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REVIEW

Does pathology of small venules contribute to cerebral microinfarcts and dementia?

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Abstract

Microinfarcts are small, but strikingly common, ischemic brain lesions in the aging human brain. There is mounting evidence that microinfarcts contribute to vascular cognitive impairment and dementia, but the origins of microinfarcts are unclear. Understanding the vascular pathologies that cause microinfarcts may yield strategies to prevent their occurrence and reduce their deleterious effects on brain function. Current thinking suggests that cortical microinfarcts arise from the occlusion of penetrating arterioles, which are responsible for delivering oxygenated blood to small volumes of tissue. Unexpectedly, pre-clinical studies have shown that the occlusion of penetrating venules, which drain deoxygenated blood from cortex, lead to microinfarcts that appear identical to those resulting from arteriole occlusion. Here we discuss the idea that cerebral venule pathology could be an overlooked source for brain microinfarcts in humans.

Keywords: microinfarct, microvascular, penetrating arteriole, vascular cognitive impairment, venous collagenosis, venule.

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Cerebral microinfarcts: small but dangerous

Cerebral microinfarcts are small (0.05–3 mm in diameter) ischemic lesions that can be found nearly everywhere in the human brain (Brundel *et al.* 2012; Smith *et al.* 2012; van Veluw *et al.* 2017) (Fig. 1a). Several different research groups have found links between greater cerebral microinfarct number and *ante-mortem* cognitive impairment (Kövari *et al.* 2004, 2007; Gold *et al.* 2007; Sonnen *et al.* 2007; Arvanitakis *et al.* 2010; Buchman *et al.* 2011). Following these initial reports, a meta-analysis of seven large clinicopathological studies revealed that individuals who died with dementia were nearly twice as likely to have microinfarcts, compared to individuals who died without dementia (Smith *et al.* 2012).

This convincing link between microinfarcts and vascular cognitive impairment and dementia (VCID) has raised many questions on how microinfarcts might contribute to cognitive decline. Recent studies estimate that the total number of microinfarcts can be in the hundreds to thousands in a single brain (Westover *et al.* 2013; Auriel *et al.* 2015). Furthermore, some clinical studies show that microinfarcts impair remote tissues by producing persistent brain inflammation (Sofroniew and Vinters 2010), lasting damage to white matter tracts (Auriel *et al.* 2014), and disorganization of axon structure in both subcortical (Hinman *et al.* 2015) and cortical tissues (Coban *et al.* 2017). These findings suggest that microinfarcts elicit secondary degeneration of

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Abbreviations used: CAA, cerebral amyloid angiopathy; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; WMH, white matter hyperintensity.

