

# Cerebral small vessel disease: Capillary pathways to stroke and cognitive decline

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## Abstract

Cerebral small vessel disease (SVD) gives rise to one in five strokes worldwide and constitutes a major source of cognitive decline in the elderly. SVD is known to occur in relation to hypertension, diabetes, smoking, radiation therapy and in a range of inherited and genetic disorders, autoimmune disorders, connective tissue disorders, and infections. Until recently, changes in capillary patency and blood viscosity have received little attention in the aetiopathogenesis of SVD and the high risk of subsequent stroke and cognitive decline. Capillary flow patterns were, however, recently shown to limit the extraction efficacy of oxygen in tissue and *capillary dysfunction* therefore proposed as a source of stroke-like symptoms and neurodegeneration, even in the absence of physical flow-limiting vascular pathology. In this review, we examine whether capillary flow disturbances may be a shared feature of conditions that represent risk factors for SVD. We then discuss aspects of capillary dysfunction that could be prevented or alleviated and therefore might be of general benefit to patients at risk of SVD, stroke or cognitive decline.

## Keywords

Cerebral small vessel disease, stroke, dementia, capillary dysfunction, oxygenation

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## Introduction

Cerebral small vessel disease (SVD) denotes a range of pathological processes, which affect the small arteries, arterioles, capillaries and small veins of the brain.<sup>1</sup> SVD is associated with small subcortical infarcts, lacunes, white matter hyperintensities, enlarged perivascular spaces, microbleeds, and cortical atrophy,<sup>2</sup> gives rise to one in five strokes worldwide, and constitutes a major source of cognitive decline, particularly in the elderly.<sup>1</sup>

Until recently, changes in capillary morphology and blood–brain barrier (BBB) function have received little attention in a etiopathogenesis of SVD and associated stroke and cognitive decline.<sup>3,4</sup> In addition, capillary flow patterns have now been shown to limit the extraction efficacy of oxygen in tissue,<sup>5</sup> and capillary dysfunction proposed as a source of stroke-like symptoms<sup>6</sup> and neurodegeneration,<sup>7</sup> even in the absence of flow-limiting vascular pathology.

Here, we briefly review the properties of capillary dysfunction and the evidence for capillary involvement

in SVD and in conditions that impose risk of SVD. We then examine whether capillary dysfunction may play a role in the aetiopathogenesis of SVD and the subsequent development of stroke or cognitive decline. Finally, we discuss whether capillary dysfunction may

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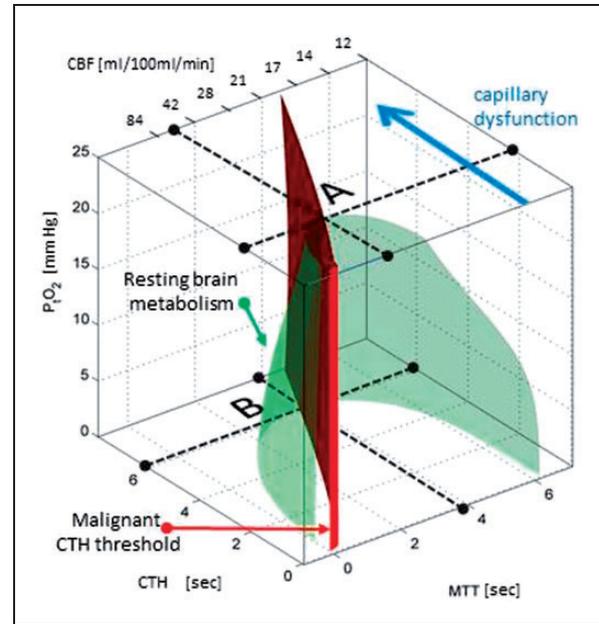
serve as a common therapeutic target in efforts to prevent or ameliorate stroke and cognitive decline across the diverse range of conditions associated with SVD.

## Capillary dysfunction

In normal brain, only 30–40% of blood's oxygen passes into the brain parenchyma from small arteries, arterioles and capillaries to fuel cerebral metabolism.<sup>8</sup> Oxygen extraction is known to be inefficient from capillaries with high flow velocities.<sup>9</sup> The heterogeneity of flow velocities across the capillary bed of normal, resting brain tissue is extremely high<sup>10–12</sup> and therefore limits net oxygen extraction<sup>5,13,14</sup> – a biophysical property referred to as *functional shunting*. In the normal brain, capillary flow patterns homogenize when cerebral blood flow (CBF) increases in relation to cortical activation<sup>10,12,15,16</sup> and thereby facilitate more efficient oxygen extraction.<sup>5,14</sup> This homogenization is partly a passive property of normal microvascular networks: as CBF increases, the blood tends to distribute in a more homogenous way across 'ideal' capillary networks.<sup>17</sup> In a later section, we discuss how cerebral pericytes regulate capillary diameter during functional activation<sup>18</sup> and possibly provide active regulation of capillary flow patterns.<sup>19</sup>

*Capillary dysfunction* refers to conditions in which changes in capillary function and/or patency, or in blood rheology, disturb either capillary flow patterns, their homogenization during hyperaemia or both. To determine the effects of capillary dysfunction on oxygen extraction, the distribution of erythrocyte velocities or transit times across the capillary bed must be known.<sup>5,14,17,20,21</sup> For convenience, we quantify capillary dysfunction by the accompanying *capillary transit time heterogeneity* (CTH) and use accepted transit time distributions for which the standard deviation describes CTH by a single parameter.<sup>5,13,14,17,21</sup> Figure 1 illustrates how CBF and its microvascular distribution (CTH), combined, affect oxygen uptake in brain tissue.

If changes in capillary patency or blood rheology become severe, our analyses predict that the flow-metabolism coupling, which is crucially required for tissue function and survival, modifies CBF in a counterintuitive direction.<sup>6</sup> While homogenization of capillary flows (CTH reduction) maintains efficient oxygen extraction during hyperaemia in normal brain,<sup>5</sup> only the *suppression of CBF* can reduce functional shunting if CTH can no longer be reduced. Attenuated CBF responses (reduced cerebrovascular reserve capacity) are therefore expected in both capillary dysfunction and flow-limiting conditions – but to represent the maintenance of flow-metabolism coupling in the former and flow limitations at the level of resistance vessels in the latter.



**Figure 1.** The green isocontour surface corresponds to all combinations of CBF, CTH, and P<sub>t</sub>O<sub>2</sub> for which brain oxygenation – according to our model<sup>5</sup> – matches the metabolic rate of oxygen in resting brain.<sup>187</sup> Transitions to combinations of CBF, CTH and P<sub>t</sub>O<sub>2</sub> that correspond to points located *outside* the resulting, bell-shaped surface are therefore predicted to result in immediate neurological symptoms, and tissue damage if they persist. The red plane marks the boundary, left of which vasodilation reduces tissue oxygen availability (dubbed malignant CTH). The maximum value that CTH can attain at a P<sub>t</sub>O<sub>2</sub> of 25 mmHg, if oxygen availability is to support the metabolic needs of resting brain tissue, is indicated by the label A. As CTH increases further (progressive capillary dysfunction), CBF must be attenuated in order to reduce the level of 'physiological shunting'. Importantly, continued tissue oxygen metabolism reduces tissue oxygen tension, and thereby improves blood-tissue concentration gradients and net extraction. As a result, the bell-shaped surface widens towards its base, reflecting that higher levels of CTH (more severe capillary dysfunction) can be accommodated by attenuating CBF and CBF responses. A critical limit is reached, however, as P<sub>t</sub>O<sub>2</sub> approaches zero – label B. At this point, the metabolic needs of tissue are met by 'delaying' mean transit time (MTT) to a threshold of approximately 4 s, corresponding to CBF = 21 ml/100ml/min. As a result, slight increases in CTH (e.g. caused by an infection or dehydration) or a slight change in CBF (small flow reductions as well as flow increases) can trigger a critical reduction in tissue oxygen availability, and thereby stroke-like symptoms. The blue arrow indicates progressive capillary flow disturbances, which cause CTH to increase and tissue oxygen availability to approach the metabolic requirements of resting brain tissue (the green isocontour). Note that the traditional notion of ischemia (which disregards capillary flow patterns) considers only a reduction in CBF (increase in MTT), that is, a transition along the x-axis in the three-dimensional plot. Source: Reproduced and modified from the literature.<sup>6</sup> CBF: cerebral blood flow; CTH: capillary transit time heterogeneity; P<sub>t</sub>O<sub>2</sub>: tissue oxygen tension.

In cerebral ischemia, tissue function and survival are threatened by tissue hypoxia as a result of *limited blood supply*, whereas in capillary dysfunction, the source of hypoxia is *inefficient oxygen extraction from the micro-circulation*. Notably, stroke-like symptoms and hypoxic tissue injury can therefore, in principle, be caused by capillary flow disturbances, in the *absence* of a flow-limiting condition.<sup>6,7</sup> We have shown that CTH can reach a critical biophysical threshold  $CTH^{max}$ , above which net oxygen extraction can no longer meet the metabolic demands of resting brain tissue, although CBF is suppressed to minimize net functional shunting.<sup>6</sup> Notably, the CBF value that optimizes net oxygen extraction for  $CTH = CTH^{max}$  seems almost identical to the classical ischemic threshold of approximately 20 ml/100ml/min,<sup>6</sup> making critical capillary dysfunction and cerebral ischemia<sup>22</sup> indistinguishable in terms of their low CBF and elevated oxygen extraction fraction (OEF).

