Stroke

AHA/ASA SCIENTIFIC STATEMENT

The Neurovasculome: Key Roles in Brain Health and Cognitive Impairment: A Scientific Statement From the American Heart Association/American Stroke Association

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BACKGROUND: Preservation of brain health has emerged as a leading public health priority for the aging world population. Advances in neurovascular biology have revealed an intricate relationship among brain cells, meninges, and the hematic and lymphatic vasculature (the neurovasculome) that is highly relevant to the maintenance of cognitive function. In this scientific statement, a multidisciplinary team of experts examines these advances, assesses their relevance to brain health and disease, identifies knowledge gaps, and provides future directions.

METHODS: Authors with relevant expertise were selected in accordance with the American Heart Association conflict-of-interest management policy. They were assigned topics pertaining to their areas of expertise, reviewed the literature, and summarized the available data.

RESULTS: The neurovasculome, composed of extracranial, intracranial, and meningeal vessels, as well as lymphatics and associated cells, subserves critical homeostatic functions vital for brain health. These include delivering O_2 and nutrients through blood flow and regulating immune trafficking, as well as clearing pathogenic proteins through perivascular spaces and dural lymphatics. Single-cell omics technologies have unveiled an unprecedented molecular heterogeneity in the cellular components of the neurovasculome and have identified novel reciprocal interactions with brain cells. The evidence suggests a previously unappreciated diversity of the pathogenic mechanisms by which disruption of the neurovasculome contributes to cognitive dysfunction in neurovascular and neurodegenerative diseases, providing new opportunities for the prevention, recognition, and treatment of these conditions.

CONCLUSIONS: These advances shed new light on the symbiotic relationship between the brain and its vessels and promise to provide new diagnostic and therapeutic approaches for brain disorders associated with cognitive dysfunction.

Key Words: AHA Scientific Statements ■ aging ■ Alzheimer disease ■ cerebrovascular circulation ■ cognition ■ dementia, vascular ■ leukoaraiosis

Dementia is one of the world's largest public health problems. In the United States, >500 000 individuals are diagnosed with dementia each year.[1] More than 1.5% of the US population is currently living with dementia.[1] The costs for providing care for individuals with dementia are enormous, exceeding those for cardiovascular disease or cancer.[2]

The predominant research focus in Alzheimer disease (AD), the most common cause of clinically diagnosed dementia in the elderly, has been on the pathobiology

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of amyloid plaques and neurofibrillary tangles, with relatively little attention paid to the neurovascular components of the disease. It is notable that disturbances in blood flow delivery[3] and blood-brain barrier (BBB) permeability[4] appear to occur before the onset of symptoms and before evidence of neurodegeneration. Furthermore, recent autopsy studies have revealed that brain infarcts are found in almost half of patients diagnosed with AD during life, and many brains also have evidence of arterial lipid deposits (atherosclerosis) or arteriolar wall thickening (arteriolosclerosis).[5] At the same time, improved cardiovascular health has been shown to correlate with improved brain health and reduced incidence of dementia later in life.[6] These converging observations highlight the critical relationship between the neurovasculature and brain health.[7] However, the mechanisms and pathogenic relevance of this apparent linkage remain elusive. Therefore, the question remains: Is cerebrovascular dysfunction an epiphenomenon of the neurodegenerative process or a pathogenic contributor?

The brain has high energy requirements that are fulfilled by oxygen and glucose delivered to a large number of functionally distinct brain regions by an intricate network of blood vessels stretching >400 miles that are densely packed into the brain substance.[8] Considering that the brain's requirements for oxygen and glucose vary in space and time depending on the level of brain activity, delivering the appropriate amount of blood to each region poses a staggering logistical challenge. Accordingly, the structural, functional, and molecular diversity of the cerebral vasculature is astounding, and the interaction between brain cells and the local vasculature is remarkably complex compared with that of other organs.[8,9]

Historically, the brain and its vasculature have been considered separate domains, with neuroscientists concentrating on brain cells and vascular biologists on blood vessels. As a result, a rigid distinction was placed between cerebrovascular diseases (eg, stroke) and neurodegenerative diseases (eg, AD), considered mutually exclusive conditions. Over the past decade, a wealth of basic science observations have shed light on the close structural and functional interaction between brain cells and vascular cells, culminating in the establishment of the neurovascular unit concept.[10] At the same time, epidemiological and clinical-pathological studies have revealed an unanticipated overlap between vascular and neurodegenerative pathologies, especially those underlying dementia, including AD.[11] In this scientific statement, we explore the emerging data on the cellular, molecular, and functional bases for these interactions in health and disease, and using this evidence, we will seek to identify unresolved issues and outstanding questions. Last, we highlight potential ramifications for preventing and treating the rapidly expanding burden of dementia in the United States and the world.

METHODS

This statement was commissioned by the American Heart Association's Stroke Council and is in accordance with the American Heart Association's conflict-of-interest and ethics policies.

Authors with expertise in vascular biology, cellular and molecular biology, neurology, neurophysiology, neuroimmunology, cognitive science, and neuropathology pertaining to the structure, function, and neurovascular pathobiology were selected to contribute to this statement. All current members of the American Heart Association/American Stroke Association were eligible for selection. Topics for the statement were identified by the chair and vice chair and revised with input from the writing group. Subgroups of experts for each of the topics were established and charged with writing the section of the statement pertaining to their expertise. Each subgroup performed a search of the relevant English literature considered for inclusion in this statement using an up-to-date search strategy of reference databases and appropriate search terms. Conflicts were resolved by group consensus. The search also included a review of bibliographies and manual searches of key articles. Drafts of each section were written and sent to the chair and vice chair of the writing group for editing and elimination of redundancy. The edited sections were returned to the group members for clarification and revisions and sent back to the chair and vice chair. The sections were then assembled in a single document that was sent back to the members for discussion and comments. On the basis of these discussions and consensus, sections were then edited accordingly by the primary author and returned to the chair and vice chair for further editing. The document was circulated among all members of the writing group, and once consensus was reached, the final document was submitted for independent peer review. After peer review, the final document was approved for publication by the relevant American Heart Association councils and the Science Advisory and Coordinating Committee.

NEUROVASCULOME AND NEUROVASCULAR UNIT

Throughout this statement, the term neurovasculome refers to the entire extracranial (aortic arch to base of the skull) and intracranial vasculature and associated cells pertaining to skull, brain, and meninges (arteries, capillaries, veins, and lymphatics). The inclusion of extracranial arteries is justified by their contribution to cerebrovascular regulation[9] and of extracranial veins for their role in cerebrospinal fluid (CSF) homeostasis and clearance.[12] Neurovascular unit refers to the specific neurovascular associations occurring at each segment of the neurovasculome. Therefore, the neurovasculome is constituted by multiple neurovascular units that vary by cell type composition, depending on the specific vascular segment.[9]

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THE NEUROVASCULOME: EMERGING MOLECULAR AND CELLULAR DIVERSITY

Single-cell and cell type–restricted molecular approaches have been increasingly applied to study the neurovasculome. Although low sensitivity for low-abundance transcripts (eg, ion channels and G-coupled receptors) is a limitation, these techniques have proven crucial for revealing the identity and heterogeneity of vascular cells in the brain. A list of the major relevant databases is provided in Supplemental Table 1.

