Brain Fluid Channels for Metabolite Removal

Marcela MALOVESKÁ[1], Filip HUMENÍK[1], Zuzana VIKARTOVSKÁ[2], Nikola HUDÁKOVÁ[1], Viera ALMÁŠIOVÁ[3], Lenka KREŠÁKOVÁ[3], Daša ČÍŽKOVÁ[1,4]

[1]*Centre of Experimental and Clinical Regenerative Medicine, University of Veterinary Medicine and Pharmacy in Kosice, Slovak Republic*

[2]*University Veterinary Hospital, University of Veterinary Medicine and Pharmacy in Kosice, Slovak Republic*

[3]*Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Kosice, Slovak Republic*

[4]*Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

Corresponding author

Daša Čížková, Centre of Experimental and Clinical Regenerative Medicine, University of Veterinary Medicine and Pharmacy in Kosice, Komenskeho 73, 041 81 Kosice, Slovak Republic. e – mail: cizkova.dasa@gmail.com

Brain Fluid Channels for Metabolite Removal

Summary

The adult human brain represents only 2% of the body's total weight, however it is one of the most metabolically active organs in the mammalian body. Its high metabolic activity necessitates an efficacious waste clearance system. Besides the blood, there are two fluids closely linked to the brain and spinal cord drainage system: interstitial fluid (ISF) and cerebrospinal fluid (CSF).

The aim of this review is to summarize the latest research clarifying the channels of metabolite removal by fluids from brain tissue, subarachnoid space (SAS) and brain dura (BD). Special attention is focused on lymphatic vascular structures in the brain dura, their localizations within the meninges, morphological properties and topographic anatomy. The review ends with an account of the consequences of brain lymphatic drainage failure. Knowledge of the physiological state of the clearance system is crucial in order to understand the changes related to impaired brain drainage.

Key words

Brain ISF; CSF; Meningeal lymphatics; Lymphatic drainage

Introduction

The high metabolic activity of the brain requires effective and fast clearance mechanisms, not only for small molecules and hydrophobic compounds, but also for large molecules with low diffusion coefficients [1]. Tissue homeostasis relies on the removal of excess fluid and interstitial solutes, and neural tissue cells are extremely sensitive to changes in their environment [2]. Interstitial fluid and metabolic waste products accumulated in the body are drained into regional lymph nodes by means of well-defined lymphatic vessels. Lymphatic capillaries and vessels are present in most mammalian organs, but the central nervous system (CNS) has long been considered an organ without conventional lymphatic vasculature [3]. This theory was challenged after the discovery of the glymphatic system [4] and the confirmation of lymphatic vessels presence in the brain dura of mice [5, 6] as well as in humans [7]. So although there are no traditional lymphatic vessels in the CNS, there exist lymphatic drainage pathways which drain CSF and ISF into the extracranial (cervical) lymph nodes. In this review we explain the communication and drainage pattern of ISF and CSF and the association of CSF with the lymphatic system. Finally, the possible impact of brain lymphatic drainage failure on the incidence of neurodegenerative diseases is briefly mentioned.

Fluid in brain parenchyma - interstitial fluid

The brain ISF bathes and surrounds neurons and glial cells and provides an immediate environment for nutrient supply, waste removal and intercellular communication [8]. Water, ions, organic molecules such as proteins, peptides and enzymes, extracellular vesicles and glycoprotein chains are components of ISF [9]. There are few studies of ISF production, but sources are still being investigated [10, 11]. The central thesis about the place of ISF production focuses on the blood-brain barrier (BBB). Particularly the capillary-astrocyte complex of the BBB has been suggested as an active producer of brain ISF. Brasnjevic in his work thoroughly described the transport mechanisms at the BBB level: the paracellular aqueous pathway, transcellular lipophilic pathway, active carrier-mediated pathway, receptor–mediated endocytosis, adsorptive endocytosis and the efflux transport pathway [12]. The second possible source of ISF could be that water is produced by brain-cell metabolism, with ions crossing the endothelium [13]. The third source of ISF may involve part of it originating from CSF [10]. The CSF produced in *ventriculi cerebri* gets into the subarachnoid space (SAS) through communication openings (foramina of Luschka and Magendie), and a portion of CSF is able to flow back into the brain tissue along the space with brain perforating arteries. It is able to percolate through the brain parenchyma and mix with ISF [14]. The part of ISF created this way represents a fraction of CSF flow. The disadvantage of this source is that it is made up of recycled CSF, which includes various excretory materials from brain tissue with which it has come into contact [15].

Fluid in brain cavities and around CNS - cerebrospinal fluid

CSF is another part of the cerebral fluid integrated system. Its basic function is to absorb mechanical vibrations of the brain and spinal cord, to relieve the weight of the brain parenchyma and also to serve as a buffer for the brain [16]. CSF is distributed between the cerebral ventricles, central canal and SAS. According to the traditional view, the choroid plexuses are responsible for the great majority (90 %) of CSF production [17]. These plexuses are enlargements of the ependymal epithelium, lining the lateral, third and fourth ventricles [18]. There are tight junctions between the choroid plexus epithelial cells representing the blood – CSF barrier, and this type of connection is thought to be the primary determinant of CSF composition. We know that CSF is produced continuously, but there is relatively little information about the regulation of its production [19]. There is constant circulation between ventricles, cisterns, SAS and blood and therefore some authors refer to the flow of CSF as the "third circulation" [9, 20, 21].

