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The Blood-CSF-Brain Route of Neurological Disease: The Indirect Pathway into the Brain.

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Abstract

The brain is protected by the endothelial blood-brain-barrier (BBB) that limits the access of micro-organisms, tumour cells, immune cells and autoantibodies to the parenchyma. However, the classic model of disease spread across a disrupted BBB does not explain the focal distribution of lesions seen in a variety of neurological diseases and why lesions are frequently adjacent to the cerebrospinal fluid (CSF) spaces. We have critically reviewed the possible role of a blood-CSF-brain route as a disease entry pathway into the brain parenchyma. The initial step of this pathway is the transfer of pathogens or immune components from the blood into the CSF at the choroid plexuses, where the blood-CSF-barrier (BCSFB) is located. The flow of CSF results in disease dissemination throughout the CSF spaces. Access to the brain parenchyma from the CSF, can then occur across the ependymal layer at the ventricular surface, or across the pial-glial barrier of the subarachnoid space and the Virchow-Robin spaces. We have reviewed the anatomy and physiology of the blood-CSF-brain pathway and the brain barriers controlling this process. We then summarised the evidence supporting this brain entry route in a cross-section of neurological diseases including neuromyelitis optica, multiple sclerosis, neurosarcoidosis, neuropsychiatric lupus, cryptococcal infection, and both solid and haematological tumours. This summary highlights the conditions that share the blood-CSF-brain pathway as a pathogenetic mechanism. These include the characteristic proximity of lesions to CSF, evidence of disruption of the brain barriers, and the identification of significant pathology within the CSF. An improved understanding of pathological transfer through the CSF and across all brain barriers will inform on more effective and targeted treatments of primary and secondary disease of the central nervous system.

Abbreviations

BBB – blood-brain barrier

BCSFB – blood-CSF barrier

CNS – central nervous system

CSF – cerebrospinal fluid

ISF – interstitial fluid

MRI – magnetic resonance imaging

MS – multiple sclerosis

NMO – neuromyelitis optica

VRS - Virchow-Robin spaces

Qalb - CSF/serum albumin ratio

Keywords

Blood-brain-barrier, blood-CSF-barrier, choroid plexus, Virchow-Robin spaces.

Key points

- The pathogenesis of many neurological diseases may involve transfer of pathogens, tumour cells or immune components via an indirect blood-CSF-brain entry route, which circumvents the blood-brain barrier.
- We review and appraise the normal anatomy, physiology and terminology regarding the blood-CSF-brain entry route, and the relevant barriers controlling this process.
- Diseases involving the blood-CSF-brain route share characteristic features including the proximity of lesions to CSF, evidence of disruption to multiple brain barriers, and the identification of significant pathology within the CSF.
- Better understanding of the blood-CSF-brain disease pathway will allow for focused investigations and treatments to be developed for conditions that are often associated with significant morbidity.

Introduction

The general understanding of the functions of the cerebrospinal fluid (CSF) is limited to its role in the protection of the brain and spinal cord from traumatic impact, and the exchange of nutrients and waste products [1]. However, the importance of CSF in brain development is now starting to be recognised [2]. Following production by choroid plexus cells, the established model of CSF dynamics is well known (Fig. 1). Solutes move via bulk flow throughout the CSF in a multidirectional manner [3]. While alterations in CSF dynamics are known to underlie conditions such as hydrocephalus [4], the role of CSF in the pathogenesis of a wide range of diseases is largely under recognised.

The idea of solute exchange between the interstitial fluid (ISF) and the CSF, facilitated by the spaces surrounding intracerebral blood vessels or the blood vessel walls, and the existence of dural lymphatics, has been known for several decades [5]. However, there is a growing interest in these concepts following the new conceptualisation of ISF-CSF exchange as the ‘glymphatic system’ [6] and the re-discovery of dural lymphatics [7, 8]. This is a topic of ongoing debate. However, there is a broad consensus that solutes and water are exchanged between the ISF and

CSF [6, 9, 10]. It is also accepted that solutes in the ISF and CSF can drain into the cervical lymph nodes before further transfer into the blood [5, 11, 12]. The exact steps of this drainage pathway are disputed. Two apparently discrepant theories are leading: i) solutes drain from the ISF via capillary basement membranes and along the basement membranes of arterial walls (the intramural perivascular drainage 'IPAD' pathway) [13, 14] or ii) alternatively via perivenous spaces (the glymphatic pathway) that are anatomically not yet defined [6]. Solutes within the CSF have been proposed to enter the cervical lymphatics via the dural lymphatics, although the mechanism by which the arachnoid barrier is crossed remains unclear, or the nasal lymphatics in the cribriform plate [15]. It has also been demonstrated that solutes in the spinal CSF spaces drain into the surrounding sacral and iliac lymph nodes [16].

