Dysfunction of the Blood-brain Barrier in Cerebral Microbleeds: from Bedside to Bench

Hai-ling Wang#, Chun-lin Zhang#, Yan-mei Qiu#, An-qi Chen, Ya-nan Li*, Bo Hu*

Department of Neurology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China.

[Received April 11, 2021; Revised May 14, 2021; Accepted May 14, 2021]

ABSTRACT: Cerebral microbleeds (CMBs) are a disorder of cerebral microvessels that are characterized as small (<10 mm), hypointense, round or ovoid lesions seen on T2*-weighted gradient echo MRI. There is a high prevalence of CMBs in community-dwelling healthy older people. An increasing number of studies have demonstrated the significance of CMBs in stroke, dementia, Parkinson’s disease, gait disturbances and late-life depression. Blood-brain barrier (BBB) dysfunction is considered to be the event that initializes CMBs development. However, the pathogenesis of CMBs has not yet been clearly elucidated. In this review, we introduce the pathogenesis of CMBs, hypertensive vasculopathy and cerebral amyloid angiopathy, and review recent research that has advanced our understanding of the mechanisms underlying BBB dysfunction and CMBs presence. CMBs-associated risk factors can exacerbate BBB breakdown through the vulnerability of BBB anatomical and functional changes. Finally, we discuss potential pharmacological approaches to target the BBB as therapy for CMBs.

Key words: Blood-brain barrier, cerebral microbleeds, cerebral amyloid angiopathy, hypertensive vasculopathy, endothelial dysfunction

1. Introduction

Cerebral microbleeds (CMBs) are designated as small (<10 mm), hypointense, round or ovoid lesions detectable by T2*-weighted gradient echo MRI, which have been increasingly detected with the widespread application of high blood-sensitive MRI techniques, such as T2*-weighted gradient-recalled echo (GRE) and susceptibility-weighted imaging (SWI) (Fig. 1) [1, 2]. Histopathological investigations have demonstrated that CMBs, which are punctate hemorrhagic lesions, contain hemosiderin deposits most likely resulting from the leakage of erythrocytes from small cerebral vessels, such as arterioles and capillaries [3, 4]. The pathophysiology of CMBs is varied with their location. The lobar microbleeds are related to cerebral amyloid angiopathy (CAA), while deep or mixed CMBs are attributable to hypertensive vasculopathy [2, 5]. Because the presence of CMBs is regarded as a precursor of both intracerebral hemorrhage (ICH) and ischemic stroke, there has been increased research on CMBs [5-7]. There is an increased prevalence of CMBs in patients with stroke. The frequency of CMBs is as high as 50% -80% in patients with primary ICH and approximately 35%~71% in patients with ischemic stroke [8]. The presence of CMBs, especially in a population with higher CMBs counts, is associated with increased...
risks of all subtypes of stroke, and this depends on whether or not the location is typically affected by CAA [5]. More remarkably, in adults with recent ischemic stroke or transient ischemic attack (TIA), the CMBs burden corresponds to a more significant relative hazard for subsequent ICH than for ischemic stroke, but a higher absolute risk of ischemic stroke than that of ICH regardless of the count and anatomical patterns of CMBs [6]. Meanwhile, CMBs can also contribute to neurologic dysfunction, and previous studies have found that increased CMBs burden is associated with cognitive deterioration and dementia [9-12]. Therefore, the pathogenesis of CMBs involves damage to the vascular wall as a result of both vascular risk factors and accumulation of β-amyloid (Aβ), and the presence of CMBs is regarded as a marker of diffuse vascular and neurodegenerative brain damage [9, 13].

Figure 1. Representative images of cerebral microbleeds (CMBs) visualized on susceptibility-weighted images (SWI). (white arrow). (A) lobar microbleeds, (B) deep cerebral microbleeds.

The risk factors contributing to microbleeds include age, blood pressure, diabetes mellitus, low serum cholesterol, smoking, and apolipoprotein E (APOE) genotype [13-16]. Among all of the above, cardiovascular risk factors contribute to deep or infratentorial microbleeds, while APOE genotype is associated with strictly lobar CMBs [15]. In addition, aging is commonly accepted as an independent risk factor related to CMBs.

At all cerebrovascular tree levels, the blood-brain barrier (BBB) is characterized as a dynamic and metabolic interface between the blood and the central nervous system (CNS). The BBB comprises a single layer of brain endothelial cells (ECs) lining up to form the cerebral blood vessels, with close communication with astrocytes, pericytes, and the basement membrane. The pericytes are embedded in the basement membrane of brain ECs, while the astrocytic endfeet almost wholly envelop the capillaries as the outer surface of the BBB [17-20]. The BBB plays an essential role in maintaining homeostasis in the CNS by preventing neurotoxic components in the blood, blood cells, and pathogens from entering the brain, a prerequisite of normal neurological function [21-23]. BBB dysfunction, with the loss of physiological functions and structural integrity, is a significant pathological characteristic of many cerebral disorders. Multiple studies have also demonstrated the existence of BBB leakage in CMBs.

Despite recognition from an increasing number of researchers regarding the crucial clinical significance of CMBs, there currently are no proper animal models for CMBs, and the etiological mechanism behind CMBs has not been elucidated clearly. In this review, we provide clinical knowledge and theories regarding CMBs, including their clinical epidemiology, risk factors, and pathogenesis. We also summarize how CMBs-related risk factors contribute to BBB dysfunction and provide additional detail regarding CMBs-related cerebrovascular dysfunction, focusing on BBB permeability, endothelial dysfunction, and molecular and cellular mechanisms of vascular disease.

2. Introduction to cerebral microbleeds

2.1 The pathogenesis of cerebral microbleeds

Cerebral microbleeds are defined as small (<10 mm), hypointense (black seen on T2*-weighted MRI), round or ovoid pathological lesions with associated blooming
detectable by T2*-weighted MRI techniques, but T1- or T2-weighted sequences [2, 24]. However, the term microbleeds was first denoted as homogeneous, small (2 to 5 mm in diameter seen on imaging), hypointense, round lesions on T2-weighted imaging by Offenbacher et al. in 1996 [25]. With the advances in MRI techniques, especially GRE and SWI with high sensitivity to the hemosiderin deposits, the detection of CMs increases rapidly, facilitating the evolution of CMBs identification criteria [2].

However, the pathogenesis of CMBs has not yet been elucidated clearly. Histopathological studies have shown that CMBs contain hemosiderin deposits or hemosiderin-laden macrophagocytes adjacent to abnormal small cerebral vessels presenting with fibrolipohyalinosis or cerebral amyloid angiopathy. CMs appear to be a marker of increased vascular fragility [3, 4]. Therefore, cerebral microbleeds seem to indicate the occurrence of previous extravasations of erythrocytes from small cerebral vessels, as a consequence of bleeding-prone cerebral arteriopathies, including hypertensive vasculopathy and cerebral amyloid angiopathy [1, 3, 4]. Contrary to ICH, the mechanical injury to brain tissue caused by microbleeds is barely measurable, due to their limited hematoma. However, products derived from blood extravasations, especially iron, can lead to a series of secondary brain injuries, such as BBB breakdown and inflammatory activation [26]. The pathophysiology of CMBs might last for an extended period, including dendritic degeneration, microglial activation, and iron deposit. Ions accumulation after multiple microhemorrhages leads to a series of secondary brain damage and impairs spatial cognition [27]. In addition, the location of CMBs corresponds to two different types of underlying vasculopathy. Strictly lobar CMBs, regardless of cerebral or cerebellar compartments, appear to result from CAA, referring to the damage of cortical and leptomeningeal vessels due to the increasing amyloid load. Population-based studies have investigated the association between CMBs and CAA locations, where the presence of new lobar CMBs was consistent with higher baseline β-amyloid load [28-30]. The presence of CMs in deep or infratentorial areas (with or without lobar CMs) is attributed to hypertensive vasculopathy and corresponds to vascular lesions within superficial perforating arterioles [31, 32]. Besides, as a bleeding-prone vasculopathy, microbleeds are regarded as a subclinical precursor of ischemic or hemorrhagic stroke. The strictly lobar CMBs, which are always present with CAA, are suggested to increase ICH risk. Simultaneously, cerebral microhemorrhage located in other regions is related to both ischemic and hemorrhagic stroke [5].

2.1.1 Cerebral amyloid angiopathy

Cerebral amyloid angiopathy is characterized by a cerebrovascular disease with the accumulation of aggregated β-amyloid protein, which selectively involves the parenchymal and leptomeningeal vessel walls. The establishment of the Boston criteria makes it possible to understand more about the pathological mechanisms of CAA [33]. Recently, a study based on a transgenic rat model of CAA (rTg-DI) showed cerebral microbleeds were present about three months after reduced cerebrospinal fluid (CSF) /plasma Aβ40 levels [34]. The primary form of vascular Aβ deposition in CAA is composed of Aβ40, which is the product of amyloid precursor protein (APP) cleaved by β-secretase 1 (BACE-1) and γ-secretases [35, 36]. Soluble Aβ is eliminated by enzymatic breakdown, BBB clearance, interstitial fluid bulk-flow clearance, perivascular drainage, phagocytosis, and CSF absorption [37]. Among all of the reported clearance pathways, perivascular drainage pathways, driven by pulsations of the blood vessel wall, are believed to contribute primarily to the pathogenesis of CAA [38, 39]. Moreover, extracellular iron dyshomeostasis might contribute to excessive amyloid plaques in CMs. This process can be explained by decreasing iron efflux mediated by the APP/Fpn1 complex and increased affinity of APP/BACE1 [40]. Additionally, Aβ deposition predominantly affects arterioles, as the major pathways for perivascular Aβ clearance [41]. The Aβ peptides are able to spread along perivascular drainage pathways to accumulate in the walls of vessels and form a self-reinforcing cycle. The vascular deposition of Aβ generates the loss of smooth vascular cells, leading to a further reduction in Aβ elimination [38, 42]. The Aβ deposition in the basement membranes of parenchymal and leptomeningeal vessels progressively replaces the smooth muscle cells located in the tunica media, until it finally composes the entire vessel wall [43]. Consequently, Aβ deposition leads to the rupture of small vessels, the accumulation of amyloid peptides in the brain, and inflammatory processes [44-48]. Moreover, these changes are suggested to influence the integrity of BBB, leading to the extravasation of proteinaceous fluid and blood cells. BBB disruption might be a contributory mechanism for CAA-related brain injury [38]. Furthermore, Aβ deposition in CAA usually affects the cortico-subcortical brain regions rather than the deep or infratentorial areas [49, 50].

Endothelial dysfunction is one of the most important characteristic features in the pathogenesis of CAA. On the one hand, Aβ deposition-induced BBB hyperpermeability may partly be attributed to endothelial death [51]. Aβ deposition may disrupt endothelial mitochondrial metabolic pathways by inhibiting the conserved metabolic
enzyme activity involved in the tricarboxylic acid cycle, electron transport chain, and oxidative phosphorylation in a manner similar to that in the mitochondria of other cell types [52, 53]. On the other hand, Aβ deposition-related BBB dysfunction is attributed to increased pinocytic vesicles and loss of tight junction (TJ) proteins [54, 55]. Aβ upregulates the endothelial expression of matrix metalloproteinase (MMP)-2 and MMP-9 through binding to RAGE, which is involved in the degradation of TJ proteins and vascular inflammation [56, 57]. Aβ deposition promotes the expression of MCP-1, GRO, IL-1β, and IL-6 in brain ECs through the JNK-AP1 signaling pathway, recruiting peripheral immune cells into the cerebral parenchyma [58]. Retraction and swelling of astrocyte endfeet were observed prior to widely spread β-amyloid plaque pathology [59]. Aβ exposure induces the progressive loss of mitochondrial membrane potential and triggers the cell death pathway in astrocytes. Loss of astrocytic endfeet and loss of Aquaporin 4, Kir4.1, and dystrophin 1 localized to the astrocytic endfeet are observed in transgenic mouse models with amyloid deposition and postmortem brain tissues from AD patients [60]. Astrocytes increase the expression and secretion of MMP-2 and MMP-9 in CAA, which degrade both Aβ and TJ proteins [61]. In CAA, reactive astrocytes also activate transcription factor nuclear factor-kappa B (NF-κB), which is followed by elevated expression of TNF-α, IL-1β, and many other pro-inflammatory factors [62-65]. Aβ stimulates the release of endothelin-1 from pericytes, which leads to contraction of pericytes and capillaries [66]. Pericyte loss and insufficient vascular platelet-derived growth factor receptor-β (PDGFRβ) signaling is a typical change that occurs during the pathogenesis of CAA [67]. Pericytes may be dead from Aβ-mediated oxidative stress, excitotoxicity, and mitochondrial dysfunction in CAA [68, 69]. The reduced number and coverage of pericytes accelerate CAA due to their active transportation of Aβ across the BBB from the brain to the blood [70, 71].

