly elevated in AD compared to Controls (all non TREM2 risk variant carriers)	Highly correlated with CSF t tau and P tau levels but not with CSF Aβ-42.	ELISA
(Gispert et al., 2016b)	Cognitively normal controls, preclinical AD, MCI-AD, and AD (mild)	Highest levels in MCI-AD; significantly higher than the Controls and preclinical AD	Positively correlated with CSF T-tau and P-tau in all diagnostic groups	ELISA (MSD platform)

8 BIOMARKERS REFLECTING NEURONAL MEMBRANE DISRUPTION (NEURODEGENERATION)

8.1 FATTY ACID BINDING PROTEIN 3 (FABP3) OR HEART TYPE FATTY ACID BINDING PROTEIN (HFABP)

8.1.1 ROLE IN AD PATHOGENESIS

The fatty-acid-binding proteins (FABP's) are transport proteins for fatty acids and other lipophilic biomolecules. FABP3 is mainly expressed in the heart and skeletal muscles but has also been isolated from the brain (Yoshimoto *et al.,* 1995). In the brain FABP's bind to long chain polyunsaturated fatty acids (PUFA), such as docosahexaenoic acid (DHA) and

arachidonic acid (ARA) and is involved in the transport of these fatty acids. These fatty acids are indispensable for maintaining neuronal membrane integrity, neurite growth and synapse formation. The DHA and ARA modulate neural membrane fluidity and permeability (Veerkamp & Zimmerman, 2001; Janssen & Kiliaan, 2014). The dietary supplementation of DHA has been found to improve spatial memory and reduce Aβ deposition in mice (Hooijmans *et al.*, 2009). DHA also prevents Aβ induced neuronal damage *in vivo* and *in vitro* (Tan *et al.*, 2016). Since HFABP or FABP3 regulates the transport of DHA and other fatty acids, it is likely to be associated with AD pathogenesis. The brain levels of FABP3 are reduced in such neurodegenerative diseases, which could be associated to altered signal transduction and membrane integrity (Cheon *et al.*, 2003). FABP's are released following a cellular injury (Glatz, 1998; Pelsers *et al.*, 2005). Therefore, like other FABP's, HFABP is likely to be associated with cellular dysfunction associated with AD. FABP3 is also associated with dopaminergic system and changes in dopaminergic system are likely to be associated with AD. It binds and regulates the dopaminergic D_2 receptors, and over expression of FABP3 promotes α-synuclein oligomerization (Shioda *et al.*, 2010; Martorana & Koch, 2014; Shioda *et al.*, 2014). The association of FABP3 with dopaminergic system also signifies the role of FABP3 in AD pathogenesis.

8.1.2 CSF BIOMARKER STUDIES PERTAINING TO FABP3

The CSF levels of FABP3 are elevated in AD and is a potential diagnostic marker for differential diagnosis of neurodegenerative diseases (Table 7). The elevated levels are significantly associated with brain atrophy in cases with low Aβ-42 and reflect on lipid dyshomeostasis in the CNS (Desikan *et al.*, 2013). Therefore, elevated FABP3 levels in CSF might be associated to brain amyloidosis. The diagnostic accuracy of the core CSF biomarkers has been found to be increased in conjunction with FABP3. Also, FABP3 and the ratio of

FABP3/Aβ−42 is useful in predicting the progression of MCI subjects to AD. (Chiasserini *et al.*, 2010; Guo *et al.*, 2013). In a recent study involving healthy aged individuals the CSF levels of FABP3 were significantly elevated in Aβ positive individuals compared to negative individuals (Hoglund *et al.*, 2017). Therefore, it is a good biomarker for predicting disease progression in early stages of disease and can help in identifying healthy aged individuals at risk of developing AD. The elevated CSF levels correlate with core markers of neuro degeneration (Table 7). The elevated levels in AD could likely be associated to the destruction of neurons.