Increased knowledge about ISF-CSF communication and the relationship with the lymphatic system has resulted in renewed and widespread interest in brain solute exchange, especially drainage. Alterations in this solute clearance route have been implicated in several conditions, including Alzheimer's disease [17]. Furthermore, clearance of myelin proteins from the brain into the cervical lymph nodes may contribute to the pathogenesis of multiple sclerosis (MS) [18]. The normal brain parenchyma lacks dendritic cells, therefore, antigen drainage into lymph nodes is potentially a pathway for antigen presentation to the peripheral immune system [19]. This route of antigen presentation may be implicated in the pathogenesis of a wider range of autoimmune conditions of the central nervous system (CNS).

The focus on brain drainage pathways has somewhat overshadowed the importance of ISF-CSF exchange as an entry route into the brain [3, 15, 20]. A blood-CSF-brain entry route could enable immune cells and inflammatory cytokines, tumour cells and micro-organisms to transfer from blood into the CSF and thus spread throughout the CSF causing brain tissue damage via ISF-CSF exchange. The importance of this indirect blood-CSF-brain route is also overshadowed in the literature by the established view of a more direct route into the brain, where pathogenic threats are believed to cross the endothelial blood-brain-barrier (BBB). The different exchange pathways of the brain are summarised in Fig. 2.

In this article, we review and appraise the normal anatomy, physiology and terminology regarding the blood-CSF-brain indirect brain entry route, and the relevant barriers controlling this process as opposed to the direct route into the brain across the endothelial BBB. We have then evaluated conditions in which a blood-CSF-brain route potentially acts as the conduit of disease and summarise the available investigations which measure disruption of this pathway. Finally, we have explored the implications of improved understanding of this entry route into the brain.

Terminology

The nomenclature used to define the spaces around the brain's blood vessels, including perivascular, paravascular and Virchow-Robin spaces (VRS) is applied inconsistently in the literature and such inconsistency is a cause for confusion [15, 21, 22]. The word 'space' is used here to refer to the location around vessels and does not necessarily imply an empty or fluid-filled region. The term 'VRS' will be used to refer to the location surrounding the penetrating arteries, but not veins, as they enter the brain prior to branching into capillaries (Fig. 3a).

Perivascular is used as to describe the area between any blood vessel and the glia limitans, including within the vessel walls themselves. The term 'paravascular' space is often used as a synonym of VRS and will therefore be avoided due to potential confusion with the perivascular space.

Central nervous system barriers

The main barriers to movement of solutes within the CNS are the BBB, the blood-CSF-barrier (BCSFB) and CSF-brain barriers. The BBB is composed of endothelial cells and joining tight junctions. Pericytes also surround the endothelial cells and their importance in mediating the BBB is increasingly recognised [23, 24] At the capillary level, blood vessels are surrounded by encircling glial end-feet termed the glia limitans [25]. In response to neuroinflammation there is upregulation of tight junctions within the glia limitans [26]. Therefore, both the BBB and glia limitans act to protect the underlying parenchyma. Lymphocytes can cross the BBB at the post-capillary venule and enter the perivascular space, prior to crossing the glia limitans [15]. A compromised BBB is frequently considered a key step in the development of pathology [27]. However, the focus on the BBB overlooks the potential importance of other brain barriers in the spread of pathogenic threats.

The BCSFB is composed of the choroid plexus epithelial cells and the tight junctions between these cells and the basement membrane. It acts to restrict the movement of solutes into the CSF [19]. The choroid plexuses are highly vascularised structures within the ventricles that are responsible for CSF production [28] (Fig. 3b). The capillaries in choroid plexus are fenestrated, allowing for transfer of cells and solutes into the surrounding extracellular fluid in the stroma. An outer arachnoid CSF-blood barrier also exists which contains the arachnoid villi. The net direction of fluid flow in the arachnoid villi is thought to be from the CSF into the blood [29]. However, the in vivo evidence for this fluid flow in humans, especially in physiological conditions, is limited [30].

The CSF-brain barrier has two components. Firstly, there is the pial-glial barrier between the subarachnoid CSF and the parenchyma (Fig. 3a). This barrier comprises the pia mater covering the outer surface of the brain and the underlying parenchymal basement membrane and glial end-feet [31]. The pia mater also extends to cover the arteries in the subarachnoid space and lines the VRS surrounding the penetrating arterioles until they branch into capillaries [31]. The anatomy of the VRS and the communication between the VRS and subarachnoid space, are disputed [22, 32]. Human post-mortem studies have questioned the existence of VRS at the brain cortex suggesting that VRS are restricted to the basal ganglia and white matter [33]. In vivo magnetic resonance imaging (MRI) has demonstrated CSF-isointense spaces along arteries, most prominent in the basal ganglia but also surrounding penetrating arteries, once they reach the subcortical white matter, and in the brainstem [34, 35]. These spaces are especially apparent using ultra-high resolution 7T MRI [36, 37]. MRI visible VRS are rare in young healthy adults, but they are more numerous with age [38] and with certain neurological diseases including MS [39] and neuropsychiatric lupus [40].

