Blood-based Biomarkers of Microvascular Pathology in Alzheimer's disease

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Abstract

SporadicAlzheimer's disease (AD) is a genetically complex and chronically progressive neurodegenerative disorder with molecular mechanisms and neuropathologies centering around the amyloidogenic pathway, hyperphosphorylation and aggregation of tau protein, and neurofibrillary degeneration. While cerebrovascular changes have not been traditionally considered to be a central part of AD pathology, a growing body of evidence demonstrates that they may, in fact, be a characteristic feature of the AD brain as well. In particular, microvascular abnormalities within the brain have been associated with pathological AD hallmarks and may precede neurodegeneration. In-vivo assessment of microvascular pathology provides a promising approach to develop useful biological markers for early detection and pathological characterization of AD. This review focuses on established blood-based biological marker candidates of microvascular pathology in AD. These candidates include plasma concentration of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) that are increased in AD. Measures of endothelial vasodilatory function including endotheline (ET-1), adrenomedulline (ADM), and atrial natriuretic peptide (ANP), as well as sphingolipids are also significantly altered in mild AD or during the predementia stage of mild cognitive impairment (MCI), suggesting sensitivity of these biomarkers for early detection and diagnosis. In conclusion, the emerging clinical diagnostic evidence for the value of blood-based microvascular biomarkers in AD is promising, hoewever, still requires validation in phase II and III diagnostic trials. Moreover, it is still unclear whether the described protein dysballances are early or downstream pathological events and how the detected systemic microvascular alterations relate to cerebrovascular and neuronal -pathologies in the AD brain.

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Keywords

Alzheimer's disease; AD; dementia; mild cognitive impairment; MCI; neurodegeneration; cerebrovascular; microvascular; vasodilation; vasoconstriction; biomarkers; early detection; diagnosis; blood; cerebrospinal fluid; CSF; vascular cell adhesion molecule-1; VCAM-1; intercellular adhesion molecule-1; ICAM-1; selectin; endotheline; ET-1; adrenomedulline; ADM; atrial natriuretic peptide; ANP; sphingolipids; ceramides

Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia. Key molecular mechanisms and histopathological hallmarks in the AD brain comprise a dynamic cascade of biochemical events including the pathological amyloidogenic cleavage of the amyloid precursor protein (APP), the generation of various beta-amyloid species including the amyloid-beta peptide ($A\beta_{1-42}$), dimers, trimers, oligomers and subsequent amyloid aggregation and deposition in plaques, abnormal hyperphosphorylation and aggregation of tau protein, progressive intracellular neurofibrillary degeneration, changes within the innate immune system and inflammation. Vascular changes are frequently observed in the AD brain as well, however, have traditionally been interpreted as an independent, separated string of "co-pathology" that may be further downstream and confound rather than support an early detection and reliable diagnosis of AD. This still presents some dilemma in objectively interpreting emerging data on vascular alterations in AD. Currently, the diagnosis of AD is precluded if the observed cognitive deficits can be explained by present cerebrovascular disease according to the widely used Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM IV) (American Psychiatric Association, 1994). However, it is increasingly recognized that cerebrovascular pathology may characteristically and perhaps even specifically ("pathognomonically") contribute to the generation of amyloid pathology and/or neurodegeneration and cognitive decline in AD, and has been estimated to at least constitute a "co-pathology" in as much as 50% of AD cases (Breteler, 2000). In fact, epidemiological studies have shown that conditions associated with pathological vascular changes, such as atherosclerosis, diabetes, or high blood pressure constitute major risk factors for late-onset AD (for review see (Breteler, 2000; de la Torre, 2000)). For example, the large, population-based Rotterdam study showed that atherosclerosis is associated with increased risk for both AD and vascular dementia; participants with severe atherosclerosis had three-fold increased odds for AD (95% CI 1.5–6.0) compared to those without (Hofman et al., 1997). Apart from macrovascular alterations affecting large brain arteries, specific microvascular changes within the extensive brain capillary system may also play an important role in AD. Preclinical studies in a transgenic mouse model of AD suggest that $A\beta_{1-42}$ may lead to reduced cerebral blood flow via microvascular endothelial dysfunction (Deane et al., 2003). Postmortem studies have found $A\beta_{1-42}$ deposition within the microvascular system (including capillaries, arterioles, and venules) and $A\beta_{1-42}$ colocalization of microhaemorrhages in blood vessels (Cullen et al., 2006). These data are consistent with the hypothesis that brain microvascular disturbances are closely related to cerebral hypoperfusion and cerebral amyloidosis in AD (de la Torre, 2004). Clinical findings suggest that microvascular damage within the brain shows an association with the severity of cognitive deficits as measured by the Clinical Dementia Rating (CDR) scale (Bailey et al., 2004). Thus, microvascular cerebral pathology may play a crucial role in the development of AD-related neurodegeneration and may contribute to late-stage clinical cognitive decline in AD.

