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The glymphatic system and cerebral small vessel disease

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ABSTRACT

Objectives: Cerebral small vessel disease is a group of pathologies in which alterations of the brain's blood vessels contribute to stroke and neurocognitive changes. Recently, a neurotoxic waste clearance system composed of perivascular spaces abutting the brain's blood vessels, termed the glymphatic system, has been identified as a key player in brain homeostasis. Given that small vessel disease and the glymphatic system share anatomical structures, this review aims to reexamine small vessel disease in the context of the glymphatic system and highlight novel aspects of small vessel disease physiology.

Materials and methods: This review was conducted with an emphasis on studies that examined aspects of small vessel disease and on works characterizing the glymphatic system. We searched PubMed for relevant articles using the following keywords: glymphatics, cerebral small vessel disease, arterial pulsatility, hypertension, blood-brain barrier, endothelial dysfunction, stroke, diabetes.

Results: Cerebral small vessel disease and glymphatic dysfunction are anatomically connected and significant risk factors are shared between the two. These include hypertension, type 2 diabetes, advanced age, poor sleep, obesity, and neuroinflammation. There is clear evidence that CSVD hinders the effective functioning of glymphatic system.

Conclusion: These shared risk factors, as well as the model of cerebral amyloid angiopathy pathogenesis, hint at the possibility that glymphatic dysfunction could independently contribute to the pathogenesis of cerebral small vessel disease. However, the current evidence supports a model of cascading dysfunction, wherein concurrent small vessel and glymphatic injury hinder glymphatic-mediated recovery and promote the progression of subclinical to clinical disease.

Introduction

The glymphatic system is a continuous network of perivascular spaces (PVSs), also called Virchow-Robin spaces, that accompany the brain's blood vessels and facilitate the convective flow of cerebrospinal fluid (CSF) from the brain's penetrating arteries into the parenchyma.[1] Traditionally, it was thought that the primary physiological function of CSF was to protect the brain from external forces by providing buoyancy to these tissues. However, recent discoveries regarding the glymphatic system challenge this view. Novel evidence has illustrated that the glymphatic system plays a prominent role in CNS fluid homeostasis, brain waste clearance, and the maintenance of proper cognition. Through this directed flow of fluids, the glymphatic system mediates the efflux of interstitial fluid (ISF), interstitial solutes, neurotoxic waste, and "dirty" CSF from the PVSs around the brain's veins into the subarachnoid space (SAS), which then acts as a waste sink.[2] These anatomical pathways exhibit structural differences along the zonation of the brain's vasculature.[3–5] Within the leptomeninges, the PVSs consist of fluid-filled spaces.[6] However, as blood vessels enter and exit the brain, their PVSs are bordered by mural cells (such as smooth muscle cells and pericytes), fibroblasts, and - on their abluminal side - astrocyte endfeet.[3,5] Though it is possible that these different cell types contribute to brain waste clearance,[3,7] an extensive number of convincing studies have established that astrocyte endfeet are a major mediator of proper glymphatic flow. At their endfeet, astrocytes express polarized orthogonal arrays of the protein Aquaporin-4 (AQP4) which mediate the flux of CSF from the

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PVS into the brain parenchyma through an undiscovered mechanism.[6]

Cerebral small vessel disease (CSVD) is a heterogeneous family of conditions characterized by dysfunction of the endothelium of the brain's small blood vessels (veins, arterioles, small perforating arteries, penetrating arteries, and capillaries) that eventually results in damage of both white and gray matter.[5] CSVD is highly prevalent, and its prevalence is only increasing in the United States as the population ages, with 5 % of those over 50 and 100 % of those over 90 affected.[8] Though the subclinical period of CSVD can last for decades, CSVD is progressive and can result in lacunar ischemic stroke, intracerebral hemorrhage, cognitive decline, and balance dysfunction secondary to white matter disease.[9,10] These diseases can be identified through neuroimaging markers visible via CT or MRI, such as extended/enlarged perivascular spaces (EPVSs), white matter hyperintensities (WMHs), cerebral microbleeds (CMBs), and lacunar infarcts.[11] Traditionally, it has been thought that the brain damage present in CSVD was a consequence of cerebral hypoperfusion and hypoxia.[9] However, recent evidence suggests that neuronal damage from CSVD is mediated by mechanisms other than hypoxia.[12] This motivated us to investigate potential connections between CSVD and the glymphatic system. We begin with a review of the literature that characterizes the glymphatic system's anatomy and physiology. After detailing glymphatic system function, we then touch upon work in both human and mice to illustrate how established CSVD risk factors and factors leading to CSVD progression coincide with glymphatic dysfunction. Finally, we discuss how CSVD itself can in turn likely lead to glymphatic impairment and how the brain damage associated with CSVD is accelerated by insufficient glymphatic waste clearance, although its role in the initial insult has yet to be determined.

