

# Regional Glymphatic Clearance and Associations with Cerebrovascular Pathology

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**ABSTRACT:** Discovered in 2012, the glymphatic system (GS) in the CNS (Central Nervous System) facilitates CSF (cerebrospinal fluid) and ISF (interstitial fluid) flow through the perivascular spaces (PVS), enabling regular clearance of proteins and neurotoxic waste to maintain GS health. Research on GS dysfunction has explored its implications for neurodegenerative diseases, such as Parkinson's (PD) and Alzheimer's (AD). Nevertheless, GS flow is not uniform across brain parenchymal spaces, as evidenced by observed areas of reduced and increased clearance of proteins and CSF. Although regional glymphatic impairment is recognized in common neurodegenerative diseases, a comprehensive understanding of its role in cerebrovascular disease (CVD) is lacking. This review examines how variations in regional glymphatic clearance may underlie differential vulnerability to CVD, and identifies key gaps through current GS mapping and imaging techniques. We analyze the mechanisms across existing studies to assess how low-clearance regions exhibit increased vulnerability to dysfunction. This vulnerability contributes to CVD progression and highlights potential targets for diagnosis and treatment.

**KEYWORDS:** Neurobiology, Cerebrovascular Disease (CVD), Glymphatic System, Regional Glymphatic Clearance, Perivascular Space (PVS).

## ■ Introduction

Since its discovery in 2012, the glymphatic system has emerged as a pathway for clearing brain waste and maintaining neurological health. While the lymphatic system facilitates waste clearance throughout the body, the glymphatic system, or the “glial-dependent lymphatic transport,” supports the CNS.<sup>1,2</sup> In particular, blood vessels in the brain parenchyma are surrounded by the endfeet of astrocytes, and between that and the vascular wall, the PVS regulates CSF and ISF flow. Furthermore, AQP4 (aquaporin-4) water channels on astrocytes regulate CSF flow while displacing ISF and metabolic waste to eventually drift out of the brain and into the lymphatic system.<sup>3-6</sup> Lack of sleep is a significant contributor to dysfunction and protein aggregation within the GS, since CSF/ISF fluid movement thrives during plentiful sleep.<sup>5,7</sup> Extensive research first established that GS dysfunction and disrupted CSF-ISF exchange contribute to the buildup of both regular and injury-associated waste – connecting these impairments to the development of neurodegenerative diseases such as AD, PD, and Huntington's.<sup>5,8,9</sup> More recently, emerging studies also underscore region-specific deficits in glymphatic clearance, highlighting the anatomical heterogeneity of GS dysfunction.<sup>9</sup> <sup>10</sup> Advances in imaging technologies – such as diffusion tensor imaging along the perivascular space (DTI-ALPS), magnetic resonance imaging (MRI), aquaporin-4 (AQP4), and fluorescent tracers indices – have linked decreased function to cardiovascular, endocrine, and neurological conditions. Notably, reduced imaging indices reflect less CSF-ISF flow and impaired clearance, serving as an *in vivo* biomarker for diagnosing glymphatic dysfunction.<sup>9</sup> However, despite these advances and emerging themes, the relationship between regional GS deficits and cerebrovascular diseases remains vague and complex. This

is particularly crucial given the enormous burden of CVD – by 2020-30, it is projected to be the leading cause of global death.<sup>3</sup> The rising burden increases morbidity rates, costs for screening, diagnosis, and treatment. Morbidity continues to rise in the aging population, with age-standardized rates higher in men. Inherited forms of CVD further extend its effects, impacting families and surrounding communities, too.<sup>11,12</sup> Moreover, low and middle-income countries, such as sub-Saharan Africa, and rural South America and Asia, are disproportionately affected, with an imbalance between and within countries.<sup>3</sup> Understanding the mechanisms that drive cerebrovascular pathology, such as regional glymphatic dysfunction, can guide future CVD prevention, diagnosis, and treatment. In the cardiovascular system, cardiac pulsatility drives CSF flow,<sup>13</sup> facilitating the clearance of metabolic byproducts and proteins from the brain.<sup>5</sup> Particularly, through the periarial spaces, arterial pulsations drive CSF from the subarachnoid space (SAS) into the brain's neuropil.<sup>14</sup> Therefore, when cerebral arterial pulsatility is reduced, cardiac diseases associated with low cardiac output are linked to impaired clearance.<sup>5</sup> Notably, both ischemic and hemorrhagic strokes disrupt GS function, contributing to critical pathological processes such as blood-brain-barrier (BBB) disruption, brain edema, and neuroinflammation.<sup>15</sup> In subarachnoid hemorrhage (SAH), dysfunction occurs when CSF circulation by the periarial influx pathways is impaired.<sup>15-18</sup> However, an effective method to prevent damage or enhance prognosis isn't prevalent. Moreover, intracerebral hemorrhage (ICH) has been linked to enlarged perivascular spaces (EVPS), since pulsatile arterial movements are impeded. Nonetheless, this relationship still requires further exploration.<sup>19-22</sup> Lastly, while rodent models simulating CVD have confirmed GS impairment, the

specific relationship between them is under-researched and warrants further study.<sup>23,24</sup>

While current literature has established the relationship between CVD and glymphatic clearance, growing evidence suggests that glymphatic clearance is not uniform across the brain. Given these unresolved links and emerging associations, this suggests that variations in regional glymphatic clearance would contribute to CVD pathology. More specifically, brain regions with lower clearance rates would be prone to dysfunction, essentially being key contributors to the development and exacerbation of CVDs. Hence, this paper aims to address the research question of how variations in regional glymphatic clearance may underlie differential vulnerability to CVD.

Firstly, through the examination of an array of studies, we will examine how using several imaging methods will visualize clearance in each disease: IS, SAH, ICH, and CSVD. These include diffusion tensor imaging along the perivascular space (DTI-ALPS), glymphatic MRI, aquaporin-4, and fluorescence imaging markers. Then, we will compare the findings of each study to pinpoint specific neural locations within the brain that display high levels of impairment. Lastly, we assess where disrupted regional glymphatic clearance in CVD codes for early diagnosis and prognosis. Targeting corresponding areas for each disease with developing treatments, including pharmacological interventions, could reboot GS and restore flow.