TABLE 7 Studies conducted to evaluate the role of FABP3 as a potential CSF biomarker

Study	***Study groups***	***CSF levels in AD***	***Association with core biomarkers/APOE genotype***	***Analysis method***
(Hoglund et al., 2017)	Healthy older individuals (divided into Aβ positive and negative)	Significantly elevated in Aβ positives group	Elevated significantly in *APOE* ε4 non carriers who were Aβ positive	Electro chemiluminescence Immunoassay (MSD)
(Bjerke et al., 2016)	Non-demented women	Elevated in those who developed AD (at follow up); higher baseline levels associated to development of dementia	Strong correlation with CSF T-tau and P-tau at baseline	Electro chemiluminescence Immunoassay (MSD)
(Biscetti et al., 2016)	AD, PD, DLB, PDD, other neurological disorders (OND) as controls.	Significantly elevated in AD, DLB compared to PD and OND	Significantly correlated with CSF T-tau levels CSF	ELISA
(Chiasserini et al., 2016)	AD, PD, OND as controls	Significantly elevated in AD compared to PD and OND	Positively correlated with CSF T-tau, P-tau but not with Aβ	Immuno assay
(Guo et al., 2013)	Healthy controls, MCI, AD	Significantly elevated in AD		Multiplex immunoassay

		compared to controls		(Luminex xMAP technology)
(Desikan et al., 2013)	Cognitively normal, amnestic MCI and probable AD	Elevated in AD and MCI compared to controls	Significantly associated with CSF P-tau	Multiplex immunoassay (Luminex xMAP technology)

9 BIOMARKERS OF NEURONAL STRUCTURE AND SIGNALING DISRUPTION (MARKERS OF NEURODEGENERATION)

9.1 NEUROFILAMENT LIGHT CHAIN PROTEIN (NFL): MARKER OF AXONAL DEGENERATION

9.1.1 ROLE IN AD PATHOGENESIS

Neurofilaments are the proteins particularly found in neuronal axons. They are 10 nm in diameter and are essential for the axonal growth and the transmission of impulses along the axons (Yuan *et al.*, 2012). These are heteropolymers composed of four subunits, namely neurofilament heavy, medium and light polypeptides and α-internexin (Yan *et al.*, 2007). Being elastic and fibrous they maintain the shape of neurons and act as neuroskeletal supports (Wagner *et al.*, 2007). NFL plays a role in protecting neurites from dystrophy and regulates pathways generating Aβ (Fernandez-Martos *et al.*, 2015). Neurofilaments are likely to be released from neuronal axons in response to neuronal damage in neurodegenerative diseases. NFL is mainly located in myelinated axons and white matter changes are associated with increased NFL levels in the CSF. Therefore, elevated levels of NFL in the CSF reflect on axonal degeneration (Sjögren *et al.*, 2001) (Figure 5). NFL is a specific biomarker of axonal degeneration, whose levels have been found to be elevated in a wide range of neurodegenerative diseases including AD. It is not a disease-specific biomarker but can aid in differential diagnosis of neurodegenerative disorders since its levels are elevated in FTD as compared to AD (Skillback *et al.*, 2014). High CSF NFL levels predict high hippocampal

atrophy rate in cognitively healthy older adults as well those at risk of AD (Idland *et al.*, 2017). In case of AD it can help in help in tracking the different dynamic changes along the disease continuum.

