Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 3818; doi:10.3390/ijms19123818

Review

Blood-brain barrier, lymphatic clearance and recovery: Ariadne's thread in labyrinths of hypothesis

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Abstract: The peripheral lymphatic system plays a crucial role in the recovery mechanisms after many pathological changes, such as infection, trauma, vascular, or metabolic diseases. The lymphatic clearance of different tissues from waste products, viruses, bacteria and toxic proteins significantly contributes to the correspondent recovery processes. However, understanding of the meningeal lymphatics functions is a challenging problem. The exploration of mechanisms of lymphatic communication with brain fluids as well as the role of the lymphatic system in the brain drainage, clearance and recovery are still in its infancy. Here we review novel concepts on the anatomy and physiology of the lymphatics in the brain, which warrant a substantial revision of our knowledge about the role of lymphatics in the rehabilitation of the brain functions after neural pathologies. We discuss a new vision on how to recruit the meningeal lymphatics by the opening of blood-brain barrier as a trigger mechanism of activation of the meningeal lymphatic drainage. This leads to innovative strategies in neurorehabilitation therapy.

Keywords: peripheral and meningeal lymphatics 1; blood-brain barrier 2; neurorehabilitation 3.

1. Interactions between the activation of the brain lymphatic clearance and recovery after changes in extracellular environment

The brain has only 2 % of the total body weight. However, the energy metabolism of the brain is extremely high. Indeed, the brain tissues utilize around 20 % of the total oxygen and the total glucose consumption. A deficit of oxygen causes loss of consciousness within few seconds and the death of neurons after about 5 minutes. The largest portion of the brain energy is used to maintain the transport of ions across the membrane involved in formation and conduction of nervous impulses.

The high metabolic rate of the brain needs a rapid clearance of metabolic products because neurons and glial cells are very sensitive to changes in their extracellular homeostasis. But these changes are a dynamic process depending on various factors. In particular, blood-brain barrier (BBB) keeps the extracellular environment in the brain strongly controlling the passage of different molecules from the blood into the brain tissues and back. However, there are many chronic diseases (dementia [1], pain [2], hypertension [3], rheumatoid arthritis [4], diabetes [5]) that we can have throughout life and that can be accompanied by an increase in the BBB permeability with significant but reversible changes of the extracellular environment in the brain. The BBB opening by the different approaches such as focused ultrasound [6-8], audible sound [9], photodynamic effects [10-12] etc., are accompanied by the BBB closing and rapid recovery of the brain tissues after a while. This means that

the brain has compensatory mechanisms underlying its recovery after changes in the extracellular environment. But, the explicit mechanisms underlying these processes are still far for being clear.

Over the last few decades the important role of lymphatics in the brain clearance and recovery has been actively debated [13-45]. The literature is filled with novel findings of a physiological relationship between the brain barriers and drainage of the brain fluids via the meningeal and extracranial lymphatics.

So, the development of vascular catastrophes, such as stroke, is accompanied by a critical disbalance in the extracellular homeostasis that activates the brain clearance from toxic blood products via the cervical lymphatics [41]. Patients suffering from brain hemorrhages demonstrated a high level of iron in the deep cervical lymph node (dcLNs) in comparison to a control group without brain hemorrhages, which supports the hypothesis about the brain clearance from the blood via the lymphatic system [41].

The BBB opening is accompanied by a significant extracellular disbalance that rapidly activates brain clearing processes [44, 45]. Indeed, in our studies on rodents we clearly showed that in 20 min after the BBB opening different tracers (dextran 70 kDa, Evans Blue, gold nanorods 92 nm), which crossed the opened BBB, are removed from the brain via the meningeal lymphatics and are drained into the dcLN [44, 45] (See section 5). The crucial role of the lymphatics in keeping the extracellular homeostasis in the brain are supported by many facts. The blockade of nasal lymphatics due to inflammation could lead to a viral invasion and infection of the brain [46]. After the obstruction of the cribriform plate on the nasal size an elevation of intracranial pressure is observed that is also found after chronical ligation of the cervical lymphatics [47-49]. Similarity, the blockade of cervical lymphatics aggravates the severity of brain edema after stroke [50]. The surgical removal of the dcLN leads to a cognitive impairment in mice [51] and necrotic changes in neurons in rabbits [52].

Thus, there are growing number of evidences suggesting about the crucial role of the lymphatic vascular system in the brain clearance and recovery after changes in the extracellular environment. However, studies of mechanisms of the lymphatic communication with brain fluids as well as the role of the lymphatic system in the brain drainage, clearance and recovery are still in its infancy. But, now is coming the new gold era for “elusive” lymphatics when considering novel conceptions about anatomy and physiology of the lymphatic vascular system in the brain will be discovered due to progress in neurophotonics and accumulation of experimental data. This warrants a revision our old knowledge about the role of lymphatics in the brain functions.

2. Where and how lymph is generated in the brain?

The lymphatic system is part of the vascular system that carries a clear fluid called lymph (from Latin, lymph meaning "water"). During day approximately 20 liters of the plasma is filtered through vascular endothelium of capillaries in the extracellular space in ϕ human and 17 liters of the filtered plasma are reabsorbed back into the venous vessels, while 3 liters remain in the extracellular or interstitial fluid (ISF). The lymphatic system is not a closed system and the lymphatic vessels are opened in the extracellular scape that provide returning of 3 liters of ISF to the blood.

