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BRAIN RESEARCH REVIEWS

Review

Plasma biomarkers for mild cognitive impairment and Alzheimer's disease

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ABSTRACT

Purpose of review: With the move toward development of disease modifying treatments, there is a need for more specific diagnosis of early Alzheimer's disease (AD) and mild cognitive impairment (MCI), plasma biomarkers are likely to play an important role in this. We review the current state of knowledge on plasma biomarkers for MCI and AD, including unbiased proteomics and very recent longitudinal studies. *Recent findings*: With the use of proteomics methodologies, some proteins have been identified as potential biomarkers in plasma and serum of AD patients, including alpha-1-antitrypsin, complement factor H, alpha-2-macroglobulin, apolipoprotein J, apolipoprotein A-I. The findings of cross-sectional studies of plasma amyloid beta (Aβ) levels are conflicting, but some recent longitudinal studies have shown that low plasma Aβ1–42 or Aβ1–40 levels, or Aβ1–42/Aβ1–40 ratio may be markers of cognitive decline. Other potential biomarkers for MCI and AD reflecting a variety of pathophysiological processes have been assessed, including isoprostanes and homocysteine (oxidative stress), total cholesterol and ApoE4 allele (lipoprotein metabolism), and cytokines and acute phase proteins (inflammation). A panel of 18 signal proteins was reported as markers of MCI and AD. *Summary:* A variety of potential plasma biomarkers for AD and MCI have been identified, however the findings need replication in longitudinal studies. This area of research promises to yield interesting results in the near future.

Contents

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1. Introduction

It is estimated that nearly 25 million people worldwide currently have dementia, and the number of people affected is projected to increase to more than 80 million by 2040 (Ferri et al., 2005). Alzheimer's disease (AD) is the most common dementia and approximately one in eight people over 65 years old are at risk. AD is an age-related and insidious-onset neurodegenerative disease (Kelley and Petersen, 2007). Disease pathogenesis may affect the brains of patients well before the clinical symptoms are fully expressed, possibly years or even decades preceding the diagnosis (Snowdon et al., 1996). Mild cognitive impairment (MCI) is proposed to be an early phase of cognitive decline that precedes dementia. MCI patients may progress to AD, vascular disease and other kinds of dementia. Rates of annual conversion from MCI to dementia are reported to range from 2.7% (Ganguli et al., 2004) to 10–15% (Petersen et al., 2001), and 56% in a follow-up period of 4 years (Rountree et al., 2007). One longitudinal study showed that people with MCI were 6.7 times more likely to develop AD than cognitively normal individuals (Boyle et al., 2006). Consequently MCI may be a useful AD prodromal phase in which to test putative biomarkers for their efficacy in early disease detection.

2. Clinical significance of circulating biomarkers

A definite diagnosis of AD can only be made by postmortem neuropathological examination, but brain tissue is inappropriate for early diagnosis of cognitive decline. Currently diagnosis of MCI and AD depends on a combination of clinical and neuropsychological tests, with no easy and effective diagnostic methods for use in the early stages of cognitive impairment. Early diagnosis may support measures to prevent disease progression from MCI to AD, and benefit the development of effective treatments.

Approaches to early diagnostic marker discovery for MCI and AD include neuroimaging, genetic testing and neurochemical testing for body fluid, such as cerebrospinal fluid (CSF), plasma, serum, urine and blood cells (Fig. 1). A variety of imaging strategies support clinical diagnosis of MCI and AD, including brain volumetric measures using magnetic resonance imaging (MRI), functional MRI, single photon emission tomography, positron emission tomography (PET), including the more recent amyloid imaging with PET using ligands such as the Pittsburgh compound B (PiB) (Hampel et al., 2008). These approaches are generally expensive, and availability of instrumentation is not widespread. In the case of genetic testing, there is a significant association of the apolipoprotein-E (ApoE) epsilon 4 (ε4) allele with late-onset AD, however ApoE genotyping is not currently recommended as a regular diagnostic strategy in general populations because of low sensitivity and specificity (Patterson et al., 2008).

Biomarkers in body fluids such as CSF, plasma and serum, could be utilised to increase the accuracy of diagnosis for cognitive decline and prediction of MCI progression. CSF presents a good resource for research into neurodegenerative diseases, but its clinical application is limited by the invasive nature of the procedure, particularly in elderly populations, and the requirement of highly trained personnel, making it unsuitable for routine application.

Plasma is a complex body fluid containing proteins, peptides, lipids and metabolites that reflect physiological activity and pathology in various body organs, including the central nervous system (CNS). In humans about 500 ml of CSF is absorbed into blood daily (Hye et al., 2006), making blood a suitable source of neurodegenerative disease biomarkers. The ease of a venepuncture compared to a lumbar puncture allows for repeatability, making it suitable for application in clinical trials to evaluate disease modifying treatments. Blood tests for diabetes, cholesterol levels, and ApoE genotyping are already available to clinicians for assessment of AD risk factors.

