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·Review·

Cerebrospinal fluid biomarkers of Alzheimer's disease

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Alzheimer's disease (AD) is a fatal neurodegenerative disorder that takes about a decade to develop, making early diagnosis possible. Clinically, the diagnosis of AD is complicated, costly, and inaccurate, so it is urgent to find specific biomarkers. Due to its multifactorial nature, a panel of biomarkers for the multiple pathologies of AD, such as cerebral amyloidogenesis, neuronal dysfunction, synapse loss, oxidative stress, and inflammation, are most promising for accurate diagnosis. Highly sensitive and high-throughput proteomic techniques can be applied to develop a panel of novel biomarkers for AD. In this review, we discuss the metabolism and diagnostic performance of the well-established core candidate cerebrospinal fluid (CSF) biomarkers (β-amyloid, total tau, and hyperphosphorylated tau). Meanwhile, novel promising CSF biomarkers, especially those identified by proteomics, updated in the last five years are also extensively discussed. Furthermore, we provide perspectives on how biomarker discovery for AD is evolving.

Keywords: Alzheimer's disease; biomarker; cerebrospinal fluid; β-amyloid; tau; proteomics

Introduction

Alzheimer's disease (AD) is becoming more prevalent due to the increasing population of people aged over 65[1]. It is estimated that it will affect 66 million by 2030 and 115 million by 2050 worldwide if no effective therapeutic strategies are found[2]. Clinically, AD is characterized by cognitive impairment, progressive disturbance of daily activities, many neuropsychiatric symptoms, and behavioral deterioration[3-8]. Pathologically, it is characterized by deposition of extracellular neuritic plaques composed of β-amyloid (Aβ)[9] and intracellular neurofibrillary tangles (NFTs) consisting of a hyperphosphorylated form of the microtubule-associated protein tau[10-12]. Besides, loss of neurons and synapses is also a pathological hallmark of AD[13-15]. The worldwide cost of dementia is huge and expected to skyrocket in the next few years. AD may become one of the most marked social, health, and economic challenges in the twenty-first century[16].

Clinically, the procedure for the diagnosis of AD is difficult and complex. Neuropsychological tests, such as the mini-mental state examination, magnetic resonance imaging of hippocampal volume, and clinical assessment, are used to aid in diagnosis. However, a definitive diagnosis of AD requires confirmation at autopsy. It is estimated to take 8–10 years or longer before mild cognitive impairment (MCI) develops into AD[17, 18]. Meanwhile, intervention therapies work most effectively in the early stage of AD. Thus, it is urgent and feasible to seek novel specific biomarkers to aid in diagnosis and the evaluation of treatments[19]. Surrounding the brain and spinal cord, cerebrospinal fluid (CSF) is an ideal source reflecting biochemical changes in the brain of the AD patient[20]. Extensive studies have focused on seeking AD biomarkers in CSF.

A comprehensive search of the Web of Science (January 1990 through November 2013) was conducted with the keywords: "Biomarker", "Alzheimer's disease" and "CSF", limited to studies in English. In this literature, the metabolism and performance of the core CSF biomarkers Aβ42, t-tau, and p-tau are reviewed. Also, emerging potential CSF biomarkers updated in last five years are discussed.

Candidate Core CSF Biomarkers

CSF Aβ42 as a Biomarker of AD

Aβ is a hydrophobic peptide of ~4 kDa and 38–42 amino-acids[21] that can aggregate automatically to form neuritic plaques in the brain. The accumulation and deposition of Aβ is regarded as the critical event contributing to the pathological processes of AD[9]. Aβ is a proteolytic cleavage product of amyloid precursor protein (APP)[22, 23]. APP is a single membrane-spanning protein with three major alternate cleavage sites and can be spliced by three proteases: α-, β-, and γ-secretases[24]. When APP is cleaved by β-secretase, it produces β-secretase-soluble APP (β-sAPP). Then β-sAPP is cleaved by γ-secretase and Aβ is produced. Alternatively, when APP is cleaved sequentially by α- and γ-secretases, Aβ is not produced. There are two dominant Aβ products, Aβ42 and Aβ40; Aβ42 has two more hydrophobic amino-acids[25, 26]. This small change enables Aβ42 to aggregate faster than Aβ40 in adequate solutions. Aβ42 is the major component of neuritic plaques, while Aβ40 predominates in the vascular system and CSF[27-30].

