Glymphatic Structure and Morphological Features of the Brain

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Disclose and conflicts of interest: none to be declared by all authors

ABSTRACT

Introduction: there is limited knowledge about the lymphatic circulatory system of brain. The process in which the solutes pass in the brain parenchyma via the interstitial space has not been fully understood up to now. The glymphatic system, which was discovered in recent years, is a waste-cleaning system using an extra-ordinary perivascular channel system, made of astroglial cells, to expel the dissolvable metabolites and proteins out of the central nervous system (CNS). Interestingly, the glymphatic system works basically while asleep and is generally impaired while awake. The metabolic wastes in the brain are cleaned by moving from the interstitial space to the cerebrospinal space. Studies in recent years have shown that some anatomical pathways in the brain facilitate the mixing of CSF (the cerebrospinal fluid) and ISF (interstitial fluid) and remove the substances solved in ISF in the brain. There are three drainage pathways in the brain; (1) Para-arterial CSF flow pathway, (2) Paravenous ISF clearance pathway, and (3) Trans-parenchymal pathway. The glymphatic cleaning system increasingly gains importance in healing some diseases like brain injury, encephalomyelitis, and Alzheimer's disease (AD). Therefore, intensive efforts have been and are still being devoted to the studies on the glymphatic system.

Keywords: Blood-brain barrier; Brain drainage; CSF; Dendritic cells; Lymphatic drainage

Introduction

This review article provides information about various proteins and the specific lymphatic protein pathways of the brain. The anatomy of the lymphatic system can be explained by that the lymph is created by the micro-circulation due to an oncotic and hydrostatic pressure difference between the blood plasma and the abluminal side of the glycocalyx living on the plasma of endothelial cells with solutes via the trans-endothelial pores and junctions (Benveniste *et al.* (2017). CSF serves in removing the metabolic waste products within the brain. However, new studies with the previously published data describe the glymphatic drainage. Also glymphatic system, a new understanding of how aging, medications, sleep and wake cycles, genetic predisposition, and even body posture affect the brain drainage system has not only changed the idea of brain fluid circulation but has also contributed to an understanding of the pathology and mechanisms of neurodegenerative diseases. Yankova *et al.* (2021).

I- Brain's Lymphatic Drainage

According to the published data, the neural cells are the most significant component of the brain (70-80%). Brain microenvironment (BME), which is formed by the vascular system and ISS (interstitial system), accounts for the remaining volume of the brain and provides an environment for neural cells to live Lei *et al.* (2017). Our knowledge about neural dissemination is able to explain the neurological and mental states of the brain

to some extent. For this reason, it is of importance to examine the effect of the ISS of the brain. The meningeal lymphatic system was first described in 2015 Aspelund *et al.* (2015). The glymphatic apparatus drains the waste from ISF to CSF. In CSF, it flows into the meningeal lymphatic system and then directs toward three groups of the deep neck lymph nodes.

Aquaporin-4, found in astrocyte feet attached to the paravascular spaces, plays a key role in clearing waste molecules such as beta-amyloid or tau proteins. The activity of this system increases during sleep, especially in the slow wave phase and unilateral sleep and exercise, and decreases with aging (Toriello *et al.* 2021)

The glymphatic system is an exclusive perivascular canal system made up of the astroglial cells with the function of efficiently eliminating the solvent proteins and metabolites accumulated in the CNS. Surprisingly, the glymphatic system is active during sleep and generally inactive when awake. Therefore, sleep is important for the brain to eliminate the neurotoxic wastes such as A β (amyloid- β) Iliff *et al.* (2012). The function of this system in the brain corresponds to the lymphatic system of other organs, and it is named "glymphatic pathway" due to its reliance on the glial cells Benveniste, Lee, & Volkow (2017). Although there is no diagnostic test designed to determine the function of this mechanism in human brain, the clinical evidence for the glymphatic pathway is as follows: the sleep-related changes in the concentration of A β and

Tau in CSF, the increase in A β due to chronic sleep deprivation, and, in elderly, the association of the shorter sleep and poor sleep quality with higher levels of A β Benveniste *et al.* (2017). The lymphatic system is found in almost all organs except privileged organs such as the CNS and bone marrow. In humans, both CSF and ISF are drained from the brain toward their lymph nodes of the cervical part Weller *et al.* (2009). It is unclear how the extracellular soluble substances in the brain pass through the interstitial space to CSF Bakker *et al.* (2016).