According to the current consensus criteria proposed by the National Institute on Aging (NIA) (Frank et al., 2003; The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group, 1998), ideal biomarkers for AD should: 1) detect the fundamental CNS pathophysiology of AD and be validated in neuropathologically confirmed cases, 2) should have a diagnostic sensitivity >85% for detecting AD and a specificity >75% for distinguishing between other dementias, 3) should detect any beneficial effects of disease modifying therapy, 4) should be reliable, reproducible, non-invasive, simple to

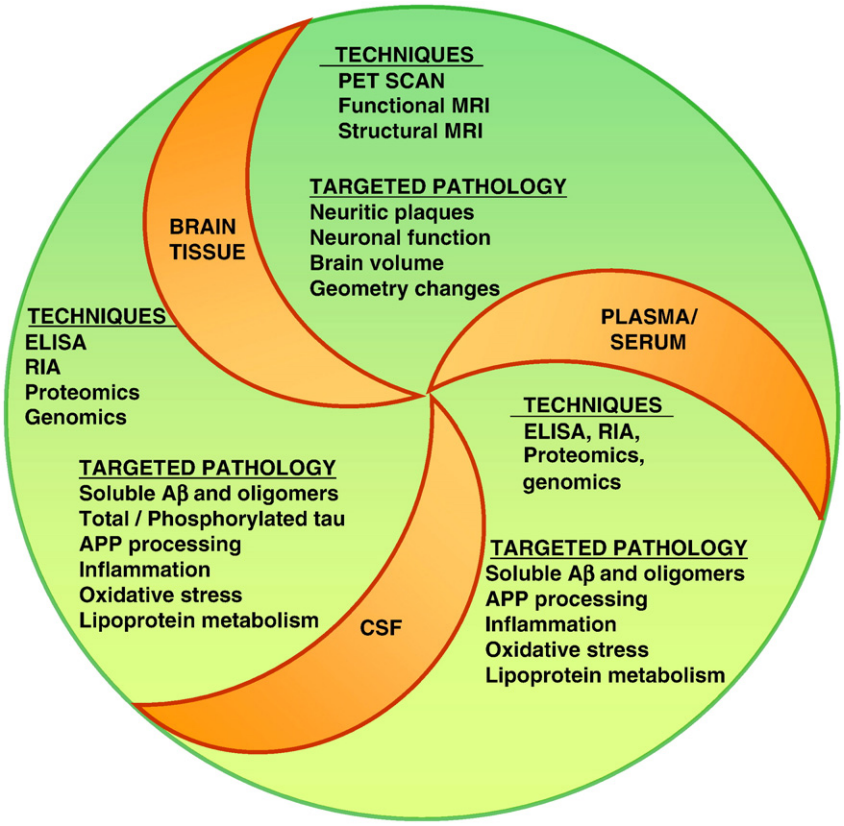

Fig. 1 – Schematic diagram of markers for MCI and AD in different tissues, targeted pathologies and techniques.

perform, and inexpensive, and 5) be confirmed by at least two independent studies conducted by qualified investigators with the results published in peer-reviewed journals. Key features of a biomarker include its ability to detect disease processes at early and preferably preclinical stages of the disease and variation with disease progression and severity. This is challenging in AD for a number of reasons: i) the neuropathology of AD represents a complex array of inter- and intra-cellular and molecular alterations, any of which is typical of AD pathology, including neuritic plaque deposits, changes in tau phosphorylation, neurofibrillary tangle formation, cerebral amyloid angiopathy, inflammation, loss of synapses, axonal atrophy, and neuronal cell death. A diversity of biochemical changes can be observed as a consequence; ii) the severity of neuropathological lesions does not always reflect the clinical diagnosis or the degree of cognitive impairment or its rate of progression; iii) a complex set of genetic and environmental factors is implicated in the etiology; iv) the brain in late-onset AD is commonly affected by multiple pathologies, most commonly vascular disease and the presence of Lewy bodies. In spite of these limitations, a number of advances have occurred toward the identification of biomarkers for AD, and its preclinical state of MCI.

2.1. *Methods*

We reviewed the current state of knowledge on plasma and serum biomarkers for MCI and AD with special emphasis placed on plasma biomarkers related to proteomics research and amyloid beta (Aβ) metabolism. For this review, we searched the following electronic databases: PubMed/Medline, Journals@OVID and PsycINFO by using keywords Alzheimer's disease or Mild cognitive impairment and plasma. According to different research fields and core biomarkers, articles were further identified with the terms proteomics, amyloid beta, oxidative stress, inflammation, Apolipoprotein E, homocysteine, cholesterol and lipoprotein. Studies were also searched through cross-references from published reviews and original papers.

3. Detection and measurement of proteome-based biomarkers for MCI and AD

Proteomics provides an unbiased approach to detecting protein biomarkers in complex sample mixtures such as plasma. It utilises mass spectrometry (MS) data coupled with protein database pattern recognition search algorithms to identify large numbers of proteins simultaneously, and has the advantage of being a discovery driven approach (Poljak et al., 2006). As such, it may allow insight into new markers and potentially lead to new hypotheses. Front end protein separation approaches include two dimensional gel electrophoresis (2 DGE), one dimensional gel electrophoresis (1 DGE), and gel-free methods, such as isotope-coded affinity tag (ICAT) and isobaric tag for relative and absolute quantitation (iTRAQ). One of the most powerful protein identification strategies is liquid chromatography (LC) separation followed

by mass spectrometric analysis (usually electrospray ionization, ESI). Another commonly utilised protein identification method is peptide mass fingerprinting using matrix-assisted laser desorption/ionization (MALDI). Surface-enhanced laser desorption/ionization (SELDI) has also been used for biomarker discovery, since changes in relative peak abundances can be observed. However this approach has a serious drawback, in that it lacks the potential to identify proteins. Only a handful of studies have utilised proteomic methods to find plasma biomarkers for AD, and research into plasma biomarkers for MCI is even more limited, making neuroproteomics an emerging technology. The proteins in plasma that have been identified by these techniques are associated with transportation of lipid or other molecules, immune regulation and inflammation, as summarized in Fig. 2 and Table 1.

