An Integrated View on Vascular Dysfunction in Alzheimer’s Disease

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Abstract

\textbf{Background:} Cerebrovascular disease is a common comorbidity in patients with Alzheimer’s disease (AD). It is believed to contribute additively to the cognitive impairment and to lower the threshold for the development of dementia. However, accumulating evidence suggests that dysfunction of the cerebral vasculature and AD neuropathology interact in multiple ways. Vascular processes even precede AD neuropathology, implicating a causal role in the etiology of AD. Thus, the review aims to provide an integrated view on vascular dysfunction in AD. \textbf{Summary:} In AD, the cerebral vasculature undergoes pronounced cellular, morphological and structural changes, which alters regulation of blood flow, vascular fluid dynamics and vessel integrity. Stiffening of central blood vessels lead to transmission of excessive pulsatile energy to the brain microvasculature, causing end-organ damage. Moreover, a dysregulated hemostasis and chronic vascular inflammation further impede vascular function, where its mediators interact synergistically. Changes of the cerebral vasculature are triggered and driven by systemic vascular abnormalities that are part of aging, and which can be accelerated and aggravated by cardiovascular diseases. \textbf{Key Messages:} In AD, the cerebral vasculature is the locus where multiple pathogenic processes converge and contribute to cognitive impairment. Understanding the molecular mechanism and pathophysiology of vascular dysfunction in AD and use of vascular blood-based and imaging biomarker in clinical studies may hold promise for future prevention and therapy of the disease.

Introduction

The brain depends on the continuous delivery of oxygen and energy substrates due to its high-energy demand and the lack of long-term energy storage. The cerebral vasculature is well suited for this purpose, where regional cerebral blood flow (rCBF) is tightly regulated and can adapt to match the local energy demands of the nervous tissue [1]. A failure in adaption or supply of substrates will result in disturbed homeostasis, tissue damage and loss of function. The cerebral vasculature constitutes the blood-brain barrier (BBB), a functional barrier that prevents entry of plasma constituents and protects the brain from infection, as well as regulates passage of oxygen and nutrients and the removal of metabolic waste products [2]. The BBB with its adhesion molecules encompasses an important access point for leukocytes during immune sur-
veillance and neuroinflammation [3, 4]. Blood vessels contain a complex suspension of cells (≈50% of volume) with erythrocytes, leukocytes, and platelets. Leukocytes, at least in humans, represent <1% of blood cells, but are critical for mounting an immune response in the brain. Platelets control the conversion of the plasma protein fibrinogen to fibrin, a key structural element in blood clot formation. Activation, aggregation and accumulation of platelets depend on local hemodynamic forces and are critical in both the normal processes of vessel haemostasis, and under pathological conditions, for example, thrombosis.

While the topology, anatomy and signalling cascades of the cerebral vasculature is unique to serve its specialized functions, its vascular bed is connected to the general circulation of the body. Thus, any change in blood content (e.g., as part of haemostasis, inflammation or infection) or haemodynamic and biomechanical changes of central blood vessel will affect the cerebral vasculature. Conversely, alterations of the cerebral vessels might have systemic effects. We know that many brain diseases are associated with vascular dysfunctions. While research in the field of cerebrovascular diseases such as stroke has mainly fuelled the interest in vascular dysfunction, it has also been implicated in the pathophysiology of neurodegenerative diseases and dementia.

Alzheimer’s disease (AD) impairs cognition, memory, and language and causes dementia. AD is defined by deposition of fibrillar amyloid-β (Aβ) plaques and neurofibrillary tangles of hyperphosphorylated tau and neurodegeneration [5, 6]. Neuropathological changes can be either documented by post-mortem examination or by cerebrospinal fluid (CSF) or positron emission tomography (PET) biomarkers [7]. However, clinically AD presents as a spectrum (termed AD clinical syndrome), where cognitive impairment culminating in dementia can be caused by other factors in absence or in addition to the defined neuropathology [6]. In this context, accumulating evidence has shown that cerebrovascular disease is a common comorbidity in the presence of AD – and can on its own cause cognitive impairment and dementia (known as vascular dementia) – that contributes (additively) to its symptomatology and lowers the threshold for the development of dementia [8]. However, given the marked structural changes of the microvasculature, an alternative hypothesis has been proposed stating that vascular dysfunction causes AD-related neuropathology and cognitive impairment (the “vascular hypothesis of AD”) [9]. Today, the picture seems more complex and far from complete. It is believed that neuropathological and vascular pathways interact synergistically and feedback to each other to potentiate AD symptoms. Here, I aim to provide a comprehensive and integrated view on the topic, using knowledge from neuropathology, neurophysiology and clinical neuroscience. It incorporates also knowledge from fields such as cardiovascular medicine, biomechanics, immunology, and hematology to explain the full breadth of vascular dysfunction in AD and their consequences for prevention and therapy.

A particular focus will be on neuroimaging studies. With recent advances in magnetic resonance imaging (MRI), PET, single-photon-emission computed tomography it has become possible to assess the structural, functional, biophysical and molecular properties of the cerebral vasculature [10–12]. These techniques offer the advantage to monitor the process under study in a spatio-temporally resolved way. In addition, such techniques can be combined with neuroimaging techniques that map neuropathology, the occurrence of lesions and gross morphological changes as well as with imaging of other vascular systems for example, the heart and peripheral vasculature, thus providing information about the pathophysiological role of processes and their interactions [7]. Complementary studies in animal models of AD have enabled the dissection of disease mechanisms underlying vascular dysfunction, the relation between vascular dysfunction and neuropathology and impairment of brain function, as for the evaluation of novel therapeutic strategies [10].