Single-Cell Methods

Single-cell RNA sequencing (RNA-seq) methods allow the transcriptome of thousands of cells to be interrogated simultaneously, making it possible to define the brain vessels at unprecedented molecular resolution.[13–15] Single-cell RNA-seq combined with bioinformatics is a robust approach to define transcriptional cell signatures, uncovering previously uncharacterized cellular diversity that, together with in situ techniques, allows the developmental, functional, and topographic classification of vascular and vascular-associated cells. In addition, it is now possible to study normal and abnormal brain vessels in postmortem human brains using single-nucleus RNA-seq,[16–18] and spatially resolved transcriptomics allows merging molecular data with tissue imaging approaches.[19] These techniques have been essential in classifying subpopulations of vascular and vascular-associated cells along the neurovascular tree[13–15] and are starting to reveal regional differences in vascular transcriptomics, for example, between the neocortex and hippocampus.[9]

Heterogeneity and Spatial Organization of Vascular and Vascular-Associated Cells

In the mouse brain, endothelial cells (ECs) exhibit significant transcriptional heterogeneity yet form a seamless continuum of shifting transcriptional states along the arteriole-capillary-venous network.[13] An initial single-cell transcriptomic study in mouse brain identified 3 EC clusters corresponding to arterial, capillary, and venous zones, and subsequent work expanded this to 7 endothelial clusters, further dividing arterial ECs into 3 subgroups and adding transitional zones between arterioles-capillaries and capillaries-venules.[20] The arterial ECs exhibit enriched expression of genes for cellular plasticity (*Mgp*, *Fbln5*, *Eln*, *Igfbp4*, *Clu*, *Sema3g*) and signal transduction (*Bmx*, *Efnb2*, *Vegfc*, *Hey1*), whereas capillary ECs express genes for transport, metabolism, and O_2 response (*Mfsd2a*, *Tfrc*, *Slc16a1*, *Fmo2*). Venous ECs exhibit heightened expression of genes for inflammatory signaling (*Cfh*, *Il1r1*, *Vwf*, *Vcam1*). Emerging evidence demonstrates regional heterogeneity also in capillary ECs. For example, a *Plvap*-positive capillary EC cluster has been identified in the circumventricular organs (CVOs) where capillaries are more permeable than in other brain regions.[21]

In contrast to the continuum of transcriptional states with ECs, murine mural cell types segregate into 2 distinct subclasses.[13] One corresponds to smooth muscle cells (SMCs) on the arterial side, which are further subdivided into arterial/large arteriole and small arteriole groups. Reflecting their dynamic roles in blood flow regulation, arterial SMCs/arteriolar SMCs express genes encoding contractility and cytoskeletal reorganization (*Acta2*, *Tagln*, *Cnn1*, *Pdlim3*). The arteriolar SMC class may be synonymous with ensheathing pericytes[22,23] or precapillary SMCs[24] found in the transition zone between arteriole and capillary branches.[25] A second class corresponds to a broad continuum of transcriptional states between pericytes and venular SMCs, which express little to no *Acta2* but are more enriched in the expression of genes for signaling (*Pdgfrb*, *Vtn*), metabolic sensing and transport (*Abcc9*, *Kcnj8*, *Atp13a5*, *Art3*, *Ggt1*, *Grm3*),[26] and cell growth and survival (*Ptn*).[27] The identification of mural class-specific markers has enabled the creation of mice for improved genetic targeting of pericyte/venous SMC subclasses.[28]

Several additional vascular-associated cell types reside within the perivascular space, delimited by the vascular basement membrane in which the mural cells are embedded and the glial basement membrane next to the astrocytic endfeet. On larger arterioles, the perivascular space is more evident, but as the diameter of vessels decreases, the glial and pial basement membrane layers fuse together, and the perivascular space disappears. Cell types within the perivascular space include perivascular fibroblasts and perivascular macrophages (PVMs). Seven clusters of fibroblast-like cells have been identified from whole-brain single-cell transcriptomic studies,[20] with 2 classes representing perivascular fibroblasts identified by studies of vascular-enriched preparations.[13] Like PVMs, perivascular fibroblasts are found on arterioles and large venules but not capillaries, except in the leaky capillaries of the CVO in the brain.[13,21,29] Like mural cells, perivascular fibroblasts express *Pdgfrβ* but are unique in their expression of genes for proteins of the extracellular matrix and basement membrane (*Col1a1*, *Col1a2*, *Col6a1*, *Col6a2*, *Lama1*, *Efemp1*) and modulators of its stability (*Itih5*, *Spp1*), suggesting a role in the regulation of vascular basement membrane. Brain macrophages reside at the border regions of the central nervous system (CNS), including the meninges, choroid plexus, CVO, and perivascular space. Similar to microglia, these border-associated macrophages, including PVMs, are derived from early erythromyeloid precursors emerging in the yolk sac[30,31] but can be distinguished from microglia throughout embryogenesis and adulthood by their location, transcriptome, and phenotype.[32] PVMs, which may

originate from neonatal meningeal macrophages,[33] are characterized by their expression of scavenger-receptors (*Mrc1*, *Cd163*, *Cd36*, *Lyve1*) not found in microglia and are distinguishable from choroid plexus macrophages by low expression of major histocompatibility complex class II genes (*H2-Aa*, *CD74*). However, bona fide parenchymal PVMs cannot be distinguished from pial macrophages by their transcriptome.[34] Topographically, PVMs are located in perivascular spaces surrounding parenchymal venules and arterioles, as well as pial vessels (pial macrophages), but are not associated with brain capillaries. In contrast, some microglial cells are located near vessels but within brain parenchyma (juxtavascular microglia) and are associated primarily with capillaries. They lie abluminal to the vessel wall and come in direct contact with the capillary basement membrane without intervening astrocytic endfeet.[35,36] These cells are prominent during embryonic development and early postnatal life when they exhibit motility along the vessel wall. In the mature mouse brain, juxtavascular microglia become predominantly stationary.[35–37]

Astrocytes have complex physiological roles in the CNS, including modulation of synapse formation, mediating immune cell interactions,[38] metabolic support, and neurovascular functions.[39] Transcriptomic studies have revealed a functional diversity among astrocytes across brain regions,[40,41] cortical layers,[42] or even the specific neural circuits in which they reside.[43] Whereas astrocytes surrounding blood vessels exhibit different phenotypes and transcriptomes depending on regional vascular anatomy,[21] diversity may also exist in distinct microvascular zones within brain regions, for example, in the CVO.[21] In addition, because astrocytes exhibit highly complex morphologies and extensive levels of ramification, whole-cell transcriptomics might not capture the complexity of astrocytic cell organization, as shown by the existence of a specialized transcriptome and proteome in astrocytic endfeet.[44,45]

Oligodendrocyte precursor cells, identified by expression of *Pdgfrα* among other genes in transcriptomic studies, are critical regulators of vascular development[46] and are occasionally found in close proximity to the vascular wall in the adult brain. Both oligodendrocyte precursor cells and mature oligodendrocytes (identified by *Opalin*) have been identified by vascular-enriched transcriptomic studies.[17]

Human Neurovasculome

Recently, the development of improved vascular-enrichment procedures made it possible to map the cerebral blood vessels in human postmortem brain and freshly isolated surgical specimen tissues with single-nucleus RNA-seq.[16–18] These studies revealed that the human cerebrovasculature recapitulates many features of murine arteriole-capillary-venous organization of neurovascular cell types, including heterogeneity of EC types, and punctuation of mural cell types into 2 categories. However, these studies also revealed greater diversity in cell types in humans such as 2 pericyte types dedicated to transport function and matrix regulation and showed that different brain regions can have distinct cerebrovascular features. It is remarkable that good correspondence was observed between freshly isolated and postmortem tissue,[16,18] but additional studies are needed to assess the influence of confounders typical of autopsy material such as postmortem intervals and other factors. The data also heed caution for the use and interpretation of murine studies because transcriptomic signatures of human vascular cell types differ in many ways from those in the murine brain.[16,18] Validation of some of these data is a critical next step in the field. The growth of this technology may reveal a wealth of information on neurovascular changes associated with diseases such as AD and paths forward for improvement of preclinical models in translational research.

