

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 (A β)^[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 A β 42, t-tau, and p-tau are reviewed. Also, emerging potential CSF biomarkers updated in last five years are discussed.

Candidate Core CSF Biomarkers

CSF A β 42 as a Biomarker of AD

A β 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 A β is regarded as the critical event contributing to the pathological processes of AD^[9]. A β 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 A β is produced. Alternatively, when APP is cleaved sequentially by α - and γ -secretases, A β is not produced. There are two dominant A β products, A β 42 and A β 40; A β 42 has two more hydrophobic amino-acids^[25, 26]. This small change enables A β 42 to aggregate faster than A β 40 in adequate solutions. A β 42 is the major component of neuritic plaques, while A β 40 predominates in the vascular system and CSF^[27–30].

As the main pathogenic factor in AD, A β is a potential biomarker. The change of total A β (t-A β), A β 42, and A β 40 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-A β 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 A β 40 are not altered or slightly elevated^[32]. Many studies demonstrated that the levels of CSF A β 42 in AD are decreased compared with controls^[34–50]. CSF A β 42 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 A β 42. The varying results may be partially ascribed to the different measurement methods in these studies. Alternatively, it may be that the levels of A β 42 are significantly decreased in early AD and increase at the severe stage of the disease. Since the level of A β 42 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, A β 40 augments the specificity and sensitivity of A β 42^[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 A β 42, t-tau, and p-tau as Biomarkers of AD

Numerous studies aimed to evaluate the combined use of A β 42, 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 A β 42/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 A β 42 has a sensitivity of 69% and a specificity of 88% in the diagnosis of AD^[55]. Shaw *et al.* revealed that the combination of A β 42, 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 A β 42, 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 A β 42 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 A β 42 and A β 40. 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 A β . 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 A β -related processes^[71].

A β 40 oligomers and A β 42 Oligomeric A β 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 A β 42, A β 40 oligomers can diagnose AD remarkably with >95% sensitivity and >90% specificity^[72].

A β 40 Although A β 42, t-tau, and p-tau can discriminate AD from controls with high sensitivity and specificity, adding A β 40 to the core CSF biomarkers boosts this discrimination^[73]. A β 40 is useful in differential diagnosis among FTD, AD, and controls.

A β 2-42 A β 2-42 and A β 1-42 are both reduced in CSF in AD, but A β 1-42 does not have sufficient specificity to exclude other dementias. A β 2-42 distinguishes AD from FTD with an accuracy of >85%, indicating that A β 2-42 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 A β , 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 A β 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 A β 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 ^[68] .
A β 42, t-tau, p-tau	2010	Pathological hallmarks of AD	Decreased A β 42, 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	A β metabolism	Increased BACE1 activity	BACE1 is the major beta-secretase involved in A β production in the brain ^[70,71] .
YKL-40	2010	Neuroinflammatory response	Increase in CSF	CSF YKL-40/A β 42 ratio could predict risk of developing cognitive impairment ^[63] .
A β 40 oligomers/A β 42	2010	A β 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 A β	Decrease in CSF	73.3% sensitivity and 75.0% specificity between MCI and other non-inflammatory neurological disease ^[76] .
A β 40	2010	A β metabolism	Decrease in CSF	CSF A β 40 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- α , 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	A β 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 A β 42 and tau ^[91] .
A β 2-42	2012	A β 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] .
Kytorphin	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 A β 42 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 A β 42 predicts cognitive impairment as well as the best CSF biomarkers (A β 42, 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/A β 42 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/A β 42 predicts cognitive impairment as well as tau/A β 42 and p-tau181/A β 42^[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 (A β 42, 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 A β 42, 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, A β , 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 A β 42 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 (A β 42, 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, phospholemmann, and chromogranin A to diagnose AD with a sensitivity of 87% and a specificity of 83%, while CSF A β 42, 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 A β 42, 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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REFERENCES

- [1] Apostolova LG, Hwang KS, Andrawis JP, Green AE, Babakchanian S, Morra JH, *et al.* 3D PIB and CSF biomarker associations with hippocampal atrophy in ADNI subjects. *Neurobiol Aging* 2010, 31: 1284–1303.
- [2] Wimo A, Prince M. World Alzheimer Report 2010: The Global Economic Impact of Dementia. London: Alzheimer's Disease International, 2010.
- [3] Kirk A, Kertesz A. On drawing impairment in Alzheimer's disease. *Arch Neurol* 1991, 48: 73–77.
- [4] Pillon B, Deweer B, Agid Y, Dubois B. Explicit memory in Alzheimer's, Huntington's, and Parkinson's diseases. *Arch Neurol* 1993, 50: 374–379.
- [5] Price BH, Gurvit H, Weintraub S, Geula C, Leimkuhler E, Mesulam M. Neuropsychological patterns and language deficits in 20 consecutive cases of autopsy-confirmed