**Cerebrovascular Comorbidities are Common in AD Brains**

Vascular lesions are common findings in patients with AD. A neuropathological study revealed that large infarcts, lacunae and multiple microinfarcts, haemorrhage, atherosclerosis, and arteriolosclerosis is prevalent in 80% of cases diagnosed with AD [8]. The concurrent cerebrovascular disease lowers the threshold for dementia due to AD. Vascular lesions in the brain of AD patients have also been described using neuroimaging. For example, white matter hyperintensities refer to regions in the white matter that appear hyperintense on T2-weighted fluid attenuation inversion recovery sequences. The etiologies of white matter hyperintensities are multifaceted (e.g., inflammation, axonal loss) [13, 14], and are presumed to reflect small vessel cerebrovascular disease [13, 15]. White matter hyperintensities are also found in normal ageing and to accompany cardiovascular disease, though their spatial
distribution differs in AD [16], implicating white matter hyperintensities as important neuroimaging biomarker. White matter hyperintensities predict accelerated cognitive decline and increased risk for AD [17]. And white matter hyperintensities have been shown to predict increased CSF total tau and progressive medial temporal lobe atrophy in AD [18], as well as PET cortical Aβ uptake in patients with preclinical AD [19] and AD [20].

Another major vascular co-morbidity observed in the AD brain is cerebral amyloid angiopathy (CAA). In CAA, Aβ accumulates in the wall of arteries, arterioles and, to a fewer extent in capillaries, in the leptomeninges and cortical areas [21]. It has been implicated to result from a failure of Aβ clearance from the brain, but its underlying mechanisms are not well understood [21]. CAA is present in the majority (~80%) of AD cases [8], but is also present in about 10–40% of the normal population [22]. Carriers of the apolipoprotein E (APOE) ε4 allele, which constitutes a well-known genetic risk factor for AD, have more severe CAA [22]. However, CAA is also a pathological feature in hereditary dementia disorders, where Aβ and non-Aβ (e.g., cystatin) forms are known, based on the accumulating peptide [23]. CAA results in the degeneration of cerebrovascular smooth muscle cells and endothelial cells, and vessel narrowing and occlusion [24–26]. In addition, the continuous remodeling of the vascular wall leads to BBB damage, microhemorrhage, intracerebral hemorrhage and infarcts, contributing to cognitive decline [22, 27, 28].

Important are microbleeds, which are caused by extravasation of red blood cells due to a loss of vascular integrity. Microbleeds can be observed non-invasively with T2*-weighted gradient echo MRI and have been widely studied in AD. Several studies indicate the appearance of microbleeds with mild cognitive impairment, early AD, and AD [29], where they are predominantly found in the occipital lobe [30]. The occurrence of microbleeds is related to the degree of cognitive impairment [29]. Microbleeds co-localize and correlate with Aβ deposition [30]. Consistent with this finding is that cerebral microbleeds are also found in animal models of cerebral amyloidosis where they are related to CAA [31–34]. Importantly, animal studies have shown that Aβ removing therapies, while lowering parenchymal Aβ load, increased CAA and microbleed load [34]. Microbleeds and vasogenic oedema have been observed with MRI in AD patients as a result of anti-Aβ targeted immunotherapy, known as amyloid-related imaging abnormalities [35]. The extravasation of red blood cells has detrimental consequences as the iron released from blood degradation products such as hæmoglobin and hemosiderin contribute to the generation of reactive oxygen species.

Intracranial vessel-associated calcifications are also common neuroimaging findings in AD patients, where they are more prevalent than in aged, cognitively healthy individuals [36]. The etiology and pathophysiology of most forms of brain calcifications are still unknown, but inflammatory and biomechanical pathways have been implicated [37]. Hippocampal calcifications were found to be correlated with cognitive ability [38]. While calcifications can present with psychiatric signs, cognitive impairment, migraine, and movement disorders their contributions to AD pathology is still debated [37]. More work is needed to clarify the pathomechanisms and functional consequences of microbleeds and calcifications on brain function in AD. Studies are currently hampered by the heterogeneity (number and topology) of their occurrence, the fact that they can also be caused by concomitant cerebrovascular and inflammatory diseases, and by methodological difficulties to detect such lesions with current neuroimaging methods.

The Interplay between Vascular Remodelling and Cerebral Hemodynamic and Biomechanics

The network organization of the cerebral vasculature and the relative density of vessels are well suited to ensure a robust blood supply and tissue oxygenation to the brain [1]. They provide also safety measures (such as collateral blood supply) to preserve perfusion in case of local vascular occlusion. The 5 vessel types: arteries, arterioles, capillaries, venules and veins have specific cell composition to serve their functions. There is a complex interplay between the composition and geometry, the blood constitution and consistence with fluid dynamics and biomechanical stress the vessel experiences (Fig. 1). Blood flow (Q) in straight vessels is laminar (Fig. 1a), but with its large concentration of cells display non-Newtonian rheological behaviour. In addition, blood flow induces biomechanical stresses on the wall in tangential (shear stress, τs) and perpendicular (circumferential stress, σθ) direction (Fig. 1a).

