Cerebral small vessel disease
A glymphopathy?
Benveniste, Helene; Nedergaard, Maiken

Published in:
Current Opinion in Neurobiology

DOI:
10.1016/j.conb.2021.07.006

Publication date:
2021

Document version
Publisher's PDF, also known as Version of record

Document license:
CC BY-NC-ND

Citation for published version (APA):
Cerebral small vessel disease: A glymphopathy?
Helene Benveniste¹ and Maiken Nedergaard²,³

Abstract
Small vessel disease (SVD) is a common instigator of dementia in the aging population. The hallmarks of SVD are enlargement of the perivascular spaces and white matter hyperintensities. The latter represents local fluid accumulation in white matter that either subsides or develops into lacunar infarcts. We here propose that failure of brain fluid transport—via the glymphatic system—plays a key role in initiation and progression of SVD. Our major case for this concept is that perivascular spaces are utilized as waterways for influx of cerebrospinal fluid. Stagnation of glymphatic transport may drive loss of brain fluid homeostasis leading to transient white matter edema, perivascular dilation, and ultimately demyelination. This review will discuss how glymphatic rodent studies of hypertension and diabetes have provided new insight into the pathogenesis of SVD.

Addresses
¹Department of Anesthesiology, Yale School of Medicine, New Haven, CT, USA
²Center for Translational Neuromedicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, 2200, Denmark
³Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester, NY, 14642, USA

Corresponding author: Nedergaard, Maiken (nedergaard@urmc.rochester.edu)

Small vessel disease is a frequent cause of dementia
According to recent reports, there are 47.5 million people worldwide who suffer from dementia. Vascular dementia is the second most common after Alzheimer disease (AD). Dementia occurs mostly in older people and mixed AD and cerebral small vessel disease (SVD) are common and contributes to at least 45% (~21 million people) of dementias worldwide [1,2]. SVD refers to abnormal appearing perforating vessels [3,4]. In SVD, penetrating arterioles undergo progressive thickening of the wall, accompanied by moderate signs of inflammation, edema, and glial scarring. In clinical terminology, SVD refers to patients with neuroimaging lesions including white matter hyperintensities (WMH), lacunes, microbleeds, visible perivascular spaces, and volume loss [5]. SVD hallmark lesions by magnetic resonance imaging (MRI) are all directly or indirectly attributed to pathology of the cerebral perforating vessels. Deterioration of the vascular bed may occur alone or in combination with other pathologies [6] and leads to progressive demyelination and loss of white matter [7]. An increase in enlarged perivascular spaces around the perforating vessels (mostly arterioles) which are fluid filled—and therefore visible on human MRI—is strongly associated with SVD [8] (Figure 1), as are subtle but increased blood-brain barrier leakage [9], circulating inflammatory markers [10], and sleep disruption [11]. Although SVD causes small, focal lesions it is important to emphasize that it is a global disease [12]. The pathological signatures of moderate inflammatory cell infiltrates in the small vessel walls, the perivascular space, and adjacent perivascular tissue have long been recognized in patients with SVD, but the sources and consequences are poorly understood [6,13]. Epidemiological studies have shown that neither carotid stenosis nor ischemic heart disease predicts SVD, suggesting that large artery atheromatous disease does not contribute significantly [14]. Furthermore, although SVD is common in hypertensive patients, many of whom are also diabetic patients or smokers, these risk factors are considered ‘accelerators’ of SVD but paradoxically are not reflecting the severity of SVD [13]. Multiple pathological mechanisms including cerebral endothelial dysfunction, impaired vasoreactivity, BBB leakage, and pericyte loss have been implicated in the cognitive decline induced by SVD [15]. This review will focus on the possible involvement of the glymphatic system and failure of brain fluid homeostasis in SVD pathobiology.