- Alzheimer's disease. *Arch Neurol* 1993, 50: 931–937.
- [6] Galton CJ, Patterson K, Xuereb JH, Hodges JR. Atypical and typical presentations of Alzheimer's disease: a clinical, neuropsychological, neuroimaging and pathological study of 13 cases. *Brain* 2000, 123: 484–498.
- [7] Esteban-Santillan C, Praditsuwan R, Ueda H, Geldmacher DS. Clock drawing test in very mild Alzheimer's disease. *J Am Geriatr Soc* 1998, 46: 1266–1269.
- [8] Greene JD, Baddeley AD, Hodges JR. Analysis of the episodic memory deficit in early Alzheimer's disease: evidence from the doors and people test. *Neuropsychologia* 1996, 34: 537–551.
- [9] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002, 297: 353–356.
- [10] Braak H, Braak E. Development of Alzheimer-related neurofibrillary changes in the neocortex inversely recapitulates cortical myelogenesis. *Acta Neuropathol* 1996, 92: 197–201.
- [11] Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci U S A* 1986, 83: 4913–4917.
- [12] Pallas M, Camins A. Molecular and biochemical features in Alzheimer's disease. *Curr Pharm Des* 2006, 12: 4389–4408.
- [13] Pappas BA, Bayley PJ, Bui BK, Hansen LA, Thal LJ. Choline acetyltransferase activity and cognitive domain scores of Alzheimer's patients. *Neurobiol Aging* 2000, 21: 11–17.
- [14] Palmer AM, Stratmann GC, Procter AW, Bowen DM. Possible neurotransmitter basis of behavioral changes in Alzheimer's disease. *Ann Neurol* 1988, 23: 616–620.
- [15] Bobinski M, Wegiel J, Tarnawski M, Bobinski M, Reisberg B, de Leon MJ, *et al.* Relationships between regional neuronal loss and neurofibrillary changes in the hippocampal formation and duration and severity of Alzheimer disease. *J Neuropathol Exp Neurol* 1997, 56: 414–420.
- [16] Ray S, Reddy PJ, Jain R, Gollapalli K, Moiyadi A, Srivastava S. Proteomic technologies for the identification of disease biomarkers in serum: Advances and challenges ahead. *Proteomics* 2011, 11: 2139–2161.
- [17] Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. *Ann Neurol* 1999, 45: 358–368.
- [18] Perrin RJ, Fagan AM, Holtzman DM. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. *Nature* 2009, 461: 916–922.
- [19] Cummings JL. Biomarkers in Alzheimer's disease drug development. *Alzheimers Dement* 2011, 7: e13–44.
- [20] Blennow K. CSF biomarkers for Alzheimer's disease: use in early diagnosis and evaluation of drug treatment. *Expert Rev Mol Diagn* 2005, 5: 661–672.
- [21] Evin G, Weidemann A. Biogenesis and metabolism of Alzheimer's disease Abeta amyloid peptides. *Peptides* 2002, 23: 1285–1297.
- [22] Sisodia SS. Beta-amyloid precursor protein cleavage by a membrane-bound protease. *Proc Natl Acad Sci U S A* 1992, 89: 6075–6079.
- [23] Jonsson T, Atwal JK, Steinberg S, Snaedal J, Jonsson PV, Bjornsson S, *et al.* A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 2012, 488(7409): 96–99.
- [24] Kang J, Lemaire HG, Unterbeck A, Salbaum JM, Masters CL, Grzeschik KH, *et al.* The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 1987, 325: 733–736.
- [25] Masters CL, Cappai R, Barnham KJ, Villemagne VL. Molecular mechanisms for Alzheimer's disease: implications for neuroimaging and therapeutics. *J Neurochem* 2006, 97: 1700–1725.
- [26] McLean CA, Cherny RA, Fraser FW, Fuller SJ, Smith MJ, Beyreuther K, *et al.* Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann Neurol* 1999, 46: 860–866.
- [27] Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). *Neuron* 1994, 13: 45–53.
- [28] Brouillette J, Caillierez R, Zommer N, Alves-Pires C, Benilova I, Blum D, *et al.* Neurotoxicity and memory deficits induced by soluble low-molecular-weight amyloid-beta1-42 oligomers are revealed *in vivo* by using a novel animal model. *J Neurosci* 2012, 32: 7852–7861.
- [29] Watts JC, Giles K, Grillo SK, Lemus A, DeArmond SJ, Prusiner SB. Bioluminescence imaging of Abeta deposition in bigenic mouse models of Alzheimer's disease. *Proc Natl Acad Sci U S A* 2011, 108: 2528–2533.
- [30] Roher AE, Lowenson JD, Clarke S, Woods AS, Cotter RJ, Gowing E, *et al.* beta-Amyloid-(1-42) is a major component of cerebrovascular amyloid deposits: implications for the pathology of Alzheimer disease. *Proc Natl Acad Sci U S A* 1993, 90: 10836–10840.
- [31] Lannfelt L, Basun H, Vigo-Pelfrey C, Wahlund LO, Winblad B, Lieberburg I, *et al.* Amyloid beta-peptide in cerebrospinal fluid in individuals with the Swedish Alzheimer amyloid precursor protein mutation. *Neurosci Lett* 1995, 199: 203–206.
- [32] Southwick PC, Yamagata SK, Echols CL, Jr., Higson GJ, Neynaber SA, Parson RE, *et al.* Assessment of amyloid beta protein in cerebrospinal fluid as an aid in the diagnosis of Alzheimer's disease. *J Neurochem* 1996, 66: 259–265.