If CBF suppression fails to compensate for capillary dysfunction, tissue hypoxia and injury can occur at CBF values above the ischemic threshold and even above normal brain perfusion. The ‘luxury perfusion syndrome’,<sup>23</sup> which is sometimes observed upon reperfusion of tissue after prolonged or severe ischemia, may represent an instance of tissue damage caused by excessive functional shunting, keeping in mind that capillary constrictions can be observed after ischemia.<sup>19,24</sup>

Reductions of CBF to levels below the ischemic threshold can cause neurological symptoms and tissue injury irrespective of whether vascular patency is reduced at the arterial or the capillary level, but some vascular causes of neurological symptoms and tissue injury are specific to capillary dysfunction: capillary dysfunction can thus cause neurological deterioration and hypoxic tissue injury (a) in the *absence* of primary, flow-limiting pathology (e.g. no severe stenosis, thrombosis, embolism) (b) under conditions of *augmented* CBF (iatrogenic or spontaneous) and (c) due to increased blood viscosity if this increases CTH beyond  $CTH^{max}$ .

We discuss additional signatures of capillary dysfunction below.

### Sources of capillary dysfunction in conditions that represent risk factors for SVD

We now review factors that may affect blood flow through individual capillaries in normal and diseased brain. In Tables 2 to 7, we list microvascular changes (middle column) in conditions considered risk factors for SVD according to Pantoni’s classification.<sup>1</sup> Cutaneous leukocytoclastic angiitis is now classified as a single-organ vasculitis<sup>25</sup> and therefore omitted from the list. We used Web of Science<sup>TM</sup> and

PubMed to search for occurrences of the terms listed in the leftmost column of Tables 2 to 7 in combination with ‘capillary’, ‘endothelium’, ‘glycocalyx’, ‘basement membrane’, ‘pericyte’ or ‘viscosity’. Examples of SVD-related infections are adapted from Younger.<sup>26</sup> Literature searches for Table 5 were conducted between 30 December 2014 and 18 January 2015, while the remaining literature searches were conducted from 21 July 2014 to 26 September 2014.

### Pericyte dysfunction

Pericytes are embedded in layers of the basement membrane that surround the capillary endothelium.<sup>27</sup> Pericytes are thought to cover most capillaries in the central nervous system where they regulate BBB function<sup>28</sup> and aspects of the brain’s immune response.<sup>29</sup> Pericytes are involved in the regulation of capillary development (angiogenesis), stabilization, maturation and remodeling<sup>30</sup> and communicate with endothelial cells through *peg-socket* contacts as they – among other functions – jointly form and maintain the basement membrane.<sup>27</sup> Neurogenic locus notch homolog protein 3 (NOTCH3) signalling is crucial for the postnatal differentiation of vascular cells into their ‘correct’ arterial, capillary and venous phenotypes<sup>27,31</sup> and their ability to adapt to changes in pressure and vascular strain.<sup>32,33</sup> While pericytes are characterized by their relation to microvessels, they share cellular and functional characteristics with vascular smooth muscle cells (VSMCs) encircling arterioles and venules. The distinction between VSMC and pericytes recently became a matter of debate with regards to the attribution of CBF-regulation<sup>19,34,35</sup> – see discussion in the literature.<sup>36</sup>

Cerebral pericytes are contractile, and they have been shown to contract and relax in response to neuro-mediators, vasoactive drugs and, importantly, to sensory stimulation in brain slices as well as *in vivo*.<sup>18,19,35</sup> Thus, cerebral pericytes have been shown to regulate capillary diameter during functional activation,<sup>18</sup> dilating about 1 s before arteriolar dilation and thereby possibly controlling both CBF and CTH.<sup>19</sup> Retinal pericytes have been characterized extensively *in vitro*: retinal pericytes constrict in response to high oxygen tension and relax in response to lactate and low pH,<sup>37</sup> possibly providing a mechanism by which capillary flows can redistribute to meet local cellular metabolic demands during activation.<sup>37</sup> Much like VSMCs, retinal pericytes react to a range of vasoactive substances, constricting when exposed to mechanical stretch, angiotensin II (AT2)<sup>38</sup> and endothelin-1 (ET1)<sup>39</sup> by a  $Ca^{++}$ -dependent mechanism<sup>40</sup> and relaxing when exposed to adenosine,<sup>41</sup> ATP<sup>42</sup> and nitric oxide (NO)<sup>43,44</sup> and in response to cholinergic<sup>45</sup> and adrenergic<sup>40</sup> stimulation. See the study by Attwell et al.<sup>46</sup> for a review of

neurovascular coupling mechanisms and the control of VSMC and pericyte tone.

Pericyte loss and basement membrane thickening are observed in conditions that represent major risk factors for SVD, such as ageing, hypertension and diabetes – see Table 2. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL – Table 3) is associated with mutations in the *NOTCH3* gene.<sup>47</sup> The receptor protein encoded by the *NOTCH3* gene is expressed in both VSMCs and pericytes,<sup>48</sup> and recent evidence suggests that CADASIL is associated not only with degeneration and damage to VSMCs in small arteries and arterioles but also to microvascular pericytes. Dziejulska and Lewandowska<sup>49</sup> observed pericyte loss and pericyte fragments within thickened basement membranes in skin and muscle biopsies in CADASIL. They reported that pericyte-endothelial peg-socket contacts were disrupted, seemingly giving rise to basement membrane thickening, endothelial swelling and protrusions into the capillary lumen.<sup>49</sup> Recent animal models of CADASIL show evidence of reduced capillary density<sup>50</sup> and pericyte degeneration<sup>51</sup> and suggest that reduced pericyte coverage is related to impaired BBB function.<sup>52</sup>

When exposed to viral and bacterial proteins, pericytes initiate inflammatory responses that facilitate the recruitment of immune cells from the blood stream – see the study by Hill et al.<sup>53</sup> for a recent review. As neutrophils pass through the surrounding basement membrane, they pass between embedded pericytes, which in turn remodel the laminin-rich basement membrane to permit extravasation.<sup>54</sup> Importantly, pericytes seemingly change their phenotype during inflammation to become migratory,<sup>54</sup> a phenomenon also observed in CNS injury.<sup>55</sup> The ability of pericytes to undertake BBB function<sup>28</sup> and capillary flow control<sup>19</sup> may therefore be compromised during infection and inflammation.

### **Pericyte exposure to parenchymal waste: Amyloid and hemoglobin**

Molecules the size of haemoglobin and amyloid  $\beta$  ( $A\beta$ ) are cleared from the subarachnoid space and brain parenchyma along the basement membranes of arteries and capillaries.<sup>56,57</sup> Indeed, impaired glymphatic clearance has recently emerged as a potential therapeutic target.<sup>58</sup> Located in the layers of capillary basement membrane, pericytes are therefore exposed to amyloid during its perivascular removal and clearance across the BBB.<sup>59–61</sup> Pericytes undergo degeneration when exposed to certain  $A\beta$  types in vitro,<sup>62,63</sup> and although the role of pericytes in the pathogenesis of cerebral amyloid angiopathy (CAA) and Alzheimer's disease (AD) remains poorly understood, evidence from  $A\beta$

transgenic mice and in vitro models suggest they may be involved in neurodegeneration.<sup>64,65</sup> For recent reviews, see the study by Hamilton et al.<sup>66</sup> and Winkler et al.<sup>67</sup>

Microbleeds are associated with SVD, and the vasoactive properties of hemoglobin breakdown-products are described in detail in the literature.<sup>68,69</sup> Briefly, spontaneous autoxidation of oxyhemoglobin (HgbO) to methemoglobin and the iron released from hemoglobin cause the release of highly reactive superoxide radicals.<sup>68,69</sup> Superoxides are thought to cause vasoconstriction by depleting vascular NO levels<sup>70,71</sup> and to induce lipid peroxidation and peroxynitrite formation, which in turn cause vasoconstriction and structural damage to cerebral microvessels, including the endothelial cell layer.<sup>72</sup> The breakdown of heme into bilirubin under such oxidative conditions results in the formation of bilirubin oxidation products (BOXes) that change the contractility, signalling and metabolism in large vessels – see also the study by Pyne-Geithman et al.<sup>73</sup>

### **Endothelial function**

Endothelial cells are mechanically coupled by tight junctions, which ensure BBB integrity and prevent leakage of toxic molecules into the brain interstitium – see discussion in the literature.<sup>3,4</sup> Endothelial cells are also electrically and metabolically coupled to each other as well as to nearby VSMCs via gap junctions composed of connexins.<sup>74</sup> This rapid, bidirectional signalling pathway seemingly provides efficient coordination of vessel function across the microvascular bed.<sup>75–77</sup> Disruption of the signalling between endothelial cells has been shown to cause profound breakdowns in vascular control across the capillary bed, resulting in extreme degrees of capillary shunting through the shortest arteriolo-venular pathways.<sup>78</sup>

In small-vessel vasculitides (Table 4) associated with antineutrophilic cytoplasmic antibodies (ANCA), neutrophils adhere to capillary endothelial cells and cause the release of reactive oxygen species (ROS) and lysosomal enzymes. This abnormal inflammatory reaction causes endothelial cells to undergo necrosis and detach from the basement membrane, after which they can be found in peripheral blood.<sup>79</sup> While the activation of neutrophils and endothelial cells in the capillary lumen may disturb capillary flow patterns in itself, the disruption of endothelial cell-to-cell signalling described above is expected to cause severe capillary dysfunction.