3.1. *Proteome-based plasma biomarkers of AD*

Using 2 DGE and liquid chromatography tandem mass spectrometry (LC/MS/MS), Liao and colleagues detected over 900 spots from silver stained 2 DGE gel images, and identified six potential plasma biomarkers, viz. α-1-antitrypsin (AAT), vitamin D-binding protein, inter-α-trypsin inhibitor family heavy chain-related protein, apolipoprotein J precursor (Apo J), cAMP-dependent protein kinase catalytic subunit alpha 1, and an orf (Liao et al., 2007). Some of these molecules are known to play important roles in CNS microglia activation, while others are involved in actin metabolism and fibrinolysis in the periphery (Liao et al., 2007). Elevated plasma AAT levels in AD patients were further validated by enzyme-linked immunoassay (ELISA) (Liao et al., 2007). AAT has been shown to be present in neurofibrillary tangles and senile plaques (Gollin et al., 1992), and as a serine proteinase inhibitor participates in the control of proteinases during inflammation, coagulation and fibrinolysis (Potempa et al., 1994). AAT has also been reported at elevated levels in AD proteomics studies of CSF (Sihlbom et al., 2008) and by other methods, such as rocket immunoelectrophoresis (Nielsen et al., 2007). Its oxidised form has also been detected in AD patients by several plasma proteomics studies (Choi et al., 2002; Yu et al., 2003).

Excluding albumin and immunoglobulin fragments, Hye and colleagues identified 11 proteins in plasma which were significantly different between AD patients and age-matched controls (Hye et al., 2006). They used image analysis of all identified proteins on 2 DGE as predictors to differentiate disease cases and control subjects, achieving 56% sensitivity and 80% specificity. Western blotting further confirmed elevated levels of complement factor H (CFH) and α-2-macroglobulin (α-2M) in plasma of AD patients (Hye et al., 2006). CFH and α-2M have also been found in amyloid plaques in AD (Bauer et al., 1991; Strohmeyer et al., 2000), and are positively associated with the hippocampal metabolite ratio N-acetylaspartate/myo-inositol (NAA/mI) (Thambisetty et al., 2008). The NAA/mI ratio is associated with cognitive decline in AD and MCI subjects, with considerably stronger correlations in AD than in the MCI group (Thambisetty et al., 2008). Another two novel biomarkers were recently reported in proteomics experiments: it was noted that serpin F1 (pigment epithelium-derived factor) and complement C1 inhibitor are down regulated in plasma from AD patients, and these observations were confirmed by specific assays (Cutler et al., 2008).

3.2. *Proteome-based serum biomarkers of AD*

Proteomics methods have been used to mine biomarkers in human serum. Liu and colleagues collected serum from 10 AD patients and sex- and age-matched controls. They selected 9 differentially expressed spots on 2 DGE gels for matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS analysis, and found low serum levels of apolipoprotein A-I (ApoA-I) in AD patients (Liu et al., 2006). In this study, polymorphism of the ApoA-I gene was not associated with the occurrence of AD. ApoA-I is the major constituent of high-density lipoprotein, has an important role in cholesterol transport out of cells and in the maintenance of lipid homeostasis, and was shown to reduce aggregation of Aβ via lipid homeostasis (Koldamova et al., 2001). However, apolipoproteins are difficult to detect in prefractionation proteomics studies, which may be due to the tendency of lipoproteins to adhere to plastic vials resulting in loss during sample transfer

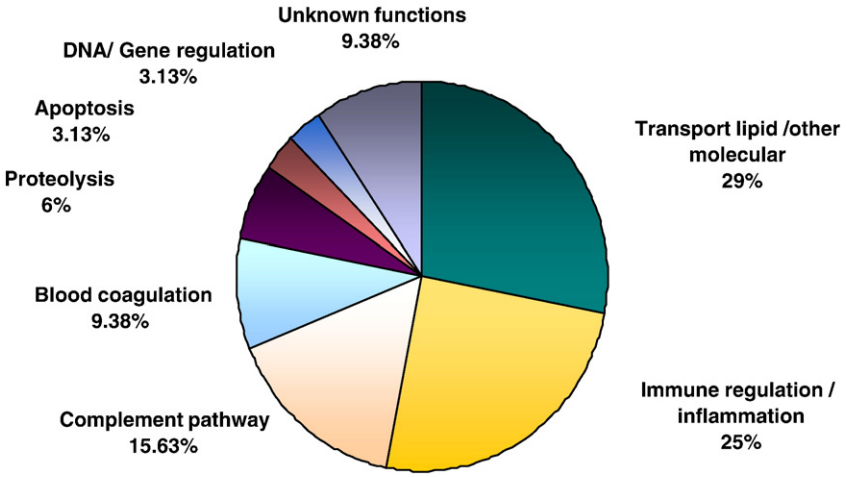

Fig. 2 – Proteins showing increased or decreased levels in the plasma of AD and MCI individuals, classified according to their function. The data are based on 32 proteins identified in plasma proteomics studies (see Table 1).