From a clinical perspective, the in-vivo detection and quantification of microvascular pathology is highly desirable, both for "detection/diagnosis/prediction" and therapy of AD. Since vascular changes are hypothesized to precede the onset of AD-related clinical dementia

(de la Torre, 2000), the measurement of microvascular cerebral damage could provide a sensitive instrument for the early detection of AD. Secondly, microvascular pathology may be an important target of specific therapeutic intervention (Zlokovic, 2008) and related biological markers (of mechanisms of action [MoA] or biological activity) would be of pivotal importance to help map disease progression and evaluate therapeutic efficacy. To date, the best established biochemical biomarkers of AD focus on the proteins total tau and p-tau (phosphorylated at different epitopes) and $A\beta_{1-42}$ measured in cerebrospinal fluid (CSF)(Blennow and Hampel, 2003; Zetterberg et al., 2009). CSF-marker candidates such as the concentration of phosphorylated tau (p-tau), total tau, $A\beta_{1-42}$, and the emerging biomarker of BACE1 activity and protein concentration show high sensitivity for the early detection of AD within the prodromal phase of the disease when mild cognitive impairment (MCI) is present (Blennow and Hampel, 2003; Hampel et al., 2009; Mattsson et al., 2009). However, CSF-derived markers are dependent upon lumbar puncture which is not common practice in the majority of settings and seems less amenable as a broadly applied screening tool for a larger at-risk population. Bloodmarkers of microvascular pathology measured in plasma or serum, however, may provide a clinically easily accessible tool for screening, early detection, diagnosis, prediction and monitoring of AD progression. In the following pages, major microvascular biomarker candidates of AD are reviewed and discussed (see table 1 for overview). Biomarkers of inflammatory and $A\beta$ phagocytosis in blood and CSF have been reviewed elsewhere (Fiala and Veerhuis, 2009).

2 Adhesion molecules

The vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) are members of the immunoglobulin gene superfamily of adhesion molecules. VCAM-1 is exclusively expressed on endothelial cells whereas ICAM-1 can also be expressed on leukocytes or fibroblasts. Cellular adhesion molecules regulate the transcapillary permeability, including the microvascular system. Levels of both VCAM-1 and ICAM-1 are upregulated by cytokines such as tumor necrosis factor-alpha (TNF-α), interferon-γ, and interleukin-1β (IL-1), and have been linked to atherogenesis (Price and Loscalzo, 1999). Inflammation-induced upregulation of cell adhesion molecules reduces the permeability of the microvasculature by interaction with leucocytes and reduces vasodilation. Increased plasma levels of VCAM-1 and ICAM-1 are correlated with microvascular impaired endothelium-dependent vasodilation, as measured by flow-mediated vasodilation (FMD), in patients with peripheral arterial disease (Brevetti et al., 2001). In elderly people, increased plasma levels of the inflammatory marker c-reactive protein (CRP) correlate with both VCAM-1 and ICAM-1 (Wilker et al.).