Targeting the Neurovasculome in Mice

The discovery of cell type– and subpopulation-specific marker genes has led to the development of mouse lines that selectively and conditionally express recombinases in various vascular cells (Figure 1), which can be used for gene deletion (Cre/lox system), activation of reporter genes for cellular tagging and fate mapping, functional transcriptomics (translatome),[47] and cell type–restricted proteomics.[48] The growth of single-cell approaches described previously will enable the development of mouse lines for improved targeting of cellular subclasses through identification of more selective gene markers or design of combinatorial approaches with multiple genes. For example, approaches that rely on Cre enzyme complementation have been developed to increase cell-type specificity and have been used to discern between microglia and PVMs.[49] A list of Cre driver mice for targeting cerebral blood vessels and associated cells is provided in Supplemental Table 2. Gene targeting of brain ECs with viral gene transfer–based approaches is also feasible and is becoming increasingly popular.[50–53]

Brain on Chip

There is also considerable interest in developing "brain on chip" microfluidic models of the BBB and neurovascular unit that commonly include human induced pluripotent stem cells,[54] with an emerging appreciation for the potential need for transcriptomic validation of vascular cell subtypes[9] because a commonly used and validated EC differentiation protocol may yield cells more closely related to epithelial cells.[55] Nevertheless, these methods have started to provide new insights into the cellular biology of the cerebral vasculature.[56] For

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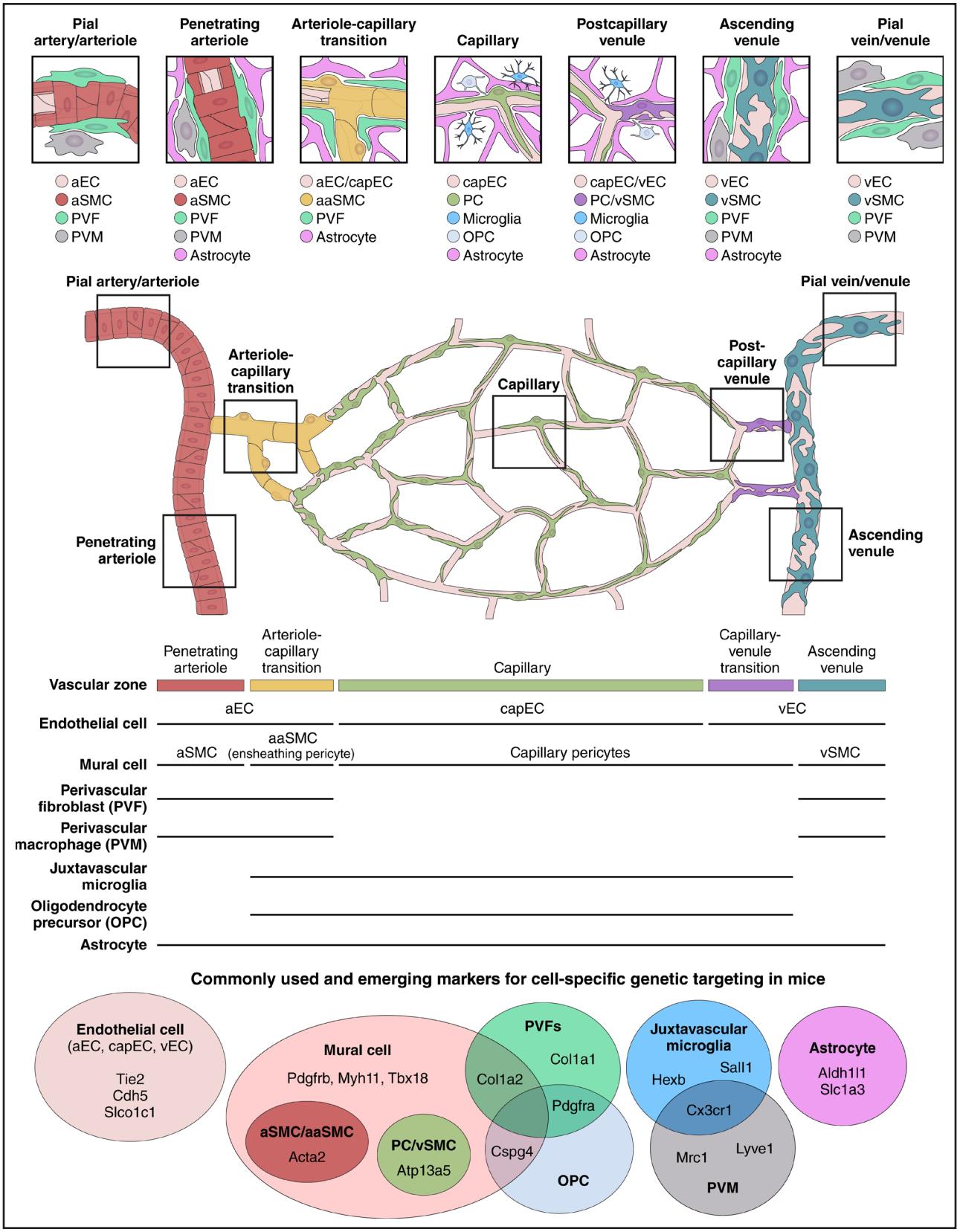

Figure 1. Vascular architecture and zonation from pial arteriole and penetrating branches diving into the brain parenchyma to ascending venule and pial venule emerging from the brain.

Schematic representation of the cerebral microvasculature and associated cells in neocortex. In the well-studied cerebral cortex, a 2-dimensional plexus of arterioles on the pial surface dives into the cortical parenchyma to form penetrating arterioles. Small offshoots emerge from penetrating arterioles to supply the capillary bed. The first few branches of these offshoots are distinct from both the penetrating arteriole and (*Continued*)

example, human induced pluripotent stem cell–derived ECs have helped establish a link between the AD risk gene *PICALM* (phosphatidylinositol-binding clathrin assembly) and enhanced amyloid-β (Aβ) clearance through BBB transcytosis.[57] Bioengineered human microvessels unveiled a role of brain apolipoprotein E and circulating high-density lipoprotein in facilitating $A\beta_{42}$ clearance from the brain.[58] Furthermore, human induced pluripotent stem cell–derived 3-dimensional microvascular cultures (EC, mural cells, and astrocytes) revealed an involvement of calcineurin–nuclear factor of activated T cells signaling in pericytes in the mechanisms by which the *ApoE4* allele may promote cerebral amyloid angiopathy.[59] Methodological developments in this area have been rapid, and more complex models involving different neuronal types, astrocytes, and microglia, in addition to vascular cells (cerebral organoids, assembloids), are fast approaching.[60]

Beyond RNA-Seq

Epigenomic DNA signatures can be studied by determining chromatin accessibility (eg, ATAC sequencing[61]) or DNA methylation state[62] at single-cell resolution, and long-read DNA sequencing of single-cell RNA libraries can be used to determine differential splicing at the single-cell level.[63] Combining these techniques together with oligonucleotide-labeled antibodies directed at cell surface proteins (cellular indexing of transcriptomes and epitopes by sequencing[64]) yields epigenomic, transcriptomic, and targeted proteomic data from individual cells. Spatial information of the brain transcriptome can be acquired by multiplexed or sequential in situ hybridization or in situ sequencing that allows targeted detection of several hundred genes at single-cell resolution.[65] Genome-wide spatial transcriptomics (slide-Seq, 10X Visium) can be studied at the level of tissue microdomains (<5 cells).[66] Although single-cell epigenomic and transcriptomic methods are now widely available and are applied to study the cerebral blood vessels in health and disease across species,[13,17,67] untargeted mass spectrometry techniques are under development to study the proteome,[68] lipidome,[69] and metabolome[70] at single-cell resolution. To date, these methods have low throughput and sensitivity and have not been applied to study the neurovasculome.