CAA is a common age-related pathology. A community-dwelling study with a well-characterized older population showed that CAA pathology was prevalent with a frequency of 85% in people investigated, and present in 94% of those with dementia and 77% of those without cognitive disorders [72]. The CAA pathology in the elderly might be related to the reduction of Aβ clearance, and may be aggravated by aging, as a result of the thickening of vessel walls, loss of vasoactivity, and alteration of basement membrane proteins [38, 73-75]. In addition, numerous studies have shown the presence of CAA is associated with spontaneous lobar ICH, Alzheimer’s disease, and cognitive impairment [76-79]. Furthermore, CAA-related brain injury is also frequently present in small vessels diseases, such as lacunar infarcts, CMBs, white matter hyperintensity (WMH) [2, 43, 80, 81]. Aβ burden is associated with increasing frequency of CMBs, with a posterior cortical predominance [2, 28]. Studies in vivo about the association between CMBs and CAA have found that Aβ deposition in vessels is not sufficient to account for vessels bleeding directly, while flow network dynamics may either make a contribution. CMBs are more likely to be present on bends and bifurcations, which are the anatomically vulnerable segments, as a consequence of continuous turbulence [41].

2.1.2 Hypertensive vasculopathy

Contrary to lobar microbleeds, deep or infratentorial CMBs, present in the cerebellum, basal ganglia, thalamus, and brainstem, are associated with hypertensive vasculopathy [2]. Hypertensive vasculopathy, also known as hypertensive small vessel disease or arteriolosclerosis, negatively influences the blood supply of deep perforators [82]. The pathological changes of hypertensive vasculopathy are comprised of fibrinoid necrosis, lipohyalinosis, microatheroma, and microaneurysms [83]. Among all of the mechanisms mentioned above, fibrinoid necrosis, characterized by the accumulation of plasma proteins in vessel walls, causes the degeneration of the muscle and collagen to produce hyalinization. Additionally, autopsy pathology evidence suggests that in lipohyalinosis, which is the deposition of fibro-hyaline materials in small perforating arteries, concentrical hypertrophy of the vessel wall and reduction of the inner arteriolar diameter occur, as a result of the loss of vascular smooth cells [84, 85]. Moreover, the narrowing of lumens plays a significant role in elevated cerebrovascular resistance, reduced autoregulatory capacity, and increased BBB leakage. In the development of hypertensive vasculopathy, the function of the microvascular endothelium decreases at the early stage due to the structural remodeling of cerebral small vessels via increased angiotensin II (Ang II). Moreover, the alteration of the basement membrane, the disruption of BBB, and the loss of autoregulation also contribute to the progress of hypertensive vasculopathy, with the entire microvessel wall damage followed [86, 87].

Dysfunction of BBB components, including endothelial cells, pericytes, and astrocytes, is well described in multiple hypertension models. Hypertension impairs the survival status, cerebral vascular blood regulating function, and barrier function of endothelial cells. First, hypertension exacerbates oxidative stress injury in endothelial cells through inhibition of the enzyme activity of superoxide dismutase and catalase, accompanied by decreased glutathione content and increased malondialdehyde level [88-90]. Transcriptomic
profile analysis of cerebral endothelial cells indicates that hypertension activates pathways related to apoptosis and mitochondrial responses [91]. Second, hypertension impairs the expression of brain-derived neurotrophic factor (BDNF) and endothelial nitric oxide synthase (eNOS) in endothelial cells, which may account for the impaired dilatory capability of endothelial cells [92]. Third, endothelial paracellular and transcellular permeability are significantly impaired in hypertension, which is related to the decreased level of TJ proteins and elevated cerebral EC endocytosis [93, 94]. Pericytes degeneration and detachment are observed during the pathogenesis of hypertension [95]. The coverage rate of pericytes is much higher in the brain of spontaneously hypertensive rats (SHR) [96]. Pericytes overlay and encircle the endothelial cells more tightly and closely under hypertensive conditions [97]. Transcription profile analysis reveals that hypertension causes upregulation of cell division signaling pathways and downregulation of cell adhesion signaling pathways in brain microvascular pericytes [98]. Swelling and pathological detachment of astrocytes endfeet were detected in hypertensive rats [95]. The astrocyte expression of AQP4 is upregulated in hypertension, which may accelerate brain edema [99]. Ang II-induced chronic hypertension mediated enhanced spontaneous Ca²⁺ events and augmented transient potential receptor vanilloid 4 channel expression in endfeet during parenchymal arteriole myogenic responses [100]. Hypertension also induces astrocyte activation and neuroinflammation in an Ang II-dependent manner [101].

Hypertension is regarded as a significant risk factor for arteriolosclerosis present in the brain and other organs, such as the kidney and retina [43]. Hypertension is the second largest risk factor of microbleeds after age. A study involving a hypertensive population without cerebrovascular disease history showed that the prevalence of CMBs was 16.1%, which was more than triples than that reported in the general population [102]. Furthermore, the presence of cerebral microbleeds plays a significant role in subsequent macrobleed and hemorrhage recurrence [2, 103]. It is reasonable to presume the similarity in the effects of hypertension on cerebral hemorrhage and microbleeds.

### 2.2 The animal models of CMBs

Due to the clinical importance of CMBs, it is urgent to find a stable and exact model to study the underlying mechanisms and related therapeutic interventions of CMBs in depth. There are several relevant experimental animal models designed in rodents from different procedures to mimic the performance of CMBs (Table 1). First, Fisher et al. observed the progressive accumulation of microbleeds with aging in Tg 2576 transgenic mice, which are characterized by amyloid deposits in their leptomeningeal and cortical artery arterioles. In that study, 24-month old animals were more than twice as likely to develop CMBs of larger size as compared to younger adult mice [105]. Since then, more aged CAA-related animal models have been applied to study the temporal and spatial development of CMBs, including APPswe/PSEN1dE9 (APP/PS1) mice and APP23-transgenic mice [41, 105, 106]. Additionally, Rosidi et al. used femtosecond laser pulses to trigger cortical microhemorrhages that occurred because of the rupture of targeted small arterioles or capillaries. The focused laser pulses specifically targeted a single cortical penetrating arteriole to produce 100 µm diameter hematoma with specific spatial and temporal distribution and minimal harm to the surrounding tissues. Combined with applying two-photon excited fluorescence microscopy, researchers can track the physiological changes after microhemorrhages, such as bleeding dynamics, tissue compression, blood flow changes, and the dynamics of multiple cells in the brain [109]. In addition to CAA-related animal models, hypertension-induced cerebral microhemorrhages are also present in aged mice. The hypertensive mice model induced by Ang II and L-NAME (inhibitor of nitric oxide synthase) was initially used by the Heistad laboratory to mimic spontaneous intracerebral hemorrhage [110]. Toth et al. confirmed that all hypertension-induced spontaneous intracerebral hemorrhage mice developed multiple histologically detectable CMBs, and the number of CMBs tended to increase with aging [111]. Furthermore, Tarantini et al. observed similar phenomena in hypertensive mice with specific knockdown of insulin-like growth factor 1 (IGF-1), an important anabolic hormone that decreases with aging. IGF-1 deficiency can mimic the aging phenotype and increase the incidence of CMBs [112]. Regardless, the CAA- and hypertension-induced CMBs models mimic two types of CMBs pathological characteristics and take approximately 15 to 24 months are required for CMBs development. Therefore, researchers tried to develop new CMBs models that are easier to establish and require less time.

Hoffmann et al. established hypoxia-reoxygenation-induced microhemorrhage models in the process of studying the pathophysiology of high-altitude hypoxic brain injury. All adult mice were exposed to normobaric hypoxia at 8% oxygen for 48 hours and then were maintained for a further 24h at room air by rapid reoxygenation. Both image and histological analyses found the presence of CMBs after hypoxic exposure, and their number and size significantly increased after 24 hours of reoxygenation, especially in the olfactory bulb.
Sumbria et al. reported an inflammation-induced mouse model of CMBs. The mice were treated with intraperitoneal lipopolysaccharide (LPS) to mimic acute and sub-acute CMBs development by adjusting the dose regimen. LPS-induced CMBs are associated with endothelial activation and BBB damage. Compared to other existing models for CMBs, the LPS-induced mouse model has its unique advantages, including simplicity, feasibility, non-invasiveness, high success rate, and low mortality [112]. Recently, Bergeron et al. developed a reproducible murine model of collagenase-induced cortical CMBs by stereotaxic cortical injection of 0.8 µU collagenases. This new CMBs model is sensitive to pharmacological modulation and presents with cognitive impairments and hypometabolism six weeks after surgery. Therefore, this model might contribute to the progress of CMB treatment strategies, especially in the fields of vascular cognitive impairment [113].

Nevertheless, none of the existing animal models mentioned above can mimic spontaneous generation of CMBs without intervention and also cover all types of clinical CMBs. Additionally, some of them might be confounded by intracerebral macrohemorrhage. Currently, there is a lack of appropriate animal models to study how CMBs contribute to cognitive disorder and cerebrovascular disease, especially ICH. Therefore, more suitable models for CMBs have yet to be developed.

Table 1. The animal models of CMBs.

| Animal models                        | Methods                                | Pathological Changes          | Advantages                                                                                           | Disadvantages                                                                                     | Refs |
|--------------------------------------|----------------------------------------|------------------------------|------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------|------|
| Aged CAA-related mouse model         | Tg 2576 transgenic mice                 | Amyloid deposits and CMBs    | Mimicking cerebral amyloid angiopathy in CMBs, allowing preclinical safety evaluation of antithrombotic therapies | Taking about 15 to 24 months for CMBs development, instability in microhemorrhage size and number | [41,104-106] |
| Laser-induced CMBs model             | Laser pulses targeted a single cortical penetrating arteriole to produce 100 µm diameter hematoma | CMBs                          | Tracking the physiological changes after microhemorrhages, having specific spatial and temporal distribution, and taking less time | Skillful technique is required, invasive, hardly forming deep CMBs                                | [107] |
| Hypertension-induced cerebral microhemorrhage model | Mice were treated with Ang II and L-NAME IGF-1 deficiency mice with angiotensin II plus L-NAME treatment | Hypertension and CMBs         | Mimicking hypertensive vasculopathy CMBs                                                              | Taking about 15 to 24 months for CMBs development, instability in microhemorrhage size and number | [108,110] |
| Hypoxia-reoxygenation-induced CMBs models | Mice were exposed to normobaric hypoxia at 8% oxygen for 48h and then kept for a further 24h at room air | CMBs                          | Mimicking the high-altitude hypoxic brain injury, easy to establish and taking less time                | Failing to mimic the major types of CMBs’ pathogenesis                                             | [111] |
| Inflammation-induced mouse model     | Intraperitoneal injection of LPS        | CMBs and BBB leakage         | simplicity, feasibility, non-invasiveness, high success rate, and low mortality                       | Unable to rule out the effects of peripheral inflammation on the behavior of experimental animals | [112] |
| Collagenase-induced cortical CMBs murine model | Stereotaxic cortical injection of 0.8 µU collagenases | CMBs, cognitive impairments and hypometabolism | Mimicking the high-altitude hypoxic brain injury, easy to establish and taking less time | Invasive, small drug concentration range, and likely to be confounded with a larger extension of hemorrhagic lesion | [113] |

CMBs, Cerebral microbleeds; CAA, Cerebral amyloid angiopathy; Ang II, Angiotensin II; L-NAME, No-nitro-l-arginine methyl ester hydrochloride; IGF-1, Insulin-like growth factor 1; LPS, Lipopolysaccharide; BBB, Blood-brain barrier.
3. The prevalence and factors influencing the clinical presentation of cerebral microbleeds

Cerebral microbleeds is a cerebrovascular disease without symptoms of acute focal neurological dysfunction, and it is linked to stroke and other neurological disorders, including dementia, Parkinson’s disease, gait disturbances, and late-life depression [9, 116-118]. Meanwhile, the presence of CMBs significantly influences the progression, treatment, and prognosis of the diseases mentioned above. Accordingly, it is vital to identify CMBs risk factors at the early phase and give reasonable intervention to reduce CMBs-related hazards. There is broad recognition in numerous cohort studies that aging, hypertension, inflammation, and APOE ε4 genotype correlate with increased risk for CMBs [15, 16, 119]. In this section, we review BBB alterations and refer to common risk factors for CMBs (Fig. 2).

![Figure 2. Schematic summarizing effects of aging, hypertension, APOE ε4, and inflammation for the clinical presentation of cerebral microbleeds in respect of BBB integrity.](image)

### 3.1 Aging

A number of studies showed that CMBs are commonly characterized as age-related cerebral microangiopathy. The prevalence of microbleeds in participants aged over 45 is 18.7%. CMBs are also found in the middle-aged population, with an increasing tendency during aging [5, 16]. The prevalence of CMBs increases with aging, from 6.5% in subjects aged 45-50 years to ~36.7% in people 80 years of age and older [16, 118]. The two types of CMBs pathogenesis, amyloid angiopathy and hypertensive vasculopathy, result in a progressive accumulative effect over time. Therefore, it is necessary to understand how aging contributes to cerebral microbleeds, especially vascular pathology. An increasing number of studies suggest that alteration of BBB permeability occurs with aging under normal and pathological conditions [119-122]. Montagne et al. found that early BBB dysfunction occurred in the hippocampus rather than other regions of the brain during normal aging via advanced dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and post-processing analysis. It might be regarded as a cue to explore the close association between CMBs and dementia [123]. The age-related BBB leakage might contribute to the occurrence and development of CMBs. The increased prevalence of CMBs associated with aging may reflect age-related neurodegeneration and decline in cerebrovascular health. The pathophysiological roles of molecular and cellular vascular disease mechanisms, including genomic instability, inflammation,
mitochondrial dysfunction, oxidative stress, and autophagy, often change with advancing age [124, 125]. Besides, cerebral microvessels are increasingly sensitive to mechanical stress with aging. However, to date, the mechanisms of aging and their underlying relationship with CMBs remain obscure and require further studies due to the deficiency of animal models suitable for this research. It might be feasible to understand how aging affects CMBs by interpreting the BBB integrity alteration under the influence of aging.