In parallel, identifying progression patterns of disease spreading over time and developing more accurate imaging methods could better determine CSF flow and GS function.<sup>9</sup> A comprehensive literature search of 71 papers was conducted in PubMed, Google Scholar, ProQuest, and JSTOR, with articles published between August 2012 and June 2025. Keywords such as “glymphatic system,” “glymphatic clearance,” “DTI-ALPS,” “cerebral small vessel disease,” “Ischemic stroke,” “intracerebral hemorrhage,” and “subarachnoid hemorrhage” were combined using Boolean operators (e.g., “and,” “or”) to refine search results. Studies were included if they involved clinical human and animal models (rodents, mice, monkeys) and review articles relevant to glymphatic dysfunction in CVD. Only peer-reviewed articles published in English were included, and full texts of eligible studies were subsequently evaluated. Titles and abstracts were screened by two independent reviewers, and full texts of relevant studies were retrieved. Studies published before 2012, not peer-reviewed, did not assess glymphatic function, and those lacking relevance to CVD were excluded. A narrative synthesis approach was employed to identify and summarize key themes and outcomes.

### ■ Regional Glymphatic System Clearance

Outlining the fundamentals of regional glymphatic clearance, the imaging modalities employed across diverse groups, and PVS fluid dynamics are key to understanding the studies in this paper. These methods are further employed within regional glymphatic clearance in neurodegenerative disease and CVD. Glymphatic clearance refers to the ability to effectively clear and distribute CSF, proteins, and substrates through the brain parenchyma. There are also distinct factors that contrib-

ute to the fluid transport patterns of each anatomical region of the GS.<sup>25</sup> CSF enters the brain via the periarterial space, driven by arterial pulsations, moving through the parenchyma through diffusion and polarized expression of AQP4. Waste-laden fluid moves through veins into the meningeal lymphatic vessels and cervical lymph nodes, with outflow influenced by respiratory-driven pressure changes, a process most effective during sleep. However, arterial pulsatility is stronger near the arteries and weaker in deep or small vessels, therefore contributing to regional clearance. Furthermore, CSF flows from the ventricular system and enters the SAS of the brain and spinal cord.<sup>26</sup> Other studies on mice, monkeys, and humans have generally found that CSF influx is inefficient and reduced in deep regions of enlarged cerebral hemispheres, while glymphatic function remains efficient in the gray matter of cerebral hemispheres.

Glymphatic circulation was also smoother in cerebral hemispheres with cortical folds (the characteristic folds and indentations of the cerebral cortex; the outer layer of the brain) than in those without.<sup>27</sup> Differences in these anatomical regions expand our knowledge; thus, various imaging approaches have been employed throughout studies to explore them. One imaging technique, diffusion tensor imaging along the perivascular space (DTI-ALPS), is a non-invasive imaging method that measures how easily water diffuses across the PVS. Water diffusivity in the PVS, used to evaluate the direction of water molecule flow, is primarily applied in deep white matter and around the lateral ventricles (medullary arteries and veins).<sup>12,28</sup> Therefore, by observing the degree of water diffusivity, studies show that differences and decreased ALPS index are associated with neurodegenerative diseases such as PD, AD, and IS.<sup>29-31</sup> DTI-ALPS is categorized into four different regions to measure ALPS index: anterior (aALPS), total (tALPS), posterior (pALPS), and middle (mALPS).<sup>9</sup> However, it is important to recognize the limited abilities of DTI-ALPS: it only maps the PVS, not actual solute clearance. Therefore, though DTI-ALPS serves as one way to map GS flow, different methods were analyzed to incorporate several angles of accuracy. In addition, AQP4 channels, located on astrocytic endfeet, are also recognized as key players in enabling the exchange between the CSF and ISF. Consequently, AQP4 facilitates the movement of water and solutes.<sup>13,14,32</sup> They play significant roles in brain edema regulation, astrocyte movement, calcium signaling, and synaptic plasticity, with varying expression in different stroke types – an indicator for glymphatic clearance. AQP4 polarization in the correct expression pattern to astrocytic endfeet is vital to efficient GS and CSF-ISF exchange throughout the system.<sup>10,14,33</sup> Thus, AQP4 polarization is widely connected to ischemic and hemorrhagic strokes – key diseases assessed in this paper. GS clearance rates vary by region, largely due to AQP4 channel density, as the absence of channels is linked to reduced CSF influx and ISF efflux. Indeed, the polarity of AQP4 in PVS is shown to decrease in all subtypes of stroke.<sup>34</sup> Therefore, AQP4 polarization serves as a meaningful indicator of regional glymphatic impairment associated with these diseases.

Additionally, contrast-enhanced glymphatic MRI is another common method for visualizing glymphatic flow throughout the GS. A study on healthy wild-type mice, using cisterna magna infusion of Gd-DTPA and serial acquisition of T1-weighted MR images over a defined period, revealed sequential flow from the ventral nuclei (pontine nucleus and pituitary recess), the caudal cortex, pineal recess, cerebellum, rostral cortex, and olfactory bulbs.<sup>35</sup> This highlights the spatial and temporal heterogeneity in clearance and explains the GS through a chronological journey. This finding aligns with another study also using standardized T1-weighted MRI scans to reveal a difference in percentage of T1 signal change in the cerebral cortex, cerebral white matter, basal ganglia, limbic structures, corpus callosum, and hippocampus.<sup>36</sup> This regional variation indicates that the clearance of proteins such as amyloid-B, tau, and CSF flow are heterogeneous. By highlighting that GS clearance and CSF flow are heterogeneous, common proteins, such as tau, amyloid-B, and CSF-ISF flow, are shown to be more susceptible to blockage in specific regions. Spatial heterogeneity may also underlie region-specific vulnerability in various neurodegenerative diseases. Several studies have confirmed the association between the caudal and rostral cortex regions with AD. In IS, AQP4 has been linked to the ipsilateral cortex and necrotic infarct core.<sup>32,35</sup> Similarly, the bilateral posterior cingulate and left lingual gyrus regions using DTI-ALPS were associated with PD.<sup>37</sup> These findings reinforce the idea that regional clearance vulnerabilities are not only anatomically present but clinically significant, demonstrating that regional clearance is a unifying factor across neurological diseases. Notably, in the forthcoming analysis of past studies, various other imaging types will be assessed. Some might be disease-specific, such as fluorescent tracers, 3D-FIESTA MRI, cerebral microbleeds, and white matter hyperintensities (for cerebral small vessel disease).