THE NEUROVASCULOME: ROLES IN BRAIN HOMEOSTASIS AND COGNITIVE HEALTH

Cerebral blood vessels have an intimate and complex relationship with brain cells that reflects their critical role in maintaining the homeostasis of the brain microenvironment. The brain's high metabolic demands, high susceptibility to energy deficit, and low regenerative capacity require a highly regulated supply of energy substrates, removal of waste, and careful regulation of the composition of the interstitial milieu. The neurovasculome achieves this goal through diverse mechanisms that engage both brain and vascular cells with segmental specificity along the cerebrovascular tree.[9] Its preeminent goals are to provide the brain with the energy substrates needed to support the functions of neurons and glia, to control the composition of the brain's microenvironment, and to enable immune surveillance. These critical functions are achieved by regulating cerebral blood flow (CBF), controlling the bidirectional molecular transfer across the cerebral endothelium through the BBB, removing the large amounts of byproducts generated by brain activity,[10,71] and directing the traffic of immune cell into and out of the brain.[72]

Cerebral Blood Flow

CBF is regulated by a wide variety of factors. Here, we briefly discuss cerebrovascular autoregulation and neurovascular coupling. More exhaustive reviews of these topics have been published recently.[71,73]

Cerebrovascular autoregulation is a relatively slow adaptive process that aims to stabilize CBF in the face of changing arterial pressure, the main determinant of cerebral perfusion pressure.[73] Within a physiologically relevant range, which varies from subject to subject and is estimated to be ±20 mm Hg mean arterial pressure,[73] increases in perfusion pressure cause resistance arteries to constrict, whereas decreases in pressure cause vasodilation, aimed at counteracting the changes in CBF resulting from changes in perfusion pressure. However, the process has a time constant on the order of seconds, and rapid changes in blood pressure escape autoregulation.[73] Cerebral autoregulation is mediated largely by the property of arterial SMCs to contract in response to increases in

Figure 1 Continued. true capillaries and are called the arteriole-capillary transition. In mice, capillaries make up >90% of the total vascular length and form a dense 3-dimensional network throughout the parenchyma.[23] Capillaries then coalesce into a larger capillary-venous transition vessel, which drains into ascending venules and eventually back into venules of the pial surface. Cell types identified by single-cell transcriptomics (Supplemental Table 1) remain to be precisely mapped onto the cerebrovascular architecture and complemented by proteomic studies. However, knowledge derived from in situ RNA hybridization, immunohistochemistry, and transgenic mouse lines begins to build the picture of where the cell types reside within the arteriole-capillary-venous circuit and their positions within the vascular wall. Available murine Cre drivers still target principal vascular and vascular-associated cell types, but mouse lines that target cell type subclusters are emerging (Supplemental Table 2). aaSMC indicates arteriolar smooth muscle cell; aEC, arterial endothelial cell; aSMC, arterial smooth muscle cell; capEC, capillary endothelial cell; PC, pericyte; vEC, venous endothelial cell; and vSMC, venous smooth muscle cell.

pressure (myogenic response). The mechanisms of the myogenic response are thought to involve pressure-sensitive ion channels and receptors on arterial SMCs, although neurogenic mechanisms mediated by the sympathetic cerebrovascular innervation, metabolic factors,[74] and possibly astrocytes[75] may modulate the response.

Neurovascular coupling is a dynamic process that provides CBF to different brain regions in response to changes in local neural activity. These mechanisms ensure a healthy supply of energy substrates and waste and heat clearance and deliver blood-borne modulatory signals such as insulin-like growth factor 1 to active regions.[9,10,76,77] It is interesting, however, that in severe acute brain injuries, neural activity induced by waves of spreading depolarizations can lead to reductions in CBF (inverse neurovascular coupling), which can induce neuronal damage.[78] Neurovascular coupling is mediated by coordinated signaling between different segments of the neurovasculome. Active neurons can trigger changes in CBF directly by releasing vasoactive molecules such as ions (eg, K^+, H^+), prostaglandins (prostaglandin E_2), epoxyeicosatrienoic acids, and nitric oxide onto SMCs to dilate arterioles.[71] Both excitatory and inhibitory neurons signal to cerebral vessels, but current evidence supports a more dominant role for inhibitory neurons, particularly nitric oxide synthase–positive interneurons.[71,79,80] Neurovascular coupling may also occur indirectly through neuronal signaling to astrocytes, which are thought to regulate the contractility of ensheathing pericytes on the arteriole-capillary transitional segment.[81] These signals can be bidirectional, with signals such as prostaglandin E_2, epoxyeicosatrienoic acids, and moderate K^+ efflux dilating and 20-hydroxyeicosatetraenoic acids and large K^+ effluxes constricting vessels.[81,82] ECs also sense neuronal or astrocytic signals to facilitate coupling of neural activity to CBF.[83] Both ECs and mural cells are important conduits that propagate vasoactive signals along the vascular tree.[84–86] Activation of inward-rectifier K^+ and transient receptor potential ankyrin 1 channels has been implicated in endothelial hyperpolarization, which propagates retrogradely to relax contractile mural cells upstream.[87,88] Coordinated dilatation of upstream and downstream vascular segments is essential for increasing flow efficiently in interconnected vascular networks.[9]

Other factors that have a profound and more global effect on CBF include arterial P_{CO_2} and P_{O_2}. Hypercapnia increases and hypocapnia decreases CBF, and hypoxia increases CBF.[73] In addition, numerous other factors can influence CBF globally, either directly or indirectly, for example, posture, circulating glucose, hormones and peptides, hematocrit, blood viscosity, and cold stress.[73,89] These regulatory mechanisms, intrinsic and extrinsic to the brain, work in concert to ensure that the brain is adequately perfused.

The BBB

The BBB is formed by the EC layer that lines the walls of brain microvessels and serves as a physiological barrier between the blood and neural tissue.[90] These specialized ECs are different from ECs in other circulatory districts because they (1) have specialized tight junctions that prohibit paracellular passage of water-soluble molecules; (2) have a low rate of vesicular transport (transcytosis) to prevent free transcellular trafficking; (3) express specific influx and efflux transporters, which allow molecule-specific exchange between the blood and the CNS; and (4) have low expression of leukocyte adhesion molecules and play a role in regulating the influx of immune cells. The restricted permeability of brain vasculature has been attributed historically to the specialized tight junctions,[91] but recent work demonstrated that vesicular transport (transcytosis) is actively inhibited in cerebral ECs.[92–95] Although CNS ECs constitute the BBB, BBB properties are not intrinsic to ECs but require active induction and maintenance from the CNS environment.[96] For example, endothelial influx and efflux transporters contribute to maintenance of brain Aβ homeostasis.[4,12] During development, Wnt from neural tissues is a key BBB induction cue and continues to play a role in BBB maintenance.[97] Pericyte and astrocyte deficiency also results in BBB leakage,[98–101] and pericyte-secreted vitronectin may regulate BBB through integrin receptors on the neighboring ECs.[102] The specific astrocytic cues that regulate the BBB are poorly understood, but in vitro data suggest a role for tissue-type plasminogen activator.[103]

