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REVIEW

Cerebrospinal fluid and blood biomarkers in Alzheimer's disease

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Abstract

Due to an ever aging society and growing prevalence of Alzheimer's disease (AD), the challenge to meet social and health care system needs will become increasingly difficult. Unfortunately, a definite ante mortem diagnosis is not possible. Thus, an early diagnosis and identification of AD patients is critical for promising, early pharmacological interventions as well as addressing health care needs. The most advanced and most reliable markers are β-amyloid, total tau and phosphorylated tau in cerebrospinal fluid (CSF). In blood, no single biomarker has been identified despite an intense search over the last decade. The most promising approaches consist of a combination of several blood-based markers increasing the reliability, sensitivity and specificity of the AD diagnosis. However, contradictory data make standardized testing methods in longitudinal and multi-center studies extremely difficult. In this review, we summarize a range of the most promising CSF and blood biomarkers for diagnosing AD.

Key words: Alzheimer's disease; Biomarker; Blood; Cerebrospinal fluid; Dementia; Plasma

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ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive neurodegenerative disorder. It is morphologically characterized by deposition of extracellular β-amyloid (Aβ)-containing plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein[1]. Neuronal loss, hippocampal and cortical atrophy, inflammation, and oxidative damage are further indications of the disease. AD leads to cognitive decline, as well as, memory loss, language problems and deterioration of executive function. Age is the major risk factor for developing AD and it is the most common form of age-related dementia affecting about 35 million people worldwide. This number is estimated to rise to 81.1 million by 2040[2,3]. AD leads to a colossal burden on AD individuals, their families, caregivers and on social and health care systems. This makes it essential to understand disease mechanisms, establish biological markers, and investigate effective therapies. To date, a definitive diagnosis of AD can only be made with both a clinical diagnosis and a post mortem histopathological examination of the brain. A clinical diagnosis of AD is based on medical records, physical and neurological examination, laboratory tests, neuroimaging and neuropsychological evaluation. Diagnosis can be made with an accuracy of over 90%[1,4]. However, neurodegeneration in AD is estimated to start 20 to 30 years before the first clinical symptoms become apparent[5]. Treatment strategies might be most

effective before pathological changes spread throughout the brain. Thus, an early diagnosis with reliable biomarkers is essential to distinguish between AD, mild cognitive impairment (MCI) and other dementia types.

DEFINITION OF A BIOMARKER

A biomarker is generally defined as a measureable substance that can be used as an indicator for ongoing physiological and pathological processes. An ideal biomarker for AD should fulfill the criteria from the consensus report by Growdon *et al*[6] (1998). It should detect AD neuropathology and should be validated by post mortem evaluation. It should have a high diagnostic sensitivity and specificity for the disease, preferably at early or presymptomatic stages. Furthermore, it should be precise, reliable, non-invasive, easy to measure, inexpensive, and adequate for routine screenings. A biomarker should provide an indication of possible drug candidates and should predict clinical outcomes[7].

BIOMARKERS IN AD

There has been an extensive search for AD-specific biomarkers over the past decade. Diagnosis of familial AD can be achieved by DNA sequencing and analysis of known genetic mutations that cause AD. These mutations occur on three genes encoding for amyloid precursor protein (APP), presinilin 1, and presinilin 2[8]. An increased risk of late onset AD is associated with environmental factors and genetic mutations including mutation of the apolipoprotein E epsilon 4 allele. Imaging techniques are relatively non-invasive, however, they are limited by availability and high cost. In addition, the accuracy of these techniques is still under debate[9-11]. Structural imaging, such as computed tomography (CT) and magnetic resonance imaging are mainly used to rule out other pathologies and to detect volumetric changes. Hippocampal atrophy reflects severe neuronal loss and decreased synaptic density, which appears relatively late in disease and makes CT an inappropriate method for the early diagnosis of AD. Single photon emission tomography (SPECT) and positron emission tomography (PET) can reveal metabolic and perfusion changes. Due to disease-specific dyes, PET can be used to detect fibrillar Aβ and tau *in vivo*[12]. Thus, a promising and cost-effective research area for diagnosing AD is the analysis of peripheral biomarkers in cerebrospinal fluid (CSF) and blood[6]. Data from various studies relating biomarker analysis from AD cases *vs* controls are summarized in Table 1.