Glymphatic system anatomy and physiology

Cerebrospinal fluid composition and production

The composition of CSF is relatively unique when compared to other liquid compartments within the human body. In contrast to plasma, CSF has relatively low concentrations of glucose and is protein sparse. While

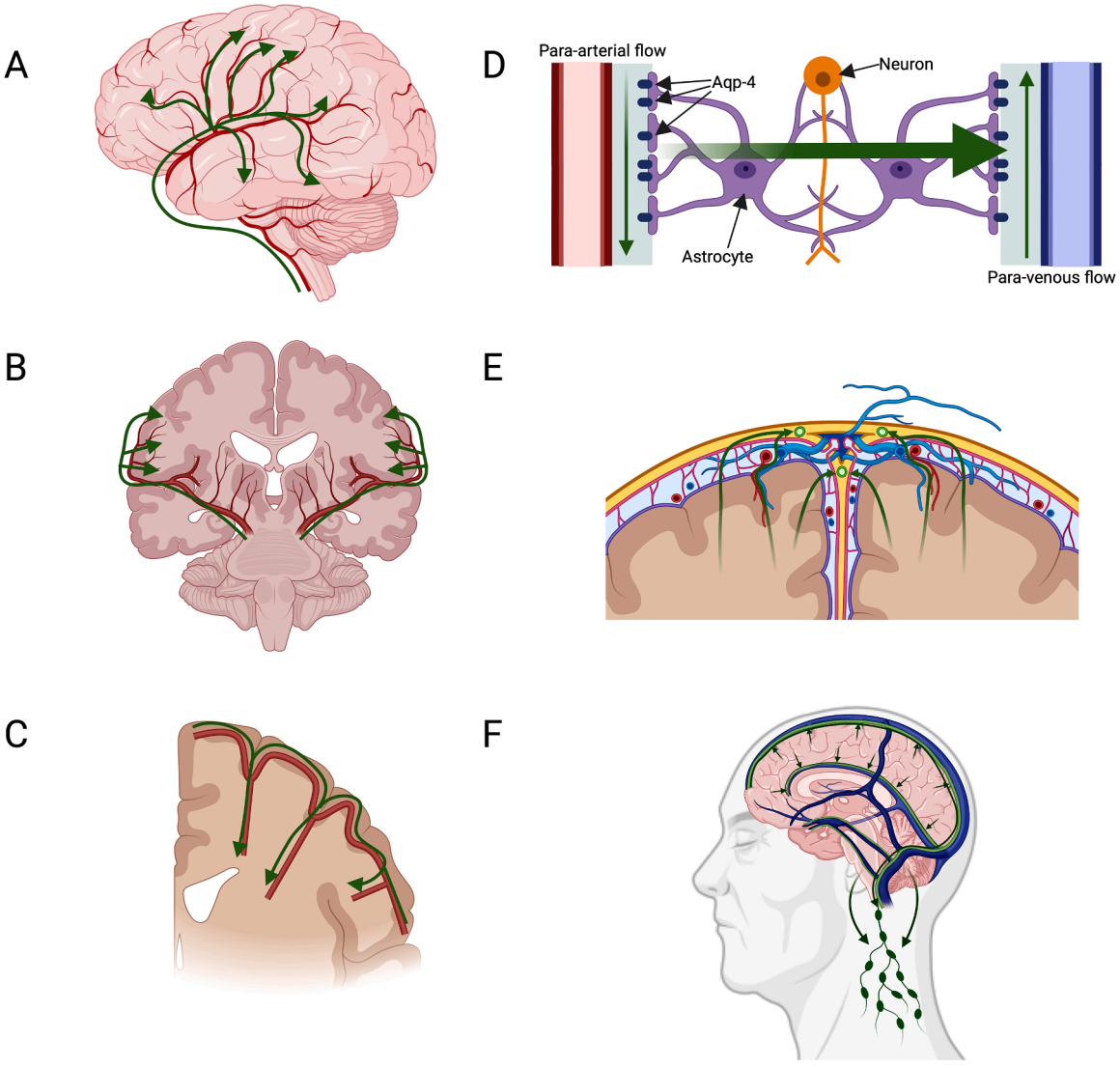

Fig. 1. The anatomy of the glymphatic system. (A) Cerebrospinal fluid (CSF) produced in the choroid plexus drains through the ventricular system to the subarachnoid space before entering the parenchyma of the brain. (B) CSF follows the major arteries of the brain via the para-arterial spaces prior to diving deep into the brain along the penetrating arteries. (C) CSF follows the direction of blood upon entering the parenchymal microvasculature. (D) Within the parenchyma, CSF from the para-arterial space flows into the extracellular matrix (ECM) via aquaporin-4 (AQP4) channels on the foot processes of astrocytes, allowing CSF to flow through and collect metabolic waste before draining via the para-venous spaces. (E) Waste CSF flows through the meningeal lymphatic vessels as a continuation of the para-venous drainage. (F) Meningeal lymphatic vessels are located adjacent to the dural sinus which drain to the local lymph nodes, as shown by tracer studies. Flow is represented by the green arrows.

blood plasma is approximately 92 % water, CSF is 99 % water.[13] This allows CSF to be an efficacious solvent of harmful hydrophilic substances within the CNS milieu, such as soluble amyloid-β oligomers and forms of Tau protein.[14,15] In addition, the concentration of K+ ions is ~ 3 mM, a concentration that is lower than that of blood and which is critical for the potassium-sensitive excitable cells of the brain. These properties are tightly regulated so they are preserved, even in the face of sustained physiologic stressors such as systemic hyperkalemia.[16]

The physicochemical properties of CSF are intrinsically tied to its production. According to the established model of CSF synthesis, the choroid plexus is the principal source of CSF, which, following its active secretion, drains through the ventricular system into the subarachnoid space (SAS) and is eventually cleared into the blood of the dural venous sinus through arachnoid granulations.[17] At the molecular level, CSF formation and secretion is primarily driven by membrane transport mechanisms that couple fluid transport to solute translocation.[1] An osmotic gradient is created by choroid plexus epithelial cells (CPECs), in which the ventricular lumen is relatively enriched in Na^+, Cl^-, and HCO_3^- ions relative to the CPEC cytoplasm. This transepithelial gradient, which is due to the action of Na^+/K^+-ATPases localized on the apical membrane of CPECs working in concert with other ion channels and transporters on both the apical and basolateral membranes, drives free water from the interstitial fluid into the ventricular system through the water channel protein.[1]