### **Glycocalyx dysfunction**

The luminal surface of the capillary endothelium is covered by a 0.5- $\mu$ m thick *glycocalyx*.<sup>80,81</sup> This

carbohydrate-rich matrix affects the passage of blood cells through the capillary bed.<sup>82</sup> reducing capillary haematocrit to 20–50% of that found in the systemic circulation. Glycocalyx damage, in turn, disrupts capillary flow regulation, causing capillary haematocrit to approach that of the systemic circulation.<sup>83,84</sup> The glycocalyx constitutes a fluid barrier in the vascular system and has been implicated in oedema formation.<sup>85,86</sup> The glycocalyx is degraded by direct oxidative stress and exposure to oxidized lipoproteins,<sup>83,87,88</sup> by hyperglycaemia<sup>89</sup> and by ischemia.<sup>88,90</sup>

### Blood viscosity

The dimensions of white blood cells (WBC) and erythrocytes exceed the average capillary diameter. Experimental studies have shown that capillary flow patterns are sensitive to the size, deformability, viscosity, number and endothelial adhesion of blood cells. In infections and low-grade vascular inflammation, blood is hence increasingly redirected to thoroughfare channels which act as functional shunts.<sup>91</sup> In a classical study, hyperviscosity was demonstrated in diabetic patients (Table 2) compared to age-matched controls and the viscosity found to correlate with the extent of microvascular diabetic complications. Accordingly, erythrocyte deformability was lower in those diabetic patients who had the most extensive microangiopathy compared to either diabetics with no complications or to controls.<sup>92</sup>

In cryoglobulinaemic vasculitis (Table 5), cryoglobulins precipitate and lead to hyperviscosity.<sup>26</sup> Haematocrit is lower in capillaries than in the systemic circulation, and since cryoglobulins precipitate more easily under conditions of serum excess, capillary flows may be particularly sensitive to this phenomenon.

### Signs of capillary dysfunction

The previous section suggests that capillary morphology and function may be disturbed in conditions considered risk factors for SVD. Neuropathological data and direct *in vivo* observations of the microcirculation in these conditions are sparse, however, and it remains unclear whether capillary dysfunction might antedate changes in the morphology and function of upstream arteries and arterioles.

The neurovascular coupling mechanisms,<sup>93</sup> which control local CBF according to the metabolic needs of tissue, are expected to account for the oxygen extraction efficacy downstream, including changes related to capillary dysfunction. Below we discuss how CBF has to be adjusted in order to compensate for the reduced oxygen extraction efficacy that accompanies various degrees of capillary dysfunction. Some CBF changes

are highly suggestive of capillary flow disturbances as opposed to a flow-limiting pathology, and neurovascular coupling studies may therefore serve as an indirect means of addressing the role of capillary dysfunction in the evolution of SVD from its risk factors. These changes are listed in the right-most column in Tables 2–7.

We used Web of Science<sup>TM</sup> and PubMed to search for occurrences of the terms listed in the leftmost column of Tables 2–7 in combination with ‘CBF’, ‘blood-oxygen-level-dependent (BOLD)’, ‘oxygen’, ‘metabolism’, ‘functional hyperemia’, ‘vasoreactivity’ and ‘stroke’. Literature searches for column 3 in Tables 2–7 were conducted concurrently with those for column 2 (previous section).

### Mild capillary dysfunction: CBF increases in conditions that represent risk factors for SVD

For mild capillary disturbances, the reduction in oxygen extraction efficacy is so small that it can be compensated for by elevated CBF. Observations of *increased* resting CBF or *increased* CBF responses early in the course of SVD precursors therefore suggest that capillary dysfunction (elevated CTH), rather than primary changes in the morphology or function of upstream arterioles, is involved in the early etiology of the condition. Meanwhile, the BOLD signal is often used to localize brain activity through its sensitivity to tissue deoxyhemoglobin concentrations [dHgb]. Mild capillary dysfunction is characterized by reduced OEF and proportionately higher CBF responses during functional activations, both of which reduce [dHgb] and thereby *increase* BOLD signal amplitudes before resting CBF and OEF become affected. In asymptomatic subjects presented with identical tasks, BOLD responses are therefore expected to be higher in those with mild capillary dysfunction than in controls, despite identical changes in metabolic activity. CBF and BOLD responses are recorded in grey matter and are hence sensitive to changes in capillary function in the cortex and subcortical nuclei. Subcortical lesions may result in secondary changes in cortical function, and, in theory, even compensatory hyperactivity in some regions.

In streptozotocin (STZ)-induced diabetes (Table 1) in rats, both total and cortical CBF values are indeed elevated compared to control animals early after induction of the disease,<sup>94–96</sup> and cortical oxygen tension elevated.<sup>96</sup> In early-stage hypertension (Table 2), elevated CBF and BOLD responses to hypercapnia have been reported in spontaneously hypertensive rats compared to age matched control animals.<sup>97</sup> The apolipoprotein (APOE)  $\epsilon 4$  allele is associated with both CAA and the development of AD<sup>98</sup> (Table 2), and carriers of

**Table 1.** Abbreviations.

A $\beta$	Amyloid $\beta$
AD	Alzheimer's disease
ANCA	Antineutrophilic cytoplasmic antibody
APOE	Apolipoprotein
AT2	Angiotensin II
ATP	Adenoside triphosphate
BBB	Blood-brain barrier
BOLD	Blood oxygen level dependent
CAA	Cerebral amyloid angiopathy
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CBF	Cerebral blood flow
CMRO <sub>2</sub>	Cerebral metabolic rate of oxygen
CNS	Central nervous system
CTC	Concentration-time curve
CTH	Capillary transit-time heterogeneity
CTH <sub>max</sub>	Maximum capillary transit-time heterogeneity
[dHgb]	Deoxyhemoglobin concentration
eNOS	endothelial nitric oxide synthase
FLAIR	Fluid attenuated inversion recovery
HCV	Hepatitis C virus
HIV-1	Human immunodeficiency Virus 1
MD	Mediterranean diet
MELAS	Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes
MRI	Magnetic resonance imaging
MTT	Mean transit time
NCM	Nailfold capillary microscopy
NIHSS	National Institute of Health Stroke Scale
NO	Nitric oxide
NOTCH3	Neurogenic locus notch homolog protein 3
OEF	Oxygen extraction fraction
PDE	Phosphodiesterase
PWI	Perfusion weighted imaging
ROS	Reactive oxygen species
STZ	Streptozotocin
SVD	Cerebral small vessel disease
VSMC	Vascular smooth muscle cell

this allele have therefore been studied extensively. In asymptomatic APOE  $\epsilon$ 4 carriers aged 19–28, both resting- and activity-related CBF levels are elevated,<sup>99,100</sup> and BOLD signal changes during memory encoding tasks are elevated in the asymptomatic APOE  $\epsilon$ 4 carriers,<sup>101–103</sup> consistent with reduced OEF and compensatory hyperaemia. Similarly, BOLD responses are elevated in patients with systemic lupus erythematosus

(SLE – Table 6) but little or no cognitive defects, compared to controls.<sup>104</sup> In asymptomatic human immunodeficiency virus 1 (HIV-1) infected patients (Table 5), elevated BOLD signals can be observed<sup>105</sup> in proportion to signs of glial activation.<sup>106</sup> The notion that viral replication in brain parenchyma is associated with mild capillary dysfunction is consistent with findings that BOLD signal amplitudes are elevated in seropositive patients with low BBB penetration of combination antiretroviral therapy, but comparable to those found in controls and in patients with high penetrance and thereby virological control.<sup>107</sup> It should be noted that *relative* BOLD signal changes depend on both resting and activation-related CBF and OEF levels. The interpretation of such changes in terms of the underlying microvascular pathology therefore requires detailed analysis of the underlying physiology and magnetic resonance signal mechanisms.<sup>17,108</sup>

### *Moderate capillary dysfunction: Transition from hyperperfusion to CBF suppression*

If capillary flow disturbances become more severe, then hyperemia fails as a means to compensate for reduced oxygen extraction efficacy. Instead, CBF responses – and ultimately resting CBF – must be attenuated in order to limit functional shunting. The transition from mild to moderate capillary dysfunction is therefore predicted to require dramatic, yet characteristic changes in CBF in order to maintain flow-metabolism coupling.

Such a transition, from hyper- to hypoperfusion, was indeed observed in a follow-up study of asymptomatic APOE  $\epsilon$ 4 carriers and controls.<sup>109</sup> At the time of their initial examination, APOE  $\epsilon$ 4 carriers showed *higher resting* CBF values in vulnerable brain regions than did control subjects, while CBF *reductions* in these regions 8 years later were significantly larger in APOE  $\epsilon$ 4 carriers than in controls.<sup>109</sup>

If flow responses are limited by a physical, flow-limiting condition, then CBF cannot increase beyond a certain upper limit, irrespective of the metabolic requirements of brain tissue. If CBF suppression instead serves to maintain flow-metabolism coupling, then CBF, tissue metabolism and the extent of capillary dysfunction determine whether flow suppression is necessary. We briefly discuss how this phenomenon might have revealed itself in studies of SVD and its precursor states.

In a mouse genetic model of CADASIL, Joutel et al.<sup>50</sup> examined CBF responses to functional activation, CO<sub>2</sub> inhalation and reduced perfusion pressure in 5- to 6-month-old animals, at a time-point where no changes in arterial structure or signs of BBB breakdown could be observed. Animals with and without

**Table 2.** Type 1: Arteriolosclerosis (age- and vascular risk-factor-related SVD). Type 2: CAA, sporadic or hereditary.