These examples are provided not as the central focus of this paper, but to illustrate that regional dysfunction has already been characterized in several neurodegenerative conditions. By contrast, fewer studies have examined whether similar region-specific clearance impairments are prominent in CVD. Therefore, this paper aims to review and analyze evidence in those terms.

## ■ Glymphatic System Relationship with Ischemic Stroke

As the second-largest cause of death in the world, accounting for more than 70% of all cases,<sup>38</sup> IS affects millions of lives every year. GS dysfunction plays a vital role in the pathophysiology of stroke, contributing to blood-brain barrier (BBB) disruption, brain edema, immune cell infiltration, and neuroinflammation. CSF-ISF flow is widely recognized to be facilitated by arterial pulsations; therefore, blood clots in IS reduce or eliminate this force. As a result, CSF flow along the PVS slows, increasing neurotoxic retention and amplifying edema.<sup>5</sup>

Additionally, the GS is unable to eliminate both injury-induced waste, regular neurotoxins, and proteins such as amyloid-B and tau proteins. Therefore, CSF flow has shown

to be impaired after ischemic stroke.<sup>5</sup> Research in past animal models contended that during hyperacute stroke, the appearance of hyperintensity in diffusion weighted images (similar to DTI-ALPS) indicated slower rates of water diffusion through ischemic parenchyma.<sup>39</sup> Water diffusivity, defined as the ease with which water molecules move through tissue, decreases during hyperacute stroke due to cellular swelling. This serves as an early imaging marker of ischemic cellular injury and glymphatic dysfunction occurring in IS.

Rodent, mouse, and human tests collectively indicate that GS dysfunction both contributes to and exacerbates IS.<sup>39, 40</sup> Lower aLPS index was measured in a set of patients with IS.<sup>58</sup> In post-stroke analysis, the ipsilateral and contralateral infarcts were examined, revealing that the aLPS index was significantly lower in stroke patients compared to the controls.<sup>41</sup> In addition, another study used DTI-ALPS index values to determine that stroke patients exhibited lower index volumes in the infarct side in comparison to the non-infarct side and non-stroke patients.<sup>42</sup> aLPS measures fluid movement along the PVS, slowing directional CSF-ISF movement. Thus, a lower aLPS index indicates that perivascular conduits are compressed or blocked, likely due to astrocytic endfeet swelling and local edema. In other words, it not only compromises local perfusion and cellular integrity but also impedes the clearance of CSF-ISF fluid and waste. Therefore, this exacerbates tissue injury and promotes neuronal swelling and inflammation. Without effective clearance, fluid accumulation in the peri-infarct region results in edema, further damaging surrounding neural tissue and worsening the overall prognosis.<sup>26,43</sup> In addition, since the ALPS index is lower in stroke patients compared to healthy individuals, its non-invasive biomarker properties allow for early detection. Early stages of ischemic injury are often challenging to identify, but aLPS provides a real-time, dynamic assessment of glymphatic dysfunction. This facilitates the monitoring of stroke progression and potentially tailored therapeutic intervention.<sup>22,42</sup>

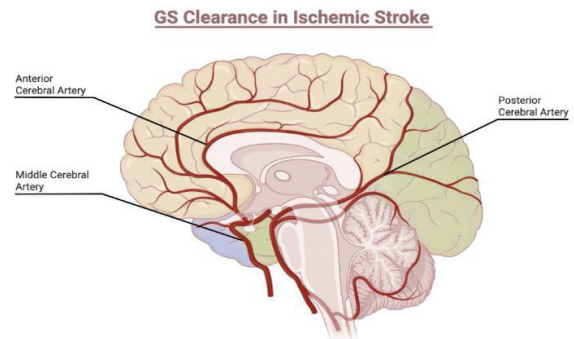
Moreover, higher stroke lesion volumes correlate with more severe stroke scores, reflecting reduced glymphatic flow. DTI-ALPS's hyperacute ability to recognize ischemia leads to effective prognosis of the severity, essentially resulting in better treatment options. By noninvasively detecting regional vulnerabilities in the brain, DTI-ALPS provides insight into regional glymphatic clearance within IS. Reduced DTI-ALPS reflects astrocytic endfeet swelling and pericyte constriction that blocks glymphatic flow. Larger lesions amplify peri-infarct fluid accumulation and edema, and pinpointing sites to restore clearance mitigates secondary injury.

Building on this evidence, regional vulnerability is reflected in early disruption of CSF inflow. GS MRI and histological examination methods show how CSF inflow into the ipsilateral cortex was impaired 3 hours after the simulation of acute ischemic stroke in mice.<sup>16</sup> Furthermore, because CSF is shown to be a primary source of edema, there was toxic buildup and fluid restriction in certain vulnerable areas, highlighting CSF entry and exit in specific brain regions during a stroke. The time frame between the simulation and the effect potentially hints at spatial heterogeneity within the brain, highlighting

the lack of uniformity in glymphatic dysfunction. ALPS index measurements in cerebral infarct regions similarly vary according to infarct volume and time after stroke onset. ALPS index in the right and left cerebral infarct differ depending on the infarct volume (degree of lesion). Infarct volume smaller than 20 cm<sup>3</sup> after 14 days post-stroke displayed a high ALPS index. This suggests another factor of regional dysfunction: the link between time and infarct size.<sup>44</sup> Injury severity correlates with clearance dysfunction, which may indirectly tie larger infarcts and smaller infarcts playing a role in regional GS clearance. Infarct expansion and reduced blood flow are most likely caused by CSF flow facilitating the swelling of astrocytic endfeet along with pericyte constriction.<sup>45,46</sup> Pericytes regulate capillary diameter; constriction reduces the size of perivascular channels, directly limiting flow and amplifying peri-infarct fluid accumulation.<sup>47</sup> Therefore, larger infarct volumes are a result of larger amounts of astrocytic endfeet swelling. Since astrocytic endfeet facilitate CSF flow, GS function is impaired, and the degree of swelling contributes to large or small infarct regions. Similar to the findings in the previous animal models discussed, this study also observed a decrease in glymphatic function in the cerebral hemisphere during IS. Regional clearance patterns across both human and rodent models provide markers for visualizing dysfunction in IS and CVD. These findings highlight the need to distinguish species-specific mechanisms for accurate comparisons and distinctions.