CSF flow through para-arterial spaces

Before entering the brain parenchyma, "fresh" CSF moves from the SAS along the PVSs of large leptomeningeal arteries (Fig. 1A, B). Both human and mouse imaging studies have illustrated that CSF not only flows through the cranium's basal cisterns, it also flows through the PVSs of the Circle of Willis and the major cerebral arteries in the same direction as blood.[18,19] As the arterial tree enters the neocortex, the leptomeningeal arteries on the brain's surface continue as penetrating arteries, which enter the parenchyma and connect leptomeningeal blood vessels on the brain's surface to the parenchymal microvasculature (Fig. 1C).[20] Seminal studies illustrating the flux of CSF into the PVS of penetrating arteries have been conducted throughout the mid- and late-twentieth century.[21] Though these works originated the idea that PVSs could be potential sites of fluid and waste exchange between the CSF and the CNS, they did not show evidence of flow into the brain. However, the recent use of *in vivo* two-photon microscopy has enabled investigators to visualize the glymphatic system by following the flow of fluorescent solutes injected into cisterna magna of mice and visualizing their entry into the PVSs of penetrating arteries and their spread into the parenchyma.[22–24]

As blood vessels carry blood towards the brain, they pulsate at a tempo set by the cardiac cycle.[24] With each heartbeat, the pulse pressure generates a pressure wave that travels through the body's arterial system. In the cranial compartment, the wave travels from the internal carotid artery, through the major cerebral arteries, and through their cortical branches.[22,25–27] There is strong evidence that the cardiac cycle and subsequent arterial pulsatility are the main drivers of convective CSF flow throughout perivascular spaces of leptomeningeal arteries. *In vivo* mouse velocimetry data indicates that the velocity of CSF flow parallels that of arterial wall pulsation, while both non-invasive MRI and *in vivo* tracer studies have shown that the periodicity of CSF flow parallels the cardiac, not respiratory, cycle.[28,29]

Importantly, the transmittance of arterial pulsatility to penetrating arteries is necessary for proper glymphatic function. When the pulsatility of penetrating arteries is diminished, the quantity of CSF that enters the brain parenchyma is diminished. Internal carotid artery ligation, which reduces the pulsatility of penetrating arteries, has been shown to hinder the glymphatic influx.[22] In contrast, use of the adrenergic agonist dobutamine in humans and mice not only increased the pulsatility of penetrating arteries, but also concomitantly increased the CSF flow into the brain by as much as 65 %.[22,29]

Astrocyte endfeet and AQP4 polarity

Fluid movement from the PVS of penetrating arteries into the brain parenchyma allows fresh CSF to mix with the brain's interstitial fluid (Fig. 1D). This in turn allows for the removal of cellular waste, such as amyloid-β, tau, and lactate, from the interstitial space.[30,31] Though there remains much to discover regarding the mechanism of CSF movement from the PVS into the brain, evidence suggests that astrocytes mediate this influx via the water channel protein AQP4. Astrocytes processes terminate at and ensheath the wall of cerebral blood vessels.[32] The endplates of these processes, termed astrocyte or vascular endfeet, are known to express AQP4 at levels many fold higher than astrocytic soma and perisynaptic processes. This localized AQP4 distribution is critical for proper glymphatic function, with development of glymphatic influx (brainstem to cortex) correlated with the polarization of AQP4 to vascular endfeet.[3,33] In addition, AQP4-null mice exhibit severely reduced glymphatic influx, with an approximately 70–80 % decrease in waste clearance – suggesting the stagnation of brain interstitial fluid.[3,33] This observation was further supported by conditional knockout of AQP4 with the glial specific promoter GFAP in mice, which demonstrated a ~31 % reduction of brain water uptake after hyperosmotic stress.[34]

Para-venous efflux of CSF and waste clearance

According to the glymphatic hypothesis, after moving through the brain parenchyma the mixture of "dirty" CSF and ISF flows along the PVSs of the brain's major veins into the SAS (Fig. 1E). This pathway was first visualized via injection of fluorescent CSF tracers into the mouse cisterna magna; analysis of *ex vivo* brain slices indicated that one hour post injection, tracer was highly concentrated along the PVSs of medial internal cerebral veins and ventral-lateral rhinal veins.[22] This data is further supported by the *in vivo* visualization of lipophilic solute efflux through mouse venous PVSs via two-photon laser scanning microscopy, as well as the finding of perivenous gadolinium clearance in a patient that received laser interstitial thermal therapy.[23,35] Finally, the SAS itself acts as a waste sink, allowing solutes within CSF to drain to the peripheral immune system.[36]

Glymphatic-meningeal lymphatic connections

Shortly after the glymphatic hypothesis was proposed, two separate groups rediscovered lymphatics along murine dural venous sinuses with the capacity to clear solutes from the brain.[37–40] Termed meningeal lymphatic vessels (mLVs), these lymphatics were later observed in both humans and non-human primates through immunohistochemistry and contrast-enhanced MRIs.[38,41] Like the glymphatic system, mLVs have been implicated in CSF drainage and the clearance of both amyloid-β and tau.[42,43] Though the exact mechanism of mLVs uptake of CSF is unknown, drainage likely involves the dura near the venous sinuses, known as the perisinus dura. Studies in humans and mice have recently shown that the perisinus dura is a site of CSF solute and CNS antigens efflux.[44,45] In addition, intravenous injections of contrast agent have shown that mLVs can drain dural interstitial fluid, suggesting that mLVs remove waste, clear solutes, and influence the immunity of the CNS through perisinus dura surveillance. Given their location and function, it is likely that meningeal lymphatics act as a circuit of CSF clearance alongside the route provided by both arachnoid granulations and the dural venous sinuses (Fig. 1F).