Changes in vascular remodelling occur during ageing, but are more pronounced in neurodegenerative diseases [39]. Early histopathological studies have revealed marked remodelling of the cerebral microvasculature in brain tissue from patients with AD. They revealed fragmented vessel and branches, thinning of microvessels (so-called string vessels), glomerular loop formation, and an in...
crease in vessel tortuosity (Fig. 1b) [39, 40]. Studies of capillary density in human AD are conflicting. While some studies found a reduction in microvessel density [39, 41], others reported an increase in vessel density [42], or did not find differences between AD and age-matched control cases [40, 43]. On the ultrastructural level thickening due to deposition of collagen fibrils and vacuolization of vascular basement membrane was also described [44]. In addition, reductions in capillary length, suggestive of endothelial degeneration, and a reduced expression of tight junction proteins have been reported [45].

Vascular remodelling has been also described to occur in animal models of cerebral amyloidosis [46–48], where some of the strains also display CAA [24, 31, 47–51]. A recent study described changes in vessel density and morphology in a transgenic model of tauopathy [52]. Interestingly, in this study vessel density was found to first increase, and then later to decrease. The change in vessel density has been attributed to angiogenesis that has also been observed to occur in humans [42]. Thus, changes in vessel density might be dynamic, and might have yielded the observed differences in autopsy studies.

The geometric changes alter the magnitudes and distributions of local biomechanical forces; however, some direct effects of these changes have been observed in other vascular beds and need to be demonstrated in the context of AD (Fig. 1a, c). Tortuosity increases the vessel length and increased blood pressure is needed to maintain flow in these vessels [53]. Both radial and axial shear
stress become bigger at the inner wall when the vessels are bent or distorted more from the circular shape, while those at the outer wall go the opposite way [54]. The high shear stress at the inner wall induces platelet activation [54]. Similarly, endothelial cells become pro-thrombotic at sites of vessel stenosis with platelet activation and fibrin formation [55]. High shear stress also increases the speed of coagulation. Thus, biomechanical forces can promote thrombosis, similar to that observed in vessel injuries. In a mouse model of tauopathy, vessel tortuosity of capillaries can restrict red blood cell movement and lead to blockage of vessels by adherent leukocytes [52]. Conversely, changes in haemodynamic function can induce vascular remodelling. Reduced blood flow has been shown to induce capillary regression by apoptosis, and promotes degeneration of endothelial cells due to loss of shear stress [56]. Changes in flow and mechanical stress lead to an altered gene expression, the expression of mitogens, cytokines, matrix metalloproteinases, and tissue inhibitors of matrix metalloproteinases [57].

Fig. 2. Arterial stiffness impact cognitive function in AD. a Scheme showing that a protective stiffness gradient between the heart and periphery is present when the arteries are elastic. Arteries become stiff during aging, which is accelerated with cardiovascular diseases. As a consequence, excessive, potentially harmful pulsatile energy is transferred to the microvasculature of vulnerable organs such as the brain, kidney and heart. In the brain, it affects vascular function and impacts the integrity of the brain, which leads to cognitive impairment. b Relationship between pulse wave velocity of the aorta, a measure of arterial stiffness, age and arterial mean pressures. The velocity of the pulse wave correlates directly with the stiffness of the aorta. From [58].

Transmittance of Pulsatile Stress to the Brain due to Stiffening of Central Arteries

The cerebral vasculature experiences an increase in biomechanical force during ageing, which has been found to significantly affect the integrity of the brain and cognitive function (Fig. 2). Under normal conditions, the pulsatile blood flow, which is created by the rhythmic action of the heart, is transferred to the aorta and its proximal branches, which are rich in elastin that dampens the pulsations through the Windkessel effect (Fig. 2a) [58]. This dampens the arterial pulse wave and transforms the pulsations into an almost continuous blood flow into the peripheral vasculature. With increasing age, the central elastic arteries become stiff caused by changes in the components of the vessel wall with addition of collagen, fibronectin and proteoglycans, a decrease and fracture of elastin, and an increase in vascular transforming growth-factor-beta, interstitial cell adhesion molecules and matrix metalloproteinases [59]. Increased aortic stiffness results in elevated aortic pulse wave velocity.
Reflected waves arrive earlier at the aorta, during systolic part of the cardiac cycle [60]. The systolic blood pressure is augmented, while diastolic blood pressure is reduced, creating an increase in pulse pressure. Thus, the protective stiffness gradient normally present between the heart and periphery is impaired and excessive, potentially harmful pulsatile energy is transferred to the microvasculature of vulnerable organs such as the brain but also in the retina, kidney and heart, causing end-organ damage [61]. Moreover, age-related remodeling of the microvasculature can affect the resistance of the vascular bed and thus renders it more vulnerable to pulsatile stress [44, 45].