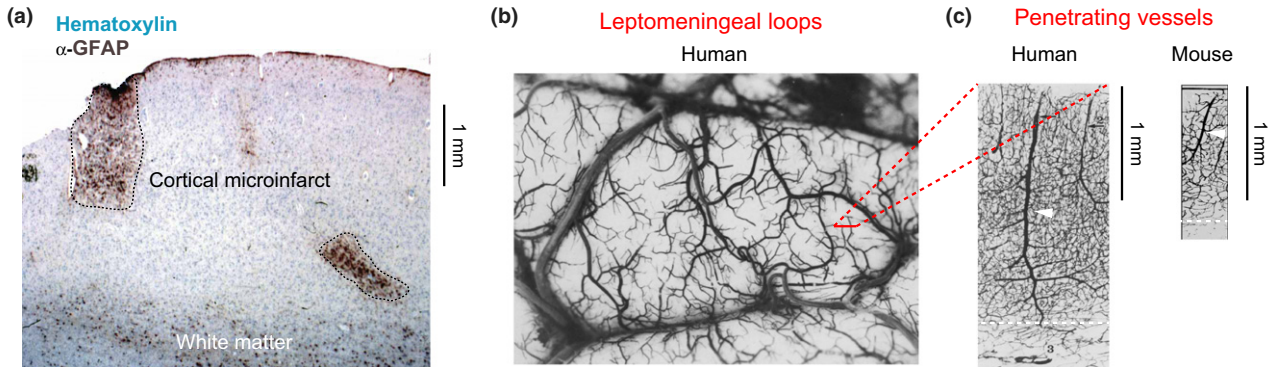

Fig. 1 Human microinfarcts in relation to the vascular anatomy of the cerebral cortex. (a) Sub-acute microinfarcts in human cortex stained with α-GFAP antibodies to show astrogliosis (Sofroniew and Vinters 2010). (b) India ink-filled vasculature shows highly interconnected pial vessels on the surface of the human cortex (Duvernoy *et al.* 1981). (c) A penetrating venule in human cortex (arrowhead) extends from the pial surface (top of image) to the white matter (below dotted white line) (Duvernoy *et al.* 1981). The size of mouse cortex and a penetrating vessel (arrowhead) is shown for comparison (Tsai *et al.* 2009). Mice can be used to study the functional and structural impact of microinfarcts.

anatomically connected brain regions, as occurs for larger infarcts (Duering *et al.* 2015; Dichgans and Leys 2017). However, the consequences of microinfarcts are difficult to determine in humans because microinfarcts are minute, widely distributed across brain regions, and often co-morbid with other pathologies like cerebral amyloid angiopathy (CAA), macroinfarcts, atherosclerosis, and arteriolosclerosis (Raman *et al.* 2014; Kövari *et al.* 2017; Arvanitakis *et al.* 2017).

To better understand how microinfarcts impair brain function, preclinical researchers have devised methods to induce microinfarcts in the rodent brain and investigate their remote effects. These studies revealed that microinfarcts can cause deficits in neural function (Summers *et al.* 2017) and glymphatic function (Wang *et al.* 2017) that extend well beyond the microinfarct lesion core. These distal effects likely contribute to the cognitive impairment that is detected in models with distributed cortical and subcortical microinfarcts (Rapp *et al.* 2008; Wang *et al.* 2012; Venkat *et al.* 2017). Together with clinical findings, this recent work has formed the compelling hypothesis that microinfarcts contribute to cognitive decline by causing cumulative, brain-wide disruptions to neural connectivity, glial dysfunction, and neuroinflammation.

The elusive etiology of microinfarcts

If we are to mitigate the impact of microinfarcts during VCID, we must first understand their etiology. Clues to their origin come from three groups of risk factors associated with higher microinfarct prevalence: (i) large vessel disease of the head and neck such as atherosclerosis (Zheng *et al.* 2013; van Veluw *et al.* 2015; Arvanitakis *et al.* 2017; Leng *et al.* 2017); (ii) small vessel diseases such as CAA, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and arteriolosclerosis (Boyle *et al.* 2015; Kövari *et al.* 2017; Reijmer *et al.* 2016; Arvanitakis *et al.* 2017); and (iii) heart disease such as atrial fibrillation (Wang *et al.* 2016) or ischemic heart disease (Hilal *et al.* 2017). These risk factors suggest a variety of causes for microinfarcts including emboli from the heart or large arteries, local thrombus formation in diseased microvessels, and cerebral hypoperfusion. It is likely that these factors collude to produce microinfarcts that are heterogeneous in brain location and appearance. For example, hypoperfusion, a common result of heart disease or atherosclerosis, and is associated with a greater number of microinfarcts in humans and animal models with CAA (Suter *et al.* 2002; Okamoto *et al.* 2012; Kövari *et al.* 2017). The location of the microinfarct is also informative, as CAA is associated with cortical microinfarcts, whereas arteriolosclerosis and atherosclerosis are associated with subcortical microinfarcts (Arvanitakis *et al.* 2017). Altogether, the existing data point to cardiac and artery/arteriolar disease as the principal causes of microinfarcts. However, a large percentage of microinfarcts may not be associated with any sign of CAA, arteriolosclerosis, or fibrin deposition (Kövari *et al.* 2017), suggesting a need to explore other mechanisms.

Here we propose that the pathology of venules is a potential mechanism of microinfarct formation. The association between microinfarcts and venular pathology has not been thoroughly described in clinical studies. Yet, it is important to consider because disease processes that affect small venules differ substantially from those affecting arterioles. Below, we discuss findings that support our hypothesis.