The process of the brain extracellular fluids drainage, especially ISF and CSF, is important not only for regulating the fluid volume but also for eliminating the wastes like A β Bakker *et al.* (2016). In a two-photon microscopic imaging technique using the fluorescent materials, it was observed that CSF entered the brain parenchyma via the perivascular spaces surrounding the penetrating arteries and then passed into the paravenous space. It was shown that the soluble substances in ISF were removed through switching to CSF in the paravenous space Plog & Nedergaard (2018). A β is drained from cerebral ISF to CSF. The recent studies assert that the process of clearing of the cerebrum takes place during sleep by a special pathway from the brain parenchyma toward the peri-vascular and para-vascular zones Bakker *et al.* (2016). The existence of A β in the glia limitans in AD is significant in deciding the cerebral amyloid angiopathy regarding the perivascular drainage failure Bakker *et al.* (2016).

II- Brain vascular system

The capillary system is not included in the organization of the glymphatic system. because the microstructure of the neurovascular unit, which enables rapid exchange between capillaries and neurons, is an axiom of neuroscience. PVS around large and moderately penetrating blood vessels is easily observed by in vivo imaging and post-mortem histology, while evidence for PVS or peri-capillary space around microvessels still requires solid evidence (Ferris 2021).

Vascular disorders in the brain can have a role in the death of nerve cells. The bloodstream toward the brain is achieved mainly via the intracranial arteries derived from the circle of Willis and the small vessels in the brain. These small arteries (pia arteries) are then branched into smaller arterioles (penetrating arterioles) that perforate into the brain parenchyma and end as a larger network of a capillary (Figure 1). Penetrating arterioles have relatively fewer branches and separate with the parenchyma (Virchow-Robin space), after which the arterioles and capillaries are covered with the astrocytic endfeet Joutel & Faraci (2014). The substance interchange takes place between the plasma and ISF in vessel capillaries Sykova & Nicholson (2008). The neurons are around 10 μ m away from the closest capillaries. This distance ensures a more efficient diffusional substance interchange between the neurons and capillaries Mabuchi *et al.* (2005). The cortical artery does not have an elastic lamina, but it has a tight perivascular space. However, the artery in SAS has not only an elastic lamina but also a big perivascular space Bakker *et al.* (2016).

Figure 1. Glymphatic transport. The medical conceptual clarification of the clearing process of the brain waste products all over cranial nerves. The front end of the glymphatic system is indicated including the peri-arterial, interstitial space, and the peri-arterial transportation of CSF (yellow arrow). The waste solutes (black points) drain along the cranial nerve or, maybe, these waste solutes with fluid enters the nerve, in which the waste solutes drain along fascicles and/or axons. AQP4, aquaporin 4 water channels. Perivascular AQP4 is critical in cleaning β -amyloid. Because vascular dementia, which develops after traumatic brain injury, will lead to the mislocalization of AQP4. (160 x 113 mm 300 DPI)

The brain perivascular spaces perform the lymphatic function. As the vessels feeding the brain tissue enter into the brain, they progress with the pia mater which is loosely connected to the vessel and forms a perivascular space between itself and the vessel. This space is found in the arteries, veins, venules, and arterioles in the brain, but not at the capillary level. Normally, brain does not have a real “perivascular space” surrounding the artery when entering the cerebral cortex (A. W. J. Morris *et al.* (2016). When molecules between 15 nm and 1 μ m were injected toward the gray matter in the brain, they were not drained through the intramural basement membranes, but they followed a path outside of the arteries and separated the glia limitans from the vascular walls Zhang *et al.* (1992). There is a relationship between the perivascular space (Virchow-Robin) and the basement membranes of the vessels for rapid drainage of CSF from the cisterna. In the studies conducted with fluorescent tracers, it was found that the molecules larger than 70 kD (500–2000 kD) accumulated in the perivascular space (Iliff *et al.* (2012).