Risk factor	Changes in capillary morphology or blood rheology	Signs of capillary dysfunction
Ageing	<i>Human brain:</i> Pericyte loss. Variable capillary diameters, increased capillary tortuosity, twisting, and looping. Thickened basement membranes with inclusions. Pericapillary fibrosis. <sup>194,195</sup> Figure 2, panel B	
Hypertension	<i>Animal brain:</i> Pericyte degeneration, swelling of endothelium and surrounding astrocyte endfeet. Thickened basement membranes. <sup>196,197</sup> <i>In vitro:</i> Angiotensin II, endothelin-1 constrict retinal pericytes. <sup>38,39</sup>	<i>Animal models:</i> Elevated CBF and BOLD responses to hypercapnia in early-stage hypertension. <sup>97</sup> Elevated resting flow at time of suppressed functional hyperemia. <sup>111</sup>
Diabetes	<i>Human:</i> Thickened basement membranes. <sup>198,199</sup> Hyperviscosity and reduced erythrocyte deformability in proportion to microvascular complications. <sup>92</sup> <i>Animal models:</i> Pericyte loss and thickening of capillary basement membrane. <sup>196,200,201</sup> Pericyte loss in STZ-induced diabetes is caused by oxidative stress in diabetic retinopathy. <sup>186</sup> Glycocalyx degradation by oxidative stress, oxidized lipoproteins, hyperglycemia. <sup>83,87,88</sup> <i>In vitro:</i> Hyperglycemia-induced oxidative stress in pericytic mitochondria cause pericyte apoptosis. <sup>202</sup>	<i>Animal models:</i> Elevated CBF early after disease induction by STZ. <sup>94–96</sup>
Cerebral amyloid angiopathy AD APOE ε4 genotype	<i>Human brain:</i> Pericyte degeneration, pericapillary fibrosis. <sup>190</sup> <i>In vitro:</i> Cultured human pericytes undergo degeneration when exposed to certain subtypes of Aβ. <sup>62</sup> Pericytes express Aβ receptors involved in amyloid internalization and pericyte death. <sup>63</sup>	<i>Human:</i> Elevated resting and activity-related CBF in young APOE ε4 carriers, <sup>99,100</sup> elevated BOLD. <sup>101,102,110</sup>

AD: Alzheimer's disease; APOE: apolipoprotein; Aβ: amyloid β; BOLD: blood oxygen level dependent; CAA: cerebral amyloid angiopathy; CBF: cerebral blood flow; STZ: streptozotocin; SVD: small vessel disease.

the CADASIL-causing *NOTCH3* point-mutation had similar blood pressure, similar resting CBF and similar CBF responses to CO<sub>2</sub> inhalation, a vasodilatory stimulus. CBF responses to functional activation, however, were attenuated in CADASIL mice.<sup>50</sup> These findings are consistent with capillary dysfunction that *only* limits tissue oxygenation during functional activation – either by deficient pericyte-mediated capillary dilation (and CTH reduction) during functional activation,<sup>19</sup> or because only the combination of elevated CBF and increased metabolic demands ‘unmasked’ capillary dysfunction at this early point in the development of characteristic disease signs.

In asymptomatic APOE ε4 carriers aged 50–65, elevated *resting* CBF values have been reported at a time where their CBF- and BOLD-responses to functional activation had been suppressed.<sup>110</sup> Suppression of CBF-responses to sensory stimulation at a time where resting CBF is elevated has also been observed in

spontaneously hypertensive rats, prior to any changes in their microvasculature.<sup>111</sup> These findings are again consistent with the gradual transition from mild to moderate capillary dysfunction which first becomes apparent in states of high metabolic demand.

The unmasking of capillary dysfunction by *combinations* of high CBF and CTH that necessitate flow suppression was recently illustrated in a study by Suri et al.<sup>112</sup> who observed flow suppression during hypercapnia (a strong vasodilator) in young APOE ε4 carriers compared to noncarriers. These young APOE ε4 carriers showed *increased* hippocampal BOLD responses to memory tasks, and the attenuation of their CBF responses during hypercapnia accounted for 70% of this increase,<sup>112</sup> consistent with the prediction that mild (asymptomatic) capillary dysfunction links the two findings.

Attenuation of functional hyperemia can also be observed immediately after administration of AT2

**Table 3.** Type 3: Inherited or genetic SVD other than CAA.

Risk factor	Changes in capillary morphology or blood rheology	Capillary dysfunction signs
CADASIL/CARASIL	<i>Human brain:</i> Deposits of N3ECD <sup>48</sup> and GOM in capillary walls, pericytes. <sup>204,49,205</sup> <i>Human skin, muscle:</i> Pericyte loss, thickened capillary basement membrane with pericyte fragments. Pericyte-endothelial peg-socket contact disruption. Endothelial swelling and luminal processes. <sup>49</sup> <i>Animal models:</i> Reduced capillary density. <sup>50</sup> Pericyte degeneration. <sup>51</sup>	<i>Mouse model:</i> Attenuated functional hyperemia prior to arteriolar damage. <sup>50</sup>
Swedish-type hereditary multi-infarct dementia <sup>203</sup>	No data available	No data available
MELAS	<i>Human:</i> Endothelial protrusions due to mitochondrial aggregates in the cerebrum <sup>192</sup> and cerebellum. <sup>206</sup> Pericytes contain aggregates of enlarged mitochondria in brain <sup>192</sup> and muscle <sup>191,207</sup> – Figure 2, panels E and F.	Globally <i>elevated</i> CBF, low OEF, and reduced CMRO <sub>2</sub> before <sup>130–133</sup> and after <sup>131,132,134</sup> stroke. Preserved CBF and vasoreactivity in lesions. <sup>135</sup>
Fabry's disease	<i>Human brain:</i> Endothelial cell swelling, vacuolization, and deposits in arteries, arterioles, capillaries and veins. <sup>208</sup> Granulomatous and 'zebra' deposits (approximately 1 μm) in endothelial cells and pericytes. <sup>209</sup> <i>Animal model:</i> In lesions, storage material in relation to pericytes. <sup>210</sup>	Hyperperfusion <sup>128</sup> and enhanced vasodilation <sup>212</sup> reversed <sup>126,127</sup> by therapy that removes capillary deposits. <sup>189</sup> Lesions develop in previously hyper-perfused tissue. <sup>129</sup>
Hereditary cerebroretinal vasculopathy	<i>Human retina:</i> Capillary obliterations with fluorescent leakage, shunt vessels with leakage. <sup>211</sup>	
HERNS	<i>Human brain:</i> Multilayered, thickened capillary basement membrane. <sup>213,214</sup> <i>Human retina:</i> Capillary obliterations with tortuous telangiectatic microaneurisms. <sup>213,214</sup> Widespread capillary closure and fluorescent leakage. <sup>213</sup>	
COL4A1 gene mutations (codes for type IV collagen α1 basement membrane protein)	<i>Human retina:</i> Arteriolar (no capillary) involvement <sup>215</sup> . <i>Animal models of collagen IV deficiency:</i> Normal vascular system, but aberrant capillary organization <sup>216</sup> during development.	
PADMAL	Small vessel (but no capillary) changes reported. <sup>217,218</sup>	

CADASIL/CARASIL: cerebral autosomal dominant/recessive arteriopathy with subcortical ischemic strokes and leuko-encephalopathy; GOM: granular osmophilic material; HERNS: Hereditary endotheliopathy with retinopathy, nephropathy and stroke; MELAS: Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes; N3ECD: *NOTCH3* extracellular domains; PADMAL: Pontine autosomal dominant angiopathy and leukoencephalopathy; SVD: small vessel disease.

and Aβ in animal models of hypertension and AD/amyloidosis, respectively (Table 2)<sup>113–115</sup> and antedates any morphological changes in the vessel wall or brain parenchyma and even the development of high blood pressure in the model of hypertension.<sup>116</sup> Although the effects of AT2 and Aβ on pericyte tone in living brain remain to be studied, these findings are consistent with capillary dysfunction caused by pericyte constrictions

and compensatory increase in VSMC tone to limit flow responses.

### Flow suppression and small vessel changes

The flow suppression observed in animal models of hypertension and AD/amyloidosis is caused by vascular production of ROS,<sup>117,118</sup> which reacts with NO to

**Table 4.** Type 4: Inflammatory and immunologically mediated SVD. Vasculitis caused by infection.

Risk factor	Changes in capillary morphology or blood rheology	Capillary dysfunction signs
Varicella Zoster	Reactivated Varicella Zoster Virus travels across axons to infect vessel walls. <sup>226</sup>	
Hepatitis C	HCV can infect capillary endothelium. <sup>227</sup> HCV-related vasculitis is often the result of cryoglobulinaemia – See Table 3.	
HIV-1	HIV infection is associated with vasculitis <sup>228</sup> and ischemic stroke, <sup>229</sup> and vasculitis is identified as the stroke mechanism in many HIV-related ischemic strokes. <sup>230</sup> The role HIV-1 virus, as opposed those of immunosuppression, coinfections, co-morbidities, and antiviral therapy, in conferring high risk of SVD <sup>231</sup> or stroke <sup>232</sup> remains controversial. HAND is associated with infection of perivascular macrophages and microglia which then release viral proteins (gp120, Tat and Vpr) which are neurotoxic in vitro. <sup>233</sup> HIV virus infects cerebral capillary endothelial cells <sup>234</sup> HIV proteins cause human brain microvascular endothelial cell apoptosis. <sup>235</sup> HIV proteins induce pericyte migration and reduce pericyte coverage. <sup>236</sup>	Elevated BOLD signals in <i>asymptomatic</i> HIV patients. <sup>105</sup> Elevated BOLD signal in seropositive patients with low CNS penetration of antiretroviral therapy compared to patients with high penetrance, and controls. <sup>107</sup> Suppression of functional hyperemia partly reversed by viscosity-lowering phosphodiesterase-inhibitor. <sup>239</sup>
<i>Treponema pallidum</i>	<i>Meningovascular neurosyphilis</i> is dominated by lymphoplasmacytic infiltrates and intima-proliferation (endarteritis obliterans) in the walls of leptomeningeal arteries, veins and vasa vasorum and associated with the development of multiple cerebral infarcts. In <i>Parenchymatous neurosyphilis</i> , meninges and nervous tissue of the brain and/or spinal cord is invaded by spirochetes parenchymal. Associated with cortical atrophy, amyloid deposits, neurofibrillary tangles and dementia. Spirochetes frequently aggregate around capillaries/microvessels. <sup>237</sup>	Significant increase in CMRO <sub>2</sub> in the absence of increases in CBF after antibacterial treatment <sup>147</sup> indicating improved oxygen extraction.
<i>Borrelia burgdorferi</i>	Similar to tertiary neurosyphilis (above), Lyme neuroborreliosis exists in a <i>meningovascular</i> form, associated with endarteritis obliterans and multiple cerebral infarcts, <sup>238</sup> and in a <i>parenchymatous</i> form, with slowly progressive dementia. Intraparenchymal, perivascular lymphoplasmacytic infiltrates is a frequent finding. <sup>237</sup>	Reduced cerebrovascular reserve capacity. <sup>240</sup> Cerebral hypoperfusion is partially reversed by antibiotic therapy. <sup>148</sup>