As the main pathogenic factor in AD, Aβ is a potential biomarker. The change of total Aβ (t-Aβ), Aβ42, and Aβ40 in AD patients at different stages of the disease has been extensively studied. However, a number of studies showed only a minor decrease of CSF t-Aβ in AD patients relative to healthy controls, while some studies found no marked difference between AD patients and the controls[31-33]. The CSF levels of Aβ40 are not altered or slightly elevated[32]. Many studies demonstrated that the levels of CSF Aβ42 in AD are decreased compared with controls[34-50]. CSF Aβ42 can differentiate AD from controls with 89% sensitivity and 90% specificity[51]. Nevertheless, some studies report an increase[52] or no change[53] in the levels of CSF Aβ42. The varying results may be partially ascribed to the different measurement methods in these studies. Alternatively, it may be that the levels of Aβ42 are significantly decreased in early AD and increase at the severe stage of the disease. Since the level of Aβ42 can be used to predict disease progression from MCI to AD[54], it may be an early biomarker of AD. In addition, as an internal standard, Aβ40 augments the specificity and sensitivity of Aβ42[55].

CSF t-tau and p-tau as Biomarkers of AD

NFTs, predominantly composed of hyperphosphorylated tau, are another pathological hallmark of AD[56]. Tau undergoes abnormal hyperphosphorylation at various sites, such as threonine 181 and 231, and serine 199, 235, 396 and 404[35]. Due to aberrant phosphorylation, tau is unable to bind and stabilize the microtubules, contributing to neuronal loss[57].

A number of studies found increased levels of CSF t-tau in AD cases relative to controls[47, 56-64]. CSF t-tau has a sensitivity of 83% and a specificity of 90% in discriminating AD from healthy controls[58]. Taking into account that CSF tau is also elevated in other neurological disorders, such as Parkinson's disease (PD) and frontotemporal dementia (FTD), it is essential to augment the specificity of CSF t-tau as a biomarker for AD.

CSF p-tau correlates well with cognitive decline and is boosted in AD patients compared with normal controls[57-59]. This may be attributed to the release of p-tau from degenerating neurons and subsequent diffusion into the CSF. At the same time, increasing p-tau could disturb the balance of binding between tau and microtubules, leading to higher levels of p-tau in the cytosol. This vicious cycle could contribute to neuronal degeneration. P-tau has 68% sensitivity and 73% specificity in differentiating AD from healthy controls, and 80–88% sensitivity and 42–52% specificity in predicting incipient AD in MCI stages[60]. Hempel *et al.* verified the accuracy of CSF p-tau231, p-tau181, and p-tau199 in discriminating AD from FTD, Lewy-body dementia, vascular dementia, and healthy controls[61]. The study revealed that each of the three p-tau markers is significantly elevated in AD compared with the other groups, while p-tau231 provides the greatest discrimination between AD and other neurological disorders, and the combination of all three does not augment the discrimination[61, 62]. Similarly, several other studies have revealed that p-tau231 and p-tau199 can discriminate AD from other dementias with sensitivities and specificities in the range of 80–90%[63-65]. It is worth noting that, as a microtubule-associated protein, hyperphosphorylation of neurofilaments potentially differentiates AD from both normal aging and other dementias[66].

Combination of Aβ42, t-tau, and p-tau as Biomarkers of AD

Numerous studies aimed to evaluate the combined use of Aβ42, t-tau, and p-tau have revealed that this diagnoses

AD with greater sensitivity and specificity than each alone. Mattsson *et al.* reported that combination of the Aβ42/p-tau ratio with t-tau discriminates incipient AD with a sensitivity of 78–88% and a specificity of 68–76%[60]. Shoji *et al.* demonstrated that combination of t-tau and Aβ42 has a sensitivity of 69% and a specificity of 88% in the diagnosis of AD[55]. Shaw *et al.* revealed that the combination of Aβ42, t-tau, and the number of apolipoprotein E4 alleles discriminates mild AD from MCI and healthy controls with a sensitivity of 98.2% and a specificity of 89.9%[67]. Mulder *et al.* revealed that Aβ42, t-tau, and p-tau diagnose AD with a sensitivity of 93.5% and specificity of 82.7%[68].