It has been posited that mLVs and the glymphatic system are connected, with meningeal lymphatics downstream of glymphatic clearance.[46] This notion is supported by functional experiments in mice showing that both systems transport solutes to a shared destination. Tracer studies illustrate that meningeal lymphatics connect to and drain

lymph to deep cervical lymph nodes (dcLNs).[42,47] Disruption of mLVs through either pharmacologic ablation or the abrogation of VEGF-C/D signaling prevents the drainage of solutes to dcLNs.[42,47] The glymphatic system also drains to the dcLNs, as shown by intracerebral injections of radioiodinated albumin and PEG-IRDye of mice and by the impaired drainage of lactate from mice brains to dcLNs with glymphatic inhibition.[31,37,48] Importantly, similarities between these two drainage routes also exist in humans. The glymphatic transport of tracer to dcLNs has been visualized in patients through MRIs, and the anatomy of mLV drainage circuits is conserved between humans and mice.[49,50]

Intriguingly, glymphatic function and the movement of CSF through mLVs are both correlated with circadian rhythms.[51] Glymphatic influx to the mouse brain was approximately 53 % greater in the day, when mice tend to sleep, than influx during the night. This was mirrored at a molecular level by AQP4 polarization to the astrocyte endfeet, which also increased during the day. Similarly, lymphatic flow to the deep cervical lymph nodes also appeared to be under circadian control. However, this rhythm was opposite to that of glymphatic influx, peaking in the night and nadiring during the day.

The flow of CSF through both systems can also be modulated by anesthesia. The sensitivity of the glymphatics to different anesthetic agents has been well characterized. Intraparenchymal clearance experiments demonstrated that, when comparing rats anesthetized with pentobarbital to others anesthetized with ketamine/xylazine (K/X), pentobarbital rats effluxed solutes at a rate that was 100 times slower.[52] Additionally, in a comparative study of different anesthetic regimens including K/X, avertin, pentobarbital, α-chloralose, isoflurane, and isoflurane-dexmedetomidine, K/X was shown to induce the highest amount of glymphatic influx.[53] This is likely due to the fact that K/X induced the greatest prevalence of delta-waves, which have been postulated to promote glymphatic flow, within the brain.[53] Meningeal lymphatic flow also dramatically increases under K/X. Interestingly, experiments examining CSF drainage in awake and K/X treated mice demonstrated that animals who received K/X had greater overall flow to their dcLNs.[54] Notably, like glymphatic flow, efflux to the perisinus dura was dramatically increased when K/X was administered in place of isoflurane.[44]

Taken together, these results show that the glymphatics and meningeal lymphatics represent a connected path of waste clearance, with flow through these systems correlated under certain contexts and disunified in others. Since the reasons for this are yet to be understood, finding the biological causes behind these changes will likely aid in elucidating governing principles of CNS homeostasis. Furthermore, understanding these changes might be of clinical import. For example, mice inflicted with cerebral ischemia *via* photothrombosis (PT), a model of CSVD induced stroke, and mice modeling major stroke through middle cerebral artery occlusion (MCAO) both display cerebral edema, glymphatic dysfunction, and a loss of AQP4 polarization.[55,56] However, a recent study has shown that PT mice exhibit dramatic lymphangiogenesis and a robust mLV network after injury.[57] This suggests that mLVs have the capacity to remodel and increase their level of uptake from the CNS to protect or heal it. This behavior has been characterized in a photothrombotic zebrafish model, which showed that mLVs can enter the brain to drain edema and act as "tracts" to help cerebral blood vessels revascularize the parenchyma.[58] Paradoxically, the mLVs of MCAO mice are largely unaltered, even though a significantly larger volume of parenchyma is affected and there is presumably more edema.[56,57] Thus, an understanding of the mechanisms driving these two different presentations might clarify subtle distinctions behind large vessel occlusion strokes and strokes caused by CSVD and help create therapeutic options for both diseases.

Shared risk factors between CSVD and glymphatic dysfunction

Hypertension

Hypertension is a risk factor for CSVD development and progression (Fig. 2A). Stage 1 and stage 2 hypertension are both associated with deep cerebral microbleeds and with occlusions of perforating arteries producing small lacunar infarcts in the brain's deep parenchyma.[59] Additionally, hypertension is associated with white matter hyperintensities (WMHs), pathological white matter changes that are visible via certain MRI scans and that represent CSVD.[59] Damage to the cerebrovascular can begin as early as the middle decades of life. In cross-sectional study of middle-aged individuals (average age 39.2, SD = 8.4) with a range of hypertension categories, increased systolic blood pressure (SBP) was linearly associated with decreased white matter integrity - a sign of vascular brain injury.[60]