After compiling and analyzing a variety of studies, we might consider how GS dysfunction is most notable in the infarct and peri-infarct – the area closest to the stroke site. Because the infarct is the area most severely impacted by the stroke, laminar GS flow is likely required the most. Notably, however, since the GS operates brain-wide, impaired clearance at a major injury site could also serve as a functional bottleneck within the system. Disruption at these main sites could impede CSF-ISF flow and metabolic waste from other brain regions as a result, since the accumulation of the neurotoxic solutes and proteins might not be limited to the infarct, but instead extend to other structurally unaffected tissue regions.<sup>5</sup> Hence, restoring flow in a central region could alleviate and reinstate order in the entire system, establishing regions of most severity, and providing a target for restoring flow beyond the infarct area. In mice, microinfarct lesions can trigger reduced global CSF-ISF flow.<sup>48</sup> Therefore, their idea that localized lesions can cause widespread clearance impairment supports this study's analysis of how the infarct region may affect global clearance. However, we must acknowledge that the infarct and peri-infarct cortex are primarily umbrella terms; therefore, we also analyzed the common areas where ischemic stroke occurs, regardless of specific connections to the GS. As represented in Figure 1, IS mainly occurs in the middle cerebral artery (MCA), anterior cerebral artery (ACA), and posterior cerebral artery (PCA) – all parts of the brain's cortical regions, including the frontal, temporal, and parietal lobes.<sup>49</sup> Although glymphatic function may partially restore itself over time, severe dysfunction warrants urgent clinical and acute intervention, as post-stroke patients remain at risk for post-stroke and vascular dementia.<sup>5</sup> Enhancing glymphatic recovery through the use of medicinal

drugs and treatments could accelerate clearance rates, particularly in critical areas. Therefore, future studies should aim to target these regions to see if GS dysfunction occurs in the peri-infarct and infarct cortex of the lesion, or if new findings emerge.



**Figure 1:** Visual diagram of the anterior cerebral artery (ACA), middle cerebral artery (MCA), and posterior cerebral artery (PCA), which supply the frontal, parietal, temporal, and occipital lobes – regions frequently impacted during IS. Because arterial pulsatility drives perivascular CSF flow, occlusion in these vessels may disrupt flow and impair regional clearance, and represent anatomical targets for investigating spatial vulnerability. Figure originally produced by the author using BioRender.com.

## ■ Glymphatic System Relationship with Subarachnoid Hemorrhage

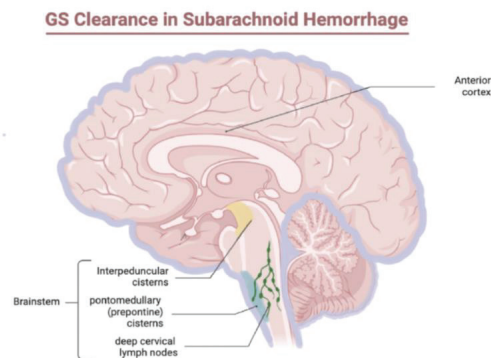
Subarachnoid Hemorrhage (SAH) is a form of hemorrhagic stroke with severe brain impairment.<sup>15</sup> In SAH, vessel rupture causes a blood leak into the SAS, mixing with CSF, ISF, and neuronal waste. Hemoglobin degradation triggers neuronal damage and inflammatory mediators, with the neutrophil influx, into the CSF.<sup>17</sup> Past studies of cerebral hemorrhage show how GS impairment leads to the accumulation of proteins, such as tau.<sup>35,50</sup> Glymphatic stagnation prevents clearance of neurotoxic proteins, creating a local microenvironment that accelerates neuronal injury. While the PVS forms a widespread network through the GS, pathological deposits such as fibrin, fibrinogen (proteins involved in blood clotting), and impaired CSF circulation following SAH, can severely occlude these spaces in specific regions. Fibrin and fibrinogen polymerize to form dense matrices within the PVS, obstructing CSF-ISF movement and creating site-specific glymphatic bottlenecks that explain regional vulnerability.<sup>51</sup> However, there currently isn't an effective method to prevent damage or enhance prognosis.<sup>16-18</sup> Therefore, this section aims to review and synthesize findings across multiple studies that have explored glymphatic clearance in SAH, with the objective of identifying regions with the most impact.

Firstly, gadolinium chelate tracers injected into the cisterna magna revealed that the induction of SAH impaired CSF circulation – particularly in the cerebral ventricles – moved into the superficial cortical regions, and ultimately deeper brain structures.<sup>18</sup> While the tracer entered the cisterna magna, *in vivo* blood from vessel rupture initially accumulated at the hemorrhage site, with downstream perivascular and cisternal magna subsequently affected. This demonstrates that obstruction at key CSF entry points reduces convective flow, leaving deeper parenchyma vulnerable to accumulation