Novel Candidate CSF Biomarkers

Although decreased Aβ42 with elevated t-tau and p-tau in CSF has high sensitivity and specificity in the diagnosis of AD, great efforts have been invested in discovering novel candidate biomarkers to improve diagnosis. In the past five years, a number of such biomarkers have been identified on the basis of AD pathogenesis, such as cerebral amyloidogenic pathology, neuronal dysfunction, synapse loss, oxidative stress, and inflammation (Table 1).

Cerebral Amyloidogenic Pathology

S100A7 S100A7 is involved in immune responses and has been reported to inhibit the production of Aβ42 and Aβ40. The underlying mechanism may be ascribed to the selective enhancement of α-secretase activity. Qin *et al.* revealed that the CSF levels of S100A7 in AD patients are markedly higher than healthy controls using a proteomic technique. S100A7 has potential to serve as a biomarker of AD[69].

Beta-site APP-cleaving enzyme (BACE1) BACE1 is responsible for the free release of Aβ. Ohno *et al.* found that CSF BACE1 activity and levels are increased in AD brains, suggesting that it may serve as a biomarker[70]. Another study reported that CSF BACE1 activity is highly correlated with hippocampal atrophy in AD and may reflect neurotoxic Aβ-related processes[71].

Aβ40 oligomers and Aβ42 Oligomeric Aβ species may correlate with the onset of disease due to their role in the pathophysiology of AD. Carol *et al.* showed that, in combination with total Aβ42, Aβ40 oligomers can diagnose AD remarkably with >95% sensitivity and >90% specificity[72].

Aβ40 Although Aβ42, t-tau, and p-tau can discriminate AD from controls with high sensitivity and specificity, adding Aβ40 to the core CSF biomarkers boosts this discrimination[73]. Aβ40 is useful in differential diagnosis among FTD, AD, and controls.

Aβ2-42 Aβ2-42 and Aβ1-42 are both reduced in CSF in AD, but Aβ1-42 does not have sufficient specificity to exclude other dementias. Aβ2-42 distinguishes AD from FTD with an accuracy of >85%, indicating that Aβ2-42 is a promising biomarker for differentiating AD from other degenerative diseases[74].

Lipoprotein receptor 11 (LR11) LR11 is a sorting protein that suppresses the production of Aβ, indicating that it is associated with the pathogenesis of AD. The levels of LR11 are higher in the CSF of AD patients than in FTD patients and controls, suggesting potential for distinguishing AD from FTD[75].

Macrophage colony-stimulating factor (M-CSF) A hematopoietic growth factor, M-CSF activates microglial cells and is involved in the phagocytosis of Aβ in the brain. The CSF levels of M-CSF are lower in AD than in other non-inflammatory neurological diseases. Further, M-CSF can distinguish MCI patients from controls with 73.7% sensitivity and 75.0% specificity, indicating that it is a potential biomarker for MCI[76].

TNF-α converting enzyme (TACE) and tumor necrosis factor receptors (TNFRs) Genetic deletion of TNFR1 reduces amyloid plaques and Aβ production through BACE1 regulation in an AD mouse model[77]. The CSF levels of TNFR1 and TNFR2 are lower in AD patients than in healthy controls. The activity of TACE and the CSF levels of soluble TNFRs are elevated in MCI relative to AD[77], suggesting that TACE activity and soluble TNFRs may be biomarkers of AD and MCI.

Synapse Loss

Neuronal pentraxin receptor (NPR) NPR belongs to the neuronal pentraxin family that is dominantly expressed in the central nervous system. Proteins in this family facilitate the uptake of synaptic materials during synapse formation and remodeling. A proteomic study by Yin *et al.* showed that the increased NPR level in the CSF of AD patients compared with normal controls is higher than that of PD patients, indicating that NPR is a possible marker for distinguishing AD from PD[78].

