ΙΑΤΡΙΚΗ ΣΧΟΛΗ

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ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

«ΕΦΑΡΜΟΣΜΕΝΗ ΝΕΥΡΟΑΝΑΤΟΜΙΑ»

ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

“Emerging Perspectives on the Life Cycle of Cerebrospinal Fluid”

«Νέες Υποθέσεις, για την Παραγωγή, Κυκλοφορία και Απορρόφηση του Εγκεφαλονωτιαίου Υγρού»

Δημήτρης Ισαακίδης

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Του Μεταπτυχιακού Φοιτητή κ. Ισαακίδη Δημήτρη

Εξεταστική Επιτροπή

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Η Τριμελής Εξεταστική Επιτροπή για την αξιολόγηση και εξέταση του υποψήφιου **κ. Ισαακίδη Δημήτρη** συνεδρίασε σήμερα 2/6/2021.

Η Επιτροπή **διαπίστωσε** ότι η Διπλωματική Εργασία του κ. Ισαακίδη Δημήτρη με τίτλο ***«Νέες υποθέσεις για την παραγωγή, κυκλοφορία και απορρόφηση του εγκεφαλονωτιαίου υγρού»*** είναι πρωτότυπη, επιστημονικά και τεχνικά άρτια και η βιβλιογραφική πληροφορία ολοκληρωμένη και εμπεριστατωμένη.

Η εξεταστική επιτροπή αφού έλαβε υπ' όψιν το περιεχόμενο της εργασίας και τη συμβολή της στην επιστήμη, με ψήφους 3/3 προτείνει την απονομή στον παραπάνω Μεταπτυχιακό Φοιτητή την απονομή του Μεταπτυχιακού Διπλώματος Ειδίκευσης (Master's).

Στην ψηφοφορία για την βαθμολογία ο υποψήφιος έλαβε για τον βαθμό «**ΑΡΙΣΤΑ**» ψήφους , για τον βαθμό «ΛΙΑΝ ΚΑΛΩΣ» ψήφους , και για τον βαθμό «ΚΑΛΩΣ» ψήφους 0. Κατά συνέπεια, απονέμεται ο βαθμός « **10.00/10.00** (**ΔΕΚΑ** στα δέκα)».

Τα Μέλη της Εξεταστικής Επιτροπής

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ΓΕΩΡΓΙΟΣ ΣΤΡΑΝΤΖΑΛΗΣ
ΚΑΘΗΓΗΤΗΣ
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PROLOGUE

Presumably, life emerged in the ocean as a cell. A simple structure for which the internal environment communicated with the external environment via the first barrier- the lipid cell membrane. During the embryonic phase, the neural tissue is derived from the ectoderm, it's "sibling", the skin. Metaphorically speaking, the nervous system is the internal skin, the layer that incorporates the internal, the external and their merging simulation of reality. Therefore, it is no surprise that the brain floats in fluid- the cerebrospinal fluid. A glimpse of this mysterious fluid and its plethora of roles, is what follows.

ACKNOWLEDGEMENTS

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ABSTRACT

This thesis discusses the newest findings related to the life cycle of cerebrospinal fluid (CSF)- its production, its circulation and its absorption. For this purpose, a systematic review of the literature was performed and basic and clinical research studies have been included. In the first part, the physiologica role of CSF is discussed. Specifically, the degree and mechanisms of CSF production from the choroid plexus but also other brain areas, CSF circulation, its intimate relationship with the interstitial fluid between the neurons and the glia, the role of the glymphatic system and its participation in metabolite and waste clearance from the Central Nervous System. Also discussed is the association of CSF function with the sleep-wake cycle, the existence of meningeal lymphatics, the role of the perineural spaces of the cerebral cortex and spinal nerves as the main drainage system, and their interaction with other variables including intracranial pressure and the immune system. In the second part, the role of CSF in pathophysiological conditions is discussed. Specifically, the role of CSF in neurological diseases and neurosurgery cases as well as its contribution to diagnosis as a source of biomarkers and via neuroimaging. Finally, new frontiers in CSF research are examined and outstanding questions and the future directions of CSF research are considered.

ΠΕΡΙΛΗΨΗ

Η διπλωματική έχει ως θεματική, τις νέες υποθέσεις που υπάρχουν στην παραγωγή, κυκλοφορία και απορρόφηση του εγκεφαλονωτιαίου υγρού (ΕΝΥ). Για αυτό τον σκοπό, πραγματοποιήθηκε μια συστηματική ανασκόπηση της βιβλιογραφίας και έχουν συμπεριληφθεί μελέτες βασικής και κλινικής έρευνας. Στο πρώτο μέρος συζητιέται ο φυσιολογικός ρόλος του ΕΝΥ. Συγκεκριμένα, ο βαθμός και μηχανισμός παραγωγής του ΕΝΥ από τα χορειοδή πλέγματα αλλά και από άλλα σημεία, η κυκλοφορία του ΕΝΥ, η σχέση του με το διάμεσο υγρό (intersistial fluid), μεταξύ των νευρώνων και των γλοιικών κυττάρων, του συστήματος της γλοίμφου (glymphatic) και η συμμετοχή του στην εκκαθάριση μεταβολικών προϊόντων και αποβλήτων από το Κεντρικό Νευρικό Σύστημα. Επίσης, συζητιέται η συσχέτιση λειτουργίας του ΕΝΥ με το τον κύκλο ύπνου-εγρήγορσης, η ύπαρξη μηνιγγικών λεμφαγγείων, ο ρόλος των περινευρικών χώρων των εγκεφαλικών συζυγιών και νωτιαίων νεύρων ως κύριο μέσου παροχέτευσης, και η αλληλεπίδραση με άλλες μεταβλητές και λειτουργίες όπως η ενδοκράνια πίεση και το ανοσοποιητικό σύστημα. Το δεύτερο μέρος αναφέρεται στον ρόλο του ΕΝΥ σε παθοφυσιολογικές καταστάσεις. Συγκεκριμένα, στην διερεύνηση των ευρημάτων σε ασθένειες της νευροχειρουργικής και της νευρολογίας καθώς και η πιθανή συμβολή του στην διαγνωστική μέσω βιοδεικτών και νευροαπεικονιστική. Τέλος, εξετάζονται τα νεότερα δεδομένα στην έρευνα του ΕΝΥ και συζητιούνται τα εκκρεμή ερωτήματα, ταυτόχρονα λαμβάνοντας υπόψη τις μελλοντικές κατευθύνσεις στην έρευνα του ΕΝΥ.

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ABBREVIATIONS

Aβ	Amyloid β
AD	Alzheimer's disease
AQP1	Aquaporin 1 channel
AQP4	Aquaporin 4 channel
BCSFB	Blood Cerebrospinal Fluid Barrier
CP	Choroid Plexus
CPE	Choroid Plexus Epithelium
CSF	Cerebrospinal Fluid
EPS	Extrapyramidal Syndrome
FTD	Frontotemporal dementia
IS	Idiopathic Scoliosis
ISF	Interstitial fluid
MND	Motor Neuron Disease
PD	Parkinson's Disease
ENY	Εγκεφαλονωτιαίο υγρό

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INTRODUCTION

One of the many mysteries of the human brain is not of the brain matter itself but of the fluid that surrounds it- the cerebrospinal fluid (CSF). Due to the obscure and dynamic nature of CSF, studying its life cycle, ie. synthesis, flow and absorption, has proven to be not only challenging but a continuous source of controversy.

Modern CSF research was facilitated by the discovery of radioisotopes after World War II. Tracers allowed the study of choroid plexus ion transport and CSF flow dynamics. Through this research, several basic CSF physiology concepts were established including: differences between the CP and blood brain barrier, CP as the primary site of CSF formation, ependymal permeability, CSF and brain interstitial fluid exchange, CSF sink action for excretion, and a quasi-lymphatic system of CSF flow/drainage to cervical lymph, coined “the glymphatic system”. These ground-breaking findings have provided the foundation on which to build fundamental models of CSF dynamics and examine its regulation with pharmacological agents to manipulate CSF formation and the role of neurotransmitters, neuropeptides, and different brain cell types.

Immunohistochemical, neuroendocrine and molecular techniques have been used the last 30 years to study the expression and function of basolateral and apical transporters at the blood-CSF interface. And even more recently, gene knock-out models and transcriptomic approaches have allowed researchers to delve even deeper into the inner workings of CP transport and metabolism. Moreover, a potential role of the CP in diseases such as stroke, intracranial hypertension, hydrocephalus, Alzheimer’s disease and Parkinson’s disease constitutes an exciting field of research

into potential therapeutic targets. Finally, expanding our understanding of CSF physiology and pathophysiology will aid improved neurosurgical practices.

The physiologic impact of CSF dynamics including CP-CSF fluid production, flow/pressure and homeostasis on the relative "newcomer" to the field of CSF research, the glymphatic system- the waste disposal or plumbing system of the brain- also comprises an intriguing research topic. Another new chapter in CSF research includes the deconstruction of the long-held dogma of brain "immune privilege". Based on the latest evidence of immune cells at the interface between the meninges and immune system, the brain's status of immune-privilege is being replaced with the term "immune-uniqueness". New advancements in imaging and computational modeling will provide the impetus for stimulating game-changing research in CSF translational neuromedicine.

OBJECTIVES

The aim of this review is to provide a comprehensive and up-to-date analysis of the current state of knowledge and emerging hypotheses relating to the life cycle of cerebrospinal fluid (CSF), from its synthesis to its absorption. This timely review highlights the new ground that has been covered, gaps in our knowledge and controversies that exist with respect to the physiology and pathophysiology of CSF.

Specifically, the issues that are addressed include the following axes: interpretation of the evidence related to ***extra-choroid plexus*** CSF production; the hypothesis of the ***glymphatic system*** in humans; the contribution of all the above in the bilateral regulation of intracranial pressure and neurovascular coupling, as well as in the postoperative, post-traumatic, post-haemorrhagic and post-infection changes to CSF dynamics.

The potential involvement of the CSF in ***diseases of the central nervous system*** including the various types of hydrocephalus (obstructive, post-traumatic, post-haemorrhagic, physiological pressure), inflammation, benign intracranial hypertension, epileptogenesis and neurodegeneration, will also be discussed. In addition, important subtopics including the role of the ciliated epithelium in hydrocephalus and scoliosis, the production and function of Reissner fibers and the consequences of craniectomy and cranioplasty time on the dynamics of the CSF cycle are examined. Finally, the advent of new ***neuroimaging techniques*** to study CSF dynamics to further our understanding of its role in health and disease and potential prospects for ***new diagnostic and therapeutic approaches*** of CSF-related CNS diseases are evaluated (summarized in Figure 1).

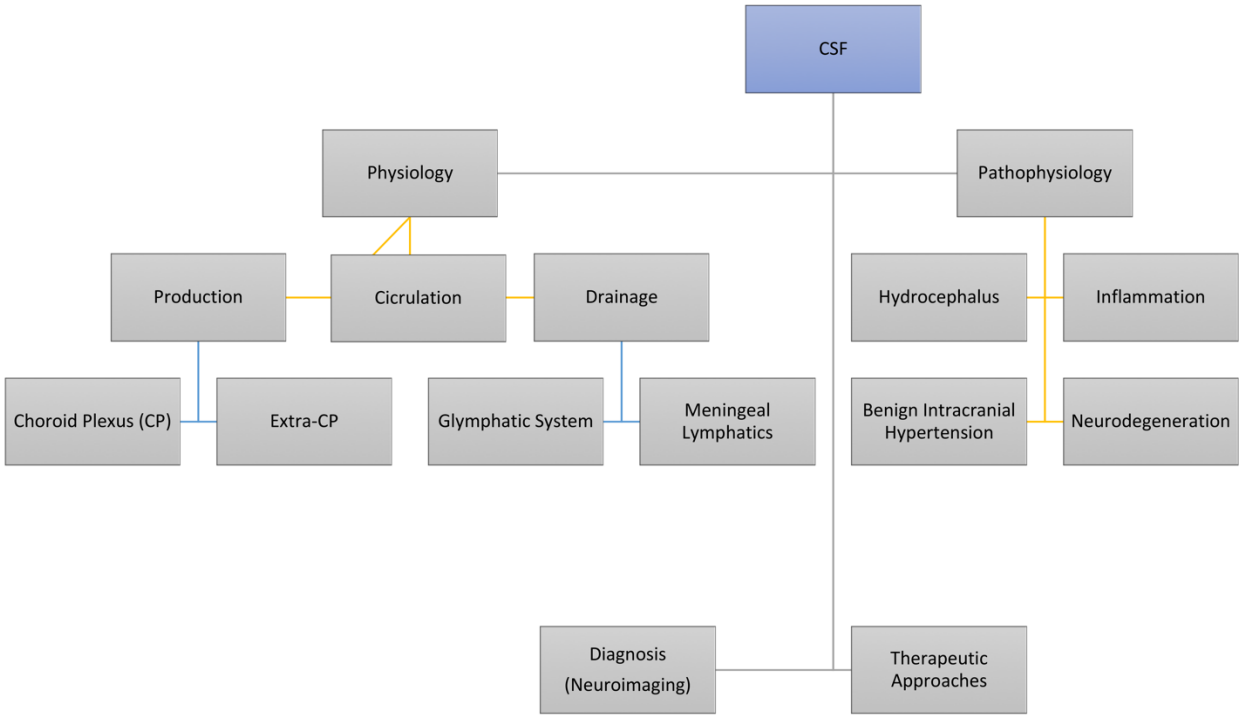

Figure 1. An overview of topics discussed in the present review and how they relate to the overarching theme of the life cycle of cerebrospinal fluid (CSF).

