Potential Blood Biomarkers in Age-related Cerebral Small Vessel Disease

Xin Cheng · Qiang Dong

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Abstract Cerebral small vessel disease is a general term used to reflect clinical, radiological, or pathological phenomena attributed to small vessels of the brain, including small arteries and arterioles but also capillaries and small veins. Age-related cerebral small vessel disease is one of its most common subtypes, and is related to age and vascular risk factors. The consequences of age-related cerebral small vessel disease are mainly lesions located in the subcortical structures such as lacunar infarcts, white matter lesions, large hemorrhages, and microbleeds, which are an important cause of stroke, dementia, and age-related disability. The causes or pathogenesis underlying these phenotypes are not completely understood, and biomarkers to predict the risk and progression are highly needed. This review aims to summarize recent knowledge regarding potential blood biomarkers in age-related cerebral small vessel disease according to the different mechanisms: endothelial dysfunction, inflammation, and β -amyloid hypothesis.

Keywords Cerebral small vessel disease · SVD · Arteriolosclerosis · Lacunar stroke · Lacunar infarcts · White matter lesions · WML · White matter hyperintensities · WMH · Leukoaraiosis · Cerebral microbleeds · CMBs · Biomarker · Endothelial dysfunction · Inflammation · β -amyloid · A β · Cerebral amyloid angiopathy · CAA

Introduction

“Cerebral small vessel disease” is a general term used to describe clinical, radiological, or pathological phenomena with various etiologies that affect the small arteries, arterioles, venules, and capillaries of the brain [1•, 2•]. It is proposed that cerebral small vessel disease (SVD) can be classified into six types according to its different etiologies, which include (1) arteriolosclerosis (or age-related and vascular risk factor-related SVDs); (2) sporadic and hereditary cerebral amyloid angiopathy (CAA); (3) inherited or genetic SVDs distinct from CAA; (4) inflammatory and immunologically mediated SVDs; (5) venous collagenosis; and (6) other SVDs [1•]. Type 1, arteriolosclerosis, is one of the most prevalent forms among the elderly, together with CAA [1•, 3]. On pathology, loss of smooth muscle cells from the tunica media, deposition of fibrohyaline material, narrowing of the lumen, and thickening of the vessel can be seen, and microatheroma and microaneurysms have been noted in some cases [4, 5].

With the aging population and the availability of improved brain imaging techniques, the high prevalence and clinical importance of cerebral SVD have been increasingly recognized in recent years. Advancing age and hypertension are the most important risk factors. Cerebral SVD can be ischemic and/or hemorrhagic, and the principal phenotypes of clinical interest are distinct lacunar infarcts, diffuse cerebral white matter lesions (WMLs, or leukoaraiosis), deep brain hemorrhages, and cerebral microbleeds (CMBs). Growing evidence shows that it is a leading cause of cognitive impairment and functional loss in the elderly population. Therefore, it would be desirable to find some ideal biomarker(s) to predict the risk and progression of cerebral SVD, and there is no doubt that a biomarker derived from a peripheral fluid would be more convenient; thus, this is an

X. Cheng (✉) · Q. Dong
Department of Neurology and Institute of Neurology,
Huashan Hospital, Fudan University,
12 Wulumuqi Zhong Road,
Shanghai 200040, China
e-mail: chengxincn@hotmail.com

Q. Dong
State Key Laboratory of Medical Neurobiology, Fudan University,
Shanghai, China

area of active investigation. This review aims to summarize recent knowledge regarding blood biomarkers in arteriolosclerosis (ie, age-related cerebral SVD).

Quite a few peripheral biomarkers have been proposed to reflect the pathogenesis and correlate with the progression of cerebral SVD, and we classified them here.

Endothelial Dysfunction

The normal endothelium plays an important role in regulation of cerebral blood flow and autoregulation and in the blood–brain barrier. Endothelial dysfunction is thought to play a pivotal role in the pathogenesis and progression of cerebral SVD, especially in lacunar stroke patients [6, 7•]. Endothelial activation is characterized by the increased or de novo expression of leukocyte adhesion molecules (E-selectin, P-selectin, intracellular adhesion molecule-1 [ICAM-1], and vascular cell adhesion molecule-1 [VCAM-1]) and a change of in phenotype from anticoagulant to procoagulant (eg, loss of surface thrombomodulin [TM]) [8, 9•], which can be assessed in vivo by measuring soluble plasma markers that are released into circulation [10]. The different molecules reflect different aspects of endothelial dysfunction.

