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New cerebrospinal fluid biomarkers in Alzheimer's disease

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In 2015, we published a review on novel cerebrospinal fluid (CSF) biomarkers for detecting Alzheimer's disease (AD) [1]. We summarized the main potential CSF biomarkers as low amyloid β_{1-42} ($A\beta_{1-42}$) and a high total tau (T-tau) and phosphorylated tau (p-tau). We discussed a number of other potential biomarkers, including those involving blood–brain barrier integrity (PDGFR- β), mitochondrial DNA (mtDNA), VEGF family members, calcium-sensor protein VILIP-1, β -site APP-cleaving enzyme 1 and the astrocyte marker YKL-40. More recently, a number of studies have focused on the potential use of other biomarkers such as TGF- β , neurofilament light polypeptide (NFL), autotaxin, α -syn and complement. These modules offer promise for earlier detection of AD and other neurodegenerative disorders including Parkinson's disease (PD) and mild cognitive impairment (MCI).

TGF- β

TGF- β are multifunctional cytokines that are secreted into the CSF to regulate the development of a number of cell types

including the differentiation, proliferation and apoptosis of neuronal cells [2]. There are three different isoforms of TGF- β in mammals: TGF- β 1, TGF- β 2 and TGF- β 3. Increased levels of TGF- β 1 have been reported in lumbar CSF from AD patients [3] and in ventricular CSF from patients with PD [4], suggesting a possible use for TGF- β 1 as a biomarker for AD. TGF- β 2 has been reported to regulate $A\beta$ -mediated neuronal death but it is unclear what precise role it plays in AD and Lewy body disease (LBD). Chong *et al.* [5] reported a significant increase in neocortical levels of TGF- β 2 in AD and LBD patients but not in PD patients. Interestingly, TGF- β 2 levels also correlated with neurofibrillary tangle scores, Lewy bodies (in the LBD group), dementia severity and soluble $A\beta$ 42 concentration. This study demonstrates that TGF- β 2 is increased in the cortex of AD and dementia with Lewy bodies (DLB) patients and correlates with neuropathological and clinical markers of disease severity; and, therefore, has potential for use as a biomarker or as a target for pharmacological

KEYWORDS

- α -synuclein • Alzheimer's disease
- autotaxin • complement
- dementia • mild cognitive impairment • neurofilament light
- Parkinson's disease • TGF- β

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“These results suggest that autotaxin could predict neuropathology and cognitive deficits in Alzheimer’s disease.”

approaches to AD and DLB. TGF- β has been shown to be increased in CSF of patients with PD and AD but is thought to be an unreliable marker for other neurodegenerative disorders, such as amyotrophic lateral sclerosis, spinocerebellar degeneration and multiple system atrophy-cerebellar subtype. For this reason, it might be a helpful biomarker that could be used to distinguish between AD and PD from other neurodegenerative disorders.

Neurofilament light polypeptide

Neurofilaments are a group of heteropolymers found in the nervous systems and are responsible for the radial growth of axons during development, maintenance of axon caliber and neural transmission. One member of the family of neurofilaments is called neurofilament light polypeptide and is thought to correlate with axonal degeneration. CSF NFL levels have been reported to be associated with age [6], white matter lesions [7], AD [8] and other neurodegenerative diseases. A recent study of CSF NFL subunit levels in patients with MCI showed a positive correlation between NFL levels and hippocampal atrophy, suggesting that CSF NFL might offer promise as a marker for progression to AD [9].

The hippocampus is one of the areas of the brain with the highest atrophy rate in aging. In a study of 144 cognitively healthy older individuals, Idland *et al.* reported that NFL levels predicted neurodegeneration as measured by hippocampal atrophy rate independently of age, β -amyloid 1–42 and p-tau [10]. They concluded that NFL was likely to be a biomarker of neurodegeneration independent of AD or age; however, further research is required to test the predictive value of NFL in the onset and progression of prodromal AD.