Table 1 – Up and down regulated proteins in AD plasma proteomics studies.

Function	Identified protein	Sample	Up down	Method	Citation
Lipid transport	Apolipoprotein J precursor	Plasma	Down	①	Corder et al. (1994)
	Apolipoprotein B100	Serum	Up	⑤	German et al. (2007)
	Apolipoprotein A–I	Plasma	Down	⑦	Ganguli et al. (2004)
	Apolipoprotein E	Serum	Up	⑧	German et al. (2007)
Copper transport	Ceruloplasmin precursor	Plasma	Down	①	Chen et al. (1995)
Thyroxine transport	Transthyretin	Serum	Up	④	German et al. (2007)
Oxygen transport	Hemoglobin alpha chain	Serum	Up	⑤	German et al. (2007)
Heme transport * oxidative stress protection	Hemopexin	Plasma	Up	②	Ferri et al. (2005)
Iron carrier protein *energy metabolism	Transferrin	Plasma	Up	②	Ferri et al. (2005)
Immune regulation	Inter-alpha-trypsin inhibitor heavy chain H4 precursor	Plasma	Down	①	Chen et al. (1995)
	CD5 antigen-like precursor	Plasma	Down	①	Chen et al. (1995)
	Serum amyloid P-component precursor	Plasma	Up	①	Chen et al. (1995)
	Factor H	Serum	Up	⑥	German et al. (2007)
Inflammation	Haptoglobin alpha 2 chain	Serum	Up	⑨	German et al. (2007)
	Alpha 1-acid glycoprotein	Serum	Down	⑩	German et al. (2007)
	Inter-alpha-trypsin inhibitor family heavy chain-related protein	Plasma	Down	①	Corder et al. (1994)
	Vitamin D-binding protein	Plasma	Up	①	Corder et al. (1994)
Complement pathway	Complement component 4 precursor	Plasma	Down	①	Chen et al. (1995)
	Complement component 4	Serum	Up	④	German et al. (2007)
	Complement component 3	Serum	Up	④	German et al. (2007)
	Complement factor H precursor	Plasma	Up	①	Chen et al. (1995)
	Complement factor H protein 2 precursor	Plasma	Up	①	Chen et al. (1995)
Proteolysis	Alpha-1-antitrypsin	Plasma	Up	①, ②, ③	Corder et al. (1994); Ferri et al. (2005); Farlow et al. (2004)
	Alpha-2-macroglobulin precursor	Plasma/serum	Up	①, ④	Chen et al. (1995); German et al. (2007)
Blood coagulation	Vitronectin precursor	Serum	Up	⑥	German et al. (2007)
	Fibrinogen r-chain precursor protein	Plasma	Up	③	Farlow et al. (2004)
	cAMP-dependent protein kinase catalytic subunit alpha isoform 1	Plasma	Down	①	Corder et al. (1994)
Apoptosis	Galectin-7	Plasma	Up	①	Chen et al. (1995)
DNA/Gene regulation	Histone H2B.a/g/h/k/l	Plasma	Down	①	Chen et al. (1995)
Unknown functions	desmoplakin	Plasma	Up	①	Chen et al. (1995)
	Unknown protein ORF	Plasma	Up	①	Corder et al. (1994)
	Histidine-rich glycoprotein	Serum	Up	⑥	German et al. (2007)

① 2-DGE LC/MS/MS.
② Glycan affinity chromatography/MALDI-TOF.
③ 2-DGE/DNP-immunostaining/MALDI-TOF.
④ DEAE/1-DGE/MALDI.
⑤ 1-DGE/MALDI.
⑥ Heparin/1-DGE/MALDI.
⑦ 2-DGE/MALDI-TOF.
⑧ DEAE/LCQ.
⑨ DEAE or Ni 2-DGE/MALDI.
⑩ ConA/LCQ.
* Additional functions.

(Davidsson and Sjogren, 2006). By using a combination of multi-dimensional liquid chromatography and gel electrophoresis coupled to MALDI quadrupole TOF MS and Ion Trap LC/MS/MS, haptoglobin, hemoglobin, vitronectin, apolipoprotein B100, fragment of factor H, and histidine-rich glycoprotein were found to be elevated in the serum of AD patients compared with controls, whereas levels of alpha 1-acid glycoprotein were lower (Zhang et al., 2004).

Carrier-protein bound peptides were separated by affinity chromatography from sera of AD patients and healthy controls, and raw mass spectra were acquired on a MALDI orthogonal TOF (o-TOF) mass spectrometer. Raw mass spectra were used to calculate the sensitivity, specificity and test efficiency (Lopez et al., 2005). A recent study used a similar method, identifying four monoisotopic peaks as potential serum markers that discriminate AD from Parkinson's disease and control subjects (German et al., 2007). However, a serious limitation of this type of analysis is the lack of sequence information, which precludes identification of the proteins from which these peptide peaks were derived.