Epidemiological studies have shown an association between increased aortic stiffness with cognitive impairment in patients with AD and other forms of dementia [62]. Furthermore, increased arterial stiffness is a significant predictor of subsequent cognitive decline [63]. Conversely, intervention studies have shown that drugs that can lower arterial stiffness have positive effects on cognitive function [64]. Neuroimaging studies have shown associations between arterial stiffness and measures of brain integrity. For example, increased pulse wave velocities were found to be associated with lower grey matter density [65]. In addition, increased pulse wave velocities were associated with white matter hyperintensities, and cerebral microbleeds in cognitively impaired people [65, 66]. Increased arterial stiffness affects the hemodynamic function of the cerebral vasculature. For example, a recent study found an association between aortic stiffening and reduced rCBF in cognitively normal older adults, without clinical presentation of stroke and dementia [67]. Interestingly, the study also demonstrated that APOE-ε4 carriers were found to have higher pulse wave velocity and lower rCBF in the whole brain and in the temporal lobe, compared to non-APOE-ε4 carriers. Similarly, a recent study has shown impairment of rCBF at rest and after stimulation and cognitive dysfunction in an experimental mouse model of arterial stiffness based on carotid calcifications [68]. Moreover, cardiovascular diseases such as hypertension, diabetes and hypercholesterolemia, which are risk factors for AD and dementia [69], are associated with increased arterial stiffness, and an amplification of the occurrence of cerebrovascular abnormalities [66] (Fig. 2a).

Importantly, arterial stiffness is partially modifiable by medication [70], and lifestyle such as aerobic exercise and dietary supplementation [71], that are known to be protective in AD. Thus, a body of evidence links arterial stiffness to cerebral damage induced by biomechanical damage, though the underlying mechanisms, and how this is aggravated by cardiovascular disease still needs to be elucidated.

**Dysregulation Regional rCBF and Volume**

The development of neuroimaging techniques such as single-photon-emission computed tomography, arterial spin labelling and dynamic susceptibility contrast MRI allow mapping of functional parameters such as rCBF and cerebral blood volume (rCBV). The application of the techniques has provided a body of evidence of aberrations in rCBF in AD patients. Reductions in rCBF have been observed in the temporoparietal and posterior cingulate regions [72, 73]. These aberrant regions are predominantly involved in the default mode and central executive networks that are implicated in the AD pathophysiology [74]. Hypoperfusion in the frontal and occipital regions, basal ganglia, thalamus, and insula have also been reported (Fig. 3a) [75, 76]. The rate of decreases in rCBF is correlated with the severity of the cognitive impairment [77]. In addition, a recent large study in healthy controls and patients with mild cognitive impairment or AD demonstrated that rCBF changes determined by arterial spin labelling are early events and arise before changes in classical AD biomarkers, Aβ and tau, occur [78]. Consistent with this finding, decreases in rCBF have been found to precede cognitive impairment and brain atrophy [79]. In preclinical and early AD phase, hyperperfusion has also been found in regions involved in memory formation such as the medial temporal lobe, posterior cingulate cortex, prefrontal cortex and hippocampus [80, 81]. Perfusion alterations are, however, variable between studies and may reflect true, yet not well-understood differences in disease pattern, patient heterogeneity (demographics, vascular comorbidities etc.) as well differences in the methodology of perfusion imaging techniques used. Perfusion imaging studies have also been performed in animal models of AD, and replicated findings from AD patients. Studies in animal models of cerebral amyloidosis have reported age-dependent reductions in rCBF (Fig. 3b) [46, 49–51, 82–84]. Interestingly, hyperperfusion was found in the rTG4510 mouse model of tauopathy [85], but have so far not been reported for models of cerebral amyloidosis.

Studies in animal models of AD collectively reported hypoperfusion in animal models of cerebral amyloidosis [46, 49–51, 82–84]. The reductions in rCBF have been found to be age-dependent (Fig. 3b) [50, 51, 82], and to
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occur prior to cerebral Aβ deposition [49]. Reductions in rCBF have been found in strains with neurodegeneration and brain atrophy [86], but also in models that do not have this phenotype [46, 50, 51]. Hypoperfusion has been attributed to an impaired vascular regulation by soluble Aβ [87]. Soluble forms of Aβ are vasoactive and have been shown to constrict arterioles [88]. Another study has shown that Aβ oligomers constrict human capillaries in AD mediated by pericytes [89]. This might explain hypoperfusion as an early event in AD. Other evidence has revealed that pericyte loss is associated with cerebral hypoperfusion [90] and that vascular inflammation can lead to the obstruction of capillaries by leukocyte adhesion [91]. Vascular amyloidosis has also been linked to reductions in rCBF [82]. However, regional hypoperfusion has been observed both in strains with extensive CAA [49–51] as well as in models where CAA is only sporadic [46, 83, 84]. This does not substantiate CAA as a single cause for rCBF reductions, though it might aggravate the condition.

Studies in animal models of AD have further shown that dysregulation of rCBF leads to distinct changes in the brain, depending on the extent and the duration of flow reduction. Even small reductions in rCBF, depending on the duration, lead to ischemic damage [24, 26]. In addition, hypoperfusion is a key modulator of AD neuropathology. For example, chronic hypoperfusion leads to an increased
Aβ aggregation by activation of β- and γ-secretases [93, 94]. Consistent with experimental studies, plasma Aβ has been reported to increase after cardiac arrest in humans [95]. Furthermore, hypoperfusion leads to an increased hyperphosphorylation of tau [96].

Hemodynamic alterations have also been observed with regard to rCBV. A reduction of rCBV was found in patients with AD and mild cognitive impairment [97, 98]. By contrast, one study used a method to assess arteriol rCBV and found an increase in several brain regions in patients with mild cognitive impairment compared to cognitively unimpaired controls [99]. This demonstrated that the contribution of different vascular compartments to rCBV changes should be more thoroughly investigated. Studies in animal models of cerebral amyloidosis showed a reduction in rCBV [46, 100, 101]. Reductions in rCBV occurred prior Aβ deposition [100], and had no correlation to parenchymal plaque deposition [101]. An impaired vascular regulation by soluble Aβ might also be responsible for alterations in rCBV [87]. On the contrary, a study in tau-overexpressing mice showed increased rCBV, though this occurred only at a late disease stage [52]. Thus, the complex remodelling and changes in regulation of the cerebral vasculature can affect rCBV differently, and the contribution of Aβ and tau and combined pathology needs to be investigated in further studies.

