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ABSTRACT

The ventricular system and subarachnoid space are filled with cerebrospinal fluid, which plays a key role in the nervous system. This fluid is produced by the choroid plexus, an organ rich in ion transporters that precisely control the transport of specific ions into the cerebrospinal fluid thanks to tight junctions between the plexus cells; these prevent the passage of substances other than the transporters, thus allowing for precise control of the fluid composition.

Cerebrospinal fluid production is based on a network of interrelationships between specific ion flows enabled by the numerous transporters. The fluid is cleaned and resorbed by the glymphatic system via multiple absorption pathways. Maintaining proper cerebrospinal fluid parameters is extremely important for proper brain function. Considering the fragility of the brain, even small fluctuations in cerebrospinal fluid composition can impair its condition. Therefore, to understand the nervous system, it is important to have thorough knowledge of the production, transport, and resorption mechanisms of cerebrospinal fluid.

The aim of this paper is to summarize the current state of knowledge about the mechanisms of production, pathways of absorption and physiological values of cerebrospinal fluid parameters; it also discusses the role of the glymphatic system in maintaining fluid homeostasis, and the changes resulting from its dysfunction as result of trauma.
Keywords: Alzheimer's disease, aquaporin, choroid plexus, glymphatic system, hemorrhagic stroke, ischemic stroke, lymphatic, nerves, traumatic brain injury

INTRODUCTION TO CEREBROSPINAL FLUID

The ventricular system and subarachnoid space is filled by cerebrospinal fluid (CSF). CSF is produced within the choroid plexus, which arises from the epithelium lining the lateral, third, and fourth ventricles [8, 40, 87].The fluid flows through the foramen of Monroe to the third ventricle and passes by the cerebral aqueduct to the fourth ventricle. It has been proposed that the flow of SCF should be known as the third circulation, with the first and second circulations referring to the blood and lymph [87]. Around 150 mL of CSF is present in a typical adult; of this, 25 mL occupies the ventricular system and 125 mL in the subarachnoid space of the brain and medulla. These values can vary and depend on age [68, 80]. Between 400 and 600 mL of fluid is produced daily, of which, 60–70% is made by the choroid plexus. Fluid production is affected by many hormones and neuropeptides; for example, natriuretic peptide increases fluid production by stimulating aquaporin1 (AQP1) [80]. Research on cats indicates that CSF production is also intertwined with the choroid plexus metabolism, which is related to body temperature, thus when body temperature decreases, so does the CSF production [87].

Cerebrospinal fluid has several functions. It protects against damage and shock by cushioning impacts. It transports hormones, neurotransmitters, and other substances to and from the brain to provide a stable environment, and ensures the maintenance and proper function of delicate neural tissue [54]. Its flow is not unidirectional, as commonly believed; rather it flows in multiple directions [65]. CSF facilitates the removal of metabolites from the brain [54]. Also, the fluid physically supports the weight of the brain (1300 g) [35] reducing its weight by more than thirtyfold due to the buoyancy it provides (25–50 g), allowing it to retain a high density despite its relatively small volume [54, 68, 87].

This review seeks to summarize the current understanding of the absorption and production of CSF while also highlighting the importance of the glymphatic system in CSF maintenance. It

also examines how disturbances in CSF homeostasis are linked to several challenging medical conditions, and how the progress of pathologies can be mitigated.

UNDERSTANDING MORPHOLOGICAL CHANGES

The glymphatic system — description and function

The impairment of glymphatic system (GS) function has been linked to serious conditions involving aggregation of products inside the brain, such as Parkinson or Alzheimer's disease (AD); it has hence become the focus of many studies of CSF physiology with the hope of finding alternative treatment options. The system is responsible for the production and purification of CSF by eliminating excessive metabolites [51]. In addition, together with the meningeal lymphatic system, the GS is responsible for immune response and drainage of antigens from the CSF [66].

The GS is made up of a network of intercellular spaces located in the brain [43, 49] that allow for the distribution of substrates and neurotransmitters, and mediate the exchange of lipids, vitamins, and folic acid via the CSF [40, 59]. Most likely, the GS is connected to the lymphatic system via lymphatic vessels present in the meninges. Its improper functioning can lead to the accumulation of protein deposits due to reduced CSF production, impaired resorption capacity and thus decreased CSF flow. This adversely affects brain functioning, causing neurodegenerative diseases, such as AD and Parkinson's disease [8, 40]. Studies on mice indicate that lymphatic vessels absorb molecules from CSF and pass them through the lymph to lymph nodes [60]. Genetically modified mice lacking lymphatic vessels in the meninges had significantly elevated levels of proteins in the fluid [1, 41]. It is hoped that it may be possible to modify the function of the GS to deliver drugs to specific structures and reduce the effects of neurodegenerative diseases [40, 59].

Glymphatic system and CSF production — circadian cycle

The function of the GS changes during the day. For example, CSF production decreases as physical activity increases and the functions of the GS are suppressed during the waking hours and increased during sleep [40] allowing the brain to cleanse itself of unnecessary substances and neurotoxins accumulated during the day. A study on mice based on injection of tracers found CSF production to differ by up to about 22% between sleeping and waking [34]. GS activity also differs during sleep induced by drugs or anesthetics e.g., pentobarbital and

tribromoethanol can lower CSF influx [34, 92]; indeed, active, sleeping, and anesthetized mice all demonstrated different levels of removal of radioactively-labeled beta amyloid. The rate of beta amyloid removal was significantly slower when the mice were awake, and almost doubled in speed when they were asleep or anesthetized; an insignificant difference was also noted between sleeping mice and those anesthetized with ketamine. Similarly, sleeping mice demonstrated more than double the clearance rate of radiolabeled inulin, and anesthetized mouse displayed noticeably faster clearance (around 20%) than in sleeping mice, further indicating the influence of specific anesthetics [84].