CEROBROSPINAL FLUID BIOMARKERS

CSF is a promising source of AD biomarkers. CSF is in direct contact with the brain's extracellular space and thus should reflect ongoing biochemical changes occurring in the central nervous system (CNS), providing a potential window for AD-related changes in the brain. There are three well- established candidate biomarkers in CSF which reflect the central AD pathology: total tau (t-tau), phosphorylated tau (p-tau), and the 42-amino acid form of Aβ ($A\beta_{1-42}$).

TAU PROTEIN

Tau is a microtubuli-associated protein, which is mainly located in neuronal axons. It plays an important role in microtubule assembly and stability. Tau is also important for axonal function and axonal transport[13]. There are six isoforms derived from alternative splicing of tau mRNA[14]. Post-translational tau can be modified by several mechanisms including: phosphorylation, glycosylation, ubiquitination, and oxidation[15,16]. Previous studies have shown that t-tau levels in CSF can be measured by ELISA techniques[17]. A strong correlation between age and t-tau in healthy individuals has been determined with a cut off value of > 500 pg/mL (> 70 years)[18]. Increased t-tau levels in CSF of AD patients (> 600 pg/mL) have been reported with an increase of 300% compared to controls. CSF t-tau levels in AD patients have a sensitivity of 90% and specificity of 81% compared to healthy elderly[19-22]. Compared to other dementias, the sensitivity and specificity drops to 50%-60%[20,23]. Similar levels of t-tau have been shown in patients with MCI. MCI converting to AD can be discriminated from stable MCI with 90% sensitivity and 100% specificity, indicating that t-tau is a good predictive marker for incipient AD[24]. In acute conditions, such as stroke, high levels of t-tau have been measured, which correlated with infarct size[25]. The highest increase in t-tau was found in Creutzfeld-Jacobs-disease (CJD) (> 3000 pg/mL)[26]. In addition, elevated concentrations have been reported in vascular dementia (VaD)[27,28], whereas, normal concentrations have been reported in depression, alcoholic dementia, and Parkinson's disease (PD)[27,29,30]. T-tau correlates with general neuronal damage and degeneration in chronic neurodegenerative disorders rather than with AD specific pathology.

PHOSPHORYLATED TAU PROTEIN

Post-translational modifications, such as phosphorylation, alter the conformation of tau and decrease its affinity to microtubules[31]. Loss of affinity to microtubules results in neuronal cytoskeleton destabilization, dysfunction of axonal transport, and neurotoxicity[32]. Stable tau dimers can form oligomers and further aggregate to neurofibrillary tangles, a hallmark of AD pathology[31]. Phosphorylation of tau takes place at the amino acids serine, threonine and tyrosine. Different phosphorylation epitopes, such as threonine 181, 231 or serine 235, can be detected by different ELISAs[27,33]. The concentration of $p\text{-tau}_{181}$ is increased in AD and yields a sensitivity of 80% and specificity of 92% in discriminating AD from healthy controls[34]. In CJD, a normal to mild increase has been detected in p-tau despite very high levels of t-tau[35]. No changes in the concentration of p-tau in CSF have been found in acute stroke, depression, PD, and other dementias (i.e. VaD, frontotemporal dementia, and Lewy Body

Table 1 Summary of biomarkers in cerebrospinal fluid and blood of Alzheimer's disease cases *vs* controls