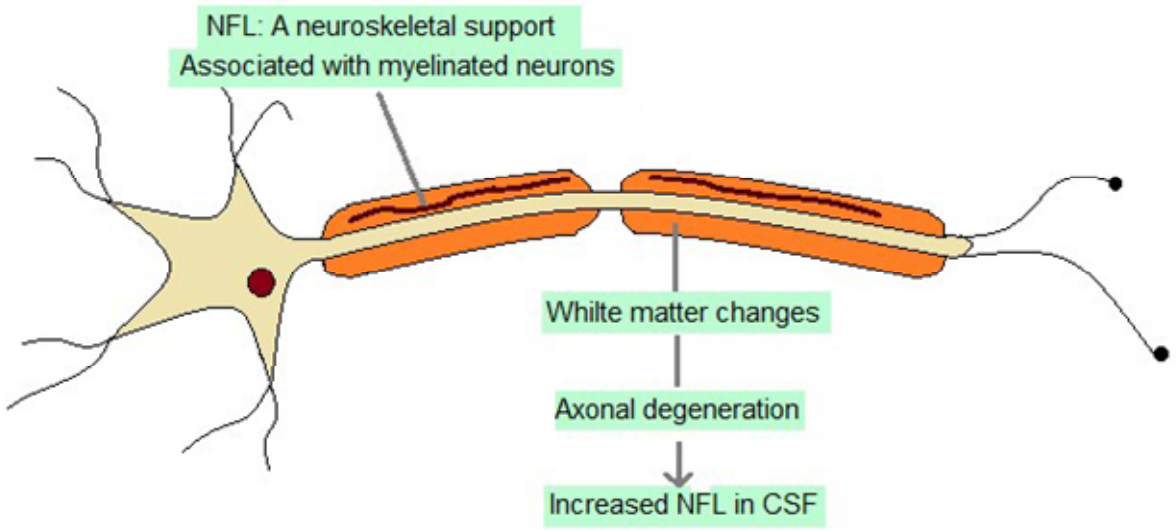

FIGURE 5: Role of NFL in AD pathogenesis. NFL: Neurofilament light chain; CSF: Cerebrospinal fluid

9.1.2 CSF BIOMARKER STUDIES PERTAINING TO NFL

The CSF levels of NFL are elevated in a wide range of neurodegenerative diseases including AD as compared to normal controls (Table 8). NFL levels are significantly elevated in AD compared to sMCI and higher CSF levels in AD are associated with cognitive decline, white matter change, brain atrophy, lower FDG-PET. The change in CSF levels and these associations are independent of Aβ positivity (Mattsson *et al.*, 2016; Zetterberg *et al.*, 2016). Therefore, NFL reflects upon neuronal or axonal degeneration independent of Aβ pathology. Since the CSF levels of NFL are significantly elevated in AD compared to sMCI and associated to brain atrophy and cognitive decline; it can be used as potential biomarker to study disease progression and severity along the AD continuum. Also, the diagnostic performance of core CSF biomarkers in differential diagnosis of early onset Alzheimer's disease (EOAD) and FTD

is improved in conjunction with the CSF levels of NFL (De Jong *et al.*, 2007). Hence, it also has a potential to differentially diagnose a range of neurodegenerative diseases. But, the potential of NFL to identify individuals at risk of developing AD or its potential to identify preclinical AD needs to be further explored.

TABLE 8 Studies conducted to evaluate the role of NFL as a potential CSF biomarker

Study	***Study groups***	***CSF levels in AD vs other study groups***	***Association with core biomarkers***	***Analysis method***
(Hoglund et al., 2017)	Healthy older individuals (divided into Aβ positive and negative)	No difference between two groups		ELISA
(Zetterberg et al., 2016)	AD, MCI and cognitively normal individuals	Significantly elevated in AD, sMCI, MCI-AD as compared to controls; AD vs sMCI	Baselines levels correlated with low CSF Aβ, T-tau and P-tau	ELISA
(Mattsson et al., 2016)	AD, MCI and controls	Elevated; Significantly predicted AD vs. controls	CSF NFL levels are not associated with Aβ positivity; correlated with T-tau	ELISA
(Scherling et al., 2014)	Normal controls, AD various other neurodegenerative disorders such as FTD, PD etc.	Elevated significantly in AD as compared to normal controls; also in all FTD subgroups compared to normal controls and AD		ELISA
(Skillback et al., 2014)	Healthy controls, EOAD, LOAD FTD, DLB , PDD, VaD, mixed AD and VaD, other dementias, dementia not specified	Elevated in all groups as compared to controls; highest in FTD; significantly elevated in EOAD and LOAD compared to controls		ELISA

Please add these papers here:

Rosengren L, Karlsson JE, Sjögren M, Blennow K, Wallin A. Neurofilament levels in CSF are increased in dementia. Neurology 1999;52:1090-1093.

Sjögren M, Blomberg M, Johnson M, Wahlund LO, Edman Å, Lind K, Rosengren L, Blennow K, Wallin A. Neurofilament protein in cerebrospinal fluid – a marker of white matter changes. J Neurosci Res 2001;66:510-516.