Thus, in peripheral tissues ISF is produced by filtration from plasma into extracellular space across the semipermeable vascular endothelium and then ISF is filtered back into the lymphatics, which returns it into the venous system. This means that the peripheral lymph is a fraction of ISF.

The situation with the brain is different due to the BBB restriction of fluid filtration from plasma into the brain. Also, the skull is incompressible, and the volume of brain fluid inside the skull is fixed.

There are several hypotheses explaining possible sources of the lymph in the brain. They explain the lymph generation as a source of ISF that is bathed neurons and glial cells or/and a source of cerebral spinal fluid (CSF).

However, in neurophysiology there is no well accepted opinion about the ISF formation in the brain. There are three discussed models [27]: i) first model, ISF is produced by brain metabolism [53]. For example, the oxidation of glucose to carbon dioxide with generation of water. But, the volume of metabolic ISF is too small (28 nlg-1 min-1around 10% of total volume of ISF that is 280 ml); ii) second model, the generation of ISF is the cerebral capillary secretion of solutes, which are moved passively

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through the endothelial cell membranes or via the tight junctions into the connective system of perivascular space from the side of arteries/arterioles to veins/venules around neuropil and axon tracts (Figure 1) [27]; iii) third model explains ISF as a fraction of recycled CSF, which flows from the choroid plexus into the subarachnoid space and then into PVS where CSF are merged with ISF generated by cerebral capillaries [27].

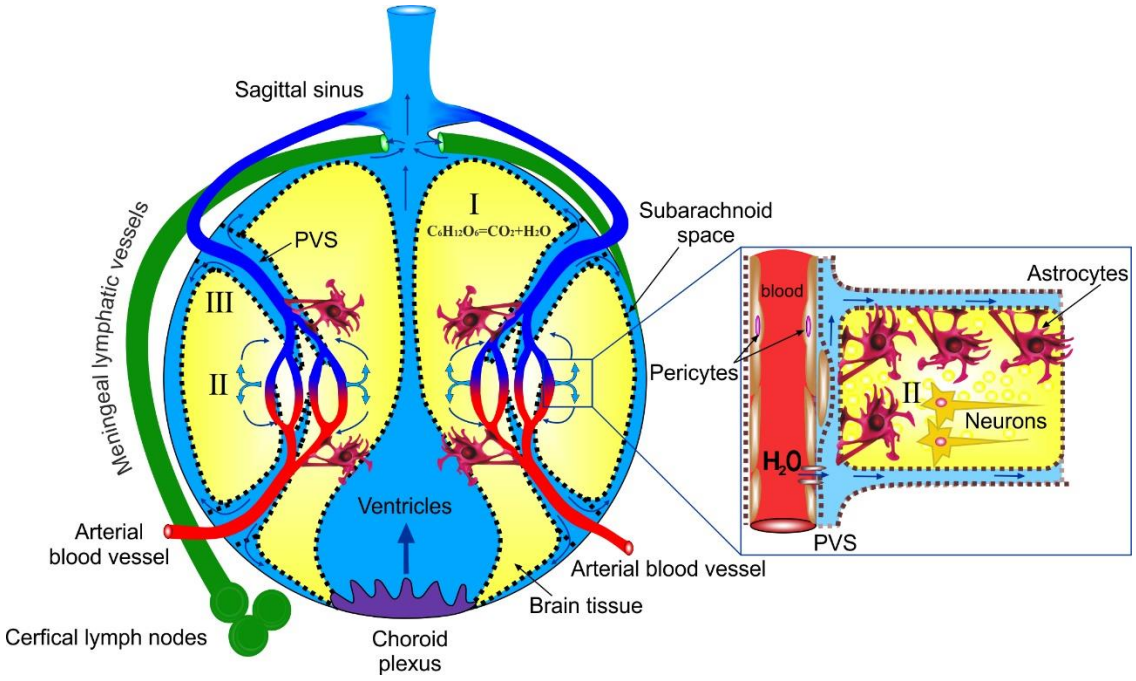

Figure 1. Schematic illustration of three models of generation and pathways of ISF in the brain. Model I explains the formation of ISF (10% of total volume of ISF) as a result of metabolic oxidation of glucose to carbon dioxide and water; Model II explains the generation of ISF as a large fraction of cerebral capillary secretion of solutes, which are driven passively by the ionic gradient through the endothelial cell membranes or via the tight junctions of BBB and formed PVS around penetrating arteries, venules and veins, and connecting with glia-lines boundaries between neuropil and regions of axon tracts; Model III explains ISF as a fraction of recycled CSF, which flows from the choroid plexus into the subarachnoid space and then into PVS where CSF are merged with ISF generated by cerebral capillaries.

It is interesting to note that sea animals (cuttlefish, mollusks, octopus, squids) who have no ventricles and CSF, have ISF with a similar flow rate of 0.1 μl g-1 min-1 as mammalians (rats, rabbits) [54]. This fact allows us to assume that capillary secretion of solutes might be a major part of ISF. However, this hypothesis needs more confirmations.

Taking together, these facts suggest that the most likely source of the lymph in the brain is two mixed components of brain fluids such as ISF and recycled CSF. However, it needs further detailed studies of the lymph formation from fraction of brain fluids such as ISF and CSF.

