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Cerebral small vessel disease: The impact of glymphopathy and sleep disorders

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Abstract

The glymphatic system, a vital brain perivascular network for waste clearance, hinges on the functionality of the aquaporin 4 (AQP4) water channel. Alarmingly, AQP4 single nucleotide polymorphisms (SNPs) are linked to impaired glymphatic clearance, or glymphopathy, which contributes to sleep disturbances and various age-related neurodegenerative diseases. Despite the critical role of glymphopathy and sleep disturbances in cerebral small vessel disease (CSVD) – a silent precursor to age-related neurodegenerative disorders – their interplay remains underexplored. CSVD is a major cause of stroke and dementia, yet its pathogenesis is not fully understood. Emerging evidence implicates glymphopathy and sleep disorders as pivotal factors in age-related CSVD, exacerbating the condition by hindering waste removal and compromising blood-brain barrier (BBB) integrity. Advanced imaging techniques promise to enhance diagnosis and monitoring, while lifestyle modifications and personalised medicine present promising treatment avenues. This narrative review underscores the need for a multidisciplinary approach to understanding glymphopathy and sleep disorders in CSVD. By exploring their roles, emphasising the necessity for longitudinal studies, and discussing potential therapeutic interventions, this paper aims to pave the way for new research and therapeutic directions in CSVD management.

Keywords

Cerebral small vessel disease, glymphatic system, sleep, aquaporin 4, glymphopathy

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Introduction

Cerebral small vessel disease (CSVD) encompasses a variety of pathological conditions affecting the small blood vessels in the brain,[1] significantly contributing to the incidence of stroke and dementia.[2] CSVD is responsible for approximately 25% of all strokes[3] and plays a crucial role in about 45% of dementia cases.[4] Alarmingly, CSVD often goes unnoticed due to its silent presentation, typically identified incidentally through neuroimaging, such as white matter

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hyperintensities (WMHs) and enlarged perivascular spaces (ePVS).[1] Although traditionally associated with aging, recent studies reveal a troubling prevalence of early CSVD markers among working-age adults (aged: 25–70), even without overt clinical symptoms.[5] Despite its prevalence and impact, the underlying mechanisms of CSVD remain poorly understood, posing a significant challenge to the development of effective treatments and preventive strategies.

Emerging research has begun to shed light on the glymphatic system, a waste clearance pathway in the central nervous system (CNS),[6,7] and its potential role in the pathogenesis of CSVD. The glymphatic system heavily relies on the aquaporin 4 (AQP4) water channel,[7] which is crucial for efficiently removing amyloid-beta (Aβ) and neurotoxic waste products during sleep by circulating cerebrospinal fluid (CSF) through the brain's perivascular spaces.[8] Evidence suggests that genetic variations in AQP4 manifested as single nucleotide polymorphisms (SNPs), may disrupt glymphatic function, hindering the brain's ability to cleanse itself during sleep and contributing to neurodegenerative disorders like Alzheimer's disease (AD),[9] schizophrenia,[10] prolonged wakefulness,[11] Parkinson's disease (PD),[12] and cognitive decline.[13]

While the clinical consequences of CSVD are well-documented,[2] the interaction between glymphatic system dysfunction (glymphopathy) and sleep disorders offers a novel and promising avenue for understanding CSVD. Recent advancements in imaging techniques have enabled more precise visualisation of the glymphatic system and its dysfunctions, providing new opportunities for early diagnosis and monitoring of CSVD.[1,14–16] Additionally, identifying biomarkers linked to glymphatic function and sleep quality could pave the way for personalised medicine approaches, tailoring interventions to individual patients based on their specific risk profiles and physiological characteristics.

Therapeutic interventions aimed at improving sleep quality, such as cognitive-behavioural therapy (CBT) for insomnia, continuous positive airway pressure (CPAP) for sleep apnoea,[17] and pharmacological treatments, may enhance glymphatic function and mitigate CSVD progression. Lifestyle modifications, including regular exercise, a healthy diet, and smoking cessation, are also crucial for maintaining both vascular and glymphatic health.[18] Despite these promising insights, several research gaps remain. Longitudinal studies are needed to track the progression of CSVD and glymphopathy over time, while clinical trials should test the efficacy of various therapeutic interventions. Additionally, understanding the molecular mechanisms linking sleep disorders, glymphopathy, and CSVD is essential for developing targeted treatments.

In summary, this review aims to explore the critical role of glymphopathy and sleep disorders in the pathogenesis of CSVD. By integrating recent findings and highlighting future research directions, we hope to advance the understanding of CSVD and contribute to the development of effective strategies for its prevention and treatment.

The glymphatic system

For decades, it was believed that the lymphatic system was absent in the human CNS. Therefore, cell debris, neurotoxic proteins, and large metabolites are thought to be eliminated through a separate pathway from brain vasculature. In 2012, Nedergaard's group[7] discovered that CSF can enter the brain parenchyma and mix with the interstitial fluid (ISF) through the AQP4 water channel on the astrocyte's end feet. This combined fluid drains waste products e.g., Aβ and tau etc. out of the brain. Recognising the lymphatic-like function and its support by astrocytes, it was named the glymphatic system, drawing from the term glia.[19] Over the past decade, this system has sparked extensive research, offering a new understanding of brain diseases by emphasizing fluid dynamics rather than focusing on isolated lesions or structures.

The glymphatic system, driven by the AQP4 water channel on the astrocytic end-feet, forms a perivascular across the brain. It delivers nutrients and neuroactive substances to the brain parenchyma through the peri-arterial CSF influx pathway and removes metabolic wastes through peri-venous clearance routes.[20] The system consists of three key elements: peri-arterial CSF influx, facilitated by astrocytic AQP4, the infusion of CSF and ISF into the brain parenchyma; and peri-venous clearance.[21] Its function is closely linked to two anatomical structures: the perivascular space and AQP4 expression on astrocytes.

Cerebral perivascular spaces

During the 19th century, Rudolf Virchow and Charles Robin identified tunnel-like spaces surrounding penetrating arterioles within the brain, naming them Virchow-Robin spaces. Later studies found that this feature surrounds all arterioles, capillaries, and venules leading to the collective term perivascular spaces (PVS).[22] PVS's inner wall mainly consists of vascular cells, including endothelial cells and smooth muscle cells, while the outer wall is formed by the pericytes and perivascular astrocytes end-feet.[23] Virchow-Robin spaces are associated with pial arteries, and the fluid within is CSF.[19] As arterioles penetrate deeper, the PVS filled with CSF establishes continuity with the basal lamina (composed of fibronectin, laminin,

heparin sulphate proteoglycan, and type IV collagen). The relaxed extracellular matrix in the basal lamina offers minimal resistance to CSF influx, which moves from the Virchow-Robin spaces along the peri-arterial space, through the basal lamina around capillaries, and exits through the perivenous space[19] (see Figure 1).

In a pre-clinical animal study, fluorescent tracers of varying molecular weights were administered into the cisterna magna of anaesthetized mice. Using *in vivo*, two-photon imaging and immunofluorescence the CSF circulation within the brain interstitial space was monitored. It shows that CSF entered the brain parenchyma through the PVS, facilitating rapid exchange with ISF. The combined CSF and ISF mixture were then efficiently cleared through the perivenous drainage pathways, facilitated by AQP4 polarized on astrocytic end-feet.[7]

Astrocytes, AQP4 water channel, and SNPs

Astrocytes connect blood vessels and neurons, acting as intermediaries that transmit neural activity from synapses to the basal lamina, which includes vascular endothelial cells, pericytes, and astrocytes.[24] Neurotransmitter-induced calcium ion elevation in astrocytes triggers the release of vasoactive substances such as glial-derived neurotrophic factor (GDNF), transforming growth factor-beta (TGF-β), interleukin-6 (IL-6), beta-fibroblast growth factor, angiopoietins, epoxyeicosatrienoic acids, prostaglandin E2, and 20-hydroxyeicosatetraenoic acid. These substances alter vascular tone, causing vasoconstriction or vasorelaxation, and leading to changes in the PVS..[25] Furthermore, 20-nanometre clefts between astrocyte end-feet around the PVS allow macromolecular solute passage.

Aquaporins, a family of membrane proteins, act as water channels, facilitating water transport across biological membranes.[26] These proteins are essential for maintaining water homeostasis in various tissues and organs by regulating the movement of water in and out of cells. In the CNS, AQP1 and AQP4 are especially significant. Notably, 90% of AQP4 proteins (encoded by the AQP4 gene on chromosome 18) are found in the CNS, primarily on the surfaces of the BBB and the CSF–brain barrier.[27] While the AQP4 gene's coding regions are highly conserved, its noncoding regions show significant sequence variation.[28] Coding nucleotide variants within AQP4 can influence protein structure, potentially modifying the functional properties.