E-selectin, P-selectin, ICAM-1, TM, Von Willebrand Factor, Tissue Factor

Selectins are transmembrane glycoproteins expressed on activated vascular endothelium (E- and P-selectin) and activated platelets (P-selectin). These adhesion molecules attract leukocytes from the circulation and promote leukocyte rolling along the endothelium [11]. ICAM-1, expressed by activated vascular endothelium and leukocytes, is a precondition for the adhesion and transendothelial migration of leukocytes [12], and increased blood levels reflect an endothelial inflammatory response. TM is a single-chain transmembrane glycoprotein produced and expressed on the endothelial cell surface, where upon binding to thrombin, it not only makes thrombin incapable of generating fibrin clot from fibrinogen, but also activates circulating protein C. Activated protein C, in turn, has an important anticoagulant function including inactivation of clotting factors Va and VIIa [13]. Increased plasma levels of TM are thought to reflect endothelial damage. Von Willebrand factor (vWF) is a large multimeric glycoprotein synthesized by endothelial cells and megakaryocytes that circulates in a noncovalent complex with factor VIII. An increase in plasma vWF is considered to be mainly a marker of endothelial damage as well [14]. Tissue factor (TF) is located at extravascular sites not in contact with the blood but, under stimulation by a variety of agents, TF can be expressed on monocytes and

endothelium. By binding to activated factor VIIa, TF initiates the extrinsic coagulation pathway, and levels of TF thus reflect prothrombotic change [15]. The physiological inhibitor of TF is tissue factor pathway inhibitor (TFPI), which is mainly present in endothelial cells and, by binding to activated factor Xa within the TF-VIIa-Xa complex, limits thrombin formation [15].

Several studies [14–20] examined different combinations of these markers in different phases of symptomatic or asymptomatic lacunar stroke (Table 1), and found elevated levels of these circulating markers suggestive of endothelial activation and dysfunction. However, due to the heterogeneity of the studies, robust conclusions are difficult to draw. Moreover, because these studies are cross-sectional, it cannot be established whether endothelial activation is causative or consecutive. Long-term follow-up of patients with intermittent brain magnetic resonance imaging (MRI) might solve this problem. Two prospective studies provide some evidence to support a causal role. After controlling for conventional risk factors and baseline lesion load, plasma ICAM-1 levels increased with the progression of white matter hyperintensities (WMHs) on MRI in a 6-year community-based population of asymptomatic cerebral SVD [21]. In another 6-year longitudinal study [22], serum ICAM-1 levels also were found to be associated with the progression of silent brain infarctions and periventricular WMLs, and this marker may predict impairment in psychomotor function in patients with type 2 diabetes. However, a recent neuropathologic study speaks against cerebral endothelial activation in the pathogenesis of cerebral SVD [23•]. In this case-control study, the authors examined whether brain endothelial ICAM-1 and TM are altered in small penetrating cerebral arteries of pathologically diagnosed cerebral SVD cases, aged control patients without cerebral SVD, young control cases with no brain pathology, and cases with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL, an early onset hereditary SVD). Interestingly, endothelial ICAM-1 was rarely seen, while robust endothelial TM expression was present in all aged cerebral SVD cases, all aged control brains, and all CADASIL cases [23•]. The findings suggest that elevated blood ICAM-1 [15, 20–22] in cerebral SVD patients was not brain-derived, and may instead derive from peripheral vasculature, while TM being upregulated in response to cerebral SVD, perhaps as a protective mechanism to guard against thrombosis of damaged vessels [23•].