III- CSF and ISF Drainage

ISF and CSF drain toward the neck lymph nodes via separate pathways. CSF goes toward the bloodstream of the venous sinuses via well-developed arachnoid granulations and villi. The CSF lymphatic drainage takes place along the cranial/spinal nerve roots via the dural and nasal lymphatics. The channels passing from the SAS through the cribriform plate make it possible for CSF to pass into the cervical lymph nodes and nasal lymphatics. The brain parenchyma-induced ISF flows via the basement membranes in the capillaries and arterial wall of the cerebrum toward the cervical lymph nodes Engelhardt *et al.* (2016). According to McKnight *et al.* (2020), it is reported that clearance of the CSF occur to the cervical lymph nodes along the cranial nerves. Similarly, CSF route to the paraspinal lymphatics along the paraspinal nerve roots.

IV- Lymphatic drainage of CSF

Drainage of CSF occurs in a two-way pattern: directly to the lymph nodes and the blood. The first drainage takes place through the nasal lymphatics while the second drainage through the arachnoid villi and granulations. The CSF lymphatic drainage is carried out mostly through the cribriform plate to the nasal lymphatics Weller *et al.* (2009). The optic nerve is a white matter tract of the CNS, not that of the cranial nerve. In light and transmission electron microscopes, the lymphatics surrounding the optic nerve are shown in the dura mater. The Indian ink injected into the optic nerve SAS was found to be present in the dural lymphatics Killer, Laeng, & Groscurth (1999). Accordingly, the CSF in the optic nerve SAS and the orbit drain through the

lymphatics in the dura mater surrounding the optic nerve Mathieu *et al.* (2017).

According to Cserr (1971), while urea needs 5.4 hours to diffuse 1 cm in the brain, albumin needs 109 hours. Based on this fact, Cserr hypothesized that the larger the brain, especially larger molecules like proteins and peptides that could not be cleared effectively by diffusion would take a long time to be efficiently cleared.

In recent studies on experimentally shaped brain edema, nanoparticle tracers injected to the lateral lobe of the brain were reported to reach both mandibular and parotid lymph nodes (Nur, (2018); Nur *et al.* (2020); Nur *et al.* 2021) (Figure 2–4). It is observed that not only glial macrophage cells but also antibodies formed in mandibular and parotid lymph nodes have a part in the defense of the brain. How antibodies reach brain tissue is still unknown. CSF moves via the ventricular apparatus to the SASs, partially flows toward the blood through the granulations in the walls of CSF, arachnoid villi, and venous sinuses in humans. The remaining part of CSF streams to the local lymph nodes through the dural/nasal lymphatics and the lymphatic vessels related to the cranial/spinal nerve roots Aspelund *et al.* (2015). In certain well-evolved creatures, it is assessed that half of CSF is drained towards lymphatics Cserr *et al.* (1992); however, this rate is still unknown in humans Johnston *et al.* (2004)

Figure 2. *In vivo* hyperspectral fluorescent images The neck region. Ventral view. The signals received from the MLN of male rat. (a) and (b) indicate the signals at the 20th and 40th min, respectively. (c) and (d) show the signals received from the MLN on both sides of the neck at the 4th hr. Scale bar, 10 mm. L, left; MLN, mandibular lymph node; R, right. L. Left. (143 x 54 mm 300 DPI).

Figure 3. *In vivo* hyperspectral fluorescent images On the right neck region (A, B, and C), on the left neck region (D, E, and F), lateral view. Lateral view. A-C shows the signals received from the right PLN and D-F from the left PLN, at the 40th minute of imaging post-injection. C and F show the strength of the signal in 3D. Scale bar. 10 mm (160 x 89 mm 300 DPI).