In summary, imaging studies in AD have shown that dysregulation for CBF and rCBV are critically involved in the pathogenesis of the disease. Changes in rCBF occur earlier than rCBV during the disease course [98], where metabolic and vascular risk factors have been implicated as a driver of hemodynamic disturbances [102, 103]. Consistent, experimental studies have shown that these risk factors compromise cerebral perfusion, contribute to cognitive impairment and enhances AD pathology [104, 105]. The variability of hemodynamic read-outs in AD and the fact that hemodynamic changes are also common in other forms of dementia limits the utility of methods for the individual diagnosis of AD patients, but the methods remain pivotal research tools.

Central Aβ Clearance Deficits and Peripheral Aβ Metabolism

Accumulation of extracellular Aβ has been hypothesized to result from an imbalance between the production, release and clearance of the protein [5]. Removal of proteins from the brain occurs via various overlapping clearance systems. This includes degradation via nrplysin and other Aβ-degrading enzymes, CSF reabsorption and removal through vascular mechanisms. For example, Aβ can be transferred to the bloodstream via the lipoprotein receptor-related protein-1 and P-glycoprotein [106]. A major pathway of Aβ clearance from brain extracellular fluids such as interstitial fluid and CSF occurs via perivascular draining [107–110]. In this clearing pathway, interstitial fluid enters the perivascular space surrounding the vascular basement membrane within the walls of capillaries and arteries by bulk flow, which ultimately drains into the cervical lymph nodes [109, 110]. Biomechanical modelling studies suggest that the motive force for perivascular drainage is the reflection wave of the arterial wall after normal cardiac pulse wave in the opposite direction of blood flow [111, 112]. The model predicts that hypoperfusion from proximal arterial stenosis or stiffening that result from ageing or cardiovascular diseases (e.g., atherosclerosis) would reduce the amplitude of vessel wall movement, thereby decreasing the efficiency of Aβ clearance and thus precipitate Aβ accumulation and CAA. CAA can further reduce perivascular clearance [108]. Alternatively, the paravascular (“lymphatic”) system has been implicated in the clearance of Aβ from extracellular fluids [113]. This involves the compartment enclosed between the pia mater and glia limitans, enclosing the vascular wall. Vascular pulsatility has been implicated as a motive force for paravascular clearance [114]. And an experimental study demonstrated that a reduction in vessel wall pulsatility of intracortical arterioles in aged mice was associated with reduced Aβ clearance [115].

The recently rediscovered meningeal lymphatic vessels might constitute another clearance route [116, 117]. It has been shown that the disruption of meningeal lymphatic vessels in transgenic mouse model of cerebral amyloidosis promoted Aβ deposition in the meninges and aggravated parenchymal Aβ accumulation [118]. The biomechanical driving forces of meningeal lymphatic clearance are however not yet known.

Physiological metabolism of Aβ occurs not only in the brain, but also in the periphery. Low concentrations of amyloid precursor protein (APP) and Aβ are found in plasma [119], though are lower (approximately tenfold greater) when compared to CSF levels and have different isoforms. Increased levels of APP of patients with moderate to severe AD were found when compared to age-matched controls [120]. Despite the fact that platelets and leukocytes have different APP isoforms and different preferred processing pathways, this shows that CNS alterations are reflected in the circulation. Platelets are a major source of Aβ in the blood but can also be released by leukoc...
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Evidence for BBB impairment in AD comes also from neuroimaging studies, such as dynamic contrast-enhanced MRI and PET with intravascular contrast agents. Early studies have found no evidence of BBB impairment in AD and dementia patients [135, 136]. This may be due to the fact that contrast agent leakage is more subtle than for example, in stroke or brain tumours, and the extent of leakage is not focal compared to these diseases [137]. Technical improvements in scanner hardware, sequence and pre- and post-processing analysis has helped to improve the detection of subtle BBB impairment [11, 138]. In more recent studies, contrast agent leakage was detected in AD patients and patient with cognitive impairment [137, 139–141]. BBB leakage as neuroimaging finding is associated with cognitive decline [139, 141]. In addition, biochemical studies measuring the level of plasma proteins such as albumin and immunoglobulin G in the CSF have also revealed increased protein extravasation in AD patients [142]. However, such studies cannot resolve the spatial extent of BBB impairment.

Animal studies using neuropathology, imaging and staining have provided evidence for BBB impairment both in transgenic mouse models of β-amyloidosis [31, 32, 143–145], and tauopathy [146]. Moreover, these studies have shown that BBB impairment occurs early during disease progression, before onset of Aβ deposition, CAA and cognitive impairment [145]. So far, the precise mechanisms of BBB impairment are not fully understood. Research has focused on the role of pericytes in BBB impairment. A correlation of pericyte loss and BBB damage has been shown in post-mortem studies [147]. Clinical studies have shown that CSF platelet-derived growth factor receptors β (PDGFRβ), which is shed from membrane of injured pericytes, levels were significantly higher in AD than in controls, and the extent correlated with CSF albumin level as a marker of BBB leakiness and with CSF t-tau and p-tau levels [90]. Increased CSF sPDGFRβ and regional BBB leakiness were also reported for preclinical AD, where CSF sPDGFRβ level predicted cognitive decline independently of CSF Aβ or tau level [141]. In the transgenic model of cerebral amyloidosis and pericyte loss, increased BBB damage and cognitive decline accelerated Aβ and tau pathology and neuronal loss [148]. An experimental study has shown that APOE, a risk factor of AD, activates the pro-inflammatory cyclooxygenin A-nuclear factor-kM-matrix-metalloproteinase-9 pathway in pericytes and that this leads to BBB impairment [149]. Taken together, these studies suggest that pericyte loss leads to BBB leakiness and contribute to cognitive decline in AD.