of neurotoxic substances. Impaired CSF flow likely reduces convective transport through PVS, limiting clearance of inflammatory mediators and neurotoxic proteins, thus contributing to secondary injury and protein aggregation.<sup>14,16</sup> Additionally, another study revealed how T1-hyperintense signals were first observed in the basal cisterna, surrounding the arteries of the circle of Willis. After a DOTA-Gd injection, the most superficial part of the brain was penetrated in just 20 minutes. Underneath the arachnoid, in the ventral part of the brain, blood was located as a result of macro- and microscopic examinations. Levels of DOTA-Gd injection were found to be stagnant in the anterior parts of the brain after SAH.<sup>52</sup> The discovery of blood beneath the arachnoid at the ventral surface of the brain is especially significant because obstruction of these superficial basal cisterns blocks downstream CSF flow. As a result, DOTA-Gd expression remained stagnant. Since DOTA-Gd is paramagnetic, DOTA-Gd shortens the T1 relaxation time in brain tissues, causing a signal change, or brightening, on T1-weighted MRI scans. Using DCE-MRI or T1 mapping, we measure and track the speed and extent of this signal change. We can consider that the GS function was impaired in the anterior region. These non-primate studies further display SAH characteristics similar to those of a human, so these findings show promise in accuracy.<sup>14,39</sup> A consistent pattern of region-specific dysfunction after SAH has been observed in non-human primate models. Superficial entry zones fail first, and deeper parenchymal regions follow. Rodent studies corroborate these findings and highlight key anatomical pathways. Fluorescent tracers such as Evans blue (EB), Alexa-labeled low molecular weight tracers (LMWS), and microspheres (MS) consistently accumulated in several regions. Overall, EB expression and fluorescence distribution across six defined segments demonstrated high expression and tracer accumulation in the pontomedullary and interpeduncular cisterns. Similarly, fluorescent imaging of the ventral brain surface is established mostly in the pontomedullary area, as compared to the rostral segments. Using two different tracers that expressed similar results strengthens the findings shown in this study. Fluorescent microspheres showed similar results to EB and Alexa as well; the distribution was predominantly expressed in the pontomedullary and interpeduncular cisterns (infratentorial cisterns), spreading along the paravascular path on the basal surface of the brain. Moreover, Alexa and microspheres were most accumulated in the basal cisterns and arteries. Lastly, after being injected into the cisterna magna, the LMWS tracer distribution penetrated the brain parenchyma, creating a gradient from the SAS, where the fluorescent dye then entered from the ventral cisterns.<sup>17</sup> Therefore, the gradient is expressed from “dark to light,” essentially brighter to dimmer fluorescence. The brighter the fluorescence, the higher the concentration, with the ventral cisterns most affected. An intersection of regions is evident, aligning with previous studies of the anterior brain region and basal cisterna. Similarly, when fresh, autologous venous blood is injected into the cisterna magna of SAH mice in another study, accumulation in the deep central lymph nodes is evident. Moreover, AQP4 polarization is impaired, resulting in tau protein accu-

mulations.<sup>53</sup> Blood clots forming in the dcLNS can partially or totally block this connective drainage route, therefore resulting in the entrance, combination, and accumulation of blood components into the PVS. Essentially, both studies targeted the cisterna magna for tracers and autologous blood injections, with the first showing LMWS tracer accumulation in the ventral cisterns, and the second observing blood clots in the dcLNS. These two regions are anatomically linked as a part of the CSF pathway, essentially supporting the notion that glymphatic dysfunction in SAH impacts shared anatomical regions. Lastly, regional variability in AQP4 expression modulates susceptibility. Analysis across dorsal, ventral, and lateral cortexes reveals significantly lower AQP4 expression in SAH mice through the injection of autologous blood into the cisterna magna, as well as a CSF tracer. Expression levels of AQP4 vary in each slice – the dorsal cortex, ventral cortex, and lateral cortex; however, it was generalized that there was significantly lower AQP4 expression in the entire brain in SAH mice. However, differences in AQP4 mRNA in the anterior, middle, and posterior cortex of the mouse cortex demonstrate that higher levels of AQP4 mRNA are shown in the anterior cortex, but not the middle or posterior. Higher AQP4 expression codes for smoother flow, whereas lower expression hinders it, compromising CSF efficiency. This zonal variability could arise from the asymmetric blood distribution during SAH, but it may also reflect inherent regional differences in GS function. Assuming the latter is true, the anterior cortex might possess greater intrinsic resilience due to higher AQP4 expression, compared to the middle and posterior cortices. This could further hint that regional glymphatic clearance may also naturally be built into the biology of the brain.<sup>54,55</sup>

Current evidence underscores the significant involvement of glymphatic dysfunction and the pathophysiology of SAH. Through the several studies assessed above, a comprehensive understanding of the regions most affected is visually represented in Figure 2: the deep cervical lymph nodes, the basal and ventral cisterns (including the pontomedullary and interpeduncular cisterns), and the anterior cortex. When mapped out, all these regions are specifically centered around the brainstem.



**Figure 2:** Visual diagram of glymphatic clearance in SAH, including interpeduncular cisterns, pontomedullary (prepontine) cisterns, deep cervical lymph nodes, and the anterior cortex. The interpeduncular cisterns, pontomedullary (prepontine) cisterns, and deep cervical lymph nodes are visually represented in an anatomically similar area: the brainstem. Figure originally produced by the author BioRender.com.

## ■ Glymphatic System Relationship with Intracerebral Hemorrhage

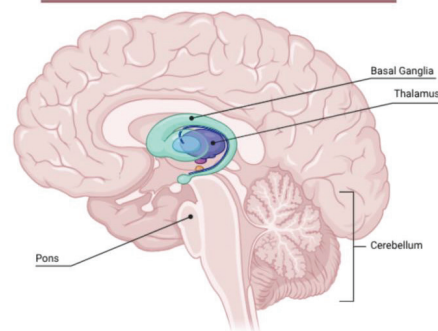
Intracerebral Hemorrhage (ICH) is another type of stroke in the hemorrhage family, linked with death and disability.<sup>19</sup> As a hemorrhage subtype, ICH occurs when bleeding in the brain rapidly forms a hematoma that tears nearby brain tissue and increases intracranial pressure. This leads to perihematomal edema (swelling around the bleed), reduced blood flow, breakdown of the BBB, and changes in the brain's intracranial environment.<sup>29,48,56</sup> While research on the relationship between GS and ICH is vague and limited,<sup>34</sup> there have been links to enlarged perivascular spaces (EVPS).<sup>20,21</sup> ICH most often occurs in the basal ganglia (the striatum). Since the basal ganglia are a deep gray matter structure, their vascular structure and dense neuronal networks make them a vulnerable target of hemorrhage and impaired GS flow.<sup>57</sup> Additionally, EVPS in the centrum semiovale is a result of vascular amyloid-B accumulation and worse outcomes.<sup>58</sup> This highlights the centrum semiovale and the basal ganglia as an important region where glymphatic dysfunction and amyloid-B accumulation can worsen disease (specifically ICH). Differences in these studies to produce different regions may result from the influencing factors, such as the type of study or outside variables affecting the outcome. Notably, unclear molecular mechanisms provide a future path for research, as well as deeper research on the GS and ICH relationship. Moreover, AQP4 expression and polarization have been found to be a determinant of GS in ICH. A recent study found that AQP4 expression was reduced in the perihematomal region post-ICH. AQP4 reduction was connected to worsened BBB, perihematomal edema (PHE), vascular leakage, and brain edema. However, we must note that AQP4 could be used as a potential therapeutic in reducing PHE.<sup>56</sup> Conversely, another study reported signs of AQP4 overexpression in the perihematomal region.<sup>59</sup> The apparent contradiction may indicate that AQP4's role is context-dependent, possibly varying with post-ICH timing, lesion severity, or specific cellular localization (e.g., endfeet compared to parenchymal). Similarly, in another study, researchers found that AQP4 expression rises significantly after about 3 days following ICH, staying elevated for up to 2 weeks.<sup>43,59</sup> This suggests that AQP4 overexpression can worsen brain edema in the surrounding perihematomal region, directly supporting the idea of AQP4 expression in the glymphatic system. Building on the contradicting findings, it is conclusive that AQP4 expression in ICH is region-based. Both the over- and underexpression of AQP4 have been linked to brain edema in ICH, clearance impairment, and exacerbating damage. In other words, after GS impairment, proteins, neurotoxins, and metabolic waste are accumulated, therefore leading to brain edema. Additionally, the PVS after glymphatic dysfunction and edema is inflamed.<sup>40</sup>