The angioarchitecture of the cerebral cortex in relation to microinfarcts

Detailed imaging studies in rodents have revealed how microinfarcts might arise with perturbation of cortical microvascular flow (Shih *et al.* 2015). The pial surface vasculature of the cerebral cortex is a highly redundant

network (Fig. 1b). If one pial arteriole becomes clogged, blood flow is maintained by rapidly re-routing through anastomotic connections (Schaffer *et al.* 2006; Blinder *et al.* 2010). In contrast, there are no anastamoses between penetrating arterioles, which descend from the pial arterioles to perfuse columns of cortical tissue (Nishimura *et al.* 2007) (Fig. 1c). This lack of collaterals makes penetrating arterioles a point of vulnerability in cortical perfusion. *In vivo* photothrombotic occlusion of single penetrating arterioles results in ischemic lesions with remarkable similarity to a subset of human cortical microinfarcts, with respect to their location, shape, and absolute volume (Shih *et al.* 2013; Summers *et al.* 2017). These similarities between rodent and human microinfarcts exist because the perfusion domains of penetrating arterioles are comparable between species, despite an approximately twofold greater thickness of the human cortex compared to rodent cortex (Fig. 1c). That is, mouse cortical vasculature is closer to a 'cropped', rather than 'scaled', version of the human vasculature (Lauwers *et al.* 2008; Blinder *et al.* 2013).

Mirroring the arteriole system is a similarly structured network of venules to drain blood from the cortex. As with pial arterioles, pial venules are resilient to localized clots because flow can be efficiently re-directed through anastomotic connections (Nguyen *et al.* 2011). However, blood emerging through the brain capillaries coalesce into penetrating venules that also form a bottleneck in perfusion, as with penetrating arterioles. In rodent cortex, there are ~2–3 times as many penetrating venules as penetrating arterioles (Nguyen *et al.* 2011; Blinder *et al.* 2013; Shih *et al.* 2013; Taylor *et al.* 2016), indicating that each penetrating venule transports only a fraction of the blood carried by a penetrating arteriole. One therefore expects that loss of flow through one penetrating venule will produce an infarct smaller than that generated by arteriole occlusion. Surprisingly, occlusion of single penetrating venules generated microinfarcts that were indistinguishable from those caused by penetrating arteriole blockade (Fig. 2) (Shih *et al.* 2013; Taylor *et al.* 2016; Summers *et al.* 2017). By examining microvascular flow *in vivo* after venule occlusion, we found that loss of flow through one penetrating venule led to gradual stagnation and thrombosis of upstream penetrating arterioles, and recruitment of the arteriolar perfusion domain into the microinfarct core (Fig. 3) (Taylor *et al.* 2016). Thus, the arteriovenous system acts as a single unit to route blood through the capillary bed, and loss of flow through either penetrating arterioles or penetrating venules produces microinfarcts of comparable size.

Differences in cortical microvasculature between rodents and humans

One aspect to consider when translating findings from rodent studies to humans is that the angioarchitecture differs between mouse and human. In human cortex, there are more penetrating arterioles than penetrating venules, which is the inverse of what is seen in rodents (Fig. 4) (Nguyen *et al.* 2011; Shih *et al.* 2013; Taylor *et al.* 2016). Using casts of human cortical vasculature, Duvernoy *et al.* (1981) described how penetrating venules formed 'units' that were surrounded by rings of penetrating arterioles (Fig. 4b). Although the exact ratio was not specified in their work, a typical penetrating venule appeared to drain blood supplied by ~4–5 penetrating arterioles. This organization of human cortical vasculature implies that occlusion of one penetrating venule would greatly increase the resistance in multiple upstream arterioles. Thus, the human cortical angioarchitecture places penetrating venules at the center of a large perfusion domain, making them a point of vulnerability during cerebrovascular disease.