BBB impairment leads to the influx of blood constituents into the central nervous system, impairs clearing mechanism and is associated with rCBF reductions [139].

Vascular Integrity in AD: The Failing Shield

The BBB by the endothelial cell layer of the tunica intima and its associated tight junctions forms a physical and functional barrier between the central nervous system and circulation. The BBB protects the brain from the influx of plasma constituents, pathogens and immune cells, while promoting the efflux of metabolic waste products that may disturb normal neuronal functions [2]. The BBB has specialized carriers and receptors at endothelial cells that regulate transport of molecules across the BBB. Thus, the BBB has an important role of safeguarding the homeostasis of the brain. In AD and other major neurological diseases, there is a dramatic transformation of the BBB. Neuropathological studies in AD patients found plasma proteins such as fibrinogen, thrombin, albumin, immunoglobulin G and red blood cell degradation products such as hemoepiderin in the brain of AD patients, mainly in the prefrontal and entorhinal cortex, and hippocampus [26, 133]. BBB impairment was more severe in APOE-ε4 carriers [134].

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Plasma proteins such as fibrin, thrombin and albumin can directly act on neurons and glia cells, promoting neuronal and axonal damage [146, 150], and are activating an immune response [151]. Moreover, BBB impairment facilitates the influx of immune cells as demonstrated by the presence of peripheral macrophages and neutrophils in the central nervous system [152, 153]. Taken together, AD is associated with molecular and cellular changes leading to BBB impairment, which can promote neuroinflammation and neuronal damage. Longitudinal studies with optimized neuroimaging methods and pharmacokinetic models are needed to further clarify the contribution of BBB impairment to cognitive impairment and loss of brain function in AD patients [11, 137, 138].

**Fig. 4.** Dysfunctional haemostasis in AD. a Dysregulation of the haemostatic system involves the activation of both the intrinsic and extrinsic coagulation pathways, which leads commonly to the production of thrombin and the deposition of fibrin. Aβ-fibrin clots are formed, which are resistant to fibrinolysis. Hemostatic dysregulation leads to a prothrombotic and proinflammatory state and affects vascular function and integrity. b Bradykinin receptors are found in the cerebral vasculature in areas of (Aβ 6E10) deposits in Tg-SwDI mice. Confocal microscopy shows bradykinin receptor (red), tomato lectin (green), 6E10 (blue). Scale bar = 10 µm. Modified from [216]. c Fibrin(ogen) accumulation at vascular Aβ deposits in TgCRND8 mice. Shown is an overlay of Aβ (red) and fibrin(ogen; green). Scale bar = 20 µm. Modified from [26]. d Recruitment of platelets to vascular Aβ deposits in APP23 mice lead to vessel occlusion. Shown is Aβ (red), platelet specific glycoprotein Ib (green) and cell nuclei (blue). Scale bar = 20 µm. Modified from [167]. e Vessel occlusion by Aβ-fibrin(ogen) deposition in vessels of arcAβ mice. Shown is Aβ (green) and fibrinogen staining (red). Scale bar = 30 µm. Modified from [24]. f Fibrinogen deposition in the entorhinal cortex of AD patient is associated with microglia (HLA-DR) clustering. Shown are microglia (red) and fibrinogen (green). Scale bar = 100 µm. Modified from [133]. Aβ, amyloid-β; BBB, blood-brain barrier.

**Dysregulated Haemostasis**

Haemostasis encompasses the finely tuned processes of blood clotting, platelet activation, and vascular repair to maintain the integrity of the vascular system. Clinical and experimental evidence have demonstrated aberrations of the hemostatic system in AD (Fig. 4). Activated factor XII, high-molecular-weight kininogen cleavage and kallikrein activity, and bradykinin have been found in the plasma of AD patients and mouse models of the disease, indicative of the activation of the factor XII-driven contact (intrinsic) system of coagulation [50, 154]. Factor XII is activated by negatively charged surfaces, but it has been shown that it can be activated by Aβ too (in
vascular dysfunction in AD and CAA. Increased levels of activated platelets have been found in AD patients [158], and correlate with the rate of cognitive decline [159]. Circulating platelets contain high amounts of APP and possess the complete enzymatic machinery to process the protein into Aβ peptides [121]. Thus, platelets are a major source of Aβ in the blood, and might contribute to the accumulation of Aβ in cerebral vessels and the brain [160].