Relationship between ISF and CSF in the brain

Because the sites of origin of ISF and CSF partially overlap, it is not possible to separate their flow paths. At this point it should be mentioned that CSF circulates in two cycles. In addition to macrocirculation (mentioned above) through the ventriculo–subarachnoid spaces to the blood (directly or indirectly by means of the lymphatic system), there is also limited microcirculation of CSF between the SAS and brain parenchyma [14, 22]. This CSF microcirculation is closely related to the brain blood vasculature, and is described as follows. Cerebral arteries change into pial arteries running in the SAS and subpial space [23]. Subsequently these brain-penetrating blood vessels issue from the subarachnoid space and the coat surrounding them delimits the space known as the Virchow-Robin space. CSF flows into this space (specifically along the arteries) and travels varying distances to the deeper brain tissue. As the penetrating arterioles branch into capillaries, the CSF-containing spaces narrow and finally disappear. At this level the perivascular space consists solely of basal lamina, which represents a negligible barrier to CSF input to the nervous tissue [19]. The blood in cerebral capillaries continues into the post-capillary venules, where the paravascular space securing the outflow pathway reappears [24]. According to this scheme, CSF flows into the paravascular space around the arteries, gathers in the brain parenchyma, becomes combined with ISF and parenchymal solutions and subsequently exits the neural tissue in paravascular spaces around the veins. This pathway, first described in 2012, was referred as the “glymphatic” system [4], consisting of the para-arterial influx and paravenous efflux of CSF [25] (Fig. 1). Studies have shown that this drainage system plays an important role in the removal of metabolic waste products secreted by neurons [26]. The similar CSF space also extends outward for varying distances in periradicular and perineural spaces along exiting cranial and spinal nerves [27].

While the pattern of CSF flow is presented uniformly, the process of ISF outflow is described in a variety of ways. Part of the ISF drains through the paravenous space of the cerebral veins (together with CSF). Previous works have described ISF outflow from the brain tissue directly in the walls of brain-penetrating capillaries and cerebral arteries [3, 28]. Thus ISF drains along basement membranes in the walls of cerebral capillaries and tunica media of the arteries and then through the vessel walls of the internal carotid artery in the neck [29, 30]. This ISF transport method is referred to as the intramural peri-arterial drainage (IPAD) system (Fig.1) [31, 32]. It is worth noting that veins themselves are not involved in the ISF drainage path. Details of CSF inflow along the Pial – Glial membrane and ISF outflow via the IPAD system are shown in Figure 2.

At this point, based on the claims of some authors, a distinction should be made between the terms “perivascular” and “paravascular” space [26, 30, 33]. The perivascular space / IPAD system, draining solutions and soluble particles from the brain interstitial system (occupied by ISF), is distinct from the paravascular space/ glymphatic system, which is primarily concerned with CSF drainage [11]. The question also arises as to whether the terms "Virchov – Robin space "and “perivascular space” are used correctly as synonyms. It is crucial to realize that German pathologist Rudolf Virchow and French anatomist Charles-Philippe Robin described this space around vessels in 1851 without the precise technical equipment offered by the 21st century. To this day, some authors do not distinguish between the names denoting these different spaces. In any case, significant controversy exists in the literature regarding this terminology.

ISF and CSF outflow

Two patterns have been proposed for ISF outflow. The first model involves direct ISF – CSF exchanges in two ways: i. into the SAS via the glymphatic system; ii. through ependymal cells present at the wall of the ventricle. The latter however is true only in the immediate vicinity of the ependymal lining, the effect decreasing away from the ventricles, and this journey ultimately also leads to the SAS [10, 34]. The second pattern of ISF outflow is via the IPAD system.

CSF outflow is described in the following ways. A portion of CSF drains back into the blood (venous system) via the cranial arachnoid granulations (CAG) and spinal arachnoid granulations (SAG) [35]. The CAG are mostly localized close to the points where the veins enter the sinuses (superior sagittal sinus), but they are also present in the convergent area of the transverse sinus and other venous sinuses [36]. This is the main site of CSF reabsorption. The other option involves connections between the olfactory nerves and nasal lymphatics. The *lamina cribrosa* of the ethmoid bone appears to be a very important anatomical structure for extra - arachnoidal cranial CSF clearance. The third drainage pathway for CSF outflow leads through the meningeal lymphatic vessels. While CSF transfer from the SAS via arachnoid granulation and the olfactory route are well-known processes, the discovery of lymphatic vasculature in the BD was confirmed only a few years ago. All three CSF outflow routes are illustrated in Figure 1.

Meningeal lymphatics

The *dura mater*, *arachnoidea*, *pia mater* and *glia limitans* together form a sophisticated set of barriers protecting the brain. Whereas the dura and arachnoid membrane are in direct contact, the pia is separated from the arachnoid by the subarachnoid space, filled with CSF and bridged by the *arachnoid trabeculae*, a dense network of connective filaments [37]. Beneath the *pia mater* begins the *glia limitans,* the brain parenchymal barrier formed by astrocytic endfeet processes [38]. The d*ura mater encephali* represents the critical site for CSF turnover and the main route for venous-CSF outflow, directly through the arachnoid granulations and indirectly by means of meningeal lymphatics. The existence of lymphatic vessels in the brain meninges was first described by Paolo Mascagni in the 18th century [39]. Despite the exceptional anatomical precision of Mascagni's wax human organ models, his claim that lymphatic vessels are present in the brain meninges was not accepted and than forgotten [40]. More than 200 years later, with the discovery of new techniques, Mascagni's original observations were confirmed by two independent researchers in the same year [5, 6]. The presence of lymphatic vascular structures in the BD was primarily described in mice, and subsequently their incidence was also reported in fish, rats, non-human primates and humans [7, 41, 42]. Because meningeal lymphatics do not enter the brain parenchyma, it is necessary to find out how they access parenchymal macromolecules, whereby the *arachnoid mater* is considered to be the barrier between the BD and cerebrospinal fluid–filled spaces [43]. Raper states that there are three possible localizations of lymphatic vessels within the meninges: (i.) protruding into the subarachnoid space, (ii.) located at the interface between the *dura mater* and *arachnoidea,* (iii.) located within the dura layers in close proximity to the venous sinuses (Fig. 3) [44]. In any case the study of this issue has confirmed that CSF is transported across the arachnoid membrane into the dura, but the unambiguous mechanism of transit is still not fully explained. In a study by Louveau, the unique character of endothelial cells in dural lymphatics was confirmed [45]. At this point, there are concerns about the morphological properties of meningeal lymphatic vessels, compared to those in the rest of the body. Lymphatic capillaries, also called initial lymphatic vessels (LVs), are thin-walled and blind-ended vessels formed by a monolayer of lymphatic endothelial cells (LECs), with an incoherent basement membrane, lack of pericytes and smooth muscle cells. The lymphatic capillaries drain into larger pre-collecting and then collecting LVs with a wider lumen, surrounded by contractile smooth muscle cells. Because these vessels have impermeable walls, they only provide passage for previously-absorbed fluids with particles, and they are equipped with intraluminal valves supporting unidirectional drainage [46, 47]. A detailed description of meningeal lymphatic vessels was presented by researchers back in 2015 [5, 6]. They state that these vessels are structurally similar to lymphatic