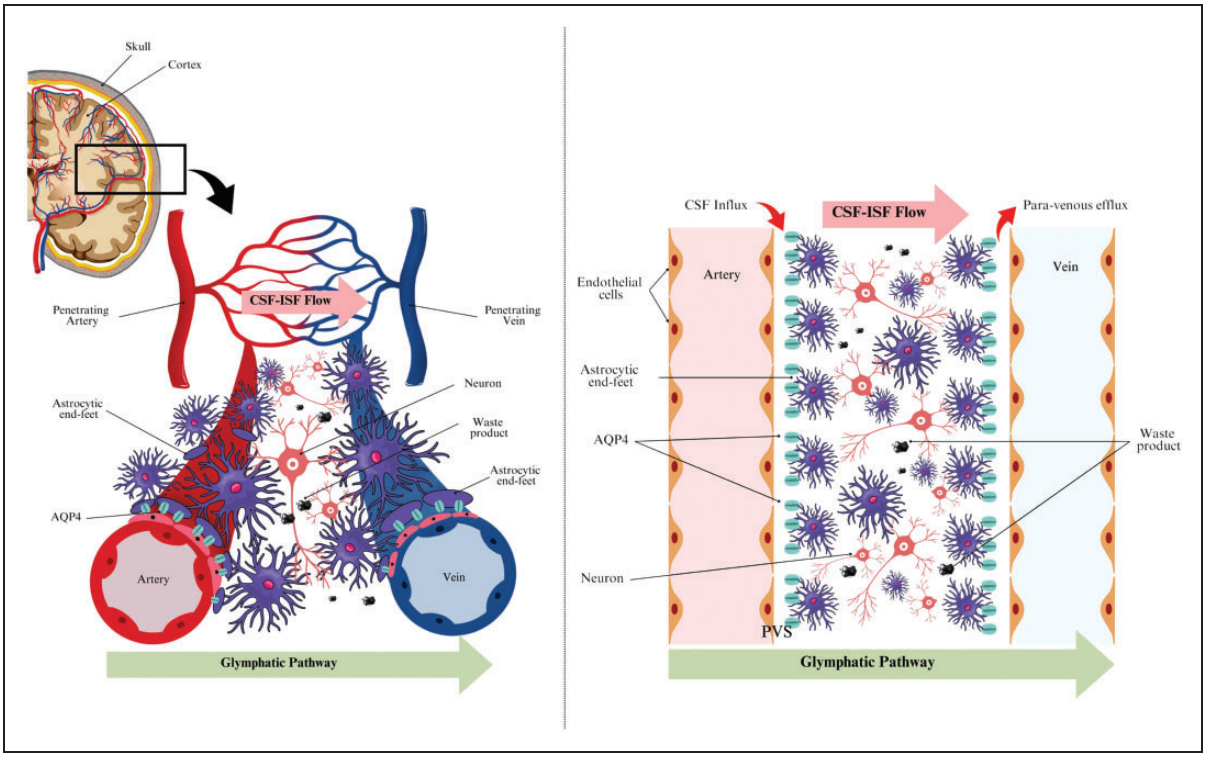

Figure 1. The schematic diagram of the constituent of the perivascular spaces (PVS) and glymphatic system. The glymphatic system is primarily constituted by astrocytes and astrocytic end-feet enveloping the vasculature. The disposal of waste products occurs through cerebrospinal fluid (CSF) transported from arteries, passing through astrocytic aquaporin-4 (AQP4), into the interstitial fluid (ISF), resulting in the amalgamation of CSF and ISF. Subsequently, waste solutes and/or by-products flow along the glymphatic pathway for absorption, contributing to further waste clearance processes.

Noncoding regions of the AQP4 gene play a key role in transcriptional and posttranscriptional regulation due to their affinity for transcription factors. The 3′ untranslated region (UTR) is a potential target for microRNAs (miRNAs), influencing gene expression.[29] The SNPs in the 5′ or 3′ UTRs of the AQP4 gene may affect protein levels and contribute to brain disorders (Figure 2).[10] Whereby, genome-wide SNPs analysis has been extensively used to study population diversity, conservation genetics, and identify functional genes linked to biological traits.[30]

As mentioned, AQP4 is specifically expressed in astrocyte end-foot processes that surround capillaries, forming a crucial barrier around the cerebral vasculature, including subpial and subependymal glial-limiting membranes.[31] A key component of astrocyte end-feet, AQP4 facilitates the movement of fluid and small molecules (approximately 0.18 molecular weight, 0.38 nm diameter) from the CSF into the brain parenchyma.[32] Studies using CSF tracers and contrast media in AQP4-deficient mice (including *Snta1* knockout mice) revealed significantly reduced CSF tracer transport, highlighting AQP4's critical role in supporting perivascular CSF influx and ISF outflow in the glymphatic system.[33]

AQP4 SNPs and neurological diseases

A genome-wide SNPs genotyping study discovered that AQP4 SNPs were linked to changes in the rates of cognitive decline following an AD diagnosis.[9] Two SNPs (rs9951307 and rs3875089) were associated with a slower cognitive decline, while two others (rs3763040 and rs3763043) were linked to a faster cognitive decline after the onset of AD. Their findings offer the initial evidence indicating that variations in the AQP4 gene, essential for the functioning of the glymphatic pathway through its gene product AQP4, might influence the trajectory of cognitive decline in neurodegenerative diseases such as AD.[9]

Moreover, AQP4 SNP rs72878794 was associated with reduced Aβ uptake as seen on neuroimaging

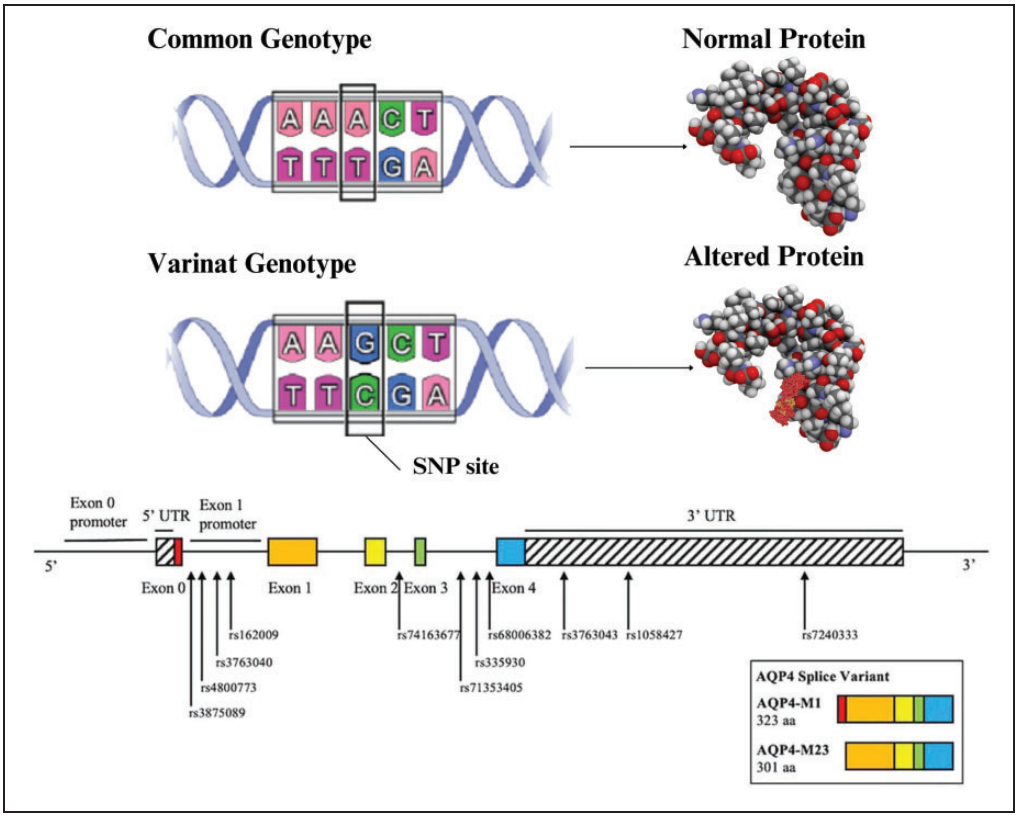

Figure 2. The schematic diagram of single-nucleotide polymorphisms (SNPs) – genetic variations that involve the alteration of a single nucleotide base in DNA. This modification can lead to changes in the amino acid sequence, potentially resulting in the malfunction of the corresponding protein. The bottom panel shows the genomic structure of aquaporin-4 (AQP4) gene, indicating exon positions and known SNP sites affecting AQP4 expression and glymphatic function. Highlighted SNPs (e.g., rs162009, rs3763040, rs68006382) have been linked to neurological and cerebrovascular conditions such as multiple sclerosis, Alzheimer's disease, ischemic stroke, and neuromyelitis optica spectrum disorder (NMOSD) respectively. The lower panel shows the genomic structure of AQP4, indicating exon positions and known SNP sites affecting AQP4 expression and glymphatic function.

(i.e., positron emission tomography, PET), while rs151244 was linked to increased Aβ uptake, a higher risk of transitioning from mild cognitive impairment (MCI) to AD, and faster cognitive decline.[34] These variations in AQP4 influence Aβ accumulation, suggesting that differences in glymphatic system performance may affect brain Aβ clearance. This makes AQP4 SNPs potential biomarkers for predicting disease burden in dementia.[34] In PD, rs2075575 is linked to an increased susceptibility to PD and rapid eye movement sleep behaviour disorder (RBD), while rs335929 is associated with memory cognition but not PD susceptibility.[12] Investigating the regulation of the glymphatic system by influencing the genetics of AQP4 could be a promising avenue for understanding and potentially intervening in the pathology of PD.

Overall, the AQP4 SNPs exhibit notable associations with various aspects of neurological health. The discussed findings underscore the potential significance of AQP4 SNPs in influencing neurodegenerative processes, with implications for understanding and predicting disease progression. Further research into the regulation of the glymphatic system through AQP4 genetics could offer valuable insights and potential biomarkers for neurological disorders on the dementia spectrum, including AD, PD, and particularly CSVD.

The glymphatic system and cerebral fluid dynamics

The brain contains four fluid types: blood, CSF, ISF, and intracellular fluid. The BBB separates blood from ISF, while the blood-CSF barrier maintains separation between blood and CSF.[35] CSF is produced by choroid plexus capillary endothelial cells in the ventricles and then moves into the ventricles and subarachnoid space for storage.[36] Solutes and water from the blood can pass through the BBB into the ISF. As pial arteries enter the brain parenchyma, they transition into penetrating arteries surrounded by PVS, through which CSF enters the brain parenchyma facilitated by AQP4 on astrocyte end feet.[37,38]

CSF integrates with ISF in the brain, demonstrating that CSF contributes to ISF formation through the glymphatic system.[39] Remarkably, CSF and ISF exit the brain parenchyma through three pathways, with the first being the perineural sheaths surrounding the head and face, whereby CSF follows the olfactory nerve sheath to the nasal mucosa, eventually reaching lymphatic vessels in the nasal mucosa and draining into cervical lymph nodes. Other perineural pathways in rodents include the trigeminal, glossopharyngeal, vagal, and spinal accessory nerves.[40] Secondly, through dural lymphatic vessels, these vessels, located in the dura mater, sigmoid sinus, retro-glenoid vein, middle meningeal artery, and pterygopalatine artery, absorb CSF and ISF and transport them to deep cervical lymph nodes through the foramina at the skull base.[41] Finally, is through arachnoid granulations, These structures in the subarachnoid space allow CSF to flow into the sagittal sinus and then into the bloodstream.[42] The cerebral fluid dynamic within the glymphatic system is shown in Figure 3.