3. The gaps in the lymphatic anatomy in the brain

We first learnt about the lymphatics in the meninges through experiments of the Italian anatomist Paulo Mascagni who described network of transparent vessels in the layers of the brain already in the 18th century [43, 55, 56]. He also developed new technique for the meningeal lymphatic visualization using intracisternal injection of mercury. Today Mascagni's models of the meningeal lymphatic vessels are presented in the Josephinum Wax Models Museum in Vienna [56]. However, Mascagni's discovery of the meningeal lymphatics was forgotten for two centuries because no one

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could repeat his experiments due to technical problems. He was even regarded by several scientists as utopian: "Mascagni is so impressed with the lymphatics that he sees the lymph vessels everywhere even where they did not exist, i.e. in the brain". Therefore, we had the dogma about the lack of lymphatic circulation in the brain despite the growing literature including qualitative and quantitative evidences supporting an integration between the brain fluids and lymphatics. However, throughout the 19th century several groups demonstrated functional connection between CBF and the lymphatics of the head. Novadays due to progress in optical techniques Mascagni's results were confirmed in two independent works of Aspelung et al. [33] and Louveau et al. [34]. In these studies, it was clearly demonstrated that the meningeal lymphatics express all of the molecular hallmarks of lymphatic endothelium (Table 1). Afterward in other studies the maturation of the meningeal lymphatics [57] and its role in the brain drainage have been shown [44, 45].

Table 1. The molecular hallmarks of the lymphatic endothelium.

LYVE-1 - Lymphatic vessel endothelial hyaluronan receptor 1	Hyaluronan (HA) is an element of skin and mesenchymal tissues that regulates cell migration in the course of wound healing, inflammation and embryonic morphogenesis [58]. LYVE-1 is a specific transmembrane receptor of HA, which was first described by Banerji in 1999 [59]. LYVE-1 was found primary on both the luminal and abluminal surface of lymphatic endothelial cells [59, 60]. The functional role of LYVE-1 is still the subject of debate, but there are evidences that LYVE-1 plays an important role in hyaluronan transport and turnover, or in hyaluronan localization to the surface of lymphatic endothelium providing for migration CD44+ leukocytes or tumor cells [61]. Notice, there are some findings where expression of Lyve-1 was also observed in the human iliac atherosclerotic arteries [62], in the embryonic blood vessels [63], in macrophages [64], the reticulo-endothelial system [65]. Therefore, the specificity of Lyve-1 as a marker for the lymphatic vessels is not strong enough.
Prox1 - Prospero homeobox protein 1	Transcription factor regulating the process of growth and differentiation of endothelial cells of lymphatic vessels [66]
CCL21-Chemokine (C-C motif ligand 21)	It is secreted by endothelial cells of lymphatic vessels and is involved in activation of T-lymphocyte movement, migration of lymphocytes to other organs and dendritic cells into lymph nodes [67].
VEGFR3 - Vascular endothelial growth factor receptor 3	VEGFR3 is a receptor that triggers the lymphangiogenesis, i.e. the formation of new lymphatic vessels [68]. In transgenic mice with absence of VEGFR3 gene, the meningeal lymphatic vessels do not develop and lymph node hypoplasia is noted [33].
PDPN - Podoplanin	PDPN is integral membrane protein, which is responsible for the normal development of the network of lymphatic vessels, providing

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	drainage of the intercellular fluid. If the synthesis is broken, lymphedema is formed [69]. The PDPN activation is accompanied by lymphangiogenesis, which is regarded as an important indicator of tumor growth [70].
ITGA9 - Integrin-α9	ITGA9 is a protein, which is a part of the valves in the lymphatic vessels [71].

But, the lymphatic anatomy still receive more open questions than answers. Following Mascagni's data, Aspelung et al. [33] and Louveau et al. [34] showed the lymphatic vessels lining the dural sinuses in the subarachnoid space but not in the brain tissues. In this aspect, it seems wrong to use the term "cerebral or brain lymphatics", as used in many related publications [19, 32, 34, 40, 72] because there is no lymphatic system in the brain parenchyma.

However, in 1979 Prineas was first who reported appearance of the lymphatic capillaries and lymphoid tissues directly in the brain and spinal cord with neurological disorders [73]. 37 years later, a group of German anatomists found the lymphatic vessels in deep areas of telencephalic hemispheres of healthy mice [13].

It is fundamentally important to fill gaps in anatomy of lymphatics in the brain to find Ariadne's thread in labyrinths of hypothesis about the physiology of lymphatics in the brain.

4. Problems with current concepts about drainage of brain fluids

There are several theories explaining the drainage of brain fluids via different extracerebral lymphatic pathways [14-18, 20-31, 35-40], which are revised now in the light of novel findings including the meningeal lymphatics [33, 34] and its interaction with BBB [44, 45] as well new anatomical data presenting the lymphatic vessels in deep brain tissues and in the nasolacrimal duct [13]. Here we give a systematization of old and novel conceptions about brain drainage pathways with a modern view on the role of "cerebral" and peripheral lymphatics in this process.