capillaries, with a spaced pattern of cell junction markers, a noncontiguous basement membrane, no smooth muscle cell lining, and a lack of lymphatic valves. The results of recent studies indicate that the morphological differences in meningeal lymphatic vessels depend on their topography in the skull. The dorsal meningeal lymphatics are mostly formed of a continuously-sealed zipper-like junctional pattern of LECs, whereas the basal meningeal lymphatics consist in principle of a discontinuously-sealed button-like junctional pattern, similar to that of initial lymphatic vessels in the peripheral organs. It is suggested that basal meningeal lymphatics are intended for taking up CSF macromolecules [48, 49]. Another point of interest is the topographic anatomy of meningeal lymphatics. Detailed description of this anatomy in rodents is presented in a review of studies led by Hershenhouse [50]. In the anterior cranium, lymphatics are found in the BD covering the olfactory bulb next to the rostral rhinal veins. Meningeal LVs are also associated with the anterior and middle meningeal arteries. In the posterior and inferior cranium, lymphatics appear with the highest density, forming an extensive network. Lymphatic vessels are found along the sigmoid sinus and the transverse sinus in the area of confluence of the sinuses. Vessels associated with the superior sagittal sinus continue rostrally. Meningeal LVs situated beside the transverse sinus are found to be larger and more complex compared with the vessels surrounding the superior sagittal sinus. They are also seen in the BD overlying the cerebellum as lymphatic vessels accompanying the branches of the transverse sinus [51]. Some authors describe the presence of lymphatic vessels in the *dural septae* entering into deeper parts of the brain tissue [52]. Lymphatic vessels leave the cranial cavity in the sheaths of cranial nerves (CN), namely the olfactory (CN I), optic (CN II), trigeminal (CN V), glossopharyngeal (CN IX), vagus (CN X) and accessory nerves (CN XI), whereby the olfactory nerve associated with lymphatics passing through the cribriform plate has special status [53]. A widely-cited review reports links between CSF and lymphatic vessels in various mammalian species [54]. Human studies (*in vivo* using MRI, and also autopsies) of meningeal lymphatics have revealed quite similar morphology and topography to the murine. Of course they differ in lumen. LVs contained within the human BD have wider diameters, ranging from 7 to 842 μm compared to mice with diameters of 20 to 30 μm [50]. The results of a study examining the direction of lymphatic flow in the meningeal LVs running alongside the superior sagittal sinus in six healthy human volunteers are worth noting. They were subjected to high-resolution MRI, which revealed that lymphatic flow was posterior to anterior, countercurrent to the direction of venous flow in the superior sagittal sinus toward the cribriform plate. These findings on flow directionality differ from those seen in a murine model [55].

Following confirmation of the existence of meningeal lymphatic vessels, an answer is sought as to which of the CSF outflow pathways is dominant: the cranial arachnoid granulation pathway, first observed by Pacchioni as early as 1721 [56], together with the spinal arachnoid granulation pathway, discovered much later in 1923 [36], or the lymphatic system pathway. Complete volumetric analysis of CSF outflow is described in the review published by Chen in 2015 [35]. We have also dealt with this issue in our workplace, when we monitored the dynamics of evans blue dye clearance from CSF (*cisterna magna*) into meningeal lymphatics vessels and deep cervical lymph nodes in rats. Our data confirmed the meningeal lymphatics function downstream in the CSF system towards corresponding extracranial structures [57]. In any case, owing to the ongoing development of increasingly effective imaging techniques, recent static and dynamic imaging studies clearly show that the predominant route of CSF outflow is via meningeal LVs [48, 58, 59].

Failure of brain lymphatic drainage

Knowing that the CNS has its own lymphatic drainage system for removing metabolic waste products and macromolecules, researchers are currently looking into the link between outflow abnormalities and the development of neurodegenerative pathologies. In the framework of neurological diseases characterized by abnormal protein aggregation in the brain parenchyma, such as Alzheimer's disease and Parkinson's disease, modulation of the meningeal lymphatics have been shown to impact disease physiopathology [60]. The question arises as to whether diseases such as Amyotrophic lateral sclerosis, Huntington's disease, Dementia with Lewy bodies, Multiple system atrophy or Frontotemporal dementia are also related to the failure of the cleansing mechanisms of nervous tissue. In his research Jaffe simulated the conditions of dysfunction of the meningeal lymph nodes (pharmacologically by injection and photo-conversion of Visudyne, or surgically by ligation of vessels afferent to the cervical lymph nodes), and found that it resulted in a significant reduction of CSF drainage [61]. Many neurological disorders have a common attribute, namely ageing [62]. Just as ageing is associated with dysfunction of the peripheral lymphatic system, the impact of senescence on the function of the meningeal lymphatic vessels is also expected [63]. In a recently-published review dealing with lymphatic senescence, a team led by Filelfi summarized the available information on how ageing leads to changes in the structure and function of lymphatic vessels in various organs, including the nervous tissue and BD (e.g. through decreased meningeal lymphatic vessel diameter). Reduced inflow of paravascular cerebrospinal fluid and reduced outflow of interstitial fluid are linked to the dysfunction of the meningeal lymphatic vessels and

their flow alone, which is also induced by age [64]. This study concludes that ageing-related deterioration affects lymphatic drainage of neural tissue at both levels (glymphatic and lymphatic vascular systems).