Multiple studies conducted with mice and humans demonstrated the increased BBB permeability by noting the occurrence of increased extrusion of plasma proteins (albumin, fibrinogen, and immunoglobulin G) and increased cerebrospinal fluid/plasma albumin ratios in vivo. There is also evidence proving that BBB alterations occur with aging, including a decreased number of endothelial cells, loss of TJ proteins, elevated activity of GFAP+ astrocytes, loss of pericytes, basement membrane thickening, and reduced microvascular density and cerebral blood flow (CBF) [120, 126-128]. It is widely recognized that senescent cells accumulate in the brain with aging [129]. A study in vitro and in vivo senescent BBB models showed the association between senescent vascular cells and the dysfunction of BBB integrity. For example, aging ECs and pericytes are associated with alteration of TJ structure and dysfunction and BBB leakage in vitro, and the reduced TJ proteins coverage in vivo [130]. Recently, studies via single-cell RNA sequencing suggested that ECs in cerebral capillaries were sensitive to age-related circulatory cues at the transcriptome level and upregulate innate immunity and oxidative stress pathways in response. For example, the occurrence of increased expression of vascular cell adhesion molecule 1 (VCAM1) was counteracted by young plasma administration to reverse these changes [120, 131, 132]. In addition, senescent astrocytes induced by oxidative stress are also present with the downregulation of pro-inflammatory genes and upregulation of genes involved in neuronal generation and differentiation, and genes related to astrocytic responses, such as GFAP and the MHC class II gene [133, 134]. Changes in the astrocyte transcriptome may lead to failure in terms of response to injury under the impact of aging.

Moreover, aging-induced microglia can upregulate their expression of inflammatory cytokines (IL-1α, TNF, and C1q). Aging microglia appeared to further induce M1-like reactive astrocytes, which lose their normal function and release neurotoxic factors to kill neurons and oligodendrocytes [135, 136]. In addition, the reduction in CBF and cerebral microcirculation might contribute to age-related loss of pericyte coverage. The pericyte loss has a significant role in pathological accumulations of neurotoxic macromolecules, such as hemosiderin, thrombin, and plasmin [137].

Apart from the direct impact of aging on BBB components, aging makes the brain more susceptible to inflammation-induced CMBs, related to microglial/macrophage activation [138]. Meanwhile, aging promotes the decline of circulating IGF-1 levels, which affects multiple aspects of brain health [139]. In IGF-1-deficient mice, Tarantini et al. observed a higher count of CMBs induced by hypertension. IGF-1 deficiency plays a significant role in promoting hypertension-induced MMP activation, impairing hypertrophy and structural remodeling [110].

### 3.2 Hypertension

Numerous population-based cohort studies indicate that hypertension, especially severe hypertension, is an independent risk factor for CMBs [5, 13, 15, 16]. More importantly, systolic blood pressure is regarded as a strong predictor of CMBs development. The new presence of CMBs occurs in those with much higher mean systolic blood pressure (163±20 mmHg) compared to those without (141±16 mmHg) [140]. Léon conducted a cohort study among hypertensive patients without a history of cerebrovascular disease through 24-hour ambulatory blood pressure monitoring. The results suggested that the prevalence of CMBs in hypertensive populations was approximately three times higher than that in the healthy population. The occurrence possibility of CMBs in patients diagnosed with nocturnal hypertension was approximately 5- to 6-fold higher [102]. Moreover, deep or infratentorial microbleeds are suggested to function as an independent indicator of hypertensive or arteriosclerotic microangiopathy [15].

In the pathogenesis of CMBs, hypertension-mediated vascular reactive oxygen species (ROS) production and redox-sensitive activation of MMPs play an essential part, which is related to the degradation of basement membrane components and extracellular matrix and further leads to BBB dysfunction [109]. Therefore, it is reasonable to believe that BBB dysfunction is linked to hypertension and CMBs development. It is valuable to understand how hypertension contributes to BBB breakdown. The pathogenesis of hypertension is closely related to the alteration of the renin-angiotensin-aldosterone system (RAAS), especially Ang II [141]. Nyúl-Tóth, Adám, et al. found that there was a higher total number of CMBs in Ang II -induced hypertensive Tg2576 mice compared to normotensive Tg2576 mice [142]. As the major effector molecule of the RAAS and a potent vasoactive peptide, Ang II mediates vascular remodeling and exerts pro-inflammatory effects, further leading to disruption of the BBB integrity [143, 144]. The impairment of Ang II in
BBB endothelial cells is closely involved in altering transcellular and paracellular permeability, which is associated with the activation of protein kinase C (PKC) [145]. Besides, the effects of Ang II also refer to cerebrovascular inflammation, which is partly related to oxidative stress within the SFO–PVN pathway. Chronic infusion of Ang II leads to a higher number of rolling and adherent leukocytes in mouse cerebral microvessels, and increased BBB permeability [146]. Mounting evidence suggests that the pro-inflammatory effects of central Ang II are likely to be exerted through the activation of microglia, which serves as a complementary mechanism of AngII-mediated BBB dysfunction. The activated microglia also function as a source of ROS, which might contribute to hypertension-mediated BBB breakdown [147].

CBF is essential for delivering oxygen and nutrients to cross BBB to keep normal cerebral function. It is regulated by the cerebral autoregulatory mechanism to sustain a relatively stable level by counteracting blood pressure fluctuations [148]. Nevertheless, the major impacts of constant elevated blood pressure on cerebral arteries and arterioles are hypertrophy and inward remodeling, leading to smaller external diameter, greater vascular resistance, and increased arterial stiffness [149]. Therefore, hypertension is involved in the extended range of cerebrovascular autoregulation in terms of reduced resting CBF. Autoregulatory dysfunction is associated with periventricular white matter injury [150]. Additionally, increased arterial stiffness contributes to an elevated level of pulse pressure (PP). Cerebral microvessels are more vulnerable to high pulse pressure-related mechanical stress due to their fragile structure. A clinical study for stroke confirmed that arterial stiffness was independently associated with CMBS [151]. High PP can lead to BBB dysfunction, the presence of microhemorrhages, and a reduction in microvessel density [152]. Furthermore, hypertension decreases the production of nitric oxide (NO) and increases shear stress, which are related to endothelial dysfunction and atheroma formation [153]. In terms of anatomy, the deep regions with CMBS are generally supplied by small perforating arteries, which are vulnerable to luminal narrowing, twisting, and looping, and might be aggravated by hypertension. Therefore, the distinctive hypertension-induced alterations in small arteries and arterioles increase cerebral small vascular disease susceptibility, especially CMBS.

### 3.3 APOE ε4

Several population-based studies have reported the association between the E4 variant of APOE (APOE ε4) carrier and the presence of cerebral microbleeds, especially in lobar distribution [13, 16, 154, 155]. Recently, a genome-wide association study (GWAS) of CMBS confirmed that APOE ε4 was an independent genetic risk factor for CMBS with OR value of 2.54, regardless of location [156]. Besides, the presence of APOE ε4 genotype plays a role in increasing brain amyloid load, indicating a higher risk of lobar CMBS [29]. Interestingly, several studies revealed the effect of APOE ε4 genotype on accelerating pericyte degeneration and BBB leakage in Alzheimer’s disease [157-159]. Similarly, Montagne provided the evidence by DCE-MRI that normal cognitive individuals carrying APOE4 showed increased BBB leakage in the hippocampus and medial temporal lobe compared to those without APOE4 (APOE3 homozygotes) [160]. Therefore, it is noteworthy whether the effect of APOE4 on BBB breakdown is involved in the occurrence and development of CMBS.

In the brain, APOE is mainly expressed by astrocytes and has a role in regulating lipid transport and cholesterol homeostasis as a ligand for lipoprotein receptors [161, 162]. Multiple studies suggest that APOE4 directly impacts BBB disruption and cerebral blood flow reduction compared to APOE2 and APOE3, which are other isoforms of human APOE [163, 164]. In APOE4 transgenic mice, the increased BBB permeability is detectable, including leakage of multiple blood-derived neurotoxic proteins, diminished pericyte coverage and microvascular length, enzymatic degradation of basement membrane proteins and TJs mediated by MMP-9, and reduction of regional CBF [164, 165]. APOE4 proteins participate in the process of BBB breakdown primarily through increasing cyclophilin A (CypA) expression in brain capillary pericytes and regulating the pro-inflammatory CypA-NF-κB-MMP9 pathway. MMP-9 is related to the degradation of the basement membranes and TJs, subsequently causing the BBB leakage [165, 166]. Moreover, APOE4 proteins contribute to cerebral Aβ accumulation in the cerebral parenchyma and microvessels through preventing Aβ clearance in the form of APOE-bound Aβ, which was confirmed as an important part of CAA [167-170]. There appears to be greater affinity between APOE4 and VLDL receptor (VLDLR) than LDL receptor-related protein 1 (LRP1), which mainly mediates Aβ clearance across the BBB. There is a much lower rate of clearance of Aβ-APOE4 complexes mediated by VLDLR than LRP1-mediated Aβ binding, endocytosis, and transcytosis, which leads to Aβ deposition in cerebral microvessels [171-173]. In vitro and vivo studies have shown that neuroinflammation and ROS are involved in TJ disruption and microglia recruitment in capillary CAA [174, 175]. Furthermore, the accumulation of Aβ in CAA contributes to the increased BBB permeability by inducing MMP-9 activity and reducing the expression of TJs [173, 176, 177].
Inflammation exerts the role as an essential part of CMBs pathogenesis and one of CMBs’ most significant risk factors. A pilot cross-sectional study showed an association between the presence of CMBs and infection with multiple pathogens, such as herpes simplex virus (HSV)-1 and HSV-2 [178]. Histopathological evidence demonstrated that hemosiderin deposits at the lesion site of CMBs are often surrounded by macrophages, a type of immune cells that further initiate inflammatory responses. The presence of CMBs is closely associated with an elevated level of circulating inflammatory biomarkers, such as high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6, IL-18, tumor necrosis factor receptor 2 (TNFR2), and myeloperoxidase [179-181]. Both vascular inflammation and systemic inflammation are reported to contribute to the occurrence and development of CMBs. Vascular inflammation seems to be related to hypertensive arteriopathy and BBB disruption, while systemic inflammation is likely involved in CAA-related microvascular pathological variation in cortical regions [181]. The inflammation-induced CMBs animal model in which the animals were treated with LPS confirmed the importance of systemic inflammation in the pathogenesis of CMBs, such as brain endothelium activation, BBB disruption, and neuroinflammation [112]. In addition to its role as one of the risk factors for developing CMBs, inflammation might also play an essential role in mediating further impairment of neuronal function after a microhemorrhage. Ahn et al. adopted in vivo 2-photon excited fluorescence microscopy to follow the inflammatory response in real-time after laser-induced cortical CMBs occurred. The study indicated that CMBs were involved in the inflammatory response, which lasted for more than a week, including activation of microglia and astrocytes, and the invasion of blood-borne CX3CR1+ and CCR2+ macrophages [182].

Despite the fact that the exact molecular mechanisms of inflammation with respect to CMBs have not yet been well clarified, loss of BBB integrity associated with inflammatory responses might potentially participate in CMBs development. Inflammation may thus lead to BBB dysfunction that gives rise to the development and progression of CMBs. The contribution of inflammatory mediators to BBB breakdown can be described by the following three aspects. First, circulating inflammation leads to microglia recruitment to cerebral vessels and elevated expression levels of paracellular TJ, which are initially conducive to BBB integrity. When persistent inflammation occurs, the microglia increase the expression of the phagocytic marker CD68 and phagocytosis of astrocyte end-feet [183]. Second, inflammation has a role in modulating TJ expression, increasing MMP-mediated enzymatic degradation of TJs, and forming vesicular transendothelial channels, which leads to reduced material transport via paracellular pathways and transcytotic vesicular pathways [184, 185]. Third, loss of endothelial integrity is commonly present in cerebral inflammation, and includes apoptotic cell death, impairment of transporter activity, and damaged organelles [185, 186]. In addition, endothelial cells are sensitive to inflammation. They increase the expression of chemokines and cell adhesion molecules to facilitate the recruitment and migration of circulating immune cells to the brain [187]. All of the mechanisms mentioned above are related to the BBB breakdown due to the interference of inflammation mediators.