The glymphatic system and its potential role in small vessel disease pathogenesis
The glymphatic system is a brain-wide perivascular fluid transport system analogous to the lymphatic system in peripheral tissues, which clears interstitial fluid (ISF) of waste products from the brain (Figure 2). Termined
‘glymphatic’ because the astroglial aquaporin-4 (AQP4) water channels expressed at high density at the vascular endfeet of astrocytes support cerebrospinal fluid (CSF)-ISF fluid exchange [16]. Astrocytic endfeet effectively enclose the vasculature thereby creating a network of interconnected tunnels around the brain’s arteries, capillaries, and veins. The existence of an astrocyte-enclosed perivascular space is now recognized as a unique anatomical feature of the central nervous system (CNS), but its importance has historically been largely dismissed due to its perceived lack of function. Functional tracking and visualization of glymphatic transport in the whole rodent brain was made possible by dynamic contrast-enhanced MRI [17]. Newer studies have now solidified that the glymphatic system uses these perivascular spaces as a pathway for fast CSF inflow, while the AQP4 water channels participate in intraparenchymal fluid dispersion [18]. Perivascular CSF influx is driven by arterial wall pulsatility initiated by the cardiac and respiratory cycles, as well as slow vasomotion [19–22]. Egress of ISF and CSF from the glymphatic system occurs along meningeal lymphatics and cervical lymphatic vessels [23,24]. Detailed anatomical mapping of lymphatic vessels was made possible by lymphatic endothelial cell-specific fluorescent reporter mice and immunofluorescent staining for LYVE-1, PROX-1, and VEGFR-3 [25] which revealed an intricate network of bona fide lymphatic vessels in the dura mater surrounding the rodent brain [23,26] and spinal cord [27,28]. A hallmark of the glymphatic system is that fluid flow peaks during sleep or with anesthetic agents, in particular those that enhance non rapid eye movement (NREM) sleep electroencephalogram (EEG) architecture in human brain [29–31]. A recent study documented that one night of total sleep deprivation suppressed molecular clearance from the human brain and that the lack of clearance was not compensated by subsequent sleep the following day [32]. Fluid transport is also circadian regulated peaking at the inactive phase [33]. Because the hallmark feature of SVD is structural remodeling of perivascular space and reversible white matter edema, it is likely that disturbances of glymphatic fluid transport contribute to SVD [34]. We will here discuss the

![Neuroimaging findings in small vessel disease](image.png)

Typical MRI finding in patients with small vessel disease. **White matter hyperintensities** are often symmetrical and visible on T2-weighted sequences, such as flair. The white matter hyperintensities are of variable volume and primarily located in the periventricular and deep white matter. **Lacunes** are round or subcortical cavitations with a diameter of 3–15 mm. The signal intensities of lacunes are similar to CSF and they can be surrounded by a hyperintensive rim. It is believed that lacunes result from acute, small subcortical infarcts or hemorrhages in the territory of a single perforating artery. **Enlarged perivascular spaces** are visible on MRI as CSF-filled spaces that follow the course of penetrating vessels with a linear or round shape depending on whether they are imaged parallel or perpendicular to the vessel. Reproduced with permission from Ref. [55].
glymphatic transport of data collected in preclinical rodent SVD models.

What has the analysis of glymphatic function in rodent small vessel disease models shown so far?

Most of the mechanistic SVD analysis of glymphatic transport is based on rodent models of hypertension and diabetes. This is far from ideal, when studying SVD, because the vascular anatomy is different, and the white matter compartment is scarce in mice and rats compared to human brain and—most importantly—the models replicate only parts of clinical SVD pathology. Indeed, the hallmark of SVD—enlarged perivascular spaces and WMH—has so far not been robustly identified in the rodent SVD models [35]. Nevertheless, rodent models with chronic hypertension have been informative for elucidating certain aspects of the cerebrovascular and glymphatic transport changes. In an initial glymphatic study, spontaneous hypertensive rats (SHR) were used as a model of SVD and studied at an age of 8 weeks (early hypertension) and 20 weeks (chronic hypertension) [36]. Glymphatic solute transport based on dynamic contrast-enhanced MRI (DCE-MRI) was analyzed at the root of the middle cerebral artery using a one-compartment model. The kinetic modeling revealed that influx of the gadolinium-based tracer gadoteric acid (Gd-DOTA) was reduced in SHR compared with Wistar-Kyoto (WKY) rats in both age groups. Notably, the suppression of CSF influx in SHR rats was not linked to significant astrogliosis or mislocation of vascular AQP4, suggesting that the upstream (parenchymal) glymphatic deficits were not secondary to reactive gliosis or neuroinflammation. Unfortunately, it was not possible to accurately define the effect of chronic hypertension on brain-wide CSF transport, because SHR rats also exhibit enlargement of their cerebral ventricles which confounds interpretation of CSF transport [36]. In a follow-up study involving another SVD rat model—the spontaneously hypertensive stroke prone (SHRSP) rat—CSF fluid dynamics and glymphatic flux were characterized in approximately 8-month-old SHRSP and normotensive WKY rats [37]. Notably, cerebral ventriculomegaly is not observed in the SHRSP rat model and CNS fluid homeostasis can be assessed without this potential confounder. Computational fluid dynamics analysis based on regularized optimal mass transport (rOMT) theory was used to
analyze the DCE-MRI data of Gd-DOTA transport across CNS tissue compartments of the SHRSP and WKY control rats. The rOMT analysis of the DCE-MRI data allows for visualization and quantification of several metrics including ‘speed’, flux, and direction of solute and fluid trajectories in the CNS [37]. The rOMT analysis of whole-brain DCE-MRI data revealed that total CSF flux in the SHRSP rats was significantly reduced in comparison to WKY rats; however, the mean relative solute speed of the total CSF compartment (subarachnoid + cerebral ventricles) was not affected. Second, the analysis also revealed that glymphatic (tissue) flux was moderately reduced (~15% whole brain) in SHRSP compared to WKY rats. Third, using higher spatial resolution DCE-MRI capturing PVS flow associated with the circle of Willis arteries and the middle cerebral artery showed that upstream solute transfer of the Gd-DOTA solute from the perivascular space surrounding the middle cerebral artery → tissue was impeded in SHRSP compared to WKY rats implying parenchymal ‘resistance’. Notably, in this study, altered perivascular AQP4 expression was documented in the brain parenchyma of SHRSP rats compared to the WKY rats [37].