BOLD: blood oxygen level dependent; CBF: cerebral blood flow; CMRO<sub>2</sub>: cerebral metabolic rate of oxygen; HAND: HIV associated neurocognitive disorder; HCV: hepatitis C virus; HIV: human immunodeficiency virus; SVD: small vessel disease.

form peroxynitrite.<sup>119</sup> Both NO depletion and peroxynitrite cause vasoconstriction by impairing normal smooth muscle cell relaxation,<sup>120</sup> but also long-term remodelling and thickening of the vessel walls and VSMC damage.<sup>121</sup> By evoking flow suppression, capillary dysfunction may therefore contribute to – or even antedate – the wall damage observed in small arteries and arterioles before SVD becomes overt. Note that

wall thickening narrows the vascular lumen and may reduce CBF and attenuate CBF responses. This ‘mechanical’ flow suppression would therefore be expected to make flow suppression by oxidative stress superfluous over time.

Oxygen diffuses through arteriolar walls to supply the capillary-free area around arterioles, and it can reach tissue with capillary supply as well. In fact,

**Table 5.** Type 4: Inflammatory and immunologically mediated SVD.

Risk factor	Changes in capillary morphology or blood rheology	Capillary dysfunction signs
ANCA-associated vasculitides: Granulomatosis with poly-angiitis (formerly Wegener's) Churg-Strauss syndrome Microscopic polyangiitis	ANCA's cause neutrophil adhesion to capillary endothelium and trigger their release of toxic proteases and oxidants in close proximity to the endothelium, resulting in endothelial cell necrosis and increased capillary permeability. <sup>79,219</sup> The diseases are dominated by capillaritis in the kidney, lung or heart, but fibrinoid necrosis of intracerebral small arteries, arterioles, capillaries and venules also occurs. <sup>26</sup>	BOLD responses to fatiguing stimulus was attenuated in patients with granulomatosis with poly-angiitis compared to controls with similar degree of fatigue. <sup>225</sup>
Henoch-Schönlein purpura	Arteriolar, capillary and venular interstitial infiltration by polymorphonuclear lymphocytes, eosinophils and mononuclear cells, with fibrinoid necrosis and perivascular granuloma formation. <sup>26</sup>	
Cryoglobulinaemic vasculitis	Cryoglobulins, composed of IgG, IgM, complement, lipoprotein and antigenic moieties, precipitate and lead to hyperviscosity in serum excess. <sup>26</sup> Intravascular activation of complement- and clotting cascades in arterioles and capillaries. <sup>26</sup>	
Primary angiitis of the CNS	Affects medium-sized arteries, arterioles, capillaries and venules of the brain parenchyma and leptomeninges. <sup>220</sup> Exists in a granulomatous form with frequent A $\beta$ deposit, in a lymphocytic form with occasional vessel destruction, and a necrotizing form with transmural fibrinoid necrosis. Strokes and transient ischemic attacks occur in 30–50% of patients. <sup>220</sup>	
Sneddon's syndrome  (Livedo reticularis)	<i>Human skin:</i> Skin discoloration caused by capillary stasis and thickening of the arteriolar wall. <sup>221</sup> Proliferating capillary endothelial cells with luminal and abluminal protrusions. <sup>222</sup> <i>Human retina:</i> Capillary occlusions/neovascularization, <sup>223</sup> arteriolar occlusions. <sup>224</sup>	

ANCA: antineutrophil cytoplasmic antibodies; A $\beta$ : amyloid  $\beta$ ; BOLD: blood oxygen level dependent; CNS: central nervous system; SVD: small vessel disease.

recent studies suggest that arterioles account for as much as 50% of the total oxygen extraction during rest, while capillaries serve as the primary site of oxygen extraction during functional hyperemia.<sup>122</sup> Arteriolar oxygen extraction is insensitive to CTH downstream, and the increased arteriolar tortuosity and length observed in some SVD precursor states<sup>123</sup> may therefore, paradoxically, increase arteriolar oxygen extraction capacity by increasing arteriolar surface area and transit time. The long, tortuous medullary arteries observed in SVD, however, are generally thought to cause hemodynamic insufficiency. Studies of retinal vessels in SVD patients suggest that venules are generally wide in SVD precursor states.<sup>124,125</sup> While the restricted venular lumen observed in venous collagenosis<sup>123</sup> (Table 7) would be expected to facilitate tissue oxygen extraction by prolonging blood's transit time

through the capillary bed, complete venous occlusions, instead, cause venous infarcts to develop.

#### *Moderate capillary dysfunction: Failure to suppress CBF*

If the microcirculation *cannot* evoke intrinsic mechanisms to limit CBF and CBF responses, then functional shunting can become so severe that stroke-like symptoms and hypoxic tissue injury may develop at CBF values well above the classical ischemic threshold.

Fabry's Disease (Table 3) is associated with cerebral hyperperfusion,<sup>126–128</sup> which is reversed or attenuated by therapies that remove the capillary deposits shown in Figure 2.<sup>126,127</sup> This hyperperfusion might, in principle, represent flow metabolism coupling, with hyperaemia compensating for mild capillary

**Table 6.** Type 4: Inflammatory and immunologically mediated SVD. Vasculitis caused by connective tissue disorders.

Risk factor	Changes in capillary morphology or blood rheology	Capillary dysfunction signs
SLE	Increased capillary length, tortuosity, looping and hemorrhage on NCM. <sup>241</sup>	Elevated task-active and task-negative BOLD responses compared to controls in childhood-onset SLE patients with little or no cognitive defects. <sup>104</sup>
Systemic sclerosis (scleroderma)	Mega-capillaries and reduced capillary density by NCM is a strong predictor of the development of systemic sclerosis in patients with Raynaud's phenomenon <sup>242</sup> . In brain, perivascular lymphocytic infiltrates <sup>243</sup> and calcifications of arterial and arteriolar walls <sup>244</sup> have been observed. The development of cerebral hypoperfusion <sup>243</sup> parallels disease development as defined by NCM in some <sup>245</sup> but not all <sup>246</sup> patients.	Negative CBF response to pain in areas with pre-existing hypoperfusion/MRI lesions. <sup>141</sup>
Dermatomyositis	Necrosis of capillary endothelium <sup>247</sup>	Negative CBF response to pain in areas with pre-existing hypoperfusion/MRI lesions <sup>141</sup> .
Sjögren's syndrome Rheumatoid vasculitis	Rare complication of longstanding, severe rheumatoid arthritis.	

BOLD: blood oxygen level dependent; MRI: magnetic resonance imaging; NCM: nailfold capillary microscopy; SLE: systemic lupus erythematosus; SVD: small vessel disease.

**Table 7.** Type 5: Venous collagenosis, and type 6: Other SVDs.

Risk factor	Changes in capillary morphology or blood rheology	Capillary dysfunction signs
Post-radiation angiopathy	Post-radiation angiopathy is associated with capillary rarefaction and tissue hypoxia and thought to be the result of endothelial vulnerability to radiation. <sup>136,137</sup>	Mineura et al. <sup>138</sup> found hyperperfusion and low OEF early after radiation therapy in humans, and Hahn et al. <sup>139</sup> demonstrated a dose-dependent CBF increase three months after irradiation, both consistent with elevated CTH caused by capillary endothelial damage.