- [33] van Gool WA, Kuiper MA, Walstra GJ, Wolters EC, Bolhuis PA. Concentrations of amyloid beta protein in cerebrospinal fluid of patients with Alzheimer's disease. *Ann Neurol* 1995, 37: 277–279.
- [34] Andreasen N, Hesse C, Davidsson P, Minthon L, Wallin A, Winblad B, *et al.* Cerebrospinal fluid beta-amyloid(1-42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. *Arch Neurol* 1999, 56: 673–680.
- [35] Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. *Lancet Neurol* 2003, 2: 605–613.
- [36] Holmes C, Boche D, Wilkinson D, Yadegarfar G, Hopkins V, Bayer A, *et al.* Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. *Lancet* 2008, 372: 216–223.
- [37] Clark CM, Xie S, Chittams J, Ewbank D, Peskind E, Galasko D, *et al.* Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? *Arch Neurol* 2003, 60: 1696–1702.
- [38] Engelborghs S, De Vreese K, Van de Castele T, Vanderstichele H, Van Everbroeck B, Cras P, *et al.* Diagnostic performance of a CSF-biomarker panel in autopsy-confirmed dementia. *Neurobiol Aging* 2008, 29: 1143–1159.
- [39] Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol* 2007, 64: 343–349.
- [40] Galasko D, Chang L, Motter R, Clark CM, Kaye J, Knopman D, *et al.* High cerebrospinal fluid tau and low amyloid beta42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E genotype. *Arch Neurol* 1998, 55: 937–945.
- [41] Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, *et al.* Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. *Neurology* 1999, 52: 1555–1562.
- [42] Ida N, Hartmann T, Pantel J, Schroder J, Zerfass R, Forstl H, *et al.* Analysis of heterogeneous A4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. *J Biol Chem* 1996, 271: 22908–22914.
- [43] Kanai M, Matsubara E, Isoe K, Urakami K, Nakashima K, Arai H, *et al.* Longitudinal study of cerebrospinal fluid levels of tau, A beta1-40, and A beta1-42(43) in Alzheimer's disease: a study in Japan. *Ann Neurol* 1998, 44: 17–26.
- [44] Kapaki E, Kilidireas K, Paraskevas GP, Michalopoulou M, Patsouris E. Highly increased CSF tau protein and decreased beta-amyloid (1-42) in sporadic CJD: a discrimination from Alzheimer's disease? *J Neurol Neurosurg Psychiatry* 2001, 71: 401–403.
- [45] Lewczuk P, Esselmann H, Otto M, Maler JM, Henkel AW, Henkel MK, *et al.* Neurochemical diagnosis of Alzheimer's dementia by CSF Abeta42, Abeta42/Abeta40 ratio and total tau. *Neurobiol Aging* 2004, 25: 273–281.
- [46] Mehta PD, Pirttila T, Mehta SP, Sersen EA, Aisen PS, Wisniewski HM. Plasma and cerebrospinal fluid levels of amyloid beta proteins 1-40 and 1-42 in Alzheimer disease. *Arch Neurol* 2000, 57: 100–105.
- [47] Skoog I, Davidsson P, Aeværsson O, Vanderstichele H, Vanmechelen E, Blennow K. Cerebrospinal fluid beta-amyloid 42 is reduced before the onset of sporadic dementia: a population-based study in 85-year-olds. *Dement Geriatr Cogn Disord* 2003, 15: 169–176.
- [48] Sjogren M, Minthon L, Davidsson P, Granerus AK, Clarberg A, Vanderstichele H, *et al.* CSF levels of tau, beta-amyloid(1-42) and GAP-43 in frontotemporal dementia, other types of dementia and normal aging. *J Neural Transm* 2000, 107: 563–579.
- [49] Sjogren M, Davidsson P, Wallin A, Granerus AK, Grundstrom E, Askmark H, *et al.* Decreased CSF-beta-amyloid 42 in Alzheimer's disease and amyotrophic lateral sclerosis may reflect mistreatment of beta-amyloid induced by disparate mechanisms. *Dement Geriatr Cogn Disord* 2002, 13: 112–118.
- [50] Rosler N, Wichart I, Jellinger KA. Clinical significance of neurobiochemical profiles in the lumbar cerebrospinal fluid of Alzheimer's disease patients. *J Neural Transm* 2001, 108: 231–246.
- [51] Andreasen N, Blennow K. beta-amyloid (A beta) protein in cerebrospinal fluid as a biomarker for Alzheimer's disease. *Peptides* 2002, 23: 1205–1214.
- [52] Jensen M, Schroder J, Blomberg M, Engvall B, Pantel J, Ida N, *et al.* Cerebrospinal fluid A beta42 is increased early in sporadic Alzheimer's disease and declines with disease progression. *Ann Neurol* 1999, 45: 504–511.
- [53] Csernansky JG, Miller JP, McKeel D, Morris JC. Relationships among cerebrospinal fluid biomarkers in dementia of the Alzheimer type. *Alzheimer Dis Assoc Disord* 2002, 16: 144–149.
- [54] Andreasen N, Vanmechelen E, Vanderstichele H, Davidsson P, Blennow K. Cerebrospinal fluid levels of total-tau, phospho-tau and A beta 42 predicts development of Alzheimer's disease in patients with mild cognitive impairment. *Acta Neurol Scand Suppl* 2003, 179: 47–51.
- [55] Shoji M, Matsubara E, Kanai M, Watanabe M, Nakamura T, Tomidokoro Y, *et al.* Combination assay of CSF tau, A beta 1-40 and A beta 1-42(43) as a biochemical marker of Alzheimer's disease. *J Neurol Sci* 1998, 158: 134–140.
- [56] Iqbal K, Alonso AD, Gondal JA, Gong CX, Haque N, Khatoon S, *et al.* Mechanism of neurofibrillary degeneration and pharmacologic therapeutic approach. *J Neural Transm Suppl*