Figure 4. SEM images of nanoparticles in rat left parietal lobe and mandibular lymph nodes.

Tracers saw in the medulla part of both lymph nodules an hour after the injection of Qdot 800 (Qdot® 800 ITK™ carboxyl quantum dots, Termofisher, Invitrogen, and Eugene, OR) as a tracer in the left parietal brain lobe of a female rat. A: left parietal brain lobe.686 MAG. B: Mandibular lymph node.1540 MAG and C: parotid lymph node,1000 MAG (166 x 146 mm 300 DPI).

CSF serves a floatability function for the brain liquid. Taking into account the fact that the brain and CSF are similar in terms of specific weight, the CSF effectively decreases brain weight. CSF is generated by actively secreting from the choroid plexus and reabsorbed by the arachnoid villi Hladky & Barrand (2014). Another noteworthy issue concerning peri and paravascular drainage is a significant difference in the direction of these two types of flow. It was depicted as the forward-backward motion of the different tracers used in the studies Ichimura, Fraser, & Cserr (1991). Other studies asserted that CSF flowed from SAS into the perivascular space of the capillaries and then arrived at the capillary basal lamina and flowed to the brain parenchyma; then, came back from brain parenchyma to the basement membrane of the venules of the peri-vascular space of the major vessels Rennels *et al.* (1985). The passage of the perivascular stream is a type of flow that utilizes the extracellular space along with the capillary membranes in the arterial wall and the capillaries originating from the brain in a reverse direction to the arterial flow of blood. While the perivascular flow is in the reverse direction of the arterial stream, the para-vascular flow may be in the same direction as blood flow (Rennels *et al.* (1985).

V- Origin of ISF and its drainage

ISF takes its origin from the metabolic action of the blood and brain parenchyma. Under normal conditions, BBB strictly regulates the entrance of solutes to CNS. Except for the CNS and eye, ISF is the main extra-cellular liquid in other organs and systems, and the majority of the organs don't have something corresponding to CSF. Early studies on rats and rabbits demonstrated that the tracers drained into the neck lymph nodes on the same side Pollay (2010). The water produced by glucose oxidation with CO₂ can contribute by 10% to the total ISF volume. A majority of ISF is obtained with the subsequent water in the vascular endothelium Na⁺/K⁺-ATPase. Within the gray matter of the brain, several small narrow spaces are associated with each other, and these spaces are loaded with ISF. These small spaces are an extracellular space and have a direct continuity with the capillary basement membranes (Figure 5) Carare *et al.* (2008).

In addition to the blood, there are 2 types of fluids linked to brain, that is, CSF and ISF. The amounts of ISF and CSF in humans are approximately 280 ml and 140 ml, respectively Bozanovic *et al.*(2001; Erdoğan *et al.*(2013). In peripheral tissues, ISF is created by filtering the plasma from the capillary endothelium,

Figure 5. The drainage process of ISF out of brain parenchyma (Engelhardt *et al.*, 2016)

Intramural perivascular interstitial fluid (ISF) drainage away from the brain. As a result of losses of arteriole tunica medialis, it begins to be a capillary, then becomes a post-capillary venule. Astrocyte tightly covers the capillaries' surface to form the glia limitans. The capillary wall is the position of the BBB for solutes. The post-capillary venular wall forms the BBB for the lymphocytes and another inflammatory cell that comes from the bloodstream to CNS; the glial (blue) and the endothelium basement membranes (green) are not combined. The ISF and solutes particles drain from the brain parenchyma extra-cellular spaces via numerous gaps located between astrocyte endfeet (yellow arrows). As a result, ISF drains towards the basement membranes between the smooth muscle cells in the medial layer of arterioles and arteries (yellow arrows): this represents the intra-mural perivascular drainage. Neither the arterioles and arteries endothelium basement membrane (light blue) nor the outer basement membranes of the arterial wall (green) participate in the intra-mural perivascular ISF drainage from CNS. (150x 62 mm 300 DPI).

which is highly permeable. Even though some of the ISF is absorbed back into the post-capillary venules, most of the ISF and even almost all of the extra-cellular proteins are collected in the primary lymphatic vessels Jessen *et al.* (2015). All pathways involved in the drainage of CSF have not been fully identified yet. But, for human beings, it is assumed that the drainage takes place equally through the spinal vessels, cribriform plate, and arachnoid granulations Veening & Barendregt (2010).