MATERIALS AND METHODS

The methodology is comprised of 1) definition of the subject area, 2) data collection 3) critical evaluation, 4) integrated extraction, 5) analytical composition, and finally, 6) presentation of the results of this study. It is a secondary qualitative method of knowledge collection. The recommendations of the Cochrane collaboration and the Center for reviews and dissemination were considered.

Data was collected through literature searches in the following online databases: Pubmed with a follow-up search of specific authors in Google Scholar, and included original articles, textbooks and expert opinions. The following keyword searches were used:

1. Blood CSF barrier (BCSFB)
2. Choroid plexus
3. Glymphatic
4. Meningeal lymphatics

The literature search was focused on the last 30 years without geographical limitations. Both clinical and preclinical studies including cellular and animal models were considered.

The following results include an analysis of the search outcomes with reference to patterns and heterogeneity, reliability of reproducibility, quality of results and good practices, evaluation of possible errors (such as overestimation of positive results, underestimation of negative results, bias of choice and other cognitive biases), followed by the extraction of and presentation of results, and examining the fields where new knowledge seems to be consolidated, areas where there are knowledge gaps and views and contradictions exchanged by the leading scientists/authors in the

field. A comprehensive overview of the literature search process including study identification, screening and inclusion, according to the PRISMA 2020 guidelines, is included in Figure 2.

In conclusion, this review provides an improvement of knowledge regarding the physiology of the CSF cycle, an overview of new research topics, and recommendations and applications in the diagnosis and treatment of diseases related to CSF cycle deregulation.

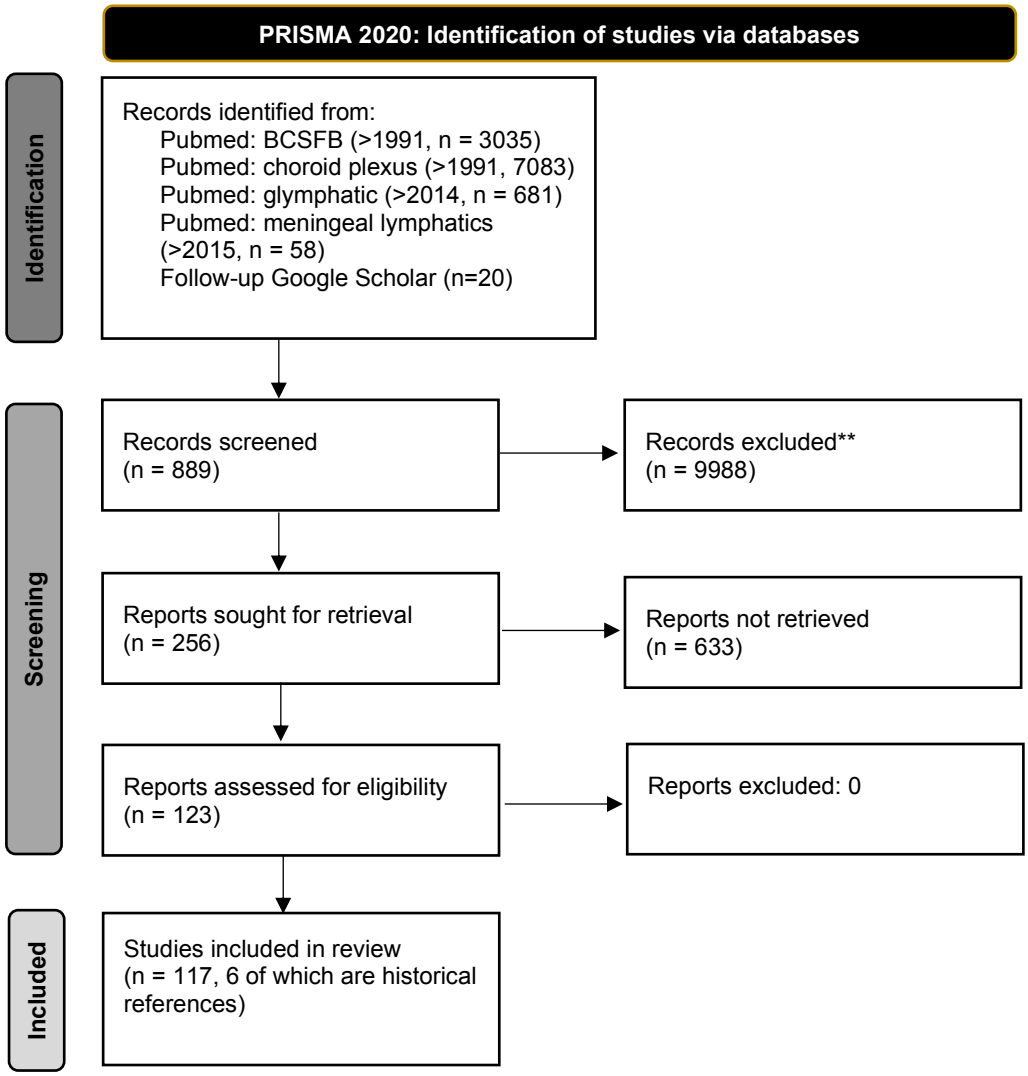

Figure 2. A PRISMA 2020 flow diagram outlining the literature search process followed including study identification, screening and inclusion (adapted from Page et al.*, 2021).*

RESULTS

CSF in Health

The History of CSF's discovery

Cerebrospinal fluid (CSF) was first described by Hippocrates, the father of medicine, as the "water surrounding the brain" (460-370 BC). Galen's work followed (129-200 AD) when he noted "excremental liquid" in the ventricles, which he believed was purged into the nose. It wasn't until the 18th century that the modern rediscovery of CSF was credited to Emmanuel Swedenborg in (written between 1741-1744 but left unpublished until 1887), who described it as a "spirituous lymph" dispensed from the roof of the fourth ventricle down to the medulla oblongata and spinal cord. Albrecht von Haller wrote in his book of physiology in 1747 that the "water" of the brain is secreted into the ventricles and absorbed in the veins, and when secreted in excess, could lead to hydrocephalus (Hajdu, 2003).

The choroid plexus (CP) was first suggested as the origin of CSF in 1854 by Faivre (Faivre, 1854), followed by Cushing in 1914 (Cushing, 1914). It wasn't until 1960, however, that the involvement of the CP in CSF secretion was demonstrated for the first time experimentally (de Rougemont *et al.*, 1960). This seminal work demonstrated that CSF is *not* a simple plasma ultrafiltrate and this is now regarded as the leading hypothesis (Praetorius and Damkier, 2017).

CSF physiology

CSF is a located within the ventricles of the brain and the subarachnoid spaces of the cranium and spine (Sakka, Coll and Chazal, 2011). Adult CSF volume is estimated to

be approximately 150 ml, with a distribution of 125 ml within the subarachnoid spaces and 25 ml within the ventricles. Its secretion varies between individuals with production, ranging between **400 to 600 ml** per day in adults. CSF is continuously secreted and thus, it is renewed 4-5 times every 24-hour period in the average young adult.

CSF is predominantly, but not exclusively, secreted by the CP epithelium (CPE), with the two lateral ventricles being the primary producers. (Cserr, 1971; Damkier, Brown and Praetorius, 2013; Spector *et al.*, 2015; Hladky and Barrand, 2016). The CP weighs approximately 1 g and consists of a specialized cuboidal epithelium of approximately 100 million cells, as a continuum of the ependymal cells lining the brain ventricles (Dohrmann and Bucy, 1970). The CPE surrounds clusters of fenestrated capillaries that allow plasma filtration (Damkier, Brown and Praetorius, 2010). The CP cellular structure consists of dense microvilli on their apical surface that are interconnected via tight junctions, thus, creating a blood-CSF barrier that regulates CSF composition. The CPE represents a more permeable counterpart to the blood brain barrier (BBB), and is thus referred to as the **blood-CSF-barrier (BCSFB)** (Figure 3).

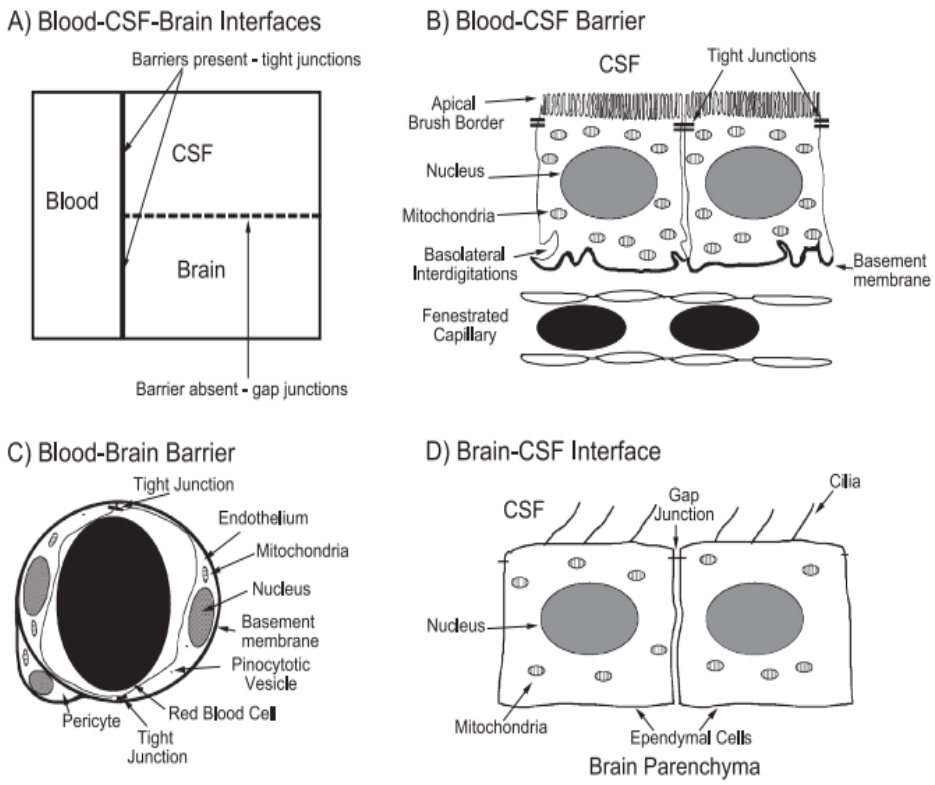

Figure 3. The blood–brain–cerebrospinal fluid (CSF) interfaces. (A) Schematic diagram of the interfaces. Unlike the blood–CSF and blood–brain interfaces that are made up of barrier cells with linking tight junctions, the CSF–brain interface is not a barrier, as the cells forming this interface are linked by gap junctions. (B) Morphology of the blood–CSF barrier. The blood–CSF barrier is formed by the choroid plexus epithelial cells and their linking tight junctions. These epithelial cells have a well-developed apical brush border and basolateral interdigitations as well as numerous mitochondria. The capillaries supplying the choroid plexus are fenestrated, thereby enhancing the movement of fluid and solutes across the endothelium. (C) Morphology of the blood–brain barrier. The endothelial cells of the blood–brain barrier are linked by tight junctions, limiting paracellular solute movement. There are few pinocytotic vesicles and more mitochondria compared to systemic endothelial cells, suggesting a relatively low rate of transcytosis and a high rate of oxidative metabolism at the blood–brain barrier. (D) Morphology of the brain–CSF interfaces. The CSF–brain interfaces are formed by ependymal cells lining the cerebral ventricles and the glia limitans (not shown) lining the surface of the brain. Neither cell type is linked by tight junctions. Ependymal cells have numerous cilia that help to move water and solutes in the CSF (Smith, Johanson and Keep, 2004, permission obtained for reuse).

The fluid production and transport rate of the CPE is incomparable to all other epithelia of the human body. The surface area of the CPE is 2 to 5 m^2 (Spector *et al.*, 2015), and the microvilli extend this area to maximally 5% of the 20 m^2 BBB area in humans (Begley and Brightman, 2003). However, as the BBB has a low permeability to ions and H_2O, the high experimental transport rate of CPE seems sufficient to

explain most of the CSF production (Praetorius and Damkier, 2017). The CPE secretes ~80% of the CSF, and thus, the remaining 20% arises from the brain interstitial fluid and thereby indirectly from the blood-brain barrier (BBB) (Redzic *et al.*, 2005).