Venous collagenosis: one established pathology of small brain venules

While there is currently little information on the relationship between microinfarcts and pathology of venules in humans, a considerable amount is known about venous collagenosis in the context of VCID. Venous collagenosis is characterized by collagen proliferation within vein or venule walls that leads to stenosis and occlusion of the lumen in severe cases (Moody *et al.* 1995). The severity of venous collagenosis is well correlated with the presence of periventricular infarcts, and with the degree of pathological changes associated with periventricular leukoaraiosis, including gliosis and myelin rarefaction in white matter without obvious infarction (Moody *et al.* 1995; Keith *et al.* 2017). Leukoaraiosis is represented on T2 MRI as white matter hyperintensities, which are putative indicators of small vessel disease and an increased risk for stroke and dementia (Gouw, et al. 2011; Debette and Markus 2010). Adding to the potential relevance of venous collagenosis as a source of subcortical microinfarcts, studies have shown that venous collagenosis occurs in small veins (< 50 μm) of individuals with Alzheimer's disease (Black *et al.* 2009; Keith *et al.* 2017) and CADASIL (Pettersen *et al.* 2017). Thus, venous collagenosis spatially overlaps with periventricular leukoaraiosis and infarcts, and occurs in the context of dementia, suggesting that this venous pathology may also lead to subcortical microinfarcts.

The mechanisms by which venous collagenosis arises and causes tissue damage remain incompletely understood. It is believed that collagenosis is a reaction to the oxidative stress caused by hypoperfusion as a result of upstream dysfunction of arteries and arterioles (Pettersen *et al.* 2017). In addition, upstream arterial stiffness can place greater pulsatile force on venules, leading to mechanical stress and damage to the venule wall (Rivera-Rivera *et al.* 2016). In line with this idea, the severity of venous collagenosis increases with the presence of arteriolosclerosis (Keith *et al.* 2017).

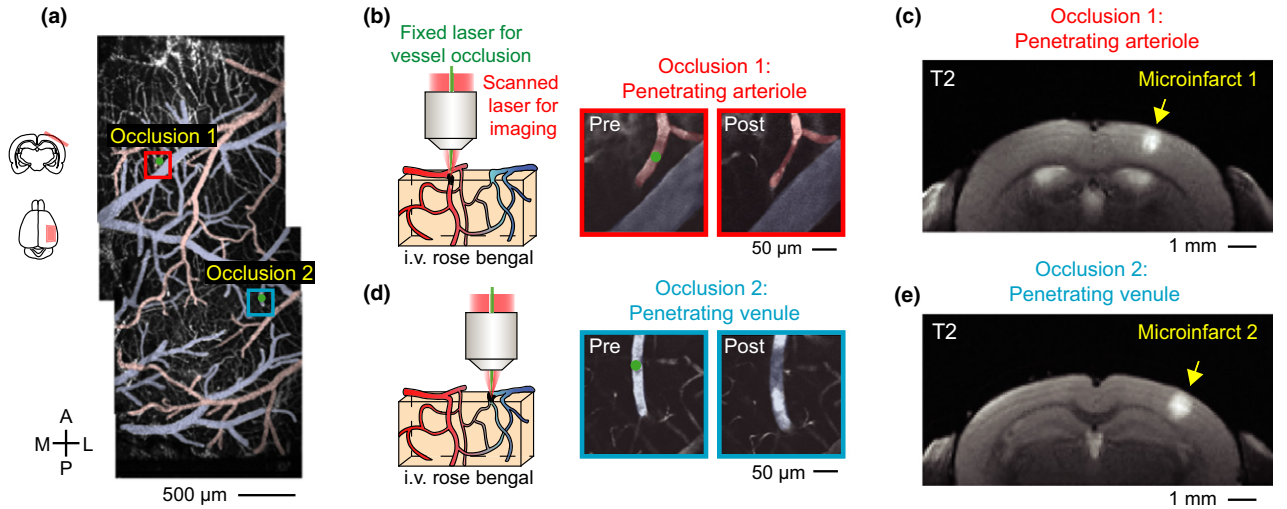

Fig. 2 Occlusion of penetrating venules in mouse cortex generates microinfarcts indistinguishable from penetrating arteriole occlusions. (a) Wide-field two-photon imaging of the pial vasculature through a thinned skull cranial window. Arterioles are pseudo-colored in red and venules in blue. A = anterior, L = lateral, P = posterior, M = medial. (b) High-resolution imaging and focal photothrombotic occlusion of a single penetrating arteriole. Green circle shows location of focused green laser irradiation, which is used to activate a photosensitizer introduced into the blood stream to induce local clotting. Figure adapted from Summers *et al.* (2017). (c) Coronal view of microinfarct resulting from occlusion of a single penetrating arteriole, viewed with T2-weighted 7T MRI at 24 h post-occlusion. (d and e) Identical procedures were used to selectively occlude a single penetrating venule. The resulting MRI-visible microinfarct is similar to that caused by penetrating arteriole occlusion.