Cerebrospinal fluid production is regulated by norepinephrine. It is probably responsible for the suppression of GS activity, with 42% reduction in CSF formation observed in rabbits injected with norepinephrine [92]. Treatment with norepinephrine led to a reduction in cerebrospinal fluid (CSF) production in mice [84], whereas inhibiting the production of norepinephrine increased CSF production. Therefore, norepinephrine plays a crucial role in governing fluid production and secretion throughout the day [40]. Individuals suffering from sleep disorders following shock or trauma may encounter impaired function of the glymphatic system (GS), causing a decline in CSF fluid production and circulation, along with its associated markers. This could considerably impact the interpretation of marker results due to dilution or concentraton of the CSF [40].

In addition, the production and clearance of CSF by the GS also varies with age. Ageing lymphatic vesicles lose their elasticity and become narrower, which may result in reduced lymphatic and glymphatic drainage. Indeed, chemical ablation of lymphatic vesicles in mice resulted in significantly lowered intellectual performance, even when blood flow remained constant [81]. Fluid production decreases with age, resulting in reduced clearance of harmful substances from the brain [40], e.g., beta amyloid removal reduced by 40% in older mice compared to younger ones [50], translating into decreased CNS functioning resulting from the accumulation of metabolic products and insufficient substrate supply. It is believed that the quantity and quality of aquaporins, particularly aquaporin 4 (AQP4), responsible for transporting water into the CS, contributes to reduced production [65].

MECHANISM OF PRODUCTION, COMPOSITION, CONTROL AND HOMEOSTASIS

Production

Most of the CSF is produced by the choroid plexus [49, 87], which is composed of highly-vascularized epithelio-endothelial convolute [18], connective tissue stroma, and many immune cells [86]. The ventricular side is composed of a single layer of cubic cells called epiplexus cells. The plexus also contains numbers of phagocytic Kolmer's cells, which find abnormal substances and remove them [86]. The choroid plexus has numerous receptors to precisely control the composition of the CSF [18, 86].

Most studies indicate that the choroid plexus produces more than 50% of the CSF volume, with the remainder coming from interstitial fluid generated by permeation of the BBB and ventricular lining cells [23, 86]. Intercellular junctions in the choroid plexus [18] strictly regulate the amounts of permeating substances. The integrity of the plexus is guaranteed by various proteins, such as claudins, occludins and zonulins [23, 86]. Choroid plexus cells are closely arranged and additionally surrounded by astrocyte protuberances, forming a tight blood-brain barrier (BBB). The brain is separated from the blood by the blood-cerebrospinal fluid barrier (BCSFB) This barrier plays a key role in preventing harmful or undesirable substances from entering the CSF and in limiting the spread of inflammatory processes [86].

It has been proposed that primary source of CSF production is the cerebral capillaries [39, 65]; however, studies have found water exchange to differ from CSF production [87]. CSF composition is managed by transporters located in the cell membranes, which allow molecules to pass from the blood to the CSF; maintaining tight control of such transport is crucial for proper nervous system function [40, 68].

The CSF is formed in three distinct stages [40]. Firstly, plasma is filtered passively along a concentration gradient, with ions entering the fluid via ion exchangers and transporters. Following this, substances are actively transported into the CSF [26, 80]. Finally, the generated concentration gradient induces water transport through dedicated transporters, mainly aquaporins (AQP). CSF production is a complex process involving multiple and interrelated ion flows [26, 40, 68, 80]. While the entire volume of CSF is exchanged four or five times per day in young adults [35, 40, 68, 80], the process slows to about three times a day with age due to reduced brain volume, requiring greater CSF volume [35], and limited fluid production [80].

Beginning of CSF production

As the embryo develops, CFS production begins with the closure of the neural groove and the formation of the neural tube [18]. When the tube closes, the amniotic fluid gets trapped inside, thus becoming the first CSF fluid [18]. In early development, the choroid plexus is not yet

active, and the pressure inside the tube increases. [18]. Hence, in this the initial stage, CSF production most likely occurs in another structure not yet determined. However, the composition and volume of CSF is strictly controlled from the beginning, as evidenced by research on chicken fetuses: non-chicken proteins injected into CSF were rapidly eliminated while endogenic proteins from the fetus increased CSF pressure; this increase was compensated for by a quick response [17, 18]. The formation of the plexus begins around day 41 in the fourth ventricle [18, 24], following the formation of the lateral ventricle plexus. It derives from neuroepithelium and mesenchymal cells from the roof of the third ventricle. The last plexus formed is the choroid plexus of the third ventricle; although it is unknown when the choroid plexus takes over fluid production, it probably starts shortly after the end of its development [18, 24, 80].