Both *in vivo* animal studies and data from human patients illustrate that hypertension is linked to glymphatic dysfunction. Data from rodents shows that hypertension likely impairs glymphatic flow by altering arterial wall dynamics. In a mouse model of hypertension, hypertension decreased the pulsatility of distal arteries and therefore decreased glymphatic influx.[28] Spontaneously hypertensive (SHR) rats, a rodent model in which arterial remodeling has been well documented, exhibit decreased glymphatic influx but normal AQP4 polarization.[61,62] These findings have been corroborated by recent work using diffusion tensor imaging along the perivascular space (DTI-ALPS), a novel non-invasive imaging method of imaging human glymphatic function, which showed that glymphatic flow is diminished in elderly hypertensive patients.[63]

Type II diabetes

Type II diabetes (T2D) is known to be a significant risk factor in the development of CSVD (Fig. 2B).[64] Though the mechanism of causality is not fully established, vascular remodeling is a prominent feature of diabetes. In mice, diabetes has been shown to induce the stiffening of major blood vessels through extracellular matrix (ECM) remodeling.[65] Arterial stiffening is also present in humans with T2D and related mechanisms of ECM remodeling have been implicated in the induction of altered arterial wall characteristics.[66] Similarly, studies in both rodents and humans demonstrate an association between T2D and glymphatic dysfunction. In a rat model of type 2 diabetes mellitus (T2DM), glymphatic efflux was reduced by two-thirds.[67] Recently, a cohort of patients with T2DM underwent DTI-ALPS imaging, which showed decreased water diffusivity along their perivascular spaces - likely reflecting glymphatic impairment.[68] Though data on arterial wall dynamics has yet to be collected in rats or in humans with T2DM, it is possible that alterations in arterial pulsatility might mediate glymphatic dysfunction as in hypertension.

Sleep disruption

The dysregulation of normal sleeping patterns has been linked to the incidence and progression of CSVD in a variety of contexts (Fig. 2C). Patients with fragmented sleep-wake cycles suffer from a greater incidence of WMHs and cerebral microbleeds.[69] When compared to controls, patients diagnosed with obstructive sleep apnea (OSA) were more likely to have WMHs, silent brain infarctions, and enlarged PVSs (EPVSs), another sign of CSVD.[70] Though the pathogenesis of EPVSs is unknown, arterial stiffening and abnormal protein aggregation be recently proposed as potential causes.[71] Additionally, in older adults, sleep quality was found to mediate the relationship between CSVD and frailty.[72]

Some of the most influential and well-cited work investigating glymphatic physiology is focused on how the system is modulated by sleep. A seminal study demonstrated that sleep is necessary for the

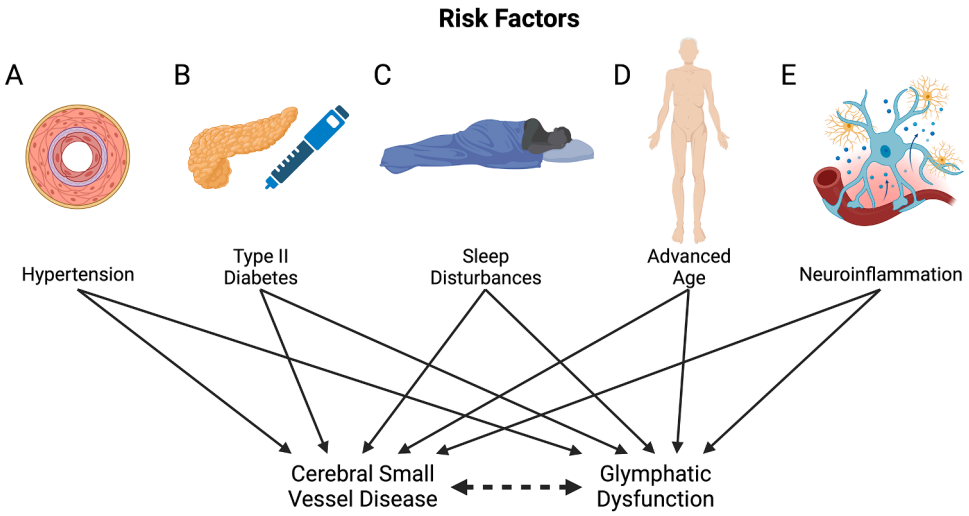

Fig. 2. Cerebral small vessel disease and glymphatic dysfunction share similar risk factors. (A) Hypertension is a known risk factor for cerebral small vessel disease (CVSD) and is associated with cerebral microbleeds, lacunar infarcts and white matter hyperintensities. *In vivo* animal studies and patient data have been shown to link hypertension to glymphatic dysfunction through decreased glymphatic influx. (B) Vascular remodeling in type II diabetes (T2D) is a significant cause of CVSD development. Arterial stiffening in T2D models have been linked to glymphatic dysfunction in animal models, highlighting decreased water diffusivity in the perivascular spaces. (C) Patients with disrupted sleep have been seen to suffer from increased white matter hyperintensities and cerebral microbleeds, signs of CVSD. Murine studies show impaired glymphatic influx and clearance of apolipoprotein E (ApoE) isoforms in sleep deprived mice, linking sleep deprivation of glymphatic dysfunction. (D) Decreased arterial pulsatility, vascular remodeling and CSVD are common in older patients. Loss of aquaporin-4 (AQP4) polarization in older mice has been shown to inhibit amyloid-β clearance. Additionally, age-related immunological changes to the central nervous system have been suggested to cause additional glymphatic dysfunction and CVSD. (E) CVSD has been linked to systemic inflammation and reactive astrogliosis. Additionally, murine models of astrogliosis have been shown to possess disrupted AQP4 polarization and impaired glymphatic flow.