Furthermore, in preclinical studies, venous collagenosis was more prominent after induction of hypertension, especially within and around spontaneously infarcted regions of tissue (Zhou *et al.* 2015). The stenosis caused by venous collagenosis presumably increases vascular resistance and exacerbates the hypoperfusion caused by arteriolosclerosis or other arteriopathies, creating a 'vicious cycle'. Furthermore, since collagen is a potent activator of platelets (Surin *et al.* 2008), up-regulation of collagen in the venule wall might also promote local thrombosis. Since venous collagenosis primarily affects veins of the periventricular tissues, it would be worth testing if a similar form of pathology in cortical venules is associated with cortical microinfarcts.

Other potential venule pathologies leading to microinfarction

Another commonly reported venous abnormality is increased tortuosity. Using 7T MRI, investigators observed that patients with mild cognitive impairment and early Alzheimer's disease had more tortuous deep medullary veins than age-matched controls (Bouvy *et al.* 2017). Another study showed that healthy middle-aged carriers of the *APOE ε4* allele had more tortuous subcortical venules than carriers of other *APOE* alleles (Shaaban *et al.* 2017). A higher number of microinfarcts has been reported in deeper nuclei of *APOE* ε4 carriers (caudate, putamen, globus pallidus, and thalamus), and venous tortuosity may be involved in this pathology (Yip *et al.* 2005). Venule tortuosity was also examined in the TgCRND8 mouse model of Alzheimer's disease, but no difference was found between transgenic and control mice (Dorr *et al.* 2012; Lai *et al.* 2015). However, mural cell defects and blunted dilatory responses to hypercapnia were observed with cortical venules in TgCRND8 mice (Lai *et al.* 2015), and more recently in the TgF344-AD rat model of Alzheimer's disease (Joo *et al.* 2017). Whether these rodent models develop spontaneous cortical microinfarcts has not been examined.

There are other mechanisms that may converge with venule pathology to induce venular obstruction and microinfarcts. One such mechanism is the hypercoagulable state produced by contact between amyloid β and clotting factors, such as factor XII, leading to augmented fibrinogen cleavage in patients and mice with CAA (Cortes-Canteli *et al.* 2010; Chen *et al.* 2017). It is conceivable for vascular amyloid and blood-borne clotting factors to interact through an impaired blood–brain barrier, inducing a hypercoagulable state that contributes to microinfarcts. The activation of clotting factors by vascular amyloid may not be potent enough to produce thrombi in fast-flowing arterioles, but could promote thrombosis of downstream venules. Another potential factor that can promote venular occlusion is capillary pathology. A role for capillary pathology in the development of dementia has been widely postulated (Østergaard *et al.* 2015; Love and Miners 2016), given reductions in capillary density in cortex and white matter of Alzheimer's disease patients (Kitaguchi *et al.* 2007; Brown and Thore 2011). Direct amyloid β