The Human Choroid Plexus

Besides the CP's role in producing and transporting CSF, it delivers local mediators and hormones to the brain parenchyma via the CSF, it regulates the ionic microenvironment, and creates a barrier against toxins, drugs, microorganisms, and immune cells. Dysfunction of the CP is associated with a wide range of clinical conditions such as aging, neurodegenerative diseases, brain edema, stroke, neoplasms, and certain types of hydrocephalus. Research focusing on CP physiology and pathophysiology should be priority in order to elucidate the mechanisms involved in abnormal CSF secretion, inflammatory processes, and toxic protein aggregation. Subsequently, this knowledge will bring with it novel treatment strategies for CP-associated diseases and more effective drug delivery methods. Finally, it is worth noting that recently, the classic theory of CSF production in the CP has been challenged and a discussion of supporting and counter evidence is warranted (see *Box 1* and *Box 2*).

Box 1: Controversies Related to the Origin of CSF- Supporting Evidence for CSF production

Evidence supporting CPE as the primary source of CSF include the following observations (Praetorius and Damkier 2017):

1) **CSF cannot be merely a plasma ultrafiltrate**: it is 5 mM hyperosmolar compared with blood plasma (Davson and Purvis, 1954), the concentrations of Na+ and HCO3- are higher in the CSF and K+ and Cl- concentrations are lower than expected from a filtration process (Hughes, Pakhomova and Brown, 2010) and there is a 5 mV lumen-positive transepithelial potential difference across the CPE (Welch and Sadler, 1965).

2) **The CPE secretes fluid**: there is a net loss of fluid from the blood during the passage of the CPE, as demonstrated by an increase in hematocrit from the arteries to the veins (Davson H, Welch K and Segal, MB, 1987), the fluid collected from the luminal surface of the CPE is practically identical in composition to the CSF in the cisterna magna in vivo (de ROUGEMONT *et al.*, 1960), the transport of Na+, Cl-, and HCO3- across the CPE is ATP dependent, the CPE secretes fluid beneath oil installed above the epithelium in vivo at a rate beyond compare with other mammalian epithelium (Damkier, Brown and Praetorius, 2013).

3) **Na+ transport and fluid secretion can be manipulated pharmacologically**. Numerous studies have demonstrated that ion transport inhibitors, such as ouabain, azetazolamide, amiloride, and DIDS, have similar effects on CSF secretion and CPE transport rates (Tschirgi, Frost and Taylor, 1954; Davson and Luck, 1957; Welch, 1963; Ames, Higashi and Nesbett, 1965; Davson and Segal, 1970; McCarthy and Reed, 1974; Zeuthen and Wright, 1978, 1978; Pollay *et al.*, 1985; Vogh and Godman, 1985; Murphy and Johanson, 1989; Johanson and Murphy, 1990; Johanson, Murphy and Dyas, 1992; Johanson, Parandoosh and Dyas, 1992);

4) **Genetically modified mice have reduced ventricle volumes**. Deficiency of proteins involved in CPE secretion, solute transport and pH homeostasis, including the Na(+)-coupled Cl(-)-HCO(3)(-) exchanger (NCBE, Slc4a10 gene), the electrogenic sodium bicarbonate cotransporter NBCe2 (Slc4a5 gene) and the water channel aquaporin 1 (AQP1), leads to a reduction in ventricle size and/or CSF secretion (Oshio *et al.*, 2005; Jacobs *et al.*, 2008; Kao *et al.*, 2011).

Box 2: Controversies Related to the Origin of CSF- Counterarguments

Despite the ample evidence for CSF production and secretion by the CPE, more recent studies have challenged this long-standing dogma. It is argued that brain interstitial fluid and CSF are continuously produced and reabsorbed by the BBB microvasculature without any contribution from the ventricles, including the CPE (Orešković and Klarica, 2014). Others believe that CSF returns to the blood via the sleeves of adventitia surrounding the cranial nerves and vasculature to the cervical lymph nodes (Bradbury, Cserr and Westrop, 1981; Szentistványi *et al.*, 1984; Cserr, Harling-Berg and Knopf, 1992). The counterarguments to CPE's role in CSF production are as follows:

1) **There is no hydrostatic pressure gradient to drive the classical CSF flow and conventional osmotic forces cannot explain the CSF production rate.** In the classical model of CSF flow, the hydrostatic pressure needs to be higher at the site of CSF synthesis, i.e. CPE, than the site of reabsorption (i.e. arachnoid villi). Global intracranial pressure is approximately 7–13 mmH_2O (Saunders, 2012) but it is inherently difficult to compare the hydrostatic pressures at these sites in animals and humans. Local pressure gradients should be sizable enough to allow fluid flow that would be influenced by body position. In cats, the intracranial pressure gradient between the lateral ventricles and the lumbar subarachnoidal space was negligible in the horizontal position, while in the upright position it was negative (Klarica *et al.*, 2014). Importantly, a similar pressure gradient was obtained in humans in the upright position (Andresen *et al.*, 2015). Following the classical model, CSF would have to flow from low pressure to high pressure, defying the laws of physics.

2) **Fluid is not directly secreted into the ventricles**. Hyperosmolar fluid injections into the ventricles of cats revealed fluid transfer from the blood to the CSF, independent of intraventricular CSF formation (Klarica *et al.*, 2013). In addition, hydrocephalus can develop from hyperosmolarity and fluid volume exchange across the BBB rather than intraventricular CSF formation (Klarica *et al.*, 2016).

CSF circulation

CSF is continuously secreted and preserves a fixed composition, thus maintaining brain homeostasis (Damkier, Brown and Praetorius, 2010). CSF flows from the site of secretion to the site of absorption, mainly by the periodic systolic pulse wave of choroidal arteries. However, CSF flow also appears to be dependent on the frequency of respiration, posture, venous pressure of the jugular vein, physical exertion, and the sleep-wake cycle (Spector *et al.*, 2015).

CSF flow is driven by motile cilia on ependymal cells of the brain ventricles and spinal canal (Grimes *et al.*, 2016). Cilia are microtubule-based, hair-like organelles that protrude from the cell surface, and play essential roles as signalling centers (primary cilia) and in fluid flow over epithelia (motile cilia) (Goetz and Anderson, 2010).

CSF is shunted throughout the ventricular system from the rostral to caudal end. CSF produced in the CPE flows through the interventricular foramina to the third ventricle, via the cerebral aqueduct to the fourth ventricle, and then through the median aperture (or the foramen of Magendie) into the subarachnoid space at the base of the brain. Once in the subarachnoid space, the CSF is freely distributed in a multidirectional fashion, equilibrating the whole brain CSF composition. The CSF flows over the surface of the brain and down the length of the spinal cord and the major efflux pathway includes the arachnoid villi that are located along the superior sagittal venous sinus, intracranial venous sinuses, and around the roots of the spinal nerves.

Arachnoid villi are protrusions of arachnoid mater in the dura mater that enter the lumen of a venous sinus. CSF absorption is facilitated via a 3 to 5 mmHg pressure

gradient between the subarachnoid space and venous sinus. Furthermore, CSF may also enter the lymphatic system via the nasal cribriform plate or spinal nerve roots.

The model of ventricular hyperosmolarity (see *Box 2*) does not directly contradict the classical model of CSF secretion by the CPE, which is insensitive to hydrostatic pressure gradients and was not shown to be directly independent of increased fluid secretion by the CPE caused by augmented osmotic gradient. These studies, however, did not compare the hydrostatic pressure with the conventional site of CSF reabsorption, the arachnoidal villi. And it is quite possible that the pressure here would be lower than that of the brain ventricle system. Thus, direct comparisons of intraventricular to cranial subarachnoidal pressures are merited to resolve this outstanding issue. In conclusion, the current paradigms of CSF circulation dynamics and CSF formation by the CPE requires further research as neither the concept of CPE as a site of CSF formation nor CSF as a plasma ultrafiltrate have been experimentally rejected.

When compared to plasma, CSF has a lower concentration of potassium and calcium and a higher concentration of sodium, chloride, and magnesium (Sakka, Coll and Chazal, 2011). In contrast to plasma, CSF has only trace amounts of cells, protein, and immunoglobulins (Spector *et al.*, 2015). Cells cannot pass through the blood-CSF barrier, although a limited number of white blood cells can be introduced to the CSF indirectly. The normal cell count of CSF is less than 5 cells/ml (Sakka, Coll and Chazal, 2011). Despite dynamic changes in blood flow and composition, the CSF's composition remains constant, in order to provide a stable intraventricular environment and maintain homeostasis and normal neural function (Damkier, Brown and Praetorius, 2010).

CSF function

The CSF performs 3 key functions in the brain: 1) protection, 2) provision of nourishment, and 3) waste removal. The vital importance of CSF is highlighted by the disruption of brain physiology that occurs with any imbalance in CSF hydrodynamics or composition (Damkier, Brown and Praetorius, 2010; Sakka, Coll and Chazal, 2011; Spector *et al.*, 2015).

Intraventricular CSF protects the brain parenchyma by creating a cushion of surrounding fluid that allows the brain to "float" in the skull. Thus, the CSF dramatically decreases the effective weight of the brain from 1.5 kg to 50 g (Klarica *et al.*, 2009; Andresen *et al.*, 2015), so that the human body can support its weight without collapsing. In addition, the reduction in weight lessens the force applied to the brain parenchyma and cerebral vessels during mechanical injury and the CSF acts as a shock absorber to protect the brain from mild traumatic injury by providing hydromechanical protection.

The nexus of the CP, CSF and extracellular space is the major conduit of nutrient supply to the brain. The brain's nutrients are transported from the blood into the CSF via the CP, and then diffuse into the extracellular space for distribution to their sites of action.

Because there is no significant barrier between the CSF and the extracellular space, the blood-CSF barrier also plays a role in regulation of the brain microenvironment (Spector *et al.*, 2015). Cells and macromolecules such as proteins and glucose cannot permeate, whereas ions and small molecules such as vitamins and nutrients pass into the CSF with relative ease (Damkier, Brown and Praetorius, 2013). Molecules that cannot pass the blood-CSF barrier but are necessary for normal brain function are

either actively synthesized on site or actively transported through the CPE into the CSF. A 5 mV lumen positive voltage potential present in CPE epithelial cell membranes drives the entry of sodium, chloride, and bicarbonate ions from the plasma into the CSF, creating an osmotic gradient that facilitates the movement of water into the CSF (Damkier, Brown and Praetorius, 2010). Water can also enter the CPE via epithelial aquaporin 1 (AQP1) channels.

Finally, CSF plays a role in the removal of metabolic waste including products of peroxidation, glycosylated proteins, excess neurotransmitters, debris from the ventricular lining, and microorganisms. New research findings support the existence of a macroscopic waste removal system, termed the glial-lymphatic or "**glymphatic system**", consisting of a unique system of perivascular channels, formed by astroglial cells that facilitate convective exchange of water and soluble contents between cerebrospinal and interstitial fluid. The recent characterizations of the glymphatic system calls for revaluation of the anatomical routes for CSF-ISF flow and the physiological role that these pathways play in brain health (Jessen *et al.*, 2015).

The Glymphatic System

The brain recycles approximately 7 g of protein daily. It is believed that the brain is the only organ that is solely responsible for this turnover, considering that, unlike the rest of the body, it does not have a lymphatic system, i.e. lymphatic vessels, and the BBB does not possess receptors for removal of protein waste.

With trillions of processes occurring simultaneously in the brain, the process of diffusion is relatively slow for the removal of waste from the CSF. However, the

process of convection, fluid flow dependent on a pressure gradient, is more efficient and does not discriminate based on protein size.

In contrast to the vasculature system of the rest of the body, the arteries and the veins of the brain vasculature do not run in parallel. 98.4% of the brain vasculature, including capillaries, arteries and veins, is surrounded by glia. This perivascular space is unique to the CNS, void of neural pathways and consisting mostly of microglia, macrophages, pericytes and fibrous tissue. Thus, the perivascular space, or Virchow-Robin space, presents a passage for relatively fast transport of fluid as there is negligible resistance. In a model proposed by Nedergaard and colleagues, the pumping action of the artery drives the CSF into the perivascular space with relatively low resistance. Injections of Dextran tracer into the cisterna magna demonstrated that the tracer enters the perivascular space rather that the arteries and the same amount of tracer enters the extracellular space and CSF, indicating direct CSF- exchange (Yang *et al.*, 2013). These studies were replicated in rats using magnetic resonance imaging, revealing a similar pattern with mice of influx from perivascular space in 30 min and then cleared out (Iliff, Lee, *et al.*, 2013; Lee *et al.*, 2015).