Biomarker	CSF	Plasma/serum	Blood cells
Total tau	Increased	-	-
Phosphorylated tau	Increased	-	-
Glycogen synthase kinase-3	-	-	Increased[73,75] Decreased[65]
β-amyloid (Aβ)	Decreased[45,46] No difference[47]	No difference[79-82]	-
$Aβ_{1-40}$	No difference[24]	No difference[83] Increased[84] Decreased[85]	-
$Aβ_{1-42}$	Decreased[24]	Increased[83]	-
$Aβ_{1-42}/Aβ_{1-40}$ ratio	Decreased[48]	No difference[83] Decreased[78,84,86]	-
APP ratio	-	-	Decreased[90-93]
Ubiquitin	Increased[24]	-	No difference[148]
BACE1	Increased[67]	-	-
Cholesterol	-	Decreased[94,95] Increased[96-98]	-
24S-hydroxycholesterol	Increased[102]	No difference[103] Decreased[104]	-
Homocysteine	-	Increased[106,107,109]	-
Epidermal growth factor	-	Decreased[111] Increased[112]	Decreased[93]
Glial cell line-derived growth factor	-	Decreased[111] No difference[112]	-
Nerve growth factor	Increased[63-65]	-	-
Platelet derived growth factor	-	Decreased[111] Increased[112]	No difference[93]
Interleukin-1β	-	Increased[70,113,114] No difference[115]	No difference[93] Decreased[116]
Interleukin-6	-	Increased[70,113,119-121] No difference[114,138]	No difference[117,118]
Interleukin-10	-	Increased[124] No difference[125]	No difference[126]
Tumor necrosis factor alpha	-	Increased[121,128] Decreased[114,129] No difference[115]	-
Monocyte chemotactic protein-1	-	Increased[111] No difference[131,132]	Decreased[133]
Monokine-induced by interferon-gamma	-	Increased[132,134]	Decreased[132]
Macrophage inflammatory protein 1δ	-	Decreased[111] Increased[112]	Increased[132]
Intercellular adhesion molecule-1	-	Increased[137] No difference[141]	No difference[93,138]
Intercellular adhesion molecule-3	-	-	Decreased[138]
Platelet endothelial cell adhesion molecule	-	Increased[137]	-
P-selectin	-	Decreased[138]	Decreased[138] No difference[93]
Vascular cell adhesion molecule -1	-	Increased[140] No difference[141]	No difference[93]
Matrix metalloproteinase-2	Decreased[143]	No difference[144]	Decreased[93]
Matrix metalloproteinase-9	-	Increased and no difference[143-145]	No difference[93]

dementia)[30,34,36,37]. Thus, p-tau reveals a higher specificity than t-tau for diagnosing AD compared to other types of dementias. In addition, MCI patients, who convert to AD, have higher p-tau levels compared to patients with stable MCI. It has also been shown that cognitive decline and tangle pathology in individuals with MCI correlates with CSF p-tau concentrations[38,39]. It seems likely that p-tau is not simply a marker for neuronal degeneration, but rather a more specific marker for AD by reflecting the phosphorylation states of tau and ultimately the formation of neurofibrillary tangles in the brain.

$Aβ_{1-42}$

Aβ is the main component of amyloid plaques seen in AD brains. It is generated from the protolytical processing of APP[40,41]. There are two different pathways for APP metabolism: the physiological pathway regulated by α-secretase (ADAM10) cleavage, and the pathological pathway, where β-secretase (BACE) is the rate-limiting enzyme. BACE cleaves APP and releases a large N-terminal fragment (sAPPβ). The membrane-anchored frag-

ment is cleaved by γ-secretase ultimately leading to Aβ release. Different truncated forms of Aβ exist including $A\beta_{1-38}$, $A\beta_{1-40}$, and $A\beta_{1-42}$, where $A\beta_{1-40}$ is the most abundant[34]. $A\beta_{1-42}$, which aggregate in extracellular plaques, aggregates more rapidly compared to $A\beta_{1-40}$ and forms soluble oligomers and fibrils[42,43]. Quantification of different Aβ forms in CSF can be determined by specific ELISAs[44]. Early studies reported controversial results involving total Aβ measurements in CSF[45-47]. Later, several reports demonstrated no changes in $A\beta_{1-40}$ CSF levels. A moderate to marked decrease in $A\beta_{1-42}$ has been detected in CSF of AD patients compared to healthy elderly with a sensitivity of 90% and specificity of 86%[24]. The ratio of $A\beta_{1-42}/A\beta_{1-40}$ shows an even stronger reduction in AD than $A\beta_{1-42}$ alone[48]. It was believed, that low levels of Aβ in CSF reflect high levels of Aβ plaque formation in the brain[44]. However, low levels of Aβ in CSF have also been reported in disease without Aβ plaque pathology (i.e. CJD, multiple system atrophy and other dementias)[49-51]. There is a strong correlation between low Aβ CSF levels and the number of plaques in specific brain regions of AD patients, such as the hippocampus, which at least partly supports this hypothesis[52]. Unchanged Aβ levels exist in depression and neurological disorders like PD[50]. It has been suggested that Aβ concentration can serve as a good predictor of AD, since reduced levels in CSF have been reported in asymptomatic healthy elderly, who go on to develop AD 1-2 years after follow-up[20]. Recent reports suggest that soluble Aβ oligomers are rather synaptotoxic and causative for AD compared to insoluble, aggregated forms of Aβ[53-55]. No correlation has been found between plaque load and degree of dementia. Some patients with assumed AD show no plaques while cognitive healthy elderly have senile plaques at autopsy[56]. However, one cannot exclude a relationship to preclinical manifestations of AD. It is assumed that the formation of plaques is a downstream event of the generation of more toxic and soluble forms of Aβ[57]. The reduction of $A\beta_{1-42}$ in CSF could result from the formation of oligomers, which are not detected by $A\beta_{1-42}$ ELISA[58]. Fukumoto *et al*[59] (2010) established a novel ELISA system that quantifies $A\beta_{1-42}$ oligomers. They reported an inverse correlation between oligomers in the CSF and severity of dementia. However, measurement of Aβ oligomers in CSF is limited by its low concentrations and must still be validated as an effective biomarker[59].