No arachnoid villi were found to be present in sections of human fetus prior to the week 39 [72]. Their absence suggests that in neonates, the primary mode of absorption is probably through olfactory nerves [15, 72]. Around week 39, the first arachnoid granules appear, which will continue to develop until about 18 months and allow absorption of most of the CSF. If the granules are absent, then other structures take over their role [80]. The granules proliferate and slowly grow into the sagittal sinus through the dura. The growth of granules is thought to be stimulated by an increase in CSF pressure [73].

The CSF of chicken [**29**], sheep [**63**] and rat [**27**] embryos were found to have higher concentrations of proteins in the early embryological stages. This concentration fell over the following developmental stages, reaching values similar to mature individuals [**27, 29, 63**].

Control

Cerebrospinal fluid secretion is controlled by the autonomic system from the superior cervical plexus. Fibers from the glossopharyngeal and vagus nerves supply the choroid plexus parasympathetically, increasing CSF secretion [21, 86]. Sympathetic innervation reduces CSF production, and hence these systems probably influence diurnal variation in CSF production [80]. An excellent example of this control method was demonstrated in rabbits: stimulation of the upper cervical ganglia resulted in a 32% reduction in CSF production, while bilateral excision of the superior cervical ganglia caused a 33% increase in production [57].

FACTORS INFLUENCING CSF PRODUCTION

Composition and changes in pathology

Cerebrospinal fluid is a derivative of plasma and hence has a similar composition. Physiologically, CSF should not contain erythrocytes [40]. It also typically demonstrates higher magnesium and chloride ion concentrations and lower sodium, calcium, potassium and protein levels [25, 26, 40, 68, 80]. CSF also has lower glucose levels, i.e. around 60% of serum, and correlate with serum glucose levels [37]. The CSF glucose values are also influenced by food intake, decreasing during periods of low food intake [91]; however, CSF glucose remains unchanged for two to four hours after digesting food [91].

Changes observed in the CSF during central nervous system (CNS) infection include: (1) increased levels of protein, (2) elevated glucose levels, particularly in the ventricular region, (3) higher white blood cell counts, and (4) elevated lactate levels resulting from anaerobic glycolysis due to hypoperfusion. Additionally, (5) positive bacterial culture may be present [31]. It's noteworthy that only one out of four patients with CNS infection had a positive CSF culture for bacteria in both ventricular and lumbar puncture fluids. This discrepancy might be attributed to the slow migration of bacteria from the ventricular area to the spinal canal. Thus, it suggests that infections located in the ventricular area may not always be detectable in samples obtained from lumbar reservoir fluid [31].

Moreover, elevated red blood cell counts have been observed, likely stemming from the fluid collection method. Lumbar puncture is more prone to causing damage to small vessels, thereby potentially inflating the results [19, 31].

Similarly, leptomeningeal metastases, a frequent complication of disseminated cancers, are almost 30% more likely to be detected in ventricular CSF samples when the lesion is located within the cranium compared to those in the lumbar cistern. Similarly, if the lesion is located within the spine, samples taken from the lumbar cistern are 30% more likely to be positive in comparison to ventricular samples. Therefore, samples should be taken from both locations to set a proper diagnosis, to confirm the response to the applied chemotherapy, and modify treatment accordingly [19]. However, this diagnostic method is effective only in the case of metastatic tumors: it is much more likely to give a positive cytology sample in such cases compared to a focal tumor. When the tumor is limited to the brain and does not breach the pia mater, it almost never yields a positive sample [31]. Alternatively, quantitative PCR could be applied as a more sensitive method [71].

TECHNIQUES FOR STUDYING MORPHOLOGICAL CHANGES

CSF reabsorption — possible pathways

Cerebrospinal fluid absorption is mediated by different structures working in parallel that complement each other. The general understanding of CSF absorption through the arachnoid granules has changed recently. It is believed that most CSF is absorbed through the olfactory bulb and along the cranial and spinal nerves [40, 43]. The proportions of absorption may change according to need; for example, as a pressure difference of 3 to 5 mmHg is required for efficient absorption, CSF resorption may be reduced when sagittal sinus pressure is elevated due to overproduction or blockage of drainage pathways [80]. Most CSF absorption studies are based on the use of contrast agents or marked proteins; however, this method runs the risk of a false result due to the proteins recirculating back into the fluid. The introduction of proteins and contrast agents into the CSF disrupts osmolarity, which is one of the key factors affecting CSF production. This can induce changes in transport intensity and inaccurate results. It is believed that there are probably other, still unrecognized, methods of CSF resorption not detectable by existing methods. Various studies [13, 43, 60, 80] indicate the existence of complementary resorption methods that work together [73]. In transgenic mice, complete agenesis of dural lymphatic vessels had no effect on intercranial pressure, suggesting that other pathways had taken over transport [4].

METHODS OF CEREBROSPINAL FLUID ABSORPTION

Arachnoid granules and absorption

Arachnoid granules are indentations of the outer part of the arachnoid epithelium that pass through the dura mater into the sinus lumen. There are several types of arachnoid granules: (I) incompletely passing through the dura mater, (II) fully penetrating the dura mater, (III) penetrating the interstitium surrounding the nerves, and (IV) existing within the subarachnoid space (V) [73]. Within the cranium, CSF absorption occurs within the venous sinuses, mainly in the superior sagittal sinus. In contrast, absorption into the supraspinal venous plexus of the spinal cord predominates in the spinal cord.