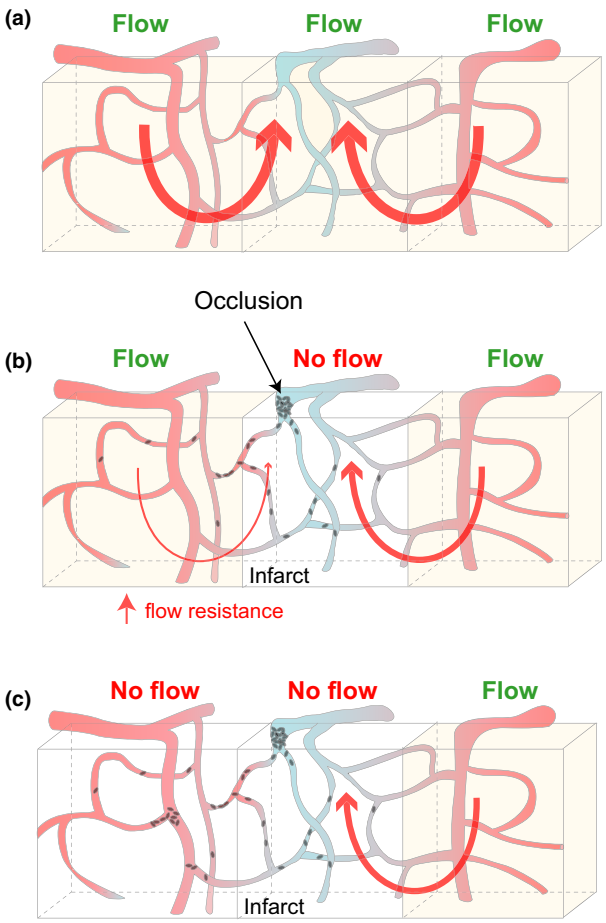

Fig. 3 Occlusion of penetrating venules leads to stagnation of flow in upstream penetrating arterioles. (a) Blood entering through penetrating arterioles flow through the capillary bed and eventually drain through penetrating venules. (b) The blockade of a penetrating venule increases flow resistance and impedes blood flow into cortex through neighboring penetrating arterioles. (c) Stagnation of flow in penetrating arterioles leads to thrombosis and lumen obstruction. The perfusion domain of the arteriole is recruited into the microinfarct core. Figure adapted from Taylor *et al.* (2016).

deposition has also been reported near deformed capillaries in Alzheimer's disease (Attems *et al.* 2010). These capillary pathologies might exacerbate hypoperfusion, inflammation, and hypercoagulability to promote thrombosis in venules.

Interestingly, cerebral microinfarcts are also common in sickle cell disease, a genetic disease resulting in vasculopathy of both large and small vessels. About 40% of children with sickle cell disease develop small ischemic lesions visible by magnetic resonance imaging (MRI), commonly referred to as silent cerebral infarcts (DeBaun *et al.* 2012). Some of these lesions are in the size range of microinfarcts, that is, < 3 mm in diameter. Microinfarcts might arise when sickled cells adhere to the endothelium activate inflammatory cells and clotting factors (Prengler *et al.* 2002). This is expected to have the greatest impact in venules, where leukocyte adhesion occurs. Small vessel stasis might also arise with exuberant endothelial proliferation, a common occurrence in sickle cell disease, which would cause narrowing of small brain vessels (Wood 1978). Recent findings from our group have revealed that Townes sickle cell mice spontaneously develop cortical microinfarcts (Hyacinth *et al.* 2017). *In vivo* high-resolution imaging of cerebral vasculopathy in these mice will provide new insight into how small brain vessels become occluded, and if venules are involved. Animal imaging studies may further help to explain a recent *in vivo* 7T MRI study that found that young adult sickle cell anemia patients possess more morphologically 'short' venules than controls, and that the proportion of short venules within a patient is associated with poorer scores on a cognition test (Novelli *et al.* 2015).

Venules are also the primary locus of leukocyte adhesion and entry into the brain under inflammatory conditions (Muller 2011). *In vivo* imaging of animal models of Alzheimer's disease has shown that the presence of pro-inflammatory molecules, such as amyloid β and endothelial dysfunction, promotes leukocyte adhesion and entry around venules (Michaud *et al.* 2013; Zenaro *et al.* 2015). Furthermore, animal studies have shown that cerebral hypoperfusion resulting from arterial stenosis, a common scenario in VCID (Wolters *et al.* 2017), can elicit marked leukocyte adhesion in venules and capillaries (Yata, *et al.* 2014). Increased leukocyte adhesion puts venules in a dangerous position, as reactive oxygen species and proteolytic enzymes derived from leukocytes can damage endothelial cells and induce clotting (Touyz and Briones 2011). During hypoperfusion, sluggish flow in venules may arise in 'watershed' regions between major cerebral arteries, where perfusion pressure is lowest, and can further reinforce leukocyte adherence and clotting. Indeed, microinfarcts have been reported to be more dense in watershed zones of individuals with Alzheimer's disease (Suter *et al.* 2002).