The common view on drainage brain fluids is based on the main drainage of CSF. CSF is formed in the choroid plexus at the rate of 350 µl/min in human (the total volume 100-140 ml [74-76]) and 0.32 µl/min in mouse (the total volume 35 µl ml [74-76]) and flows through the system of the four ventricles in the subarachnoid space (SAS), which is the final side of CSF reabsorption in the venous circulation (Figure 1). The main reabsorption of CSF occurs through the arachnoid villi and granulations that projects into the dural venous sinuses and additionally through the nasal lymphatics or along cranial nerve sheaths that play a supporting role.

But, there are some problems with this concept. The mechanisms underlying absorption of CSF from SAS into the blood stream has remained speculative over many decades of investigations. Jonston et al. discussed possible connective pathways between CSF and the arachnoid structures such as cell phagocytosis or pinocytosis via giant arachnoid vacuoles or passive transport through the extracellular arachnoid labyrinths (open tubes) [23]. However, whether arachnoid elements absorb CSF is unknown and some arachnoid projections are not associated with dural sinuses [23]. Furthermore, the arachnoid system does not appear to exist prenatally. At around the time of birth, the arachnoid villi and granulations star to become visibly, in infants they increase in number and only in adults they exist in abundance [77]. Therefore, the drainage of CSF via the arachnoid projects may be important in adulthood. Nevertheless, the choroid plexus produces CSF from the third gestational month, which suggests that the fetuses and neonates need an effective absorption of CSF [78]. It seems that other mechanisms excepting the arachnoid system might be important for drainage of CSF.

Weller et al. discussed that there are significant differences between drainage of CSF in animals and humans [28]. So, up 50% of CSF in most animals drains into extracerebral lymphatics, while in humans directly into venous circulation through the arachnoid system [28 ,31]. The proportion of CSF drainage into lymphatics remains unknown [79].

The concept about the role of the nasal lymphatics and the cribriform plate as a main structure in this brain drainage pathway was demonstrated in 1912 in human [80] and was later confirmed in

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several animal studies that used an injection of different tracers into the CSF (the ventricles or the SAS) or the brain parenchyma and observation of tracers in the dcLN – the first anatomical station of CSF exit from the brain [22, 29-31, 35-38, 81]. The time for the CBF drainage via the cervical lymphatic system in animals is presented in Table 2.

Table 2. The time for the CBF drainage via the cervical lymphatic system in animals [30, 81].

Objects	The side of injection of tracer (radio-iodinated albumin)	Time of lymph collection (h)	Lymph recovery (%)*
Rabbit	Caudate nucleus	25	47
	Internal capsule	25	22
	Brain	25	18
	CSF	6	30
Cat	CSF	8	14
Sheep	CSF	26	32

*- The lymph recovery is given as percent of the total lymph outflow from the CNS.

However, in a recent study of Lohrberg M. and Wilting J. with using of serial paraffin section of mouse head and specific labelling of lymphatic endothelium by Lyve-1 and Prox-1/CD31 showed missing of the lymphatic system in the nasal mucous membrane excepting the basal part of the interior turbinates [13]. The authors hypothesized that the nasal lymphatics is not a main pathway for drainage of CSF and mostly plays role of moisturization of the respiratory air. They proposed a new pathway for the main lymph drainage pathway along the nasolacrimal duct (NLD) and the optic nerve connected with the eye lids and conjunctiva, which further drain lymph into the cervical lymph nodes. The NLD is presented in the embryonic period [82] and can be an alternative pathway for the arachnoid villi in embryos and neonates, which do not have the well-developed arachnoid system. These findings support the view of drainage of CSF via the arachnoid villi along the optic nerve of eyeballs. To confirm this theory, the authors discuss the presence of metastasis of ocular tumors in the cervical lymph nodes that might be explained by drainage of metastatic cells [83-85].

The ISF drainage is remain still unclear and highly debated issue due to technical problems of quantitative measurements of ISF movement in very small extracellular spaces (ESC); i.e. the extracellular space in the in vivo rat cortex lies between 38-64 nm [86].

Recently the glymphatic system was proposed as a possible pathway for ISF and waster clearance of the brain [38-40]. Glymphatics utilizes the PVS tunnels, which are connected with astroglial cells to promote an elimination of metabolic proteins from the brain. The hypothesis about glymphatics is based on experimental data using labelling of CSF with fluorescent tracers injected into the cisterna magna. The tracers (dextran 2000 kDa and 3kDa) were observed along the cortical pial arteries 100 μm below the cortical surface in 10-30 min after their injection with further diffusion into the brain parenchyma and elimination from the brain along the cerebral veins [38]. Using radiolabeled amyloid β1-40 that was injected into the brain parenchyma the authors showed that aquaporin-4 (AQP4) knockout mice revealed a significant reduction of the CSF flux and clearance of β-amyloid. It was, therefore, proposed that ISF drains by an AQP4-depended bulk through the brain parenchyma from the PVS of the cerebral arteries to the PVS of cerebral veins. Therefore, they proposed term “Glymphatics”, i.e. ISF drainage through glia cells.

However, Abbott stated that the neuropil has a very narrow space between cells that makes difficult to permit significant bulk flow, especially proteins [27]. If traces are cleared by diffusion, the small molecules would be expected to be cleared faster. But, in fact both small and large molecules are cleared in similar rates. So, Cserr and coworkers using tracers with a range of molecular weights showed that they all cleared with a single rate constant [20, 87, 88]. They argued that these facts are against diffusion as a clearing mechanism. If diffusion is the dominant process, small and large molecules should have individual effective diffusion coefficients.