Early research was based around injecting a dye into the CSF and observing its migration, its concentration around the arachnoid villi and passage through to the sagittal sinus. This research has highly influenced the view of CSF resorption [94]. One of the most criticized points of the research is that the dye was injected under high pressure, which might have influenced on the results [16]. This aspect is essential since the other researchers have suggested that like the valves located in the canal of Schlemm, arachnoid villi can work as one-way valves which open under elevated pressure. Indeed, electron microscopy performed

on arachnoid villi confirmed the presence of microscopic valves which work by increasing the volume of a vacuole inside the cell. This leads to opening of a transcellular canal which allows for outflow of excessive CSF fluid [16, 89]. This observation was supported by tomography electron microscopy (TEM) and scanning electron microscopy (SEM) data in monkeys, indicating changes in arachnoid villi under different CSF pressures. The SEM data confirmed that villi taken from subjects with elevated CSF pressure were distended with higher numbers of intracellular pores. This mechanism works only under elevated CSF pressure, and is associated with four- to ten-times greater CSF absorption over an elongated period [53]. However, no similar relationship was noted on different species, probably due to each species having a unique CSF absorption rate, which influences the point at which valves open [53]. Blockage of this transport pathways can occur following fibrosis of the arachnoid granules, which can increase the risk of hydrocephalus; however, this is rare [80].

Absorption via the lymphatic system

Lymphatic vesicles in the brain are also referred as meningeal lymphatic vesicles. They have a similar composition to other lymphatic vesicles, although some differences are visible. They are smaller and concentrated tightly, whereas those outside the brain are more dispersed [60]. The lymph is drained to deep cervical nodes only: not into superficial cervical nodes.

Currently, the lymphatic vesicles are believed to play a key role in clearing the CSF and absorbing it [60]. Rats, sheep and rabbits have demonstrated that 30–50% of CSF is resorbed via the lymphatic system [85]. Despite the presence of basal and dorsal lymphatic vesicles inside the brain, studies based on injecting tracers and observing their flow indicate that most drainage occurs through the basal vesicles [1]. They are most commonly present in the dura mater, but nearly absent in the pina and arachnoid mater. Lymphatic vesicles are present at the highest densities around large blood vesicles, like the superior sagittal sinus, vesicles of the dura matter [60] and the middle meningeal artery [76].

The number of vesicles falls with age, as does the rate of flow, which may lead to lowered cleansing. To compensate, the vesicles therefore tend towards hyperplasia. A section of the basal lymphatic vesicles of aged mice found the number of valves to decrease with age, while the structure of collagen IV becomes more disturbed. Additionally, the expression of genes responsible for maintaining the valve structures reduced with aging [1]. Moreover, the junctions between lymphatic endothelial cells get weaker, and their numbers decrease; for example, the amounts of zipper type junctions decreases by 40.5% in older mice, lowering

their transport efficiency [1]. Hence, it can be seen that lymphatic drainage obstruction is a risk factor for neurodegenerative diseases.

Absorption along nerves

Cerebrospinal fluid absorption along the nerves increases under the influence of gravity when in an upright position [80]. The absorption of fluid into the intercellular space along the cranial and spinal nerves occurs through the Virchow-Robin spaces. Absorption processes are also observed around the vagus, sublingual, and vestibulocochlear nerves [59], and are particularly enhanced around the olfactory, trigeminal nerves [80]. CSF flow into the subarachnoid space of newborn sheep was visualized by injecting Microfil, a yellow tracer, and observing its location during a section. Traces of Microfil were located in the endoneurium of cranial nerves [97], confirming that the epineurium is involved in transporting substances from CSF into distant lymph nodes [72]. The findings also indicate that this transport intensifies during elevated cranial pressure; this was confirmed by sealing the cribriform plate of sheep, thus closing the main drainage route, leading to increased cranial pressure and greater transport along the nerves [72].

Optic nerve

Absorption along the optic nerve is possible and it can be severely enhanced during hydrocephalus. The presence of orbital interstitial fluid can be noted in almost all T2 weighted magnetic resonance images of children below three years of age; this should not be considered a pathological state, but rather possible route of CSF reabsorption in underage children [79]. Currently, it is still unclear whether resorption occurs in the vicinity of the nerve or in its hollow [59]. It has been suggested that resorption may occur by fluid entering the nerve and being absorbed along the nerve or axons [43].

Other research has focused on impairment of function in pathological states such as glioma in mice. In one study, tracers were injected into the CSF and its flow was observed. Surprisingly, the tracer concentration in the region of the optic nerve was about 2.5 times smaller in mice bearing glioma than in control models [62]. Moreover, after injecting tracers into the cisterna magna and observing them using time of flight MRI, a high concentration was noted around the optic nerve. However, the concentration remained constant over time, unlike in the control group, where it degraded over time. This empathizes that CSF reabsorption by perineural route is severely impaired during pathological states. To compensate for this impairment, CSF is more intensely reabsorbed in regions which are not affected by trauma. This was evidenced

in MRI scans of the spinal region which found eight out of 12 mouse subjects to demonstrate an elevated number of tracers in the thoracic/sacral lymph nodes [62].