It is worth noting that CSF tracer distribution studies in post-mortem tissue are prone to artifacts; ie. CSF movements that occurred after death. For example, cardiac arrest or stroke abruptly initiate a pathological influx of cerebrospinal fluid (CSF) (Ma *et al.*, 2019; Mestre *et al.*, 2020). Such substantial differences between live vs. post-mortem brain studies, emphasizing the importance of preforming these experiments in live animals and humans (Figure 4).

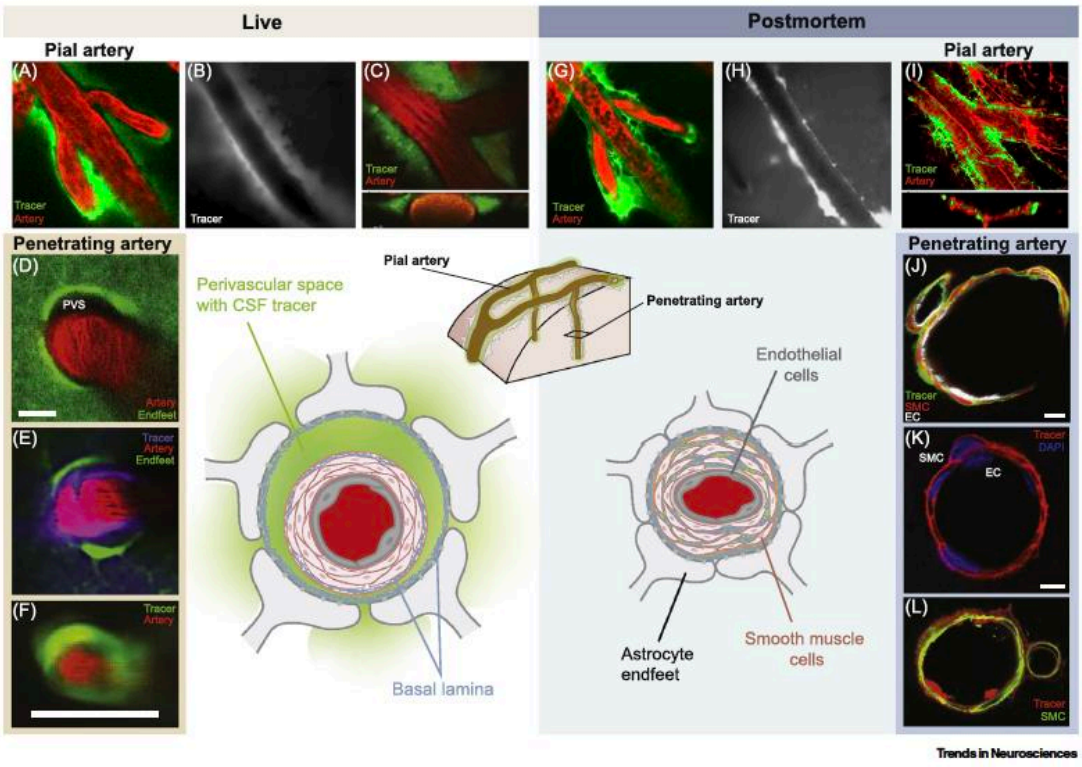

*Figure 4. Summary of differences between live and postmortem relocation of periarterial Cerebrospinal Fluid (CSF) Tracers. In the **live brain** (left), (A)–(C): the pulsatility of pial arteries drives CSF tracers along large surface perivascular spaces (PVS). The tracers are confined to the PVS on each side of the vessel and do not enter the compact smooth muscle cell layer [(C) inset, orthogonal projection]. (D)–(F): CSF tracers enter the neuropil by leaving the PVS of penetrating arteries via gaps between the vascular astrocytic endfeet. In the **postmortem** brain (right), (G)–(I): the arteries collapse, and the fluid-filled PVS largely disappears – compare with (A)–(C); and tracers relocate into the smooth muscle cell (SMC) layer, basal lamina, and endothelial cell (EC) basement membrane (J)–(L). Abbreviation: DAPI, 4',6-diamidino-2-phenylindole (a blue-fluorescent DNA stain) (adapted from Mestre, Mori and Nedergaard, 2020, available under the Creative Commons CC-BY-NC-ND).*

The Role of the Aquaporin 4 Channel

AQP4 are expressed only in astrocytes and primarily in the astrocyte vesicular end feet that are polarized, i.e. only expressed at high densities on the side facing the vessel wall. The question thus arises as to why the brain requires so many water channels in this specific area since one of the characteristics of brain endothelial cells is that they are devoid of water channels (Verkman, 2002). On the contrary, endothelial cells of peripheral organs express AQP1. There is limited water flowing

into the brain for this reason. Fluid pumped out of perivascular space thus facilitates water influx into the brain. Along these lines, reduced brain efflux of radiolabeled mannitol and Aβ were observed in AQP4-deficient mice with decreased glymphatic flow (Iliff *et al.*, 2012).

Based on these findings, Needergard and colleagues defined a new system that supports a central role of CSF in the garbage disposal/plumbing system of the brain or a "quasi-lymphatic system" and named it the "glymphatic" system.

This system consists of a highly-polarized water transport where CSF surrounds the pial arteries and the pumping action of circulation drives the CSF into the perivascular space. From the perivascular space, it leaves via convective flow to enter the brain tissue, facilitated by the expression of AQP4, without discrimination of size or composition; allowing fluid flow from the extracellular space to the perivenous space (Figure 5). This fluid is believed to exit the brain via perivascular spaces to enter the lymphatic system.

Glymphatic clearance serves various CNS functions. First, it is involved in the delivery of nutrients from the CSF to the brain, specifically glucose (Lundgaard *et al.*, 2015). Secondly, the glymphatic transporting system facilitates the circulation and distribution of choroid plexus/CSF-derived apolipoprotein E to neurons and ISF clearance- both of which are suppressed by sleep deprivation (Achariyar *et al.*, 2016). Furthermore, paravascular circulation enables selective and rapid transport of lipid molecules and widespread glial calcium signalling (Rangroo Thrane *et al.*, 2013). Finally, its primary role is that of a brain "lymphatic" system that clears extracellular metabolites and waste products from the parenchyma into the CSF (Louveau *et al.*, 2017).

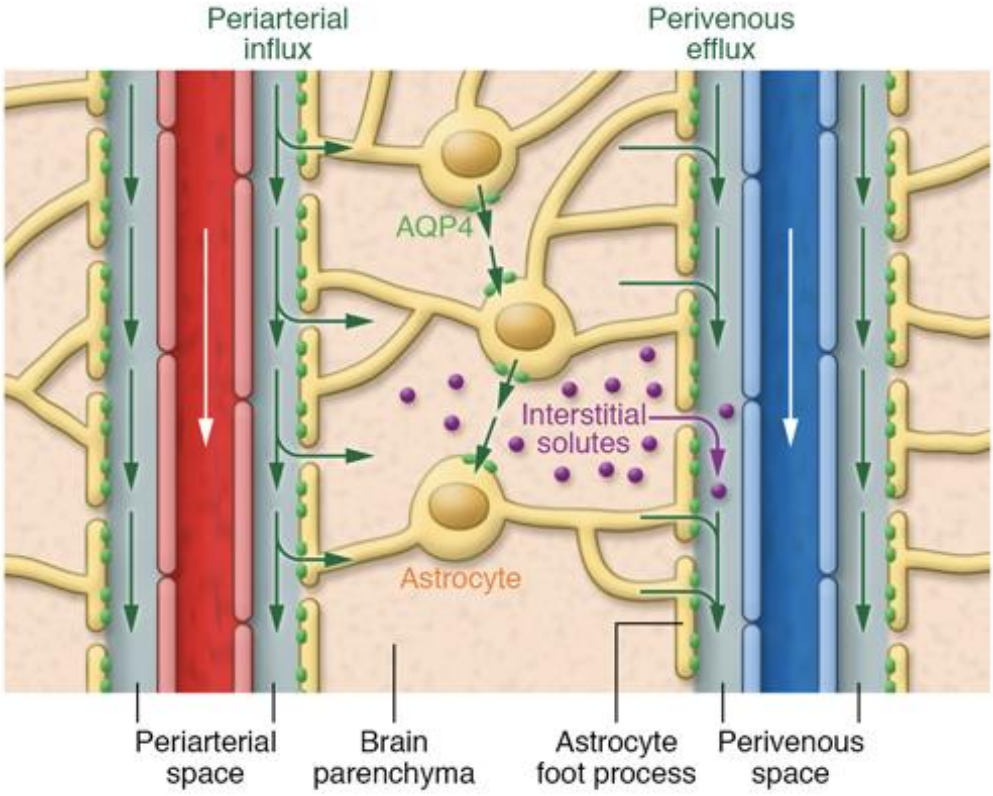

Figure 5. Circulation of CSF and ISF through the glymphatic pathway. (A) CSF within the subarachnoid and cisternal spaces flows into the brain via periarterial spaces and then exchanges with ISF via aquaporin-4 (AQP4) water channels that are located on the perivascular astrocyte end-foot processes. The bulk movement of CSF into the brain drives the convective flow of ISF and interstitial solutes through the extracellular space to ultimately be absorbed into the perivenous space (Louveau et al.*, 2017, permission obtained for reuse).*

The regulation of the Glymphatic System in the sleep-wake cycle

Magnetic resonance imaging (MRI) studies demonstrate that approximately 20% of contrast agents injected into the cisterna magna permeate the brain in anesthetized rats (Lee *et al.*, 2018). Sleep drives metabolite (i.e. interstitial lactate) clearance as measured by a consistent increase in extracellular space volume (~ 60%), facilitating CSF entry, in the transition from wakefulness to sleep or under anesthesia (Xie *et al.*, 2013). Recent evidence collected in awake rats using contrast-enhanced MRI shows that the glymphatic system is also under circadian control (Cai *et al.*, 2020)- awake rats demonstrate minimal influx of tracer whereas mice that are asleep show the same pattern as anesthetized mice (Figure 6). Circadian control of CSF distribution has also

been demonstrated in AQP4 deficient mice in which the day-night difference in both glymphatic influx and lymphatic drainage is lost (Hablitz *et al.*, 2020). In addition, this concept is supported by MRI studies which have demonstrated that water diffusivity is restricted during wakefulness (Demiral *et al.*, 2019). More recently, a seminal study demonstrated that a prime function of sleep is to "reset" synapse strength via synaptic scaling, highlighting the detrimental effects of sleep deprivation (de Vivo *et al.*, 2017). Considering the energetic demands of the brain during wakefulness, this intricately orchestrated circadian control of brain waste removal during sleep is both logical and fascinating.