Generally, the fluid dynamics in the glymphatic system are influenced by pressure differentials, arterial pulsations, and respiration.[43] CSF production creates a pressure gradient that moves fluid from the ventricles to the subarachnoid space. Respiration affects CSF flow, with deep inhalations increasing flow and breath-holding decreasing it, likely due to changes in chest pressure.[44] Additionally, ligation of the internal carotid artery reduces arterial pulsations and slows CSF-ISF exchange. Conversely, systemic administration of the adrenergic agonist dobutamine increases arterial pulsations and accelerates CSF-ISF exchange in perivascular regions.[45] These findings opened a new insight into how arterial pulsations play a crucial role in facilitating CSF flow within the glymphatic system through the PVS.

Glymphatic system and the solutes transport in the brain

Two classifications of solutes are conveyed within the glymphatic system: (1) Nutrients, including glucose and lipids,[46] alongside neuroactive substances such as apolipoprotein E;[47] (2) Metabolites found in the CNS, such as Aβ and α-synuclein. Various studies have indicated that glucose, growth factors, lipids, neuroactive substances, neurotransmitters, and pharmaceuticals administered into the CSF, including anti-tumour medications, can be conveyed to the brain via the influx of CSF.[46,47] Particularly in lipid transport, the glymphatic system assumes a pivotal role in the brain. Metabolites present in the CNS, including Aβ, α-synuclein, tau protein, and lactic acid, can also be expelled from the brain via the glymphatic system.[48]

Moreover, Aβ, a protein associated with AD, accumulates abnormally in the brain ISF, contributing to neurodegenerative disorders.[31] Research indicates that Aβ is transported along the glymphatic system within the CNS. A previous study showed that the knockout of AQP4, a critical component of the glymphatic system, results in reduced clearance of Aβ. This reduction is due to fluid congestion, as Aβ is primarily cleared through the bulk flow of the glymphatic system rather than localized clearance at the BBB.[7] For solutes that cannot cross the BBB, the glymphatic system provides the primary pathway for their

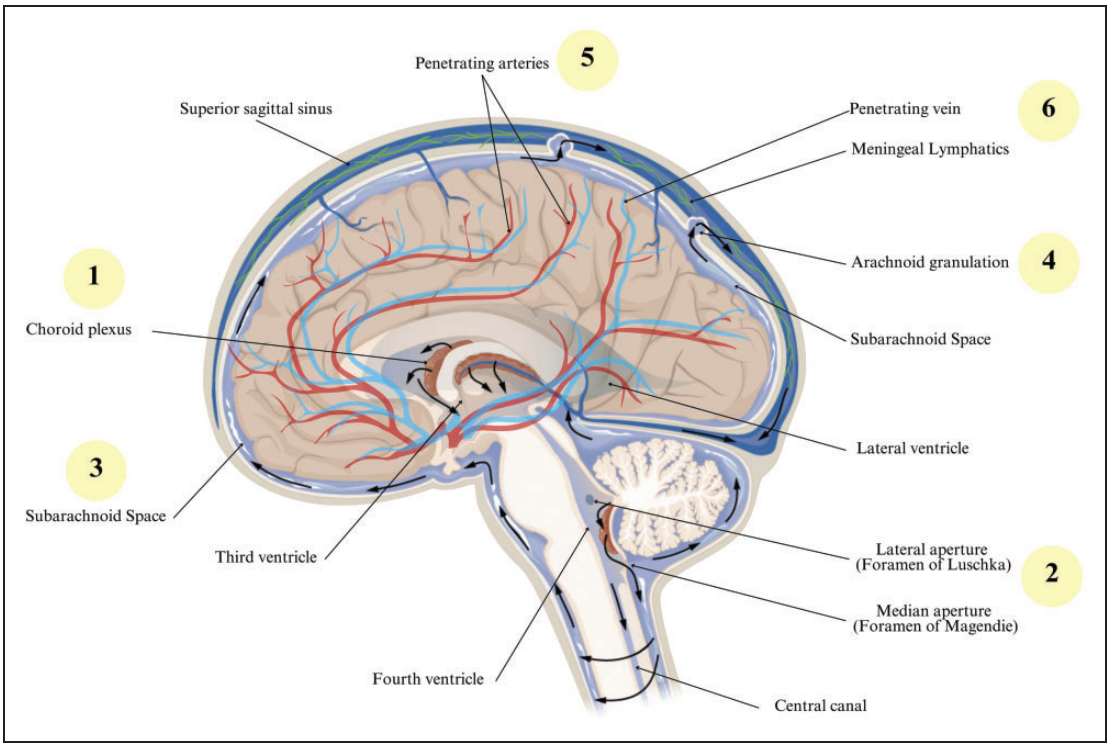

Figure 3. The schematic diagram of the fluid dynamics within the glymphatic system. The model involves (1) cerebrospinal fluid (CSF) secretion by blood through choroid plexus capillary endothelial cells distributed in the lateral, third, and fourth ventricles. (2) CSF progresses from the ventricular system (ventricular bulk flow) through the lateral aperture and median aperture into the (3) subarachnoid space of the brain and spinal cord. Within the subarachnoid space, (4) CSF efflux via subarachnoid granulations and nerve roots (5) some CSF enters the brain parenchyma through perivascular spaces (PVS) surrounding penetrating arteries, facilitated by AQP4 on the end feet of astrocytes. Furthermore, CSF intermingles with interstitial fluid (ISF) in the brain parenchyma, and the amalgamation subsequently enters the (6) perivenous space and meningeal lymphatics. Egress points for cranial fluid, are depicted by black arrows. Ultimately, the combined CSF and ISF mixture is drained to the cervical lymph nodes.

movement into and out of the brain parenchyma. Understanding the glymphatic system's role is crucial for developing strategies to enhance drug delivery to the brain. Investigating how the glymphatic system facilitates the removal of pathogenic factors can lead to new therapeutic approaches for neurodegenerative diseases.

Glymphatic system and neurodegenerative disorders

It has been suggested that the breakdown of brain fluid transport (fluidopathy),[6,49] specifically through the glymphatic system, is essential in neurodegenerative disorder. The primary rationale behind this proposition is the utilization of PVS as conduits for the influx of CSF. The impeded or dysfunction of transport within the glymphatic system (glymphopathy) could potentially disrupt the equilibrium of brain fluid, resulting in transient white matter oedema, ePVS, and ultimately leading to demyelination.[49] Various studies have documented the significance of glymphopathy and neurodegeneration.

AD is a progressive neurodegenerative disorder marked by memory impairment and a reduction in brain volume.[50] Its pathogenesis involves the development of senile plaques due to the accumulation of Aβ,[51] the formation of neurofibrillary tangles from abnormal tau protein,[52] and changes in aquaporin-4 (AQP4) expression in astrocytes. Specifically, in AD, AQP4 expression shifts from the astrocytic end-feet to the entire cell body, a phenomenon known as AQP4 depolarization.[53] In APP/PS1 mice, a recognized model for AD, the accumulation of toxic Aβ, including soluble oligomers, is intricately linked to the dysfunction of glymphatic system transport. This dysfunction is assessed by quantifying the inflow of solutes into the brain from CSF and their clearance from the brain using radio-labelled tracers. Importantly, a decline in glymphatic system transport is correlated with the entry and buildup of Aβ40 in the PVS, a phenomenon associated with AQP4 depolarization.[7] Consequently, the malfunction of the glymphatic system precedes significant deposits of Aβ, potentially serving as an early indicator of AD.[54]

Moreover, in PD – a condition associated with an imbalanced production and clearance of α-synuclein.[55] In a PD model using A53T mice, where meningeal lymphatic drainage was impeded by ligating the deep cervical lymph nodes, there was a reduction in the glymphatic inflow of CSF tracer in the mice's brains. This reduction led to increased accumulation of α-synuclein, heightened neuroglial activation, increased inflammation, loss of dopaminergic neurons, and the onset of dyskinesia.[56] The absence of AQP4 hindered the clearance of α-synuclein in the brain, resulting in an increase in protein monomers without a corresponding increase in oligomers.[57] These findings suggest that AQP4 and meningeal lymphatic drainage play a partial role in the clearance of soluble monomeric forms of α-synuclein, indicating a potentially promising avenue for alleviating PD symptoms.

Studies show a significant link between glymphatic dysfunction and stroke. Stroke, including haemorrhagic (cerebral and subarachnoid) and ischemic types (thrombosis and infarction), disrupts cerebral blood flow and causes localized nerve dysfunction.[58] Following 24 hours of subarachnoid haemorrhage, immunohistochemical findings revealed that fibrin clots obstructed the PVS of cerebral cortical vessels.[59] The functionality of the glymphatic system could be restored through intraventricular injection of tissue plasminogen activator (t-PA),[60] suggesting that subarachnoid haemorrhage induces disorders in the glymphatic system. Importantly, targeted enhancement of cerebral glymphatic clearance may emerge as a novel strategy for treating haemorrhagic stroke. In the ischemic stroke model, pathological alterations resembling those in subarachnoid haemorrhagic stroke are evident, including the ePVS and a shift in AQP4 polarity distribution from the typical perivascular pattern to a scattered parenchymal pattern.[61] Therefore, preserving and sustaining the normal function of the glymphatic system holds promise for mitigating stroke-induced injuries and improving cognitive impairment resulting from stroke.