glymphatics to efficiently clear interstitial solutes and amyloid-β from the brain and that the glymphatic system is dramatically suppressed when mice are awake.[73] Through the usage of real-time tetramethylammonium diffusion and two-photon microscopy, this group illustrated that the parenchymal interstitial space is ~60 % larger in sleep than in the awake state and that increased interstitial volume was correlated with a high prevalence of delta-waves within the brain.[74] Similar observations using coupled EEG, BOLD, and CSF flow measurements have been made in human patients. As in mice, sleep and slow-delta waves were correlated with glymphatic influx.[75] Interestingly, the EEG readings suggested that slow-delta waves preceded glymphatic influx and a reduction in blood volume. This suggests that during slow wave sleep, glymphatic flow is facilitated by the outflow of blood from the parenchyma and that CSF influxes into the parenchyma to maintain a constant pressure.

Given the importance that sleep plays in glymphatic function, it is not surprising that a lack of sleep impairs glymphatic flow. After sleep deprivation, mice retained lactate in their cortex, demonstrating a failure of glymphatic clearance.[31] These findings are corroborated by work showing that both the influx and the clearance of ApoE isoforms are impaired in mice after sleep deprivation.[76] Evidence suggests that poor sleep or sleep distribution disturbs human glymphatic clearance as well. Sleep disorders are common among patients with Alzheimer's disease, and sleep deprivation has been shown to increase the brain's accumulation of amyloid-β, even after one night.[77]

Advanced age

Aging is one of the most significant risk factors for CSVD and CSVD progression (Fig. 2D). Alterations to arterial dynamics and age-related glymphatic decline have been documented in mice, suggesting that parallel disease mechanisms could be contributing.[78] When compared to young (2, 3 months) mice, the arterial pulsatility of older mice was decreased by 27 %.[24] In addition, older mice exhibited a loss of AQP4 polarization, inhibited CSF influx, and a 40 % reduction in intraparenchymal amyloid-β clearance.[24] Recent studies in humans also suggest that CSVD, altered arterial pulsatility, and vascular remodeling are all linked.[79]

Importantly, age-related glymphatic dysfunction might be mediated by structural changes to components of the brain or the meninges. Recently, it has been shown that the architecture of the pia, the innermost layer of meninges, can influence glymphatic function.[80] In young mice, the pial architecture of periarterial spaces varies, with CSF influx greatest at the architectural subset found predominantly at the ventral aspect of the brain. However, in aged mice where the pia is denuded, the proportion of periarterial spaces with this type of pial structure is diminished.[80] Furthermore, novel work has characterized the glymphatic system in pigs. Like humans, pigs are gyrencephalic. Thus, patterns of glymphatic flow in these animals are likely more relevant for human CSF dynamics. Tracer experiments revealed that the sulci enhanced the influx of CSF.[81] Since sulci have been shown to widen and alter in their structure with increasing age, these changes could potentially mediate reduced glymphatic flow in older humans.[82]

Finally, aging-associated changes to the immunological milieu of the CNS have been implicated in impaired glymphatic flow. A subpopulation of parenchymal border macrophages (PBMs) are closely associated with cerebral arteries, and these cells have the capacity to regulate CSF flow through the modulation of arterial motion.[83] Even though the quantity of PBMs is stable throughout age, the subpopulation of PBMs that mediates CSF flow was reduced in aged mice. Astoundingly, glymphatic flow was restored in animals after intra cisterna magna injections of macrophage-colony stimulating factor (M-CSF), a cognate ligand for the receptor Csf1r, which is expressed by PBMs and promotes growth.[83] It has also been shown that old mice exhibit a loss of another subpopulation of cells, meningeal Ccr7+ T-cells.[84] Behavior testing and assessment of glymphatic influx and in *CCR7-/-* mice showed that when compared to aged-matched controls, *CCR7-/-* animals performed worse in novel location recognition and spatial memory tests and exhibited decreased glymphatic influx, suggesting that the age-related loss of Ccr7+ T-cells contributes to cognitive decline and glymphatic dysfunction.[84] Interestingly, knockout of Ccr7 in a mouse model of Alzheimer's resulted in an increased amyloid-β burden compared to

Alzheimer's disease (AD) mice with normal Ccr7 signaling – further implicating Ccr7 in glymphatic efflux.[84]

Neuroinflammation

Evidence suggests that consistent states of systemic inflammation can affect the CNS and induce disease processes linked to CSVD (Fig. 2E). Microglia are immune cells that surveille the CNS and have been tied not only to the clearance and containment of neurotoxic metabolites, but also with various features of CSVD.[85–87] For example, in a human study, areas of microglial activation displayed blood-brain barrier breaks, a hallmark of CSVD.[88] CSVD has also been tied to reactive astrogliosis, another form of neuroinflammation that is thought to indicate cell stress and is related and is marked by morphological changes within astrocytes such as increased size and decreased branching.[86,89] While examining the pathophysiology of WMHs, investigators noticed that these lesions were marked by astrogliosis.[87]