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Cserr et al. [87, 88] discussed that the PVS or the Virchow-Robin space surrounding the capillaries restricts the bulk flow. Abbott [27] in her review of bulk flow also provided that the Virchow-Robin space could be a significant mechanism for ISF drainage that, however, is hard to quantify. The osmotic gradient might be a driven pressure for solutes movement, where PVS is a low resistance pathway, while extracellular spaces in the neuropil are too narrow to permit a fluid flow. However, some authors state that diffusion through ECS provides ISF drainage [42, 90, 91].

One of the important factors that define the transport speed in ECS is the relative proportion of the extracellular volume. Note that the study of the structure of ECS and the assessment of its volume are complicated by the fact that these characteristics may change significantly during the preparation of tissue samples. Thus, early measurements gave an ECS fraction of about 5% [92-94]. However, as was established later [95, 96], this was the effect of the specific tissue preparation technology, and now it is assumed that ECS occupies from 10% to 30% of the total volume.

In the classical understanding of diffusion as a physical process, changes in ECS would affect the current concentration of ISF components, but not the speed of their propagation. However, the actual process in the parenchyma is much more complicated. It may involve the temporary binding of substances to receptors or elements of the extracellular matrix, as well as its temporary trapping (see [97] and references there).

A change in the extracellular volume may alter these processes. So there are many reasons to expect a strong dependence of the transport rate on the current value of ECS.

In this regard, recent data on the dynamic regulation of the extracellular volume are of great importance.

It has long been known that an intense neural activity leads to a noticeable change in ionic gradients, and therefore to the osmotic flow of water. However, neurons were found to be relatively resistant to osmolarity differences in terms of the regulation of their volume. Pasantes-Morales and Tuz [98] reported that hypo-osmolarity causes a change in the degree of excitability of brain cortex neurons, but has little effect on their volume, a sharp change of them is usually associated with cell death. Zhou et al. [99] described the rapid but reversible change in the neural volume with spreading depolarization, which can be classified as an extreme physiological state.

However, with astrocytes the situation is different. Risher et al. [100] showed that the volume of astrocytes quickly followed changes in the ionic composition of ISF, as expected, through a large number of functional AQPs in their plasma membrane, which neurons do not have. Note that probably for the same reason, astrocytes much better recover from a brief ischemic insult in cortical slices.

Pannasch et al [101] noted that a regulation of the volume in the network of astrocytes is considerably different from what a single cell shows, due to the redistribution of absorbed (locally excessive) potassium, over many coupled cells.

It was found that a relatively small physiological increase in the concentration of extracellular potassium (3 mM, such changes accompany a temporary increase in the activity of neurons), causes almost 20% increase of the astrocyte volume [102]. Murphy et al. [103] discussed contribution of the regulation of the astrocyte volume in generation of epileptic seizures. During stress the same basic regulatory mechanisms as glutamate uptake, extracellular potassium buffering, and brain water regulation that provide the tight junction control over neuronal excitability, may also be actively involved in seizure generation.

In the light of the above, astrocytes (not neurons) are mainly responsible for modulating the volume of ECS in response to, for example, adrenergic signaling, which triggers rapid changes in neural activity, which in turn can modulate the volume of ECS [104].

In relation to astrocytes, new data on the dynamic regulation of their volume were obtained in connection with attempts to clarify the differences in the intensity of drainage processes during sleep and wakefulness. It was found that astrocytes are responsible for the observed sensitivity of the extracellular volume to change of the ionic composition (rather than neuronal activity) that can be observed during sleep-awake transitions. Ding et al. (2016) [105] showed that ECS increased by more than 30% when artificially prepared “sleep” CSF was applied to the mice cortex, despite the mice remaining awake and mobile.

Evidently, all this should strongly affect the process of metabolite clearance from the brain. As reported in Xie et al. [106], "natural sleep or anesthesia are associated with a 60% increase in the interstitial space, resulting in a striking increase in convective exchange of CSF fluid with ISF".

In general, a detailed understanding of how quantitative, and possibly qualitative (topology, connectivity) characteristics of ECS vary depending on the mode of functioning of the brain, is extremely important in connection with the discussion of recently proposed alternative drainage mechanisms.

The proposed hypothesis about the glymphatic system successfully linked a number of experimental observations and suggested a rather simple mechanism. For this reason, it was readily accepted and various publications appeared based on the glymphatic mechanism.

Diem et al. [107] suggested a computational model in order to describe the process of periarterial drainage in the context of diffusion in the brain. This model shows that the periarterial drainage along basement membranes is very rapid compared with diffusion.

Nakada et al. [108] discussed the specific organization of flows through AQPs. However, his hypothesis is only partially consistent with the originally proposed mechanism of glymphatics.

Bezerra et al [109] proposed that the glymphatic dysfunction is identified as a major pathogenetic mechanism underpinning idiopathic intracranial hypertension.

However, a critical examination of the glymphatic hypothesis indicates that not all links of the mechanism are explained unambiguously. In particular, the proposed propulsive function of pulsations of penetrating arterial vessels was questioned. In the comprehensive review by Hladky and Barrand [110] several possible options for the organization of flows are considered, being alternatives to the glymphatics.