Autotaxin

Ectonucleotide pyrophosphatase/phosphodiesterase 2, also known as autotaxin, is produced by beige adipose tissue and acts to regulate energy metabolism. Autotaxin gene expression was increased in the frontal cortices of AD patients compared with non-AD patients, with a tendency for higher gene expression in patients with the ApoE e3/e4 genotype compared with the ApoE e3/e3 genotype [11]. Recently, elevated autotaxin levels have been found in the CSF of MCI and AD patients compared with healthy controls [12]. Interestingly, autotaxin was

also positively correlated with levels of T-tau, p-Tau181, T-tau/A β 1–42 as well as p-Tau181/A β 1–42, although to a lesser degree. These results suggest that autotaxin could predict neuropathology and cognitive deficits in AD.

α -syn

α -syn is involved in the regulation of synaptic plasticity, dopamine synthesis and neuronal differentiation. Abnormal accumulation of α -syn in the brain is a pathological hallmark of PD and DLB, and can be found in the brains of individuals with AD. Therefore, there has been recent interest in developing an assay for the detection of α -syn as a biomarker for neurodegenerative disorders. A simple ELISA-based method of detecting total α -syn in CSF has recently been developed and validated using electrochemiluminescence technology in 50 CSF samples [13]. This assay offers promise for use as a biomarker not only in AD, but also in other neurodegenerative disorders including PD and prion disease [14]. As α -syn is found in other neurodegenerative conditions, its use as a marker to distinguish AD from other diseases is probably restricted.

Complement

Complement is an integral part of the innate immune system whose role is to recognize a target and opsonize it for engulfment and destruction by phagocytes. This is achieved by complement assembling the membrane attack complex which acts to create holes in a foreign pathogen’s protective membrane. One major fault of this system is that complement can mistakenly attack host tissue which is known as bystander lysis. This mechanism can possibly contribute to the pathogenesis of AD.

Three decades ago, it was discovered that complement could opsonize A β deposits in the brain of AD patients [15] and, soon after, it was reported that the membrane attack complex could attack dystrophic neurites [16]. The genes for C1q, C3 and C4 are expressed in the brain and have been reported to be upregulated more than threefold in AD [17]. Rogers *et al.* subsequently reported that C1q binds strongly to the A β peptide suggesting that the classical pathway could be activated by any tissue component recognized by complement [18]. Extracellular A β deposits in the brain have been reported to directly activate the complement system by associating with the opsonizing components

of complement. Specifically, C1q can bind to A β and its N-terminal fragments which can potentially trigger the inflammatory cascade of AD [19]. Moreover, levels of complement components C3 and C4 and complement receptor 1 (CR1) have been reported to be elevated in the CSF of AD patients [20]. Daborg *et al.* reported that CSF levels of C3 and C4 were elevated in AD patients when compared with MCI patients who progressed to AD [20]. In addition, CSF levels of CR1 were elevated after combining MCI and AD groups compared with controls. These results, while promising, were deemed not useful for diagnostic purposes due to the small changes detected and could be strengthened by involving larger numbers of patients in future studies.

Conclusion

There have been some interesting and promising recent advances in detecting and validating potential CSF biomarkers for use in AD and other neurodegenerative disorders. A recent emphasis has been on immunological factors. TGF- β is reported to be increased in the cortex of AD and DLB patients and correlates with neuropathological and clinical markers of disease severity, and therefore has potential for use as a biomarker for AD. NFL could potentially be developed as a biomarker for neurodegeneration

which is independent of age or AD status; therefore, it might be helpful to distinguish AD from other neurodegenerative disorders. Recent studies report increased gene expression of autotaxin in the frontal cortex of AD patients and increased levels in the CSF of MCI and AD patients which could predict neuropathology and cognitive deficits in AD. α -syn has been shown to be increased in the CSF of AD, PD and prion disease patients, and is, therefore, a useful marker for neurodegeneration but might not help in distinguishing these conditions. Other studies have demonstrated increased levels of complement C3 and C4, as well as CR1, in the CSF of AD patients. Taken together these studies not only offer hope for the development of biomarkers to detect AD and its progression, but also the advancement of therapeutic targets to limit disease progression.

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