Second, the analysis also revealed that glymphatic (tissue) flux was moderately reduced (~15% whole brain) in SHRSP compared to WKY rats. Third, using higher spatial resolution DCE-MRI capturing PVS flow associated with the circle of Willis arteries and the middle cerebral artery showed that upstream solute transfer of the Gd-DOTA solute from the perivascular space surrounding the middle cerebral artery → tissue was impeded in SHRSP compared to WKY rats implying parenchymal ‘resistance’. Notably, in this study, altered perivascular AQP4 expression was documented in the brain parenchyma of SHRSP rats compared to the WKY rats [37].

Diabetes is another risk factor for SVD [38]. However, the majority of clinical SVD randomized clinical trials include subjects with multiple comorbidities and risk factors such as diabetes and therefore there is a gap in knowledge on the impact of diabetes (type 1 as well as type 2) on SVD as a ‘single comorbidity’. Several recent reviews on diabetes and brain health reported that SVD MRI biomarkers such as WMH and lacunar infarcts are indeed affected by and related to decrements in type 2 diabetes [38–41]. From a preclinical study conducted in a rat model of streptozotocin-induced type 2 diabetes, different aspects of CSF flow dynamics and glymphatic transport were revealed supporting a role for

Glymphatic flux, CSF solute flux, and speed in WKY and SHRSP rats by regularized optimal mass transport analysis. The figure illustrates dynamic contrast-enhanced MRI data acquired from WKY and SHRSP rats processed by the rOMT computational analytical framework. Data are from Koundal et al. [37]. The rOMT analysis explicitly includes the two key transport processes of computational fluid dynamics (advection and diffusion) and determines a unique representation of the flow driven by the data. Trajectories of fluid and the solute (Gd-DOTA = ‘solute’) movement—the so-called ‘pathlines’—are calculated over a finite tracer circulation time using the Lagrangian approach for the description of fluid flow [37]. Specifically, for each tissue compartment the rOMT analysis derives two different measurements that capture solute and fluid transport dynamics over an approximately 2-h timeframe: (1) pathline volume = total solute flux and (2) pathline speed = relative mean solute speed within the pathline network. Three-dimensional (3D) volume-rendered glymphatic flux maps (green masks) from a WKY (a) and a SHRSP (b) rat, illustrating that reduced glymphatic flux in the SHRSP compared to WKY rat. (c, d) Corresponding 3D CSF flux maps from the same two rats showing reduced CSF flux in the SHRSP rat compared to the WKY rat.
Proposed model for why glymphatic dysfunction in the setting of hypertension and diabetes predispose to SVD. In hypertension, abnormal arterial wall pulsatility is linked to a decrease in influx of CSF along the periarterial spaces. The stiffening of the vessel wall results in transmission of an increased pulse pressure that alter CSF inflow, by increasing backflow resulting in reduced net forward flow [44]. Transmission of the pulse pressure waves to the surrounding tissue along with reduced fluid flow results in local inflammation, reactive micro- and astrogliosis that further decreases CSF inflow into the brain parenchyma. In diabetes, accumulation of AGEs may be responsible for faster CSF inflow kinetics of perivascular flow. However, the inflammatory changes characteristic of diabetes ultimately lead to a reduction in CSF influx into the parenchyma. In both hypertension and diabetes, the atrophy and stagnation of perivascular fluid will eventually dilate the perivascular spaces and lead to fluid accumulation—the two hallmark features of SVD. In addition, the interruption of polarized glymphatic flow accelerates accumulation of protein aggregates explaining, at least in part, why both hypertension and diabetes increase the risk of developing neurodegenerative diseases.