OTHER BIOMARKERS IN CSF

An intense search for new probable biomarkers in CSF is ongoing. Several disease-related proteins are under investigation including: ubiquitin, nerve growth factor (NGF) and BACE1. Ubiquitin is involved in protein degradation by tagging target proteins. In AD, paired helical filaments of neurofibrillary tangles are ubiquitinated and increased levels of ubiquitin have been correlated with total tangle formation in the brain[60,61]. Elevated concentrations of free and conjugated ubiquitin in CSF have been detected by a specific ELISA in AD cases[24]. However, to validate ubiquitin as a potential diagnostic marker further studies are needed. NGF maintains cholinergic neurons in the basal forebrain, which have been shown to be primarily affected in AD[62]. Numerous studies have reported higher NGF levels in the brain and CSF in AD patients compared to healthy controls[63-65]. Furthermore, a down-regulation of the NGF receptor has been reported[66]. A dysfunctional neurotrophin system may lead to an accumulation of NGF in the brain and can be detected in the CSF[63]. Measurement of NGF levels in CSF is restricted due to its low concentration and the fact that NGF might accumulate only at a certain stage in AD[64,65]. BACE1 is involved in APP processing of the amyloidogenic pathway in AD and has been discussed as a promising biomarker candidate[34]. Upregulation of BACE1 in the brain and increased BACE activity in CSF have been reported in AD and MCI that progresses to AD suggesting that the upregulation of BACE is an early event in AD pathology[34,67-69]. The advantage of these and other markers is still questionable and further studies are needed to confirm their potential as prognostic or diagnostic biomarkers in AD.

BLOOD BIOMARKERS

Lumbar puncture is an invasive process and the collection of CSF does not seem feasible as a routine procedure. For the growing AD population, the collection of blood, however, by venepuncture is a simple, non-invasive, inexpensive and time-saving method. Thus, a blood-based biomarker would have more potential for routine screenings with repeatable measurements. This type of screening would provide a good chance for early detection, diagnosis and monitoring of the disease, and treatment effects. Peripheral blood has no direct contact with the brain and its delimitation by the blood-brain barrier (BBB) limits the usefulness of markers[70]. However, in humans, CSF is constantly exchanged and cleared *via* the blood[71] suggesting blood could reflect pathological changes in the brain and thus provides a good source of AD biomarkers. In plasma, serum and blood cells (i.e. erythrocytes, leukocytes, platelets) various proteins, lipids and other metabolic products can be examined. Plasma is a highly complex fluid with thousands of proteins available for potential biomarker evaluation. Several candidate biomarkers in blood and blood cells have been introduced, but their lack of sensitivity, specificity, and true relation to brain mechanisms remain unclear. Altogether, the discovery of a single blood-based biomarker in AD has thus far failed and further intense investigations are needed.