While the presence of pulsations can be regarded as a proven fact [111, 112], their ability to create a directional fluid flow along the arterial PVS is not obvious. In Asgari et al. [113], an analysis of the proposed glymphatic mechanism by mathematical modeling was carried out and it was concluded that the presence of a bulk flow is doubtful, and that dispersion, rather than convection, is the most probable mechanism for transporting tracer to the parenchyma. Diem et al. [114] come to the conclusion that arterial pulsations cannot drive intramural periarterial drainage. Smith et al. [115] detected the declared flow patterns in the parenchyma, but they also found that (i) the transport of fluorescent dextrans in brain parenchyma depends on the dextran size in a manner consistent with diffusive rather than convective transport; (ii) transport of dextrans in the parenchymal ESC, measured by 2-photon fluorescence recovery after photobleaching, was not affected just after cardiorespiratory arrest; and (iii) AQP4 gene deletion did not impair a transport of fluorescent solutes from SAS to the brain of mice or rats. In a further work Smith et al. [116] concluded that "the theoretical plausibility of glymphatic transport has been questioned, and recent data have challenged its experimental underpinnings".

One of the serious reasons for doubts in the propulsive work of the pulse wave is related with its wavelength, which can be estimated on the basis of the previously reported pulse wave velocity for small vessels, which is as low as 10 cm per second [117,118]. With this, the length of the pulse wave is more than ten times larger than the working distance, which is less than 1 mm. Therefore, the cardiac rhythm should cause an almost simultaneous, non-directional, change in the volume of PVS, rather than a running pulse.

In this regard, a discussion of the role of AQP4 also looks ambiguous. There is a general agreement on their important role in the flow of water in the parenchyma, including the dynamic regulation of the astrocyte volume, as mentioned above. In the framework of the glymphatic hypothesis, they are assigned the role of the main conductor of the glymphatic flow. Indeed, in the work of Asgari et al. [119], the fundamental possibility of such a flow through the astrocyte network was shown, but it was presumed that there is a pressure gradient. Nakada et al. [108] hypothesized that AQPs deliver water to the near-capillary region but the fluid flow there is significantly limited by BBB. This hypothesis needs to be further justified, since the trans-network transmission of a considerable amount of water might overload the mesh of thin astrocyte processes, where the gap junctions between astrocytes are located.

Bacyinski et al. [120] concluded that there is currently significant controversy in the literature regarding both the direction of waste clearance as well as the pathways by which the waste-fluid mixture is cleared. Benveniste et al. [121], the inconsistencies in the data of various papers are analyzed, pointing to the imperfection of experimental techniques.

In general, at present one can observe a fight between two opinions. In a recent review by Plog and Nedergaard [122] a convincing collection of facts that support the glymphatic hypothesis is described. However, another, and not the less convincing review and discussion are presented in Abbott et al. [123], where they argue, that recent evidence suggests important amendments to the 'glymphatic' hypothesis.

In summary, the existence of a perivascular fluid system, whereby CSF enters the brain via flow through PVS is supported both by new as well as key historical studies. The specific moving pattern of fluid in PVS, directed or oscillating with a low or vanishing net flow, still needs to be justified, but the latter one seems to be more consistent with physical laws. This is what is referred as “dispersion” being the combination of a directed flow together with diffusion. This mechanism, even with zero net flow, is able to provide a much faster transport of different substances, than can be achieved with conventional diffusion process. Interestingly, this mechanism that recently gained much attention, is consistent with the 27 years-old observation of Ichimura et al. [124] who observed that the direction of the flow was variable, with a vector into the brain along one segment of an artery and out of the brain in a more distal segment.

In the light of BBB related issues, every aspect discussed above is important, since it can appear, that substances, which penetrated the opened BBB will be transported to the surface layers of the cortex and further to the lymphatic vessels, rather than to the deep parts of the parenchyma.

Potentially, this is the second challenge after overcoming BBB in solving the problems of drug delivery, and therefore progress in understanding this issue is in great demand.

The recently presented the lymphatic vessels penetrating into the brain from the meninges [13,73] might be the connective bridge between ISF and CSF drainage that needs to be reviewed with more details and if it will be confirmed, it will shed light on new approaches for the study and therapy of neuroinflammatory and neurodegenerative diseases.

5. Alternative notions for brain drainage: recruitments of the meningeal lymphatics by extracellular changes

Czerr [30] proposed in 1992 an intrigue idea about the relationship between BBB and lymphatic drainage of the brain. Recently in the light of renovation of interest to the meningeal lymphatics, Czerr’s idea was modified in the hypothesis that contrary to the peripheral lymphatics, the meningeal lymphatic system is like by to be exposed to changes in the extracellular environment and the effects of physical properties that might be a reason for activation of drainage of brain fluid [125]. In our experimental work we have found confirmation of these proposals [44, 45, 126].