Olfactory nerves and nasal mucosa

An important site of absorption is the region of the cribiform plate. Removal of the olfactory nerves and closure of the cribriform plate was found to result in a significant increase in intracranial pressure in animal studies [13, 67, 72]. When the cribriform plate is sealed accompanied by closure of the spinal cord connection, a significant increase in intracranial pressure, to almost twice the normal value (15–30 cm^3 H_20), occurs four to five hours after the procedure [67]. A similar increase of intercranial pressure was also noted following closure of the cribriform plate [42].

Injection of India ink as a marker into the cisterna magna in rats resulted in high concentrations being noted around the cribriform plate, followed by the nasal submucosa and lymphatic vessels of the plate. Small traces were also noted in the deep cervical lymph nodes [47]. Studies in sheep also found that when intercranial pressure is elevated, the amount of radioactive protein tracers injected into the CSF increases inside the cervical lymph nodes [14]. This observation indicates that CSF absorption is the product of several mechanisms, of which lymphatic absorption is paramount and arachnoid granule-mediated absorption plays a secondary role [13, 80]. This pathway is much more active in neonates and results from immature arachnoid granules. It is also more important in the elderly due to the decreased efficiency of granule-mediated absorption [80].

TRANSPORTERS

The formation of CSF depends mainly on the transport of Na^+, K^+, $Cl^–$, $HCO_3^–$ ions and the water accompanying them. This transport is facilitated by numerous transporters present within the choroid plexus [8, 86], e.g., Na^+, K^+, $Cl^–$ (cotransporter — NKCC1), K^+, $Cl^–$ (cotransporter — KCC), Na^+/H^+ (exchanger — NHE1), channels for $Cl^–$ ions, Na^+-K^+-ATPase (responsible for formation of about 50–60% of the CSF [58]), and aquaporins including AQP1 (water transport) [13, 46, 55, 56].

Table 1. Transporters located on the cell membrane of choroid plexus cells facing the cell lumen.

Ion exchanger	**Na^+/K^+-ATPaza**	**AQP1**	**NKCC1**	**NHE**	**K^+ channels**

Inside	3 Na^+	H_2O	–	Na^+	K^+
Outside	2 K^+	–	Na^+, K^+, 2Cl^-	H^+	–

*Outside indicates into the cell lumen; *inside* indicates into the cell; NHE — sodium hydrogen exchanger [13, 46, 55, 56].

Table 2. Transporters located on the cell membrane of choroid plexus cells targeting the vascular lumen.

Ion exchanger	**AE2**	**AQP1**	**NBC**	**KCC2**
Outside	$HCO3^-$	–	–	Cl^-, K^+
Inside	Cl^-	H_20	Na^+, HCO_3^-	

**Outside* indicates into the vessel lumen; *inside* indicates into the cell interior; AE2 — epithelial anion exchanger; KCC2 — potassium chloride cotransporter; NBC — sodium bicarbonate coexhanger; NHE — sodium hydrogen exchanger; NKCC1 — sodium potassium chloride cotransporter.

Cerebrospinal fluid plays a key role in lipid transport to CNS structures. The secretion of lipoproteins occurs mainly via apolipoproteins E and J; apolipoproteins are also involved in the clearance of CSF from oxidized cholesterol and beta amyloid remnants [40]. NKCC1 is responsible for the production of approximately half of the CSF by creating an ion gradient to draw in water [13, 86]. Water transport is mediated by AQP transporters which enable rapid water transport consistent with the concentration gradient. AQP 1, 4, 9 are present in the brain [23, 56] and AQP1 and AQP4 have a major role in water transport [65]. AQP1 is responsible for up to 25% of water transport into the CSF of knocked out mice [69]. The transporters vary in location throughout the ventricular system, e.g. AQP1 is mainly present in the choroid plexus and astrocytes, whereas AQP4 localized in the plexus of third and fourth ventricle and astrocytes [23, 56].

In addition to ion transport, other transporters responsible for selective release of substances into the CSF play an essential role in detoxification processes and metabolite and xenobiotic exchange. Many of the transporters located on the surface of the choroid plexus are responsible for maintaining CSF parameters; however, many of these remain unknown. Although the transporters are distributed throughout the surface of the plexus cells, most are present in the apical part than the basolateral part [13].

CLINICAL RELEVANCE AND APPLICATIONS

Injuries

Brain damage following an accident can result in premature onset of several abnormalities, with even a single accident affecting later brain condition. Why some people are individually more resistant to brain damage than others, remains unexplained [40].

A clear indication of pathology is a change of color, as healthy CSF is clear. When blood enters the CSF, the color changes to pink, subsequently shifting to orange and yellow as the blood is broken down; similar discolorations can also be linked to hyperbilirubinemia. A green tint can indicate a bacterial infection with the presence of pus, whereas brown is associated with meningeal melanomatosis [82].

Injury stimulates increased production and release of beta amyloids and C-tau protein, a marker of brain damage [40, 78]. It is assumed that C-tau protein is responsible for damaging the brain [96], because it stimulates the formation of fibrillary aggregates that can induce the formation of neurofibrillary tangles [40, 61]; these cause the spread of pathologies resembling prion diseases, resulting in the formation of astroglial scars. The developing inflammatory process leads to a significant slowdown in GS function, and CSF production. GS impairment persists for at least 28 days because excess C-tau protein impairs the production and absorption of CSF [40]. The inflammation that appears after injury has both negative and positive effects. Microglia and macrophages protect neuronal cells and promote their recovery, stimulate neuro and angio genesis and accelerate formation of synapses. However, they also stimulate various inflammatory substances that can damage neurons, such as TNF and IFN [44]. A detailed understanding of these mechanisms may provide ways to reduce the post-accident damage caused by these inflammatory processes [40, 44].