4. The vulnerability of the BBB to cerebral microbleeds

An increasing number of clinical studies have demonstrated the association between the presence of CMBs and the BBB breakdown through various biomarkers existing in CSF and serum, including increased serum VEGF and fibrin levels, reduced MMP-9 levels in CSF, elevated levels of contrast agent leakage, and altered CSF/serum albumin ratio [48, 188]. Meanwhile, numerous animal studies have verified the correlation between CMBs development and BBB dysfunction via contrast agent leakage, plasma protein extravasation, gelatinase expression alteration, and IgG deposition [189]. Due to the barrier effect of the BBB, the increased permeability of the BBB plays an essential role in reducing cerebral blood flow and impairing hemodynamic responses. Particularly, neuroinflammation significantly contributes to the breakdown of the BBB, which is recognized as an essential activation event during the early phase of neurological system diseases, such as stroke, Alzheimer’s disease, and multiple sclerosis [185, 190].

Under the impact of multiple risk factors, the structure and function of the BBB are damaged due to the alteration of endothelial cells, pericytes, astrocytes, and the basement membrane, which causes small vessels to rupture in the context of CAA or hypertension. The increased BBB permeability leads to erythrocyte exudation from small cerebral vessels, which is the primary characteristic of CMBs. Additionally, dysfunction of the BBB enables neurotoxic blood-derived components, blood cells, and pathogens to enter the brain tissues, which causes deterioration of the cerebral environment, further brain tissue injury, and aggravation of CMBs development. However, the cellular and molecular mechanisms underlying BBB dysfunction associated with CMBs development remain to be identified. Here, we provide an overview of existing...
studies on pathological structural alterations of BBB components and their roles in the occurrence and development of CMBs (Fig. 3).

Figure 3. Schematic representation of blood-brain barrier (BBB) alteration in cerebral microbleeds (CMBs). Cell-cell interactions in the neurovascular unit indicate the breakdown of BBB and promote CMBs development. VEGF, CRP, sICAM, MPO, IL-6, and E-selectin contribute to endothelial dysfunction. Serum Response Factor (SRF) and its MRTF cofactors play a vital role in cerebral microvascular integrity through regulating EC junction components and basement membrane proteins. Matrix metalloproteinases (MMPs) derived from microglia and astrocytes are associated with the degradation of TJ and ECM, exacerbating the injury of the vascular wall. S100B derived from astrocytes is able to promote the release of oxidative stress mediators and pro-inflammatory cytokines, resulting in further BBB breakdown and the development of CMBs. At the same time, the cytokines derived from microglia and astrocytes play a role in endothelial dysfunction, such as glia-derived neurotrophic factor (GDNF), fibroblast growth factor (FGF), angiopoietin 1 (Ang1), and IFN-γ. Recent studies also suggest a role of pericytes in the development of CMBs through the bone morphogenetic protein 4 (BMP4) pathway, which is related to astrogliogenesis and inhibits oligodendrocyte differentiation.

4.1 Endothelial dysfunction

Compared with endothelial cells in the peripheral tissue, the continuous endothelial monolayer within the BBB lacks fenestrations and has the capability to strictly regulate the efflux and influx of ions, toxins, blood cells, nutrition, and pathogens by its unique permeability properties. The adjacent ECs are linked by the junction complex predominately comprising TJs and adherens junctions at the ultrastructural level, which contribute to limiting the diffusion of most hydrophilic molecules from plasma to the CNS through the paracellular pathway and the subsequent creation of high transendothelial electrical resistance (1500–2000 omega•cm²) of the BBB [20, 191-193]. In addition, the maintenance of normal BBB physiological function is inseparable from the unique transport systems, including influx and efflux transporters, limited transcytosis rate, and low level of leukocyte adhesion molecules [17, 190].

Endothelial cells are essential for maintaining vascular homeostasis due to their capability of perceiving alterations in the hemodynamic forces and blood-derived factors. They can give a timely response through releasing substances involved in different pathways of endothelial functions, including regulating vascular tension, participating in inflammatory responses, regulating fibrinolysis and coagulation pathways, and playing a role in vessel formation, repair, and remodeling [194-196]. Considering the extensive effects of ECs, it is no wonder that endothelial dysfunction takes center stage in the pathogenesis of cerebrovascular diseases, CMBs included. Weinl found that Srj<sup>ECKO</sup> mice, in which serum
response factor (SRF) is depleted, are prone to develop macro- and microhemorrhages. SRF and its MRTF cofactors play a vital role in cerebral microvascular integrity by regulating EC junction components, such as claudins, ZO adapter proteins, actin, and basement membrane proteins [197]. Alomar et al. observed increased levels of BSA-FITC leakage from arterioles with diameters of 20-50 μm in type 1 diabetic rats, which resembled CMBs. In contrast, BSA-FITC transcytosis was blunted by reduced methylglyoxal under the regulation of methylglyoxal-degrading enzyme glyoxalase-1 (Glo-I) in smooth muscle cells in cerebral arterioles [198]. However, there are few directly relevant studies on transcellular leakage during the development of CMBs, which requires further exploration.

Clinical studies revealed exclusive associations between CMBs and endothelial dysfunction markers, E-selectin, and vascular endothelial growth factor (VEGF), rather than other cerebral small vessel disease (cSVD) markers [179, 181, 199]. E-selectin is a glycoprotein adhesion molecule that is specifically expressed in activated ECs. It promotes the migration of leukocytes into the arterial wall and mediates inflammatory cascades [200]. There is a significant correlation between serum VEGF level and the number of CMBs in patients with Alzheimer’s disease [201], while similar results were also found in patients with acute ischemic stroke [202]. As an essential effector in regulating microvascular density and permeability, VEGF can impair the BBB integrity and further disrupt CNS homeostasis [203]. VEGF-A inhibits the expression of claudin-5 and occludin in brain microvessel endothelial cell cultures in vitro and in the CNS in vivo, which causes increased paracellular permeability [204]. The levels of serum soluble intercellular adhesion molecule 1 (sICAM-1) significantly correlate with the presence of CMBs and the hemorrhagic transformation risk, and sICAM-1 plays a role in endothelial dysfunction and inflammatory responses [205]. Higher levels of circulating inflammatory biomarkers, such as TNFR2, myeloperoxidase, CRP, and IL-6, have been previously reported in patients with CMBs [179, 206]. Because the multiple endothelial circulating biomarkers cannot exactly reflect the alteration of the brain endothelium, it is essential to find specific biomarkers corresponding to cerebral endothelial dysfunction. Moreover, the issue of causality between endothelial dysfunction and the presence of CMBs remains to be further explored.

Apart from inflammation responses and TJ breakdown, endothelial erythrophagocytosis might also contribute to the occurrence of CMBs. Multiple studies have shown endothelial cells are involved in the phagocytosis of aged or apoptotic erythrocytes upon phosphatidylserine exposure [207]. Chang et al. studied the relationship between the phagocytosis of red cells and CMBs based on a cerebral microbleeds model in vitro. There was more significant cerebral endothelial phagocytosis of erythrocytes exposed to oxidative stress compared to the control group. The promoted endothelial erythrophagocytosis was mediated by the passage of hemoglobin across brain endothelial cells without any alteration in monolayer integrity [208]. Additionally, a portion of endothelial cells showed signs of apoptosis after the phagocytosis of red cells, which might affect intracellular processes or the release of oxidized free heme [209]. However, the detection of CMBs mainly depends on hemosiderin’s paramagnetic properties, and thus, the presence of CMBs can be recognized by any passage of hemosiderin or erythrocytes through endothelial cells. Therefore, it is worth considering the potent mechanisms of CMBs without microvessel rupture.

4.2 Cross-talk among the BBB cellular components in CMBs

Pericytes are located adjacent to tight junctions and gaps between endothelial cells, and they function as a ‘gatekeeper’ in the BBB. Pericytes possess contractile properties that directly regulate the CBF through the constriction of capillaries, which is initiated by pericytes under the stimulation of ATP and noradrenaline [210]. Pericytes can regulate gene expression in endothelial cells and contribute to BBB permeability, for example, by upregulation of TJ proteins and upregulation of Mfsd2a to suppress endothelial transcytosis [17]. An increasing number of studies have recently identified the multipotential stemness of pericytes, and especially their capacity to differentiate into neural and vascular lineage cells under ischemia/hypoxia. Furthermore, pericytes are considered as an underlying resource that can be used for restoration of the BBB after brain damage [211]. Pathological analysis of post-mortem brain specimens demonstrated pericyte involvement in 2 out 22 cases with CMBs that were immediately adjacent to endothelial TJs [212]. The activation of the CypA–MMP-9 pathway in pericytes promotes the degradation of TJ proteins and basement membrane proteins. Recently, in a spontaneous cSVD animal model with partial eNOS deficiency, there was increased BBB breakdown in aged mice, and pericyte-derived bone morphogenetic protein 4 (BMP4) in eNOS-deficient mice was elevated. BMP4 accelerates astrogliogenesis and inhibits oligodendrocyte differentiation, which further leads to microbleeds, white matter pathology, and neurodegeneration [213].

Astrocytes play a vital role in narrowing the gap between capillaries and neurons due to their contribution to synapse formation, BBB formation and maintenance,
and CNS homeostasis [214]. Astrocytes contribute to BBB permeability mainly through releasing a series of cytokines, such as glia-derived neurotrophic factor (GDNF), fibroblast growth factor (FGF), and growth factors such as Ang-1, that affect TJ expression and EC activity [215-217]. Microglia act as continuous immune surveillant cells in the brain. They can monitor the CNS environment for pathogens and be activated by stimuli to protect the brain. Activated microglia have the ability to engulf microorganisms, transform to an M1/M2 phenotype, and release a series of inflammatory mediators [218]. Furthermore, activated microglia can trigger reactive astrocytes and amplify neuroinflammation, while astrocytes are able to sensitively identify minor changes in neurons and vessels, and deliver signals to microglia [219]. In the laser-induced microhemorrhage model, microglia migrated to the lesion during the early stages, while the activation of astrocytes was delayed for several days [182]. In the LPS-induced animal model, the CMBs burden was significantly associated with total Iba1- and GFAP-positive immunoreactive areas and ICAM-1-positive areas, which was related to the activation of microglia, astrocytes, and endothelial cells [112]. Furthermore, in diabetic mice, there were reduced microglial polarization and accumulation near the microvascular injury, lesions, concomitant with increased BBB leakage. The inhibition of IFN-γ promoted microglial function impairment and reduced BBB dysfunction [220].

Apart from animal studies, a clinical study confirmed the role of astrocytes in the presence and number of deep CMBs, with reduced soluble receptors for advanced glycation end products (sRAGE) levels and increased S100B levels [221]. S100B is a member of S100 protein family of Ca²⁺-binding proteins, mainly expressed in astrocytes [222]. S100B plays a neurotrophic role in facilitating neuronal proliferation, oligodendrocyte differentiation, and astrocyte and microglia migration, with concentrations in the nanomolar range. In contrast, micromolar concentrations of S100B can induce pro-inflammatory effects through the activation of RAGE. The high concentrations of S100B derived from astrocytes lead to astrocyte and microglial activation and neuronal death. S100B can also promote the release of oxidative stress mediators and pro-inflammatory cytokines, resulting in further BBB breakdown and the development of CMBs [223]. More importantly, multiple studies reported the correlation between CMBs development and MMPs, especially MMP-2 and MMP-9 [61, 224, 225]. MMPs are a family of zinc-dependent endopeptidases that can be partially secreted by astrocytes and microglia [224]. MMPs activation is commonly observed in CMBs animal models under the inducement of oxidative stress. Activated MMPs can damage the basal lamina and degrade TJs and the extracellular matrix (ECM), exacerbating vascular wall injury.

5. Potential pharmacological approaches to target the BBB as therapy for CMBs

An increasing number of studies have noted the significance of CMBs in the prevention and treatment of stroke, which includes antihypertensive treatment, antiplatelet therapy, thrombolysis, anticoagulant therapy, and statin therapy. The presence of CMBs is closely related to subsequent ICH and recurrent ischemic stroke in patients with recent ischemic stroke or TIA under antithrombotic treatment [6]. Additionally, CMBs might participate in the progression of dementia, which seems to be delayed with early intervention to CMBs. Therefore, it is essential to devise potential pharmacological interventions to inhibit or reduce CMBs development. In particular, BBB is a promising therapeutic target for CMBs.

A growing number of studies have indicated that vascular ROS production and redox-sensitive activation of MMPs are essential elements of CMBs development, which is interrelated with damage to the basal lamina and degradation of TJs and ECM. Thus, antioxidants and MMPs inhibitors are a likely option for the treatment of CMBs. The two different anti-ROS interventions, apocynin and tempol, played a significant role in improving cerebrovascular function via attenuating CAA formation and CAA-induced vasomotor dysfunction in aged Tg2576 mice. More importantly, the NADPH oxidase inhibitor, apocynin, reduced CAA-related CMBs [68]. Furthermore, resveratrol treatment has protective effects in the development of hypertension-induced CMBs in aged mice. Resveratrol reduces vascular ROS production via the downregulation of the NADPH oxidase subunits and disruption of MMPs activation [109]. A study in aged Tg2576 mice indicated that chronic minocycline treatment inhibited MMP-2 and -9 activity, and attenuated gliosis, gelatinase activity, and inflammation, which further resulted in reduced hemorrhage frequency [226].