- 2000, 59: 213–222.
- [57] Mandelkow EM, Mandelkow E. Tau in Alzheimer's disease. *Trends Cell Biol* 1998, 8: 425–427.
- [58] Hampel H, Teipel SJ, Fuchsberger T, Andreasen N, Wiltfang J, Otto M, *et al.* Value of CSF beta-amyloid1-42 and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. *Mol Psychiatry* 2004, 9: 705–710.
- [59] Hu YY, He SS, Wang X, Duan QH, Grundke-Iqbal I, Iqbal K, *et al.* Levels of nonphosphorylated and phosphorylated tau in cerebrospinal fluid of Alzheimer's disease patients : an ultrasensitive bienzyme-substrate-recycle enzyme-linked immunosorbent assay. *Am J Pathol* 2002, 160: 1269–1278.
- [60] Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, *et al.* CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. *JAMA* 2009, 302: 385–393.
- [61] Hampel H, Buerger K, Zinkowski R, Teipel SJ, Goernitz A, Andreasen N, *et al.* Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. *Arch Gen Psychiatry* 2004, 61: 95–102.
- [62] Brys M, Pirraglia E, Rich K, Rolstad S, Mosconi L, Switalski R, *et al.* Prediction and longitudinal study of CSF biomarkers in mild cognitive impairment. *Neurobiol Aging* 2009, 30: 682–690.
- [63] Itoh N, Arai H, Urakami K, Ishiguro K, Ohno H, Hampel H, *et al.* Large-scale, multicenter study of cerebrospinal fluid tau protein phosphorylated at serine 199 for the antemortem diagnosis of Alzheimer's disease. *Ann Neurol* 2001, 50: 150–156.
- [64] Kohnken R, Buerger K, Zinkowski R, Miller C, Kerkman D, DeBernardis J, *et al.* Detection of tau phosphorylated at threonine 231 in cerebrospinal fluid of Alzheimer's disease patients. *Neurosci Lett* 2000, 287: 187–190.
- [65] Buerger K, Zinkowski R, Teipel SJ, Tapiola T, Arai H, Blennow K, *et al.* Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231. *Arch Neurol* 2002, 59: 1267–1272.
- [66] Hu YY, He SS, Wang XC, Duan QH, Khatoon S, Iqbal K, *et al.* Elevated levels of phosphorylated neurofilament proteins in cerebrospinal fluid of Alzheimer disease patients. *Neurosci Lett* 2002, 320: 156–160.
- [67] Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Petersen RC, *et al.* Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* 2009, 65: 403–413.
- [68] Mulder C, Verwey NA, van der Flier WM, Bouwman FH, Kok A, van Elk EJ, *et al.* Amyloid-beta(1-42), total tau, and phosphorylated tau as cerebrospinal fluid biomarkers for the diagnosis of Alzheimer disease. *Clin Chem* 2010, 56: 248–253.