Clearance Mechanisms and Proteostasis

Besides meeting the high metabolic demand of the brain by delivering oxygen and nutrients to the tissue through blood flow, the neurovasculome also plays a major role in conveying proteins and metabolites from the brain parenchyma and to the lymphatic system. Various clearance mechanisms include transport across the BBB, clearance through dural lymphatic vessels, and drainage of soluble waste products along the perivascular spaces surrounding penetrating blood vessels.[12] The relative contribution of each of these pathways in health and disease remains to be defined, but the role of perivascular clearance has received increasing attention as a potential key mediator in the pathophysiology of AD and related dementias. Although there are outstanding controversies concerning the exact anatomic pathways of perivascular clearance and how they function, the leading paradigms in the field suggest that soluble waste products travel along spaces surrounding individual penetrating vessels (Figure 2). According to the glymphatic model, CSF flows inward through arteriolar perivascular

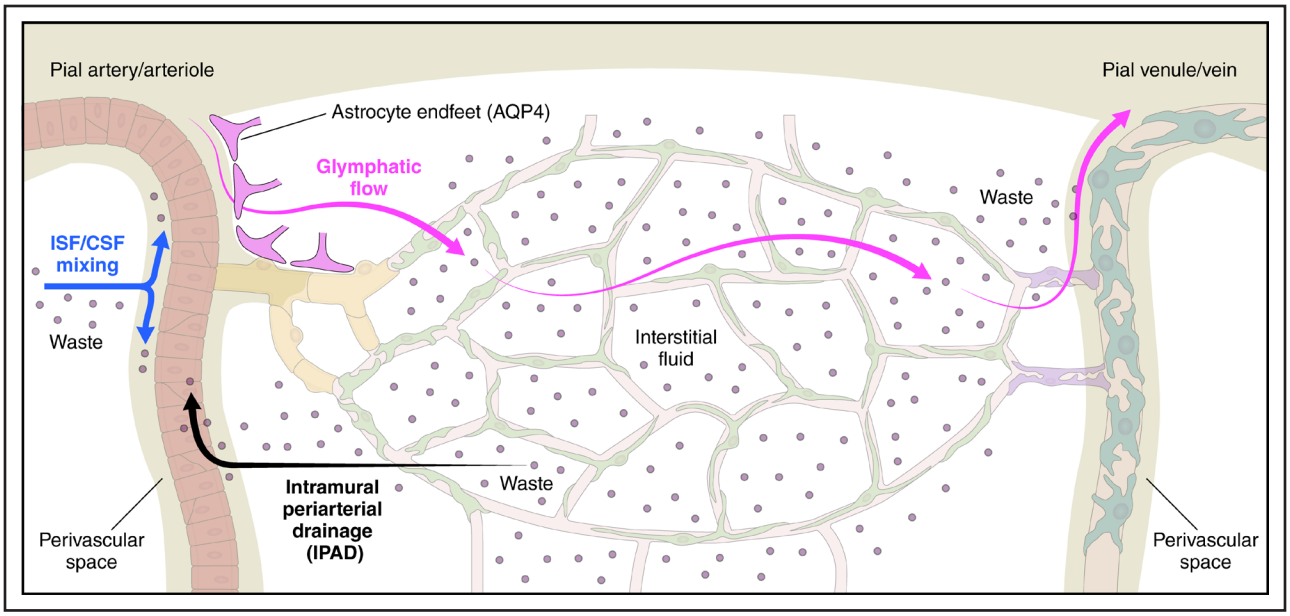

Figure 2. Schematic representation of neurovascular clearance pathways from the brain.
Simplified depiction of the major clearance pathways operating through the neurovasculature. These include intramural periarterial drainage, mixing between cerebrospinal fluid (CSF) and interstitial fluid (ISF) and glymphatic flow. See text for details and references.

spaces, where it mixes with interstitial fluid and drains along perivenular spaces in the same direction as blood flow.[104] Glymphatic clearance is most active during sleep, and impaired clearance of toxins, possibly caused by disordered sleep structure, has been implicated in neurodegenerative diseases.[105] In contrast, the intramural periarterial drainage model suggests that solutes within the interstitial fluid drain along the basement membranes of the same arterioles in the opposite direction of blood flow.[106] Whether perivascular pathways for fluid also exist along the smallest blood vessels in the brain, including the arteriole-capillary transition and at the level of the capillaries, remains unclear. However, these small vessels represent the majority of total vascular length and may have a role in the drainage of interstitial fluid. The microarchitecture of the perivascular spaces along different vascular zones, as well as the cell types that contribute to the formation of the spaces, remains poorly defined. More recently, an alternative model was proposed that does not rely on perivascular drainage routes. It suggests that there is mixing of CSF and interstitial fluid at the pial surface, which allows soluble waste products to be cleared without the need for unidirectional flow.[107] Additional experiments designed to test assumptions underlying these proposed models are needed next steps to harmonizing conflicting experimental observations that may partially be attributable to heterogeneous experimental setups across laboratories.

Another area of ongoing debate concerns the driving forces that move solutes along various proposed perivascular clearance routes. Several studies have suggested that arterial pulsations, generated by the heartbeat and breathing, can generate unidirectional fluid flow in perivascular spaces.[108,109] In direct imaging, fluorescently labeled tracers injected into the cisterna magna were observed to travel into the brain along periarteriolar spaces in a pulsatile manner in phase with the cardiac cycle.[109] Whether these rather subtle arteriolar wall displacements are strong enough to drive fluid out of the brain along perivenular or intramural pathways has been disputed.[110]

An alternative candidate to drive fluid efflux is vasomotion, which is observed during resting wakefulness as spontaneous large fluctuations in arterial diameter that occur at low frequency (0.1 Hz).[111–113] Low-frequency hemodynamics have been found to increase during sleep, which may facilitate waste drainage during the night. Of note, preclinical studies investigating perivascular fluid flow often rely on invasive techniques (including cranial window surgeries and injection of tracers) and the use of anesthesia, which interfere with the brain's natural homeostatic environment and cerebral hemodynamics.[114] Thus, there is a great need for the development of noninvasive imaging techniques to track fluid flow dynamics, preferably in the awake resting or sleep state.

Immune Cell Trafficking

Immune cell trafficking into the brain is restricted by tight junctions in ECs, choroid plexus epithelium, arachnoid cells, and CVO tanycytes, each acting as a barrier.[115–117] However, tight junctions are largely absent

in blood vessels of the dura mater, choroid plexus, and CVOs, resulting in free bidirectional exchange of macromolecules and immune cells between the blood and the surrounding tissue. In particular, choroid plexus and dura mater are well perfused[118] and have emerged as key sites of neuroimmune regulation.[115–117]

Several immune cells are found in the extravascular space of the dura mater, including dendritic cells, monocytes, neutrophils, natural killer cells, T cells, and B cells.[34] Cell tracking studies have shown that intestinal immune cells contribute to the pool of dural immune cells.[119] Besides extravasating from dural vessels, immune cells might also migrate directly from the skull bone marrow along penetrating diploic veins[120] and bridging vessels[121] that connect subarachnoid vessels to dural lacunar veins and the skull bone marrow.[121–125] Under pathological conditions, they can penetrate the arachnoid layer and enter the brain parenchyma.[121,122]

Compared with other brain myeloid cells, stromal choroid plexus macrophages, also yolk sac derived,[126] are short-lived (weeks in the adult mouse) and are at least partially replaced by blood monocyte–derived macrophages.[30,127] Other cells found in the choroid plexus stroma are lymphocytes, natural killer cells, monocytes, and dendritic cells.[34] Peripheral immune cells are found in CVOs under homeostatic conditions.[128]

The normal CSF contains mainly memory T cells.[129] Under homeostatic conditions, CSF T cells are transitory[129] and egress from the CSF through dural lymphatic vessels to reach deep cervical lymph nodes and through transcribrosal lymphatics to reach the superficial cervical lymph nodes.[130] Extravasation of effector T cells from pial vessels and choroid plexus and transarachnoid migration from the dural extracellular space have been observed in diverse pathologies.[116] Resident brain immune cells such as microglia and macrophages also interact with infiltrating inflammatory cells in the setting of brain diseases and can lead to pathology by producing cytokines, chemokines, and other proinflammatory and toxic molecules.[32]

These observations collectively highlight the diverse nature and mechanistic heterogeneity through which the neurovasculome maintains the homeostasis of the working brain.