Activation of hemostatic system has various consequences to the vascular system, the brain parenchyma and at the systems level (Fig. 4a). Activation of the factor XII-driven contact system triggers the plasma kallikrein-mediated cleavage of high molecular-weight kininogen to release kinins, in particular bradykinin, which act through the activation of 2 G protein-coupled bradykinin receptors (B1 and B2 receptor, Fig. 4b). Increased bradykinin receptor densities have been found after chronic infusion of Aβ [161]. Bradykinin acts on smooth muscle contraction or relaxation and vasodilatation through activation of the bradykinin 2 receptor and release of endothelial-derived nitric oxide [162]. Transgenic mouse models of cerebral amyloidosis display regional hypoperfusion and an impaired vascular reactivity and it has been shown that pharmacological blockade of bradykinin receptors in these models restores hemodynamic function [50, 163]. In addition, activation of bradykinin receptors leads to a downregulation of the tight junction protein claudin-5 and BBB permeability, and leukocyte recruitment to the brain [164]. Apart from the vasculature, bradykinin receptors are also found on astrocytes and microglia and interaction of the receptor is known to activate these cells. However, reports on pharmacological blockade of bradykinin receptors on neuroinflammation have yielded contradictory results [50, 163].

An important consequence of the dysregulated haemostasis in AD is the induction of a pro-thrombotic state. The coagulation cascade involves the sequential activation of a series of factors, leading to the production of thrombin, which mediates the final step, that is, the conversion of fibrinogen into fibrin [154]. Increased pro-thrombin fragment 1 and 2 levels were detected in AD patient serum [157]. Plasma levels of fibrinogen, in particular α- and γ-chains, were found to be increased in AD patients, and have been shown to be associated with cognitive decline in mild cognitive impairment patients [165], increased risk of AD [166]. Studies in animal models have shown that fibrin forms complexes with Aβ that are deposited in cerebral microvessels (Fig. 4c, e) [24, 26]. The Aβ-fibrin clots are structurally abnormal and are more resistant to fibrinolysis, [26] and leads to persistent narrowing and occlusion of the cerebral microvasculature (Fig. 4e) [24]. In addition, platelets show an increased adhesion in blood vessels of mice with cerebral amyloidosis and accumulate at Aβ deposits (Fig. 4d) [25, 167]. Experimental studies have demonstrated a greater susceptibility of transgenic mouse models of cerebral amyloidosis to thrombus formation than non-transgenic controls [25, 26]. However, if an overlap between cerebrovascular diseases and AD is due to an aberrant hemostasis, this still needs to be investigated.

Activation of hemostatic system creates also a pro-inflammatory environment by exerting an array of effects on different cell types. Direct effects of thrombin on vascular smooth muscle cells and endothelial cells have been reported [4]. For example, thrombin increases the expression of adhesion molecules such as vascular cell adhesion molecule-1, intracellular adhesion molecule-1 (ICAM-1), E-selectin, and P-selectin, which facilitate migration of leukocytes from the vessels [4]. Fibrin also mediates leukocyte recruitment through the interaction with the αMβ2 (mac-1) integrin receptor [3]. Activated platelets that adhere to the vascular wall release cytokines, growth factors, and numerous pro-inflammatory mediators such as interleukin (IL)-1 and CD40L [168]. Moreover, mediators of the coagulation cascade can extravasate via a leaky BBB and stimulate neuroinflammation by direct interaction with glial cells in the brain parenchyma, stimulating synaptic remodelling and neurodegeneration [150]. Thrombin can activate microglia via protease-activated receptors [169], while fibrinogen has been shown to cause a significant rearrangement of the microglia cytoskeleton and an increase in cell size and phagocytic activity (Fig. 4f) [170]. Plasmin can activate the complement cascade, and complement factors can activate microglia via their complement receptors [171]. Exposure of microglia or astrocytes to thrombin results in increased release of noxious reactive oxygen species and matrix metalloproteinases [172]. Conversely, experimental studies have shown that pro-inflammatory cytokines like tumor necrosis factor-α and IL-6 and complement proteins are substrates for coagulation factors and enhance fibrin deposition, which might initiate a vicious cycle between coagulation and inflammation [173, 174].

Although the contact activation system is primarily thought to function in the circulatory system, its media-
Vascular Inflammation

There is evidence that inflammatory processes may have a key role in the etiology of AD, and even precede Aβ-related neuropathology [188]. Inflammation also affects the cerebral and systemic vasculature. Microvessels from AD patients express high levels of vascular adhesion molecules such as monocyte chemoattractant protein-1 (MCP-1), ICAM-1 and cationic antimicrobial protein 37 kDa [189–191]. They also release significantly higher levels of pro-inflammatory proteins including thrombin, tumor necrosis factor-α, IL-1, IL-6, IL-8, and matrix metalloproteinases [189, 192, 193]. In addition, blood-based biomarker studies revealed increased levels of soluble vascular cell adhesion molecule-1 [194] and ICAM-1 [195] in AD patients. Levels of circulating monocytes were found decreased in AD patients, while lymphocyte counts were decreased [128, 196]. Furthermore, lymphocytes from AD patients have functional deficits [197, 198].