In addition, over the last two years studies have appeared regarding COVID - 19 and its possible relation to glymphatic - lymphatic drainage disorders [65, 66].

Conclusion

In this review we have presented the existing body of evidence regarding the communication channels between ISF and CSF at brain parenchyma level, as well as the communication between CSF and the lymphatic system. We have briefly described their sources and overlapping flow paths, as well as the ISF/CSF outflow scheme. Another part of the review is devoted to meningeal lymphatics, their localizations within the meninges, their morphological properties and topographic anatomy. In conclusion, although research into the brain cleansing system has a long history, advanced methods and technology in neuroscience research will hopefully deal with new challenges and subsequently provide some long-awaited answers.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by grants APVV-19-0193, VEGA 1/0376/20 and VEGA 1/0285/22.

The list of abbreviations

BBB - blood-brain barrier; BD - brain dura; CAG - cranial arachnoid granulations; CN – cranial nerve; CNS – central nervous system; COVID -19 - Coronavirus disease 2019; CSF – cerebrospinal fluid; IPAD – intramural peri-arterial drainage; ISF - interstitial fluid; LECs – lymphatic endothelial cells; LVs - lymphatic vessels; MRI - magnetic resonance imaging; SAG - spinal arachnoid granulations; SAS - subarachnoid space

References

1. Syková E, Nicholson C. Diffusion in Brain Extracellular Space. Physiol Rev. 2008;88(4):1277-1340. doi:10.1152/physrev.00027.2007

2. Bradbury MW. Physiopathology of the blood-brain barrier. Adv Exp Med Biol. 1976;69:507-516. doi:10.1007/978-1-4684-3264-0_37

3. Weller RO, Djuanda E, Yow HY, Carare RO. Lymphatic drainage of the brain and the pathophysiology of neurological disease. Acta Neuropathol (Berl). 2009;117(1):1-14. doi:10.1007/s00401-008-0457-0

4. Iliff JJ, Wang M, Liao Y, et al. A Paravascular Pathway Facilitates CSF Flow Through the Brain Parenchyma and the Clearance of Interstitial Solutes, Including Amyloid β. Sci Transl Med. 2012;4(147):147ra111. doi:10.1126/scitranslmed.3003748

5. Aspelund A, Antila S, Proulx ST, et al. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med. 2015;212(7):991-999. doi:10.1084/jem.20142290

6. Louveau A, Smirnov I, Keyes TJ, et al. Structural and functional features of central nervous system lymphatics. Nature. 2015;523(7560):337-341. doi:10.1038/nature14432

7. Absinta M, Ha SK, Nair G, et al. Human and nonhuman primate meninges harbor lymphatic vessels that can be visualized noninvasively by MRI. eLife. 2017;6. doi:10.7554/eLife.29738

8. Howell MD, Gottschall PE. Lectican proteoglycans, their cleaving metalloproteinases, and plasticity in the central nervous system extracellular microenvironment. Neuroscience. 2012;217:6-18. doi:10.1016/j.neuroscience.2012.05.034

9. Brinker T, Stopa E, Morrison J, Klinge P. A new look at cerebrospinal fluid circulation. Fluids Barriers CNS. 2014;11:10. doi:10.1186/2045-8118-11-10

10. Lei Y, Han H, Yuan F, Javeed A, Zhao Y. The brain interstitial system: Anatomy, modeling, in vivo measurement, and applications. Prog Neurobiol. 2017;157:230-246. doi:10.1016/j.pneurobio.2015.12.007

11. Shetty AK, Zanirati G. The Interstitial System of the Brain in Health and Disease. Aging Dis. 2020;11(1):200-211. doi:10.14336/AD.2020.0103

12. Brasnjevic I, Steinbusch HWM, Schmitz C, Martinez-Martinez P, European NanoBioPharmaceutics Research Initiative. Delivery of peptide and protein drugs over the blood-brain barrier. Prog Neurobiol. 2009;87(4):212-251. doi:10.1016/j.pneurobio.2008.12.002

13. Abbott NJ. Evidence for bulk flow of brain interstitial fluid: significance for physiology and pathology. Neurochem Int. 2004;45(4):545-552. doi:10.1016/j.neuint.2003.11.006

14. Rennels ML, Gregory TF, Blaumanis OR, Fujimoto K, Grady PA. Evidence for a 'Paravascular' fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space. Brain Res. 1985;326(1):47-63. doi:10.1016/0006-8993(85)91383-6

15. Khasawneh AH, Garling RJ, Harris CA. Cerebrospinal fluid circulation: What do we know and how do we know it? Brain Circ. 2018;4(1):14-18. doi:10.4103/bc.bc_3_18

16. Brodbelt MA, Stoodley M. CSF pathways: a review. Br J Neurosurg. 2007;21(5):510-520. doi:10.1080/02688690701447420

17. Oreskovic D, Klarica M. The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations. Brain Res Rev. 2010;64(2):241-262. doi:10.1016/j.brainresrev.2010.04.006

18. Damkier HH, Brown PD, Praetorius J. Cerebrospinal fluid secretion by the choroid plexus. Physiol Rev. 2013;93(4):1847-1892. doi:10.1152/physrev.00004.2013