HOW DOES THE NEUROVASCULOME INFLUENCE COGNITIVE FUNCTION

Alterations of structure and function of the neurovasculome have a profound effect on brain health, and a growing body of evidence suggests a link between neurovascular dysfunction and cognitive impairment in humans.

Flow-Dependent Mechanisms Causing Ischemia and Brain Injury

Hypoperfusion is a key feature of vascular cognitive impairment and dementia (VCID) and leads to ischemic injury to the white matter (WM) and gray matter. WM lesions (WMLs) are commonly seen in aging and, when the burden becomes high, are a substrate for VCID. Data from epidemiological and postmortem pathological studies show that vasculopathy and reduced WM perfusion are significant features of WMLs,[131] which tend to accumulate in the deep watershed territories of the WM and are a major contributor to increased risk for dementia.[131] There are also data to suggest flow-independent mechanisms that stem from the injured cerebral endothelium and result in inflammation and BBB leakage within and adjacent to WMLs.[132] Epidemiological data show a strong association between WMLs and vascular risk factors such as hypertension and diabetes but also vascular diseases such as aortic stiffness and atrial fibrillation without stroke.[133] Postmortem studies demonstrate extensive pathological changes across the WM microvascular bed.[134] These include wall thickening by hyalinosis and fibroid necrosis of the arterioles, SMC loss, rarefaction of the capillaries, and thickening of the venule walls by collagen deposition (venous collagenosis), which are associated with pallor of adjacent perivascular myelin and astrogliosis.[135,136] Perivascular accumulation of axonal structural proteins, neurofilament heavy in particular, has also been reported.[137]

Recent imaging and postmortem pathological studies highlight changes in astrocytes and pericytes that are shared across various neurological conditions associated with WMLs and cognitive dysfunction. Fragmentation of the distal processes and swollen cell bodies of astrocytes (clasmatodendrosis)[138] may be observed in neuropathological specimens, but their contribution to the development of cognitive dysfunction in disorders associated with WMLs such as stroke and AD remains unclear. Earlier changes such as swollen cell bodies, cytoplasmic vacuolation, and amoeboid shape[139] could indicate that astrocytes are dysfunctional and no longer able to maintain the homeostatic environment of the surrounding WM. In addition, experimental studies have suggested a role of reactive astrocytes in neuroinflammation and Aβ production in AD models.[140] Similarly, capillary pericytes are decreased in the deep WM, but not the overlying cortex, in age-related dementias, including poststroke dementia.[141] The emerging picture suggests disrupted gliovascular and neurovascular interactions with compensatory microvascular remodeling as a substrate for a disconnection syndrome that manifests as cognitive impairment and dementia in individuals with WMLs. The interplay among WM injury, network connectivity, and cognition is supported by data from multimodal

brain imaging studies that include structural connectivity, resting-state functional magnetic resonance imaging (MRI), and WML segmentation.[142] Studies that use transcranial Doppler ultrasound or arterial spin labeling–MRI to measure CBF and neurovascular coupling in individuals with WML provide additional support for neurovascular interactions and network connectivity as a substrate for preserving healthy brain aging.[143]

Hypoperfusion in the gray matter may cause ischemic injury in the form of microinfarcts, which easily escape detection on conventional MRI because of their small size (on average, ≈200 μm on postmortem examination).[144] They are twice as frequent in individuals with dementia as in those who die without dementia.[145] Clinical and animal studies suggest that small-vessel disease (including venular disease), hypoperfusion, and embolism can cause them.[146,147] Although the volume of each individual microinfarct is miniscule, the fact that they can number in the thousands in an individual brain[148] and have effects on connectivity and neurovascular function beyond their borders[149] suggests that they can have direct adverse effects on brain networks subserving cognition.

Flow-Dependent Mechanisms Not Related to Ischemia

Recent experimental evidence has raised the possibility that neurovascular dysfunction has the potential to also alter cognitive function by disrupting the homeostasis of neuronal proteins or failing to deliver neurotrophic factors needed to maintain a healthy cognition. For example, a salt-rich diet induces cognitive impairment in mice in the absence of elevated blood pressure.[150] Cognitive dysfunction depends on the gut-brain axis through which gut Th17 lymphocytes elevate circulating levels of interleukin-17, leading to a deficit in cerebral endothelial nitric oxide, endothelial dysfunction, and reduced resting CBF.[150] However, these hemodynamic alterations do not play a role in cognitive dysfunction, which is instead mediated by a deficit in endothelial nitric oxide, which, in turn, leads to hyperphosphorylation and aggregation of tau, a protein involved in AD.[150] Other studies have suggested that the increase in CBF induced by brain activity (functional hyperemia) does more than deliver oxygen and glucose to the brain. For example, hippocampal neurogenesis, which is essential for cognitive health, relies on functional hyperemia to deliver angiocrine factors to the neurogenic niche to support neuroblast survival.[77,151] Failure of delivery of endothelial or circulating neurotrophic factors through blood flow has also been implicated in cognitive deficits in a model of autism (6p11.2 deletion syndrome).[152] The relevance of these mechanisms to cognitive impairment in humans awaits further study.

Failure of Clearance Mechanisms

Impaired perivascular clearance has been implicated in the pathophysiology of various neurological disorders. Reduced tracer movement was observed in experimental models of cerebral amyloid angiopathy,[112,153] microinfarcts,[154] apolipoprotein E isoforms trafficking,[155] and hypertension.[109] What these models have in common is impaired vessel function. In patients with cerebral small-vessel disease, loss of vessel function may contribute to the accumulation of proteins in the walls of blood vessels such as Aβ in cerebral amyloid angiopathy.[156] This could lead into a feed-forward cycle of SMC loss, impaired clearance, and continued protein buildup, eventually resulting in vessel wall breakdown and ischemia or hemorrhage. A deeper understanding of perivascular waste clearance systems may elucidate their involvement in cognitive dysfunction and provide novel targets for intervention.

Neuroimmune Mechanisms

Innate and adaptive immunity can have profound effects on cognitive function. Neutrophils, innate immune cells, have been shown to contribute to capillary stalling in a mouse model of AD, and blocking neutrophil-endothelium interactions improved cognitive function.[157–159] In humans, hyperactivated neutrophils correlate with AD progression, whereas a higher neutrophil-to-lymphocyte ratio correlates with any dementia.[160–162] PVMs are increased in aged and hypertensive rats[160] and contribute to neurovascular and cognitive dysfunction in models of hypertension or amyloid accumulation through oxidative stress.[52,163] Microglia are involved in the deleterious effects of amyloid and tau pathology and neuroinflammation in mouse models.[164] Increased reactive oxygen species and proinflammatory cytokines, coupled with impaired migratory capacity and reduced Aβ phagocytosis, were found in blood monocytes of individuals with mild cognitive impairment and AD.[165] As for adaptive immunity, loss of naive T cells during aging decreases T-cell diversity and favors the expansion of memory T cells, including memory effector T cells. The number of adaptive immune cells in brain increases with age. For example, age-related increases in vascular-associated T cells were observed within the WM of monkeys.[166] Clonally expanded interferon-γ–producing $CD8^+$ T cells have been described in the subventricular zone of aged mice.[167] Similarly, a higher proportion of activated $CD8^+$ T cells are found in the CSF of patients with AD compared with healthy age-matched control subjects, which correlated with AD-related cognitive decline.[168] An increased lymphocyte-to-monocyte ratio was positively correlated with dementia incidence.[169] In conclusion, although few animal studies have suggested a clear cause-effect relationship between increased immune cell trafficking to the brain and cognitive decline, correlative studies support an association of activated innate and