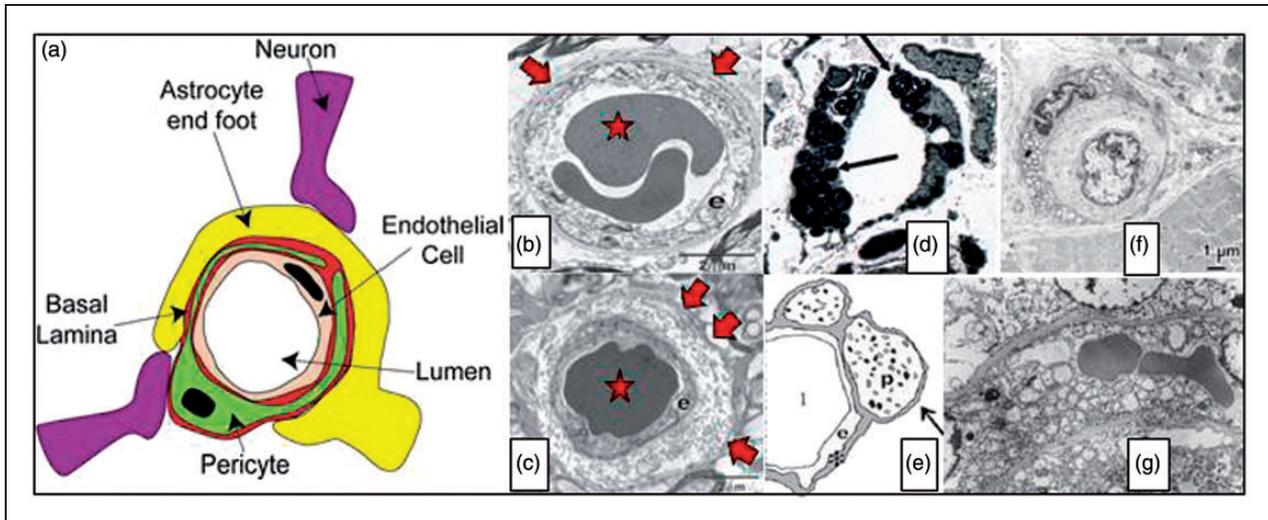
CTH: capillary transit time heterogeneity; OEF: oxygen extraction fraction; SVD: small vessel disease.

dysfunction. The relative hyperemia is, however, observed in patients with severe neurological symptoms<sup>127</sup> and seemingly antedates the development of white matter lesions that may occur in the disease,<sup>129</sup> suggesting that tissue damage is the result of insufficient suppression of excessive vasodilation and functional shunting in this disease.

Mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS – Table 3) is also associated with severe hyperperfusion, which seemingly contributes to tissue infarction. Studies have revealed globally elevated CBF, low OEF and reduced cerebral metabolic rate of oxygen (CMRO<sub>2</sub>) in MELAS patients both before<sup>130–133</sup> and after<sup>131,132,134</sup> stroke. In these patients, the mitochondrial enzymes needed for

oxidative phosphorylation are deficient, and it is therefore unclear how tissue levels of oxygen, ATP and lactate affect neurovascular coupling. The lack of metabolic feedback mechanisms to limit excessive vasodilation in this condition is underscored by the finding that normal vasodilation is preserved, even in hyperperfused stroke lesions.<sup>135</sup>

Vascular endothelium is extremely vulnerable to radiation and post-radiation angiopathy (Table 7) is associated with capillary rarefaction and tissue hypoxia.<sup>136,137</sup> Mineura et al.<sup>138</sup> found hyperperfusion and low OEF early after radiation therapy in humans, and Hahn et al.<sup>139</sup> demonstrated a dose-dependent CBF increase three months after irradiation. If this hyperaemia was a compensatory response to



**Figure 2.** Panel (a) illustrates the organization of endothelial cells, basement membrane and pericytes in the vessel wall. Capillaries are ensheathed by astrocytic endfeet, and neuronal terminals are closely apposed to capillaries and pericytes.<sup>66</sup> Source: Reproduced from Hamilton et al.<sup>66</sup> according to the Creative Commons terms. Panel (b) shows a cross section of normal capillary with a thin basement membrane (arrow) and normal appearing endothelial cell (e). In ageing (Panel (c)), thickened basement membranes (arrows), pericapillary fibrosis and pericyte loss are often found. Source: Panels (b) and (c) are reproduced from Farkas et al.<sup>188</sup> Panel (d) shows a capillary cross section from the skin of a patient with Fabry's disease. Note the lamellar sphingolipid inclusions in the capillary endothelium (arrow). These inclusions disappear upon enzyme replacement therapy. Source: Reproduced from Eng et al.<sup>189</sup> Panel (e) shows typical cerebral capillary wall pathology in human AD. The arrow indicates pericyte degeneration. The symbols denote lumen (l), endothelial cell (e), basement membrane (\*) and pericyte (p). Source: Reproduced from Farkas et al.<sup>190</sup> Panel (f) shows a cross section of a muscle capillary from a patient with MELAS. Note the thickened basement membrane and increased number and size of mitochondria in the pericyte. Source: Reproduced from Sakuta and Nonaka.<sup>191</sup> Panel (g) shows a cross section of a cerebral capillary from the motor cortex of a MELAS patient with accumulation of mitochondria in the endothelial cell. Source: Reproduced from Ohama et al.<sup>192</sup> with permission from the publisher. AD: Alzheimer's disease; MELAS: mitochondrial encephalopathy with lactic acidosis and stroke-like episodes.

mild capillary dysfunction, then tissue oxygen tension would be *elevated* rather than reduced and hyperaemia limited by flow-metabolism coupling rather than radiation dose. Instead, these observations suggest that microvascular signalling is severely disturbed, possibly due to a loss of endothelial regulatory signalling. After radiation exposure, initial hyperperfusion is often followed by normalization of CBF,<sup>138</sup> but OEF remains low,<sup>138</sup> indicating reduced tissue metabolism as a result of tissue damage. Hahn et al.<sup>139</sup> observed that neuropsychological performance deteriorated when the dose-dependent CBF increase three months after irradiation was followed by a reduction in CBF by six months. These findings suggest that uncontrolled hyperemia contributes to on-going tissue damage at least three months after radiation, but also lends hope to the notion that dose fractionation might ameliorate tissue damage and cognitive decline.

### Severe capillary dysfunction

In severe capillary dysfunction, oxygen availability gradually approaches the metabolic needs of brain

tissue. The parallel reduction in tissue oxygen tension, in turn, is highly conducive to BBB breakdown, A $\beta$  deposition and loss of trophic support. See literature<sup>7,140</sup> for a discussion of capillary dysfunction in dementia and preliminary data.

If capillary changes become so severe that CTH approaches CTH<sup>max</sup>, then even increases in viscosity might reduce tissue oxygenation enough to trigger neurological symptoms or tissue injury. This suggests a mechanism by which dehydration or bacterial infections (during which WBC count is elevated) can cause neurological deterioration or stroke-like symptoms in patients with preexisting, capillary dysfunction.<sup>6</sup> We discuss therapeutic implications of this pathophysiological process below.

In severe capillary dysfunction, resting CBF is predicted to approach the classical ischemic threshold at which oxygen extraction is optimal, even for high CTH values. This biophysical feature implies that, paradoxically, tissue oxygenation may be improved to meet the metabolic demands of functional activation by *reducing* CBF to reduce physiological shunting. Indeed, Ferraccioli et al.<sup>141</sup> observed inverted CBF responses

to painful stimuli (Raynaud's phenomenon) in the sensorimotor cortex of patients with scleroderma or SLE vasculitis (Table 6).

### What determines the course of tissue damage in SVD?

The analysis presented here suggests that progressive capillary dysfunction inevitably leads to tissue damage – either gradually, by loss of trophic support, amyloid pathology, hypoxic injury or inflow of toxic substances through a failing BBB – or suddenly, in relation to stroke-like episodes. The precise cause of the small subcortical infarcts, lacunes, white matter hyperintensities, microbleeds and cortical atrophy that accompany SVD has been the subject of intense scrutiny – see recent work by Wardlaw et al.<sup>3,4,142</sup>

Hassan et al.<sup>143</sup> showed that the intron 4ab polymorphism of the endothelial NO synthase (eNOS) gene protects against the development of SVD in the form of symptomatic, small subcortical infarctions, but not of SVD with white matter hyperintensities, suggesting that insufficiency of NO may be associated with the development of small subcortical infarctions. Similarly, the intron 4aa genotype appears to be protective for lacunar infarctions.<sup>144</sup> In mammals, dietary nitrite is a major source of NO,<sup>145</sup> and eNOS may be involved in the conversion of nitrate to NO in tissue.<sup>146</sup> The finding that blood nitrite levels depend on endothelial nitric oxide gene haplotype<sup>143</sup> may indicate that interactions between genotype and dietary habits (see below) is one of many factors which determine whether SVD progresses along a 'stroke-like' clinical presentation or along one of white matter hyperintensities and cognitive decline.

### Cognitive decline or stroke-like phenotype?

Both capillary dysfunction and arteriolar narrowing can lead to hypoxic tissue injury – but what determines whether a SVD precursor state develops along a 'dementia-like'<sup>7</sup> or a 'stroke-like'<sup>6</sup> pathway? Although 'mixed' cases exist, chronic spirochete infections by *Treponema pallidum* and *Borrelia burgdorferi* both come in a *meningovascular* form, which is dominated by lymphoplasmacytic infiltrates and intima-proliferation in the walls of leptomeningeal arteries, veins and *vasa vasorum* and associated with the development of multiple cerebral infarcts, that is, a stroke-like phenotype. In their *parenchymal* form, spirochetes invade brain tissue to aggregate around microvessels, and particularly capillaries.<sup>147</sup> In this form, the infection is instead associated with cortical atrophy and dementia – and often amyloid deposits and neurofibrillary tangles. In severe neurosyphilis, antibacterial

treatment in some cases leads to significant increases in CMRO<sub>2</sub> without increases in CBF from its near-ischemic levels.<sup>147</sup> Such an 'isolated' improvement of OEF and cerebral metabolism is difficult to reconcile with flow-limiting SVD, or the reversal of infection-specific suppression of neuronal function.<sup>147</sup> We speculate that the pericapillary invasion of spirochetes may be associated with severe capillary dysfunction, causing CTH to exceed CTH<sup>max</sup> in general paresis. Reduction of capillary flow disturbances by antibiotic therapy would then be expected to improve CMRO<sub>2</sub> and neurological function, but only to normalize CBF if CTH was reduced considerably. In patients with neuroborreliosis and less severe neurological symptoms, cerebral hypoperfusion is partially reversed by antibiotic therapy.<sup>148</sup>

Similar correlates between microvascular histopathology and the progression along pathways dominated by stroke-like symptoms or cognitive decline may exist for other SVD risk factors and help elucidate what separates these etiologically related, but clinically separate, presentations.