19. Jessen NA, Munk ASF, Lundgaard I, Nedergaard M. The Glymphatic System: A Beginner's Guide. Neurochem Res. 2015;40(12):2583-2599. doi:10.1007/s11064-015-1581-6

20. Barshes N, Demopoulos A, Engelhard HH. Anatomy and physiology of the leptomeninges and CSF space. Cancer Treat Res. 2005;125:1-16. doi:10.1007/0-387-24199-x_1

21. Milhorat TH. The third circulation revisited. J Neurosurg. 1975;42(6):628-645. doi:10.3171/jns.1975.42.6.0628

22. Proescholdt MG, Hutto B, Brady LS, Herkenham M. Studies of cerebrospinal fluid flow and penetration into brain following lateral ventricle and cisterna magna injections of the tracer [14C]inulin in rat. Neuroscience. 2000;95(2):577-592. doi:10.1016/s0306-4522(99)00417-0

23. Zhang ET, Inman CB, Weller RO. Interrelationships of the pia mater and the perivascular (Virchow-Robin) spaces in the human cerebrum. J Anat. 1990;170:111-123.

24. Engelhardt B, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. Trends Immunol. 2012;33(12):579-589. doi:10.1016/j.it.2012.07.004

25. Natale G, Limanaqi F, Busceti CL, et al. Glymphatic System as a Gateway to Connect Neurodegeneration From Periphery to CNS. Front Neurosci. 2021;15. doi:10.3389/fnins.2021.639140

26. Bacyinski A, Xu M, Wang W, Hu J. The Paravascular Pathway for Brain Waste Clearance: Current Understanding, Significance and Controversy. Front Neuroanat. 2017;11:101. doi:10.3389/fnana.2017.00101

27. Proulx ST. Cerebrospinal fluid outflow: a review of the historical and contemporary evidence for arachnoid villi, perineural routes, and dural lymphatics. Cell Mol Life Sci. 2021;78(6):2429-2457. doi:10.1007/s00018-020-03706-5

28. Carare RO, Bernardes-Silva M, Newman TA, et al. Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. Neuropathol Appl Neurobiol. 2008;34(2):131-144. doi:10.1111/j.1365-2990.2007.00926.x

29. Morris AWJ, Sharp MM, Albargothy NJ, et al. Vascular basement membranes as pathways for the passage of fluid into and out of the brain. Acta Neuropathol (Berl). 2016;131:725-736. doi:10.1007/s00401-016-1555-z

30. Ueno M, Chiba Y, Murakami R, Matsumoto K, Kawauchi M, Fujihara R. Blood-brain barrier and blood-cerebrospinal fluid barrier in normal and pathological conditions. Brain Tumor Pathol. 2016;33(2):89-96. doi:10.1007/s10014-016-0255-7

31. Albargothy NJ, Johnston DA, MacGregor-Sharp M, et al. Convective influx/glymphatic system: tracers injected into the CSF enter and leave the brain along separate periarterial basement membrane pathways. Acta Neuropathol (Berl). 2018;136(1):139-152. doi:10.1007/s00401-018-1862-7

32. Carare RO, Aldea R, Agarwal N, et al. Clearance of interstitial fluid (ISF) and CSF (CLIC) group—part of Vascular Professional Interest Area (PIA). Alzheimers Dement Diagn Assess Dis Monit. 2020;12(1):e12053. doi:10.1002/dad2.12053

33. Bakker ENTP, Bacskai BJ, Arbel-Ornath M, et al. Lymphatic Clearance of the Brain: Perivascular, Paravascular and Significance for Neurodegenerative Diseases. Cell Mol Neurobiol. 2016;36(2):181-194. doi:10.1007/s10571-015-0273-8

34. Bedussi B, van Lier MGJTB, Bartstra JW, et al. Clearance from the mouse brain by convection of interstitial fluid towards the ventricular system. Fluids Barriers CNS. 2015;12:23. doi:10.1186/s12987-015-0019-5

35. Chen L, Elias G, Yostos MP, Stimec B, Fasel J, Murphy K. Pathways of cerebrospinal fluid outflow: a deeper understanding of resorption. Neuroradiology. 2015;57(2):139-147. doi:10.1007/s00234-014-1461-9

36. Pollay M. The function and structure of the cerebrospinal fluid outflow system. Cerebrospinal Fluid Res. 2010;7:9. doi:10.1186/1743-8454-7-9

37. Papadopoulos Z, Herz J, Kipnis J. Meningeal Lymphatics: From Anatomy to Central Nervous System Immune Surveillance. J Immunol. 2020;204(2):286-293. doi:10.4049/jimmunol.1900838

38. Quintana FJ. Astrocytes to the rescue! Glia limitans astrocytic endfeet control CNS inflammation. J Clin Invest. 2017;127(8):2897-2899. doi:10.1172/JCI95769

39. Bucchieri F, Farina F, Zummo G, Cappello F. Lymphatic vessels of the dura mater: a new discovery? J Anat. 2015;227(5):702-703. doi:10.1111/joa.12381

40. Lukić IK, Glunčić V, Ivkić G, Hubenstorf M, Marušić A. Virtual dissection: a lesson from the 18th century. The Lancet. 2003;362(9401):2110-2113. doi:10.1016/S0140-6736(03)15114-8

41. Bower NI, Koltowska K, Pichol-Thievend C, et al. Mural lymphatic endothelial cells regulate meningeal angiogenesis in the zebrafish. Nat Neurosci. 2017;20(6):774-783. doi:10.1038/nn.4558

42. Jung E, Gardner D, Choi D, et al. Development and Characterization of A Novel Prox1-EGFP Lymphatic and Schlemm's Canal Reporter Rat. Sci Rep. 2017;7. doi:10.1038/s41598-017-06031-3

43. Rua R, McGavern DB. Advances in Meningeal Immunity. Trends Mol Med. 2018;24(6):542-559. doi:10.1016/j.molmed.2018.04.003