TAU

T-tau and p-tau are established markers for diagnosing AD in CSF, while tau levels in blood have not been investigated. Recently, a new sandwich ELISA for $p\text{-tau}_{231P}$

detection in serum was developed. However, reliable data regarding its use for AD diagnosis is still lacking[72]. Studies, instead, have been concentrated on protein kinases and phosphatases, whose alterations are associated with tau pathology. It has been shown that glycogen synthase kinase-3 (GSK3) activity is increased in AD brains. GSK3 contributes to hyperphosphorylation of tau and increased GSK3 levels have been reported in the leukocytes of AD patients. However, these elevated levels were not found in peripheral-blood mononuclear cells of AD patients indicating a high variability in leukocyte subpopulations[73,74]. In addition, increased GSK3 activity has been reported in the platelets of AD and MCI patients compared to controls[75]. Protein phosphatase-2A dephosphorylates tau and a decrease in its activity and expression in AD brains has been reported[76,77]. However, the usefulness of tau-related biomarkers in blood, such as alterations of peripheral kinases and phosphatases, needs further validation.

Aβ PEPTIDES

Peripheral Aβ is not only generated from peripheral tissues and organs, but also from the brain. Aβ is transported over the BBB by RAGE (receptor for advanced glycation end products) and LRP-1 (low-density lipoprotein receptor-related protein-1)[78]. Several assays for $A\beta_{1-40}$, $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio have been developed. In familial AD cases, total Aβ and $A\beta_{1-42}$ plasma levels are elevated[79]. In sporadic AD, several cross-sectional studies report no significant difference in plasma Aβ concentrations in general compared to controls[79-82]. Unfortunately, longitudinal studies have shown high data variability. Schupf *et al*[83] (2008) reported enhanced levels of $A\beta_{1-42}$, but not $A\beta_{1-40}$ and $A\beta_{1-42}/A\beta_{1-40}$ ratio at baseline. They also showed a decline in $A\beta_{1-42}$ levels over time, which was associated with a higher risk of AD incidence. Another study reported increased $A\beta_{1-40}$ baseline levels and linked it to an increased risk of dementia[84]. In contrast, Sundelöf *et al*[85] (2008) associated lower $A\beta_{1-40}$ levels at baseline with a higher risk for subsequent AD. Multiple studies have associated a higher baseline $A\beta_{1-42}/A\beta_{1-40}$ ratio to a reduced risk of dementia at follow-up or rather a lower ratio of $A\beta_{1-42}/A\beta_{1-40}$ to a higher risk for MCI conversion to AD[84,86,87]. It was found that serum Aβ autoantibodies are decreased, however, another study reported no difference between plasma Aβ autoantibodies in AD and controls[88,89]. Taken together, these results reveal poor consistency between research groups, possibly caused by the influence of medication, by different ELISA techniques, by detection of different Aβ conformations, or by the adherence of Aβ to other proteins, e.g. albumin. In the periphery, platelets express high levels of APP and an altered pattern of APP isoforms (130 - 110 - 106 kDa) has been reported in the platelets of AD patients[90-93]. The APP ratio in MCI and AD, but not in other dementias, is reduced compared to controls with a sensitivity and specificity between 70% and 95%, correlating with AD severity[90-92]. Information about platelet-generated APP and its contribution to AD pathology is still lacking and the potential of the APP ratio as a biomarker is still unclear.

CHOLESTEROL AND 24S-HYDROXYCHOLESTEROL

High cholesterol levels are associated with increased β-secretase activity leading to an enhanced production of pathogenic Aβ from its precursor protein. In AD patients, decreased levels of total cholesterol have been detected[94,95]. However, high serum total cholesterol concentrations are associated with an increased risk of developing AD[96-98]. Controversial results have been reported in patients treated with cholesterol-lowering drugs (statins). Rockwood *et al*[99] (2002) associated cholesterol-lowering drugs with a decreased risk of AD, while other trials did not show any effects[100]. It has been suggested that only mid-life high total cholesterol is a risk factor for AD, however, increased total cholesterol levels at late-life seem to be associated with a reduced risk[95,101]. Cholesterol from damaged neurons is metabolized into 24S-hydroxycholesterol and then transferred across the BBB. Most 24S-hydroxycholesterol in plasma derives from the brain, thus it is believed that plasma levels of 24S-hydroxycholesterol reflect brain cholesterol catabolism, and thereby ongoing neuronal damage. Increased CSF 24S-hydroxycholesterol levels weakly correlate with 24S-hydroxycholesterol plasma levels and plasma levels have been found to be inconsistently increased[102,103]. In addition, it has been reported that plasma concentrations of 24S-hydroxycholesterol are decreased in dementia disorders[104]. Clearly, further studies are needed to investigate the correlation between cholesterol and AD, and the potential of cholesterol and 24S-hydroxycholesterol levels as prospective biomarkers.