The tracers injected into the rabbit/rodent brains were found to be at the arterial adventitial layer in the Willis polygon at the brain base; however, they were not observed to be in the neck segment of the carotid arteries. The abrupt termination of the presence of tracer in the carotid artery adventitia at the base of the skull suggests that the dissolved substances and ISF are drained from the arterial walls to the neck lymphatics. The flow that occurs following each beat is in the reverse direction and likely to remove perivascular lymphatic drainage from the brain Schley *et al.* (2006).

The relationship between ISF to CSF is a physiological one, just like waste removal and lipid metabolism. The edema in the brain during brain traumas or cerebral ischemia disrupts this entire functional structure, like in the case of the accumulation of certain proteins in the neuroinflammatory disease such as multiple sclerosis (MS). In an *in vivo* study carried out on mice, it was reported that 5 minutes after the injection, the tracers reached the perivascular space and, approximately 25 minutes later, they gradually progressed toward the brain parenchyma in the perivascular space of penetrating arteries Plog & Nedergaard(2018).

The tracers advance along with the perivascular space around the penetrating cerebral arteries perpendicular to the brain parenchyma within CSF Drewes (2004). In electron microscope studies, leptomenigeal artery and veins form a perivascular space (Virchow Robin space) within the surrounding the piamater layer, in other words, in the elastic lamina of the artery Lei *et al.*(2017). The Virchow Robin space, an expansion of SAS, is known to contain a fluid with a composition similar to CSF Bell & Zlokovic(2009). Also, the SAS has direct continuity with the perivascular spaces; however, the penetrating arterial perivascular spaces were separated from the subpial and subarachnoid spaces by a membrane of the leptomenigeal cells that were the continuation of the piamater Preston, *et al.*(2003).

VI- Glia limitans' role in drainage

Glia limitans is thicker in humans compared to rodents and composed of the different layers of astrocytes. Even though the lymphocytes and antigen-presenting cells (APC) effectively pass through the sensitive glia limitans around the capillaries in the brain of humans, it is much more difficult for the inflammatory cells to pass through the denser glia limitans on the surface of the brain or spinal cord. For instance, in MS, demyelinating plaques on the cerebral cortex surface might be linked to the inflammatory cells in the underlying SAS. But, these cells don't enter the glia limitans. It was reported that if the inflammatory cells were added to CSF, the demyelination sites on the brain surface enlarged and the cytotoxic agents could pass from the brain to CSF Howell *et al.* (2015).

VII- Effect of Sleep on the Drainage of Brain

In the glymphatic system, the main function of sleep, rather than being awake, is to drain ISF from the brain and push CSF through the peri-vascular space. It was determined that 90% of the fluorescent substance delivered into the brain tissue of the mouse during sleep passed through the SAS. The fluorescent tracers accumulate 2 times more in ISF in wakefulness compared to sleep, thus supporting the glymphatic drainage function in the amount of extracellular fluid between sleep and wakefulness. In the electrophysiological studies, it was shown that there was a 65% change in the extracellular fluid of a dormant brain compared to an awake brain. The increase in the amount of extracellular fluid clarifies that the ISF is pushed from the brain parenchyma to the perivascular space in a more rapid way Xie *et al.*(2013). Another research reported that position of head while asleep altered the flow along the pathway. The enhanced contrast MRI was used in the study and it was found that the viscosity of the ISF was lower when the mice took the lateral recumbency compared to the side-by-side or supine positions. Furthermore, it was shown in mice that the fluorescent CSF tracer flux in the cerebrum was greater in those positioned laterally compared to those positioned prone. So, it is obvious that some other factors such as posture and gravitation also have a role in regulating the glymphatic passageway Lee *et al.* (2015).