Few clinical studies have examined blood levels of these markers in patients with AD (see table 1). Zuliani and colleagues found that plasma levels of VCAM-1 were increased in both 60 patients with AD and 80 patients with vascular dementia compared to normal elderly controls, independently upon the presence of small or large vessel disease (Zuliani et al., 2008). The cognitive status of these controls was unfortunately not reportedIn a population based setting however, plasma levels of ICAM-1 and VCAM-1 were not associated with an increased risk of AD as assessed in the Rotterdam study including a subset of 727 subjects selected randomly out of a total of 7050 subjects. The rate ratio for ICAM-1 associated with AD was 0.87 (95% confidence interval = 0.63 – 1.21) and for VACM-1 the rate ratio was 1.02 (0.75 – 1.38). Yet, inflammatory peripheral markers including CRP and interleukin 6 (IL-6) receptor complex were associated with an increased risk of AD in both univariate and multivariate models controlling for other vascular risk factors including tobacco smoking, body mass index, diabetes mellitus, use of anti-inflammatory medication, and atherosclerosis (Engelhart et al., 2004). It should be noted, however, that the control subjects may have had mild cognitive impairment (MCI), a clinical syndrome associated with increased risk of AD

(Petersen et al., 2001). Thus, the unclear cognitive status of the controls that may have included MCI could potentially have reduced the power to detect an AD related association with ICAM-1 and VCAM-1 plasma levels.

Selectins constitute another class of adhesion molecules that have been associated with microvascular damage. Selectins are exclusively shed by endothelial cells and mediate leucocyte adhesion at sites of inflammation or injury (Price and Loscalzo, 1999). The subtype E-selectin is exclusively expressed within the endothelium and, similar to VCAM-1 and ICAM-1, induced by cytokines and involved in atherogenesis. Increased plasma levels of E-selectin, in addition to VACM-1 and ICAM-1, have been observed to be associated with increased risk of diabetes, a condition that is commonly associated with cardiovascular disease (Meigs et al., 2004) and increased risk of AD.

Findings of clinical studies in AD, however, suggest that blood-levels of E-selectin are not significantly altered. In the same AD and vascular dementia patients who showed increased blood concentration of VCAM-1 plasma levels of E-selectin were not significantly altered (Zuliani et al., 2008). Similarly, in contrast to increased serum concentration of ICAM-1, serum concentration of E-selectin was not found to be elevated within AD patients, when compared to controls who had either headache, depression, or anxiety disorder (Rentzos et al., 2004). The discrepancy between the findings of alterations on ICAM-1 and VCAM-1 versus E-selectin may be due to different sources of origin, as ICAM-1 and VCAM-1 may stem more from cerebral parenchymal sources (Rentzos et al., 2004).

3. Vasodilators and Vasconstrictors

Regulators of endothelial function and vasodilation include endothelin-1 (ET-1), atrial natriuretic peptide (ANP), and adrenomedullin (ADM).

ET-1, a 21 amino acid peptide, is the most abundant member of a family of endothelins. It is primarily derived from peripheral and central endothelial and vascular smooth muscle cells, but is also synthesized by astrocytes and neurons. ET-1 exerts a vasoconstrictive effect and has been implicated in the development of hypertension, chronic heart failure, and myocardial infarction. The atrial natriuretic peptide (ANP) is found in neurons and astrocytes in the CNS, especially in the hypothalamus, and acts as a vasodilator. Adrenomedullin (ADM) is, among others, produced by peripheral and central endothelial and vascular smooth muscle cells as well as neuronal and glial cells. With its potent vasodilating activity, the production of ADM assures blood supply to the individual organs.

While these molecules have been extremely difficult to measure in the blood, due to the rapid clearance and a half-life from 1 to 22 minutes, assays have recently been developed to detect precursor fragments of these bioactive peptides, a concept well known (e.g. for the relationship of insulin and the so called C-peptide precursor fragment). These assays measure within plasma the C-terminal endothelin-1 precursor fragment (CT-proET-1), midregional pro-adrenomedullin (MR-proADM), and midregional pro-atrial natriuretic peptide (MR-proANP) as functionally inactive surrogates of the active substances (Morgenthaler et al., 2005; Morgenthaler et al., 2004; Papassotiriou et al., 2006).