3.3. *Proteome-based biomarkers of MCI-to-AD progression*

Proteomic analysis is also used to study the progression from MCI to AD. Seventeen potential protein biomarkers were differentially expressed in the CSF of patients with stable MCI and those who progressed to AD (Simonsen et al., 2007). Five up-regulated proteins in this group were associated with AD neuropathology, including C3a anaphylatoxin des-Arg, C4a anaphylatoxin des-Arg, phosphorylated osteopontin C-terminal fragment, ubiquitin and β2-microglobulin (Simonsen et al., 2007). C3a anaphylatoxin des-Arg and C4a anaphylatoxin des-Arg are related to inflammation (Gasque et al., 2000), and ubiquitin is implicated in protein degradation in brain, as well as formation of senile plaques and neurofibrillary tangles (Perry et al., 1987). Biomarkers which reflect longitudinal changes in disease may further increase confidence in their diagnostic efficacy, but currently there is no published data relating to proteome-based plasma biomarkers and the likelihood of progression from MCI to AD. Longitudinal studies utilising discovery based approaches are therefore critically needed.

3.4. *Proteome-based biomarkers and AD treatment efficacy*

Proteomics methods have been utilised in clinical trials to seek and evaluate AD biomarkers in treatment efficacy. In one study, although no statistically significant difference was observed in change from baseline in AD Assessment Scale-Cognitive (ADAS-Cog) at 24 weeks between the placebo and the rosiglitazone groups, α-2M, complement C1 inhibitor, CFH and ApoE expression showed a correlation with change in ADAS-Cog score in rosiglitazone groups compared with placebo (Akuffo et al., 2008). Another recent analysis of peripheral leukocytes in AD patients treated for 4 weeks with divalproex sodium identified 10 significantly different proteins related to the cytoskeleton, cell signaling, apoptosis, and intracellular redox status functions which may explain the potential therapeutic mechanism of valproic acid (VPA) on the CNS (Mhyre et al., 2008). However, the therapeutic effect of VPA in AD is not clear; VPA may reduce agitation associated with dementia (Porsteinsson et al., 2003; Sival et al., 2002), but these observations are not unanimous in the literature (Tariot et al., 2005), and additional work is required.

3.5. *Proteomics of oxidised plasma proteins in AD*

A number of studies have explored post-translational modifications such as those related to oxidative stress in relation to cognitive decline, using 2, 4-dinitrophenylhydrazine (DNP) antibodies to monitor carbonyl groups in oxidised proteins as potential biomarkers in plasma. Yu et al. combined affinity column chromatography, 2 DGE, anti-carbonyl western blotting and MALDI-TOF MS analysis, and found three oxidised glycoproteins in AD plasma; transferrin, hemopexin and AAT (Yu et al., 2003). Another study identified seven oxidised protein spots in plasma of AD patients (Choi et al., 2002), including isoforms of fibrinogen c-chain precursor protein and, as with the previous study AAT precursor. Both of which are associated with inflammation and senile plaques of AD (Choi et al., 2002). To date, no published work has used similar approaches to detect oxidised proteins in plasma of MCI patients.

In summary, relatively few proteomics studies of AD and MCI are available, and novel proteome-based plasma biomarkers need to be confirmed independently by several groups to improve confidence in the reproducibility of the findings. Different experimental approaches and designs may partly explain some of the variability, and include methodological variables such as different prefractionation steps and methods of MS. Some studies have evaluated qualitative changes, such as post-translational modifications, whereas other studies purely focus on up or down regulation of protein expression levels. Once a panel of proteins is discovered, further validation is necessary to determine the specificity and sensitivity of detected proteins as biomarkers, and to establish their predictive value in large numbers of samples and in longitudinal studies.

4. Plasma biomarkers related to Aβ metabolism

Extra-cellular amyloid-containing senile plaques and intracellular neurofibrillary tangles are considered to be the central pathologic features of AD (Selkoe, 1994b), and as such are natural biomarker targets. Aβ peptides, total tau and phosphorylated tau have all been quantified in CSF, however, the concentration of tau in plasma is below the limit of detection of most analytical techniques (Tang and Kumar, 2008), so most research has focused on verifying whether Aβ peptides are effective biomarkers in MCI and AD plasma. Two peptides of 40 and 42 amino acids in length, amyloid beta 1–42 (Aβ1–42) and amyloid beta 1–40 (Aβ1–40), derived from amyloid precursor protein (APP), are the main components of amyloid senile plaques (Selkoe, 1994a). Aβ1–42 deposits first in the course of the disease and constitutes the predominant component of senile plaques, whereas Aβ1–40 deposits later (Younkin, 1995). The large majority of Aβ in plasma is bound to albumin, and very little Aβ is free (Biere et al., 1996). Other than Aβ1–40 and Aβ1–42, there are at least six other Aβ isoforms in human plasma: C-truncated Aβ peptides Aβ1–37, Aβ1–38, Aβ1–39, Aβ1–41, and the N-truncated Aβ2–40, Aβ2–42 (Maler et al., 2007). The precise form of Aβ identified in a particular ELISA assay is determined by the specificity of the antibody pair, and whether they are monoclonal or polyclonal. In some studies, Aβ40 and Aβ42 are the full length Aβ1–40 and Aβ1–42 peptides, whereas in other assays a variety of N-terminally truncated forms may be recognised.