Methods for finding a link between venule pathology and microinfarcts

Despite the many possibilities described above, to date there have been few studies that have considered venule pathology as a source for microinfarcts. This may in part be due to difficulties in differentiating between venules with thickened walls and arteriole hyalinization using routine stains such as hematoxylin and eosin (Moody *et al.* 1995; Brown and Thore 2011). Methods to unambiguously identify venules would be important in future studies on microinfarcts and their spatial overlap with venule pathology. Incorporating additional stains to differentiate arterioles from venules would be key, including alkaline phosphatase (Moody *et al.* 1995), α-smooth muscle actin (Fig. 5) (Keith *et al.* 2017), or

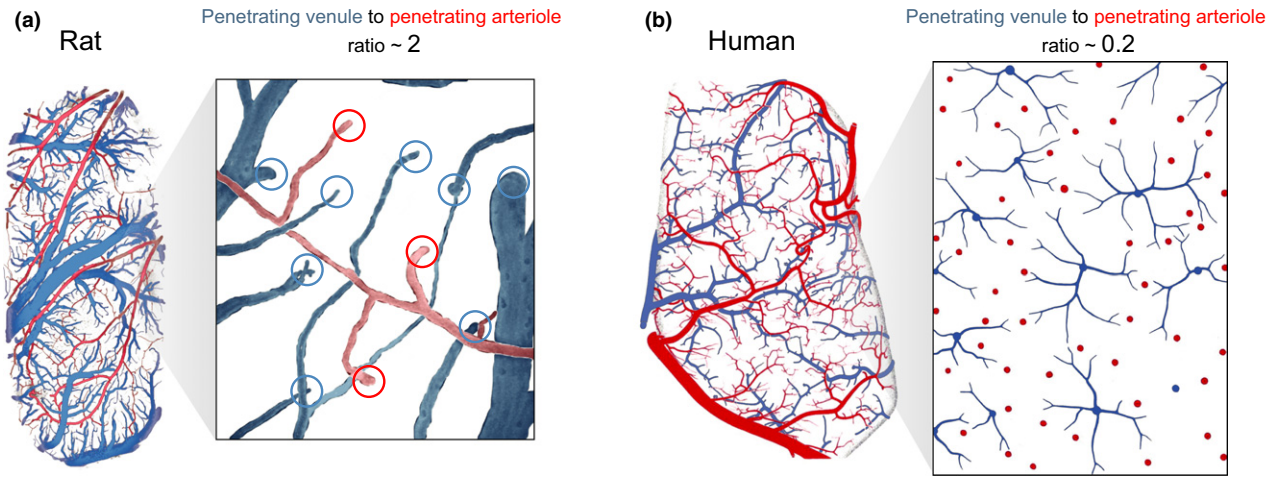

Fig. 4 Arteriole–venule ratios in rat versus human cortex. (a) The location and number of penetrating arterioles and ascending venules in rat cortex visualized through a cranial window using *in vivo* two-photon microscopy. A magnified view from the cranial window shows penetrating arterioles (red circles) and ascending venules (blue circles) at the pial surface, which appear to end, but actually descend into cortex. Note that this image shows roughly twofold more ascending venules than penetrating arterioles in one field of view, which is consistent with our previous data (Shih *et al.* 2013). (b) India ink tracing studies by Duvernoy *et al.* (1981) show the arrangement of pial venules (blue) and arterioles (red) in human cortex. A magnified inset shows arrangement of penetrating arterioles (red) and ascending venules (blue) in a histological section below the cortical surface. Note the relatively low number of venules compared to arterioles, which is the inverse of what is observed in rat cortex.

possibly a new fluorescent dye (Alexa 633 hydrazide), which labels elastin in arteries and large arterioles (Shen *et al.* 2012). However, these approaches are not without limitations, as smooth muscle cells and elastin can degenerate during small vessel disease, potentially leaving nothing to stain.

Another way to distinguish between small arterioles and venules *ex vivo* is to follow their connections to larger upstream arterioles or venules where vessel type can be more easily distinguished by morphological and staining features. While this is difficult with thin tissue sections, new tissue clearing and optical imaging protocols (Ke *et al.* 2013; Susaki *et al.* 2014; Murray *et al.* 2015; Seo *et al.* 2016) allow one to visualize the vascular network in larger volumes (Hartmann *et al.* 2015). Furthermore, this approach makes it possible to quantify changes in vascular branch pattern and vessel tortuosity.