Recently, we examined in a first study these novel potential biomarkers of microvascular changes in 94 patients with probable AD and 53 elderly cognitively normal healthy controls (Buerger et al., 2009). For the diagnosis of AD, the NINCDS-ADRDA criteria (McKhann et al., 1984) were used. The HC subjects were physically and cognitive healthy, and scored within one standard deviation of the norm on all subtests of the CERAD cognitive subtests (Morris et al., 1989). The blood was obtained from non-fasting subjects in the morning between 8.00 and 12.00 am. The blood was collected in ethylenediaminetetraacetic acid (EDTA) containing

tubes and immediately centrifuged at 2000 g for 10 min. at +4°C. The supernatant was extracted with a pipette and stored at −80°C until analysis of the microvascular marker candidates. The plasma levels of MR-proADM, MR-proANP, and pro CT-proET-1 were measured by chemiluminescence sandwich immunoassays as previously described (Morgenthaler et al., 2005; Morgenthaler et al., 2004; Papassotiriou et al., 2006). Results showed that in AD patients the plasma concentration of the vasodilators MR-proADM and MR-proANP were increased but the vasoconstrictor CT-proET-1 decreased. Controlling for age, CRP levels, blood pressure, ApoE genotype, and intake of diuretics, increased levels of the vasodilator MR-proANP in blood was the strongest predictor for the classification of AD (vs. healthy controls), with a sensitivity of 62.8% at a specificity of 81.1%. When the ratio of MR-proANP/CT-proET-1 was taken as the predictor, the sensitivity yielded 76.6% and specificity of 81.1% (Buerger et al., 2009). The blood concentration of MR-proADM showed a lower classification accuracy with a sensitivity of 46.8% at a specificity of 81.1 % and the ratio of MR-proADM/CT-proET-1 showed sensitivity of 66% at a specificity of 81.1% for the detection of AD. These first results suggest that these microvascular blood-markers demonstrate a clinically relevant diagnostic accuracy. Preliminary results from another study suggest that these marker candidates may be already altered in the prodromal stage of AD. The concentration of MR-proANP predicted significantly the time to conversion from MCI to AD within a clinical follow-up time window between 4–6 years, when controlling for age, gender, systolic and diastolic blood pressure, as well as APOE ε4 carrier status (Bergmann et al., 2008). These preliminary findings are currently further evaluated in validation studies.

4. Sphingolipids

Sphingolipids are a class of lipids derived from the amino alcohol sphingosine and include sphingomyelins (SM), ceramides, and sphingosine-1-phosphate (S1P). Sphingomyelins (SM), give cells their asymmetric shape and marked curvature, directly effecting structure and permeability of the cell membrane. There is strong evidence from cultured cell studies that cholesterol and SM preferentially interact in plasma neuronal membranes to form lipid rafts (Slotte, 1997). Ceramides can be created as a product of SM hydrolysis by sphingomyelinases or synthesized *de novo* by ceramide synthases. Ceramides are pro-apoptotic and pro-atherogenic. The exogenous application of short-chain ceramide analogues can induce apoptosis in vascular wall cells, including cerebral endothelial cells. Ceramides are also involved in the development of atherosclerosis. Stress stimuli such as TNF-α, oxidized low density lipoprotein (LDL) and IL-1, induce ceramide generation in endothelial cells. Ceramide is subsequently found to promote IL-6 and CRP, thereby enhancing pro-inflammatory effects and further contributing to the development of the atherosclerotic process. Further, ceramide-induced oxidative stress reduces NO levels in endothelial cells and increases peroxynitrite, superoxide and hydrogen peroxide damages to endothelial cells and astrocytes in the brain (Chen et al., 2005). This cascade leads to a leaky blood brain barrier that may attract macrophages and neutrophils, further enhancing the atherogenic process and also affecting blood-brain barrier (BBB) permeability. Lastly, the effects of the neuropeptide endothelin-1 (ET-1) in altering BBB permeability may be mediated through ceramide production (Catalan et al., 1996).