There are some known factors that affect plasma Aβ metabolism in the human body, such as advanced AD-related pathology, age, cerebrovascular disease, liver catabolism and renal excretion (Lopez et al., 2008). In the CNS, Aβ is generated in brain, transported into the peripheral vascular system across the blood–brain barrier (Zlokovic, 2004), and also secreted by platelets in blood (Chen et al., 1995). Peripheral Aβ is uptaken and degraded in the liver, and excreted through the kidneys (Ghiso et al., 2004). Further, cerebrovascular disease is associated with levels of plasma Aβ (van Dijk et al., 2004), which were shown to be elevated in ischemic stroke patients (Lee et al., 2005). Levels of plasma Aβ also increase

with age (Fukumoto et al., 2003; Mayeux et al., 2003). Soluble Aβ in plasma is measured by sandwich ELISA, but with much more difficulty than in CSF, because lipoprotein and Fc-binding proteins in plasma might influence immunological detection (Kawarabayashi and Shoji, 2008) and levels of plasma Aβ peptide are about 10 fold lower than in CSF. The quantification of plasma Aβ may also be confounded by the diversity of ELISA antibodies, with their varying sensitivities and sometimes poorly defined specificities.

There is growing evidence that low Aβ1–42 and high tau in CSF could be biomarkers for the diagnosis of AD. Many studies have shown that the concentration of Aβ1–42 in CSF is low in AD patients compared with healthy controls (Andreasen et al., 2001; Gloeckner et al., 2008; Sjogren et al., 2000), and the level of Aβ1–42 in CSF significantly declines in MCI (Andreason et al., 2001). Utilising 17 studies of CSF Aβ and 34 studies of CSF tau, a meta-analysis showed that levels of CSF Aβ1–42 were significantly lower, and CSF tau were significantly higher in AD patients (Sunderland et al., 2003). However, decreased Aβ1–42 levels in CSF are seen not only in AD patients, but also in other dementias, including Lewy body dementia, fronto-temporal dementia (Gloeckner et al., 2008), and white-matter dementia (Sjogren et al., 2000). A recent study found that levels of CSF Aβ1–42 were lower in AD patients carrying the ApoE var ε4 allele than those without the ε4 allele (Smach et al., 2008).

In cross-sectional studies, there is conflicting evidence as to whether plasma Aβ peptide is a useful diagnostic marker. Some early studies did not find that Aβ peptides in plasma differed significantly between AD patients and control groups (Fukumoto et al., 2003; Kosaka et al., 1997; Tamaoka et al., 1996). One study showed a significant increase of Aβ1–40 in AD, but with a substantial overlap between AD and control groups (Mehta et al., 2000).

Some recent follow-up studies have shown that declining plasma Aβ peptide levels may be related to disease progression. Mayeux et al. found that levels of plasma Aβ1–42, but not Aβ1–40, decreased over time in patients with newly acquired AD, and may be associated with mortality in AD patients, whereas plasma Aβ1–40 was stable or increased in prevalent and incident AD cases (Mayeux et al., 2003). Plasma Aβ1–42 levels reduced in a 4 year longitudinal study, and high baseline Aβ1–42 levels and a decrease during follow-up were associated with decline in Mini-Mental State Exam (MMSE) scores (Pomara et al., 2005). In a recent longitudinal study, AD outpatients were followed up for more than 4 years, and low plasma levels of Aβ1–40, Aβ1–42 and high-sensitivity C-reactive protein were associated with rapid cognitive decline (Locascio et al., 2008). Locascio et al. hypothesised that before Aβ deposition commences in the brain, a high plasma Aβ level may reflect a genetic predisposition to increased production, or reduced clearance of Aβ (Locascio et al., 2008). With the initiation of Aβ deposition, plasma Aβ levels decline, and once the disease is established, the lowest Aβ levels seem to predict more rapid progression (Locascio et al., 2008). Sequestration of Aβ in neuritic plaques also explains the low Aβ1–42 levels in CSF of AD patients; however this hypothesis does not explain how plasma Aβ levels can influence AD risk and AD progression (Locascio et al., 2008). A 2.5 years cohort study showed cognitively healthy (CH)-to-MCI and CH-to-AD converters had significantly increased plasma Aβ1–42 levels as compared to subjects who remained cognitive healthy, and a logistic regression analysis showed that increased plasma Aβ1–42 significantly predicted the conversion from CH to MCI, but not the conversion from CH to AD (Blasko et al., 2008). Although not significant, plasma Aβ1–42 tended to decrease in MCI-to-AD converters compared to CH-to-AD converters (Blasko et al., 2008). These results also showed that plasma Aβ levels are high in the preclinical stages and drop as the disease progresses. A replication of this study is needed before these findings can be used to inform the pathogenesis of AD.

Some longitudinal studies have shown a marginal association between plasma Aβ levels and cognitive decline. In an unadjusted prospective model in normal subjects, both Aβ1–40 and Aβ1–42 levels at baseline were associated with incident AD in longitudinal analysis, but in the fully adjusted multivariate model neither Aβ1–42 and Aβ1–40, nor their ratio, was associated with incident AD (Lopez et al., 2008). One cohort study compared Aβ levels both in plasma and CSF to evaluate plasma Aβ isoforms as predictors of conversion to AD in patients with MCI, and showed that decreased Aβ1–42 in CSF but not plasma was associated with increased risk of future AD (Hansson et al., 2008).