44. Raper D, Louveau A, Kipnis J. How do meningeal lymphatic vessels drain the CNS? Trends Neurosci. 2016;39(9):581-586. doi:10.1016/j.tins.2016.07.001

45. Louveau A, Herz J, Alme MN, et al. CNS lymphatic drainage and neuroinflammation are regulated by meningeal lymphatic vasculature. Nat Neurosci. 2018;21(10):1380-1391. doi:10.1038/s41593-018-0227-9

46. Alderfer L, Wei A, Hanjaya-Putra D. Lymphatic Tissue Engineering and Regeneration. J Biol Eng. 2018;12(1):32. doi:10.1186/s13036-018-0122-7

47. Petrova TV, Koh GY. Biological functions of lymphatic vessels. Science. 2020;369(6500):eaax4063. doi:10.1126/science.aax4063

48. Ahn JH, Cho H, Kim JH, et al. Meningeal lymphatic vessels at the skull base drain cerebrospinal fluid. Nature. 2019;572(7767):62-66. doi:10.1038/s41586-019-1419-5

49. Cheng Y, Wang YJ. Meningeal Lymphatic Vessels: A Drain of the Brain Involved in Neurodegeneration? Neurosci Bull. 2020;36(5):557-560. doi:10.1007/s12264-019-00456-8

50. Hershenhouse KS, Shauly O, Gould DJ, Patel KM. Meningeal Lymphatics: A Review and Future Directions From a Clinical Perspective. Neurosci Insights. 2019;14:1179069519889027. doi:10.1177/1179069519889027

51. Antila S, Karaman S, Nurmi H, et al. Development and plasticity of meningeal lymphatic vessels. J Exp Med. 2017;214(12):3645-3667. doi:10.1084/jem.20170391

52. Lohrberg M, Wilting J. The lymphatic vascular system of the mouse head. Cell Tissue Res. 2016;366(3):667-677. doi:10.1007/s00441-016-2493-8

53. Ma Q, Schlegel F, Bachmann SB, et al. Lymphatic outflow of cerebrospinal fluid is reduced in glioma. Sci Rep. 2019;9:14815. doi:10.1038/s41598-019-51373-9

54. Koh L, Zakharov A, Johnston M. Integration of the subarachnoid space and lymphatics: is it time to embrace a new concept of cerebrospinal fluid absorption? Cerebrospinal Fluid Res. 2005;2:6. doi:10.1186/1743-8454-2-6

55. Kuo PH, Stuehm C, Squire S, Johnson K. Meningeal Lymphatic Vessel Flow Runs Countercurrent to Venous Flow in the Superior Sagittal Sinus of the Human Brain. Tomogr Ann Arbor Mich. 2018;4(3):99-104. doi:10.18383/j.tom.2018.00013

56. Brunori A, Vagnozzi R, Giuffrè R. Antonio Pacchioni (1665-1726): early studies of the dura mater. J Neurosurg. 1993;78(3):515-518. doi:10.3171/jns.1993.78.3.0515

57. Maloveska M, Danko J, Petrovova E, et al. Dynamics of Evans blue clearance from cerebrospinal fluid into meningeal lymphatic vessels and deep cervical lymph nodes. Neurol Res. 2018;40(5):372-380. doi:10.1080/01616412.2018.1446282

58. Kutomi O, Takeda S. Identification of lymphatic endothelium in cranial arachnoid granulation-like dural gap. Microsc Oxf Engl. 2020;69(6):391-400. doi:10.1093/jmicro/dfaa038

59. Pal S, Rao S, Louveau A. Meningeal lymphatic network: The middleman of neuroinflammation. Clin Exp Neuroimmunol. 2020;11(1):21-25. doi:10.1111/cen3.12563

60. Frederick N, Louveau A. Meningeal lymphatics, immunity and neuroinflammation. Curr Opin Neurobiol. 2020;62:41-47. doi:10.1016/j.conb.2019.11.010

61. Jaffe RJ, Dave RS, Byrareddy SN. Meningeal lymphatics in aging and Alzheimer's disease. Ann Transl Med. 2019;7(Suppl 1):S2. doi:10.21037/atm.2019.01.06

62. Da Mesquita S, Louveau A, Vaccari A, et al. Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease. Nature. 2018;560(7717):185-191. doi:10.1038/s41586-018-0368-8

63. Jakic B, Kerjaschki D, Wick G. Lymphatic Capillaries in Aging. Gerontology. 2020;66(5):419-426. doi:10.1159/000508459

64. Filelfi SL, Onorato A, Brix B, Goswami N. Lymphatic Senescence: Current Updates and Perspectives. Biology. 2021;10(4):293. doi:10.3390/biology10040293

65. Bostancıklıoğlu M. SARS-CoV2 entry and spread in the lymphatic drainage system of the brain. Brain Behav Immun. 2020;87:122-123. doi:10.1016/j.bbi.2020.04.080

66. Wostyn P. COVID-19 and chronic fatigue syndrome: Is the worst yet to come? Med Hypotheses. 2021;146:110469. doi:10.1016/j.mehy.2020.110469