In the first study to examine peripheral ceramides in relation to cognitive impairment, we examined serum ceramides among a group of 100 women, aged 70–80 at baseline, and followed up to 6 times over 9 years in the Women's Health and Aging Study (WHAS) II. Blood was drawn from non-fasting participants and serum was centrifuged at 3000 rpm for 15 minutes at +4°C and frozen at −80°C until processing. Total lipids were extracted from plasma samples according to a modified Bligh and Dyer procedure (Bandaru et al., 2007). The quantification of sphingolipids (described in (Bandaru et al., 2007)) was first based on high performance liquid chromatography (HPLC) for temporal resolution of compounds with subsequent

introduction into the mass spectrometer for detection and quantification by mass/charge. The detection, and quantitation of each analyte (in cycles per second; cps) was carried out by ESI/ MS/MS in multiple reaction monitoring mode (MRM) of the parent compound, and products by ion scan (m/z 264.4, 266.4 for ceramides). Lipids were analyzed in tertiles. We found that low levels of serum ceramides were cross-sectionally associated with memory impairment (delayed and immediate recall), but high levels predicted *future* impairment (Mielke et al., 2008a). In fact, none of the participants in this study who had levels of ceramide C22:0 in the lowest tertile developed impairment in HVLT-delayed recall over 9-years of follow-up. In a second independent study we examined ceramide levels in a cross-sectional clinical sample of 25 HC, 17 amnestic MCI subjects (diagnosed according to Petersen criteria; (Petersen, 2004)), and 20 early probable AD patients (diagnosed according to NINCDS-ADRDA criteria (McKhann et al., 1984)). Examining plasma ceramides, using the above described method (Bandaru et al., 2007) we replicated our previous finding such that amnestic MCI cases had lower mean ceramide C22:0 and C24:0 levels compared to both cognitively normal controls and AD patients (Mielke et al., 2008b). Notably, there were no differences in mean ceramide levels between NC and AD groups, suggesting that these lipids are altered in the early stages of AD (see table 1). Among the MCI group, higher plasma levels of both C22:0 and C24:0 were cross-sectionally associated with reduced brain white matter integrity in the splenium and posterior cingulate. Thus, while mean ceramide levels were lower in amnestic MCI participants, higher ceramide levels were found detrimental with regard to white matter integrity in this group. We are currently examining the longitudinal predictive value of these ceramide biomarker candidates in amnestic MCI subjects and validate the relationship between blood and brain ceramide levels.

5. Conclusions

In summary, data derived from the emerging development of microvascular biomarkers and current approaches on the blood-based assessment of microvascular changes for the detection and early diagnosis of AD are promising. Further validation in larger and independent samples is warranted. For a variety of candidate markers, such as adhesion molecules ICAM-1 and VCAM-1, as well as vasodilators and vasoconstrictors including CT-proET-1, MR-proADM, MR-proANP there is growing evidence for significant AD related changes. The clinical diagnostic utility of these biomarkers, however, will depend upon the established sensitivity and specificity for the early detection and diagnosis of AD, i.e. at a presymptomatic prodromal stage when clinical manifestations of AD are not yet present. The evidence of the utility of the blood-based biomarkers discussed here to predict the progression to AD in subjects with MCI is sparse yet. The microvascular biomarkers including CT-proET-1, MR-proADM, MR-proANP and ceramides show first evidence for a predictive value, however, the full clinical value of these markers awaits further establishment.

It should be noted that it is currently unclear whether the reported protein dysbalances are products of key initiating molecular events or rather downstream phenomena and to what extent systemic microvascular blood biomarkers specifically reflect microvascular change confined in the brain compartment or perhaps predominantly mirror systemic microvascular changes (Rentzos et al., 2004). Microvasculature is also found in adipose tissue of the body and the biomarker measurements may be influenced by changes in such types of tissue. Testing the association between blood-based biomarkers and neuroimaging of white matter hyperintensities (Mills et al., 2007), cerebral diffusion and perfusion as an indirect measure of cerebral microvascular change may be a suitable methodological approach to test the construct validity of the blood-based markers as measures of microvascular function within the in-vivo brain.