Other neurological disorders related to glymphopathy have also been documented such as brain oedema,[31] traumatic brain injury that mediated neurodegeneration,[62] amyotrophic lateral sclerosis,[63] and migraine.[64] Moreover, glymphopathy has also been associated with cognitive impairment, for example, individuals with diabetes commonly experience cognitive and memory decline and are at an elevated risk of vascular diseases and AD.[65] A previous imaging study on type-2 diabetic rats revealed that the clearance rate of the contrast agent Gd-DTPA in CSF from the interstitial space in the hippocampus was three times slower compared to non-diabetic rats in a diabetes rat model. This observation was further validated through fluorescence imaging analysis[66] indicating type-2 diabetes has the potential to hinder the exchange flow of CSF and ISF, resulting in glymphopathy attributed to the ePVS. The accumulation of metabolic wastes in the ePVS is likely to trigger an inflammatory reaction, leading to further progression of ePVS.[67] Therefore, the investigation of the glymphatic system offers a new perspective on understanding cognitive impairment, although its mechanism remains unclear and necessitates further experimental exploration.

Sleep and age-related glymphopathy

The glymphatic system's activity is modulated by the sleep-wake cycle, with its functionality significantly heightened during sleep. Initial experiments on the glymphatic system used mice under anaesthesia with ketamine/xylazine, revealing that glymphatic function differs markedly between sleep and wakefulness.[7] Specifically, glymphatic influx of solutes into the brain increased by about 80% during sleep compared to wakefulness, suggesting minimal glymphatic activity when awake.[8] Additionally, Aβ clearance from the brain was roughly 40% more efficient during sleep or anaesthesia compared to wakefulness. A substantial rise in ISF volume fraction during sleep, regulated by norepinephrine, was linked to improved glymphatic function, indicating less constrained solute transport in the ISF during sleep.[8]

The enhancement in glymphatic transport with ketamine/xylazine anaesthesia was attributed to xylazine's effect on reducing central norepinephrine levels, rather than ketamine, which increases these levels.[8,68] Xylazine, an α-2agonistr agonist, inhibits central norepinephrine release,[69] resembling the actions of the sedative dexmedetomidine[70] used in clinical settings for sedation and as an adjunct for general anaesthesia. Supporting this assertion, it was also demonstrated that anaesthesia with dexmedetomidine and low-dose isoflurane doubled glymphatic system transport compared to isoflurane alone.[71] Moreover, insufficient sleep has been reported to contribute to a reduction in the clearance rate of metabolic proteins within the brain parenchyma, leading to the deposition of proteins such as Aβ and tau protein.[72] In summary, extending sleep duration or enhancing sleep quality has the potential to improve CSF inflow and metabolite clearance.

Interestingly, body position during sleep or anaesthesia affects glymphatic function. Dynamic-contrast-enhanced MRI and kinetic modelling demonstrated lateral positioning in anaesthetized rats resulting in higher glymphatic transport efficiency compared to supine and prone positions. In prone rats, the tracer in the brain entered and cleared slowly in the

glymphatic system.[73] Optical imaging and radiotracer studies confirmed that the transport of the glymphatic system and the clearance rate of Aβ were faster in the lateral and supine positions.[74] Changes in body position may affect the cardiovascular and respiratory systems, subsequently impacting pulsation and CSF-ISF dynamics.[73] This mechanism requires further exploration. These findings suggest that the lateral position during sleep/anaesthesia may be advantageous in removing brain metabolic wastes, including Aβ. In the future, attention should be given to the placement of test animals in the experimental design of the glymphatic system.

Multiple neurodegenerative diseases, including AD, PD, amyotrophic lateral sclerosis, and others, frequently manifest in middle and old age, with their pathogenesis linked to the accumulation of waste products such as Aβ and tau protein.[19] Aβ and tau protein can be removed from the brain parenchyma through CSF outflow in the glymphatic system. A comparison of interstitial solute clearance rates between young (2–3 months old) and old (18–20 months old) wild-type mice revealed a 40% reduction in Aβ b clearance in the old mice. This reduction was accompanied by a 27% decrease in the pulsatility of the vascular wall of the arterioles in the old mice cortex and a widespread loss of AQP4 polarization along the penetrating artery.[75] Additionally, ageing contributes to artery stiffening, which can diminish arterial pulsatility in the cerebral cortex of the elderly, thereby hindering CSF inflow from the perivascular space to the brain parenchyma.[76] These findings indicate that as individuals age, the functions of the glymphatic system tend to deteriorate, impacting the clearance of metabolic wastes in the brain.

Preclinical methods for glymphatic fluid dynamics

Ex vivo imaging is commonly conducted using light sheet fluorescence microscopy on brain or spinal cord sections obtained from optically cleared mouse brains, which were injected with CSF tracers while under anaesthesia.[77] In this manner, high-resolution 3D imaging of tracer distribution within cells and PVS, with a capability of up to 200 nm, can be observed.[78] Coronal sections of the brain or spinal cord, which are fixed, are commonly paired with immunohistochemistry to assess CSF flow in correlation with the expression patterns of relevant proteins. Nevertheless, Panoptic imaging of transparent mice has become increasingly utilized in recent years to analyse the distribution of tracers in the brain.[79]

In vivo imaging was employed to observe CSF flow by introducing a CSF tracer into the cisterna magna. Methods include two-photon fluorescence imaging (TPI),[80,81] near-infrared fluorescence (NIRF),[82] transcranial optical imaging,[83] and MRI,[84] allowing viewing up to 240 mm below the cortical surface, which is useful for examining CSF tracer diffusion along cortical arteries and arterioles.[7] However, TPI is invasive, can damage the skull, and has a limited field of view, restricting observations to subcortical areas.[85] In contrast, transcranial optical imaging is non-invasive, can image up to 5–6 mm below the cortical surface, and allows comprehensive observation of the dorsal perivascular region in living mice.[86] Nonetheless, it is limited to the dorsal cortex and cannot image the ventral cortex.[87]

Interestingly, MRI can dynamically monitor the entire brain in real-time, facilitating the study of CSF flow in the glymphatic system. NIRF enables non-invasive measurement of human brain fluid dynamics, offering the potential for long-term brain monitoring, including during sleep, and can be integrated with various neuroimaging technologies.[88] NIRF allows real-time dynamic detection of CSF tracer *in vivo*, but its limited spatial resolution hinders the analysis of fluid flow in PVS.[89] MRI,[37] single-photon emission computerized tomography (SPECT), and PET combined with CT[90] can be employed to generate brain-wide 4D images of tracer movement *in vivo* in experimental animals. Importantly, these methods are readily translatable to clinical neuroimaging studies. Information regarding preclinical approaches for investigating the glymphatic system can be found in Table 1.

CSVD and glymphopathy

The pathophysiological foundation of CSVD encompasses alterations in the structure and function of the microvasculature within the deep subcortical regions. These alterations primarily affect arteries, including tributaries of the middle cerebral artery, and arterioles, resulting in phenomena such as fibrinolysis, lipohyalinosis, necrosis, and microthrombosis.[3,92] CSVD becomes increasingly prevalent with age and is frequently encountered as an incidental discovery during neuroimaging. This condition is frequently underestimated by healthcare professionals because of its covert (silent) nature, as it often presents without symptoms. Current neuroimaging indicators (or manifestation) of CSVD based on Standards for Reporting Vascular Changes on Neuroimaging 2 (STRIVE-2) encompass recent small subcortical infarcts, WMHs of presumed vascular origin, lacune infarcts (of presumed vascular origin), ePVS, cerebral microbleed, cortical superficial siderosis, brain atrophy, and cortical cerebral microinfarct[1] (Figure 4). Frequent cardio-cerebrovascular risk factors for sporadic CSVD, including ageing, type 2 diabetes, hypertension, smoking, and

Table 1. Information regarding preclinical methodologies for studying flow dynamics in the glymphatic system.

Method	Application	Pros	Cons
Ex Vivo			
Sections of the brain or spinal cord that are fixed in a coronal (frontal) orientation	The penetration of CSF tracers into the brain parenchyma was evaluated across multiple slices.	Employed in conjunction with immunohistochemistry to juxtapose CSF flow with the expression patterns of related protein	Issues related to CSF tracer studies: the misplacement of tracers caused by the collapse of PVS in deceased animals, tissue damage during dissection, and the time-consuming nature of the process.
Panoptic imaging (3DISCO) of transparent mice[91]	Capturing images of the entire rodent's head or body.	Visually demonstrating the direct connection between the brain and meningeal structures	Challenges associated with CSF tracer studies: the misplacement of tracers due to the collapse of PVS in deceased animals, time-consuming processes with limited quantifiability, and difficulty in immunolabeling.
In Vivo			
TPI	To monitor the spread of CSF tracer along the outer surfaces of cortical arteries and penetrating arterioles.	Precise or fine spatial resolution	Invasiveness to the study object, coupled with a restricted field of vision and a limited imaging depth, constrains the observation of subcortical brain regions, rendering it impossible to observe the entire glymphatic system across the whole brain field.
NIRF	Achieve continuous, real-time detection of CSF tracer in living animals	Address the requirements of both *in vitro* and *in vivo* research.	Due to its low spatial resolution, the analysis of fluid flow in PVS is hindered.
Transcranial optical imaging	Extensive imaging of CSF flow in the dorsal perivascular region of the cerebral cortex in living mice.	Preservation of skull integrity.	It is limited to observing the dorsal region of the cerebral cortex and cannot capture images of the ventral area.
The combination of PET or SPECT with CT[90]	The entry of tracers into sizable CSF spaces, their penetration into the brain, and the subsequent exit from the brain parenchyma.	Quantitative and dynamic imaging of the entire CNS and body.	Limitations of low spatial resolution. CT primarily offers anatomical information for hard tissue (e.g., bone), with constrained options for physiological interventions and monitoring during dynamic scans.
MRI			
1. DCE-MRI	Captures the inflow and outflow of CSF in the glymphatic system, assessed through the rate of the contrast agent entering and exiting the brain parenchyma.	Visualizing the three-dimensional flow of CSF throughout the entire brain, offering simultaneous time and spatial information.	The spatial resolution is insufficient for micro-scale analysis, real-time monitoring of tracer distribution in the brain is not possible, and prolonged deposition of the contrast agent in the brain parenchyma may lead to adverse effects.
2. DTI	To investigate the flow of fluids in PVS	No contrast agent is necessary; Non-invasive imaging.	Limited spatial resolution, preventing microscopic analysis, and inability to capture real-time images.
3. CEST-MRI	Capture the alterations of information at the molecular level, such as changes in glucose and protein levels.	It can capture information regarding proteins and other molecules, enabling measurements of metabolic functions.	The specific absorption rate is high, posing potential risks in human body research; Low resolution, incapable of conducting microscopic analysis.