FIGURES

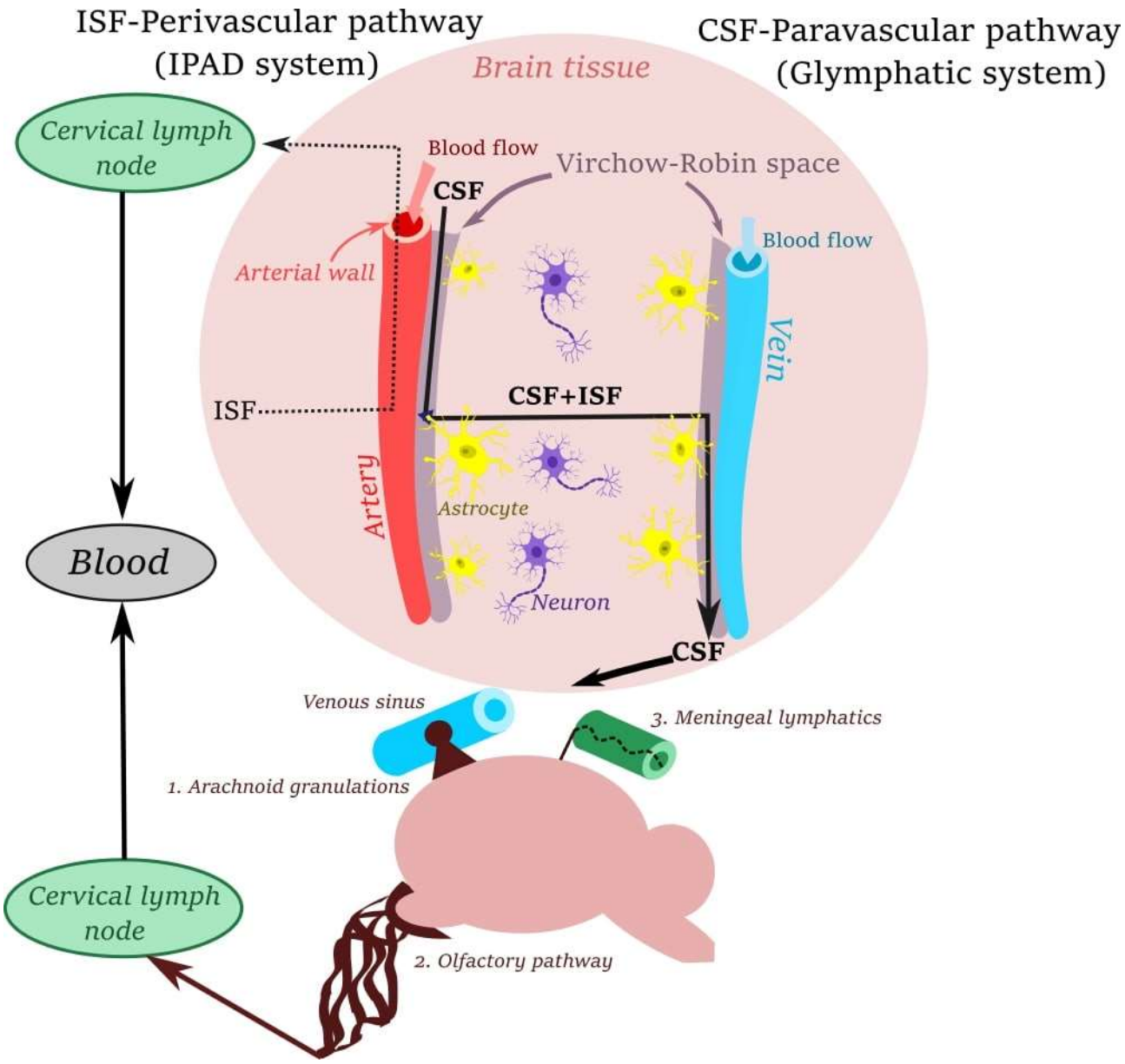

Fig. 1 The ISF and CSF flow paths in brain tissue (top of the image) and routs of CSF outflow from SAS (bottom of the image)

ISF - perivascular drainage channel /IPAD system (shown by dotted lines) – elimination of ISF along the basement membrane of brain penetrating capillaries and arteries

CSF - paravascular drainage channel/ glymphatic system (shown by thick lines) - CSF flows into the paravascular space around arteries, is combined with ISF and parenchymal solutions in brain parenchyma and subsequently it exits the brain in paravascular space around veins

CSF outflow from SAS via: 1. the arachnoid granulations of the dural sinus; 2. connections between the olfactory nerves and nasal lymphatics; 3. by means of the meningeal lymphatic vessels.