9.2 VISININ LIKE PROTEIN 1 (VILIP-1): MARKER OF NEURONAL INJURY

9.2.1 ROLE IN AD PATHOGENESIS

VILIP-1 belongs to a large family of calcium binding proteins called neuronal calcium sensors (NCS's) (Burgoyne & Weiss, 2001). The VIPIL-1 protein is encoded by the visinin like 1 (VSNL1) gene and contains 191 amino acids and weighs 22 kDa (Groblewska *et al.*, 2015). VILPI-1 is distributed in different regions of the brain (Bernstein *et al.*, 1999). The Calcium ions (Ca^{2+}) are involved in neuronal signalling and the NCS's mediate the action of these ions. In response to a high intracellular concentration of Ca^{2+}, VILIP-1 gets reversibly translocated to the membrane components of the cell. This reversible interaction of VILIP-1 modulates signalling cascade in the neurons via activation of specific membrane bound targets (Spilker *et al.*, 2002a; Spilker *et al.*, 2002b). Therefore, VILIP-1 plays an important role in neuronal signalling. The VILIP-1 regulates neuron ion channels, neuronal growth, survival, synaptic plasticity and activates cyclic adenosine monophosphate (cAMP) and cyclic guanine monophosphate (cGMP) signalling pathways (Groblewska *et al.*, 2015). Neurodegenerative disorders such as AD are associated with disturbed Ca^{2+} homeostasis in the neurons, which affect neuronal signalling by causing excessive activation of receptors, weakening the Ca^{2+} buffering capacity of neurons and deregulating the Ca^{2+} channels (Marambaud *et al.*, 2009). Aβ modulates this disturbed Ca^{2+} homeostasis by increasing the influx of Ca^{2+} by forming channels (Arispe *et al.*, 1993). The NCS's such as VILIP-1 play a significant role in AD pathogenesis. The intracellular expression of VILIP-1 is reduced in AD brains as compared to controls. VILIP-1 has been found to be associated with extracellular plaques and NFT's in the

brains of AD patients and its expression is associated with enhanced hyper phosphorylation of tau protein and cell death (Braunewell *et al.*, 2001; Braunewell, 2012). In mild AD there is a considerable loss of neurons in the entorhinal cortex (Gomez-Isla *et al.*, 1996; Price *et al.*, 2001). The levels of VILIP-1 are reduced in the entorhinal cortex of AD patients (Kirkwood *et al.*, 2016). Therefore, it is a marker of neuronal injury. Figure 6 depicts the role of VILIP-1 in AD pathogenesis.

This signifies that VILIP-1 is neurotoxic under a disturbed Ca^{2+} homeostasis. In AD its intracellular expression is reduced. Increased expression promotes hyperphosphorylation and cell death which is reduced by calcium buffer protein. A disturbed Ca^{2+} balance causes the loss of vulnerable neurons and thereby, the release of VILIP-1 extracellularly. (Braunewell *et al.*, 2001; Schnurra *et al.*, 2001; Braunewell, 2012; Groblewska *et al.*, 2015).