CEST-MRI: Chemical exchange saturation transfer- magnetic resonance imaging; CT: computed tomography; DCE-MRI: Dynamic Contrast-Enhanced magnetic resonance imaging; DTI: Diffusion Tensor Imaging; MRI: magnetic resonance imaging; NIRF: Near-infrared Fluorescence imaging; PET: positron emission tomography; SPECT: single-photon emission computed tomography; TPI: Two-photon Fluorescence Imaging.

dyslipidaemia, elevate the risk of pathological alterations in arteries and arterioles, potentially resulting in vessel blockage, which in turn leads to the development of arteriosclerosis and arteriolosclerosis.[3]

Various etiopathogenic classifications exist for CSVD. Nevertheless, the most widely acknowledged categories of CSVD include amyloidal CSVD (e.g., sporadic, and hereditary cerebral amyloid angiopathy)

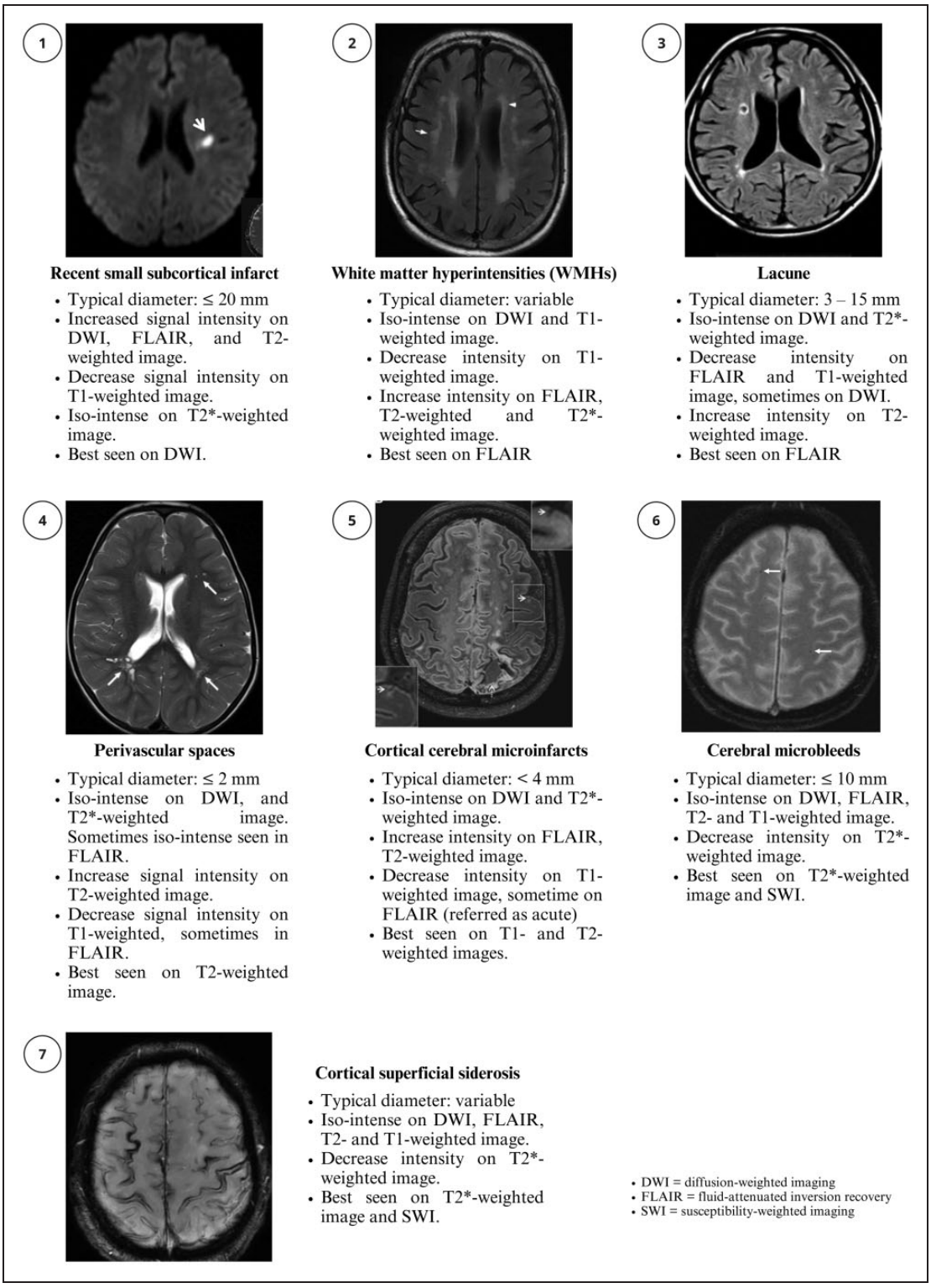

Figure 4. Neuroimaging (i.e., magnetic resonance imaging, MRI) manifestations of lesions associated with cerebral small vessel disease (CSVD) are depicted in the figure. The upper section provides examples of CSVD features, while the middle portion illustrates their corresponding schematic representation. The lower part summarizes the MRI sequence characteristics. DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; SWI, susceptibility-weighted imaging.

and non-amyloidal CSVD, which encompasses age-related and small vessel disease related to vascular risk factors (such as arteriolosclerosis and ageing).[93] Meanwhile, the less prevalent categories of CSVD encompass inherited or genetic (monogenic) CSVD, which exhibits distinct characteristics separate from cerebral amyloid angiopathy for example, Fabry's disease and cerebral autosomal dominant arteriopathy with subcortical ischemic strokes and leukoencephalopathy (CADASIL), as well as inflammatory and immunologically-mediated CSVD, venous collagenases, and other forms of CSVD, including non-amyloid micro-vessel degeneration in AD and post-radiation angiopathy.[94] Various pathomechanism and molecular cascades have been proposed for the onset and progression of CSVD, and most of them are intercurrent in the majority if not all categories of CSVD.

Current knowledge of pathomechanism of CSVD

In general, it is widely recognized that the pathomechanism of cerebrovascular disease linked to hypertensive alterations in the vasculature leads to the formation of arteriosclerosis or lipo-hyalinosis (thickening and/or damage to the walls of arterioles), fibrohyalinosis, and the subsequent blockage of cerebral penetrating arteries.[95] These pathologies are believed to relate to the CSVD manifestation and the increased growth of connective tissue fibres. This, in turn, leads to a decline in vascular contractility before advancing into vascular and/or microvascular sclerosis.

Apart from that, pathological alterations associated with CSVD may occur when a cerebral small vessel becomes blocked due to age-related arteriolar contortion, venous collagenases, demyelination, and glial cell loss.[96] These changes can be exacerbated by inflammaging, a concept introduced recently to characterize age-related, persistent, low-grade inflammation that arises because of extended immune system activity.[97] These enduring effects encompass a range of molecular and cellular mechanisms, including dysfunction in mitochondria, imbalance in gut microbiota, meta-inflammation, immune-senescence, and cellular senescence. Therefore, it can be argued that inflammaging may potentially encompass various other factors tied to DNA repair, the release of pro-inflammatory cytokines, and stem cell senescence.[97]

Recent studies using arterial spin labelling (ASL)-based imaging have shown a correlation between cerebral blood flow (CBF) values and CSVD manifestations like WMHs and ePVS. This correlation may serve as a biomarker for vascular cognitive impairment in CSVD cases.[98] Additionally, the breakdown of the BBB can be worsened by hypercoagulation, which increases platelet deposition and microthrombi formation associated with arteriosclerosis or arteriolosclerosis.[99] Moreover, inflammatory signals cause damaged endothelial cells to release von Willebrand factors, enhancing platelet activation and adherence. Subsequently, activated platelets produce vasoconstrictive compounds like thromboxane A2 and adenosine diphosphate, promoting further platelet activation and aggregation.[100] These processes are indicators of arteriosclerosis progression and contribute to CSVD development.

These pathophysiological reactions result in additional insult to the cerebral tissue, leading to various issues such as axonal injury, neuronal apoptosis, demyelination, and damage to oligodendrocytes. This damage gives rise to clinical symptoms and a range of complex neuroimaging manifestations, including silent CSVD.[101] However, it is worth noting that a significant portion of current therapeutic understandings appears to be derived from the pathomechanisms of sporadic CSVD. These mechanisms involve molecular and cellular outcomes related to several systemic dysregulations, including coagulopathy, heightened microthrombosis, increased cellular activation, inflammation, and oxidative stress. Some or all these processes can lead to microstructural changes in cerebral parenchyma, which are well-known characteristics of CSVD. These changes encompass endothelial dysfunction, alterations in CBF, and the impairment of the BBB.[101]

ePVS and CSVD

Based on Figure 4, PVS are fluid-filled structures with round, ovoid, or linear shapes, reflecting the trajectory of small perforating vessels through white or deep grey matter.[1] The STRIVE 1 consensus recommends using "PVS" for dilated perivascular spaces on MRI, avoiding "enlarged," and this has been widely adopted.[102] However, this review will continue using "ePVS" to describe abnormal PVS dilation. On brain MRI, ePVS have a signal intensity similar to CSF and typically lack a T2-hyperintense rim unless in white matter hyperintensities (WMHs). They usually have a maximum axial diameter of less than 3 mm and are most prominent in the basal ganglia and centrum semiovale.[103] It's important not to confuse them with developmental perivascular invaginations in the inferior basal ganglia. MRI-visible ePVS are primarily periarteriolar, as shown by T2-weighted, SWI sequences, and 7-Tesla MRI.[104]