The ependymal layer is the second CSF-brain barrier and separates the CSF in the ventricles and brain tissue. (Fig. 3b).It lines the ventricles and consists of specialised cuboidal cells that

are connected by gap and adhesion junctions [41]. It is absent in the normal fetal brain at the level of the frontal and occipital horns of lateral ventricles, and the temporal horn overlying the alveus of the hippocampus [42]. Tanycytes are elongated cells that are located within the ependymal layer and are in contact with the CSF, blood vessels and subcortical nuclei[43]. They may represent remnants of radial glia [44]. Subcortical nuclei can also contain CSF contacting neurons, which have dendrites or axons that penetrate the ependymal layer [45]. The circumventricular organs are unique structures that include the subfornical organ, the vascular organ of the lamina terminalis, the pineal gland, the subcommissural organ (SCO), and the median eminence/neurohypophysial complex. They contain fenestrated capillaries and therefore lack a complete BBB. However, they are separated from the CSF by tight junctions between ependymal cells [46]. The lack of tight junctions between the ependymal cells elsewhere indicates that the ependymal layer does not constitute a true barrier and solutes can transfer between the CSF and parenchyma [47]. However, it has been argued that the ependymal layer may present a partial barrier [48]. The ependymal cells have cilia toward the ventricular surface that beat synchronously to remove particles. They also have the ability to phagocytose and break down proteins, and they contain proteins that can bind toxic metal ions [48]. Ependymal cells have a limited potential to regenerate. Following injury, gaps in the ependymal layer are filled by reactive astrocytes that form glial nodules [41]. The loss of the partial barrier function caused by ependymal cell death could potentially result in increased susceptibility of the periventricular region to pathology. This is supported by the finding that widespread subependymal gliotic changes occur following ependymal damage even under areas of intact ependyma layer [41]. Additionally, the presence of blood vessels in glial nodules may allow for movement of pathogens into the perivascular space.

Physiology of the blood-CSF-brain entry route

As has been reviewed elsewhere, water, electrolytes and various proteins are transferred across the BCSFB within the choroid plexus [49, 50]. The course of CSF solute flow after crossing the BCSFB has been demonstrated in humans using intravenous injection of the contrast agent gadolinium and delayed sequential MRI [51]. Contrast was detected within the choroid plexus, the ventricles, cortical subarachnoid spaces and the VRS as would be expected in the classic model of CSF flow. Additionally, when ^{3}H-inulin was injected into the lateral ventricles or cisterna magna of dogs there was preferential tracer movement into the spinal subarachnoid space [52]. This evidence suggests that solutes enter the CSF via the choroid plexus. Transit is followed by rapid transfer throughout the CSF spaces, including accumulation in the VRS and subarachnoid space.

The CSF is not an immune-privileged environment [15]. In healthy individuals, proteins of varying molecular weight, albeit in small quantities, can enter the CSF resulting in a serum/CSF gradient. For example, IgG is approximately 800 times more concentrated in the serum compared to the CSF [53]. How antibodies enter the CSF from the blood is uncertain. However, antibodies, especially IgG, are found in the choroid plexus epithelial cells which could suggest exchange across the BCSFB [49]. Intrathecal antibody synthesis can also occur. Immune cells are detectable in the CSF, such as T-lymphocytes which make up over 80% of CSF cells [54]. Lymphocytes migrate from the blood into the choroid plexus, especially following peripheral

immune stimulation [55]. Lymphocytes can potentially cross into the CSF at the choroid plexus, as demonstrated using an animal model of MS [56] and an in vitro BCSFB model [57]. Lymphocytes are also detectable in the VRS and perivascular spaces, even in the absence of known neurological disease [58]. The transfer of antibodies and immune cells into the CSF plays an important protective role against infections but can also have implications in autoimmune conditions.

Transfer of solutes from the CSF into the brain has been demonstrated in mouse models where a fluorescent tracer was shown to cross both the pial-glial barrier and the ependymal layer [6, 10]. Transfer of gadolinium through the ependymal layer has also been demonstrated in vivo using high-resolution MRI in rats [59]. After crossing the ependymal layer, the contrast was shown to spread preferentially along the perivascular spaces deeper into the parenchyma. Furthermore, it has been demonstrated that intrathecally injected antibodies and smaller antigen-binding fragments can enter the parenchyma from the CSF, with the spinal cord and brainstem demonstrating higher rates of influx [60]. Cytokines can also enter the parenchyma from the CSF [61, 62].