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adaptive immune cells with cognitive dysfunction. Furthermore, the recent discovery that CNS antigens accumulate in dural sinuses and are presented to circulating lymphocytes, a process disrupted during neuroinflammatory states and aging, suggests the possibility that alteration of the cross-talk between CNS and systemic immunity may have a role in brain disease.[170]

THE NEUROVASCULOME IN STROKE, AD, AND RELATED DEMENTIAS

Abundant epidemiological evidence implicates neurovascular dysfunction as a risk factor for age-related cognitive decline, vascular cognitive impairment, AD, and related dementias. Of the 12 modifiable risk factors identified by the Lancet Commission with the strongest evidence for potential prevention of dementia, 9 are also risk factors for cardiovascular disease.[171] Hypertension, diabetes, smoking, obesity, and hypercholesterolemia alter neurovascular regulation[172,173] and increase the risk for dementia.[11] It is likely that neurovascular dysfunction mediates at least some of the associations of these risk factors with dementia. For many of these risk factors, the elevation in risk can be detected in midlife,[174] suggesting that early neurovascular dysfunction begins several decades before clinical symptoms.

Stroke

Symptomatic stroke is a major cause of VCID.[175] Cerebrovascular disease can, in addition to stroke, cause covert vascular damage to the brain, detectable on neuroimaging as lacunar infarcts, WMLs, cortical microinfarcts, and cerebral microbleeds. In patients with cerebral small-vessel disease, damage to the cerebral WM may begin with increased BBB permeability, followed by suppression of cerebrovascular responses to CO_2 and then ischemia,[176] although other studies suggest that ischemia in watershed areas between cortical and subcortical arterial territories is the initial pathogenic event.[172,173]

Cognitive Decline

Stroke and covert cerebrovascular damage are not the only ways by which neurovascular dysfunction causes cognitive decline. Evidence from human studies suggests that neurovascular dysfunction occurs early in the AD pathophysiological process, when it may possibly serve as an initiator or accelerator of AD pathology. For example, a study in the general population found that higher total vascular risk factor burden was associated with greater burden of Aβ 20 years later.[177] A drop in cerebral perfusion is one of the earliest detectable alterations in the brains of individuals with AD.[3] Many, but not all, studies suggest that cerebrovascular reactivity to CO_2 is reduced in AD.[178] Decreased clearance of Aβ is hypothesized to be the initial step in vascular Aβ formation; human studies suggest that sporadic AD is the result of failure to adequately clear Aβ peptide from the extracellular space into the CSF or the blood.[179] This suggests a potential role for neurovasculome-related clearance mechanisms in the initiation of AD, although the exact causes of this failure to clear Aβ and tau remain elusive. Studies in animal models strongly implicate failure of perivascular, glymphatic, and dural lymphatic pathways in the clearance impairment.[105,112,180,181] A growing ability to use biomarkers of neurovascular and clearance functions in living human subjects, discussed in more detail in the subsequent section, may further clarify which mechanisms are at play in the pathogenesis of AD and related dementias.

NEUROVASCULAR BIOMARKERS OF COGNITIVE DYSFUNCTION

Emerging technologies are increasingly offering new windows into human neurovasculome function, either directly in the brain—often through neuroimaging—or by identifying peripheral surrogates of these processes and linking them with cognitive decline. Some of these biomarkers are summarized in the Table, focusing on those associated with specific neurovasculome cell types and neurovasculome function. These biomarkers are in various stages of validation, but all of them have some evidence of proof of concept (ie, that they measure a change related to a process) and proof of principle (ie, that they have been associated with cognitive decline or with cognitive disorders such as AD).

Many studies have examined cerebral and systemic vascular hemodynamic measures, typically derived from ultrasonography or MRI, in relation to cognition. These studies demonstrate that impairments in several vascular hemodynamic measures are associated with cognitive function, cerebral small-vessel disease, and CSF tau, Aβ, and inflammatory biomarkers.[178,188,204,205] Pilot studies using various MRI techniques to measure bulk movement of CSF, which may play a role in Aβ clearance, are beginning to emerge.[195] Fluid biomarkers of lipid metabolism—including ceramides, cholesterol esters, low-density lipoprotein C, apolipoprotein B, and lipoprotein(a)—have been linked to cerebral WM changes and cognition.[199,200] Putative blood markers of microvascular dysfunction and inflammation have been associated with cerebral small-vessel disease and cognitive decline.[198,203] Soluble platelet-derived growth factor receptor β, released by pericytes when under stress, has been detected in CSF in patients with AD.[183] Circulating microparticles released from ECs are an emerging vascular biomarker class and seem to be elevated in patients with AD with vascular risk factors compared with patients with AD without vascular risk factors, although they are not associated with cognitive decline.[202] Despite the growing panoply of useful biomarkers, they still fail to explain all of the associations between vascular risk and cognitive decline,[206] indicating

Table. Selected In Vivo Human Neuroimaging and Fluid Biomarkers of Neurovasculome Dysfunction and Cognitive Decline

Pathophysiological process	Biomarker	
	Imaging	Biofluid
Astrocyte dysfunction	…	Glial fibrillary acidic protein[182]
Pericyte stress	…	Platelet-derived growth factor β[183]
Functional connectivity	Resting-state fMRI[184] Task-related fMRI[185]	…
Low CBF	ASL MRI[186] ^{15}Oxygen PET[187]	…
Altered cerebrovascular reactivity to CO_2	Evoked TCD flow velocity[178] Evoked MRI ASL or fMRI BOLD responses[188]	…
Vascular stiffness and pulsatility	Ankle-brachial index[189] Pulse-wave velocity measurements[190] Phase-contrast MRI[191]	…
BBB permeability	MR DCE and DSC permeability[192]	CSF:serum albumin ratio[193] Matrix metalloproteinases[194]
CSF bulk flow and clearance	Ultralong echo time diffusion MRI[195] Phase-contrast MRI[196] BOLD MRI[197]	…
Inflammation	TSPO PET (microglial activation)[197]	Biochemical markers, including C-reactive protein and interleukins[198]
Lipid dysmetabolism	…	Blood cholesterol[199,200] Blood lipidomics[199]
Endothelial dysfunction	Brachial flow-mediated dilation[201]	Circulating microparticles[202] Various blood markers (including fibrinogen, PAI, ICAM-1, ADMA, and others)[203]

Emerging biomarkers of vascular-mediated processes, measured in the brain or in the periphery, that have been associated with cognitive decline, dementia, clinically diagnosed Alzheimer disease, or mixed dementia.

ADMA indicates asymmetric dimethylarginine; ASL, arterial spin label; BBB, blood-brain barrier; BOLD, blood oxygen level dependent; CBF, cerebral blood flow; CSF, cerebrospinal fluid; DCE, dynamic contrast enhanced; DSC, dynamic susceptibility contrast; fMRI, functional magnetic resonance imaging; ICAM-1, intercellular adhesion molecule 1; MR, magnetic resonance; MRI, magnetic resonance imaging; PAI, plasminogen activator inhibitor type 1; PET, positron emission tomography; TCD, transcranial Doppler; and TSPO, translocator protein.

that either the biomarkers are insufficient markers of the underlying processes or additional processes remain to be discovered.