It was reported that in slow-wave sleep/general anesthesia, the clearance rate of dissoluble, transportable, and soluble A β increased due to the increase in cortical ISF by 60%. In awake mice, the proportion of ISF is 14% in the cortical area. During sleep, this rate reaches 23% with an increase of 60%. It was observed that the transport of glymphatic soluble substances increased during sleep/anesthesia Xie *et al.* (2013). Moreover, the drugs that increase sleep or prolong sleep time prevent amyloid accumulation in the brain Roh *et al.*(2014).

Major proteins of brain drainage

I- Aquaporin-4 (AQP-4) Protein

This protein is responsible for the selective delivery of water Zador *et al.*(2007). Aquaporins (AQPs) play an important role in the cleansing system of the brain. In particular, AQP4 and AQP1 are prominent in the circulation of glymphatic fluid in the histopathology of the brain. (de Laurentis *et al.* 2020)

AQP-4 activators are involved in the clearance of the vasogenic component of the edema. In contrast, AQP-4 inhibitors have a brain-protective effect on cytotoxic edema Donkin & Vink(2010). AQP-4 in the CNS is an aquatic channel expressed at the perivascular astrocytes endfeet that delimit the entire cerebrovasculature as well as astrocytic processes forming the subpial and subependymal glia limitans membranes Nagelhus & Ottersen (2013). The void of

tracers entering the brain through the CSF occurs by paravenous flow into either CSF or neck lymph nodes. The immunological value of the conventional drainage system is not clear. The relationship between the ISF and CSF drainage systems has not been clarified in detail yet Rennels *et al.*(1985).

II- Amyloid beta (A β) Protein

Amyloid- β originates from a trans-membrane protein named "amyloid precursor protein (APP)". APPs appear in the proximal dendrites, axons, and cell bodies, and are transported toward the axons and endpoints via the rapid anterograde axonal transporting system Carare *et al.*(2013). Normally, A β is also released toward the CSF. Even though there is limited knowledge about how A β physiologically functions, its indissoluble forms have an important pathological function in the prevention of the ISF and soluble substances drainage from the brain. As a result of the A β accumulation in the cerebral capillary vessels, the vascular basement membranes get thinner and divide, leading to the destruction of the BBB. The BBB failure disrupts cerebral metabolism, minimizes the cerebral blood flow, and leads to neuronal damage Rostagno & Ghiso(2008). In an AD accelerated mouse model, the cerebral hypoperfusion was shown to cause A β deposition in the leptomeningeal vessel wall Okamoto *et al.*(2012). Neurodegenerative diseases do not only negatively affect the glymphatic system, but can also cause an effect on glaucoma, extending to blindness. Some studies have shown that A β deposition causes apoptosis of retinal ganglion cells (Oliveira *et al.* 2018).

III- Tau Protein

Tau is a neuronal microtubule associated protein and mainly found at the neuron axons. Tau provides the formation and fixation of the cellular microtubules and supports the development of neural extensions and neuronal cell polarization Buee *et al.*(2000). Tau is considered a phospho-protein, and phosphorylation organizes its biological activities. Tau phosphorylation diminishes as age increases Drewes(2004). Since the ordinary role of Tau is organized by phosphorylation, the failure of this organization brings about an abnormality in Tau aggregation. Tau accumulation with no amyloid pathology is enough to lead to dementia in human beings. The impaired functioning of tau protein leads to neurodegeneration or dementia. An increase in the amount of Tau disrupts the regulation of the microtubule network for neurons. Furthermore, the excess Tau protein may also interfere with the exchange of vesicles and organelles in neurons Erdoğan *et al.*(2013).