### SVD risk factors versus comorbidities

Many regard hypertension as the single strongest risk factor for SVD, but not all patients with SVD have hypertension or diabetes, and it has been debated whether hypertension might be secondary to impaired cerebral perfusion. Here we briefly discuss whether hypertension and type-2 diabetes are risk factors for SVD – or whether they themselves reflect evolving capillary dysfunction.

We argued above that the attenuation of functional hyperemia observed after administration of AT2 – a likely source of capillary dysfunction<sup>38</sup> – may represent flow suppression to maintain flow-metabolism coupling in brain tissue. This phenomenon was shown to antedate the development of high blood pressure in AT2 animal models of hypertension.<sup>116</sup> Is it conceivable that hypertension itself represents a systemic response to maintain tissue oxygenation in certain organs as their level of capillary dysfunction reaches the thresholds at which flow-suppression becomes necessary? If so, which organs? Dickinson proposed that essential hypertension serves to overcome increased vascular resistance in the brain's large, feeding vessels.<sup>149</sup> While studying large vessel resistance in hypertension, however, he points out that histopathological studies show early changes in capillaries and veins, rather than small arteries and arterioles, in brain tissue from hypertensive patients.<sup>149,150</sup> These observations are consistent with capillary changes as a primary event in hypertension and with the notion that capillary dysfunction require proximal adjustments of vascular resistance to preserve brain oxygenation.

If hypertension represents an early sign of capillary dysfunction, either in brain or other in other organs, then antihypertensive therapy may be warranted at an early stage in patients at risk of SVD.<sup>151</sup> Further studies should address whether the antihypertensive effects of AT2 inhibitors, angiotensin converting enzyme (ACE) inhibitors and Ca<sup>++</sup> channel blockers are linked to inhibition of AT2 and Ca<sup>++</sup>-dependent pericyte constriction. Specific antihypertensive agents may also prove useful in targeting organ- and cell-specific aspects of capillary dysfunction.

Measurements of glucose-analogue extraction in the human brain suggest that capillary transit time heterogeneity limits glucose extraction.<sup>152</sup> Although glucose extraction differs somewhat from that of oxygen,<sup>153</sup> our analysis suggests that capillary dysfunction also limits the clearance of glucose from blood.<sup>5,154</sup> In prediabetic rats, impaired glucose tolerance is indeed accompanied by reduced oxygen extraction efficacy,<sup>155</sup> and the beneficial effects of Mediterranean Diet (MD) (see discussion below) extend to both ambulatory blood pressure and blood glucose levels.<sup>156</sup> Our preliminary analysis indicates that capillary dysfunction favours the extraction of glucose over that of oxygen.<sup>157</sup> As capillary dysfunction becomes severe, tissue is therefore predicted to reveal some degree of aerobic glycolysis.<sup>157</sup> This property may contribute to findings of reduced respiratory coefficient in patients with severe hypertension.<sup>149</sup>

## Therapeutic implications

Despite the heterogeneity and complexity of the conditions known to contribute to the development of SVD and to subsequent stroke or cognitive decline, we speculate that capillary flow disturbances may be a shared feature of some if not most of these conditions. Below, we therefore discuss aspects of capillary dysfunction that can be prevented or alleviated and therefore might be of general benefit to patients at risk of SVD, stroke or cognitive decline.

### Blood viscosity

Dehydration and infection-induced leukocytosis increase blood viscosity. These common occurrences can therefore reduce the brain's oxygen supply by increasing CTH. Influenza vaccinations are offered to the elderly in order to reduce the incidence of influenza and secondary infections, and this is known to reduce the number of stroke deaths.<sup>158–160</sup> Infections may trigger thromboembolism by destabilizing atheromatous plaques in the walls of cerebral vessels, but we speculate that increases in blood viscosity may elicit stroke-like symptoms in patients with preexisting, severe capillary dysfunction as well. Therefore, influenza vaccinations

may be beneficial for SVD patients, irrespective of age. The impact of bacterial infections on cerebral oxygenation and thereby cognition may also be reflected in the observation that eradication of chronic helicobacter pylori infections in AD patients dramatically improves their cognitive scores and overall survival.<sup>161,162</sup>

CTH may be reduced by lowering blood viscosity, and aggressive management of hyperlipidaemia may therefore be warranted in SVD patients. Similarly, high homocysteine levels increase blood viscosity, increase the adhesion of monocytes to the capillary wall and cause oxidation of low-density lipoproteins<sup>163</sup> and could therefore represent a source of capillary dysfunction. Indeed, Hassan et al.<sup>164</sup> showed that elevated homocysteine levels represent an independent risk factor of SVD. A meta-analysis has suggested that homocysteine-lowering therapy may reduce stroke risk in regions where folate levels are low.<sup>165</sup>

Phosphodiesterase (PDE) inhibitors reduce platelet aggregation,<sup>166</sup> decrease blood viscosity<sup>167</sup> and increase the flexibility of erythrocytes.<sup>167</sup> While reduced platelet aggregation might increase bleeding risk, the haemorrhagic PDE inhibitor effects would be expected to reduce CTH and thereby improve tissue oxygenation. For a recent review of pharmacological approaches to SVD management, see literature.<sup>168</sup>

Nicotine up-regulates the expression of adhesion molecules in the capillary endothelium<sup>169</sup> and increases leukocyte rolling,<sup>170</sup> keeping in mind that the latter is observed mainly in post-capillary venules where selectin density and glycocalyx properties provide optimal adhesion for leukocyte recruitment.<sup>171</sup> In addition to nicotine's effects on larger vessels and large vessel atheromatous plaques, smoking would therefore be expected to worsen capillary flow disturbances, to accelerate the development of SVD from its risk factors and to increase the risk of an ischemia-like event. Indeed, pack years of smoking are associated with an increased risk of stroke in CADASIL<sup>172</sup> and with a higher burden of SVD lesions in patients with sporadic lacunar stroke.<sup>173</sup> Patients with SVD precursor states should therefore receive help to reduce not only smoking, but also nicotine consumption in general, in order to reduce their risk of later cognitive decline or stroke.

### Hyperaemia, anaemia and poor oxygen saturation

Hyperaemia is predicted to be harmful in patients with capillary dysfunction.

Obstructive sleep apnea (OSA) is associated with periods of severe nocturnal hypercapnia and hypoxemia, both of which cause dramatic increases in CBF in the normal brain. In addition, reductions in oxygen saturation cause a proportionate reduction in the net oxygen metabolism that can be supported for a given

level of capillary dysfunction – increasing the risk of neurological symptoms. The risk that hyperaemia poses to patients who suffer from capillary dysfunction may in part be reflected in the observation that continuous positive airway pressure (CPAP) treatment reduces the incidence of strokes.<sup>174</sup>

The vulnerability of patients with capillary dysfunction to increased blood viscosity and reduced arterial oxygen content may contribute to the strong association between delirium and dementia:<sup>175</sup> declining haemoglobin levels, dehydration and minor infections are known to herald delirium in the elderly. In patients with severe capillary dysfunction, small reductions in haemoglobin concentration or blood saturation, as well as increases in blood viscosity, may trigger regional cerebral hypoxia and thus, in principle, contribute to delirium symptoms. For patients with SVD precursor states, stricter definitions of anaemia and special attention to pulmonary function may therefore be warranted to reduce the risk of delirium and to alleviate the long-term consequences of chronic brain tissue hypoxia.

### NO depletion

Capillary NO depletion due to oxidative stress and tissue hypoxia may represent a modifiable aspect of capillary dysfunction. Mediterranean diet is rich in green-leafed vegetables, a major source of dietary nitrate (see above). One might expect this diet to offer some protection towards NO depletion in patients with capillary dysfunction, and hence the development of SVD pathology. Preference for MD is indeed associated with a lower burden of white matter hyperintensities<sup>176</sup> and, more generally, with a lower risk of ischemic stroke.<sup>177,178</sup> Keeping in mind that this protective effect may be attributable to other MD constituents, these observations may warrant further studies of dietary interventions in SVD and its risk factors. See also the study by Bath and Wardlaw.<sup>168</sup>