Another approach to quantification of Aβ peptides is the Aβ1–42/Aβ1–40 ratio. A recent cohort study supported the possibility that this ratio in CSF was significantly decreased in the MCI patients developing AD, compared to cognitively stable MCI patients and MCI patients developing other dementias (Hansson et al., 2007). Two other studies found similar results in the plasma of patients with MCI and AD. One case-cohort study reported that a lower ratio of Aβ42/Aβ40 and a higher level of Aβ1–40 at baseline were associated with increased risk of developing dementia, and this association was independent of the ApoE 4 allele (van Oijen et al., 2006). Another recent study combined aMCI and AD as follow-up endpoints from cognitively normal subjects, and showed that a low plasma Aβ42/Aβ40 ratio was associated with conversion to MCI or AD, whereas the levels of Aβ42 and Aβ40 on their own did not (Graff-Radford et al., 2007).

In summary, there is no definitive conclusion as to whether plasma Aβ reflects the changing level of central amyloid, and research reports are conflicting. Some recent studies have shown that low Aβ1–42 or Aβ1–40 levels, or the ratio of Aβ1–42/Aβ1–40, are risk factors for MCI and AD (Blasko et al., 2008; Graff-Radford et al., 2007; Hansson et al., 2007; Locascio et al., 2008; Mayeux et al., 2003; Pomara et al., 2005; van Oijen et al., 2006). Longitudinal studies using large sample sizes and reliable measurement techniques may be an effective approach to address this issue, and measurement of the Aβ1–42/Aβ1–40 ratio is warranted. Furthermore, combination of Aβ peptides with other potential plasma biomarkers achieved high sensitivity (91%) and specificity (71%–99%) in one study (Ait-ghezala et al., 2008).

5. Biomarkers of oxidative stress

There are two hypotheses relating peripheral oxidative stress to neurodegeneration. The first suggests that oxidative stress initially develops in the periphery with a variety of possible causes, and results in reduction of CNS antioxidants, finally

leading to oxidative damage and neurodegeneration (Pratico, 2005). The second is that the CNS is the original place where oxidative stress begins, and then several different metabolic end-products are formed and transported into the periphery (Pratico, 2005). Lipid peroxidation (malondialdehyde, 4-hydroxynonenal, F2-idoprostanes), protein carbonyls (protein carbonyls and nitrotyrosine) and DNA oxidation (8-hydroxy-2-deoxyguanosine, single-strand breaks) in the AD brain have been reported as markers of oxidative damage (Solfrizzi et al., 2006). However, oxidative DNA damage is not an effective diagnostic biomarker for AD patients (Pratico, 2005). As described above, results of proteomics studies provide evidence that oxidised proteins in plasma may be useful biomarkers in MCI and AD. Using ELISA based studies, levels of protein carbonyls in AD patients were found to be lower in serum but not in CSF and plasma, and levels of nitrotyrosine did not differ between AD patients and controls (Korolainen and Pirttila, 2009).

Isoprostane is one of the products of lipid peroxidation, formed by free radical-mediated peroxidation of poly-unsaturated fatty acid (Lovell and Markesbery, 2007). Although isoprostane is not neurotoxic, elevated levels of F2-isoprostane (F2-IsoP) have been observed in AD CSF and brain (Montine et al., 2007; Pratico et al., 2000). In a longitudinal study, levels of CSF F2-IsoPs in AD patients were significantly increased during the follow-up period, and also significantly declined in patients accepting anti-oxidant treatment (Quinn et al., 2004). The significance of F2-isoprostane in AD and MCI plasma is still controversial. Pratico et al. found high levels of F2-IsoP in plasma, CSF and urine of MCI patients (Pratico et al., 2002), and the same research group showed similar results in AD patients (Pratico et al., 2000). In a recent study, mean plasma F2-IsoP levels were not increased in AD or MCI, but a high percentage of anti-oxidant use in MCI (74%) and AD (88%) in this research may have influenced the F2-IsoP concentrations in plasma (Irizarry et al., 2007). Other research has reported that plasma and urine F2-IsoP did not accurately reflect CNS levels in AD patients (Montine et al., 2002). More work will be needed to make firm conclusions as to the validity of F2-Isop as a plasma biomarker.

6. Biomarkers of inflammation

It has been established that molecules representing inflammatory processes occur in the AD brain (Akiyama et al., 2000; Solfrizzi et al., 2006), and the presence of activated microglia and astrocytes increases the level of pro-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor α (TNF-α), as well as acute phase proteins, such as C-reactive protein (CRP) and α1-antichymotrypsin (ACT) (Akiyama et al., 2000; Yasojima et al., 2000). There is experimental as well as clinical evidence to support the hypothesis that inflammatory processes might be involved in the early stages of AD, even before amyloid deposition or the appearance of clinical symptoms (Engelhart et al., 2004; Schuitemaker et al., 2008; Veerhuis et al., 2003). However, the results on inflammatory markers in the peripheral circulation of AD and MCI patients are controversial. Several longitudinal studies have shown that inflammatory markers in serum or plasma are related to cognitive decline. High serum ACT was associated with an increased risk of cognitive decline (Dik et al., 2005), and high-sensitivity CRP significantly increased risk of combined dementias, AD, and vascular dementia (Schmidt et al., 2002). Serum IL-6 and CRP were prospectively related to cognitive decline in well-functioning elders (Yaffe et al., 2003) and high levels of ACT, IL-6 and CRP in plasma were associated with an increased risk of AD respectively (Engelhart et al., 2004).