Alarmingly, the number of ePVS increases with age and vascular risk factors and is associated with cognitive disturbances.[5,105] These spaces are highly heritable,[106] and their associations with age and risk factors depend on their location.[104] According to the Boston 2.0 criteria for cerebral amyloid angiopathy, a high

burden of ePVS in the centrum semiovale, combined with lobar haemorrhagic features (e.g., intracerebral haemorrhage or CMBs), suggests a probable diagnosis of cerebral amyloid angiopathy.[107] While a few ePVS can be normal, a significant burden is linked to neurological diseases such as stroke, cerebral amyloid angiopathy, and AD.[105,107] ePVS may appear on MRI before lacunes, CMBs, or WMHs[108] and can signal impaired ISF clearance, especially during sleep.[109]

The PVS and ePVS are assessed using visual rating scales, which are convenient for clinical research but may lack sensitivity.[110] To date, various computational methods for quantifying various parameters related to ePVS are now accessible.[111] These computational approaches exhibit enhanced sensitivity in detecting associations with WMHs, retinal vessel diameters (e.g., smaller arterioles and wider venules, serving as proxies for cerebral small perforating arterioles and venules), or genetic variants indicative of relevant vascular dysfunction mechanisms. While high-resolution T2-weighted images are recommended for optimal (i.e., most sensitive) ePVS detection, T1-weighted images can also be utilized.[111]

Role of the glymphatic system in CSVD – evidence from animal model

A key characteristic of the glymphatic system is its peak fluid flow during sleep or under anaesthesia which promotes non-REM sleep EEG patterns.[78] A recent study shows that one night of total sleep deprivation impairs molecular clearance from the brain, and this deficit is not fully addressed by subsequent sleep.[112] Fluid transport also follows circadian rhythms, peaking during the inactive phase. Given that CSVD involves structural changes in PVS and reversible white matter oedema, disruptions in glymphatic fluid transport may contribute to CSVD.[113]

Most mechanistic analyses on CSVD related to glymphatic transport use rodent models of hypertension and diabetes. However, rodent models, particularly mice and rats, have limitations due to differences in vascular anatomy, smaller white matter compartments, and incomplete replication of human CSVD pathology. Key features of CSVD pathology like ePVS and WMHs, are not consistently identified in rodent models.[114] Despite limitations, chronic hypertension rodent models, particularly spontaneous hypertensive rats (SHR), have provided insights into cerebrovascular and glymphatic transport changes. In a previous study, SHRs were examined at 8 weeks (early hypertension) and 20 weeks (chronic hypertension).[115] Using DCE-MRI and kinetic modelling, studies revealed a reduction in the influx of gadolinium-based tracer gadoteric acid (Gd-DOTA) in SHR compared to Wistar-Kyoto (WKY) rats, without significant astrogliosis or AQP4 depolarization, indicating upstream glymphopathy was not associated with reactive gliosis or neuroinflammation.[115]

Regrettably, accurately determining the impact of chronic hypertension on brain-wide CSF transport was hindered by the fact that SHR also displays enlargement of their cerebral ventricles, introducing ambiguity in the interpretation of CSF transport.[115] In a subsequent investigation utilizing another rat model of CSVD, specifically, the spontaneously hypertensive stroke-prone (SHRSP) rats, CSF fluid dynamics and glymphatic flux were examined in approximately 8-month-old SHRSP and normotensive WKY rats.[116] Significantly, the SHRSP rat model does not exhibit cerebral ventriculomegaly, providing an opportunity to assess CNS fluid homeostasis without this potential confounding factor. Additionally, the analysis employed computational fluid dynamics based on regularized optimal mass transport (rOMT) theory has been used to scrutinize the DCE-MRI data related to Gd-DOTA transport within the CNS tissue compartments of both the SHRSP and WKY rats.[116]

The rOMT analysis of DCE-MRI data provided insights into metrics such as 'speed,' flux, and fluid trajectories in the CNS.[116] It revealed significant reductions in total CSF flux in SHRSP rats compared to WKY rats, though the mean relative solute speed remained unchanged. Glymphatic flux showed a moderate decrease ($\sim$15% in the whole brain) in SHRSP rats. High-resolution DCE-MRI further indicated impediments in solute transfer from the PVS to the tissue in SHRSP rats, suggesting parenchymal resistance. Additionally, altered AQP4 expression was observed in SHRSP rats compared to WKY rats.[116]

Diabetes is recognized as a significant risk factor for CSVD[120], but most clinical trials include subjects with multiple comorbidities, including diabetes, creating a gap in understanding diabetes as a sole comorbidity for CSVD. Recent reviews have linked type 2 diabetes with CSVD MRI biomarkers like WMHs and lacunar infarcts.[117,118] Preclinical studies using a streptozotocin-induced type 2 diabetes rat model revealed disrupted CSF dynamics and glymphatic transport, suggesting impaired CNS homeostasis and solute transport. Notably, diabetic rats showed a fluid compartment associated with PVS of large pial arteries, characterized by rapid CSF currents and solute influx,[67] alongside slower contrast clearance from the brain, potentially involving outflow to meningeal lymphatics and cervical lymph nodes. Additionally, older hypertensive rats also displayed diminished solute clearance.[115] These studies suggest that glymphatic flow in rat models of chronic hypertension and diabetes displays similar patterns, characterized by reduced glymphatic transport and

clearance (glymphopathy). However, the CSF fluid flux signatures differ between the two conditions. In diabetes, CSF transport along the subarachnoid space and ePVS shows rapid influx kinetics, while in chronic hypertension, CSF flow is reduced, likely due to increased arterial stiffness and decreased CSF pulsatility.[119]

However, in diabetes, unaccompanied by hypertension, vascular stiffness may not play a dominant role in the early stages of CSVD pathophysiology. In diabetes, the accumulation of advanced glycation end (AGE) products primarily affects the vasculature, leading to inflammation, reactive alterations, and disruption of the BBB.[120] The advanced glycation end products also induce the release of vascular endothelial growth factor from endothelial cells, potentially increasing the volume of the PVS through angiogenesis.[121] The subsequent decrease in glymphatic flux and the stagnation of the contrast agent in the PVS could result from heightened tissue resistance to parenchymal exchange and clearance due to reactive gliosis and depolarization of AQP4 observed in both hypertension and diabetes. In any case, the stagnation of glymphatic flux with secondary ePVS emerges as a common factor in the long-term pathophysiology as CSVD progresses in both conditions (Figure 5).

Finally, it is essential to note that CSVD risk factors like hypertension and diabetes are linked to a higher risk of neurodegenerative diseases such as AD and early cognitive decline. Glymphopathy, which involves stagnant ISF flow, increases susceptibility to protein aggregation. For example, in a murine AD model, AQP4 deletion worsened Aβ aggregation without affecting Aβ-related protein levels.[122] Similarly, obstructing meningeal or cervical lymphatic vessels accelerates AD pathology and cognitive decline.[123] In mice, glymphatic clearance decreases before Aβ deposition and worsens with plaque formation and reactive gliosis. Even introducing Aβ into the CSF impairs glymphatic flow,[124] indicating that multiple pathophysiological events contribute to cognitive decline in CSVD.

Glymphopathy, sleep, and CSVD intercurrent relationship

The removal of AQP4 has been shown to disrupt the circadian rhythm in glymphatic fluid transport. Since AQP4 are highly polarized in astrocytic end-feet, hence they play a crucial role in modulating bulk fluid movement, exchange between CSF and ISF, and clearance of solutes, whereby all are under the influence of circadian rhythm.[78] Additionally, there is evidence suggesting that astrocytes inhibit suprachiasmatic nucleus neurons and, through glutamate signalling, regulate circadian timekeeping.[125] Consequently, astrocytes and AQP4 act as critical checkpoints for the proper functioning of the glymphatic system, especially during sleep i.e., in deep sleep (or slow-wave sleep, SWS).

Sleep disturbances are increasingly acknowledged as risk factors for stroke and dementia.[126] One potential mechanism linking sleep to neurodegenerative diseases, and cognitive outcomes is its recently unveiled role in glymphatic clearance.[8] The increased interstitial spaces during sleep contribute to the removal of metabolic waste products through perivascular pathways. Enhanced glymphatic clearance, particularly associated with deeper sleep, is characterized by increased CSF flow time-locked with slow oscillations.[127] Consequently, poorer sleep lacking in SWS may be correlated with disrupted fluid exchanges among CSF, interstitial spaces, and PVS, leading to less effective clearance of waste products from the brain. Consequently, impaired waste product clearance following sleep disturbances and shorter SWS durations could contribute to ePVS. A recent study by Baril and colleagues documented that lighter sleep is correlated with a greater burden of CSVD (i.e., ePVS) in middle-aged and elderly individuals.[128]

Moreover, changes in sleep patterns, including increased sleep fragmentation and reduced deep sleep (in terms of SWS), are characteristic signs of age-related alterations in sleep.[129] The degeneration of neural pathways regulating sleep-wake patterns and sleep architecture, as well as potential somatic or psychiatric connections between sleep characteristics and cognitive decline in the elderly, have been proposed.[130] There is a suggested link between sleep disturbances and cognitive decline, and notably, many of the disorders mentioned are recognized risk factors for the development of CSVD i.e., the precursor for AD, stroke, and PD. Alarmingly, disturbances are not exclusive to individuals with major neurodegenerative disorders mentioned; they are also prevalent in patients with mild cognitive impairment.[131] This condition has significant implications for both patients and caregivers and is considered a major risk factor for early institutionalization.