Table 1. Novel candidate CSF biomarkers of AD

Biomarker	Year	Pathogenic process	Changes in biomarker level in AD	Comment
S100A7	2009	Immune response	Increase in CSF	Promotion of S100A7 expression in the brain may selectively promote α-secretase activity in the brain of AD, precluding the generation of amyloidogenic peptides[69].
NPR	2009	Synapse loss	Increase in CSF	A higher level of NPR in AD serum[78].
Dkk-3	2009	Unknown function	Increase in CSF	Significantly increased Dkk-3 levels in plasma and CSF in AD patients compared with healthy subjects but not patients suffering from MCI or depression[86]
Aβ42, t-tau, p-tau	2010	Pathological hallmarks of AD	Decreased Aβ42, increased t-tau and p-tau	Discriminating AD from non AD neurological comparison group with 93.5% sensitivity and 82.7% specificity[68].
BACE1	2010	Aβ metabolism	Increased BACE1 activity	BACE1 is the major beta-secretase involved in Aβ production in the brain[70,71].
YKL-40	2010	Neuroinflammatory response	Increase in CSF	CSF YKL-40/Aβ42 ratio could predict risk of developing cognitive impairment[83].
Aβ40 oligomers/ Aβ42	2010	Aβ metabolism	Increase in CSF	95% sensitivity and 90% specificity between AD and non AD neurological comparison group[72]
LR11	2010	Lipid metabolism	Increase in CSF	Limited diagnostic value for individual patients[75]
Sphingomyelin	2010	Lipid metabolism	Increase in CSF	Sphingomyelin is phospholipid[87].
MCSF	2010	Phagocytosis of Aβ	Decrease in CSF	73.3% sensitivity and 75.0% specificity between MCI and other non-inflammatory neurological disease[76]
Aβ40	2010	Aβ metabolism	Decrease in CSF	CSF Aβ40 levels added to the conventional CSF biomarkers increases the potential to discriminate subjects with dementia from controls[73].
PEDF/ haptoglobin/ tau	2011	Unknown function	Unknown	Improving the diagnostic accuracy of AD[88]
Chemokines	2011	Inflammation	Increase in CSF	Chemokines are up-regulated in resident CNS cells during AD, which may contribute to plaque-associated inflammation and neurodegeneration[82].
Twelve-protein panel	2011	Unknown function		Cystatin C, VEGF, TRAIL-R3, PAI-1, PP, NT-proBNP, MMP-10, MIF, GRO-alpha, fibrinogen, FAS, eotaxin-3[92]
Synaptic proteins	2011	Synapse loss	Unknown	Distinguishing early AD from MCI with a sensitivity of 87% and a specificity of 83%[93]
TACE activity and soluble TNFRs	2011	Aβ metabolism	Decrease in CSF	Distinguishing MCI from healthy controls[77]
VILIP-1	2011	Neuroinflammation	Increase in CSF	Discriminating AD from controls[84,85]
F2-isoprostanes	2011	Oxidative stress	Increase in CSF	Increasing CSF F2-isoprostanes across the human lifespan[81]
Four-protein panel	2011	Unknown function		Improving the diagnostic accuracy of Aβ42 and tau[91]
Aβ2-42	2012	Aβ metabolism	Decrease in CSF	Differentiating AD from other degenerative dementias[74]
Seven-protein panel	2012	Unknown function		Classifying AD cases from controls with median accuracy of 84.5% (sensitivity 93.3%, specificity 75.7%)[90]
Alpha-synuclein	2013	Synapse loss	Increase in CSF	CSF alpha-syn and calculation of alpha-syn-p-tau(181)-Mis improves the diagnostic sensitivity/specificity of classic CSF AD biomarkers[79]
Kyotorphin	2013	Neuronal death	Decrease in CSF	Significant difference between p-tau/KTP values in AD and control groups[80]
hFABP/VEGF	2013	Unknown function		Classifying AD cases from controls with a sensitivity of 83% and a specificity of 86% in combination with Aβ42 t-tau and p-tau[89]

BACE1, beta-site APP-cleaving enzyme; CSF, cerebrospinal fluid; Dkk-3, Dickkopf homolog 3; hFABP, heart-type fatty acid binding protein; LR11, lipoprotein receptor 11; MCSF, macrophage colony-stimulating factor; NPR, neuronal pentraxin receptor; PEDF, pigment epithelium-derived factor; TACE, TNF-α converting enzyme; TNFRs, tumor necrosis factor receptors; VEGF, vascular endothelial growth factor; VILIP-1, visinin-like protein-1.