TRAUMATIC BRAIN INJURY (TBI)

Even one accident resulting in traumatic brain injury (TBI) can induce neurodegeneration, whose effects may last for a long time [77]. Moreover, sports with a predisposition to head injury, such as martial arts, can result in with repeated small or mild brain injuries that can also trigger neurodegeneration [41]. However, patients who perform more physical activities tend to have a shorter recovery period due to their overall better condition. Even so, studies have shown that the severity of TBI increases the risk of a more negative recovery outcome [12]. In addition, beta amyloid plaque formation was noted in around 30% patients suffering TBI, suggesting that TBI may be correlated with the development of AD. The first plaques

appear within hours after the accident and tend to locate in the encephalon, being rarely present in the region of diencephalon and cerebellum, and absent in the midbrain, pons and white matter; they are typically only observed in the gray matter [32, 41]. Elevated levels of Tau proteins and Tau aggregates are also noted after a TBI. They are corelated to a faulty mechanism which attempts to stabilize microtubules, which are damaged by the trauma. Another hypothesis suggests that beta amyloid aggregates induce improper Tau phosphorylation, leading to aggregation [12].

Physiological changes also affect the inflammatory response. Around the first day after the injury, microglia are rapidly activated to repair the damaged brain tissue. Furthermore, in the case of severe TBI, a significant rise in cytokines is observed, resulting in intense inflammation. It is possible that modulating this response may be a supplementary treatment in TBI; however, several treatments have proved ineffective, most likely due to low penetration resulting in inappropriate concentration. However, subcutaneous administration of interleukin-1 receptor agonist in contradiction to intravenous administration has a positive effect on reduction of the neuroinflammatory response due to higher penetration to the brain extracellular space [12, 38].

Patients with TBI in most cases develop edema [13, 70]. This can develops due to cytotoxicity resulting from disruption of Na^+/K^+ homeostasis, disruption of osmosis and increased permeation of brain vesicles following damage [90]. All these factors disrupt intracranial pressure, resulting in cell damage [30]. In addition, AQP4 and AQP9 stimulation has been noted after TBI, often accompanied by damage to the BBB. This leads to an infiltration of proteins and ions into the CSF, disturbing its balance and resulting in an increase in intracranial pressure.

An excessive increase of intercranial pressure may greatly increase pressure in the brain blood vessels, successively reducing their lumen and preventing the supply of substrates [13, 30]. Reducing pressure with classic drugs based on differences in substrate concentrations between blood and CSF may be ineffective, as these substrates may permeate the BBB following its rupture. An additional problem is the infiltration of immunoglobulins and immune cells which were previously absent in the brain; these aggravate the imbalance and extend the inflammatory process [13]. One clinical study found anti-inflammatory steroids to be effective in lowering the edema in all of nine tested patients with mild and moderate edema after TBI [74].

TBI can be detected using various markers, with the most commonly-used ones being UCH-L1 and NSE, indicating damage to neuronal cells, S100B and GFAP, indicating damage to

astroglial cells, Tau proteins, associated with axonal injuries, and NF-L (neurofilament light) [38, 93]. A recent study confirmed that the levels of brevican and neurocan fragments, these veing peptides associated with neurons, increase after an injury and could also be used as markers for TBI [64].

Some markers, like NF-L, are not suitable for diagnosing mild TBI in athletes who frequently suffer axonal damage (e.g., boxing), due to their high sensitivity. A good alternative, whose concentration corresponds with the severity of TBI, is neuron specific enolase (NSE): an enzyme abundant inside neurons. However, despite being a good indicator, this enzyme is also prevalent inside erythrocytes, and samples contaminated with blood can give false positive results [12].

ISCHEMIC AND HEMORRHAGIC STROKE AND ITS EFFECT ON CHOROID PLEXUS

Ischemic stroke

Ischemia results in impaired choroid plexus function, partial apoptosis, or cell necrosis. Depending on the degree of ischemia, it can be divided into global and local ischemia (focal).

The global form is characterized by a reduction of blood flow throughout most of the brain, resulting in necrosis of the choroid plexus and compromised continuity and tightness of the BBB. Local ischemia on the other hand, is characterized by a reduction of blood flow in one sector. It is milder because part of the inflow can be compensated by anastomoses from other vessels [86]. The first signs of plexus repair appear around the day three after the incident and most of them resolve at around 14 days [95].

Shortly after a stroke, the space between the choroid plexus cells widens, resulting in the infiltration of undesirable and/or previously absent substances into the CSF. Despite the absence of BBB breakdown, cytokine and chemokine levels increase. Levels of the neurodegenerative chemokine CCL2 [86] increases substantially. Due to the temporary ischemia of the choroid plexus cells, fluid secretion decreases. This drastically affects the transport and functional physiology of the entire system. In studies conducted on pigs, the transient receptor potential vanilloid 4 (TRPV4) mechanosensory ion channel, present in the choroid plexus, was proved to be stimulated by a ischemic incident. This transporter allows the transport of Ca ions into the choroid plexus cells [13] activating the Ca-dependent KCNN4 channels [36] and causing further disruption of homeostasis; however, its activity in stroke has not been studied in humans. Animal studies have demonstrated that regulation of TRPV4 helps alleviate symptoms following cardiac ischemia, thus suggesting it may have a

role in elevating brain ischemia [13, 36]. However, the usage of receptor agonists is challenging since improper dosing on porcine choroid plexus colonies have resulted in complete disintegration within minutes. Wrong dosage in other animal models resulted in endothelial barrier disruption leading to circulatory failure [75].