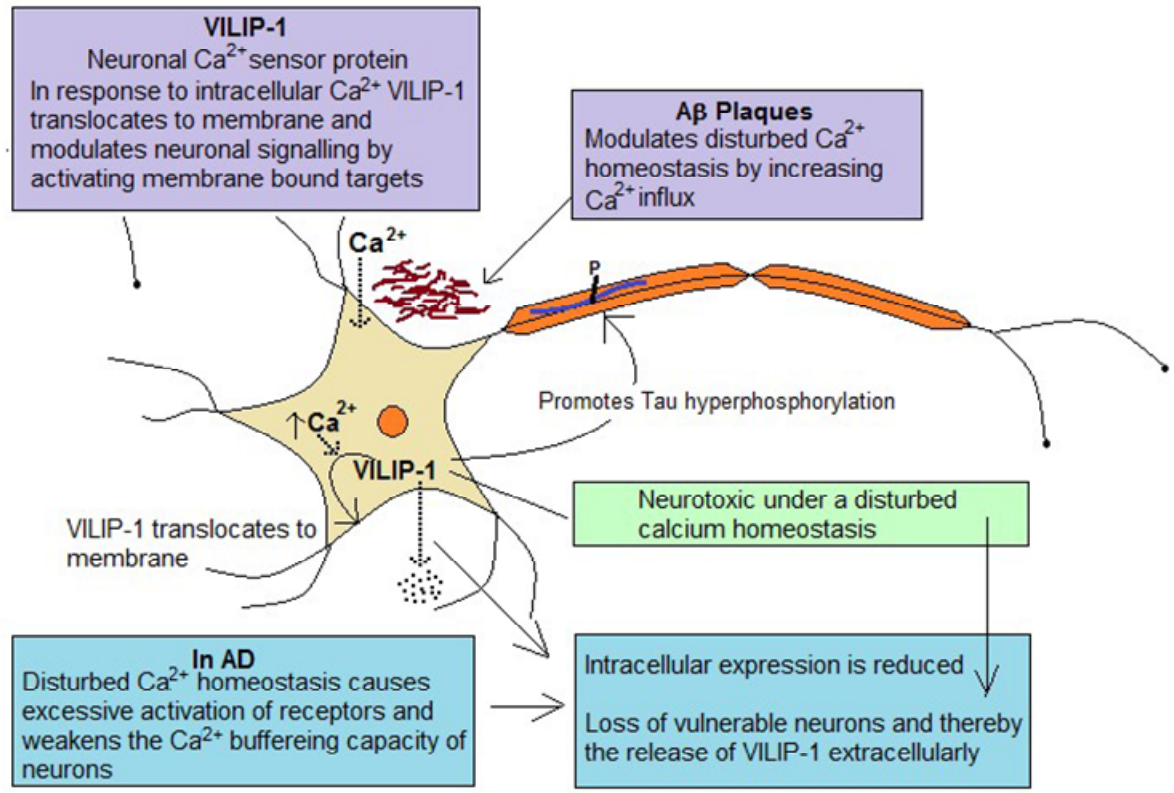

FIGURE 6: The role of VILIP-1 in AD pathogenesis. Aβ: Amyloid beta, AD: Alzheimer's disease

9.2.2 CSF BIOMARKER STUDIES PERTAINING TO VILIP-1

Numerous studies have been conducted to illustrate its role as a potential CSF diagnostic, prognostic and a differential biomarker. CSF levels of VILIP-1 aid in the early diagnosis of AD, distinguish AD from MCI, helps in identifying the patients with MCI likely to progress to AD, and in differentiating AD from other forms of dementia (Table 9). When used in combination with the core CSF markers the diagnostic performance is improved (Lee *et al.*, 2008). VILIP-1 and VILIP-1/Aβ-42 ratio negatively correlates with MMSE (Lee *et al.*, 2008; Babic Leko *et al.*, 2015). Baseline CSF levels of VILIP-1 are associated with rate of whole brain and regional brain atrophy in AD. VILIP-1 and the ratio of VILIP-1/Aβ-42 correlate significantly with the brain amyloid load. Therefore, VILIP-1 and the ratio of VILIP-1/Aβ-42 help in predicting the future cognitive decline. (Tarawneh *et al.*, 2011; Tarawneh *et al.*, 2012; Luo *et al.*, 2013; Mroczko *et al.*, 2015; Tarawneh *et al.*, 2015). VILIP-1 can be used as a surrogate marker of neurodegeneration but, larger longitudinal studies are needed to validate the same. It can help in tracking the protective effects of neuroprotective therapeutic interventions.

TABLE 9 Studies conducted to evaluate the role of VILIP-1 as a potential CSF biomarker

Study	***Study groups***	***CSF levels in AD vs other study groups***	***Association with core biomarkers***	***Analysis method***
(Hoglund et al., 2017)	Healthy older individuals (divided into Aβ positive and negative)	No difference between two groups	Significantly elevated in *APOE* ε4 non carriers who were Aβ positive	ELISA
(Mroczko et al., 2015)	Cognitively normal controls, AD, MCI	Significantly elevated in AD vs. controls and MCI; also elevated in MCI vs. controls but not significantly	Significantly correlated with CSF P tau in AD and controls; with	ELISA