IV- Astrocytes

Astrocytes show many different morphologies compared to other cell types depending on their

location and relationship. Approximately 11 distinct phenotypes can be easily distinguished and eight of them are related to the blood vessels Abbott, Ronnback, & Hansson(2006). Astrocytes reach 10 Microns in diameter and have the ability to expand much when exposed to excitation. Astrocytes, the essential type of glial cells, are star-shaped and have numerous processes originating from the body of the cell. They are located around most of the microvessels and capillaries and react to the endothelial cells along the endfeet of cellular processes in the neurovascular unit Oberheim *et al.*(2009). Moreover, just a single astrocyte is able to communicate with thousands of capillaries and synapses and by means of their processes Haydon & Carmignoto (2006). In this way, astrocytes ensure a cellular connection called “neurovascular binding” between the neuronal activity and blood vessels. Astrocytes have a key role in the regulation of the bloodstream of the cerebrum in response to the activities of neurons by signaling Gordon, Howarth, & MacVicar (2011). Furthermore, the astrocytes contribute to preserving the BBB role by creating barrier characteristics and carrier polarization. However, a previous research suggests that a BBB role already exists in the embryo-genesis phase, even before the formation of astrocytes (Daneman *et al.* (2010).

V- Pericytes

Pericytes are the wall cells that cover 30-90% of the abluminal surface of microvessels with their extensions. They communicate with the vascular wall cells and control numerous microcirculation functions Attwell *et al.*(2010). The endfeet of both pericytes and astrocytes in the neurovascular unit are embedded in the basement membrane. In cases where the pericytes cannot connect or contact the endothelial layer of the vessel due to the basement membrane, they can also be connected with [peg-and -socket] interdigitations in areas without the basement membrane. Also, N-cadherin and connexin-43 proteins enable the pericytes to link to the endothelial cells Dalkara(2015). In the previous studies, it was reported that the pericytes controlled not only the angiogenesis but also the formation of the extra-cellular matrix and the function of BBB in both developing and adult brains. Furthermore, the pericytes contribute to controlling the flow of blood via regulating the capillary diameter of the vessels with contraction property Peppiatt *et al.*(2006).

Pericytes have a key function in preserving the macro stability of the vessels and also serve a function in regulating the brain's flow of blood. There is a close relationship among the endothelial vessels, pericytes, and astrocytes in terms of the development and working of the neuro-vascular unit and BBB Armulik *et al.*(2010). The number of pericytes for each endothelial cell and the vascular wall surface area

that the pericytes cover are the determinant factors for the permeability of capillaries. Therefore, the pericytes dysfunction or failure increases the BBB's permeability Armulik *et al.*(2010). Pericytes are capable of responding to neurotransmitters and vasoactive mediators released by the adjacent neurons/astrocytes through expressing various vasoactive receptors. These pericytes may be necessary for the tissues that have a higher functionality as in the case of the brain and retina.

VI- Neurovascular Unit

When the pial artery/arteriolar covering the brain surface passes to the brain tissue, the penetrating artery gives branches to arterioles and then into a capillary network. The capillary length in the human cortex of 1 mm³ is approximately 50 cm, but 2.5% - 3% of a human cortex is occupied by vessels Lauwers *et al.* (2008). A neurovascular unit is formed with the participation of pericytes, astrocytes, and neurons (Figure 6). The types of cell and basement membranes in the neurovascular unit have a vital function in the proper induction and preservation of the role of the BBB Dalkara (2015).

Figure 6. The neurovascular unit.

The glymphatic system (in orange) is located between the basement membrane of the central nervous system's blood capillaries and the astrocytes layer. The characteristic features of the neurovascular unit permit the bi-directional connection among the microvasculature and neurons, and the astrocytes play an intermediary role. The Virchow-Robin space is the name given to the perivascular space around penetrating arteries. As the results of the branching of the penetrating arteries to arterioles and capillaries, the CSF-involving Virchow-Robin spaces get narrow and eventually disappear. Astrocytic vascular endfeet named aquaporin-4 (AQP4) surround the whole vasculature and form the boundaries of the peri-vascular spaces. (160 x 174 mm 300 DPI).