Tissue Plasminogen Activator and Plasminogen Activator Inhibitor-1

Tissue plasminogen activator (tPA), also synthesized and released by endothelial cells, is the primary mediator of

Table 1 Selected papers on blood biomarkers of endothelial dysfunction in age-related cerebral SVD

Study	Year	Endothelial function markers	Time from last ischemic event	Patients, <i>n</i>	Control patients, <i>n</i>	Findings
Kozuka et al. [14]	2002	E-selectin, P-selectin, TM, vWF (activity)	≤48 h, 1 month	27 lacunar stroke	25: other ischemic stroke; 86: healthy age-matched controls	vWF activities and P-selectin levels elevated during both acute and subacute phases in lacunar stroke vs. control; TM & E-selectin levels elevated only in acute phase in lacunar stroke vs control
Hassan et al. [15]	2003	ICAM-1, TM, TF, TFPI	2 month	110 lacunar stroke (47 ILA; 63 WML)	50: healthy age- and sex-matched controls	ICAM-1, TM, and TFPI elevated in lacunar stroke vs controls; lower levels of TFPI and higher TF/TFPI ratio in WML vs ILA
Cherian et al. [16]	2003	E-selectin, P-selectin, vWF (concentration)	≤7 day, 3–6 month	67 small artery ischemic stroke	133: other ischemic stroke; 205: healthy age- and sex-matched controls	E-selectin, P-selectin: increased within 7 day, fell at 3–6 month; independent association between E-selectin and small artery ischemic stroke
Nomura et al. [17]	2004	TM	≤48 h, 1 month	35 lacunar stroke	48: other ischemic stroke; 66: healthy age- and sex-matched controls	TM levels elevated only in acute phase in lacunar stroke vs cardioembolic stroke; TM levels elevated in both acute and subacute phases with no significance in lacunar stroke vs controls
Han et al. [20]	2009	ICAM-1		175 asymptomatic patients ages ≥60 y		ICAM-1 positively associated with grades of WML; higher ICAM-1 levels, age, and hypertension are independent risk factors associated with the presence and severity of WML
Knotfnerus et al. [18]	2010	tPA (activity), PAI-1, tPA-PAI-1 complex, vWF (concentration), TF, TM	≥3 month	Lacunar stroke subtypes: 43 ILA; 53 WML; 53 aLAC		tPA activity elevated and PAI-1 levels decreased in WML vs ILA; after multivariable analysis, association between WML and PAI-1 remained significant
Rouhl et al. [19]	2011	ICAM-1, VCAM-1, E-selectin, P-selectin	3 month	163 lacunar stroke	183 hypertension patients; 43 control patients;	ICAM-1 and VCAM-1 elevated in patients with extensive WMLs and aLAC; higher E-selectin independently correlated with higher number of CMBs

SVD small-vessel disease; TM thrombomodulin; vWF Von Willebrand factor; ICAM-1 intracellular adhesion molecule-1; TF tissue factor; TFPI tissue factor pathway inhibitor; ILA isolated lacunar infarct; WML white matter lesions; tPA tissue plasminogen activator; PAI-1 plasminogen activator inhibitor-1; aLAC asymptomatic lacunar infarcts; VCAM-1 vascular cell adhesion molecule-1; CMB cerebral microbleeds

local intravascular fibrinolysis. Vascular injury may induce an acute release of local tPA to mediate the dissolution of arterial thrombus, and a long-term change in the rate of synthesis of tPA, which is a marker of atherosclerotic burden [24]. Therefore, a low capacity for rapid tPA release is likely to predispose to ischemic stroke. The possession of a thymidine (T) allele (–7351C/T polymorphism) is associated with reduced DNA transcription, leading to about half the tPA release observed in those homozygous for the cytosine (C) allele [25]. Because small vessels predominantly express tPA within the brain [26], it is found that the tPA –7351C/T polymorphism is an independent risk factor for lacunar stroke [27]. However, levels of tPA were not examined in this study; therefore, the influence of the polymorphism on levels of tPA could not be determined. A recent study showed that higher levels of tPA were found in patients with concomitant extensive WMLs than in those with isolated lacunar infarct, and low levels of plasminogen activator inhibitor-1 (PAI-1), the principal inhibitor of tPA, also were associated with extensive WMLs [18]. Unfortunately, because the study only selected patients with lacunar stroke, levels of tPA among different subtypes of ischemic stroke could not be compared. Moreover, it is a cross-sectional study, and the causal relationship has to be established by long-term follow-up studies.