It was previously noted that controlled cooling also has a neuroprotective effect. Controlled hypothermia decreases the brain metabolism, prevents apoptosis, and suppresses the inflammatory response, which in return gives a higher recovery rate from ischemia [22]. The volume of the choroid plexus has been found to differ before and after ischemia. A study of ischemia patients found consistent enlargement to be maintained three and 12 months after the incident compared to controls. Further studies can establish whether choroid plexus enlargement can be used as a marker indicating a previous stroke [28].

AQP1 expression decreases immediately after global ischemia, but then increases after about two days [86]. This is explained by the proliferation of plexus cells and barrier renewal, with the reconstructed barrier not being as tight as the final form for some time. The proliferation of cells is stimulated by autocrine or paracrine secretion of TGFbeta1 [9, 86], BDNF and other growth factors that enable reconstruction. Mediators of the inflammatory process, *viz.* chemokines and cytokines, also play a role. A crucial role in limiting the extent of inflammation is played by IL-10: studies have found larger areas of the brain to be affected by ischemia in mice lacking IL-10 [33]. Increased expression of proteins such as VCAM1, MAdCAM1 and C3CL1 is noted in choroid plexus cells; these enable the adhesion and penetration of macrophages through the barrier and into the cerebrospinal fluid [86].

A good CSF and serum marker of ischemic stroke is neurofilament light (NFL) and phosphorylated neurofilament heavy (pNFH). Both markers have similar growth tendencies, being at their lowest level the day after the stroke, rising significantly on days two to three, peaking around three weeks later, and the returning to normal values between three and five months [38].

Hemorrhagic stroke

Hemorrhagic stroke may have many origins: it can result from trauma inducing vascular damage, spontaneous rupture of a vascular malformation or as a complication after a surgical intervention [20]. Following damage to the BBB, blood and its components enter the CSF, resulting in elevated protein levels of around 2.7 g per liter.

Elements derived from the breakdown of thrombi caused by hemorrhage induce inflammatory reactions via damage associated molecular patterns (DAMPs) [6, 86] which cause a cascade of inflammatory reactions via toll like receptors (TLRs) [48, 86].

This results in increased NKKC1-mediated CSF secretion, together with elevated chemokine and cytokine levels, which stimulates macrophage migration into the choroid plexus [86]. Excessive CSF production in these conditions may be a component of intracranial hypertension resulting from hematomas. The hematoma not only decreases cranial volume, but increases pressure due to overproduction of CSF. Hydrocephalus after hematoma was thought to arise from blocked circulation of CSF, and decreased absorption due to the blockage of resorption pathways [86]. Moreover, a subarachnoid hemorrhage (SAH) can also spread and enter the ventricles, becoming an intraventricular hemorrhage (IVH). IVH was linked with a higher possibility of developing hydrocephalus. Only 27% of patients with SAH suffered from hydrocephalus compared to 62% of patients with SAH and IVH [95].

Current research suggests that the mechanism may be based on overproduction of CSF due to overstimulation of NKCC1. It has also been suggested that this may be due to overstimulation of the glossopharyngeal nerve and the vagus nerve, while another hypothesis proposes that it may be due to increased expression of AQP1 after hemorrhage [86]. Moreover, studies on mice found that macrophages within the brain rapidly respond to presence of hemoglobin and its derivatives by increasing the phenotypes responsible for its detoxification and removal. The levels of macrophages peak probably around three to four days after the bleeding, since at that moment, bilirubin also reaches its peak with subsequent increase of biliverdin [2]. Hemoglobin and the byproducts of its breakdown act as a vasoconstrictors, and have a high oxidation potential, leading to oxidation of lipids and neuronal damage increasing secondary damage [2].

PATHOLOGICAL PROCESSES: DISEASES

Alzheimer’s disease

Beta amyloids are produced by neurons and oligodendrocytes during the daytime, and under physiological conditions, they should be removed by the CSF. However, the clearance of beta-amyloid decreases with age due to the gradual failure of the cleansing mechanisms [7] and the lowering of the CSF circulation, resulting in beta amyloid protein accumulation and neurodegenerative changes. This is a self-perpetuating phenomenon since the accumulation of proteins obstructs the circulation of fluid, which further impairs their removal and exacerbates the pathology [40, 59]. AD is characterized by the overproduction and excessive accumulation

of beta-amyloid in brain tissue, which stimulates neurodegenerative processes [86]. These result in the loss of neurons in specific regions, including the temporo-parietal association cortex and the medial temporal lobes [62]. There is growing concern that the condition may be exacerbated by abnormalities in choroid plexus function and CSF production [86].