### Stroke management in SVD patients

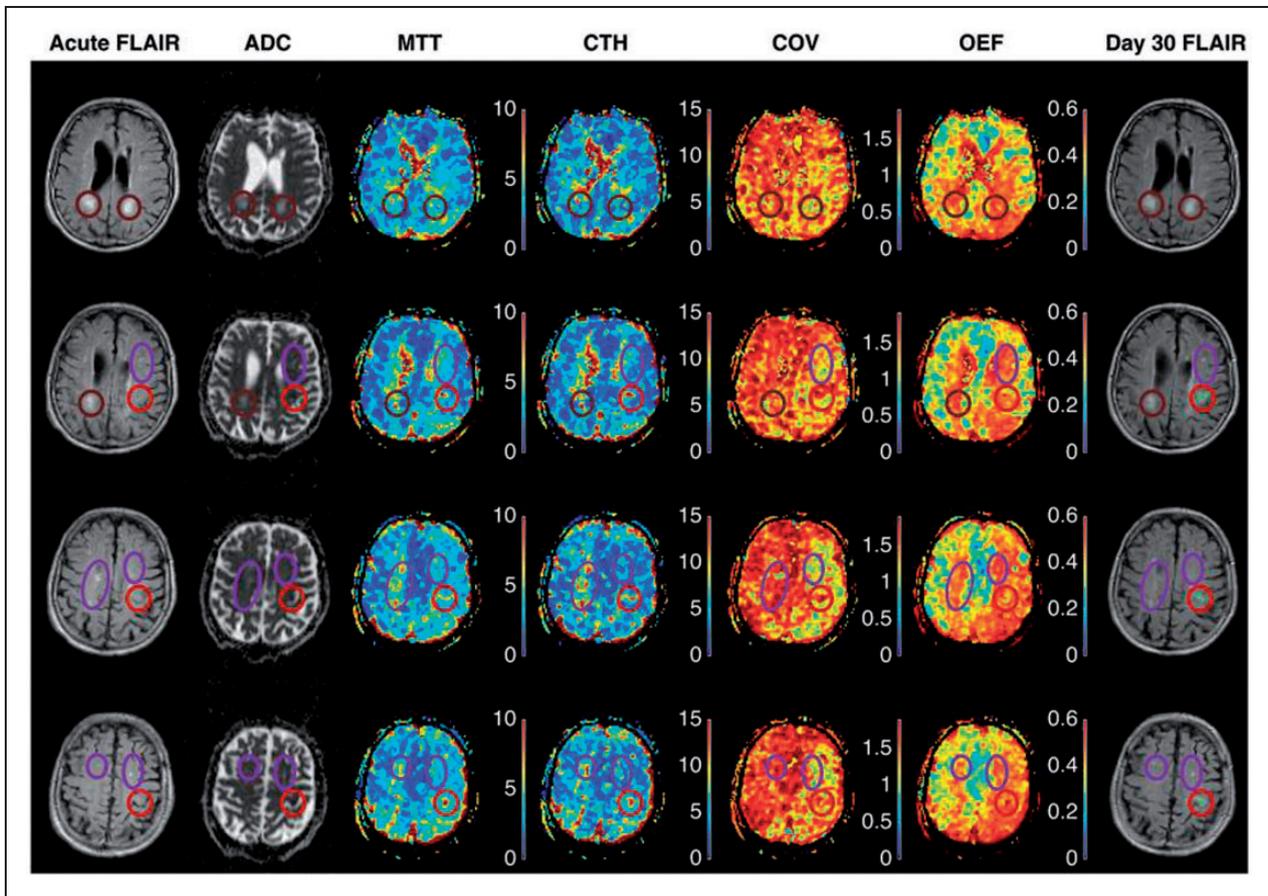
Given the deleterious effects of dehydration and poor saturation on tissue oxygenation, pre-hospital rehydration and efforts to reach full blood saturation may be warranted in patients with capillary dysfunction. While recanalization therapy clearly limits infarct size if large vessel occlusion is the cause of tissue hypoxia, it is important to keep in mind that means of restoring capillary flow patterns should also be explored.<sup>19,24,179</sup> Since SVD risk factors are generally prevalent in the stroke population irrespective of mechanisms of individual events, these interventions may be of general benefit.

## Diagnostic considerations

Flow-limiting conditions and moderate capillary dysfunction are both predicted to reduce cerebrovascular reserve capacity and increase OEF. We showed recently that elevated CTH can contribute significantly to the elevated OEF observed in patients with carotid stenosis<sup>140</sup> and proposed that concurrent capillary dysfunction may contribute to the non-superiority of bypass surgery over aggressive cardiovascular risk factor management for stroke prevention in patients with severe carotid disease.<sup>180</sup> Assessment of CTH and thus the ‘capillary contribution’ to elevated OEF may therefore be warranted when revascularization therapy is considered in patients with carotid stenosis and SVD progenitor states such as diabetes and hypertension.

The detection of capillary dysfunction may also prove important in the management of acute stroke in SVD patients. In acute ischemic stroke, perfusion weighted imaging (PWI) is widely used in the identification of salvageable tissue prior to recanalization therapy. Based on dynamic computerized tomography (CT) or magnetic resonance imaging (MRI) during bolus injection of an intravascular contrast agent, concentration–time curves (CTC) can be estimated in each image voxel and corrected for arterial contrast profile in each patient. The resulting transit time metrics, such as the mean transit time (MTT) and time-to-maximum ( $T_{max}$ ), can then be compared to thresholds above or below which literature studies have shown high likelihood of infarction in the absence of recanalization. Such studies of the relation between perfusion metrics and tissue outcome have found inconsistent transit time thresholds.<sup>181</sup> We speculate that this finding relates to the fact that tissue outcome depends on the extent of capillary dysfunction in ‘hypoperfused’ tissue: first, the tissue CTC recorded during PWI depends on both CBF and CTH, but the PWI algorithms used in studies so far cannot disentangle the two.<sup>182</sup> The success of current recanalization therapies, however, clearly hinges on whether hypoperfusion is the result of a vascular occlusion, exacerbated capillary dysfunction, or both. Differences in the proportion of stroke subtypes and the incidental dependence of preferred transit time metric on oxygen extraction efficacy<sup>6</sup> may therefore explain the difficulty in establishing ‘universal’ transit time thresholds to define tissue-at-risk in acute stroke patients from cohort studies.

Figure 3 shows acute PWI maps from an 84-year-old man with a history of hypertension and smoking who presented with mild (NIHSS 4) stroke symptoms. We note that MTT was prolonged and OEF predicted to be high in relation to tissue that subsequently went on to infarction (red circle). Prolonged MTT and high OEF



**Figure 3.** The two leftmost columns in Figure 3 show acute FLAIR and ADC at four identical slice-positions in an 84-year-old patient who presented with acute stroke symptoms three hours earlier. Acute ischemic changes are visible as areas of low ADC (red circles), consistent with reduced extracellular water diffusion and often ascribed to anoxic depolarizations. The FLAIR images show discrete (purple ellipses) and confluent (brown circles) white matter hyperintensities. The rightmost column show FLAIR images in the same slice positions 30 days later. The green overlays on bright tissue lesions (within the red circles) indicate tissue that infarcted in relation to the stroke episode. Note that areas of elevated CTH and MTT are observed in relation to the area of low ADC.

The COV is relatively independent of CBF in normal microvascular network, and this map therefore helps visualize areas where CTH are higher or lower than expected.<sup>17</sup> Note that COV is elevated in the tissue areas with elevated ADC, indicating that microvascular flow patterns are disturbed beyond what would be expected based on reduced CBF alone. It should be kept in mind that PWI is sensitive to the tracer retention in a large tissue volume, in which small arteries/arterioles, capillaries and venules/small veins each take up roughly one-third of the blood volume. The gradient-echo pulse sequence used in this study is equally sensitive to tracer in these vessels, irrespective of their size, while PWI by spin-echo MRI is weighted towards capillary-size vessels.<sup>193</sup> Our preliminary experience shows that disease may alter COV, as determined by gradient- and spin-echo PWI, respectively, in opposing directions (results not shown). We speculate that areas of reduced COV in this patient may reflect that flow through small arteries and arterioles become more uniform as their walls undergo morphological changes in chronic SVD. The OEF as determined by our biophysical model<sup>5</sup> is also shown. Widespread areas of elevated white matter OEF are noted, especially in the hemisphere affected by the stroke. Detailed studies of well-characterized SVD patients are clearly needed to understand how changes in capillary morphology and local tissue oedema (elevated ADC) affect CTH values determined by PWI methods. ADC: apparent diffusion coefficient; CBF: cerebral blood flow; MTT: Mean Transit Time; COV: CTH/MTT ratio; CTH: capillary transit-time heterogeneity; FLAIR: fluid attenuated inversion recovery; MRI: magnetic resonance imaging; OEF: oxygen extraction fraction; PWI: perfusion weighted imaging; SVD: small vessel disease.

are indeed central characteristic of penumbral tissue,<sup>22</sup> yet areas with white matter hyperintensities showed similar changes in this patient. Our preliminary data suggest that knowledge of CTH is necessary to predict

OEF as obtained by positron emission tomography (PET).<sup>140</sup> Needless to say, further studies are needed to disentangle the effects of limited flow (low CBF) and capillary dysfunction (high CTH relative to

MTT) on oxygen extraction in SVD and SVD-related strokes.

## Conclusion

The morphology and function of cerebral capillaries in conditions considered risk factors for SVD remains relatively understudied. This review suggests that capillary dysfunction may be an early and shared feature of SVD risk factors, and a source of neurodegeneration, stroke and cognitive decline, despite considerable differences in the aetiologies and clinical presentations of these syndromes. We propose that the study of parallel changes in capillary and arteriolar morphology and function may represent an important area of preclinical SVD research. Except for studies in animal models of hypertension, diabetes and CADASIL and in human APOE-ε4 carriers, we identified few reports of neurovascular coupling in the early or presymptomatic phase of SVD risk factors. Given that altered neurovascular coupling may reveal capillary dysfunction before symptoms are predicted to arise, studies using BOLD contrast or CBF-sensitive methods might provide new insights into the aetiopathogenesis of SVD. Similarly, direct measurements of CTH as an index of capillary dysfunction should be applied to SVD risk factors to test the sensitivity of PWI as an investigative or diagnostic tool.

In this review, the pericyte emerged as a critical determinant of several aspects of capillary function. Recent breakthroughs in the understanding of this cell<sup>19,24,64,67,183–185</sup> have already contributed to our understanding of neurodegeneration and stroke. Studies in stroke<sup>179</sup> and diabetic retinopathy<sup>186</sup> lend hope to the notion that pericytes and other components of the neurovascular unit that are affected in SVD may represent targets in future efforts to prevent capillary dysfunction.

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## Declaration of conflicting interests

MBH owns stock in COMBAT Stroke.

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