HOMOCYSTEINE

Homocysteine (Hcy) is a methionine-derived amino acid. High levels of Hcy are not only a risk factor for vascular disease, but also for cognitive impairment and AD[105]. Elevated plasma Hcy levels and reduced vitamine B 12 and folate levels may indicate VaD and helps distinguish AD cases from healthy controls[106]. High Hcy levels in plasma increase the risk for developing AD by two-fold[107]. It has also been shown that controls who develop AD, have higher plasma Hcy levels compared to controls who convert to MCI[108], and MCI subjects who convert to AD, have higher baseline plasma Hcy levels than stable MCI patients[109].

GROWTH FACTORS

Growth factors support cell survival and play an important role in the regulation of cellular growth in the CNS and periphery. The growth factor NGF is the most

potent factor in counteracting cholinergic cell death[110]. Growth factors have been considered as possible treatments for AD[110]. However, results from plasma growth factor levels have been conflicting. One study reported decreased concentrations of platelet derived growth factor (PDGF), glial cell line-derived growth factor , and epidermal growth factor (EGF), while another study reported increased or unchanged plasma levels in AD patients compared to controls[111,112]. PDGF levels in platelets were similar in AD subjects and controls. However, EGF was decreased in the platelets of AD patients, indicating an enhanced release of platelet EGF into plasma[93].

CYTOKINES AND CHEMOKINES

Previous studies have suggested that chronic inflammation in the brain contributes to AD. However, an adequate reflection of brain cytokine and chemokine levels in peripheral blood is unclear since many of these proteins cannot easily cross the BBB. Several proteins with a putative role in AD pathology have been examined in plasma, serum and blood cells, but the results on protein levels have been highly contradictory. Some examples are given below:

Interleukin (IL)-1β is increased in the plasma and serum of AD patients compared to controls. However, IL-1β was unchanged in other studies, including longitudinal studies[70,113-115]. In platelets, no difference in IL-1β levels between AD, MCI and controls was found[93]. Lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMC) revealed lower IL-1β levels in AD[116]. In contrast, elevated concentrations or no changes in IL-6 have been reported when PBMCs were stimulated with LPS[117,118]. Several studies reported increased IL-6 levels in the serum and plasma of AD patients, while other studies found no differences[113,114,119-122]. Sun *et al*[123] (2003) found a correlation between IL-6 levels in CSF and serum. Higher mean levels of anti-inflammatory IL-10 in patients with dementia have been detected[124]. It has also been reported that serum and LPS-stimulated blood cell IL-10 concentrations were unchanged in AD[125,126]. Studies have demonstrated that tumor necrosis factor α (TNFα) is produced by activated microglia in response to Aβ, but TNFα-levels also increase in serum with age[127]. Results from TNFα measurements in AD patients compared to controls are divergent. Studies show increased, decreased and no changes in serum TNFα levels[114,115,121,128,129]. Paganelli *et al*[129] (2002) showed lower TNFα levels in mild and moderate AD compared to patients with severe AD, pointing to changes in the cytokine profile during the course of the disease. Monocyte chemotactic protein-1 (MCP-1) plays a role in inflammatory processes in the CNS. MCP-1 production can be stimulated by Aβ and are influenced by age[64,130]. In plasma, higher and unchanged MCP-1 levels have been found in AD[111,131,132]. Stimulated PBMCs show decreased levels of MCP-1 in AD compared to controls[133]. Monokine-induced by interferon-γ (MIG) plasma levels are higher in AD *vs* MCI and controls[132,134]. In monocytes, we found lower MIG levels, suggesting an enhanced release from monocytes into the blood[132]. Macrophage inflammatory protein 1δ (MIP-1δ) is expressed by monocytes in the periphery, and reduced plasma MIP-1δ concentrations have been reported[111]. In our study, we found reduced levels of MIP-1δ in monocytes, but enhanced MIP-1δ levels in plasma. These data support similar results obtained by Marksteiner *et al*[112] (2011)[132].