A randomized controlled trial suggested that cilostazol, a type III phosphodiesterase (PDE3) inhibitor, significantly reduced the incidence of cerebral hemorrhage compared with aspirin in patients with multiple CMBs [227]. The post hoc analysis of this trial showed that there was lower ICH risk with the use of cilostazol, and it tended to reduce the composite of major vascular events compared to aspirin in the CMBs subgroup [228]. Studies in vivo demonstrated that cilostazol appeared to provide protective effects to BBB properties. Cilostazol decreased paracellular and
transcellular permeability, promoted the expression of claudin-5, and regulated actin cytoskeleton rearrangement [229]. However, Sumbria et al. found that pharmacological inhibition by cilostazol failed to modulate CMBs development in both LPS-induced models and CAA-related models. The failure appeared to be consistent with no reduction in endothelial, astrocyte, or microglial activation, or BBB injury [230].

Histologically, the vascular rupture in CMBs leads to the leakage of erythrocytes from small cerebral vessels, and the formation of hemosiderin deposits near the lesions [4]. The iron derived from heme degradation is closely related to the secondary brain damage mediated by excessive production of free radicals [40]. Iron dyshomeostasis induces BBB dysfunction and microglial activation, and further leads to dendritic degeneration. Deferoxamine is approved for the treatment of chronic iron overload and acute iron intoxication. CMBs induced by two-photon lasing were ameliorated by deferoxamine treatment, which was associated with a reduction in iron deposits and reactive microglia [27]. Therefore, deferoxamine has significant potential to be used as a treatment for CMBs.

The pharmacological approaches targeting the BBB mentioned above provide potential novel interventional strategies for CMBs. However, most of these interventions are still at the preclinical phase, and their treatment effects might vary unpredictably when further applied to large numbers of patients. Therefore, further investigation is needed to translate these laboratory findings to the clinic. CMBs are related to multiple alterations of the BBB components in various pathways, and single-target drugs appear to have limited impact. Thus, the application of the combination of vasoprotective drugs and other interventions may be more effective. Moreover, the relationship between CMBs and the side effects of common antithrombotic therapies remains controversial. Further preclinical studies are required to improve our understanding of the diagnostic and therapeutic significance of CMBs when they occur under multiple pathological conditions.

Conclusion

Although there have been numerous studies on CMBs, the existing research has concentrated on the clinical significance of CMBs. The exact mechanisms of vascular pathology and BBB alterations are still far from clear. There also exist a number of controversies on prevention and management, including the use of therapies with antiplatelet, anticoagulant, lipid-lowering, and thrombolytic actions. Furthermore, greater emphasis on understanding CMBs-related clinical consequences, especially stroke and dementia, is necessary, as well as additional interventional strategies to ameliorate or prevent CMBs. Considering their clinical significance, it is necessary to place greater emphasis on studying CMBs and finding potential preventive and treatment strategies. BBB dysfunction is considered to initiate the occurrence of CMBs. However, the main difficulty in investigating CMBs is the deficiency of ideal animal models, and thus, it is difficult to conduct additional exploration to elucidate the advanced mechanisms of BBB that contribute to CMBs. Additional definitive studies are needed to understand how CMBs contribute to the different outcomes of ischemic stroke under antithrombotic therapy.

In conclusion, despite many details that still require study, considerable evidence suggests that BBB dysfunction appears to play a significant role in the development and progression of CMBs. Risk factors for CMBs can exacerbate BBB breakdown through the vulnerability of the BBB to anatomical and functional changes. To reduce the burden of CMBs, it is necessary to increase awareness of BBB alterations and perform additional research to increase our knowledge regarding their relationship with CMBs.

Acknowledgements

This work was supported by National Natural Science Foundation of China (no. 82071336 to YNL), Natural Science Foundation of Hubei Province (no. 2020CF763 to YNL), National Key R&D Program of China (no. 2018YFC1312200), National Natural Science Foundation of China (no.82090044 to BH, no. 8182018010 to BH, no. 81901212 to YFZ, no. 81901214 to YW and no. 82001271 to JHW).

Conflict of interest

The authors declare that they have no competing interests.

References

[1] Haller S, Vernooij MW, Kuijper JPA, Larsson EM, Jager HR, Barkhof F (2018). Cerebral Microbleeds: Imaging and Clinical Significance. Radiology, 287:11-28.
[2] SM G, MW V, C C, A V, R A-SS, S W, et al. (2009). Cerebral microbleeds: a guide to detection and interpretation. Lancet Neurol, 8:165-174.
[3] Fazekas F, Kleinert R, Roob G, Kleinert G, Kapeller P, Schmidt R, et al. (1999). Histopathologic analysis of foci of signal loss on gradient-echo T2*-weighted MR images in patients with spontaneous intracerebral hemorrhage: evidence of microangiopathy-related microbleeds. AJNR Am J Neuroradiol, 20:637-642.
Cerebral microbleeds: histopathological correlation of neuroimaging. Cerebrovasc Dis, 32:528-534.

Akoudad S, Portegies ML, Koudstaal PJ, Hofman A, van der Lugt A, Ikram MA, et al. (2015). Cerebral Microbleeds Are Associated With an Increased Risk of Stroke: The Rotterdam Study. Circulation, 132:509-516.

Wilson D, Ambler G, Lee KJ, Lim JS, Shiozawa M, Koga M, et al. (2019). Cerebral microbleeds and stroke risk after ischaemic stroke or transient ischaemic attack: a pooled analysis of individual patient data from cohort studies. Lancet Neurol, 18:653-665.

Wilson D, Ambler G, Shakeshaft C, Brown MM, Charidimou A, Al-Shahi Salman R, et al. (2018). Cerebral microbleeds and intracranial haemorrhage risk in patients anticoagulated for atrial fibrillation after acute ischaemic stroke or transient ischaemic attack (CROMIS-2): a multicentre observational cohort study. Lancet Neurol, 17:539-547.

Kim BJ, Lee S-H (2013). Cerebral microbleeds: their associated factors, radiologic findings, and clinical implications. J Stroke, 15:153-163.

Akoudad S, Wolters FJ, Viswanathan A, de Bruijn RF, van der Lugt A, Hofman A, et al. (2016). Association of Cerebral Microbleeds With Cognitive Decline and Dementia. JAMA Neurol, 73:934-943.

Charidimou A, Jager HR, Werring DJ (2012). Cerebral microbleed detection and mapping: principles, methodological aspects and rationale in vascular dementia. Exp Gerontol, 47:843-852.

PB G, MU F (2016). Cerebral Microbleeds, Cognition, and Therapeutic Implications. JAMA Neurol, 73:908-910.

MM P, MA I, A vdL, A H, WJ N, GP K, et al. (2012). Cerebral microbleeds are associated with worse cognitive function: the Rotterdam Scan Study. Neurology, 78:326-333.

Vernooij MW, van der Lugt A, Ikram MA, Wielopolski PA, Niessen WJ, Hofman A, et al. (2008). Prevalence and risk factors of cerebral microbleeds: the Rotterdam Scan Study. Neurology, 70:1208-1214.

Thorn LM, Shams S, Gordin D, Liebkind R, Forsblom C, Summanen P, et al. (2019). Clinical and MRI Features of Cerebral Small-Vessel Disease in Type 1 Diabetes. Diabetes Care, 42:327-330.

Poels MM, Ikram MA, van der Lugt A, Hofman A, Krestin GP, Breterler MM, et al. (2011). Incidence of cerebral microbleeds in the general population: the Rotterdam Scan Study. Stroke, 42:656-661.

Poels MM, Vernooij MW, Ikram MA, Hofman A, Krestin GP, van der Lugt A, et al. (2010). Prevalence and risk factors of cerebral microbleeds: an update of the Rotterdam scan study. Stroke, 41:e103-106.

Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV (2015). Establishment and Dysfunction of the Blood-Brain Barrier. Cell, 163:1064-1078.

O’Brien NM, Pfau SJ, Gu C (2018). Bridging barriers: a comparative look at the blood-brain barrier across organisms. Genes Dev, 32:466-478.

Obermeier B, Daneman R, Ransohoff RM (2013). Development, maintenance and disruption of the blood-brain barrier. Nat Med, 19:1584-1596.

Langen UD, Ayloo S, Gu C (2019). Development and Cell Biology of the Blood-Brain Barrier. Annu Rev Cell Dev Biol, 35:591-613.

Zlokovic BV (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron, 57:178-201.

Ballaith P, Braun A, Nedergaard M (2004). The blood–brain barrier: an overview: Structure, regulation, and clinical implications. Neurobiol Dis, 16:1-13.

Sweeney MD, Sagare AP, Zlokovic BV (2018). Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol, 14:133-150.

WM J, ES S, BJ G, C C, F F, R F, et al. (2013). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol, 12:822-838.

HH O, FF R, MS M, KG F, PK P (1996). MR of cerebral abnormalities concomitant with primary intracerebral hematomas. AJNR Am J Neuroradiol, 17:573-578.

Gu Y, Hua Y, Keep Richard F, Morgenstern Lewis B, Xi G (2009). Deferoxamine Reduces Intracerebral Hematoma-Induced Iron Accumulation and Neuronal Death in Piglets. Stroke, 40:2241-2243.

He XF, Lan Y, Zhang Q, Liu DX, Wang Q, Liang FY, et al. (2016). Deferoxamine inhibits microglial activation, attenuates blood-brain barrier disruption, rescues dendritic damage, and improves spatial memory in a mouse model of microhemorrhages. J Neurochem, 138:436-447.

Graff-Radford J, Botha H, Rabinstein AA, Gunter JL, Przybelski SA, Lesnick T, et al. (2019). Cerebral microbleeds: Prevalence and relationship to amyloid burden. Neurology, 92:e253-e262.

Graff-Radford J, Lesnick T, Rabinstein AA, Gunter J, Aakre J, Przybelski SA, et al. (2020). Cerebral microbleed incidence, relationship to amyloid burden: The Mayo Clinic Study of Aging. Neurology, 94:e190-e199.

Jung YH, Jang H, Park SB, Choe YS, Park Y, Kang SH, et al. (2020). Strictly Lobar Microbleeds Reflect Amyloid Angiopathy Regardless of Cerebral and Cerebellar Compartments. Stroke, 51:3600-3607.

Park JH, Seo SW, Kim C, Kim GH, Noh HJ, Kim ST, et al. (2013). Pathogenesis of cerebral microbleeds: In vivo imaging of amyloid and subcortical ischemic small vessel disease in 226 individuals with cognitive impairment. Ann Neurol, 73:584-593.

Graff-Radford J, Simino J, Kantarci K, Mosley TH, Jr., Griswold ME, Windham BG, et al. (2017). Neuroimaging Correlates of Cerebral Microbleeds: The ARIC Study (Atherosclerosis Risk in Communities). Stroke, 48:2964-2972.

Kunieda KA, Rosand J, Karluk D, Greenberg SM (2001). Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria.
Amyloid Angiopathy. Dement Geriatr Cogn Disord, 44:343-353.

Freeze WM, Bacskai BJ, Frosch MP, Jacobs HL, Backes WH, Greenberg SM, et al. (2019). Blood-Brain Barrier Leakage and Microvascular Lesions in Cerebral Amyloid Angiopathy. Stroke, 50:328-335.

J R, A M, A K, JJ W, EE S, RA B, et al. (2005). Spatial clustering of hemorrhages in probable cerebral amyloid angiopathy. Ann Neurol, 58:459-462.

Ritter MA, Droste DW, Hagedus K, Szepesi R, Nabavi DG, Csiba L, et al. (2005). Role of cerebral amyloid angiopathy in intracerebral hemorrhage in hypertensive patients. Neurology, 64:1233-1237.

Hsu MJ, Hsu CY, Chen BC, Chen MC, Ou G, Lin CH (2007). Apoptosis signal-regulating kinase 1 in amyloid beta peptide-induced cerebral endothelial cell apoptosis. J Neurosci, 27:5719-5729.

Parodi-Rullán R, Sone JY, Fossati S (2019). Endothelial Mitochondrial Dysfunction in Cerebral Amyloid Angiopathy and Alzheimer's Disease. J Alzheimers Dis, 72:1019-1039.

Teng T, Ridgley DM, Tsoy A, Sun GY, Askarova S, Lee JC (2019). Azelnidipine Attenuates the Oxidative and NFκB Pathways in Amyloid-β-Stimulated Cerebral Endothelial Cells. ACS Chem Neurosci, 10:209-215.

Cheng X, He P, Yao H, Dong Q, Li R, Shen Y (2014). Occludin deficiency with BACE1 elevation in cerebral amyloid angiopathy. Neurology, 82:1707-1715.

Claudio L (1996). Ultrastructural features of the blood-brain barrier in biopsy tissue from Alzheimer's disease patients. Acta Neuropathol, 91:6-14.

Du H, Li P, Wang J, Qing X, Li W (2012). The interaction of amyloid β and the receptor for advanced glycation endproducts induces matrix metalloproteinase-2 expression in brain endothelial cells. Cell Mol Neurobiol, 32:141-147.

Lee JM, Yin KJ, Hsin I, Chen S, Fryer JD, Holtzman DM, et al. (2003). Matrix metalloproteinase-9 and spontaneous hemorrhage in an animal model of cerebral amyloid angiopathy. Ann Neurol, 54:379-382.