			Aβ-42 in controls	
(Babic Leko et al., 2015)	Healthy controls, AD, MCI, VaD, FTD, DLB	Significantly elevated in AD vs. controls, MCI and DLB; no difference in AD vs. VaD and FTD	Positive correlation with CSF T-tau and P-tau in a mixed group of (healthy controls, AD, MCI) as well as in AD and MCI	ELISA
(Kester et al., 2015b)	Cognitively normal, MCI, AD	Elevated in AD and MCI vs. normal controls but not significantly; baseline levels in MCI predicted progression to AD. Longitudinally increased in AD and MCI		Microparticle based immuno assay (Erenna Singulex)
(Tarawneh et al., 2015)	Normal controls (CDR 0) and AD	Significantly elevated in AD vs. controls		Microparticle based immuno assay (Erenna Singulex)
(Sutphen et al., 2015)	Cognitively normal middle aged individuals (CDR 0), classified as early, mid and late	Significantly elevated in late middle aged individuals as compared to early and mid in non ε4 carriers;	Biomarker changes more evident in ε4 carriers longitudinally	Microparticle based immuno assay (Erenna Singulex)
(Luo et al., 2013)	Normal controls, AD, DLB	Significantly elevated in AD vs. controls and DLB	Positively correlated with T-tau and P-tau	ELISA
(Tarawneh et al., 2012)	Normal controls (CDR 0) and AD (CDR 0.5 and 1)	Significantly elevated in AD vs. controls		Microparticle based immuno assay (Erenna Singulex)
(Tarawneh et al., 2011)	Cognitively normal controls, AD, other dementias	Significantly elevated in AD vs. controls and other dementias	Correlated with T-tau and P-tau	Microparticle based immuno assay (Erenna Singulex)

Please add this paper here:

Lee JM, Blennow K, Andreasen N, Laterza O, Modur V, Olander J, Gao F, Ohlendorf M, Ladenson JH. The Brain Injury Biomarker VLP-1 Is Increased in the Cerebrospinal Fluid of Alzheimer's Disease Patients. Clin Chem 2008;54:1617-1623.

(VLP-1 and VILIP-1 is the same protein)

10 CONCLUSION

The multifaceted AD dementia is an amalgam of different pathological changes in the brain. The different pathological changes may represent a hierarchy of events that occur one after another or may follow their own trajectory, which ultimately leads to dementia due to AD. In order to get a deeper insight into different aspects of disease pathogenesis biomolecules/proteins involved in the associated biochemical pathways need to be explored and evaluated as disease biomarkers for disease diagnosis, prognosis and therapy. The CSF biomarkers would serve as reliable measures, to assess the time course of AD and the associated pathological changes along the continuum of the disease. A number of biomarkers in relation to different AD associated pathological changes have been discussed in the current manuscript. They together or alone can aid in an accurate AD diagnosis starting from the preclinical phase and thereby, can give a clear picture of the pathological changes that occur across the disease continuum. The use of multiple biomarkers can help in understanding the association of individual pathologies (Melah *et al.*, 2016), and may provide an understanding about how one pathological change influences the other. Hence, these biomarkers in conjunction can improve the accuracy of diagnosis. It has been found that a biomarker model consisting of the biomarkers T-tau, NFL, neurogranin reflecting upon neurodegeneration, axonal damage and synaptic dysfunction respectively; has a higher diagnostic accuracy (area under the receiver operating curve (AUC) 85.5%) in classifying AD and controls (Mattsson *et al.*, 2016). The

combination of CSF biomarkers, including YKL-40 could distinguish cognitively normal participants with clinical dementia rating (CDR) score of 0 from those with CDR > 0 with AUC 0.896 (Perrin *et al.*, 2011).