Before entering the brain parenchyma, the cerebral arteries lose the external nerve source in the Virchow Robin space. Accordingly, the majority of the intrinsic innervation ends at the astrocytes covering the penetrating arterioles or micro-vessels Cipolla, Li, & Vitullo (2004). In contrast to the endothelium in other organs, the vascular endothelium supplying the brain tissue is characterized by the leakage of the fenestrations and has a little number of pinocytotic vesicles. The neurovascular unit makes up of all essential brain cellular elements such as the brain neurons, astrocytes, endothelial of the brain, pericytes, and vascular smooth muscle cells [VSMCs], microglia, and peri-vascular macro-phages. The cerebral arteries are composed of three tunicae; tunica intima [endothelial], tunica medium [TM], tunica adventitial including collagen fibroblasts, and activity nerves. TM controls the cerebral blood flow, which is vital in terms of maintaining the neurovascular unit. The penetrating arteries branch out in the form of a capillary microcirculatory system (with a diameter of 6 -10 μ) consisting of the endothelium and pericytes partially divided by the basal laminate of the extracellular matrix which supports the capillaries and arterioles. In human brains, capillaries reach 400 miles in length. The surface area of capillary suitable for molecule transportation is approximately 20 m^2 Erdoğan *et al.* (2013). In humans, the total length of the microvessels within the brain cortex of 1 mm^3 forms a surface of about 10 mm^2 , which means that there is a comprehensive microcirculation network. Thanks to this network, there is a surface area that consumes a significant amount of oxygen (O_2) and creates Nitric oxide (NO) Wei, Dawson, & Zweier (1999).

In the studies on the mouse brain, no A-V anastomosis was observed and there were no interconnections between the vessels or arteries until the pre-capillary level was reached, and there were no interconnections between the perforating arteries. Furthermore, the vascular anastomosis capillary network provided by different perforating arteries was not mentioned to be present Rosenblum & Zweifach (1963).

The diseases causing intracranial pressure, the cases damaging the vascular system, and/or the illnesses

disturbing the sleep mechanism affect the glymphatic system. In other words, the disorders in the glymphatic system will cause many neurodegenerative and autoimmune diseases. Although there is no conventional brain lymphatic pathway, the physiological examinations have demonstrated the presence of a rich and immunologically important lymphatic draining system extending from the brain toward the neck lymph nodes Engelhardt *et al.* (2016). In MS, the neural antigens are found in proinflammatory APC of the neck lymph nodes; however, in the general of myelin-including cells, they are anti-inflammatory. Whereas the chemokine receptor 7 [CCR7] cannot be expressed in cells including neuronal antigens in human neck lymph nodes, it is expressed *in situ* and *in vitro* studies of myelin antigens. $A\beta$ is a peptide that can be found in forms of dissolvable, oligomeric, unsolvable, and β -fold plate fibrils in brain parenchyma extracellular spaces and cerebral arterial and capillaries wall in aging people Zwam *et al.* (2009). In AD, $A\beta$ originates from the APP and seems to be created by most cells in the brain and other organs. Depending on the age and AD, $A\beta$ accumulates in the intramural basement membranes of the arteries/capillaries in the brain. Aggregated changes in the cerebral arteries disrupt the intramural perivascular draining of ISF Hawkes *et al.* (2011), and this may trigger the amyloid deposition, homeostasis failure, and the spread of Tau protein in the brain Weller *et al.* (2015).

Conclusion

This review provides information about CNS-draining lymph nodes in both experimental animals and humans. New imaging agents can be developed to show the perivascular drainage pathway from the brain toward the lymph nodes. Neurological diseases can be treated with various agents that can be sent to nerve tissue from parotid and mandibular lymph nodes. In other words, it is foreseen that in the management of and protection from both AD and various brain diseases like multiple sclerosis, new treatments can be developed to facilitate the removal of $A\beta$ and Tau from the environment.

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Received: August 12, 2021

Accepted: August 25, 2021

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