Pathogenesis

The excessive presence of beta-amyloids influences the immune response. Increased levels of IgG antibodies and their excessive concentration results in vascular damage and the formation of fibrous lesions [61, 86]. Moreover, accumulation induces the production of pro-inflammatory cytokines and metalloproteinases. This reduces the production of claudins, occludins and zonulins, which compromises the tightness of the blood-CSF-barrier and allows the permeation of substances that are not present in the CSF under physiological conditions. The choroid plexus and BCSFB are involved in beta-amyloid removal via the presence of the megalin a multi ligand endocytic receptor [5, 86], which enables the uptake and transport of beta-amyloid from the fluid across the barrier into the blood. In AD patients megalin secretion decreases which lowers beta-amyloid clearance [45, 86]. In addition, deposit formation is also known to be promoted by oxidative stress, mitochondrial defects, and morphological changes in the choroid plexus; these result in fibrosis, vascular sclerosis, accumulation of lipofuscin granules, plexus cell atrophy and decreased numbers of transporters. These all translate into decreased production and altered quality composition of CSF [86].

In patients with AD, the CSF is characterized by elevated levels of Aβ42 around 25 years before AD symptoms appear, however this level later decreases [7]. Subsequently increased formation of fibrillar amyloid deposits is noted, together with elevation of tau protein levels in the CSF, which impairs the CSF homeostasis and brain functionality [7].

Risk factors

The primary risk factor for AD is age. It is estimated that 30–50% of people of aged above 85 years have some form of AD compared to around 19% among those aged from 75 to 84.

Gene mutation is also a risk factor of AD. In particular, AD is associated with mutations [3] in amyloid precursor protein (APP), a key component required for proper cell growth, adhesion as well as modulation of mitochondrial activity. It is also crucial for neuron functioning since APP knock out leads to neuronal apoptosis. Beta amyloid is a byproduct of APP proteolysis, thus APP mutation may result in formation of beta amyloid deposits [3, 88]; APP missense mutation in transgenic mouse also results in age-related deposition of beta amyloid, in a

similar way as in human patients [88]. It has also been associated with changes in presenilin protein (PSEN 1/2), responsible for forming an endoplasmatic enzyme required for APP processing. Improper functioning may result in loss of function thus leading to improper APP metabolism. Also, mutations in apolipoprotein e (APOE) involved with cholesterol transferal have been found to be a risk factor in AD. Some variations of this allele, like E4 in heterozygotes, result in a two- to three-times higher possibility of AD [3, 88], and even 10-times higher probability in homozygotic organisms [88].

Other factors increasing the probability of developing AD include diet, which should be balanced and fulfil basic micro and macro element requirements, and have an optimal caloric amount. A high-fat diet has also proven to stimulate AD by overproduction of beta amyloids and overexposure to certain metals, *inter alia* zinc, aluminum, copper, mercury and magnesium, has been connected to AD by influencing the metabolism of APP [3].

Hence, the only way to effectively reduce the likelihood of developing AD is the early implementation of a permanent lifestyle change. Young adults with high fitness level tend to be less at risk of chronic disease, including AD: around 60% of fit young adults have one or fewer chronic diseases in old age compared with those with low fitness levels, 20% of whom had four or more chronic disease in their last five years [3, 11].

Diagnostic

On February 2017, The Alzheimer's Association published a list which indicated when a CSF diagnosis for AD should be performed. It includes patients with subjective cognitive declines or with a progressive unexpected cognitive decline, those in a group with elevated AD risks; patients with symptoms of AD; patients with mild cognitive disorders at a young age; patients with AD symptoms at a typical onset age; and patients with unexplained behavioral changes when AD is considered [83].

Currently several markers are used for detection of AD; however, Beta Amyloid T-tau and P-tau are the most sensitive CSF markers, with a sensitivity above 80% [10]. Moreover, there is an inverse correlation between the amount of beta amyloid plaques and CSF levels of Beta Amyloid $_{1–42.}$ Correlation of these markers in contradiction to each other can help to distinguish between types of dementia. Beta Amyloid $_{1–42}$/T-tau and Beta Amyloid $_{1–42}$/ P-tau $_{181}$ ratio can be used to discriminate between AD dementia and non-AD dementia [10].

CONCLUSIONS

Insights, perspectives, and future

Cerebrospinal fluid is a key component of normal brain function. Recent interest in understanding the function of CSF, and its maintenance by the GS and resorption pathways, has indicated ways to raise medical standards and develop alternative treatments for previously untreatable diseases. The understanding of CSF drainage has changed in recent years. The main role of the arachnoid granules is now believed to be associated with the previously overlooked lymphatic system, with the recognition that the granules have a supporting role. In addition, other pathways have been proposed, including absorption along nerves and through the nasal mucosa. Further research may help find alternative drugs that do not affect cerebrospinal fluid homeostasis, making them more effective while avoiding potential negative effects.

The development of AD is much more complex than previously thought and may have several causes, including previous brain injury. However, modulating GS function may help alleviate the negative symptoms of some neurodegenerative diseases, as well as promote recovery in other conditions. Therefore, further research is needed to fully understand the complex way the brain functions.

Article information and declarations

Data availability statement

Please contact authors for data requests (Łukasz Olewnik, PhD — e-mail address: lukaszolewnik@gmail.com.

Authors' contributions

Hubert Paszkowycz — project development, data collection and management, data analysis and manuscript writing. Łukasz Olewnik — data collection, data analysis and manuscript editing. Bartosz Gonera — data analysis and manuscript editing. Robert Haładaj — data analysis and manuscript editing. Nicol Zielinska — data collection, data analysis and manuscript editing. All authors have read and approved the manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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