The CSF levels of these biomarkers change likely with the pathological change or event in the AD brain. The elevated CSF levels of clusterin can highlight upon the role of clusterin in binding with Aβ and preventing its fibrillization or its role in promoting the formation of soluble toxic Aβ oligomers. An elevated CSF levels of biomarkers YKL-40 and MCP-1 highlight upon neuroinflammation as a protective response to brain damage. These proteins are expressed by the astrocytes, which are activated in response to neurodegeneration and thereafter, release inflammatory mediators. Elevated levels of sTREM2 highlight upon brain microgliosis as a response to phagocytise accumulated Aβ. Therefore, these novel biomarkers can help in tracking inflammatory processes related to AD neurodegeneration. They can help in tracking stage and state associated neuroinflammation in AD and combating the same with the therapeutic agents. Inflammation is associated with a number of psychiatric disorders (Réus *et al.*, 2015). These biomarkers can help in understanding the association of psychiatric disorders such as depression with AD. The dynamic changes in levels of VILIP-1, a biomarker of neuronal injury and NFL, a biomarker of axonal damage can alone or in conjunction provide an insight into the longitudinal cognitive changes associated with neurodegeneration. The cognitive decline associated with synaptic degeneration can be well accounted via CSF measure of neurogranin.

Hence, it can be concluded that the CSF biomarkers will certainly benefit in diagnosing AD at an early stage with much higher diagnostic accuracy either alone, together or in conjunction with the core CSF biomarkers. This would also aid in understanding the disease pathogenesis and progression. They can account for the lag between preclinical and clinical AD, and can act as indices of pathological change. They can serve as end point measures in

clinical trials and accelerate the drug development process through the design of new drug molecules that can be targeted on the right individuals at the right stage. The complex nature of AD, definitely directs us toward a strong rationale to use multiple biomarkers for understanding disease pathogenesis, and for a successful and accurate preclinical diagnosis, prognosis and treatment.

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12 CONFLICT OF INTEREST

The author have no conflicts of interest to declare.

KB has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Novartis, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

HZ has served at scientific advisory boards of Eli Lilly, Roche Diagnostics, Samumed, CogRx and Wave, has received travel support from Teva and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

13 AUTHOR CONTRIBUTIONS

14 ABBREVIATIONS

AA: Alzheimer's Association; AD: Alzheimer's disease; ADAM 10: A disintegrin and metalloproteinase domain containing protein 10; ApoE: Apolipoprotein E; APP: Amyloid precursor protein; ARA: Arachidonic acid; AUC: Area under the receiver operating curve; Aβ: Amyloid beta ; Bax: Bcl-2 associated X; cAMP: Cyclic adenosine monophosphate; CDR: Clinical dementia rating; cGMP: Cyclic guanine monophosphate; CHI3L1: Chitinase-3-like-1 protein; CNS: Central Nervous System; CSF: Cerebrospinal fluid ; DAP12: DNAX activating protein of 12 kDa ; DHA: Docosahexaenoic acid; DLB: Dementia with Lewy bodies; ELISA: Enzyme linked immuno sorbent assay; EMA: European Medicine Agency ; EOAD: Early onset Alzheimer's disease; FABP3: Fatty acid binding protein 3; FDA: Food and Drug Administration; FTD: Fronto temporal dementia; HC gp-39: Human cartilage glycoprotein-39; HFABP: Heart type fatty acid binding protein; IWG: International working group; LOAD: Late onset Alzheimer's disease; MCI-AD: MCI who later developed AD; MCP-1: Monocyte chemo attractant protein 1; MDD: Major depressive disorder; MMSE: Mini Mental Status

Examination; MRI: Magnetic resonance imaging; MSA: Multiple system atrophy; NCS's: Neuronal calcium sensors; NFL: Neurofilament light chain protein; NF-κB: Nuclear factor kappa light chain enhancer of activated B cell; NIA: National Institute on Aging; ODD: Other dementia disorders; OND: Other neurological disorders; PD: Parkinson's disease; PDD: Parkinson's disease dementia; PET: Positron emission tomography; PSP: Progressive supranuclear palsy; P-tau: Phosphorylated tau; PUFA: Polyunsaturated fatty acids; SMC: Subjective memory complainers; sMCI: Stable MCI; sTREM2: Soluble ectodomain of triggering receptor expressed on myeloid cells; TREM2: Ectodomain of triggering receptor expressed on myeloid cells; T-tau: Total tau; VaD: Vascular dementia; VSNL1: Visinin like 1; FDG-PET: 18F-2-fluoro-2-deoxy-D-glucose positron emission tomography

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