Clinical studies have shown that neurodegenerative diseases like AD begin more than 20 years before extracellular misfolded protein deposits are identified and clinical cognitive decline starts.[132] Thus, the preclinical stage of CSVD is critical for developing effective interventions. A recent study used a mouse model of AD and sleep fragmentation to replicate intermittent awakenings.[133] It's reported that sleep fragmentation accelerated AD progression in 5×FAD mice and affected cognitive behaviours, particularly learning and memory, in wild-type mice. These effects were linked to AQP4 modulation, with sleep fragmentation upregulating AQP4 in younger animals but not affecting older ones.[133] In wild-type mice, sleep fragmentation

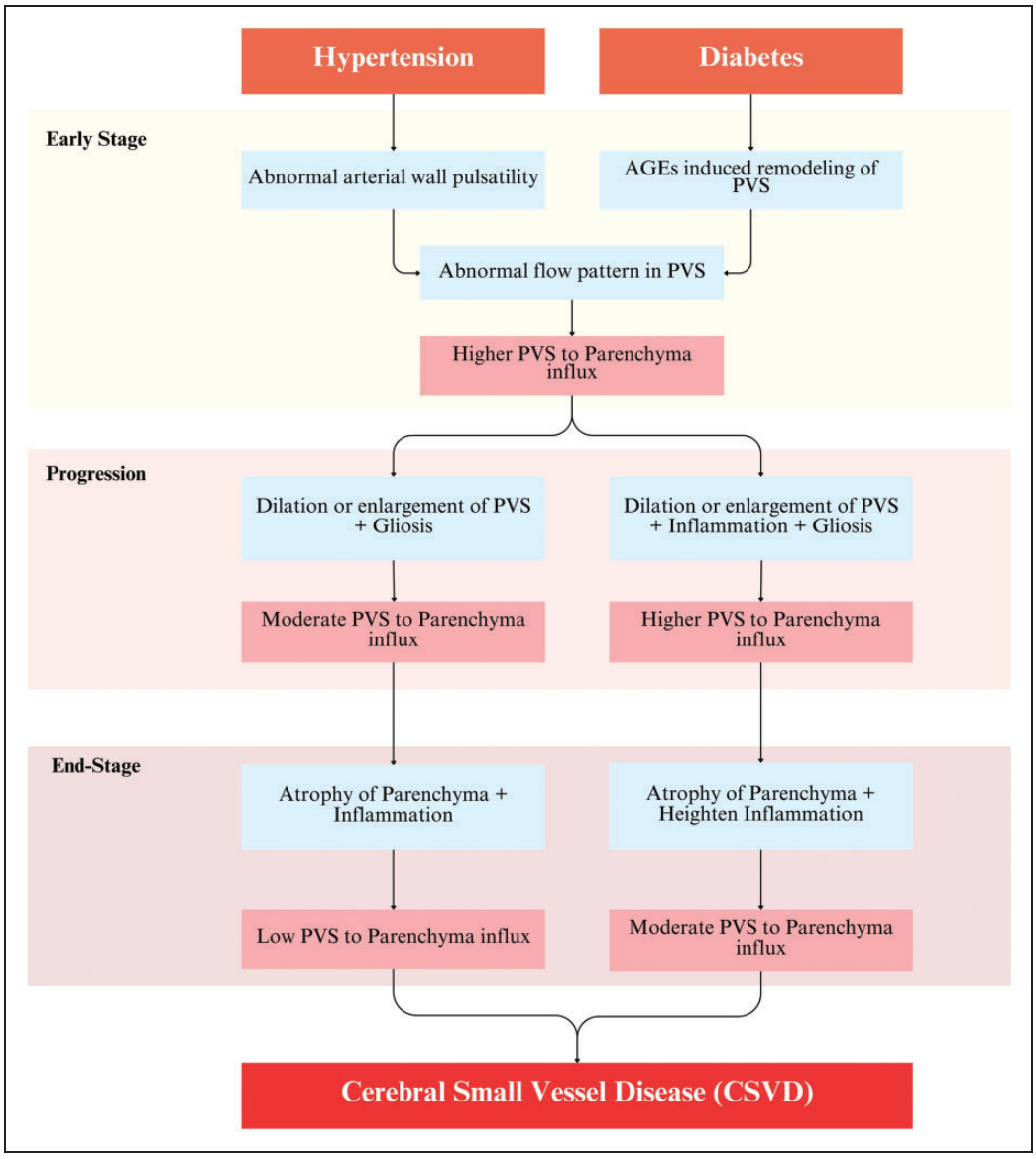

Figure 5. Hypothesized model explaining how glymphopathy in the presence of cerebral small vessel disease (CSVD) risk factors such as hypertension and diabetes predisposes to pathogenesis and progression of CSVD and relation with enlarged perivascular spaces (ePVS).

reduced AQP4 expression and glymphatic activity, impairing cognitive functions. Further research is needed to clarify AQP4's role and its potential as a link between CSVD and sleep disorder mechanisms.

Self-reported sleep disturbances, such as those measured by the Pittsburgh Sleep Quality Index, have been linked to a higher burden of ePVS.[134] Studies also show that increased ePVS is associated with disrupted sleep, including shorter sleep efficiency and duration, reduced SWS and REM sleep, and greater sleep fragmentation. Specific sleep disorders like insomnia, obstructive sleep apnoea, and periodic limb movements during sleep are similarly linked to a higher ePVS burden.[135] Despite these findings, which are based on small clinical samples with varied results, more research is needed to understand the broader relationship between sleep architecture, glymphatic function, and ePVS burden in both general and at-risk populations.

Method used to study sleep-related CSVD

Sleep, characterized as an intricate behaviour, is defined as a reversible state of perceptual disengagement and unresponsiveness from the environment.[136] Beyond perceptual disengagement, typical sleep involves closed eyes, postural recumbency, and relative stillness.[136] Despite the elusive primary function of sleep, various theories propose its role in the repair of bodily 'wear

and tear,' memory encoding, and learning processes. However, accurately measuring sleep presents challenges. Studying the relationship between sleep and CSVD involves various methods to assess sleep patterns and the presence of CSVD-related changes in the brain. Various methods are widely utilized for diagnosing sleep disorders, assessing treatment effectiveness, and outlining the investigative approaches employed by sleep researchers. The most utilized subjective and objective sleep measurements are summarized in Table 2 for an overview.

Polysomnography is a comprehensive sleep study that records various physiological parameters during sleep, including brain wave activity (through electroencephalography, EEG), eye movement (through electrooculogram, EOG), muscle activity (through electromyogram, EMG), heart rate, and respiratory patterns. This method helps identify different sleep stages and disturbances, which have been linked to CSVD.[137] Based on Table 2, polysomnography and multiple sleep latency tests are the gold standard for sleep study. However, both possessed multiple drawbacks especially if considering certain research settings that have limited access to the instruments and the use of vulnerable groups of populations.

To date, sleep questionnaires and diaries have been widely used to assess sleep behaviour. Participants may keep sleep diaries or respond to questionnaires about their sleep habits and quality, which, combined with objective data, provide a comprehensive understanding of sleep. Key questionnaires include the Pittsburgh Sleep Quality Index (PSQI), extensively used to evaluate sleep quality with over 5,000 citations;[138] the Epworth Sleepiness Scale (ESS), which assesses excessive daytime sleepiness;[139] the Functional Outcomes of Sleep Questionnaire (FOSQ), used to profile functional status related to sleep deprivation and obstructive sleep apnoea;[140] the Flinders Fatigue Scale (FFS), which measures daytime fatigue;[141] the Daytime Insomnia Symptom Scale (DISS), comparing sleep, rhythms, and mood in individuals with insomnia versus healthy sleepers;[142] and the Insomnia Severity Index (ISI), a brief tool assessing insomnia severity and its daytime impact.[143] Recent findings suggest that glymphatic system dysfunction contributes to sleep disturbances in young adults, with correlations observed between PSQI scores and MRI-based diffusivity indices along the perivascular space (ALPS index).[144]

Non-invasive neuroimaging techniques to measure glymphatic system activity

Structural and functional MRI can be used to assess brain changes associated with CSVD. Specific MRI sequences can reveal white WMHs, ePVS, lacunes, and other signs of small vessel damage. Combining sleep data with MRI findings allows researchers to investigate correlations between sleep quality and CSVD-related brain changes. Table 3 summarises the comparative analysis of neuroimaging techniques in human sleep research.

Applying glymphatic fluid dynamic knowledge in humans is invasive, involving the administration of tracers such as gadolinium-based contrast medium. As a result, a standardized method for assessing the glymphatic system in humans has not been established. Hence, a novel non-invasive approach was recently developed called diffusion tensor image analysis along the perivascular space (DTI-ALPS).[16] This method was developed to assess the activity of the glymphatic system in the human brain, through MRI-based diffusion images. In this approach, the movement of water molecules toward the PVS was assessed by measuring diffusivity through the diffusion tensor method (see Figure 6).

Principally, at the level lateral ventricle body, the medullary veins exhibit a perpendicular orientation to the ventricular wall.[145] The PVS aligns itself with the medullary veins, specifically in the right-left direction (x-axis). In this plane, projection fibres traverse in the head-foot direction, predominantly adjacent to the lateral ventricle, while superior longitudinal fascicles (SLFs), representing association fibres, extend in the anterior-posterior direction outside the projection fibres. Subcortical fibres, situated beyond the SLFs, primarily follow the right-left direction within subcortical areas. Consequently, within this region, the PVS runs perpendicular to both the projection fibres and SLFs.

This specific configuration of the PVS and major fibre tracts in this region facilitates nearly independent analysis of diffusivity along the perivascular space's direction. Notably, major fibre tracts do not parallel the PVS's direction. In the event of histological changes along the right-left direction (x-axis), such alterations will equally impact both projection and association fibres. Therefore, when observing such changes in both fibre bundles, it is reasonably safe to assert that at least a portion of this alteration stems from pathology involving the PVS, specifically the glymphatic system. Hence Taoka and colleagues reported that reduced diffusivity along the perivascular space observed in DTI-ALPS appears indicative of glymphatic system impairment.[16,49] This methodology holds promise for assessing glymphatic system activity.

Following the introduction of DTI-ALPS, multiple studies have employed this approach, finding it promising for observing glymphatic system activity in various neurological diseases such as ePVS-related

Table 2. Summary of the approaches employed in sleep assessment.