Investigators have utilized mice models to study astrogliosis and have found that these changes inhibit the glymphatic function of astrocytes; reactive astrogliosis disrupts AQP4 polarization and subsequently diminishes glymphatic flow.[89] In line with this, astrogliosis has been seen in mouse models of closed-skull traumatic brain injury and subarachnoid hemorrhage, two pathologies with impaired glymphatic function.[90–92] Investigators have also found that inhibition of astrogliosis via administration of the anti-scarring agent, olomoucine, improved glymphatic function in a mouse model of post-hemorrhagic hydrocephalus.[93]

Cerebral small vessel disease as a mediator of glymphatic dysfunction

Given the relative novelty of the glymphatic hypothesis, investigations are still evaluating the role of glymphatic function in a range of disease states. Intriguingly, a myriad of CSVD animal models display signs of glymphatic impairment, while pathophysiology of CSVD alters structures within the human brain that are part of the anatomy of the glymphatic system. We review this evidence in depth below, with an eye toward illustrating how CSVD might induce glymphatic dysfunction and subsequentially lead to CNS damage and neuronal dysfunction.

Cerebral microinfarcts

Cerebral microinfarcts are microscopic, sharply delineated, and focal brain lesions that are characterized by cell death, necrosis, and potentially by cavitation.[94] These lesions have a similar histological appearance to ischemic infarctions, but unlike macroscopic brain infarcts, they are typically not visible via MRI. As a result, most knowledge regarding cerebral microinfarcts is derived from post-mortem autopsy studies. Though the pathological causes of microinfarcts are not fully understood, they have been tied to small vessel pathologies such as cerebral amyloid angiopathy (CAA) and are common among patients with Alzheimer's and vascular dementia and are correlated with age-related cognitive decline (Fig. 3A).[94,95]

Since these lesions are difficult to identify in the clinical environment, the mechanisms in which cerebral microinfarcts impair proper cognition are unclear. However, microinfarct animal models have provided potential insights into the pathology and consequences of these lesions. Experiments using CSF tracers show trapping within areas of microinfarction in injured mice, suggesting that microinfarcts create focal areas of glymphatic dysfunction.[96] Further evidence of glymphatic impairment stemming from microinfarcts was discovered in a mouse model that relies on laser-invoked arteriole occlusion. Here, investigators found that microinfarcts suppressed the glymphatic clearance of intracerebrally injected solutes and that glymphatic influx could be rescued through the overexpression of *Slit2*.[97]

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy

Cerebral autosomal dominant arteriopathy with subcortical infarcts (CADASIL) is the most common monogenetic inherited stroke disorder.[98] This condition is a dominantly inherited form of vascular dementia that arises from *NOTCH3* mutations.[98] Clinically, CADASIL can manifest as severe migraines, recurrent ischemic strokes, and psychiatric disturbances.[99] These manifestations are a frequent cause of dementia,

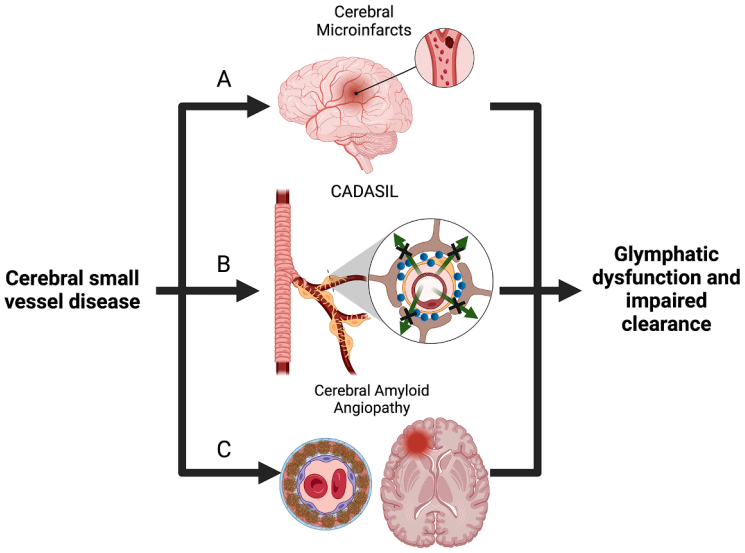

Fig. 3. Cerebral small vessel disease plays a possible role as mediator of glymphatic dysfunction. (A) Animal models of cerebral microinfarcts using cerebrospinal fluid (CSF) tracers have shown trapping within the area of infarction, suggesting a localized area of glymphatic dysfunction. (B) Histopathology of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) patients have shown dysfunctional cerebrovascular structures associated with the glymphatic system. Patients with CADASIL often show accumulation of granular osmiophilic material (GOM) in their perivascular spaces (PVS) which could lead to glymphatic dysfunction. It is suggested that the elevated iron accumulation in CADASIL patients could also demonstrate a link to a lack of glymphatic clearance. (C) Deposition of amyloid in the cerebral vasculature could mediate glymphatic dysfunction. Brown deposits represent the amyloid in the vasculature that can lead to intracranial hemorrhages, characteristic of cerebral amyloid angiopathy (CAA). CAA rat models have been shown to have increased CSF shunting away from the brain, leading to impaired clearance of metabolic wastes and decreased glymphatic transport.

disability, and death.[100] As a result, the average life expectancy of CADASIL patients is 64.6 years amongst men and 70.7 years in women.[101]