From these studies we conclude that glymphatic (tissue) flux in rat models of chronic hypertension and diabetes exhibits grossly similar patterns with regard to reductions in glymphatic transport and clearance. However, the overall signature of CSF fluid flux in the two conditions may likely differ. In diabetes, CSF transport along the subarachnoid space including large perivascular spaces appears to display fast influx kinetics while in chronic hypertension CSF flows measured by rOMTIs reduced (Figure 3). A likely scenario in chronic hypertension therefore involve decreased CSF fluid flux secondary to increased stiffness of the large arterial vasculature and reduced CSF pulsatility [43]. Specifically, constriction of the arterial wall in response to acute hypertension, was shown to reduce the forward solute movement by almost 50% in young mice, likely by stiffening the vessel wall [44]. Indeed, high-resolution 2-photon line scanning showed that acute hypertension made arteries expand and contract faster and increased the maximal negative wall velocity thereby accelerating solute backflow in the perivascular spaces by more than 20% [44]. The altered flow pattern in the perivascular space resulted in an overall reduction in forward speed of approximately 40% in acute hypertension [44]. On the other hand, in diabetes not accompanied by hypertension, vascular stiffness would likely not dominate the pathophysiology at least early on. In diabetes, accumulation of advanced glycation end products (AGEs) is known to affect primarily the vasculature and is associated with inflammation, reactive alterations, and BBB disruption [45,46]. AGEs also trigger the release of vascular endothelial growth factor from the endothelial cells, possibly expanding the perivascular space volume secondary to angiogenesis [47,48]. The subsequent glymphatic flux reduction and stagnation of the contrast agent in the perivascular space could be a consequence of increased tissue resistance toward parenchymal exchange and clearance due to the reactive gliosis and...
mislocation of AQP4 observed in both hypertension and diabetes. Regardless, in both conditions, stagnation of lymphatic flux with secondary perivascular dilation emerges as SVD progresses and represents a ‘common denominator’ in the pathophysiology long-term (Figure 4).

It is important to note that both hypertension and diabetes are associated with an increased risk of AD and early cognitive decline [49,50]. Lymphatic dysfunction is linked to stagnant ISF flow and an increased risk of protein aggregation. For example, deletion of AQP4 in a murine model of AD aggravated amyloid-β aggregation without altering the expression levels of proteins associated with amyloid-β formation and degradation [51]. A similar accelerated time course of Alzheimer pathology and cognitive decline has been observed after blocking either meningeal [52] or cervical lymphatic vessels [53]. In mice, lymphatic clearance is suppressed before amyloid-β deposition and further reduced upon plaque formation and reactive astro- and microgliosis. In fact, simply delivering amyloid-β into CSF reduces lymphatic flow [54]. Thus, multiple pathophysiological events may in parallel contribute to cognitive decline in SVD.

Conflict of interest statement
Nothing declared.

References
Papers of particular interest, published within the period of review, have been highlighted as: * of special interest

21. In vivo study introducing Gd-based contrast administration into CSF in combination with dynamic contrast enhanced MRI to visualize whole brain lymphatic transport in the rat brain.
23. In vivo study introducing Gd-based contrast administration into CSF in combination with dynamic contrast enhanced MRI to visualize whole brain lymphatic transport in the rat brain.
Cerebral small vessel disease Benveniste and Nedergaard


Human neuroimaging study showing that clearance of cingrat agent dilivered into CSF is significantly suppressed by sleep deprivation. Subsequent sleep failed to restore clearance of the contrast agent


MRSI study showing that influx of contrast agent from the perivascular space into the neureoip is reduced in hypertensive compared with normotensive rats


Study of CSF fluid flow and glymphatic transport using optimal mass transport analysis of MRSI data showing that CSF and glymphatic solute flux – but not CSF solute speed – into the neureoip is reduced in hyperpensive compared with normotensive rats


Two-photon particle tracking velocimetry of microspheres moving in the periarnteral spaces of pial arteries demonstrating that arterial pulsatility drives peri-arterial CSF influx. Acute hypertension reduced CSF transport by almost 50% prehaps explaining why hypdration is linked to an increased risk of developing Alzheimer disease