Lastly, DTI-ALPS imaging further substantiates region-specific clearance. In acute spontaneous ICH, human patients display signs of a lower DTI-ALPS index on the ipsilateral than contralateral side.<sup>60</sup> While this isn't necessarily correlated to a specific region, rather a more generalized one, it is notable to recognize these studies' establishment suggest-

ed that GS dysfunction may contribute to the exacerbation of this disease, and as a therapeutic target. Notably, prior studies have observed specific regions of impaired glymphatic flow – such as the basal ganglia. Therefore, the observation in the present study that ICH most prominently affects the ipsilateral hemisphere may align with the involvement of ipsilateral basal ganglia structures. Similarly, another article indicated significant accumulation in the deep cervical lymph nodes. By injecting another fluorescent tracer, called FITC-d4000, into the right basal ganglia, this even indicated extracranial circulation through these nodes.<sup>57</sup>

Overall, the exact brain regions affected remain unclear and vary across multiple studies assessed. The observation in the present study demonstrates that ICH most prominently affects the ipsilateral hemisphere and may align with the involvement of ipsilateral basal ganglia structures. However, even with an extensive search of the literature, the specific relationship between ICH and regional glymphatic clearance remains vague. ICH is most commonly known to occur in the basal ganglia, thalamus, pons, and cerebellum, as represented by Figure 3.<sup>61</sup> Therefore, since the common site of ICH is known, extensive research is needed to verify the relationship of ICH glymphatic clearance. These discrepancies and regional variations likely result from multiple factors, such as heterogeneity in hemorrhage severity and location, differences in tests and models, variation in time post-ICH examination, and diverse methodologies employed to assess clearance. Therefore, to advance the understanding of regional glymphatic clearance, future studies should focus on standardized, region-specific, and longitudinal approaches to consider and reconcile these variables by carefully focusing on hemorrhage location, severity, and imaging differences. Ultimately, such refined tests are essential to elucidate the nuanced temporal and spatial dynamics of glymphatic dysfunction in ICH.

### GS Dysfunction During ICH



**Figure 3:** Visual diagram of glymphatic clearance in ICH, including the basal ganglia, thalamus pons, and cerebellum. The lack of spatial overlap among these regions indicates regional heterogeneity, limiting direct inference of a unified anatomical pattern. Further investigation is required to elucidate potential links between regional glymphatic system dysfunction and ICH. Figure originally produced by the author using BioRender.com.

## ■ Glymphatic System Relationship with Cerebral Small Vessel Disease

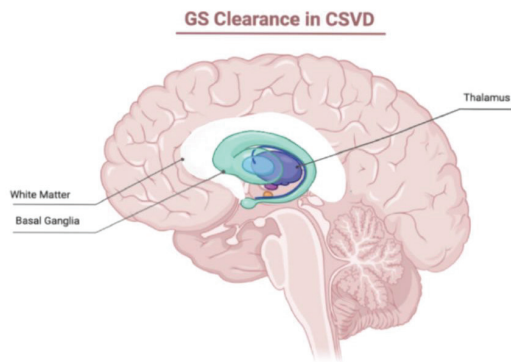
CSVD is a chronic disorder affecting the arteries, small veins, arterioles, and capillaries. The main connection between many

CSVD and GS studies all explore DTI-ALPS and its impacts on CSVD markers. CSVD's detection markers, such as white matter hyperintensities (WMH), cerebral microbleeds (CMB), PVS, cerebral microbleeds (CMBs), brain atrophy, and lacunes, are associated with secondary neurodegenerative diseases like AD and stroke.<sup>62</sup> While the relationship between CSVD and the GS has been studied using various imaging techniques, such as AQP4 expression, DTI-ALPS, and the measurement of white matter hyperintensities (WMH),<sup>63</sup> research on how regional glymphatic clearance affects CSVD has not received scholarly attention. Through reduced expression of any imaging marker, this section aims to review studies establishing the relationship between GS and CSVD. Glymphatic dysfunction and high-grade EPVS are strongly linked to increased BBB leakage, emphasizing the idea that weakened BBB integrity may be driven by impaired glymphatic clearance.<sup>15,64</sup> Glymphatic failure plays a mechanistic role in the pathophysiology of cerebrovascular disease – in this case, CSVD. MRI scans have also revealed how larger basal ganglia and thalamic ePVS volumes were linked to larger total WMH volume, increased chance of cerebral microbleeds, and lower WMFA. CMB is a CSVD marker, as it disrupts blood vessels in the BBB, causing fluid leakage, buildup of toxic proteins, and inflammation. On special MRI scans, CMBs appear as small black spots, indicating vulnerable and damaged areas – essential for identifying region-based glymphatic-CMB vulnerability.<sup>11,62,65</sup> The basal ganglia and the thalamus show enlarged PVS, which is an indicator of impaired glymphatic clearance. Basal ganglia, white matter, and the thalamus are major regions affected by CSVD, and through ePVS and AQP4 expression in rodent models, we can conclude that these regions show the greatest GS-related changes in CSVD.