Histopathological investigations into CADASIL patients have revealed that the disease alters cerebrovascular structures that are part of the glymphatic system (Fig. 3B). Though the phenotypic manifestations of CADASIL can vary, patients can present with enlarged perivascular spaces and smooth muscle deterioration in their small blood vessels.[102] Importantly, most patients with CADASIL exhibit the accumulation of granular osmiophilic material (GOM) within their PVSs.[9] These large protein depositions are crucial for the CADASIL progression and are thought to arise from the secretion of abnormal Notch3 isoforms into the PVS by pericytes.[103] Though there is a lack of data regarding glymphatic function in CADASIL patients and CADASIL animal models, it is possible that depositions of GOM might obstruct the PVS and prevent glymphatic flow. Somewhat supporting this are findings illustrating that brain iron deposition might be a hallmark of glymphatic dysfunction and that CADASIL patients have elevated amounts of iron within their basal ganglia.[104,105] Additionally, the TgNotch3^{R169C} mouse model of CADASIL demonstrates astrocyte endfeet detachment secondary to pericyte loss.[103] This disruption of endfeet attachment undoubtably produces glymphatic dysfunction.

Cerebral amyloid angiopathy

Cerebral amyloid angiopathy (CAA) is a type of CSVD in which amyloid beta is deposited along vessel walls. It is often associated with cognitive impairment and increased risk of intracerebral hemorrhages and also contributes to the pathophysiology of aging and Alzheimer's (Fig. 3C).[106] Prior to the discovery of the glymphatic system, research on CAA in Alzheimer's disease identified impaired perivascular drainage of amyloid-beta as a contributor to amyloid beta deposition.[107] Coincident with the recent elaboration of the glymphatic system and meningeal lymphatics, an increasing amount of research on the relationship between perivascular spaces and CAA pathophysiology has occurred over the past decade.

Multiple studies have linked EPVS and glymphatic dysfunction to CAA and markers of CAA. These markers of CAA include intracerebral hemorrhages, cerebral microbleeds, and cortical superficial siderosis visualized via MRI or CT.[108] Many of these imaging studies have identified EPVSs within white matter tracts, particularly the centrum semiovale, as a potential novel marker of CAA.[109] EPVSs in the centrum semiovale differentiate CAA from other cerebral vasculopathies, such as hypertensive arteriopathy, which are instead linked to EPVSs in the basal ganglia. Given these studies, the location of EPVSs has been proposed as a way to differentiate the causes of spontaneous intracerebral hemorrhage.[108] Other studies have found that the cerebral microbleeds associated with CAA also colocalize with enlarged juxtacortical perivascular spaces (jPVSs) and that the number and degree of dilation of these jPVSs were also associated with CAA severity.[110]

Recent studies focusing on the changes in glymphatic function that are associated with CAA have found that glymphatic transport is decreased in CAA. In a rat model of severe type 1 CAA, rats with CAA had higher velocity movement of CSF and solutes along the peri-arterial influx routes to the glymphatic system, but these CSF currents were diverted away from the brain, decreasing overall glymphatic transport and lymphatic drainage to deep cervical lymph nodes.[111] Of note, this study also found that perivascular AQP4 polarization was decreased in these rats compared to wild-type rats. In another recent study, Xu et al. quantified glymphatic function in humans with CAA by using the ALPS index, an index that uses MRI to quantify diffusivity along perivascular spaces.[112] In this study, CAA patients were found to have decreased glymphatic function compared to controls, and lower glymphatic function in CAA patients was associated with increased EPVSs in the basal ganglia, increased white matter hyperintensities, increased lacunes, greater cerebral small vessel disease burden, and lower cognitive function.

Conclusion

Overall, review of recent studies indicates that both understanding of and appreciation for the role of the glymphatic system have grown rapidly amongst clinicians and researchers in the last decade. Evidence clearly shows that the glymphatic system – a name that reflects its dependence on the astrocyte endfeet of glial cells and its role that parallels that of the lymphatic system in the rest of the body – is critical for modulating the accumulation and clearance of waste. It is also intricately regulated, with its flow directed by arterial wall pulsatility initiated by the cardiac cycle and associated with perivascular CSF influx. Moreover, there is clearly overlap between glymphatic system dysfunction and CSVD, a set of pathologies with significant morbidity and mortality. Prevalent and known risk factors for CVSD (hypertension, diabetes, aging, sleep disruption, and neuroinflammation) are clearly implicated in the development of glymphatic dysfunction. Studies have demonstrated that chronic hypertension and diabetes impair glymphatic clearance via altered arterial pulsatility, while aging and sleep disruption are also associated with reduced glymphatic activity, albeit through still emerging mechanisms. Additionally, experimental data from mouse models reveal observations of CSVD disease states causing glymphatic dysfunction. These findings suggest that in cases of CSVD, damage to the CNS might result from the glymphatic impairment and the subsequent accumulation of neurotoxic waste. Thus, maintaining optimal glymphatic function may be an important factor in preventing or slowing the progression of neurologic disease. Interestingly, some hypothesize that the glymphatic dysfunction initiates, not just exacerbates, CSVD. While the deposition of amyloid could be secondary to decreased glymphatic flow like in CAA, other disease case studies do not yet give weight to this theory. Overall, although more needs to be done to parse the interconnection between the glymphatic system and pathogenesis of CSVD, the importance and dynamicism of their relationship are undeniable.

CRediT authorship contribution statement

Phillip S. Ang: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Douglas M. Zhang:** Formal analysis, Visualization, Writing – review & editing, Writing – original draft. **Saara-Anne Azizi:** Formal analysis, Writing – original draft, Writing – review & editing. **Salvador A. Norton de Matos:** Visualization, Writing – review & editing. **James R. Brorson:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

Declaration of competing interest

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