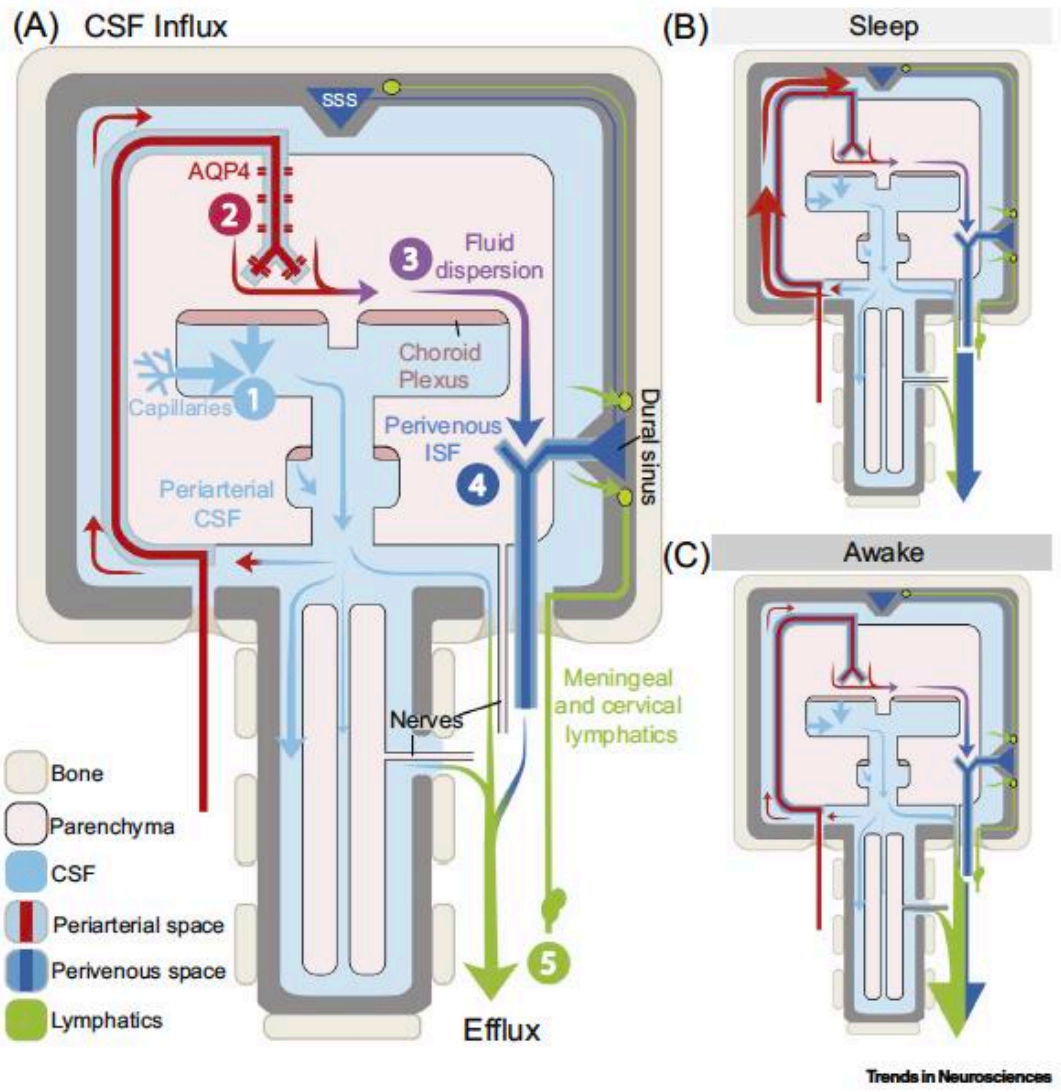

Figure 6. Schematic model of the Glymphatic–Lymphatic System and sleep–wake cycle control. (A) The fluid transport pathway is divided into five distinct sections: (1) cerebrospinal fluid (CSF) is produced mainly by the choroid plexus (~80%) but also by other sources (ie. capillary influx and metabolic water production); (2) arterial wall pulsatility shunts CSF into the brain along perivascular spaces; (3) CSF enters the brain parenchyma via aquaporin-4 (AQP4) water channels and disperses within the neuropil; (4) interstitial fluid (ISF) mixes with CSF and accumulates in the perivenous space and drains from here out of the brain via (5) meningeal and cervical lymphatic vessels as well as along cranial and spinal nerves. The two models on the right map the routes of CSF flow during the sleep and wakefulness states: during sleep (B), CSF enters the brain via glymphatic transport, but during wakefulness (C), it is mostly excluded and shunted out via lymphatic vessels. CSF may also exit via arachnoid granulations (not included in this model) but this theory is highly debated (Koh, Zakharov and Johnston, 2005; Miyajima and Arai, 2015). Abbreviation: SSS, superior sagittal sinus. (Mestre, Mori and Nedergaard, 2020, available under the Creative Commons License CC BY-NC-ND 4.0).

The discovery of a lymphatic system not within the brain but in the surrounding meninges provided a link with the findings of CSF outflow, suggesting an explanation for the transport of the impressive volume of 500 ml of fluid out of the brain on a

daily basis. The theory of the glymphatic system thus became complete when studies revealed that there are lymphatic capillaries positioned around the perivenous system, primarily around the superior sagittal sinus and traverse sinus (Aspelund *et al.*, 2015; Louveau *et al.*, 2015). They form "traditional" lymphatic capillaries that merge onto lymphatic precollector and collector vessels to dump out the waste products of the lymph into the systemic circulation to be degraded by the liver (Louveau *et al.*, 2017).

These studies were further complemented by experiments in a transgenic model of Alzheimer's disease (Pappolla *et al.*, 2014). This research demonstrated amyloid-β deposition at equally high concentrations in the brain and axillary lymph as well as significant expression in the cervical lymph node, providing evidence of lymphatic amyloid-β clearance derived from the brain.

Meningeal Lymphatics

As already mentioned, the glymphatic system controls the flux of interstitial fluid from the brain parenchyma into perivascular spaces, forming a continuum with the CSF-filled subarachnoid space. CSF mixed with interstitial fluid is absorbed into meningeal lymphatic vessels in dural blood vessels to drive metabolite/waste

clearance (Figure 7).

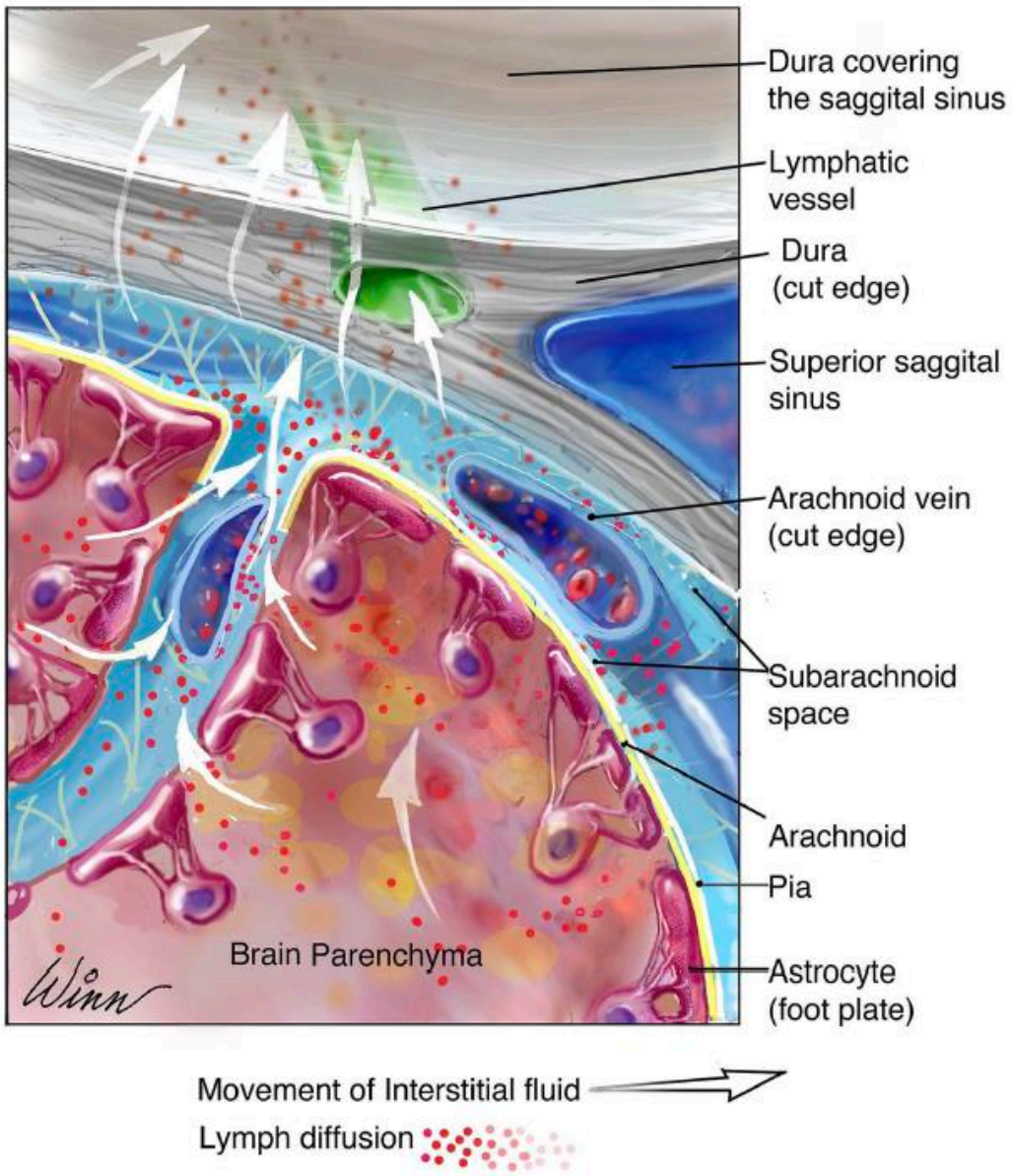

Figure 7. The drainage pathway of interstitial fluid from the brain parenchyma to the meningeal lymphatic system. The glymphatic system controls the flux of interstitial fluid from the brain parenchyma into perivascular spaces that form a continuum with the CSF-filled subarachnoid space. CSF mixed with interstitial fluid is absorbed into meningeal lymphatic vessels in dural blood vessels. (Hershenhouse et al.*, 2019, available under the Creative Commons License CC BY-NC-ND 4.0).*

The brain is constantly undergoing immune surveillance that takes place within the meningeal compartment, however, until recently, the mechanisms of transcerebral

immune cell transport remained an enigma. In 2015, functional lymphatic vessels discovered along the lining of the dural sinuses. These structures express all of the molecular hallmarks of lymphatic endothelial cells, are able to carry both fluid and immune cells from the CSF, and are connected to the deep cervical lymph nodes (Louveau *et al.*, 2015). Dural lymphatic vessels absorb CSF from the adjacent subarachnoid space and brain interstitial fluid (ISF) via the glymphatic system. Dural lymphatic vessels transport fluid into deep cervical lymph nodes via foramina at the base of the skull (Figure 8). In a transgenic mouse model devoid of dural lymphatic vasculature, solute clearance from the brain was attenuated and transport from the subarachnoid space into deep cerebral lymph nodes was abolished. Furthermore, surgical ligation of the lymphatic vessels prevented tracer accumulation in the lymph node, and increased meningeal T cell numbers and tracer filling of the dural lymphatic network in the basal parts of the skull (Aspelund *et al.*, 2015; Louveau *et al.*, 2015). Suppression of the glymphatic transport system with AQP4 deficiency, CSF volume reduction, or altered posture, all result in enhanced brain and reduced cervical lymph node lactate levels (Lundgaard *et al.*, 2017), even in an anesthetized state that promotes clearance (Xie *et al.*, 2013).

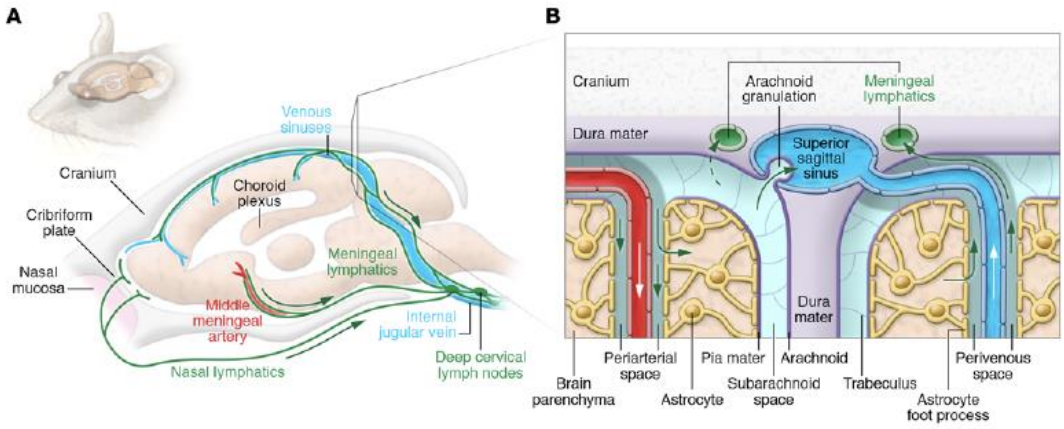

Figure 8. Glymphatic and lymphatic drainage pathways from the brain to the cervical lymph nodes. (A) Schematic illustration of the meningeal lymphatic vessel system in mouse cranium. The dural lymphatic vessels align with dural blood vessels and cranial nerves and exit the cranium via the foramina together with the venous sinuses and arteries. The nasal lymphatic vessels traverse the cribriform plate with the olfactory nerves. Tracers injected into either brain parenchyma or subarachnoid space drain via the dural lymphatic vessels into deep cervical lymph nodes located next to the jugular vein. (B) A close-up view of interstitial fluid and cerebrospinal fluid circulation. The perivascular glymphatic drainage system *transports* ***CSF and solutes*** into *the brain via a periarterial pathway, while* ***ISF and solutes*** *are removed from the brain via the* perivenous glymphatic pathway. *CSF can enter the venous system via arachnoid granulations/villi, and CSF solutes and immune cells are transported mainly along the dural lymphatic vessels into the lymph nodes and extracranial systemic circulation (adapted from Louveau* et al.*, 2017, permission obtained for reuse).*

The close proximity of the lymphatic vessels to the subarachnoid space suggests that they absorb CSF from the arachnoid mater. Nonetheless, the mechanism of fluid and solute uptake into dural lymphatics remains to be discovered. It has been hypothesized that a portion of the CSF transported across the arachnoid barrier layer accumulates in the dural border interstitium, a sort of "buffer zone", consisting of non-epithelial channels within this collagen-rich network, to then be transported or absorbed into the endothelial-lined parasagittal plexus (Pollay, 2010).

The significance of the lymphatic outflow route in maintaining normal intracranial fluid homeostasis has been shown by obstruction of different outflow routes. Surgical ligation of the perineural cribriform pathway led to a prompt and sustained increase in intracranial pressure (Mollanji *et al.*, 2002). The meningeal lymphatic vessels also

appear to play a vital role in the clearance of subdural hematomas (SDHs). In mice and rats, SDHs were drained into the cervical lymph nodes of the meningeal lymphatic vessel pathway while the haematomas themselves impede meningeal lymphatic drainage (Liu *et al.*, 2020).