Homocysteine

Homocysteine is a dietary sulphur-containing amino acid derived as an intermediate during the metabolism of methionine [28], and genetic factors (most often methylenetetrahydrofolate reductase [MTHFR] C677T polymorphism), systemic disorders (such as renal failure), nutritional status (folate, vitamin B12, and vitamin B6 deficits) or medications can cause hyperhomocysteinemia [29], which can lead to endothelial dysfunction [30]. Therefore, homocysteine may represent an important endothelial toxin in cerebral SVD [6]. Many studies have shown that hyperhomocysteinemia is an independent risk factor for SVD, particularly WMLs [31–50]. Even in patients with symptomatic atherosclerotic (macrovascular) disease, higher homocysteine levels are associated with higher WML volume, presence of lacunar infarcts, and slightly worse cognitive function [51]. After controlling for endothelial dysfunction markers ICAM-1 and TM, the association between homocysteine and SVD was no longer significant, suggesting hyperhomocysteinemia mediates its effect via endothelial dysfunction [37]. It is of particular interest because homocysteine levels can be regulated by folic acid, vitamin B6, and B12, and therefore could be a potential target to prevent stroke [52]. Although homocysteine-lowering treatments in several large randomized trials have thus far failed to provide evidence of

benefit for ischemic stroke prevention, a closer look at the data suggests that there may well be an effect in patients with symptomatic cerebral SVD causing lacunar infarction or intracerebral hemorrhage [52, 53••]. Meanwhile, there are no data showing that lowering homocysteine levels improves white matter disease or slows its progression. Larger longitudinal studies in patients with true hyperhomocysteinemia focusing on progression of both lacunar stroke and WMLs are warranted to improve our understanding of the role of homocysteine in the pathophysiology of cerebral SVD.

Asymmetric Dimethylarginine

Nitric oxide (NO) is one of the most important vasoactive substances released by the endothelium, using the L-arginine as substrate. Various pharmacological and physical stimuli lead to the release of NO. Synthesized by endothelial constitutive NO synthase, NO results in vasodilatation and increased blood flow [9•]. Asymmetric dimethylarginine (ADMA) competes with L-arginine as an endogenous non-isoform-specific inhibitor of endothelial NO synthase (eNOS), and it may play a role in endothelial dysfunction [54, 55]. Therefore, increased plasma ADMA is hypothesized to be a marker for stroke risk prediction and has been associated with other traditional cardiovascular risk factors including hypertension, diabetes, hyperhomocysteinemia, left ventricular hypertrophy, and hypercholesterolemia [56–59]. Furthermore, blood levels of ADMA were elevated in symptomatic SVD patients and correlate with the degree of leukoaraiosis, compared to community control patients free of symptomatic cerebrovascular disease [45]. No association was found between plasma ADMA and homocysteine levels, suggesting that ADMA might be independently associated with SVD [45]. An independent association of the L-arginine/ADMA ratio with microangiopathy-related cerebral damage (lacunar infarction and WMH) also was observed in 712 patients [60]. The Framingham Offspring Study evaluated plasma ADMA concentrations from 2013 stroke-free individuals for whom simultaneous neuroimaging studies were available [61]. ADMA was independently associated with an increased prevalence of silent brain infarcts, most of which (74% to 86%) are lacunar infarcts [62]. Therefore, plasma ADMA may be a potentially useful new biomarker of subclinical vascular brain injury. Because ADMA appears to be a novel biomarker linked to overall cardiovascular mortality, risk of stroke, and endothelial dysfunction, future prospective studies ideally should include control patients with symptomatic atherosclerotic disease (eg, coronary and carotid disease) and asymptomatic control patients with a similar risk factor profile to better elucidate its value as a biomarker in cerebral SVD.

Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) have emerged as a new marker of endothelial dysfunction until recent years. Derived from immature stem cells, EPCs are maturing cells that circulate in peripheral blood. EPCs possess functional and structural characteristics of both stem cells and mature endothelial cells [63]. The number of EPCs can be quantified by cell culture, which depends on the EPC function, and by flow cytometric analysis, which does not depend on the EPC function. Therefore, results are not exchangeable between the two quantification techniques due to this difference [63]. Besides, the function of EPCs is determined by the tests of colony formation, proliferation, and migration among others. EPCs are involved in repair of endothelial damage [63] and are possibly also involved in improving endothelial cell function [64]. Lower EPC numbers and disturbed EPC function relate to different atherosclerotic risk factors [64–66] and a poor prognosis regarding functional recovery [67, 68], which reflect a higher consumption of EPCs for restoring the damaged endothelium. Moreover, EPC cluster numbers differed between stroke subtypes: patients with cardioembolic stroke had higher numbers than patients with large or small vessel stroke [68]. Patients with a first-ever lacunar stroke, especially those with silent ischemic lesions, also have impaired cluster-forming potential [69]. Individuals with hypertension with cerebral SVD (WMLs, asymptomatic lacunar infarcts, or CMBs) had lower EPC numbers than control patients with hypertension without cerebral SVD, independently of blood pressure levels [70]. Regulating factors of EPC number or cluster-forming potential in cerebral SVD are largely unknown, and recent studies suggest that different haptoglobin phenotypes [69] and angiogenic T cells [70] are likely causative factors. However, the definition of EPC remains controversial [63], and the mentioned studies of EPC in cerebral SVD are all cross-sectional with a relatively small number of patients.

Although the pathogenesis of cerebral SVD remains poorly understood, considerable evidence suggests that endothelial dysfunction, which results in cerebral hypoperfusion and impaired autoregulation and/or increased blood–brain barrier permeability [71], may play an important role in cerebral SVD. The reviewed blood biomarkers of endothelial dysfunction are found to be related to the severity and even progression of cerebral SVD. However, current data do not confirm that these markers are specific to cerebral SVD because they are involved in cerebral large artery disease as well, and may simply reflect exposure to vascular risk factors and having a stroke. Besides, endothelial function also can be assessed by the vascular response to hypercapnia (CO₂) or infusion of acetazolamide or L-arginine, or brachial artery flow-mediated dilatation (FMD), as reviewed by Stevenson et al. [72•]. Genetic predisposition involved in

endothelial function has been implicated as well (reviewed in [6]). These are beyond the scope of this review.

Inflammation

Inflammatory processes are implied in the pathogenesis of atherosclerosis [73–75, 76•], and several lines of evidence suggest that inflammation is involved in cerebral SVD as well. The acute-phase reactant C-reactive protein (CRP) has proven to be a sensitive systemic marker of inflammation and to be involved in the endothelial inflammatory response [77, 78]. The population-based Rotterdam Scan Study of 1033 nondemented elderly individuals showed that higher CRP levels were associated with presence and progression of WMLs, particularly with marked lesion progression, independent of cardiovascular risk factors and carotid atherosclerosis [79]. This finding was later confirmed not only in whites but in blacks as well in the Cardiovascular Health Study [80]. There was no relation between any CRP polymorphism or haplotypes and measures of cerebral SVD [80, 81], suggesting that CRP is not causally involved in the pathogenesis of cerebral SVD. However, several studies failed to build the association between CRP and WMLs, especially in Asian population [82–84], and no significant relationship between CRP levels and WML progression was demonstrated in patients with type 2 diabetes mellitus [22]. The differences may probably be attributed to the different criteria for classification of WMLs, different ages of study population, and racial differences. On the other hand, in the Rotterdam Scan Study, higher plasma CRP levels were marginally associated with greater prevalence and incidence of lacunar infarcts [79]. In two independent cohorts of elderly Japanese individuals, CRP levels were elevated in patients with silent brain infarctions [85, 86]. Moreover, baseline CRP levels were significantly associated with progression of silent brain infarctions at 3-year follow-up in patients with type 2 diabetes mellitus [22]. Thus, elevated CRP levels may be related to WMLs and lacunar infarcts via different mechanisms. More recently, elevated CRP levels were found to be associated with CMBs, another important manifestation of cerebral SVD, independent of cardiovascular risk factors, carotid atherosclerosis, WMLs, and silent lacunar infarcts [87].

As a main inducer of hepatic production of CRP, interleukin-6 (IL-6) is a multifunctional cytokine that plays important roles in the regulation of the immune responses and inflammation. Same as CRP, IL-6 could predict future risk of recurrent ischemic stroke and cardiovascular events [88, 89]. Besides, the Cardiovascular Health Study showed that plasma IL-6 levels were significantly associated with the presence of WMLs and brain infarcts in whites and blacks, while common haplotypes of the IL-6 gene were

significantly associated with WMLs and brain infarcts only in whites [80]. IL-6 levels also were found to be independently related with CMBs [87].