- [69] Qin W, Ho L, Wang J, Peskind E, Pasinetti GM. S100A7, a novel Alzheimer's disease biomarker with non-amyloidogenic alpha-secretase activity acts via selective promotion of ADAM-10. *PLoS One* 2009, 4: e4183
- [70] Barao S, Zhou LJ, Adamczuk K, Vanhoutvin T, van Leuven F, Demedts D, *et al.* BACE1 levels correlate with phospho-tau levels in human cerebrospinal fluid. *Curr Alzheimer Res* 2013, 10: 671–678.
- [71] Mulder SD, van der Flier WM, Verheijen JH, Mulder C, Scheltens P, Blankenstein MA, *et al.* BACE1 activity in cerebrospinal fluid and its relation to markers of AD Pathology. *J Alzheimers Dis* 2010, 20: 253–260.
- [72] Gao CM, Yam AY, Wang XM, Magdangal E, Salisbury C, Peretz D, *et al.* A beta 40 Oligomers identified as a potential biomarker for the diagnosis of Alzheimer's disease. *PLoS One* 2010, 5.
- [73] Verwey NA, Kester MI, van der Flier WM, Veerhuis R, Berkhof H, Twaalfhoven H, *et al.* Additional Value of CSF Amyloid-beta(40) Levels in the Differentiation between FTLD and Control Subjects. *J Alzheimers Dis* 2010, 20: 445–452.
- [74] Bibl M, Gallus M, Welge V, Esselmann H, Wolf S, Ruther E, *et al.* Cerebrospinal fluid amyloid-beta 2-42 is decreased in Alzheimer's, but not in frontotemporal dementia. *J Neural Transm* 2012, 119: 805–813.
- [75] Ikeuchi T, Hirayama S, Miida T, Fukamachi I, Tokutake T, Ebinuma H, *et al.* Increased levels of soluble LR11 in cerebrospinal fluid of patients with Alzheimer disease. *Dement Geriatr Cogn Disord* 2010, 30: 28–32.
- [76] Laske C, Stransky E, Hoffmann N, Maetzler W, Straten G, Eschweiler GW, *et al.* Macrophage colony-stimulating factor (M-CSF) in plasma and CSF of patients with mild cognitive impairment and Alzheimer's disease. *Curr Alzheimer Res* 2010, 7: 409–414.
- [77] Jiang H, Hampel H, Prvulovic D, Wallin A, Blennow K, Li RN, *et al.* Elevated CSF levels of TACE activity and soluble TNF receptors in subjects with mild cognitive impairment and patients with Alzheimer's disease. *Mol Neurodegener* 2011, 6: 69.
- [78] Yin GN, Lee HW, Cho JY, Suk K. Neuronal pentraxin receptor in cerebrospinal fluid as a potential biomarker for neurodegenerative diseases. *Brain Res* 2009, 1265: 158–170.
- [79] Toledo JB, Korff A, Shaw LM, Trojanowski JQ, Zhang J. CSF alpha-synuclein improves diagnostic and prognostic performance of CSF tau and A beta in Alzheimer's disease. *Acta Neuropathol* 2013, 126: 683–697.
- [80] Santos SM, Garcia-Nimo L, Santos SS, Tavares I, Cocho JA, Castanho M. Neuropeptide kyotorphin (tyrosyl-arginine) has decreased levels in the cerebro-spinal fluid of Alzheimer's