Vukic V, Callaghan D, Walker D, Lue LF, Liu QY, Couraud PO, et al. (2009). Expression of inflammatory genes induced by beta-amyloid peptides in human brain endothelial cells and in Alzheimer's brain is mediated by the JNK/AP1 signaling pathway. Neurobiol Dis, 34:95-106.

Merlini M, Meyer EP, Ulmann-Schuler A, Nitsch RM (2011). Vascular β-amyloid and early astrocyte alterations impair cerebrovascular function and cerebral metabolism in transgenic arcAb mice. Acta Neuropathol, 122:293-311.

Wilcock DM, Vitek MP, Colton CA (2009). Vascular amyloid alters astrocyte water and potassium channels in mouse models and humans with Alzheimer's disease. Neuroscience, 159:1055-1069.

Hernandez-Guillamon M, Martinez-Saez E, Delgado P, Dominguez-Montanari S, Boada C, Penalba A, et al. (2012). MMP-2/MMP-9 Plasma Level and Brain Expression in Cerebral Amyloid Angiopathy-
Associated Hemorrhagic Stroke. Brain Pathol, 22:133-141.

Chakraborty A, Kamermans A, van Het Hof B, Castricum K, Aanhané E, van Horssen J, et al. (2018). Angiopoietin like-4 as a novel molecular mediator in capillary cerebral amyloid angiopathy. Brain, 141:3377-3388.

Qin H, Benveniste EN (2012). ELISA methodology to quantify astrocyte production of cytokines/chemokines in vitro. Methods Mol Biol, 814:235-249.

Garwood CJ, Poorer AM, Atherton J, Hanger DP, Noble W (2011). Astrocytes are important mediators of Aβ-induced neurotoxicity and tau phosphorylation in primary culture. Cell Death Dis, 2:e167.

Miao J, Xu F, Davis J, Otte-Höller I, Verbeek MM, Van Nostrand WE (2005). Cerebral microvascular amyloid beta protein deposition induces vascular degeneration and neuroinflammation in transgenic mice expressing human vasculotrophic mutant amyloid beta precursor protein. Am J Pathol, 167:505-515.

Soto-Rojas LO, Pacheco-Herrero M, Martínez-Gómez PA, Campa Córdoba BB, Apártiga-Pérez R, Villegas-Rojas MM, et al. (2021). The Neurovascular Unit Dysfunction in Alzheimer's Disease. Int J Mol Sci, 22.

Giannoni P, Arango Medina MC, Baranger K, Rivera S, et al. (2016). Cerebrovascular pathology during the progression of experimental Alzheimer's disease. Neurobiol Dis, 88:107-117.

Han BH, Zhou ML, Johnson AW, Singh J, Liao F, Vellimana AK, et al. (2015). Contribution of reactive oxygen species to cerebral amyloid angiopathy, vasomotor dysfunction, and microhemorrhage in aged Tg2576 mice. Proc Natl Acad Sci U S A, 112:E881-890.

Salmina AB, Komleva YK, Lopatina OL, Birbrair A (2019). Pericytes in Alzheimer's Disease: Novel Clues to Cerebral Amyloid Angiopathy Pathogenesis. Adv Exp Med Biol, 1147:147-166.

Tachibana M, Yamazaki Y, Liu CC, Bu G, Kanekiyo T (2018). Pericyte implantation in the brain enhances cerebral blood flow and reduces amyloid-β pathology in amyloid model mice. Exp Neurol, 300:13-21.

Sagare AP, Bell RD, Zhao Z, Ma Q, Winkler EA, Ramanathan A, et al. (2013). Pericyte loss influences Alzheimer-like neurodegeneration in mice. Nat Commun, 4:2932.

Arvanitakis Z, Leurgans SE, Wang Z, Wilson RS, Bennett DA, Schneider JA (2011). Cerebral amyloid angiopathy pathology and cognitive domains in older persons. Ann Neurol, 69:320-327.

Kress BT, Iliff JJ, Xia M, Wang M, Wei HS, Zepeñfeld D, et al. (2014). Impairment of paravascular clearance pathways in the aging brain. Ann Neurol, 76:485-861.

CA H, W H, J K, R S, RO W, JA N, et al. (2011). Perivascular drainage of solutes is impaired in the ageing mouse brain and in the presence of cerebral amyloid angiopathy. Acta Neuropathol, 121:431-443.

Shin HK, Jones PB, Garcia-Alloza M, Borrelli L, Greenberg SM, Bacskai BJ, et al. (2007). Age-dependent cerebrovascular dysfunction in a transgenic mouse model of cerebral amyloid angiopathy. Brain, 130:2310-2319.

SM G, ME G, J R, EE S (2004). Amyloid angiopathy-related vascular cognitive impairment. Stroke, 35:2616-2619.

Thal DR, Griffin WS, de Vos RA, Ghebremedhin E (2008). Cerebral amyloid angiopathy and its relationship to Alzheimer's disease. Acta Neuropathol, 115:599-609.

Viguier A, Raposo N, Patsoura S, Calviere L, Albucher JF, Ruidavets JB, et al. (2019). Subarachnoid and Subdural Hemorrhages in Lobar Intracerebral Hemorrhage Associated With Cerebral Amyloid Angiopathy. Stroke, 50:1567-1569.

Rodrigues MA, Samarasekera N, Lerpiere C, Humphreys C, McCarron MO, White PM, et al. (2018). The Edinburgh CT and genetic diagnostic criteria for lobar intracerebral haemorrhage associated with cerebral amyloid angiopathy: model development and diagnostic test accuracy study. Lancet Neurol, 17:232-240.

Pasi M, Boulouis G, Fotiadis P, Auriel E, Charidimou A, Halek Y, et al. (2017). Distribution of lacunes in cerebral amyloid angiopathy and hypertensive small vessel disease. Neurology, 88:2162-2168.

van Veluw SJ, Charidimou A, van der Kouwe AJ, Lauer A, Reijmer YD, Costantino I, et al. (2016). Microbleed and microinfarct detection in amyloid angiopathy: a high-resolution MRI-histopathology study. Brain, 139:3151-3162.

Tsai HH, Pasi M, Tsai LK, Chen YF, Lee BC, Tang SC, et al. (2019). Microangiopathy underlying mixed-location intracerebral hemorrhages/microbleeds: A PiB-PET study. Neurology, 92:e774-e781.

L P (2010). Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol, 9:689-701.

Lammie GA (2002). Hypertensive cerebral small vessel disease and stroke. Brain Pathol, 12:358-370.

Cipolla MJ, Liebeskind DS, Chan SL, et al. (2018). The importance of comorbidities in ischemic stroke: Impact of hypertension on the cerebral circulation. J Cereb Blood Flow Metab, 38:2129-2149.

Rosenblum WJ (2008). Fibrinoid necrosis of small brain arteries and arterioles and miiliary aneurysms as causes of hypertensive hemorrhage: a critical reappraisal. Acta Neuropathol, 116:361-369.

Chillon JM, Baumbach GL (1999). Effects of an angiotensin-converting enzyme inhibitor and a beta-blocker on cerebral arterioles in rats. Hypertension, 32:370.

Chillón JM, Baumbach GL (1999). Effects of an angiotensin-converting enzyme inhibitor and a beta-blocker on cerebral arterioles in rats. Hypertension, 32:370-376.

Stanzione R, Bianchi F, Cotugno M, Marchitti S, Forte M, Busceti C, et al. (2017). A Decrease of Brain MicroRNA-122 Level Is an Early Marker of Cerebrovascular Disease in the Stroke-Prone Spontaneously Hypertensive Rat. Oxid Med Cell Longev, 2017:1206420.
[89] Mohammadi MT, Dehghani GA (2014). Acute hypertension induces brain injury and blood-brain barrier disruption through reduction of claudins mRNA expression in rat. Pathol Res Pract, 210:985-990.

[90] Suzuki K, Nakazato K, Kusakabe T, Nagamine T, Sakurai H, Takata M (2007). Role of oxidative stress on pathogenesis of hypertensive cerebrovascular lesions. Pathol Int, 57:133-139.

[91] Guo S, Deng W, Xing C, Zhou Y, Ning M, Lo EH (2019). Effects of aging, hypertension and diabetes on the mouse brain and heart vasculatures. Neurobiol Dis, 126:117-123.

[92] Monnier A, Garnier P, Quire J, Pernet N, Demougeot C, Marie C, et al. (2017). Effect of short-term exercise training on brain-derived neurotrophic factor signaling in spontaneously hypertensive rats. J Hypertens, 35:279-290.

[93] Li CC, Chen WX, Wang J, Xia M, Jia ZC, Guo C, et al. (2020). Nicotinamide riboside rescues angiotensin II-induced cerebral small vessel disease in mice. CNS Neurosci Ther, 26:438-447.

[94] Guillot FL, Audus KL (1990). Angiotensin peptide regulation of fluid-phase endocytosis in brain microvessel endothelial cell monolayers. J Cereb Blood Flow Metab, 10:827-834.

[95] Tagami M, Nara Y, Kubota A, Fujino H, Yamori Y (1990). Ultrastructural changes in cerebral pericytes and astrocytes of stroke-prone spontaneously hypertensive rats. Stroke, 21:1064-1071.

[96] Herman IM, Jacobson S (1988). In situ analysis of microvascular pericytes in hypertensive rat brains. Tissue Cell, 20:1-12.

[97] Herman IM, Newcomb PM, Coughlin JE, Jacobson S (1987). Characterization of microvascular cell cultures from normotensive and hypertensive rat brains: pericyte-endothelial cell interactions in vitro. Tissue Cell, 19:197-206.

[98] Yuan X, Wu Q, Liu X, Zhang H, Xiu R (2018). Transcriptomic profile analysis of brain microvascular pericytes in spontaneously hypertensive rats by RNA-Seq. Am J Transl Res, 10:2372-2386.

[99] Ishida H, Takemori K, Dote K, Ito H (2006). Expression of glucose transporter-1 and aquaporin-4 in the cerebral cortex of stroke-prone spontaneously hypertensive rats in relation to the blood-brain barrier function. Am J Hypertens, 19:33-39.

[100] Díaz JR, Kim KJ, Brands MW, Filosa JA (2019). Augmented astrocyte microdomain Cat(2+) dynamics and parenchymal arteriole tone in angiotensin II-infused hypertensive mice. Glia, 67:551-565.

[101] O’Connor AT, Clark MA (2018). Astrocytes and the Renin Angiotensin System: Relevance in Disease Pathogenesis. Neurochem Res, 43:1297-1307.

[102] Henskens LH, van Oostenbrugge RJ, Kroon AA, de Leeuw PW, Lodder J (2008). Brain microbleeds are associated with ambulatory blood pressure levels in a hypertensive population. Hypertension, 51:62-68.

[103] Pasi M, Charidimou A, Boulouis G, Auriel E, Ayres A, Schwab KM, et al. (2018). Mixed-location cerebral hemorrhage/microbleeds: Underlying microangiopathy and recurrence risk. Neurology, 90:e119-e126.

[104] Fisher M, Vasilevko V, Passos GF, Ventura C, Quiring D, Cribbs DH (2011). Therapeutic modulation of cerebral microhemorrhage in a mouse model of cerebral amyloid angiopathy. Stroke, 42:3300-3303.

[105] Reuter B, Venus A, Heiler P, Schad L, Ebert A, Hennerici MG, et al. (2016). Development of Cerebral Microbleeds in the APP23-Transgenic Mouse Model of Cerebral Amyloid Angiopathy-A 9.4 Tesla MRI Study. Front Aging Neurosci, 8:170-170.

[106] Marinescu M, Sun L, Fatar M, Neubauer A, Schad L, van Ryn J, et al. (2017). Cerebral Microbleeds in Murine Amyloid Angiopathy: Natural Course and Anticoagulant Effects. Stroke, 48:2248-2254.

[107] Rosidi NL, Zhou J, Pattanaik S, Wang P, Jin W, Brophy M, et al. (2011). Cortical microhemorrhages cause local inflammation but do not trigger widespread dendrite degeneration. PLoS One, 6:e26612.

[108] Wakisaka Y, Chu Y, Miller JD, Rosenberg GA, Heistad DD (2010). Critical role for copper/zinc-superoxide dismutase in preventing spontaneous intracerebral hemorrhage during acute and chronic hypertension in mice. Stroke, 41:790-797.

[109] Toth P, Tarantini S, Springer Z, Tucek Z, Gautam T, Giles CB, et al. (2015). Aging exacerbates hypertension-induced cerebral microhemorrhages in mice: role of resveratrol treatment in vasoprotection. Aging Cell, 14:400-408.

[110] Tarantini S, Valcarcel-Ares NM, Yabluchanskiy A, Springer Z, Fulop GA, Ashpole N, et al. (2017). Insulin-like growth factor 1 deficiency exacerbates hypertension-induced cerebral microhemorrhages in mice, mimicking the aging phenotype. Aging Cell, 16:469-479.

[111] Hoffmann A, Kunze R, Helluy X, Milford D, Heiland S, Bendszus M, et al. (2016). High-Field MRI Reveals a Drastic Increase of Hypoxia-Induced Microhemorrhages upon Tissue Reoxygenation in the Mouse Brain with Strong Predominance in the Olfactory Bulb. PLoS One, 11:e0148441.