Additionally, deep white matter hyperintensities (DWMH) and EVPS are frequently co-localized, suggesting that impaired GS function in deep white matter may contribute to the development of lesions.<sup>66</sup> While DWMH doesn't directly measure glymphatic flow, it serves as a marker for deep brain regions via MRI, such as the basal ganglia, where poor glymphatic clearance, as measured by DTI-ALPS, is evident. This imaging technique, commonly used in CSVD, has been linked to GS dysfunction, suggesting that these figures might indirectly influence fluid clearance, particularly in the deep brain regions such as the basal ganglia. Additionally, in another study of 330 CSVD patients, deep white matter hyperintensities, the number of microbleeds and lacunas, were shown to be connected to the mALPS-index. This study had a two-part test: the first was comparing imaging techniques, specifically DTI-ALPS, mALPS, and Glymphatic MRI (using gadolinium as a tracer). They calculated the ALPS and mALPS index by measuring the diffusion of water in directions that align with perivascular flow in projection and association fibers. mALPS was measured the same way, but uses a brain template to place ROIs (regions of interest). This study demonstrated that patients with low mALPS and ALPS index scores indicated worsened glymphatic clearance. Secondly, EVPS in the basal ganglia was found to be correlated with GS dysfunction (measured through mALPS), but EVPS in the centrum

semivole (a white matter region) wasn't. This may be because mALPS was shown to measure clearances in deep medullary veins (which drain the basal ganglia) but not regions drained by superficial veins (centrum semiovale).<sup>67</sup> These varying regions support the idea of how GS dysfunction is regional, not uniform. This pattern likely reflects the anatomical organization of perivascular pathways and the vulnerability of deep perforating vessels to CSVD pathology, suggesting glymphatic failure contributes to lesion growth.<sup>23,66</sup> This study also points out the limitations that mALPS imaging only captures a part of GS function, emphasizing the importance of anatomical context when interpreting regional GS impairment. Additionally, a study assessing glymphatic clearance with CSVD imaging markers proved that the relationship is complex and influenced by other regions. The DTI-ALPS index of CSVD patients was negatively correlated with imaging features. Essentially, negatively correlated is when a relationship between two variables in which the value of one variable increases as the value of the other decreases (APA Dictionary). More specifically, by analyzing participants with CSVD and the control group, the DTI-ALPS index in the bilateral hemisphere of CSVD patients was negatively correlated with the grade of EVPS in the basal ganglia. The right DTI-ALPS index was negatively correlated with the deep white matter hyperintensities (DWMHs), and the left was negatively correlated with the lacunas.<sup>68</sup> Essentially, the negative correlations between the DTI-ALPS index and imaging markers in patients with CSVD (EVPS, lacunas, and DWMHs) suggest that when the DTI-ALPS index is lower, the severity of EVPS is higher. When the severity of lacunae increases, DWMH's severity is higher. Since worsened glymphatic clearance is evident in this study of CSVD patients, there is a more pronounced expression of EVPS in the basal ganglia region, a prevalence of extensive white matter abnormalities (larger areas of white matter damage), and an increase in lacunar lesions.<sup>63</sup> The exacerbation of these CSVD imaging markers is an indicator of impaired glymphatic clearance in CSVD; more specifically, basal ganglia regions were highlighted with DTI-ALPS being negatively correlated with EVPS.

Ultimately, current evidence supports a strong association between GS impairment and CSVD, with the basal ganglia, thalamus, and deep white matter emerging as key regions of impairment. This can be seen in Figure 4. Most, if not all, studies assessed strongly linked glymphatic impairment of CSVD in the basal ganglia, not only reinforcing how GS function is non-uniform, but also a novel target for future treatments to pinpoint the basal ganglia in CSVD.



**Figure 4:** Visual diagram of glymphatic clearance in CSVD, including deep white matter, the basal ganglia, and the thalamus. The deep white matter, basal ganglia, and thalamus are all visually represented anatomically surrounding one another. Figure originally produced by the author using BioRender.com.

## ■ Conclusion

In conclusion, by reviewing and synthesizing studies spanning scientific literature and using several different GS imaging and mapping techniques through a systems neuroscience angle, this study has provided valuable insight into the topic of regional glymphatic clearance within CVDs. We aimed to observe how brain areas with reduced glymphatic clearance are more prone to GS dysfunction, highlighting regional vulnerability as a significant factor in the onset and progression of cerebrovascular diseases. Therefore, by presenting a valuable focus on targeting these regions, future treatments can be precisely directed to restore flow in profoundly impaired areas.

In IS, while GS clearance is shown to be most impaired in the infarct and peri-infarct cortex, these regions can vary, so we suggest that the infarct regions of the middle cerebral artery, anterior cerebral artery, and posterior cerebral artery are where dysfunction is most notable. In SAH, glymphatic clearance is mostly impaired in the deep cervical lymph nodes, basal and ventral cisterns (pontomedullary and interpeduncular cisterns), and the anterior cortex – regions largely centered around the brainstem. In ICH, while some studies show GS clearance to be mostly impaired in the basal ganglia, perihematomal regions, the centrum semiovale, and potentially the ipsilateral hemisphere, these findings vary due to differences in hemorrhage location, severity, and imaging methods. Therefore, future research on human patients should aim to measure GS clearance specifically around areas most prone to ICH. Lastly, in CSVD, glymphatic clearance is consistently impaired in the basal ganglia, with the exception of the thalamus and deep white matter.

Our research has revealed that through different GS imaging and mapping techniques (DTI-ALPS, WMH, AQP4 expression, FLAIR MRI, Glymphatic MRI, fluorescent tracers, etc.), not only do we identify specific regions of glymphatic flow susceptibility, but we also intertwine our findings and overlap them with other diseases. Essentially, this hints at an interconnected relationship between IS, ICH, SAH, and CSVD, enabling broad-spectrum therapies. Rather than creating separate treatments for each disease, streamlining shared ones that can be implemented in clinical applications serves as a future direction for CVD. In general, restoring arterial pulsa-

tility, such as pulsation enhancers, aids in the restarting of flow. While experimental manipulations in rodents build a foundation, demonstrating that enhancing pulsation can improve CSF entry and tracer movement, translations to humans must account for vascular differences. Importantly, interventions that preserve or restore physiological pulsatility, such as maintaining vascular elasticity, promoting sleep-driven oscillations, and controlling hypertension, may represent strategies to optimize clearance.<sup>69</sup> Arterial pulsatility drives glymphatic clearance, pushing CSF from the subarachnoid space into perivascular and interstitial spaces.<sup>69-71</sup> To illustrate, in IS and CSVD, enhanced arterial pulsatility may generally be beneficial, improving waste clearance and mitigating secondary injury in IS. However, in the context of ICH and SAH, increased pulsatile forces could exacerbate hematoma expansion or perihematomal edema, since blood vessels are already compromised.<sup>19,72</sup>