It is approximated that between 14% and 47% of albumin tracer injected into the brain parenchyma or CSF passes through the meningeal lymphatic system (Cserr, Harling-Berg and Knopf, 1992). However, the exact proportion of clearance is not yet known and may vary between species and depend on the functional dynamics of other drainage routes (Louveau *et al.*, 2017). Furthermore, brain ISF pressure and water content were unaffected in mice devoid of meningeal lymphatic vessels, indicating the existence of compensatory or adaptive mechanisms (Aspelund *et al.*, 2015).

The discovery of the brain's meningeal lymphatic system warrants a re-examination of the basic assumptions in neuroimmunology and provides insight into the aetiology of neuroinflammatory and neurodegenerative diseases.

The Glymphatic System, Meningeal Lymphatics and "Immune-Uniqueness"

One hundred years ago, Yoko Shirai transplanted tumor tissue into a mouse's body and reported that the tissue was destroyed by its immune system. However, when tumors were grafted in the mouse's brain, they grew. Tumors seemed to be able to escape the immune system's surveillance when hidden inside the brain. Similar findings accumulated and in and in the late 1940s, the "father of transplantation", Peter Medawar, coined the term "immune privilege" to describe a tissue or organ in which the introduction of foreign antigen does not elicit an immune response. He observed that skin allografts implanted into the brain parenchyma elicit a delayed

graft rejection (Medawar, 1946). Interestingly, if an immune response toward allograft antigens has already been established in the periphery prior to grafting into brain parenchyma, the brain will mount an immune response, similar to the peripheral organs' response (Medawar, 1946); thus, antigens within the brain may not prime an immune response but they can maintain and amplify it.

CNS immune privilege was partially attributed to a lack of lymphatic drainage from the CNS, with a subsequent lack of antigen presentation on antigen-presenting cells (e.g. dendritic cells). However, it was observed that a normal immune response could be provoked when the allograft was implanted close to the ventricles or within the subarachnoid space, indicating that the meningeal spaces are more interactive with peripheral immunity (Mason *et al.*, 1986; Nicholas *et al.*, 1987); acting as "border patrol". More recently, an involvement of the meningeal immune compartment in neuronal function was demonstrated (Kipnis, 2016). Specifically, production of interleukins IL-4 and IL-13 by meningeal T cells appears to be implicated in cognitive function in mice (Derecki *et al.*, 2010; Brombacher *et al.*, 2017). In addition, interferon-γ (IFN-γ) production in the subarachnoid space unexpectedly regulates social behavior in mice (Filiano *et al.*, 2016).

The brain's parenchymal and CSF/meningeal compartments possess different properties that define each compartment's immune status. Under physiological conditions, various immune cells perform CNS surveillance and occupy the CSF/meningeal compartment (Derecki *et al.*, 2010; Ransohoff and Engelhardt, 2012; Louveau *et al.*, 2015; Kipnis, 2016). Meningeal endothelial cells lack astrocytic end-feet and as a result, the blood-meningeal barrier is more penetrable than the BBB for immune cells; allowing them to enter and circulate within the meninges under homeostatic conditions (Shechter, London and Schwartz, 2013). On the other hand,

the ability of such meningeal immune cells to penetrate the brain parenchyma under patho/physiological conditions remains to be elucidated (Schläger *et al.*, 2016).

ISF-CSF exchange occurs primarily via the glymphatic system (Iliff *et al.*, 2012) as demonstrated with tracers injected into the brain parenchyma that also drain into the cervical lymph nodes (Cserr, Harling-Berg and Knopf, 1992; Iliff *et al.*, 2014). In both rodent and primate models of multiple sclerosis, brain antigens were increased in the cervical lymph nodes (de Vos *et al.*, 2002; van Zwam *et al.*, 2009). Following stroke, brain injury, or oligodendrocyte death induced by diphtheria toxin, myelin antigens have also been found to accumulate in the cervical lymph nodes (Locatelli *et al.*, 2012; Urra *et al.*, 2014; Traka *et al.*, 2016). Finally, lymphatic drainage of the brain antigen Aβ, was demonstrated as it was detected in the cervical lymph nodes in murine models of Alzheimer's disease (Pappolla *et al.*, 2014).

The perivascular space does not appear to allow entry of immune cells (Carare *et al.*, 2008). The cribriform plate was proposed as the route by which immune cells exit the brain via the nasal lymphatics (Goldmann *et al.*, 2006; Kaminski *et al.*, 2012). It is debatable as to whether T cells and dendritic cells in the brain parenchyma can recirculate under physiological or pathophysiological conditions. The parenchymal environment is hostile towards T cells and dendritic cells, promoting death rather than recirculation (Bauer *et al.*, 1998; Choi and Benveniste, 2004). Recent findings demonstrate that endogenous CD11c+ cells might recirculate from the CNS during inflammation, but the exact source of these cells (i.e. meningeal or parenchymal) remains unknown (Schiefenhövel *et al.*, 2017).

Available evidence suggests that brain antigens can enter the cervical lymph nodes, and in doing so, potentially induce an immune response. Recent findings

demonstrated that induction of massive death of oligodendrocytes leads to the production of autoreactive T cells that can initiate multiple sclerosis–like symptoms in mice (Locatelli *et al.*, 2012). Furthermore, expression of a foreign antigen on oligodendrocytes induces T cell response in the draining lymph nodes (Harris *et al.*, 2014). These findings demonstrate that brain antigens can drain into cervical lymph nodes and elicit a restricted immune response. This specialized immune response may progress slowly, require enhanced antigen release, or a secondary signal (e.g. danger- or pathogen-associated molecular patterns (DAMPs or PAMPs) to be drained alongside the synthesized antigens- perhaps rationalizing the phenomenon of increased allograft survival in the brain parenchyma (Louveau *et al.*, 2017).

Based on the above evidence, the unique nature of brain lymphatic drainage could be the main driver of immune privilege. Lymph-node resident lymphatic endothelial cells are implicated in regulation of the immune response (Card, Yu and Swartz, 2014; Betterman and Harvey, 2016; Randolph *et al.*, 2017), thus, it is quite possible that meningeal lymphatic endothelial cells are also implicated in initiation and regulation of immune tolerance. Alternatively, the cervical lymph nodes may play a role in tuning down the mounted immune response to CNS antigens. Strategic placement of macrophages and dendritic cells in lymph node capsules allows for the timely coordination of T and B cell induction in the lymph nodes (Gerner, Torabi-Parizi and Germain, 2015).

Overall, the existence of the meningeal lymphatic drainage system doesn't contradict the central theory of immune privilege, but on the contrary, provides a convincing mechanistic explanation for CNS immune privilege that perhaps should be more correctly termed "**immune uniqueness**" (Louveau *et al.*, 2017).

CSF in Disease

CSF Leak

CSF Leak occurs when CSF leaks from the subarachnoid space through a hole in the surrounding dura. The volume of CSF lost in a leak is very variable, ranging from inconsequential to significant amounts. If the loss of CSF is great enough, spontaneous intracranial hypotension (SIH) may occur. SIH most often presents with a positional headache caused by downward displacement of the brain due to loss of buoyancy previously provided by the CSF. Posterior neck stiffness, nausea, and vomiting are also common symptoms. The incidence of SIH is estimated to be 5/100,000 annually. Women are twice as likely to be affected with a peak age at around 40 years.

Diagnosis is aided by typical MRI findings, such as an increase of intracranial venous volume, pituitary hyperemia, enhanced pachymeninges, and descent of the brain. Many cases of SIH resolve without any treatment. Conservative approaches such as bed rest, hydration, and increased caffeine intake may also prove to be effective; however, more drastic measures may be necessary. An epidural blood patch, where blood is injected into the spinal epidural space, may relieve CSF hypovolemic symptoms by replacing lost CSF volume with blood volume. Surgical repair of the CSF leak via a suture or metal aneurysm clip is relatively safe and usually effective in providing relief (Schievink, 2006).

Hydrocephalus

Hydrocephalus is the abnormal accumulation of CSF caused by 1) increased CSF production, 2) blockage of flow through the ventricles, or 3) decreased reabsorption. The ventricles swell to accommodate elevated CSF volumes, potentially causing brain damage due to the pressing of cerebral tissue against the skull. Hydrocephalus may be congenital or acquired. It can be caused by genetic defects, infection, brain haemorrhage, trauma, or tumours. **Non-communicating, or obstructive, hydrocephalus** occurs when the flow of CSF becomes blocked due to a mass such as a tumor or an abscess located within a foramen. Because CSF secretion and flow are constant processes, an obstruction will lead to CSF build up in ventricles anterior of the blockage. For example, one of the most common causes of obstructive hydrocephalus, stenosis of the cerebral aqueduct, results in swelling of both lateral ventricles as well as the third ventricle. **Communicating, or non-obstructive hydrocephalus** occurs when CSF flow becomes obstructed outside the ventricles, in either the subarachnoid space or arachnoid villi.

Symptoms of hydrocephalus include headache, convulsions, nausea, vomiting, visual disturbances, and mental deterioration. Diagnosis is usually made with imaging techniques such as ultrasound, computed tomogram (CT), or magnetic resonance imaging (MRI).

The most common treatment is shunt insertion, which diverts CSF away from the ventricles to a lower area of the body where it can be absorbed into circulation. Other more invasive treatment options include endoscopic third ventriculostomy, a procedure in which a hole is created in the floor of the third ventricle allowing CSF to bypass an obstruction, and cauterization of CP sections, effectively decreasing CSF production and secretion. Untreated hydrocephalus poses serious health risks to the

patient including physical and cognitive disturbances and even death (Orešković and Klarica, 2011; Kahle *et al.*, 2016).

Subarachnoid Haemorrhage

Subarachnoid Hemorrhage (SAH) is the leakage of blood into the subarachnoid space which leads to mixing of blood with CSF. SAH may occur as the result of head trauma or a ruptured cerebral aneurysm. 80% of nontraumatic SAHs are associated with cerebral aneurysm rupture and other nontramautic causes include arteriovenous malformations and vasculitis. Spontaneous SAH is rare, with only 30,000 cases worldwide annually. Symptoms of SAH include a severe headache of rapid onset, vomiting, decreased level of consciousness, fever, sometimes seizures and death. Non-contrast head Computerized Tomography (CT) is useful in diagnosis as is a lumbar puncture that is positive for erythrocytes (Abraham and Chang, 2016).

The Role of CSF in Meningitis

Meningitis is an inflammation of the meninges, the protective coverings of the brain and spinal cord. Meningitis can be classified as aseptic and bacterial. Aseptic meningitis typically is the results of a viral infection of the CSF but can also be due to fungi, medications, injury or cancer metastasis. Symptoms include fever, headache and nuchal rigidity. Other symptoms include confusion or altered consciousness, vomiting, and photophobia and hyperacusis. Young children often exhibit only nonspecific symptoms, such as irritability, drowsiness, or poor feeding. Diagnosis is made via lumbar puncture and analysis of CSF. Treatment is usually supportive, controlling fever and pain levels.

Bacterial meningitis has a much lower incidence than aseptic meningitis but is much more serious and can be life-threatening. Additional symptoms include seizures and focal neurologic signs. Some forms of meningitis are preventable by immunization with the meningococcal, mumps, pneumococcal, and *Haemophilus influenzae* type B vaccines. The CSF sample is usually cloudy in appearance, with a low glucose level, and potential positive gram stain and culture. Patients believed to have bacterial meningitis are typically receiving broad-spectrum antibiotics immediately to prevent clinical deterioration and once culture results return, the clinician can adjust the antibiotic regimen. Most patients with bacterial meningitis who receive appropriate treatment recover without complications (Putz, Hayani and Zar, 2013).

Benign Intracranial Hypertension

Idiopathic intracranial hypertension (IIH) or Pseudotumor Cerebri Syndrome (PTCS) is a rare medical condition in which intracranial pressure is raised without the occurrence of ventriculomegaly or brain tumors. This condition occurs approximately 9 in 1,000,000 in the general population annually. Interestingly, before puberty, both females and males are equally affected, but after puberty, women are affected nine times more often, signifying the existence of a sex difference. PTCS most commonly affects obese women of childbearing age. Women between the ages of 20 and 44 years, weighing 20% more than their ideal body weight have an incidence rate of 19.3/100,000. Pseudotumor cerebri literally means “false brain tumor” because the signs and symptoms are similar to those of a large brain tumor but the pathogenesis is still not well understood. The dominating theory of PTCS proposes decreased absorption of CSF at the arachnoid villi or the olfactory lymphatics. CSF analysis is critical in the diagnosis of PTCS. A CSF pressure greater than 250 mm CSF in adults

and 280 mm CSF in children and adolescents is indicative of a PTCS diagnosis. Headache is the most common symptom and asymptomatic patients may present with papilledema detected during routine eye exams. Other symptoms of PTCS include pulsatile tinnitus, and transient visual disturbance (e.g. double vision, vision loss). Treatment options include diuretic medications like acetazolamide that decrease CSF secretion from the choroid plexus and surgery for patients experiencing worsening vision caused by papilledema. Surgical options include optic nerve sheath fenestration and ventriculoperitoneal or CSF shunting. Most patients with PTCS have a good outcome, although a small percentage of patients may continue to experience persistent headaches or blindness (Friedman, 2014).