CONCLUSIONS AND FUTURE DIRECTIONS

This overview provides a glimpse of the great strides that have been made in defining the critical role of the neurovasculome in brain health and its pathogenic involvement in brain diseases. Proteomic and transcriptomic studies have revealed a remarkable molecular diversity of vascular and vascular-associated cells. Functional roles of cerebral blood vessels beyond the delivery of O_2 and critical nutrients have been identified such as clearance of toxic byproducts of brain activity, regulation of neuroimmune trafficking, and provision of factors essential for maintaining neuronal homeostasis. Failure of these mechanisms has been convincingly linked to brain diseases in animal models, especially those involving impaired cognition. These advances have also unveiled an unexpected level of complexity that raises additional questions, requiring new approaches and strategies to find answers. Some of these are listed here.

Single-Cell Studies

Cell type– and subtype-specific marker genes have generally been defined by studying healthy young mice, and more work is needed to confirm their validity across brain regions, age, sex, species, and disease states.[17,67,207] Although cell type–specific Cre-driver mouse lines have become indispensable in neurovascular studies, to date, they lack the specificity that would allow targeting of subclusters of vascular cell types. Intersectional approaches using mice harboring >1 recombinase are still needed to obtain cellular specificity for some vascular cell types.[27,49] Furthermore, new neurovascular omics studies are likely to provide new markers for specifically targeting vascular cell subpopulations. In particular, investigations of the proteome, epigenome, lipidome, and metabolome, in different brain regions, as well as gray matter versus WM, would be valuable. These approaches are essential to elucidate the functional role of vascular cell subclusters identified

by single-cell–single-nuclei RNA-seq studies and will be facilitated by methods for spatially resolved omics.[208,209]

Neurovascular Coupling

A major question in the field is how vasoactive signals from neurons, astrocytes, ECs, pericytes, and perivascular cells are integrated to shape the highly orchestrated segmental hemodynamic adjustments underlying the coupling response. For example, astrocytes may not be necessary for the CBF response to occur, but they may trigger a larger increase in CBF when engaged.[210] Perhaps they are engaged only during more sustained neural activity[211] or to help restore resting diameter.[212] Similarly, the mechanisms of the retrograde propagation of vasoactive signals from microvessels within the activated brain region to pial vessels upstream, needed for the full expression of functional hyperemia, remain to be elucidated. The relative contribution of each cell type to the propagation and expression of vasodilatation and to focusing the vasodilatation strictly to vessels within activated regions remains to be assessed. Regional differences in CBF regulation, already suggested by some studies, need to be explored at the cellular and molecular levels.[213–215] New imaging technologies (3-photon microscopy, optical coherence tomography, functional ultrasound imaging) will provide the tools to explore neurovascular function in regions located deep in the brain that are not accessible noninvasively by conventional imaging methods.[216–218] Answering these questions opens the way to elucidating which aspect of neurovascular coupling is altered in disease states and to defining its specific contributions to brain dysfunction at the regional level.

Clearance

The involvement of the cerebrovascular and lymphatic vessels in brain protein clearance implicates the neurovasculome in proteostasis. Because accumulation of protein aggregates is a critical pathogenic mechanism in a large number of brain diseases, there is a great need to gain a better understanding of the underlying pathways. What is the relative contribution of the different pathways thus implicated (Figure 2)? Are they preferentially active at specific neurovascular segments? What is the anatomy of the perivascular compartments across these segments? What are the dominant driving forces of clearance? How are they influenced by biological rhythms (sleep-awake cycle, estrogen cycle)? How do these pathways interact, and how do they ultimately drain into the lymphatic system? How do these pathways contribute to the overall fluid dynamics of the brain, from CSF and interstitial fluid production, bidirectional transfer at the BBB, and lymphatic drainage? The development of noninvasive imaging techniques of fluid flow dynamics in animal models, preferably in the awake state or sleep, is sorely needed. Such approaches will also facilitate translation of experimental insights to the human brain now that examination of fluid flow dynamics with noninvasive brain imaging is becoming a reality.[219–221]

Models of VCID and AD and Related Dementias

There is a pressing need to develop clinically relevant animal models to study the role of the neurovasculome in cognitive health. No single model to date recapitulates the comorbidities underlying cognitive impairment on vascular or neurodegenerative bases. Models are needed to reproduce the multiplicity of vascular lesions underlying VCID (microinfarcts, microhemorrhages, hypertensive lipohyalinosis, arteriolosclerosis, atherosclerosis, cerebral amyloid angiopathy, vascular inflammation). These need to be combined with models of neurodegenerative pathologies (Aβ, tau, TDP-43, α-synuclein) to reproduce mixed causes of cognitive impairment, as well as with aging, metabolic disorders (eg, obesity and diabetes), and lifestyle (eg, diet and physical activity) and genetic (*ApoE4, TREM2*) risk factors[213,222] common to both neurodegenerative and vascular pathologies. Models to investigate WM pathology, a major contributor to cognitive impairment on vascular and neurodegenerative bases, are also needed, the development of which will be facilitated by emerging technologies to image the deep cortical WM in vivo[213,217] and for gene targeting WM and associated cells.[223,224]

Prevention and Therapy

Identifying and treating vascular risk factors, which should preserve neurovasculome function, are widely acknowledged to be key components for public health strategies to optimize brain health.[7] However, definitive evidence for dementia prevention from large randomized trials is not yet available. The FINGER study (Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability) showed that an intervention package that included vascular risk reduction, healthy diet, and physical activity enhanced performance on neuropsychologic testing in older individuals but was not designed to have the sample size to detect reduction in dementia.[225] The results of other multidomain prevention trials have been mixed,[7] potentially indicating that the manner in which the interventions are applied and to what populations may be important. Hypertension is the strongest risk factor for stroke and is also a strong risk factor for dementia.[226] In the MIND substudy (Memory and Cognition in Decreased Hypertension) of the SPRINT trial (Systolic Blood Pressure Intervention Trial), intensive systolic blood pressure lowering to a target of <120 mm Hg reduced the combined incidence of mild cognitive impairment and dementia.[227] Lowering blood pressure also reduced the progression of WML[228] and improved CBF,[229] but whether these were the reasons

for the reduced incidence of mild cognitive impairment and dementia has not been determined. Nevertheless, currently, the best clinical evidence for preventing dementia is treating elevated blood pressure. A more nuanced understanding of how risk factors for cognitive impairment affect the neurovasculome in humans would provide new leads that may unveil new preventive strategies and, possibly, therapeutic targets.

Need for Integrated Approaches

Neurovascular biology in relation to cognitive impairment and neurodegenerative diseases has been explored only in discrete studies and specialized contexts. Consequently, a comprehensive understanding of the neurovascular underpinnings of neurodegeneration has remained elusive. We are now at the point where technical advances to explore neurovascular function in vivo at the cellular and molecular levels, coupled with cutting edge imaging modalities to probe both cerebrovascular and brain function in humans, provide the bases for potentially transformative leaps in elucidating the role of vascular health in brain health at the mechanistic level. An ideal research approach would be sufficiently comprehensive to support exploration of the integrated brain system together, rather than maintaining the shortcomings of research to date, whereby specialists focus on their own individual elements: neurons, astrocytes, vascular cells, inflammation, and cell lineage tracing. The resulting discoveries could inspire human interventions in collaboration with vascular neurologists, neurointensivists, and neurosurgeons. The great opportunity is to look to neurovascular interactions from the developmental-cellular-molecular level to the network-system-organ level in health and disease, bringing together technologies, approaches, and expertise from different fields to gain an understanding unattainable within a single discipline.

ARTICLE INFORMATION

The American Heart Association makes every effort to avoid any actual or potential conflicts of interest that may arise as a result of an outside relationship or a personal, professional, or business interest of a member of the writing panel. Specifically, all members of the writing group are required to complete and submit a Disclosure Questionnaire showing all such relationships that might be perceived as real or potential conflicts of interest.

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