In contrast, modulation of AQP4 channels on astrocytic endfeet can enhance interstitial fluid movement without altering vascular pressures. Therefore, this still benefits IS and CSVD with regional glymphatic impairment by enhancing CSF-ISF fluid exchange, since upregulating AQP4 enhances CSF-ISF exchange, accelerating the clearance of neurotoxic waste.<sup>32,40,56,57,70</sup>

Moreover, utilizing anti-edema strategies potentially offers complementary solutions by acting on both solute clearance and interstitial fluid accumulation. Osmotic agents increase plasma osmolality, therefore creating a transvascular gradient that pulls fluid (water) out of swollen brain tissue back into circulation, reducing intracranial pressure and secondary injury.<sup>41</sup> In parallel, combining this with the glymphatic system's primary function of clearing neurotoxic waste and solutes provides potential treatment for hemorrhages. Collectively, these avenues for treatment provide shared as well as disease-specific approaches for restoring glymphatic function through targeting clearance pathways and modulating driving forces. In addition, throughout this paper, ICH and CSVD both showed signs of impaired glymphatic clearance in the basal ganglia. However, future studies should aim to investigate deeper into this connection, since multiple factors can affect this outcome, such as sample size, different imaging methods, and different molecular factors depending on the study. Therefore, this could effectively cause us to rewire and rethink the effects of GS and GS impairment on cerebrovascular diseases.

Furthermore, identifying regional glymphatic impairment also serves as a predictive biomarker for early detection or prediction of any cerebrovascular disease. From the findings in this paper, we can conclude that regional glymphatic clearance is a vital factor in neurodegenerative diseases. These findings together point the way toward novel treatment targets for CVD.

## *Limitations:*

It must be recognized that strokes occur almost anywhere in the brain, but we have analyzed some of the most common regions. In terms of ischemic stroke, SAH, and ICH, it remains unclear whether impaired glymphatic areas undergo structural remodeling in CSVD, IS, SAH, or ICH, which could support the idea of cognitive deficits post-stroke. Additionally, this pa-

per analyzes and synthesizes studies from a variety of animal models: rodents, humans, and nonhuman primates; therefore, the implementation in a more complex brain – a human – is required for future translational therapeutic strategies.

Solely identifying impaired glymphatic regions that are associated with the CVD mentioned in this paper for humans is a highly significant area of research. Furthermore, this review is constrained by the limited novels directly exploring glymphatic clearance in CVDs, and there is an opportunity and encouragement to investigate with a larger sample size of papers.

Lastly, targeting these brain regions might differ from individual to individual when applying this to clinical scenarios. An individual's medical history can play a significant role in the accuracy of the findings in this paper, as taken into account in several of the studies analyzed. However, understanding the mechanisms and foundation of regions' glymphatic clearance in cerebrovascular disease also proves worthy of identifying which patients may be at a higher risk of developing or exacerbating these diseases.

#### **Future Research:**

This paper highlights regional anatomy and fluid dynamics when identifying regional glymphatic clearance. Further research should define the molecular and cellular drivers of region-specific clearance – particularly AQP4 expression regulators such as dystrophin, syntrophin, AQP4 isoforms, genetics, and PVS remodeling. Accordingly, the aim is to connect these mechanisms to CVD phenotypes by imaging the specific molecular mechanisms underlying regional dysfunction and determining their correspondence to CVD. Future research should delve deeper into further affirming this process.

Additionally, we suggest that future research aims to apply the same process to other families of diseases, such as dementia, AD, PD, Huntington's, and multiple sclerosis, to create a cohesive understanding of the differences and similarities between impaired glymphatic regions. Comparative analysis across these diseases may reveal similar patterns of regional susceptibility and clearance bottlenecks, essentially offering a different approach to addressing these diseases.

Furthermore, integrating genetic, proteomic, and biomarker profiling may identify molecular signatures linked to regional glymphatic impairment, such as dysregulation of dystrophin-associated scaffolding protein<sup>54</sup> or pericyte-mediated vascular control.<sup>47</sup> This provides predictive markers for disease detection and progression. Moreover, developing other GS imaging techniques expands our understanding and accuracy of defining the glymphatic system and utilizing our understanding to further the scientific field. DTI-ALPS imaging is a mere representation of tissue pathology using brain structure, rather than a measurement of specific GS function, and therefore, a more accurate method will prove useful in understanding the underlying mechanisms of GS function.<sup>19</sup> Additionally, utilizing non-enzymatic breaking of AGE cross-links has been shown to temporarily increase arterial wall flexibility and compliance during sleep in the elderly, to amplify CSF flow without affecting systemic blood pressure.<sup>72</sup> Further research is needed to observe this potential therapy across age, gender,

demographics, etc., as the direct glymphatic connection is limited. Furthermore, the GS operates on a circadian rhythm, with activity peaking during rest or sleep, and clearance kinetics being reduced during wakefulness. Since sleep can effectively reduce GS dysfunction, the risk of glymphatic-linked diseases decreases – essentially serving as a natural remedy. Therefore, the question arises of whether time spent in different stages of sleep can be linked to glymphatic clearance. In general, papers have studied sleep architecture, including REM and NREM, in both young and old individuals. It is notable to recognize that sleep quality decreases as a function of normal aging. As insomnia grows more frequent with increasing age, sleep duration shortens and is more interrupted.

Consequently, poor sleep quality has been epidemiologically linked to an increased incidence of cardiovascular diseases, particularly stroke.<sup>73</sup> Further research should aim to identify how preceding sleep patterns and routines affect GS recovery after cerebrovascular diseases.

Ultimately, high quality of sleep, exercise, dietary changes, and even managing hypertension may confer resilience to the GS, essentially preserving its functional integrity as much as possible.<sup>32</sup>

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