We used two methods for the BBB opening – a loud audible sound that induces a mild BBB opening and the photodynamic (PD) method causing a strong BBB leakage with accumulation of extensive fluids in PVS [9, 10, 44, 45, 127]. Indeed, using confocal imaging of dextran extravasation and spectrofluorimetric assay of the Evans Blue albumin complex level in the brain tissues, we showed that opening of BBB for these tracers was more pronounced in the PD group vs. the sound group. These changes stimulated the brain drainage with an increase in the volume of CSF in the cisterna magna that was also more pronounced in the PD group vs. the sound group. The BBB-mediated activation of the brain drainage can be explained by the hypothesis of Alexander Monro and Kellie [31]. The constitutions of the brain (blood, CSF, ISF) create a state of volume equilibrium such that any increase in the volume of one of the cranial constitutions would be compensated by a distribution of the volume of another. The BBB opening is accompanied by an influx of fluids and different molecules in the brain tissues that in some cases (PD-induced opening of BBB) causes a vasogenic edema or an accumulation of extensive fluids in the brain parenchyma. These changes stimulate the brain drainage of fluids in a way to keep the extracellular homeostasis. Our results clearly show that PD caused the strong the BBB leakage that was associated with vasogenic edema and with a significant increase in the CSF volume in the cisterna magna vs. the control group. The

mild BBB disruption by sound was not associated with visible changes in PVS [45] and changes in the CSF volume in the cisterna magna were within a normal range [128]. We hypothesize that mild changes in BBB might be completely compensated by an activation of the brain drainage and clearing system as in the case with sound-related opening of BBB. While a strong BBB leakage (PD-induced opening of BBB) causes a quick and significant fluid influx in the brain parenchyma that cannot be compensated immediately by the drainage system of the brain and requests longer time for recovery [10].

Our results confirm the hypothesis about the recruitment of the meningeal lymphatics and its involvement in the brain drainage. So, a comparison between the PD/sound groups clearly demonstrate that the BBB opening is accompanied by a significant increase in the diameter of the meningeal lymphatic vessels that is equivalent to the intensity of BBB disruption and to the CSF accumulation in the cisterna magna [126]. Changes in the lymphatic vessels associated with the BBB opening might be explained by a similar structure of the lymphatic vessels and BBB. Indeed, the lymphatic vessels are composed by tight junction proteins such as claudin-5, family of ZO proteins, endothelial selective adhesion molecule; adherents junction proteins (VE-cadherin); junctional adhesion molecule-A and Platelet/Endothelial Cell Adhesion Molecule 1 / the C-terminus 31 (PECAM-1/CD31) [130, 131]. The opening of the BBB is accompanied by a disbalance in expression of indicated proteins [132] that can influence on the complex of these proteins in the lymphatic system. However, this hypothesis requires further more detailed studies for confirmation. The activation of nitric oxide (NO) production in the lymphatic endothelium might be a reason for increasing of the diameter of the meningeal lymphatic vessels after opening of the BBB. The NO is responsible for the reduction in the tone of the lymphatic vessels via opening of the ATP-sensitive $K(^{+})$ channels [131-136]. The impact of NO on lymphatic tone has been extensively studied using pharmacological modulation of NO synthase [131, 132, 137-138]. However, the NO effects on the lymphatic endothelium strongly depend on the initial conditions [136, 139]. The relaxation of the lymphatic vessels also depends on the oxygen level in the lymphatic system. The low oxygen tension (8-35 mmHg) in lymphatics vs. surrounding tissues promotes an increase in the NO bioavailability and dilation of the lymphatic vessels [134].

We also revealed that the meningeal lymphatics is the pathway in the brain clearing. The confocal imaging showed the presence of dextran in the meningeal lymphatics after its crossing of the BBB from the blood and accumulation in the brain parenchyma [44, 45]. Aspelung et al. also showed the involvement of the meningeal lymphatics in the brain clearing from high weight molecules [33]. This group was not focused on the study of interactions between the BBB and the meningeal lymphatics but they used an injection of tracers directly into the brain parenchyma that might destroy BBB structure and stimulate brain drainage as well.

Using of optical coherent tomography imaging of gold nanorods (GNRs) accumulation in the dcLN after their crossing of BBB in the PD/sound groups, we uncovered positive correlation between the intensity of the BBB-stimulated brain drainage and the GNRs level in the dcLN. These data suggest about the anatomical and functional connection between the meningeal and cervical lymphatics, which are both involved in brain drainage system (Figure 2).

Although that the important role of the nasal and cervical lymphatics in the brain drainage has been discussed by Schwalbe [36] et al., Czerr and Knopf [30], Kida [22] et al., Koh [140] et al., Abbott [27], and more recently by Aspelung [33] et al. and Louveau [34] et al., no anatomical pathway for the brain drainage and clearing has still been clearly demonstrated. It is more likely that the meningeal lymphatics and the cerebral lymphatic vessels [13] are the most prominent pathway for the brain drainage and clearing as well as the connective bridge between the meningeal/cerebral and peripheral lymphatics (Figure 2).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 November 2018 doi:10.20944/preprints201811.0051.v1

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 3818; doi:10.3390/ijms19123818