[112] Sumbria RK, Grigoryan MM, Vasilevko V, Krasieva TB, Scadeng M, Dvornikova AK, et al. (2016). A murine model of inflammation-induced cerebral microbleeds. J Neuroinflammation, 13:218-218.

[113] Bergeron S, Chen Y, Auger F, Deguill J, Durieux N, Skrobala E, et al. (2019). Role of cortical microbleeds in cognitive impairment: In vivo behavioral and imaging characterization of a novel murine model. J Cereb Blood Flow Metab, 39:1015-1025.

[114] Hatate J, Miwa K, Matsumoto M, Sasaki T, Yagita Y, Sakaguchi M, et al. (2016). Association between cerebral small vessel diseases and mild parkinsonian signs in the elderly with vascular risk factors. Parkinsonism Relat Disord, 26:29-34.

[115] de Laat KF, van den Berg HA, van Norden AG, Gons RA, Olde Rikkert MG, de Leeuw FE (2011). Microbleeds are independently related to gait disturbances in elderly individuals with cerebral small
vessel disease. Stroke, 42:494-497.

[116] van Agtmaal MJM, Houben A, Pouver F, Stehouwer CDA, Schram MT (2017). Association of Microvascular Dysfunction with Late-Life Depression: A Systematic Review and Meta-analysis. JAMA Psychiatry, 74:729-739.

[117] Ding J, Sigurdsson S, Garcia M, Phillips CL, Eiriksdottir G, Gudnason V, et al. (2015). Risk Factors Associated With Incident Cerebral Microbleeds According to Location in Older People: The Age, Gene/Environment Susceptibility (AGES)-Reykjavik Study. JAMA Neurol, 72:682-688.

[118] I-A-S, S T, AJ dc, AC vE, JW J, DJ S, et al. (2011). Cerebral microbleeds are predictive of mortality in the elderly. Stroke, 42:638-644.

[119] Nahimey PC, Reeson P, Brown CE (2016). Ultrastructural analysis of blood-brain barrier breakdown in the peri-infarct zone in young adult and aged mice. J Cereb Blood Flow Metab, 36:413-425.

[120] Goodall EF, Wang C, Simpson JE, Baker DJ, Drew DR, Heath PR, et al. (2018). Age-associated changes in the blood-brain barrier: comparative studies in human and mouse. Neuropathol Appl Neurobiol, 44:328-337.

[121] Costea L, Mészáros A, Bauer H, Bauer H-C, Traweger A, Wilhelm I, et al. (2019). The Blood-Brain Barrier and Its Intercellular Junctions in Age-Related Brain Disorders. Int J Mol Sci, 20:5472.

[122] Farrall AJ, Wardlaw JM (2009). Blood–brain barrier: Ageing and microvascular disease – systematic review and meta-analysis. Neurobiol Aging, 30:337-352.

[123] Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. (2015). Blood-brain barrier breakdown in the aging human hippocampus. Neuron, 85:296-302.

[124] Donato AJ, Machin DR, Lesniewski LA (2018). Mechanisms of Dysfunction in the Aging Vasculature and Role in Age-Related Disease. Circ Res, 123:825-848.

[125] Ungvari Z, Tarantini S, Donato AJ, Galvan V, Csiszar A (2018). Mechanisms of Vascular Aging. Circ Res, 123:849-867.

[126] Brown WR, Thore CR (2011). Review: cerebral microvascular pathology in ageing and neurodegeneration. Neuropathol Appl Neurobiol, 37:56-74.

[127] Jucker M, Battig K, Meier-Ruge W (1990). Effects of aging and vincamine derivatives on pericapillary microenvironment: stereological characterization of the cerebral capillary network. Neurobiol Aging, 11:39-46.

[128] Farkas E, de Vos RA, Donka G, Jansen Steur EN, Mihaly A, Luiten PG (2006). Age-related microvascular degeneration in the human cerebral periventricular white matter. Acta Neuropathol, 111:150-157.

[129] Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM (2006). Cellular senescence in aging primates. Science, 311:1257.

[130] Yamazaki Y, Baker DJ, Tachibana M, Liu C-C, van Deursen JM, Brott TG, et al. (2016). Vascular Cell Senescence Contributes to Blood-Brain Barrier Breakdown. Stroke, 47:1068-1077.

[131] Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KL, Luo J, et al. (2014). Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat Med, 20:659-663.

[132] Yousef H, Czupalla CJ, Lee D, Chen MB, Burke AN, Zera KA, et al. (2019). Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1. Nat Med, 25:988-1000.

[133] Chen JJ, Rosas HD, Salat DH (2011). Age-associated reductions in cerebral blood flow are independent from regional atrophy. NeuroImage, 55:468-478.

[134] Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ (2018). The Aging Astrocyte Transcriptionome from Multiple Regions of the Mouse Brain. Cell Rep, 22:269-285.

[135] Liddelow SA, Guttenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. (2017). Neurotoxic reactive astrocytes are induced by activated microglia. Nature, 541:481-487.

[136] Clarke LE, Liddelow SA, Chakraborty C, Münch AE, Heiman M, Barres BA (2018). Normal aging induces A1-like astrocyte reactivity. Proc Natl Acad Sci U S A, 115:E1896-E1905.

[137] Bell RD, Winkler EA, Sagare AP, Singh I, LaRue B, Deane R, et al. (2010). Pericytes control key neurovascular functions and neuronal phenotype in the adult brain and during brain aging. Neuron, 68:409-427.

[138] Sumbria RK, Grigoryan MM, Vasilevko V, Paganini-Hill A, Kilday K, Kim R, et al. (2018). Aging exacerbates development of cerebral microbleeds in a mouse model. J Neuroinflammation, 15:69.

[139] Sonntag WE, Deak F, Ashpole N, Toth P, Csiszar A, Freeman W, et al. (2013). Insulin-like growth factor-1 in CNS and cerebrovascular aging. Front Aging Neurosci, 5:27-27.

[140] Gregoire Simone M, Brown Martin M, Kallis C, Jäger HR, Yousry Tarek A, Werring David J (2010). MRI Detection of New Microbleeds in Patients With Ischemic Stroke. Stroke, 41:184-186.

[141] Forrester SJ, Booz GW, Sigmund CD, Coffman TM, Kawai T, Rizzo V, et al. (2018). Angiotensin II Signal Transduction: An Update on Mechanisms of Physiology and Pathophysiology. Physiol Rev, 98:1627-1738.

[142] Nyul-Tóth Á, Tarantini S, Kiss T, Toth P, Galvan V, Tarantini A, et al. (2020). Increases in hypertension-induced cerebral microhemorrhages exacerbate gait dysfunction in a mouse model of Alzheimer's disease. Geroscience, 42:1685-1698.

[143] Marchesi C, Paradis P, Schiffrin EL (2008). Role of the renin-angiotensin system in vascular inflammation. Trends Pharmacol Sci, 29:367-374.

[144] Te Riet L, van Esch JH, Roks AJ, van den Meiracker AH, Danser AH (2015). Hypertension: renin-angiotensin-aldosterone system alterations. Circ Res, 116:960-975.
Angiotensin II modulates BBB permeability via activation of the AT(1) receptor in brain endothelial cells. J Cereb Blood Flow Metab, 29:640-647.

Zhang M, Mao Y, Ramirez SH, Tuma RF, Chabrashvili T (2010). Angiotensin II induced cerebral microvascular inflammation and increased blood–brain barrier permeability via oxidative stress. Neuroscience, 171:852-858.

Biancardi VC, Stern JE (2016). Compromised blood–brain barrier permeability: novel mechanism by which circulating angiotensin II signals to sympathoexcitatory centres during hypertension. J Physiol, 594:1591-1600.

Nagata K, Yamazaki T, Takano D, Maeda T, Fujimaki Y, Nakase T, et al. (2016). Cerebral circulation in aging. Ageing Res Rev, 30:49-60.

Mulvany MJ, Baumbach GL, Aalkjaer C, Heagerty AM, Korsgaard N, Schiffrin EL, et al. (1996). Vascular remodeling. Hypertension, 28:505-506.

Faraco G, Iadecola C (2013). Hypertension: a harbinger of stroke and dementia. Hypertension, 62:810-817.

Seo WK, Lee JM, Park MH, Park KW, Lee DH (2008). Cerebral microbleeds are independently associated with arterial stiffness in stroke patients. Cerebrovasc Dis, 26:618-623.

de Montgolfier O, Pinçon A, Poulilot P, Gillis MA, Bishop J, Sled JG, et al. (2019). High Systolic Blood Pressure Induces Cerebral Microvascular Endothelial Dysfunction, Neurovascular Unit Damage, and Cognitive Decline in Mice. Hypertension, 73:217-228.

Humphrey JD (2008). Mechanisms of arterial remodeling in hypertension: coupled roles of wall shear and intramural stress. Hypertension, 52:195-200.

Loehrner E, Ikram MA, Akoudad S, Vrooman HA, van der Lugt A, Niessen WJ, et al. (2014). Apolipoprotein E genotype influences spatial distribution of cerebral microbleeds. Neurobiol Aging, 35:899-905.

Sveinbjörnsdóttir S, Sigurdsson S, Aspelund T, Kjarðansson O, Einríksdóttir G, Valtýsdóttir B, et al. (2008). Cerebral microbleeds in the population based AGES–Reykjavik study: prevalence and location. J Neurol Neurosurg Psychiatry, 79:1002-1006.

Li H-Q, Cai W-J, Hou X-H, Cui M, Tan L, Yu J-T, et al. (2020). Genome-Wide Association Study of Cerebral Microbleeds on MRI. Neurotox Res, 37:146-155.

Hultman K, Strickland S, Norris EH (2013). The APOE varepsilon4/varepsilon4 genotype potentiates vascular fibrinogen deposition in amyloid-laden vessels in the brains of Alzheimer's disease patients. J Cereb Blood Flow Metab, 33:1251-1258.

Halliday MR, Rege SV, Ma Q, Zhao Z, Miller CA, Winkler EA, et al. (2016). Accelerated pericyte degeneration and blood–brain barrier breakdown in apolipoprotein E4 carriers with Alzheimer's disease. J Cereb Blood Flow Metab, 36:216-227.

Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hulette CM, et al. (2007). Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. Neurobiol Aging, 28:977-986.

Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, et al. (2020). APOE4 leads to blood–brain barrier dysfunction predicting cognitive decline. Nature, 581:71-76.

Grehan S, Tse E, Taylor JM (2001). Two distal downstream enhancers direct expression of the human apolipoprotein E gene to astrocytes in the brain. J Neurosci, 21:812-822.

Flowers SA, Rebeck GW (2020). APOE in the normal brain. Neurobiol Dis, 136:104724.

Nguyen D, Dhanasekaran P, Nickel M, Nakatani R, Saito H, Phillips MC, et al. (2010). Molecular Basis for the Differences in Lipid and Lipoprotein Binding Properties of Human Apolipoproteins E3 and E4. Biochemistry, 49:10881-10889.

Nishitsuji K, Hosono T, Nakamura T, Bu G, Michikawa M (2011). Apolipoprotein E regulates the integrity of tight junctions in an isoform-dependent manner in an in vitro blood-brain barrier model. J Biol Chem, 286:17536-17542.

Bell RD, Winkler EA, Singh I, Sagare AP, Deane R, Wu Z, et al. (2012). Apolipoprotein E controls cerebrovascular integrity via cyclophilin A. Nature, 485:512-516.

Profaci CP, Munji RN, Pulido RS, Daneman R (2020). The blood–brain barrier in health and disease: Important unanswered questions. J Exp Med, 217.

Bu G (2009). Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. Nature reviews. Neuroscience, 10:333-344.

Zlokovic BV (2013). Cerebrovascular effects of apolipoprotein E: implications for Alzheimer disease. JAMA Neurol, 70:440-444.

Thal DR, Larionov S, Abramowski D, Wiederhold K-H, Van Dooren T, Yamaguchi H, et al. (2007). Occurrence and co-localization of amyloid β-protein and apolipoprotein E in perivascular drainage channels of wild-type and APP-transgenic mice. Neurobiol Aging, 28:1221-1230.

Utter S, Tamboli IY, Walter J, Upadhyaya AR, Birkenmeier G, Pizartik CU, et al. (2008). Cerebral Small Vessel Disease-Induced Apolipoprotein E Leakage Is Associated With Alzheimer Disease and the Accumulation of Amyloid β-Protein in Perivascular Astrocytes. J Neuropathol Exp Neurol, 67:842-856.

Deane R, Wu Z, Sagare A, Davis J, Du Yan S, Hamm K, et al. (2004). LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. Neuron, 43:333-344.

Deane R, Sagare A, Hamm K, Parisi M, Lane S, Finn MB, et al. (2008). apoE isoform-specific disruption of amyloid beta peptide clearance from mouse brain. J Clin Invest, 118:4002-4013.

Bell RD, Zlokovic BV (2009). Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. Acta Neuropathol, 118:103-113.