Other markers of inflammation, such as monocyte [90] and leukocyte [84] counts, monocyte/macrophage activation (neopterin) [19], and myeloperoxidase (MPO) [91], have been identified to independently predict the presence of lacunar infarcts or WMLs. Although markers of inflammation are not disease-specific but are sensitive markers produced in response to tissue injury, infectious agents, immunological stimuli, and inflammation, the characteristics desired of inflammatory markers of cardiovascular diseases are as follows: independence from established risk factors, well-established associations with cardiovascular diseases in observational studies and clinical trials, the availabilities of population norms to guide the interpretation of results, the applicabilities of results to different population groups, the availability of a standardized assay with acceptable reproducibility, and an acceptable assay cost [92]. Hopefully, the ongoing Levels of Inflammatory Markers in the Treatment of Stroke (LIMITS) study [93] will identify novel inflammatory biomarkers with the aforementioned characteristics for use in risk prediction and treatment selection in patients with cerebral SVD.

β -Amyloid Hypothesis

Plasma β -amyloid peptide ($A\beta$) is a peptide consisting of either 42 ($A\beta_{1-42}$) or 40 ($A\beta_{1-40}$) amino acids derived from a proteolytic processing of the amyloid precursor protein. Insoluble $A\beta$ fibrils are the predominant constituents of senile plaques, one of the pathological hallmarks of AD, and of cerebrovascular amyloid in the related condition of CAA. Plaque amyloid is primarily comprised of $A\beta_{42}$, whereas vascular amyloid is formed by $A\beta_{40}$ species [94]. $A\beta$ has emerged as a potential mediator of microvascular dysfunction. In vitro studies have suggested direct physiologic or toxic effects of $A\beta$ on the blood vessel wall [95]. Plasma $A\beta_{40}$ and $A\beta_{42}$ levels are strongly associated with the presence of lacunar infarcts and severity of WMLs in nondemented elderly patients who carry an apolipoprotein E (APOE) $\epsilon 4$ allele in the Rotterdam cohort [96]. In patients with AD, mild cognitive impairment (MCI), or CAA, plasma $A\beta_{40}$ concentration is independently associated with extent of WMHs [97]. Furthermore, a recent study demonstrated that plasma $A\beta_{40}$ levels, but not $A\beta_{42}$, are strongly associated with the diffuse SVD subtype (ie, leukoaraiosis [98]). Plasma $A\beta_{40}$ levels are also found to be markedly elevated in patients with ischemic stroke, and patients with cardioembolic and large-artery atherosclerotic infarcts had higher levels than those with SVD infarctions [99]. However, this study included only 12 patients classified as

cerebral SVD and the presence of WMLs was not analyzed.

Taken together, both experimental and clinical studies have suggested the pathophysiological role of $A\beta_{40}$ in disrupting microvascular function, and circulating $A\beta$ might be a novel marker of cerebral SVD. Prospective studies are needed to confirm its role. Moreover, because AD and cerebral SVD, especially age-related cerebral SVD, usually coexist in elderly patients, it also would be interesting to compare plasma $A\beta$ levels in patients with vascular cognitive impairment with no dementia (VCI-ND), vascular dementia (VaD), MCI, and AD in future studies.

Conclusions

Age-related cerebral SVD is a common condition in elderly patients and has been strongly associated with risk of incident stroke, dementia, and age-related disability. Endothelial dysfunction, inflammation, and $A\beta$ are suggested to be involved in the pathogenesis of cerebral SVD. Although the investigations of blood biomarkers regarding these mechanisms are quite diverse and active, uniform and robust conclusions are difficult to draw due to the heterogeneity of the studies. Future studies should precisely classify the different phenotypes of cerebral SVD using established clinical criteria combined with MRI findings, include symptomatic control patients with atherosclerotic disease and asymptomatic control patients with similar risk factor profiles, and follow up patients with repeated MRI and blood collection. Meanwhile, more attention and targeted efforts are needed to identify novel biochemical biomarkers that shed light on the multifactorial pathogenesis and offer potential therapeutic targets.

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