Vascular inflammation results from aging [199, 200] and can be enforced by metabolic and cardiovascular diseases [201, 202]. An important mediator of vascular inflammation is thrombin, which can directly activate endothelial cells and enhance the expression of pro-inflammatory proteins such as ICAM-1 and MCP-1, angiopoietin-2 release and the up-regulation of αVβ3 integrin [189]. Inflammation-induced up-regulation of cell adhesion molecules and release of inflammatory mediators can affect BBB permeability by interaction with leukocytes, and reduce vasodilation [203]. Adhering leukocytes stall blood flow in capillaries [91]. And adhesion molecule expression and BBB impairment facilitate the recruitment of leukocytes to the brain [152, 153, 204, 205]. When inflammatory cytokines and chemokines reach the brain parenchyma, they can be neurotoxic [206], and spur local inflammation by activation of microglia and astrocytes [170, 172]. Moreover, activation of glia cells can increase Aβ secretion and cleavage [207]. Conversely, exposure of endothelial cells to Aβ promotes the release of a large variety of inflammatory mediators such as MCP-1, IL-1β and IL-6 [208] and results in an increase in prostaglandin production [209]. Interaction of Aβ with the receptor for advanced glycation end products upregulates C-C chemokine receptor type 5 expression and promotes leukocyte recruitment [204]. Leukocytes express and secrete APP, with substantial amounts in the form of L-APP-KPI1, and Aβ [122, 123] and the levels of this APP can be increased when the cells are exposed to activation signals such as the mitogenic lectins or antibodies to the antigen receptors of B and T cells [210]. Thus, they can be another source of Aβ in the blood. These findings point to a molecular connection between vascular inflammation, hemodynamic dysfunction, and neuropathology; vascular inflammation is an early event where AD neuropathology in turn promotes the release of inflammatory mediator, thus initiating a vi-
Central circulation changes
- Increased central artery stiffness
- Cardiovascular diseases
- Renal dysfunction

Hematological abnormalities
- Soluble Aβ
- APP expression and processing on platelets
- Reduced Aβ-binding proteins and cells

Dysregulated hemostasis
- Intrinsic and extrinsic coagulation cascade activation
- Activated factor VII
- Increased platelets count, platelet activation
- Hypercoagulation

Systemic vascular inflammation
- Plasma pro-inflammatory cytokines
- High monocyte count
- Low lymphocyte count
- Lymphocyte functional deficits

Cerebral vascular abnormalities

Cerebrovascular diseases
- Infarcts, microinfarcts
- Microbleeds, haemorrhage
- Atherosclerosis
- Small vessel cerebrovascular disease
- Calcification

Vascular remodeling
- Fragmented vessels and branches
- Stenosis
- Glomerular loop formation
- Increased vessel tortuosity
- CAA

Hemodynamic dysfunction
- Reduced rCBF, rCBV, vascular reactivity

Protein clearance
- Plasma protein extravasation

BBB impairment
- Plasma protein extravasation

Thrombosis
- Aβ-fibrinogen clots
- Increased susceptibility

Vascular inflammation
- Adhesion molecule expression
- Leukocyte adhesion

Brain pathology

Neuronal damage
- Synaptic and axonal damage
- Inhibition of protein synthesis
- Disturbance of energy metabolism
- Ischemic damage

Neuroinflammation
- ROS production
- Microglia activation
- Astrocyte activation
- Complement activation
- Leukocyte recruitment

AD neuropathology
- Production and cleavage Aβ processing
- tau processing and aggregation
- Aβ accumulation

Protein clearance
- Plasma protein extravasation

Thrombosis
- Aβ-fibrinogen clots
- Increased susceptibility

Vascular inflammation
- Adhesion molecule expression
- Leukocyte adhesion

Fig. 5. An integrated view on vascular dysfunction in AD. The cerebral vasculature is the locus where multiple pathogenic processes converge. Dysfunction of the cerebral vasculature and brain pathology interact in multiple ways. Changes of the cerebral vasculature are triggered and driven by systemic vascular abnormalities that are part of aging, and which can be accelerated and aggravated by cardiovascular diseases. Processes contribute synergistically to cognitive impairment and dementia in the clinical spectrum of AD. Aβ, amyloid-β; APP, amyloid precursor protein; BBB, blood-brain barrier; CAA, cerebral amyloid angiopathy; rCBF, regional cerebral blood flow; rCBV, cerebral blood volume; ROS, reactive oxygen species.

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Cognitive cycle. Vascular inflammation might also constitute a pathway by which systemic infection, known to aggravate AD symptoms, might contribute to cognitive impairment [211].

Conclusion

In summary, accumulating evidence demands to include vascular factors into the complex and multi-factorial pathophysiology of AD (Fig. 5). Cerebral vascular abnormalities are highly prevalent in AD patients and can result in cognitive impairment and dementia, and thus can add to the symptomatology caused by AD pathology. However, many vascular processes directly affect and modulate, and often proceed AD neuropathology, the most important one being BBB impairment and hemodynamic dysfunction. This implicates vascular dysfunction as an integral part of AD etiology and pathophysiology. The interaction is bidirectional, where AD neuropathology can also lead to changes in vascular function. In addition, many changes observed to occur at the cerebral vasculature are related to systemic vascular abnormalities, which occur during aging and can be accelerated and aggravated by cardiovascular diseases. Thus, the cerebral vasculature is the locus where multiple pathogenic processes converge and contribute to cognitive impairment. Strategies that promote vascular health by managing vascular risk factors, changes in life style and medication can significantly reduce the prevalence of AD, which has been demonstrated indeed in some smaller studies [212, 213]. Other strategies may be aimed to target vascular inflam-
mation and mediators of the coagulation system. We should invest more in research that aims to understand the role of vascular dysfunction in AD and how this can be targeted therapeutically. Given the variety of processes, research across fields and medical disciplines need to be encouraged. Moreover, vascular imaging and blood-based biomarkers should be included in clinical trials of AD [214].

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