Schreibelt G, Kooij G, Reijerkerk A, van Doorn R,
Gringhuis SI, van der Pol S, et al. (2007). Reactive oxygen species alter brain endothelial tight junction dynamics via RhoA, PI3 kinase, and PKB signaling. FASEB J, 21:3666-3676.

Carrano A, Carrano A, Hoozemans JJM, van der Vies SM, van Horssen J, de Vries HE, et al. (2012). Neuroinflammation and Blood-Brain Barrier Changes in Capillary Amyloid Angiopathy. Neurodegener Dis, 10:329-331.

Zipfel GF, Han H, Ford AL, Lee J-M (2009). Cerebral amyloid angiopathy: progressive disruption of the neurovascular unit. Stroke, 40:S16-S19.

Hartz AMS, Bauer B, Soldner ELB, Wolf A, Boy S, Backhaus R, et al. (2012). Amyloid-β contributes to blood-brain barrier leakage in transgenic human amyloid precursor protein mice and in humans with cerebral amyloid angiopathy. Stroke, 43:514-523.

Fan F, Yang C, Zhu X, Liu Z, Liu H, Li J, et al. (2021). Association between infectious burden and cerebral microbleeds: a pilot cross-sectional study. Ann Clin Transl Neurol, 8:395-405.

Shoamanesh A, Preis SR, Beiser AS, Vasan RS, Benjamin EJ, Kase CS, et al. (2015). Inflammatory biomarkers, cerebral microbleeds, and small vessel disease: Framingham Heart Study. Neurology, 84:825-832.

Miwa K, Tanaka M, Okazaki S, Furukado S, Sakaguchi M, Kitagawa K (2011). Relations of Blood Inflammatory Marker Levels With Cerebral Microbleeds. Stroke, 42:3202-3206.

Low A, Mak E, Rowe JB, Markus HS, O’Brien JT (2019). Inflammation and cerebral small vessel disease: a systematic review. Ageing Res Rev, 53:100916.

Ahn SJ, Anrather J, Nishimura N, Schaffer CB (2018). Diverse Inflammatory Response After Cerebral Microbleeds Includes Coordinated Microglial Migration and Proliferation. Stroke, 49:1719-1726.

Haruwaka K, Ikegami A, Tachibana Y, Ohno N, Konishi H, Hashimoto A, et al. (2019). Dual microglia effects on blood brain barrier permeability induced by systemic inflammation. Nat Commun, 10:5816-5816.

Erickson MA, Banks WA (2018). Neuroimmune Axes of the Blood-Brain Barriers and Blood-Brain Interfaces: Bases for Physiological Regulation, Disease States, and Pharmacological Interventions. Pharmacol Rev, 70:278-314.

Varatharaj A, Galea I (2017). The blood-brain barrier in systemic inflammation. Brain Behav Immun, 60:1-12.

Cardoso FL, Kittel Á, Veszelska S, Palmela I, Tóth A, Brites D, et al. (2012). Exposure to Lipopolysaccharide and/or Unconjugated Bilirubin Impair the Integrity and Function of Brain Microvascular Endothelial Cells. PLoS One, 7:e35919.

Smyth LCD, Rustenhoven J, Park TII, Schweder P, Jansson D, Heppner PA, et al. (2018). Unique and shared inflammatory profiles of human brain endothelia and pericytes. J Neuroinflammation, 15:138-138.

Wang HL, et al. BBB dysfunction in cerebral microbleeds

Shams S, Granberg T, Martola J, Li X, Shams M, Fereshtehnejad S-M, et al. (2015). Cerebrospinal fluid profiles with increasing number of cerebral microbleeds in a continuum of cognitive impairment. J Cereb Blood Flow Metab, 36:621-628.

Freeze WM, Jacobs HIL, Schreuder FHB, van Oostenbrugge RJ, Backes WH, Verhey FR, et al. (2018). Blood-Brain Barrier Dysfunction in Small Vessel Disease Related Intracerebral Hemorrhage. Front Neurol, 9:926-926.

Daneman R, Pratt A (2015). The blood-brain barrier. Cold Spring Harb Perspect Biol, 7:a020412-a020412.

Abbott NJ, Patabendige AAK, Dolman DEM, Yusof SR, Begley DJ (2010). Structure and function of the blood–brain barrier. Neurobiol Dis, 37:13-25.

Ribatti D, Nico B, Crivellato E, Artico M (2006). Development of the blood-brain barrier: A historical point of view. Anat Rec B New Anat, 289B:3-8.

Butt AM, Jones HC, Abbott NJ (1990). Electrical resistance across the blood-brain barrier in anesthetized rats: a developmental study. J Physiol, 429:47-62.

Poggesa A, Pasi M, Pescini F, Pantonl I, Inzitari D (2016). Circulating biologic markers of endothelial dysfunction in cerebral small vessel disease: A review. J Cereb Blood Flow Metab, 36:72-94.

Michiels C (2003). Endothelial cell functions. J Cell Physiol, 196:430-443.

Deanfield JE, Halcox JP, Rabelink TJ (2007). Endothelial function and dysfunction: testing and clinical relevance. Circulation, 115:1285-1295.

Weinl C, Castaneda Vega S, Riehle H, Stritt C, Calaminus C, Wolburg H, et al. (2015). Endothelial depletion of murine SRF/MRTF provokes intracerebral hemorrhagic stroke. Proc Natl Acad Sci U S A, 112:9914-9919.

Alomar F, Singh J, Jang HS, Rozanzki GJ, Shao CH, Padanilam BJ, et al. (2016). Smooth muscle-generated methylglyoxal impairs endothelial cell-mediated vasodilatation of cerebral microvessels in type 1 diabetic rats. Br J Pharmacol, 173:3307-3326.

Huang Z, Yin Q, Sun W, Zhu W, Li Y, Liu W, et al. (2013). Microbleeds in ischemic stroke are associated with lower serum adiponectin and higher soluble E-selectin levels. J Neurol Sci, 334:83-87.

Ma XJ, Cheng JW, Zhang J, Liu AJ, Liu W, Guo W, et al. (2012). E-selectin deficiency attenuates brain ischemia in mice. CNS Neurosci Ther, 18:903-908.

Zhang JB, Li MF, Zhang HX, Li ZG, Sun HR, Zhang JS, et al. (2016). Association of serum vascular endothelial growth factor levels and cerebral microbleeds in patients with Alzheimer's disease. Eur J Neurol, 23:1337-1342.

Dassan P, Brown MM, Gregoire SM, Keir G, Werring DJ (2012). Association of Cerebral Microbleeds in Acute Ischemic Stroke With High Serum Levels of Vascular Endothelial Growth Factor. Arch Neurol, 69:1186-1189.

Lange C, Storkebaum E, de Almodóvar C, Dewerchin M, Carmeliet P (2016). Vascular endothelial growth
factor: a neurovascular target in neurological diseases. Nat Rev Neurol, 12:439-454.

[204] Argaw AT, Gurfein BT, Zhang Y, Zameer A, John GR (2009). VEGF-mediated disruption of endothelial CLN-5 promotes blood-brain barrier breakdown. Proc Natl Acad Sci U S A, 106:1977-1982.

[205] Wu BN, Wu J, Hao DL, Mao LL, Zhang J, Huang TT (2018). High serum sICAM-1 is correlated with cerebral microbleeds and hemorrhagic transformation in ischemic stroke patients. Br J Neurosurg, 32:631-636.

[206] Gu Y, Gutierrez J, Meier IB, Guzman VA, Manly JJ, Schupf N, et al. (2019). Circulating inflammatory biomarkers are related to cerebrovascular disease in older adults. Neurrol Neuroimmunol Neuroinflamm, 6:e521.

[207] Fens MHAM, Storm G, Pelgrim RCM, Ultee A, Byrne AT, Gaillard CA, et al. (2010). Erythrophagocytosis by angiogenic endothelial cells is enhanced by loss of erythrocyte deformability. Exp Hematol, 38:282-291.

[208] Chang R, Castillo J, Zambon AC, Krasieva TB, Fisher MJ, Sumbria RK (2018). Brain Endothelial Erythrophagocytosis and Hemoglobin Transmigration Across Brain Endothelium: Implications for Pathogenesis of Cerebral Microbleeds. Front Cell Neurosci, 12:279-279.

[209] Fens MHAM, van Wijk R, Andringa P, van Rooijen N, Fens MHAM, Storm G, van Wijk R, Andringa G, van Rooijen N, Peppiatt CM, Howarth C, Mobbs P, Attwell D (2012). A role for activated endothelial cells in red blood cell clearance: implications for vasopathology. Haematologica, 97:500-508.

[210] Peppiatt CM, Howarth C, Mobbs P, Attwell D (2006). Bidirectional control of CNS capillary diameter by pericytes. Nature, 443:700-704.

[211] Nakagomi T, Kubo S, Nakano-Doi A, Sakuma R, Lu S, Narita A, et al. (2015). Brain Vascular Pericytes Following Ischemia Have Multipotential Stem Cell Activity to Differentiate Into Neural and Vascular Lineage Cells. Stem Cells, 33:1962-1974.

[212] Fisher M, French S, Ji P, Kim RC (2010). Cerebral microbleeds in the elderly: a pathological analysis. Stroke, 41:2782-2785.

[213] Liao F-F, Lin G, Chen X, Chen L, Zheng W, Raghow R, et al. (2021). Endothelial Nitric Oxide Synthase-Deficient Mice. Am J Pathol:S0002-9440(0021)00102-00104.

[214] Tsai H-H, Li H, Fuentealba LC, Molofsky AV, Taveira-Marques R, Zhuang H, et al. (2012). Regional astrocyte allocation regulates CNS synaptogenesis and repair. Science, 337:358-362.

[215] Abbott NJ, Ronnback L, Hansson E (2006). Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci, 7:41-53.

[216] Daneman R, Zhou L, Kebede AA, Barres BA (2010). Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature, 468:562-566.

[217] Engelhardt S, Patkar S, Ogunshola OO (2014). Cell-specific blood-brain barrier regulation in health and disease: a focus on hypoxia. Br J Pharmacol, 171:1210-1230.

[218] Dudvarski Stankovic N, Teodorczyk M, Ploeg R, Zipp F, Schmidt MHH (2016). Microglia-blood vessel interactions: a double-edged sword in brain pathologies. Acta Neuropathol, 131:347-363.

[219] Liu LR, Liu JC, Bao JS, Bai QQ, Wang GQ (2020). Interaction of Microglia and Astrocytes in the Neurovascular Unit. Front Immunol, 11:1024.

[220] Taylor S, Mehina E, White E, Reeson P, Yongblah K, Doyle KP, et al. (2018). Suppressing Interferon-γ Stimulates Microglial Responses and Repair of Microbleeds in the Diabetic Brain. J Neurosci, 38:8707-8722.

[221] Xiao L, Sun W, Lan W, Xiong Y, Duan Z, Zhang Z, et al. (2014). Correlation between cerebral microbleeds and S100B/RAGE in acute lacunar stroke patients. J Neurosci, 34:208-212.

[222] Michetti F, D’Ambrosi N, Toesca A, Puglisi MA, Serrano A, Marchese E, et al. (2019). The S100B story: from biomarker to active factor in neural injury. J Neurochem, 148:168-187.

[223] Barateiro A, Afonso V, Santos G, Cerqueira JJ, Brites D, van Horsen J, et al. (2016). S100B as a Potential Biomarker and Therapeutic Target in Multiple Sclerosis. Mol Neurobiol, 53:3976-3991.

[224] Duits FH, Hernandez-Guillamon M, Montaner J, Goos JD, Mañonella A, Wattjes MP, et al. (2015). Matrix Metalloproteinases in Alzheimer’s Disease and Concurrent Cerebral Microbleeds. J Alzheimers Dis, 48:711-720.

[225] Koh SH, Park CY, Kim MK, Lee KY, Kim J, Chang DI, et al. (2011). Microbleeds and free active MMP-9 are independent risk factors for neurological deterioration in acute lacunar stroke. Eur J Neurol, 18:158-164.

[226] Yan P, Zhu A, Liao F, Xiao Q, Kraft A, Gonzales E, et al. (2015). Minocycline reduces spontaneous hemorrhage in mouse models of cerebral amyloid angiopathy. Stroke, 46:1633-1640.

[227] Kim Bum J, Kwon Sun U, Park J-H, Kim Y-J, Hong K-S, Wong Lawrence KS, et al. (2020). Cilostazol Versus Aspirin in Ischemic Stroke Patients With High-Risk Cerebral Hemorrhage. Stroke, 51:931-937.

[228] Park HK, Lee JS, Kim BJ, Park JH, Kim YJ, Yu S, et al. (2020). Cilostazol versus aspirin in ischemic stroke with cerebral microbleeds versus prior intracerebral hemorrhage. Int J Stroke:1747493020941273.

[229] Liu S, Yu C, Yang F, Paganini-Hill A, Fisher MJ (2012). Phosphodiesterase inhibitor modulation of brain microvascular endothelial cell barrier properties. J Neurol Sci, 320:45-51.

[230] Sumbria RK, Vasilevko V, Grigoryan MM, Paganini-Hill A, Kim R, Cribs DH, et al. (2017). Effects of phosphodiesterase 3A modulation on murine cerebral microhemorrhages. J Neuroinflammation, 14:114-114.