A recent study used ELISA to measure 120 known signaling proteins involved in central and peripheral immune and inflammatory mechanisms in plasma from AD subjects and nondemented controls (Ray et al., 2007). Two groups of subjects were divided equally into a training set and a test set. By analyzing the training set with the predictive analysis of microarrays, a panel of 18 signal proteins was certified as effectively predictive for diagnosis of MCI and AD. Researchers used 18 predictor proteins to classify the test set of AD and control subjects, and reported 90% positive agreement and 88% negative agreement with clinical diagnosis. It also effectively predicted progression from MCI to AD with 91% positive agreement with clinical diagnosis. The biological functions of these markers are associated with hematopoiesis, inflammation, neuroprotection, apoptosis and energy homeostasis in AD (Ray et al., 2007).

7. Biomarkers for lipoprotein metabolism

7.1. *Apolipoprotein E*

Three common isoforms of ApoE are ApoE2, ApoE3 and ApoE4, which are coded by three alleles, ApoE ε2, ApoE ε3 and ApoE ε4 (Sando et al., 2008). ApoE protein is a major component of very low-density lipoproteins (VLDL), and is involved in cholesterol transport centrally as well as peripherally. ApoE ε4 allele is a significant genetic risk factor for sporadic AD (Petersen et al., 1995; Saunders et al., 1993), and may also impair memory function in MCI patients (Albert et al., 2007; Farlow et al., 2004). It is also the only established genetic risk factor for sporadic onset of AD. Compared with controls, the prevalence of the ApoE ε4 allele is increased not only in AD and MCI patients, but also in individuals reporting subjective complaints (Ramakers et al., 2008). The negative role of the ApoE ε4 allele in MCI and AD is related with its effect on cholesterol metabolism, which increases levels of total and low-density lipoprotein cholesterol (Kulminski et al., 2008; Sorli et al., 2006). Longitudinal studies have shown the ApoE ε2 allele to be a protective factor for AD (Corder et al., 1994) and cognitive decline (Blacker et al., 2007; Wilson et al., 2002). The protective mechanism of the ApoE ε2 allele may be the reverse of the ε4 allele, *i.e.* decreased levels of total and low-density lipoprotein cholesterol (Kulminski et al., 2008; Sorli et al., 2006). ApoE ε3 allele appears to be neutral for AD development.

7.2. *Cholesterol and lipoprotein*

There are many other risk factors for vascular disease that are also related to increased risk of AD, such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and lipoprotein

A (Solfrizzi et al., 2006). Longitudinal studies have shown that high serum TC at midlife is a risk factor for dementia/Alzheimer disease. A 21 year follow-up study recently showed that high midlife TC represented a risk factor for more severe cognitive impairment later in life, and a moderate decrease in serum TC from midlife to late life (0.5 to 2 mmol/L) was significantly associated with a more impaired late-life cognitive status, even after adjusting for multiple variables, such as age, follow-up time, sex, education, cholesterol, body mass index, ApoE ε4 genotype, medical history, and lipid-lowering treatment (Solomon et al., 2007). However, another follow-up study showed that higher levels of TC and LDL were associated with a decreased risk of total MCI in models adjusted for age and sex (Reitz et al., 2008). These associations were attenuated after adjusting for ethnicity, education, ApoE ε4 and vascular risk factors, and there was no association between lipids and the risk of amnestic or nonamnestic MCI, nor was there an effect of lipid-lowering treatment on MCI risk (Reitz et al., 2008).

8. Homocysteine

Homocysteine, a sulfur-containing amino acid derived from methionine, is a risk factor of developing vascular disease, brain atrophy, cognitive impairment and AD (Sachdev, 2005). Deficiency of Vitamin B12, Vitamin B6 or folate results in high plasma homocysteine levels (Selhub et al., 1993), and homocysteine may be a modifiable risk factor (Kidd, 2008). High plasma homocysteine over 14 μmol/L doubled the risk of AD in an eight year longitudinal study (Seshadri et al., 2002). A recent cohort study showed that subjects who converted from cognitive health to AD had a higher increment of homocysteine compared to cognitive health to MCI converters and subjects remaining cognitively healthy (Blasko et al., 2008). A three year follow-up study showed that MCI subjects who converted to dementia, with evidence of impairment in memory and non-memory cognitive domains, had significantly higher baseline plasma total homocysteine levels than non-converters (Gabryelewicz et al., 2007). High levels of homocysteine might predict cognitive decline (Tucker et al., 2005) and the conversion from cognitive health or MCI to AD (Blasko et al., 2008). Whether lowering homocysteine levels could improve cognitive impairment, or prevent dementia development should be evaluated further (Seshadri, 2006).

9. Conclusion

A variety of potential plasma biomarkers for AD and MCI have been identified. So far there has been no single plasma biomarker that comes close to NIA criteria, especially in relation to sensitivity and specificity. However, a panel of plasma proteins achieved a higher level of specificity (Ray et al., 2007), and combination of plasma biomarkers increased sensitivity (91%) and specificity (71%) (Ait-ghezala et al., 2008). For a complex disease such as AD, combining multiple biomarkers from different metabolic pathways may increase the sensitivity and specificity of diagnosis of cognitive decline, and is possibly the way for the future (Solfrizzi et al., 2006). All identified biomarkers are still in the preclinical testing stage, and need much more validation in large population based longitudinal studies. This area of research promises to yield interesting results in the next few years.

10. Conflicts of interest

None of the authors have any conflicts of interests with regard to this work.

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