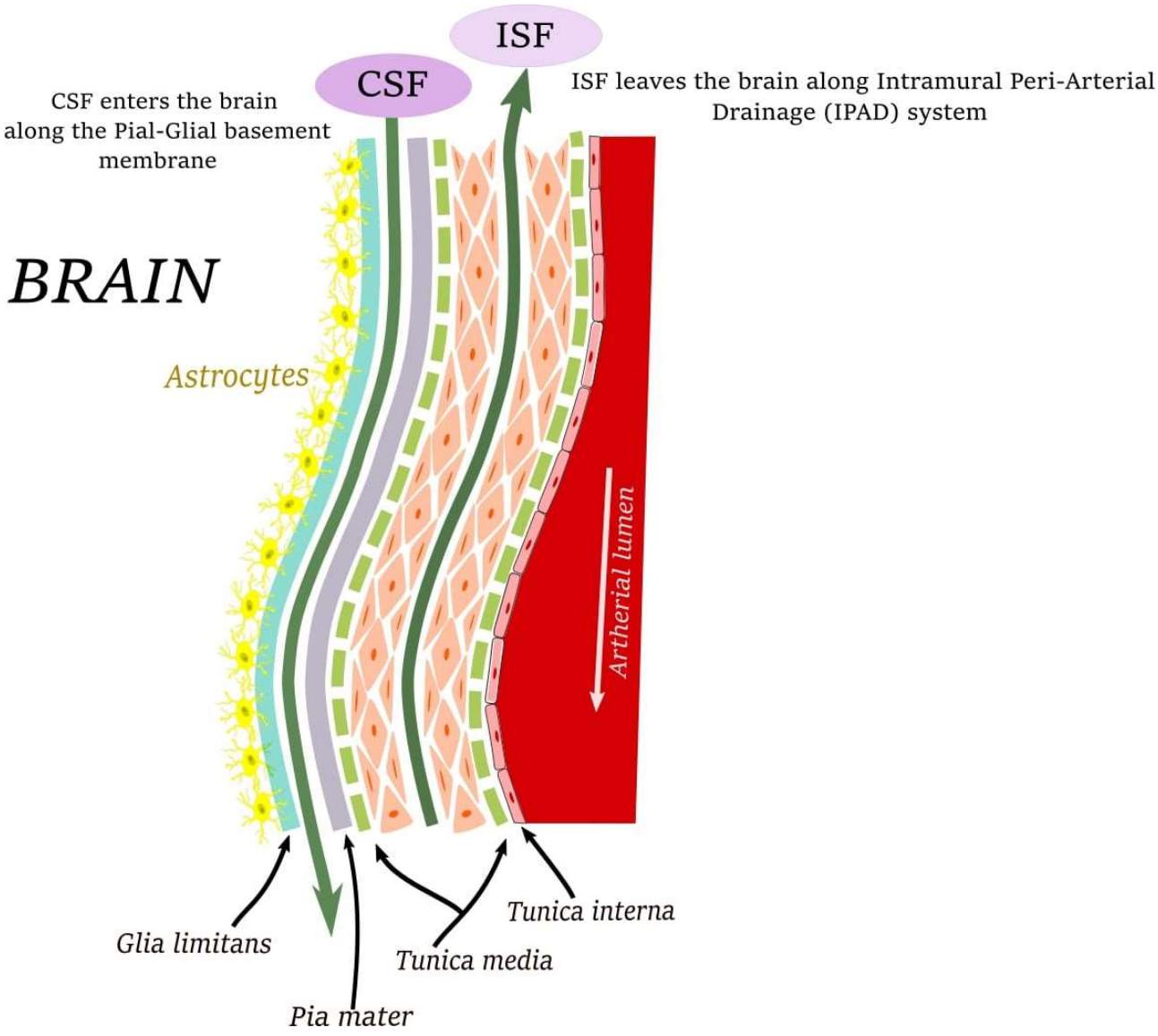

Fig. 2 Detail of CSF inflow along the Pial – Glial basement membrane and ISF outflow via Intramural peri-arterial drainage (IPAD) system.

CSF enters the brain between the pia mater and glia limitans. ISF and solutes pass out the brain along basement membranes surrounding smooth muscle cells in the tunica media of arteries, what is referred to as the Intra mural Peri - Arterial Drainage (IPAD) system.

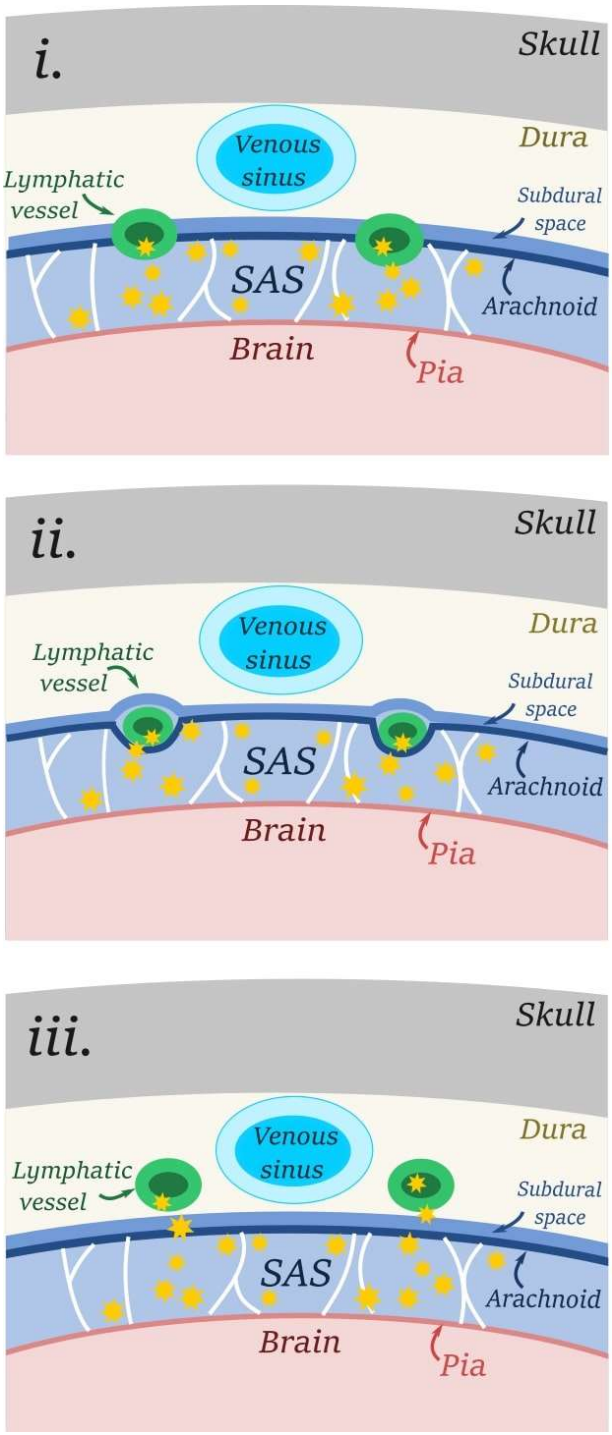

Fig. 3 Possibility of meningeal lymphatic vessels localization within the subarachnoid space The meningeal lymphatic vessels are located within the subarachnoid space: (i.) they are directly "bathed" by CSF, therefore the products of brain metabolism (yellow stars) could easily diffuse into the meningeal lymphatic vasculature, (ii.) the lymphatic vessels are located at the interface between the brain dura and arachnoid layer, (iii.) they are positioned directly inside in brain dura. The figure is modified image of Raper [44].