Alpha-synuclein Alpha-synuclein plays an important role in maintaining the supply of synaptic vesicles to presynaptic terminals. Toledo *et al.* discovered that alpha-synuclein is strongly correlated with t-tau in controls, as well as in patients with AD and MCI; similarly, a higher p-tau181 level together with lower alpha-synuclein levels was found in a subset of patients in the Alzheimer's Disease Neuroimaging Initiative[79]. Alpha-synuclein enhances the sensitivity and specificity of t-tau and p-tau in diagnosing AD and improves the prediction of longitudinal cognitive decline.

Kyotorphin (KTP) KTP is an endogenous neuroprotective and neuromodulatory analgesic dipeptide (Tyr-Arg). Santos *et al.* reported that it has an inverse correlation with p-tau, and that p-tau/KTP values differ significantly between AD cases and controls[80]. Thus, KTP has potential utility in diagnosing AD.

Oxidative Stress

F2-isoprostanes Both the early and severe stages of AD feature increased free-radical injury to different regions of the brain. F2-isoprostanes are biomarkers of such injury in various diseases. The levels of CSF F2-isoprostanes increase with aging in the healthy population, and they are increased in AD relative to healthy controls[81], indicating that they have potential to serve as biomarkers of AD.

Inflammation

Chemokines Chemokines and chemokine receptors are up-regulated in brain cells during AD, contributing to plaque-associated inflammation and neurodegeneration. The expression of CSF chemokine ligand 2 (CCL2), a member of the chemokine family that plays a significant role in inflammatory processes, is increased in AD and is positively correlated with p-tau levels[82]. CCL2 may serve as a potential biomarker to monitor the progression of AD.

YKL-40 YKL-40, also known as chitinase 3-like 1, is a secreted 40-kDa glycoprotein with a role in inflammation and tissue remodeling, but its physiological functions remain unclear. The expression of YKL-40 in the CSF is elevated in early AD. The ratio of YKL-40 to Aβ42 predicts cognitive impairment as well as the best CSF biomarkers (Aβ42, t-tau, and p-tau)[83], suggesting potential as a biomarker for preclinical AD.

Visinin-like protein-1 (VILIP-1) VILIP-1 is a potential marker of neuronal injury. Tarawneh *et al.* investigated CSF VILIP-1 and the VILIP-1/Aβ42 ratio as diagnostic and prognostic markers in early AD. It was concluded that CSF VILIP-1 levels can differentiate individuals with AD from cognitively normal controls and patients with other dementias, and that CSF VILIP-1/Aβ42 predicts cognitive impairment as well as tau/Aβ42 and p-tau181/Aβ42[84, 85].

Other Potential Biomarkers

Dickkopf homolog 3 (Dkk-3) Dkk-3 is a novel potential biomarker for AD. Significantly elevated Dkk-3 levels in CSF were found in AD patients compared with healthy individuals but not in patients suffering from MCI or depression[86], indicating that elevated Dkk-3 levels are specifically associated with AD.

Sphingomyelin (SM) SM is one of the major phospholipid classes and increasing evidence demonstrates that lipids are critical in AD. SM levels in CSF of probable AD patients are higher than in controls[87], indicating that SM could serve as a biomarker of AD.

Pigment epithelium-derived factor (PEDF), haptoglobin, and tau PEDF and haptoglobin in CSF identified by proteomic analysis differ between healthy controls and AD patients. In combination with tau, PEDF and haptoglobin could improve the differential diagnosis of AD, especially in cases with moderate to severe dementia. PEDF, haptoglobin, and tau are potential markers for diagnosing AD[88], while needing further investigation in larger population.