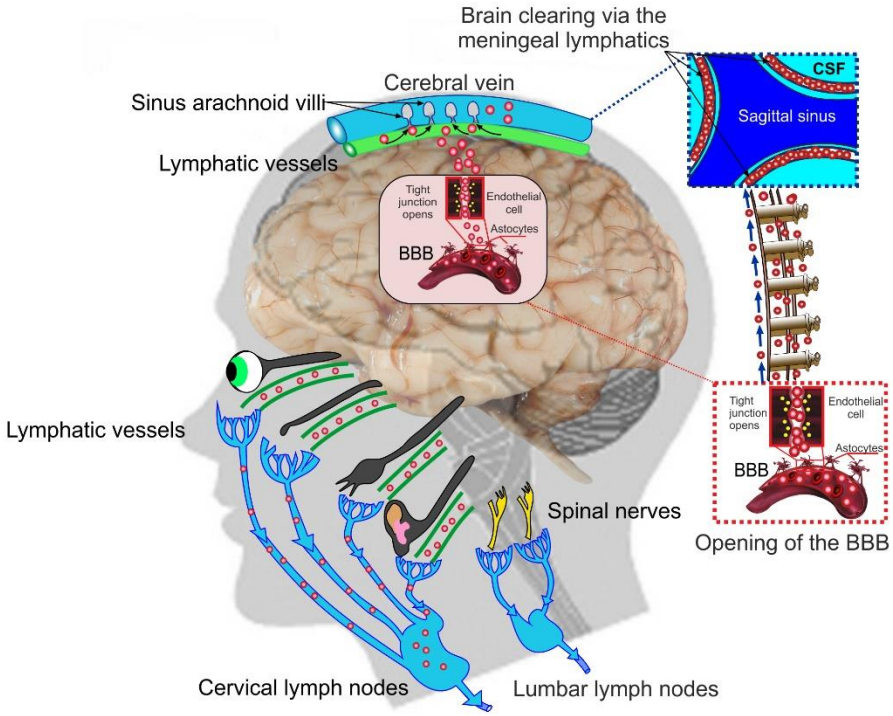

Figure 2. Schematic illustration of recruitment of the meningeal lymphatics by BBB: the opening of BBB is accompanied by the brain clearing from molecules, which crossed BBB; about 50% of them in the adult brain absorb from SAS into the venous system via the sinus arachnoid villi [28, 31]; other molecules (the proportion remain unknown) move into the opened meningeal lymphatic vessels, which draw them into the cervical lymphatics [44, 45, 126]. However, there is a significant gap in a clear understanding of mechanisms underlying the movement of molecules from the brain parenchyma into SAS and then into the meningeal lymphatics that requests further detailed studies.

Thus, our results give initial hints for the recruitment of the meningeal lymphatics to homeostasis of extracellular environment and the BBB-related stimulation of the meningeal lymphatic pathway for the brain drainage of fluids.

We believe that the recruitment of the meningeal lymphatics by BBB might be a crucial mechanism of the brain recovery after brain injuries such as stroke, trauma, subdural and subarachnoid hemorrhages, brain edema, neurodegenerative diseases.

To confirm our idea, we analyzed the clearance of the brain from toxic products of blood after different hemorrhagic events in human using of immunohistochemical assay and atomic abortion spectroscopy (not published data). In all cases we found the presence of the hemosiderin/iron in the meningeal lymphatic system and in the dcLN. These data shed light on the role of the meningeal lymphatics in the mechanisms underlying the brain recovery after hemorrhagic injuries and open novel strategies for a non-invasive stimulation of endogenous processes of lymphatic brain drainage by using of lasers (see Review "Non-invasive photonic technologies with computer adaptive control for neurorehabilitation therapy").

In summary, a better understanding of anatomy and physiology of the lymphatics in the brain will give new knowledge about the role of lymphatics in the rehabilitation of the brain functions after neural pathologies. The ability to stimulate the lymph flow in the brain, it is likely to play an important role in developing future innovative strategies in neurorehabilitation therapy.

Funding: This work was supported by Grants of Russian Science Foundation № 17-15-01263 (O.S-G. and J.K.) and partly (discussion of dynamic regulation of extracellular volume in 4th part of review) by Russian Science Foundation № 16-15-10252 (D.P. and O.S-G.).

Acknowledgments: We thank Saranceva Elena for preparation of figures of this review.

Author Contributions: Oxana Semyachkina-Glushkovskaya conceived the presented idea and took the lead in writing the manuscript. Jurgen Kurths contributed to the style and design of review as well as English grammar. Dmitry Postnov wrote part of review about modeling and mathematical approaches in the study of lymphatic physiology in the brain tissues and meninges in 4th part of review. All authors discussed the results and contributed to the final manuscript.

Conflicts of Interest: The authors declare that there are no conflicts of interest related to this article.

Abbreviations

APQ(s)-4	Aquaporin(s)-4
ATP-K(+) channels	Adenosine Tri-Phosphate Potassium Channels
BBB	Blood-brain barrier
CCL21	Chemokine (C-C motif ligand 21)
CSF	Cerebral Spinal Fluid
ECF	Extracellular Fluid
GNRs	Gold Nano Roads
HA	Hyaluronan
ISF	Interstitial Fluid
ITGA9	Intergrin-α9
LYVE-1	Lymphatic Vessel Endothelial Hyaluronan Receptor 1
NO	Nitric Oxide
NCD	Nasolacrimal Duct
PECAM-1/CD31	Platelet/Endothelial Cell Adhesion Molecule 1 / the C-terminus 31
PD	Photodynamic
PDPN	Podoplanin
PVS	Perivascular Space
VEGFR3	Vascular Endothelial Growth Factor Receptor 3

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