- disease patients: potential diagnostic and pharmacologic alimplications. *Front Aging Neurosci* 2013, 5.
- [81] Montine TJ, Peskind ER, Quinn JF, Wilson AM, Montine KS, Galasko D. Increased cerebrospinal fluid F-2-isoprostanes are associated with aging and latent Alzheimer's disease as identified by biomarkers. *Neuromolecular Med* 2011, 13: 37–43.
- [82] Correa JD, Starling D, Teixeira AL, Caramelli P, Silva TA. Chemokines in CSF of Alzheimer's disease patients. *Arq Neuropsiquiatr* 2011, 69: 455–459.
- [83] Craig-Schapiro R, Perrin RJ, Roe CM, Xiong CJ, Carter D, Cairns NJ, *et al.* YKL-40: A novel prognostic fluid biomarker for preclinical Alzheimer's disease. *Biol Psychiatry* 2010, 68: 903–912.
- [84] Tarawneh R, D'Angelo G, Macy E, Xiong CJ, Carter D, Cairns NJ, *et al.* Visinin-like Protein-1: diagnostic and prognostic biomarker in Alzheimer disease. *Ann Neurol* 2011, 70: 274–285.
- [85] Tarawneh R, Lee JM, Ladenson JH, Morris JC, Holtzman DM. CSF VILIP-1 predicts rates of cognitive decline in early Alzheimer disease. *Neurology* 2012, 78: 709–719.
- [86] Zenzmaier C, Marksteiner J, Kiefer A, Berger P, Humpel C. Dkk-3 is elevated in CSF and plasma of Alzheimer's disease patients. *J Neurochem* 2009, 110: 653–661.
- [87] Kosicek M, Kirsch S, Bene R, Trkanjec Z, Titlic M, Bindila L, *et al.* Nano-HPLC-MS analysis of phospholipids in cerebrospinal fluid of Alzheimer's disease patients-a pilot study. *Anal Bioanal Chem* 2010, 398: 2929–2937.
- [88] Abraham JD, Calvayrac-Pawlowski S, Cobo S, Salvatat N, Vicat G, Molina L, *et al.* Combined measurement of PEDF, haptoglobin and tau in cerebrospinal fluid improves the diagnostic discrimination between alzheimer's disease and other dementias. *Biomarkers* 2011, 16: 161–171.
- [89] Guo LH, Alexopoulos P, Perneczky R. Heart-type fatty acid binding protein and vascular endothelial growth factor: cerebrospinal fluid biomarker candidates for Alzheimer's disease. *Eur Arch Psychiatry Clin Neurosci* 2013, 263: 553–560.
- [90] Vafadar-Isfahani B, Ball G, Coveney C, Lemetre C, Boocock D, Minthon L, *et al.* Identification of SPARC-like 1 protein as part of a biomarker panel for Alzheimer's disease in cerebrospinal fluid. *J Alzheimers Dis* 2012, 28: 625–636.
- [91] Perrin RJ, Craig-Schapiro R, Malone JP, Shah AR, Gilmore P, Davis AE, *et al.* Identification and validation of novel cerebrospinal fluid biomarkers for staging early Alzheimer's disease. *PLoS One* 2011, 6: e16032.
- [92] Craig-Schapiro R, Kuhn M, Xiong CJ, Pickering EH, Liu JX, Misko TP, *et al.* Multiplexed immunoassay panel identifies novel CSF biomarkers for Alzheimer's disease diagnosis and prognosis. *PLoS One* 2011, 6: e18850.
- [93] Jahn H, Wittke S, Zurbig P, Raedler TJ, Arlt S, Kellmann M, *et al.* Peptide fingerprinting of Alzheimer's disease in cerebrospinal fluid: identification and prospective evaluation of new synaptic biomarkers. *PLoS One* 2011, 6: e26540.