Neurodegenerative diseases

The reduction of CSF turnover may contribute to the accumulation of metabolites and protein aggregates seen in aging and neurodegenerative diseases. Aging is a major risk factor for neurodegenerative diseases. The commonality amongst the neurodegenerative diseases is the accumulation of protein aggregates; e.g. α-synuclein in Parkinson's disease, amyloid and tau in Alzheimer's disease, and huntingtin in Huntington's disease (Soto, 2003). The reduction of CSF turnover may contribute to the accumulation of metabolites seen in aging and neurodegenerative diseases. Perhaps the importance of glymphatic clearance is best demonstrated by the fact that its dysfunction plays a role in the pathophysiology of various neurodegenerative diseases, as we will see below.

Perivenous fluid (and solute) drain from the brain predominantly alongside ventral veins. Once within the subarachnoid CSF, solutes such as Aβ can exit the brain via

arachnoid villi or meningeal lymphatic vessels or along cranial and spinal nerves. However, a proportion can recirculate into brain via periarterial spaces. Periarterial solute may seed and accumulate within the base membranes of smooth muscle cells, precipitating conditions such as cerebral amyloid angiopathy (Kim *et al.*, 2020).

In aged mice and in transgenic mice modeling Alzheimer's disease, glymphatic CSF influx is reduced and the clearance of amyloid-β is significantly impaired (Kress *et al.*, 2014; Peng *et al.*, 2016). Intriguingly, treating young wild-type mice with Aβ suppressed glymphatic influx, suggesting that there is a bidirectional feedback loop of toxic Aβ aggregation because of a glymphatic flow dysfunction but the increased Aβ burden may also be an independent cause of glymphatic dysfunction (Peng *et al.*, 2016).

Furthermore, glymphatic impairment is a characteristic feature of cerebrovascular disease, including subarachnoid hemorrhage and acute ischemic stroke (Gaberel *et al.*, 2014). Microinfarcts associated with age-related dementia also appear to be related to glymphatic dysfunction as a murine model demonstrated disruption of glymphatic function focally around microinfarcts, suggesting that microlesions may trap proteins within the brain parenchyma, enhancing the risk of amyloid plaque formation (Wang *et al.*, 2017). In addition, traumatic brain injury in mice reduced glymphatic function by ~60%, causing reduced clearance of cortical interstitial solutes such as Aβ (Iliff *et al.*, 2014).

Parkinson's Disease (PD) is characterized by debilitating motor and non-motor symptoms and the hallmark pathological features include the accumulation of the neuronal protein α-synuclein that form aggregates called Lewy bodies in advanced disease, especially in the substantia nigra where there is also the characteristic loss of

dopaminergic neurons. A handful of studies has also implicated meningeal lymphatic drainage dysfunction in disease models and more recently, in humans. In mice overexpressing mutant A53T α-synuclein, blocking meningeal lymphatic drainage via ligation of deep cervical lymph nodes resulted in reduced glymphatic influx of CSF tracer, accompanied by perivascular aggregation of α-synuclein and impaired polarization of AQP4 expression in the substantia nigra. In addition, it led to exacerbated glial activation, inflammation, dopaminergic neuronal loss and motor deficits (Zou *et al.*, 2019). Similarly, in mice injected with α-synuclein preformed fibrils, the development of α-synuclein pathology resulted in delayed meningeal lymphatic drainage, loss of tight junctions among meningeal lymphatic endothelial cells and increased inflammation of the meninges. Finally, blocking flow through the meningeal lymphatic vessels in these mice led to increased α-synuclein pathology and exacerbated motor and memory deficits (Ding *et al.*, 2021). These findings were extended to humans where the researchers used dynamic contrast-enhanced MRI to assess meningeal lymphatic flow in cognitively normal controls and patients with idiopathic Parkinson's Disease (PD) or atypical Parkinsonism. Fascinatingly, they observed that patients with idiopathic PD exhibited significantly reduced flow through the meningeal lymphatic vessels along the superior sagittal sinus and sigmoid sinus, in addition to a significant delay in deep cervical lymph node perfusion, compared to patients with atypical Parkinsonism (Ding *et al.*, 2021), demonstrating that it aggravates α-synuclein pathology and contributes to the progression of PD.

Finally, CSF-ISF exchange within the glymphatic pathway can be enhanced by: 1) boosting cerebral arterial pulsatility (Iliff, Wang, *et al.*, 2013), 2) during sleep (Gaberel *et al.*, 2014), and 3) a lateral head position during sleep (Lee *et al.*, 2015). However, none of these factors have yet to demonstrate improved waste clearance in

any of the abovementioned diseases. Therefore, two critical gaps to be filled in future glymphatic research is to assess its potential as an early biomarker of neurological diseases and to identify novel therapeutic targets that can modify glymphatic flow with the goal of enhancing clearance of toxic extracellular solute (ie. protein aggregate) accumulation.

CSF as a source of biomarkers

CSF levels and composition are strictly maintained, thus any deviation from the physiological levels (i.e. intracranial pressure and constituent levels) can theoretically be used as biomarkers for disease diagnosis (Ma *et al.*, 2019; Mestre *et al.*, 2020).

Lumbar puncture, also known as a spinal tap, is a relatively safe, invasive procedure in which CSF is removed from the subarachnoid space. LP is commonly used to collect CSF for diagnostic purposes (ie. infection, haemorrhage, tumors and autoimmune diseases like multiple sclerosis). For example, the presence of xanthochromia, a yellow-orange discoloration of CSF caused by red blood cell degeneration, indicates the possibility of a subarachnoid hemorrhage. Elevation in concentrations of immunoglobulins, termed oligoclonal bands, may indicate the presence of a systemic infection or an autoimmune disease like Multiple Sclerosis. LP is also used to measure intracranial pressure in order to diagnose, and in some cases, relieve hydrocephalus. It can also be used to deliver drugs intrathecally, typically for epidural anesthesia but also for analgesia, chemotherapy and more recently, for the delivery of the antisense oligonucleotide, nuriseren, for the treatment of spinal muscular atrophy (Finkel *et al.*, 2016). During an LP, the patient is placed in the lateral recumbent (fetal) position. A sterile spinal needle is then slowly inserted between vertebrae, usually at the level of L3/4 or L4/5, into the subarachnoid space.

Contraindications of LP include increased intracranial pressure, bleeding disorders, and local skin infection. Rare complications of LP include infection, bleeding, radicular pain, or cerebral herniation. The most common complication is a post-LP headache with symptom onset within 24 hours that lasts up to 10 days (Doherty and Forbes, 2014).

A high unmet medical need for neurodegenerative diseases is the identification of biomarkers for early detection and assessment of disease progression that would aid diagnosis and prognosis, respectively. CSF provides a window into the health, and consequently, the disease status of the brain. Thus, CSF biomarkers holds invaluable potential as prospective future biomarkers for diseases such as Alzheimer's disease (AD), frontotemporal dementia (FTD), Prion Disease, Motor Neuron Disease (MND), Parkinson's Disease (PD) and Extrapyramidal Syndrome (EPS) (summarized in Table 1).

Specifically, AD pathology, linked to amyloid-β (Aβ) and tau pathology is associated with elevated CSF Aβ42, decreased CSF T-tau and decreased CSF P-tau (Ritchie *et al.*, 2017). However, measurements of ratios such as CSF Aβ42/Aβ40 are becoming more prevalent and may prove to be more superior in terms of predictability (Blanco-Cantó *et al.*, 2017). Potential biomarkers such as YKL-40 (marker of glial inflammation), VILIP-1 (marker of neuronal damage), NFL (marker of neurodegeneration), Ng (synaptic protein), and UCH-L1 (neuron-specific cell cycle enzyme) are also being investigated for various neurodegenerative diseases (Robey and Panegyres, 2019). With respect to FTD, disease heterogeneity is a major challenge in identifying specific markers but some success has been achieved with tau ratios, for example, decreased CSF P-tau181/T-tau, but further work is required (Hu *et al.*, 2013; Borroni *et al.*, 2015). Regarding Prion disease, the development of a new

assay technique called real-time quaking-induced conversion, has enabled the detection of pathological prion proteins (PrP^{Sc}) in biological samples such as CSF (Atarashi *et al.*, 2011). The 14-3-3 protein has also been a popular biomarker for prion disease; however, its specificity and sensitivity are questionable. However, newer data indicates that 14-3-3 protein levels may be combined with other biomarkers (e.g. Aβ42, T-tau, NFL, etc.) to provide a more improved readout (Matsui *et al.*, 2011). As far as MND is concerned, relatively fewer studies have been performed. The most promising biomarkers appear to be NFL and pNFH, however, the specificity of elevated NFL and elevated pNFH for MND is uncertain (Li *et al.*, 2016). Newer biomarkers such as lipids and Sonic Hedgehog protein have shown promising results (Robey and Panegyres, 2019). With respect to PD, most work has been done with α-synuclein, demonstrating for the most part decreased CSF levels, but some patterns with nonspecific markers such as NFL may also prove to be useful but further work is required (Hong *et al.*, 2010). Other biomarker studies include examining PD-associated genes (e.g. PARK5, PARK7, LRRK2, GBA), but additional studies are needed. Furthermore, oxidative stress markers, Aβ42, T-tau or P-tau are also currently being investigated (Robey and Panegyres, 2019). Finally, EPS includes neurodegenerative diseases characterized by differing pathologies (e.g. α-synucleinopathies, tauopathies) with overlapping clinical features such as include multiple system atrophy (MSA), corticobasal degeneration (CBD) or Progressive supranuclear palsy (PSP). Some attempts have been made to identify patterns of both specific and nonspecific biomarkers but findings need to be verified.

Overall, CSF biomarkers demonstrate great potential as future diagnostic and prognostic tools in neurodegenerative disease research but further studies are needed before clinical application. Another potential approach in exploiting the contents of

the CSF for diagnosis is the use of CNS-derived exosomes that cross the blood-brain-barrier into the bloodstream. CNS-derived exosomes have drawn considerable attention recently as a source of biomarkers for various neurodegenerative diseases as they can be isolated via a minimally invasive blood draw and reflect the biochemical status of the CNS (Hornung, Dutta and Bitan, 2020). The future diagnosis and treatment of neurodegenerative diseases is reliant on a combination of brain imaging and CSF (as well as other biofluid) biomarkers to more accurately predict disease onset, track progression and thus, yield maximal clinical and therapeutic benefit. This emerging field of research includes the development of theranostics, drugs that can serve as both diagnostics (e.g. radioactive tracers) and therapeutics; currently focused on cancer but nonetheless this technology holds exciting possibilities for the future diagnosis and treatment of neurological diseases as well.

Table 1. Potential cerebrospinal fluid biomarkers in neurodegenerative disorders. Each column represents a specific disease (AD: Alzheimer's disease; FTD: Frontotemporal dementia; Prion Disease; MND: Motor neuron disease; PD: Parkinson's disease; EPS: Extrapyramidal syndrome) and each row a potential biomarker (Aβ: amyloid; T-tau: total-tau; P-tau: phosphorylated tau; NFL: neurofilament light protein; PrP: prion protein; pNFH: phosphorylated neurofilament heavy chain); Red boxes: useful biomarkers; gold boxes: promising biomarkers, further study required (Robey and Panegyres, 2019, available under the Creative Commons License CC BY-NC-ND 4.0).

	AD	FTD	Prion Disease	MND	PD	EPS
$A\beta_{42}/A\beta_{40}$						
$A\beta_{42}$						
T-tau						
P-tau						
NFL						
YKL-40						
VILIP-1						
$P\text{-tau}_{181}/T\text{-tau}$						
14-3-3 protein						
PrP^{sc}						
pNFH						
α-synuclein						

A novel gene target for congenital hydrocephalus

Recent research in mice points to a critical role of Geminin coiled-coil domain-containing protein 1, encoded by the GemC1 gene, in the regulation of neural stem cell generation and multiciliated ependymal cell differentiation in the subventricular zone. GemC1 knockout mice demonstrate a hydrocephalic phenotype with dome-shaped heads and dilation of lateral ventricles (Figure 9). These findings provide evidence for the involvement of GemC1 in the pathogenesis of congenital hydrocephalus in humans (Lalioti *et al.*, 2019).