CELL ADHESION MOLECULES

Cell adhesion molecules (CAMs) are involved in the transmigration of monocytes across the BBB and become activated during the inflammatory and neurodegenerative responses[135,136]. In plasma, higher levels of soluble platelet endothelial CAM and intercellular adhesion molecule (ICAM) in AD patients compared to controls have been observed[137]. We found no difference in monocyte and platelet ICAM-1 levels between AD and controls[132,138]. In monocytes, ICAM-3 and P-selectin levels were lower compared to controls[138]. In plasma, P-selectin was also reduced in demented patients, while no changes were found in P-selectin and vascular CAM (VCAM) concentrations in platelets[132,139]. In plasma, VCAM-1 levels increased in AD and VaD[140]. However, another study reported no association between plasma VCAM-1 and ICAM-1 and an increased risk of developing AD[141].

MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMPs) are involved in cell-cell and cell-extracellular matrix interaction and have been implicated in AD pathophysiology. MMP-2 exhibits α-secretase activity and is responsible for the cleavage of APP[142]. MMP-2 is decreased in platelets in MCI and AD patients, which is in line with reduced MMP-2 CSF levels[93,143]. However, plasma MMP-2 levels are unchanged in AD and MCI patients[144]. Contradictory results have also been published for MMP-9 plasma levels. It has been reported that MMP-9 plasma levels are increased or unchanged in AD patients[143-145]. In platelets, MMP-9 levels were unaltered in AD patients compared with controls[93]. The correlation between MMPs and AD remains unclear. For instance, upregulation of MMP-9 could result from oxidative stress and inflammation, and thus one could hypothesize that the course of the disease varies throughout[146]. In addition, MMPs may play a role in BBB breakdown and cerebrovascular dysfunction in AD and contribute to disease pathology. However, longitudinal studies must be performed in order to validate their potential as reliable biomarkers.

MULTIPLEX APPROACHES

Contradictory results in peripheral protein levels might be due to varying methological techniques and the lack of standardized tests. None of the above-mentioned proteins serves as an exclusive, consistent, and reliable biomarker for early AD diagnosis. Nevertheless, analysis

of markers can further improve our knowledge on the ongoing pathological mechanisms and diagnostic accuracy in AD. Furthermore, these proteins might serve as interesting biomarker candidates for multiple biomarker strategies. Some multiplex approaches are already underway. Ray *et al*[111] (2007) examined the levels of 120 signaling plasma proteins. They identified an 18 plasma protein signature to discriminate AD patients from controls with an accuracy of 90%. They further discriminated MCI converters from stable MCI patients or patients who converted to other dementias. Marksteiner *et al*[112] (2011) examined 16 of these proteins using Searchlight multiplex ELISA and found five elevated plasma proteins with a sensitivity and specificity of 65%-75% and 52%-63%. Other studies examined the same protein in plasma using alternative approaches[71], however, replication of the results from Ray *et al*[111] (2007) in other cohorts was less successful. In our laboratory, we examined the monocytic levels of MIP-1δ and the tumor suppressor protein, p21, in combination with the clinical marker 'Mini-Mental State Examination', providing us with a good tool to differentiate AD patients from healthy controls[132].

CRITERIA FOR VALIDATING NOVEL BIOMARKERS IN AD

Cross-institutional standards must be employed in order to validate a novel biomarker. Sample collection, transport, processing, storage, and analysis interpretation must be optimized for widespread and efficient use[147]. The first step in searching for a successful biomarker is the inclusion of age-matched healthy controls, which include the same sex, comparable education and life-style. It is also important to have a reliable clinical diagnosis that is applicable worldwide and comparable between institutions. Furthermore, the achieved data must be reproducible by other researches and must be published in peer-reviewed journals[6].

CONCLUSION

$A\beta_{1-42}$, t-tau and p-tau in CSF provide the most reliable, most sensitive and specific biomarkers for AD today. However, the collection of CSF is an invasive procedure, therefore, the development of new methods and identification of blood-based biomarkers are needed. Many biomarkers in blood have been identified, however, no single candidate biomarker demonstrating reliability, sensitivity and specificity for AD has thus far been found. Currently, the most promising approach to diagnosing AD is the combination of multiple markers. However, these multimarker approaches remain in the preclinical phase and further investigation and validation is needed before premortem diagnosis can be achieved.

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