Approach	Application	Pros	Cons
Polysomnography	Quantitative evaluation of sleep and sleep disorders	• Benchmark assessment for sleep and sleep disorders	• High cost, • Limited accessibility • Discomfort • First-night effect • Requires anaesthesia
Multiple sleep latency test	Quantitative assessment of daytime sleepiness	• Benchmark assessment for the severity of daytime sleepiness • Utilized in the diagnosis of narcolepsy	• Preceding polysomnography is necessary. • In-lab evaluation with a trained staff member • Challenging to obtain or access. • Ceiling effect observed. • Limited ecological validity • Mandates thorough analysis and interpretation
Maintenance of wakefulness test	Quantitative assessment of wakefulness and alertness in sedative environments	• No ceiling effect and does not require polysomnography. • Improved ecological validity over multiple sleep latency test	• In-lab evaluation conducted by a trained staff member. • Challenging to obtain or access. • Demands subsequent analysis and interpretation.
Actigraphy	Quantitative assessment of movement as an indicator of sleep-wake patterns	• Exhibits correlations with Polysomnography. • Suitable for long-term use. • Possesses ecological validity. • Identified as a treatment target.	• Constrained by battery duration. • Demands subsequent analysis and interpretation.
Clinical Interview	Quantitative assessment of movement as an indicator of sleep-wake patterns	• Easily accessible. • Clinically valid. • Valuable for pre-treatment or research purposes.	• Unstandardized. • Restricted in efficacy without additional sleep measures.
Sleep diary	Self-reported assessment of sleep-wake patterns	• Easily accessible. • Identified as a treatment target. • User-friendly and cost-effective.	• Limited correlation with polysomnography. • Relies on user memory for completion. • Subjective evaluation of sleep. • Involves subsequent analysis and interpretation.
Questionnaires	Self-reported assessments of sleep, wakefulness, health, and cognition	• Easily accessible. • Identified as a treatment target. • User-friendly and cost-effective.	• Susceptible to bias. • Demands subsequent analysis and interpretation.
Brain imaging	Quantitative measure of the brain and its functioning	• Non-invasive approach for brain investigation. • Applicable during sleep. • Capable of measuring brain activation and metabolism.	• High cost. • Challenging to obtain or access. • Limited temporal resolution. • Lack of validity. • Demands subsequent analysis and interpretation.
Cortisol	Hormones are utilised as an indicator of stress.	• Quantitative assessment. • Strongly associated with the sleep cycle.	• Necessitates analysis. • Challenging to obtain or access. • The test requires additional validation.

(continued)

Table 2. Continued.

Approach	Application	Pros	Cons
Melatonin	Hormone utilised to assess circadian synchronization.	• Quantifiable assessment. • Can be employed to signify changes with treatment. • Strongly associated with the sleep cycle.	• Demands analysis. • Challenging to obtain or access. • The test necessitates additional validation.

Table 3. Comparative analysis of neuroimaging techniques in human sleep research.

Modality	Principle	Space Resolution	Acquisition Time	Details	Cost	Environment & requirements
DWI/DTI	Water diffusion based on tissue structural properties	1–3 mm	Minutes	Tissue structure	High	• Necessitates a fixed head. • Faces limitations in in-scanner space. • Introduces acoustic noise, disrupting EEG acquisition.
fMRI	Blood deoxyhaemoglobin concentration dependent NMR relaxation	1–3 mm	Seconds	Vascular blood flow	High	• Demands a fixed head, • Encounters spatial constraints within the scanner. • Produces acoustic noise. • Interferes with EEG acquisition.
fNIRS	Blood oxygenation and blood volume-dependent absorption of near-infrared light	Centimetre	Seconds	• Cerebral blood volume, • Blood oxygenation	Low	• Required light avoidance
PET	Gamma radiation level secondary to positron emission from blood-injected tracers	2–3 mm	Seconds to Minutes	Regional CBF (perfusion)	High	• Injection of radioactive tracer
sMRI	Density and NMR relaxation properties of water protons	0.5–1 mm	Seconds to Minutes	Tissue composition	High	• Necessitates a fixed head. • Faces limitations in in-scanner space. • Introduces acoustic noise, disrupting EEG acquisition.
SPECT	Radiation level from gamma-emitting injected blood-injected tracers	6–8 mm	Minutes	Regional CBF (perfusion)	Medium	• Injection of radioactive tracer

Note: DWI/DTI: diffusion-weighted imaging/diffusion tensor imaging; EEG: electroencephalography; fMRI: functional Magnetic Resonance Imaging; fNIRS: functional Near-infrared Spectroscopy; PET: Positron Emission Tomography; CBF: cerebral blood flow; sMRI: structural Magnetic Resonance Imaging; SPECT: Single-Photon Emission Computed Tomography.

cognitive decline,[146] ePVS-related PD,[147] sleep-related ePVS (also use PSQI),[144] restless legs syndrome[148] and many more. Given reports of gymphopathy in neurodegenerative disease from animal experiments, it is timely to conduct a comprehensive analysis of varying degrees of glymphatic system activity employing diffusion tensor studies on cases with CSVD with distinct levels of severity particularly in asymptomatic cohorts.

However, despite the advancement in DTI-ALPS techniques, several studies have upraised scrutiny regarding its specificity and accuracy in reflecting glymphatic function. Some limitations may involve its indirect measurement approach, potential confounding effects from vascular and IF movements, and variability in anatomical structures that may influence diffusion measurements.[50,149] Moreover, while DTI-ALPS correlates with known glymphatic-associated disorders, it does not directly visualise CSF-ISF exchange, a key process in glymphatic clearance.[150]

Current advancements intended to refine the DTI-ALPS technique through the integration with other complementary imaging techniques including DCE-MRI and intrathecal contrast-enhanced MRI may enhance its validity.[90] Even with these challenges, DTI-ALPS may remain a valuable tool for assessing glymphatic dysfunction in a clinical setting due to its

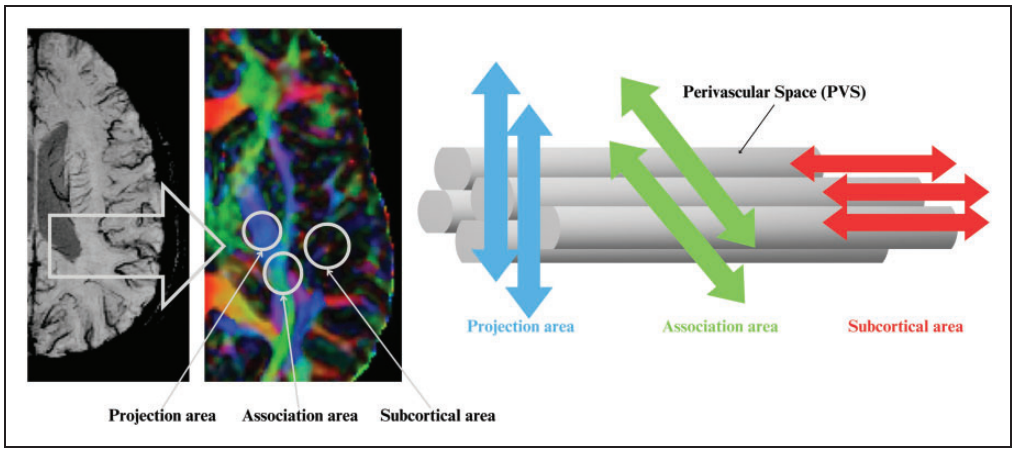

Figure 6. Conceptual framework for diffusion tensor image analysis along the perivascular space (DTI-ALPS) method (Taoka et al., 2017). From left to right - radiograph of an axial brain slice, displaying parenchymal vessels running horizontally within the slice (highlighted by the grey box) at the lateral ventricle with lateral orientation (along the x-axis) of parenchymal vessels; diffusion tensor imaging (DTI) with a colour display [illustrating the distribution of projection fibres (z-axis: blue), association fibres (y-axis: green), and subcortical fibres (x-axis: red). Three regions of interest (ROIs) are positioned in the projection area, association area, and subcortical area to quantify diffusivities in the three directions (x, y, z)]; and finally, the schematic illustrating the correlation between the direction of the perivascular space (grey cylinders) and the orientations of the fibres. It is noteworthy that the perivascular space's direction is perpendicular to both projection and association fibres.

accessibility and non-invasive nature. Future studies should focus on cross-validating DTI-ALPS findings with alternative neuroimaging approaches to consolidate its role in glymphatic research.

Future direction and research gaps

Despite significant advancements, several research gaps and future directions need to be addressed to fully understand and manage CSVD. Firstly, longitudinal studies are essential to track CSVD and glymphopathy progression over time, identify early biomarkers, and assess the long-term effects of therapies. Further research should also elucidate the molecular mechanisms connecting glymphopathy, sleep disorders, and CSVD, which can help in developing targeted treatments. Additionally, rigorous clinical trials are needed to test the efficacy of pharmacological agents, lifestyle changes, and sleep therapies for both prevention and treatment of CSVD. Advanced imaging techniques must be developed and refined to improve the visualization of glymphatic function and CSVD, enhancing accuracy and clinical accessibility.

Identifying specific biomarkers for glymphopathy and CSVD is crucial for early diagnosis and monitoring, with a focus on biomarkers related to sleep quality and genetic factors. Lastly, a multidisciplinary approach integrating neurology, cardiology, sleep medicine, and imaging technology is vital for driving innovation and improving patient outcomes. By addressing these research directions, we can enhance the understanding and management of CSVD, leading to better prevention, diagnosis, and treatment strategies.

Conclusion

CSVD significantly contributes to stroke and dementia. This review highlights the critical role of glymphopathy and sleep disorders in CSVD. Advanced imaging techniques and personalized interventions targeting glymphatic health and sleep quality show promise for early diagnosis and effective treatment. Addressing research gaps through longitudinal studies and clinical trials is essential. A multidisciplinary approach integrating neurology, cardiology, sleep medicine, and imaging technology is vital. Advancing our understanding of these mechanisms can lead to innovative strategies, improving brain health and quality of life for individuals with CSVD.

Data availability

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Declaration of conflicting interests

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