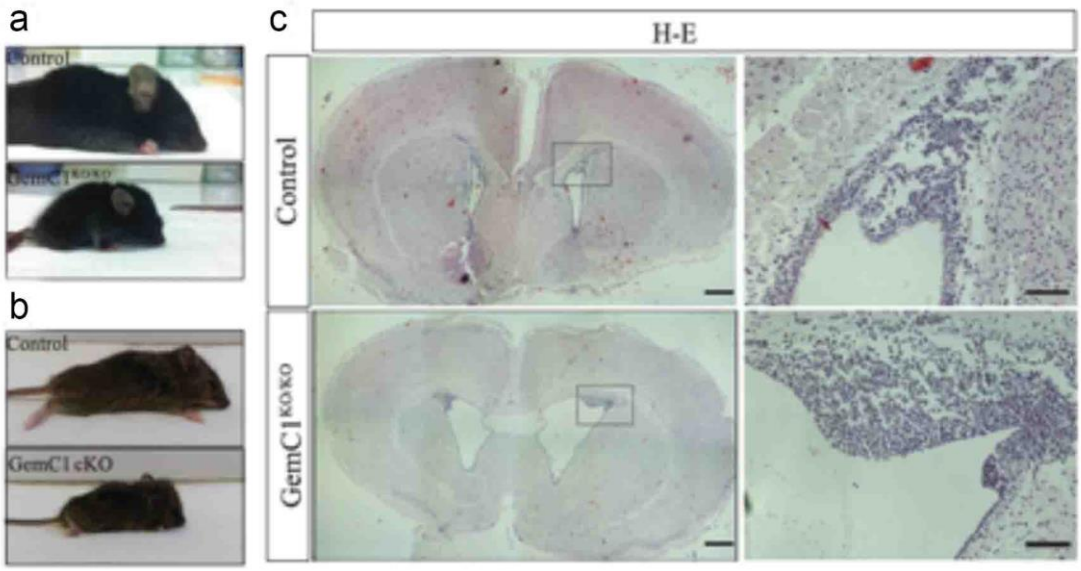

Figure 9. GemC1 knockout mice have a hydrocephalic phenotype. Lateral views of postnatal day 29 GemC1 homozygous knockout (KO/KO) (a) and conditional KO (cKO) (b) (and their control littermates), displayed a dome-shaped head, characteristic of hydrocephalus. Histological analysis of mouse brain coronal sections from postnatal day 9 GemC1KO/KO mice stained with Hematoxylin–Eosin (H–E), demonstrates dilation of the lateral ventricles (adapted from Lalioti et al., 2019, permission obtained for reuse).

Cilia, CSF and idiopathic scoliosis

Recent findings support a role of motile cilia and/or CSF flow defects in idiopathic scoliosis (IS) (Grimes *et al.*, 2016; Boswell and Ciruna, 2017). Population-based association studies and next-generation sequencing approaches have identified numerous disease-associated genetic loci. However, appropriate animal models are lacking. Zebrafish demonstrate a natural susceptibility towards developing spinal curvatures and numerous zebrafish mutants have recently been used to successfully model human IS. These zebrafish studies demonstrate a novel role for motile cilia-directed CSF flow in physiological spine morphogenesis, and implicate cilia motility and CSF flow defects as the underlying biological cause of spinal curvatures. Whole-exome sequencing studies have identified IS-associated variants in the centriolar protein POC5 (Patten *et al.*, 2015). Since ciliary basal bodies are recycled centrioles, this finding supports a link between cilia defects and human IS. On the other hand, patients with primary ciliary dyskinesia lack functional motile cilia (Knowles *et al.*, 2013) and have an increased incidence of scoliosis (5–10%) (Engesaeth, Warner and Bush, 1993; Knowles *et al.*, 2013). However, if motile cilia dysfunction were the primary cause for IS, this number would be expected to be higher, albeit, hydrocephalus, i.e. CSF flow dysfunction, is also not common among patients with primary ciliary dyskinesia. In humans, CSF flow is driven by external forces, such as gravity, respiration, movement, and even heartbeat (Simon and Iliff, 2016) and because CSF is produced and absorbed locally in the ventricular system, cilia may have a more passive role in regulating CSF dynamics when compared to zebrafish. But while mechanisms driving CSF flow differ between humans and zebrafish, it is hypothesized that CSF composition and functions and hydrodynamic forces, are conserved across taxa.

Several independent studies support a conserved role for CSF flow in mammalian spine development. Experimental manipulation of CSF flow by kaolin injection into the subarachnoid space has been shown to cause scoliosis in both dogs (Chuma *et al.*, 1997) and rabbits (Turgut *et al.*, 2005). Developmental scoliosis is also observed in several human conditions related to CSF flow dysfunction, including neural tube closure defects, spinal canal cysts, and Chiari malformations (i.e., herniation of the cerebellum through the base of the skull) (Goel, 1999; Ozerdemoglu, Denis and Transfeldt, 2003; Verhoef *et al.*, 2004; Kelly, Guillaume and Lenke, 2015). Interestingly, scoliosis associated with Chiari malformation (with an incidence as high as 60%) is often resolved following decompression surgery (Kelly, Guillaume and Lenke, 2015). Notably, the ptk7 locus associated with IS (Hayes *et al.*, 2014), whose zebrafish mutant exhibits distinct features of IS (Grimes *et al.*, 2016; Boswell and Ciruna, 2017) (Figure 10), has also been independently linked to neural tube defects in humans (Wang *et al.*, 2015)- suggesting that IS could potentially result from neural tube defects (e.g. dural tears) that disrupt CSF homeostasis and/or dynamics.

Finally, the mechanism of cilia-driven CSF flow appears to involve the generation of adrenergic signals that induce urotensin neuropeptides in CSF-contacting neurons along the spinal cord to regulate axial straightening (Zhang *et al.*, 2018). In addition, a recent study has suggested that the Reissner fiber also plays a role in axial morphogenesis (Cantaut-Belarif *et al.*, 2018). The Reissner fiber is a dynamic extracellular proteinaceous structure formed within the brain ventricles and central canal and SCO-spondin is the major protein component of the Reissner fiber. SCO-spondin can bind and transport adrenaline and other monoamines (Caprile *et al.*, 2003), thus, implicating the Reissner fiber in the regulation of axial straightening via the adrenergic pathway.

Currently, the only treatment options available for IS are supportive braces or surgery to correct the spinal deformities. Hence, the urotensin signalling pathway holds promise for future potential pharmacological treatment of IS.

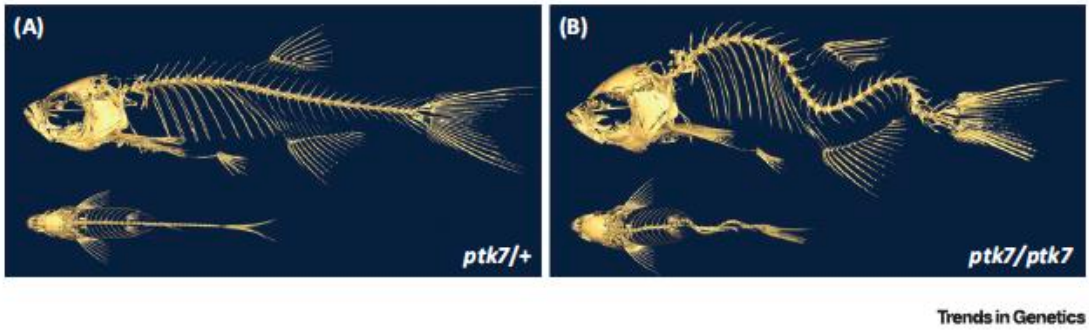

Figure 10. Visualization and analysis of idiopathic spinal deformity in adult zebrafish by microComputed Tomography (microCT). Protein tyrosine kinase 7 (A) Lateral microCT image of protein tyrosine kinase 7 (ptk7) heterozygous sibling and (B) ptk7 mutant zebrafish (dorsal view in lower left corner). These mutant fish exhibit distinct features of idiopathic scoliosis (IS), including no observable vertebral defects during curve onset, complex 3D rotational deformity of the spine, and late onset of spinal curvature (Boswell and Ciruna, 2017, permission obtained for reuse).

CSF in space

Little is known about the consequences of long durations of spaceflight on the human brain. A recent study compared MRI scans from astronauts on 6 month and 12 month missions, pre- and post-flight. Their results revealed that spaceflight causes enhanced ventricular volume and these changes may endure for long periods after flight (Hupfeld *et al.*, 2020). Furthermore, in a retrospective study of diffusion pre- and post-flight MRI scans in astronauts, intracranial extracellular free water shifts and changes in brain white matter diffusion were analysed. Overall, free water volume increased in the frontal, temporal, and occipital lobes and decreased in the posterior aspect of the vertex after spaceflight (Lee *et al.*, 2019). The water redistribution may reflect headward fluid shifts occurring in microgravity as well as an upward shift of the brain within the skull. White matter changes were of a greater magnitude than those typically seen during the same period with healthy aging and were associated

with balance changes in astronauts. With the prospect of human missions to Mars closer than ever before with the recent successful landing of the rover *Perseverance* and robotic, coaxial helicopter, *Ingenuity*, as part of NASA's Mars Exploration Program, studying the long-term effects of space travel on the human brain has now become a pressing research need.

DISCUSSION

Despite the leaps in progress made regarding our understanding of CSF dynamics in the last 50 years, there is still much ground to cover in terms of validation and ultimate application in neurology and neurosurgery. Some of the priorities in CSF research worth considering for future studies follow.

With the technology toolbox available to basic research scientists, including -omics and gene deletion and editing, a priority of CSF research should include the clarification and if possible, quantification of the different CNS fluids (i.e. CSF and ISF) and their barriers in health and disease; mainly trauma, tumors, hydrocephalus, edema, infection, ischemia, aging, and degeneration.

With respect to CNS metabolite and waste clearance and their dysfunction in neurological diseases, we must appreciate the different processes that help clearance, the role of the glymphatic system, lymphatic system, pericytes, myeloid cells, and BBB.

Studies on the immune surveillance of the CNS are also warranted. Particularly, investigation of the lymphatic vessel's presence and the movement of immune cells from the CNS to the periphery and vice versa should be a prime target. For example, why is it that multiforme glioblastoma, an extremely infiltrating brain tumor does not produce extra-CNS metastases?

Interesting clinical cases that may provide some more clues about CSF based on their pathophysiology are dilated Virchow-Robin spaces (when located at basal ganglia they are called état crible/status cribrosum), idiopathic intracranial hypertension, normal pressure hydrocephalus, and arrested hydrocephalus. For example, what is the

role of inflammation in producing hydrocephalus after infection, subarachnoid hemorrhage, or trauma?

In the clinical setting, we must strive to further our understanding of pathophysiological processes and clinical entities, and correct reference values clinical definitions and indications, where needed. For example, vascular problems acutely lead to infracts or hemorrhage but chronically may cause degeneration and neuronal malfunction that can be depicted in the CSF consistency/ biochemical status. Another example includes our treatment of ICP. When it is persistently above 15 to 20 mmHg, pharmaceutical or surgical interventions are applied. If the limit is lowered, a greater proportion of patients will be treated, however, the negative side effects of the intervention simultaneously increases. Suggested adjustments include normal values up to 5 - 10 mmHg, a “buffer zone” at 10 to 20 mmHg, currently considered pathological but the patient usually compensates without adverse effects. Thus, clinical intervention would be adjusted to an ICP above 20 mmHg.

Further exploration of CSF biomarkers (molecules, exosomes, liquid biopsies) and their clinical relevance is also relevant to early and correct diagnosis. In addition, consideration of age and sex adjustments will allow for greater precision of diagnosis and care, bringing us closer to personalized medicine approaches.

Additional clinical insights include the mapping of lymphatic vessels to strengthen the minimal invasive approach in neurosurgery where both the meninges and their vasculature are respected. Furthermore, early cranioplasty after craniectomy helps the restoration of CSF dynamics. Subsequently, ICP measurement, CSF production and absorption, and evaluation of parenchyma compliance may become less invasive, similar to dynamic contrast enhanced MR perfusion and MRI elastography.

Cisternostomy may need re-evaluation with respect to its possible benefit in a subgroup of edema patients. Documentation of the long-term effects of shunting or endoscopic ventriculostomy will also aid our understanding of CSF dynamics. Finally, our knowledge of CSF function and dysfunction should be applied not only in the clinical setting but in public health policy. Based on the established importance of brain waste clearance and the importance of sleep, campaigns should enforce the notion of healthy sleep habits and measures should be taken to protect occupations with inherent chronic sleep deprivation.

CONCLUSIONS

It seems that in medicine, research already conquered the majority of "low hanging fruits", namely the main causative factors of different biological phenomena with high clinical yield. Nowadays we struggle to unravel complex, multifactorial functions with great intrapersonal variability. Trying to navigate through such multifarious mazes requires multidisciplinary approaches with sincere cooperation between basic and clinical scientists, from the bench to the bedside and back. This epoch of accelerated big data generation demands complementary measures so that one can shift focus from the over specialized to the bird's view of the examined fields. CSF and brain barrier interfaces are an exemplary case.

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