The structure and function of small venules can also be examined *in vivo* with ultra-high field MRI. As mentioned above, venule structure in deeper tissues has been examined with susceptibility weighted imaging (Bouvy *et al.* 2017; Shaaban *et al.* 2017). Novel methods are also emerging for the measurement of blood flow velocity and pulsatility in very small cortical and subcortical perforating vessels (Geurts *et al.* 2017). Complementing clinical studies are preclinical techniques with impressive spatiotemporal resolution for imaging microvasculature *in vivo*. Multi-photon microscopy has been used to study cortical venule structure (Lai *et al.* 2015) and leukocyte adhesion in models of Alzheimer's disease (Michaud *et al.* 2013). Ultrafast ultrasound imaging allows rapid non-invasive assessment of arterioles and venules down to < 10 μm in diameter in both cortical and deep brain regions (Errico *et al.* 2015). Furthermore, fMRI has achieved resolutions necessary to visualize hemodynamics in individual cortical penetrating arterioles and venules (Yu *et al.* 2016). These techniques can be used to understand the vascular basis of microinfarcts in animal models that develop microinfarcts spontaneously (Okamoto *et al.* 2012; Holland *et al.* 2015; Tan *et al.* 2015; Hyacinth *et al.* 2017).

Conclusions and future directions

Pre-clinical data have confirmed that venule occlusion causes microinfarcts that are remarkably similar to those found in clinicopathological human studies (Smith *et al.* 2012). The vascular architecture of the human cortex further suggests that each penetrating venule could be a locus of vulnerability for perfusion, since multiple arterioles rely on a single venule for drainage. Thickening of venular walls, leukocyte adhesion, capillary pathology, and hypercoagulability caused by amyloid β may cooperate to increase blood flow resistance and venule thrombus. When considering these factors in the context of small vessel disease and cerebral hypoperfusion, we see a potential for venules to become occluded. There is very limited data on the relationship between venular pathology and microinfarct burden in humans. Novel approaches to image vasculature of *post mortem* tissues in 3D, and recent advances in ultrahigh-field MRI, may aid in

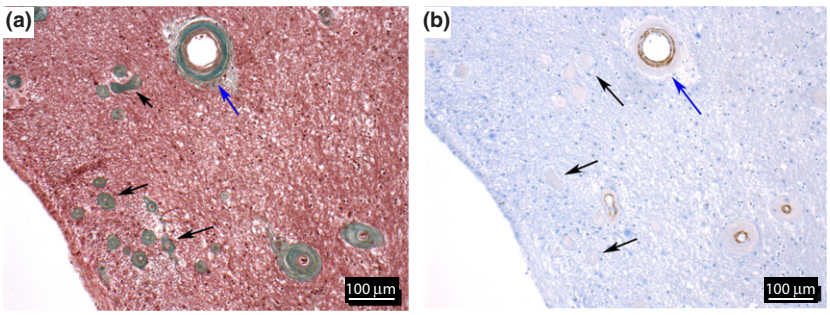

Fig. 5 Identifying venous collagenosis of small caliber vessels. (a) Severe collagenosis of small vessels in periventricular white matter visualized in Gomori trichrome-stained sections. (b) Immunostaining for α-smooth muscle actin in an adjacent section helps to distinguish venules (black arrows) from arterioles that are α-smooth muscle actin-positive (blue arrow). Figure reproduced with permission from Keith *et al.* (2017).

the identification of venules in clinical studies on microinfarcts.

Animal models of cerebral microinfarcts can provide new mechanistic insight, but also have some limitations. For example, injection of microemboli into the carotid artery produces distributed microinfarcts, including subcortical microinfarcts, but leads to only arteriolar occlusions. Direct optical occlusion of single venules is useful for generating spatiotemporally controlled microinfarcts, but the occlusion method differs substantially from the slower developing partial obstructions that might arise with venous collagenosis. There is also currently no method for targeted occlusion of single subcortical vessels, which makes it difficult to study the impact of microinfarcts on subcortical and white matter integrity. However, emerging animal models that develop spontaneous microinfarcts, in combination with novel high-resolution preclinical imaging methods, will be useful for understanding the potential role of venular pathology in microinfarct development.

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