Heart-type fatty-acid-binding protein (hFABP) and vascular endothelial growth factor (VEGF) With proteomic techniques, the levels of hFABP and VEGF markedly differ between healthy controls and AD patients. Combined with the three core biomarkers (Aβ42, t-tau, and p-tau), hFABP and VEGF can distinguish AD dementia from healthy controls with 83% sensitivity and 86% specificity. hFABP also predicts the progression from MCI to AD dementia. The study by Guo *et al.* supports the potential of hFABP and VEGF in CSF as AD biomarkers in combination with Aβ42, t-tau, and p-tau181[89].

CSF Proteomic Biomarkers of AD

Multiple techniques have been applied to exploring biomarkers of AD, including proteomics and microarray chips. Proteomic analysis, especially with quantitative methods, identifies informative proteins that may serve as biomarkers.

Seven-protein panel Vafadar-Isfahani *et al.* investigated

the diagnosis of AD with CSF proteomic fingerprinting samples from 33 AD patients, 20 age-matched controls, and 10 patients with MCI. They found that a panel of seven peptides (SPARC-like 1 protein, fibrinogen α-chain precursor, Aβ, apolipoprotein E precursor, serum albumin precursor, keratin type I cytoskeletal 9, and tetranectin) was able to discriminate AD patients from controls with a median accuracy of 95% (sensitivity 85% and specificity 97%)[90].

Four-protein panel Perrin *et al.* took advantage of proteomics to identify four novel potential biomarkers (NrCAM, YKL-40, chromogranin A, and carnosinase I) that could improve the diagnostic accuracy of Aβ42 and tau. The panel of six biomarkers describes six clinicopathological stages from normal to mild dementia, including stages defined by increasing risk of cognitive decline, and might improve the efficiency of clinical trials by monitoring disease progression[91].

Twelve-protein panel Craig-Schapiro *et al.* used targeted proteomic screening to identify novel candidate biomarkers (cystatin C, VEGF, TRAIL-R3, PAI-1, PP, NT-proBNP, MMP-10, MIF, GRO-α, fibrinogen, FAS, and eotaxin-3) that augment the core CSF biomarkers (Aβ42, t-tau, and p-tau) for distinguishing very mildly/mildly demented from cognitively normal individuals[92]. In addition, calbindin may be a novel biomarker.

Synaptic proteins Jahn *et al.* used capillary electrophoresis–mass spectrometry to detect low-molecular-weight peptides identified as synaptic proteins like proSAAS, ApoJ, neurosecretory protein VGF, phospholemman, and chromogranin A to diagnose AD with a sensitivity of 87% and a specificity of 83%, while CSF Aβ42, t-tau, and p-tau diagnosed AD with a sensitivity of 88% and a specificity of 67% in the same sample[93]. Such specific peptide fingerprints may allow early differential diagnosis of various dementias and distinction of incipient AD from MCI.

Conclusions and Perspectives

Although numerous studies have shown that the well-established CSF biomarkers Aβ42, t-tau, and p-tau can differentiate AD from MCI and predict the progression of AD, these biomarkers cannot discriminate AD from other dementias with satisfactory specificity. More specific biomarkers are required to optimize the diagnosis. Since it is almost impossible to diagnose AD using a single biomarker, it is most promising for a panel of highly sensitive and specific biomarkers to work together to achieve the goal of good performance. New informative techniques such as isobaric tags for relative and absolute quantitation (iTRAQ), two-dimensional gel electrophoresis (2D-DIGE), tandem mass spectrometry (MS/MS), and label-free methods can be used to develop a panel of novel promising biomarkers for AD, especially for early AD. In this case, an optimized panel of biomarkers reflecting cerebral amyloidogenic pathology, neuronal dysfunction, synapse loss, oxidative stress, and inflammation could facilitate the diagnosis of AD, predict its progression, and monitor the effects of therapeutic drugs. Since a number of promising findings can not be transferred to clinical testing due to the limitation of small sample size, future studies should also focus on validation of the previous promising studies with larger sample sizes.

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