Furthermore, blood-based biomarker candidates are influenced or directly regulated by inflammatory molecules such as CRP or cytokines (Price and Loscalzo, 1999). Most studies that examined AD-related changes of the microvascular changes controlled for inflammatory processes by including measures such as CRP, suggesting an at least partially independent contribution of the microvascular markers for the detection of AD. For the optimization of a microvascular prediction model, the relation between the different types of microvascular and inflammatory biomarkers could be of interest. It remains also to be investigated whether microvascular pathology constitutes a core pathology in AD or may be present only in a subgroup of AD as this may likely determine the utility of potential microvascular damage targeting drug treatments. It will be also of interest to examine whether recently discovered polymorphisms within the clusterin encoding gene CLU and the clathrin protein related PICALM gene are associated with microvascular changes in AD (Harold et al., 2009). Clusterin/ApoJ has been shown to bind to the brain capillary system where it may be involved in regulation of Aβ clearance (Zlokovic, 1996). An essential next goal could be the generation of a microvascular biomarker profile and pathological signature in AD using multimodal neurochemical, genetic and imaging derived assessments.

Acknowledgments

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Table 1

Mean and (standard deviation) of blood-based microvascular biomarkers and clinical study data in AD and HC.

Biomarker	Mean (SD) concentration in AD	Mean (SD) concentration in HC	N (AD/HC)	Sample type	AD vs HC	Ref.
VCAM-1	708 ng/ml (544–1143)*	562 ng/ml (471–676)*	60/30	plasma	elevated by 1.3 x	Zuliani et al. (2008)
	na	na	140/539	plasma	not changed	Engelhart et al. (2004)
ICAM-1	368.5 ng/ml (335.8)	193.3 ng/ml (103.6)	25/15	serum	elevated by 1.9 x	Rentzos et al. (2004)
	na	na	140/539	plasma	not changed	Engelhart et al. (2004)
E-selectin	27.5 ng/ml (20.8)	38.6 ng/ml (29.5)	25/15	serum	not changed	Rentzos et al. (2004),
	37 ng/ml (26.1–70)*	44.1 ng/ml (32.3–78)*	60/30	plasma	not changed	Zuliani et al. (2008)
CT- proET-1	65.2 pmol/L (19)	73.2 pmol/L (15.7)	94/53	plasma	reduced by 1.1 x	Buerger et al. (2009)
MR- proADM	0.7 nmol/L (0.3)	0.6 nmol/L (0.2)	94/53	plasma	elevated by 1.2 x	
MR- proANP	119.6 pmol/L (77.6)	70.7 pmol/L (29.2)	94/53	plasma	elevated by 1.7 x	
Ceramide C22:0 **	1.28e+07 cps (5.49e+06)	1.21e+07 cps (4.18e+06)	25/20	plasma	not changed ***	Mielke et al. (2008)
Ceramide C24:0 **	6.44e+07 cps (2.09e+07)	6.44e+07 cps (1.68e+07)	25/20	plasma	not changed ***	Mielke et al. (2008)

VCAM-1 = vascular cell adhesion molecule-1, ICAM-1 intercellular adhesion molecule-1, CT-proET-1 = C-terminal endothelin-1 precursor fragment, MR-proADM = midregional pro-adrenomedullin, MR-proANP = midregional pro-atrial natriuretic peptide, N = sample size, na = not available, Ref. = Reference

*
median (interquartile range) is indicated

**
Results are reported in units of cycles per second (cps) reflecting areas under the curve using ES/MS/MS. This is common among these methods because, until recently, standards were not available for all the chain lengths.

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While there were no mean differences between HC and AD subjects, MCI cases had significantly lower levels of both ceramides C22:0 and C24:0 compared to both HC and AD subjects at p<0.01.