It is therefore clear that solutes can cross the BCSFB, spread throughout the CSF and enter the parenchyma across the CSF-brain barriers. It is also apparent that components of the immune system gain access to the CSF as part of immune surveillance and that antibodies and cytokines can potentially move from the CSF into the parenchyma across the permeable CSF-brain barriers.

Pathological involvement of the blood-CSF-brain route.

The key methodologies to evaluate the significance of this route include pathological assessment, CSF sampling, MRI, in vitro barrier models and animal models (Table 1). Neuroinflammatory conditions, in particular antibody-mediated diseases such as neuromyelitis optica (NMO), are likely to involve the blood-CSF-brain pathway. Micro-organisms can cross from the blood into the CSF at the choroid plexus [63]; cryptococcus can also enter the brain parenchyma from the CSF. Primary and secondary CNS lymphoma and leptomeningeal carcinomatosis are potential examples of tumour spread via the blood-CSF-brain route. The supportive evidence for the involvement of the blood-CSF-brain route across a spectrum of neurological diseases is summarised in Table 2. The following features of these conditions would implicate spread of disease involving the blood-CSF-brain route:

- Characteristic disease distribution**.** Lesion location can be determined using neuroimaging and by post-mortem pathological assessment. Within the conditions described in Table 2 there is a predominance of lesions within CSF-adjacent locations. Periventricular and cortical lesions suggest compromise of the ependymal layer or the pial-glial barrier respectively. The optic nerves, spinal cord and brainstem are additional CSF-adjacent regions that are commonly affected. Involvement of the deep white matter, which is located away from the CSF, is relatively spared in these diseases.

- Evidence of BCSFB dysfunction. The conditions described in Table 2 frequently exhibit raised CSF/serum albumin ratios (Qalb) which indicates increased permeability of the

BCSFB. Moreover, in NMO, MS and neurosarcoidosis the rise in Qalb has been shown to correlate with clinical relapses, implicating BCSFB disruption in disease pathogenesis. The BCSFB is located within the choroid plexus. Disease infiltration or damage to the choroid plexus is detectable in many of the conditions described. Furthermore, tumour cells have been shown to transfer across in vitro BCSFB models and lymphocytes have been shown to enter the CSF via the choroid plexus in animal models of MS.

- Significance of CSF pathology. CSF biomarkers such as detection of neurofilament light chains due to parenchymal tissue damage can reflect changes in the parenchyma [64]. Evaluation of the CSF can also identify immune cells, tumour cells, or micro-organisms, suggesting disease propagation within the CSF. Intrathecal antibody synthesis can also be detected in the CSF. The importance of CSF involvement in causing disease is supported by the following evidence:
  i) Longitudinal changes in CSF pathology correlate with disease status more closely than serum pathology.
  ii) Greater sensitivity of CSF compared to serum/plasma investigations for disease diagnosis.
  iii) Passive transfer of autoantibodies from human to animal CSF causes a similar disease phenotype.

  All of these situations are supported by examples in Table 2.

- Evidence of pathological involvement of the CSF-brain barriers. Barrier disruption and disease infiltration across the ependymal lining and pial-glial barrier is detectable at pathological examination and with MRI in many of the conditions described in Table 2. Accumulation of immune cells, micro-organisms and tumour cells is also detectable within the VRS and in some conditions the number of VRS detectable on MRI increases with disease progression. Leptomeningeal gadolinium enhancement on MRI is common in these conditions and suggests disease involvement of the pial-glial barrier. Furthermore, release of brain-derived proteins into the CSF is suggestive of CSF-brain barrier breakdown.

- Lack of evidence of BBB disruption also characterises several of the conditions described in Table 2. Parenchymal contrast enhancement on MRI is not seen in cell-surface antibody mediated autoimmune encephalitis [65] and is infrequent in NMO [66] and neuropsychiatric lupus [67].

Discussion

We have reviewed the evidence for an indirect pathway entry into the brain. The blood-CSF-brain route, which bypasses the direct route across the BBB (Fig. 4). Transfer across the BBB does not adequately explain the distribution of lesions seen in many of the conditions listed in this paper. The choroid plexuses act as an entry route for micro-organisms and tumour cells into the CSF. The immune system protects against these threats, primarily in the form of antibodies and lymphocytes entering the CSF. However, the ability of the immune cells and antibodies to access the CSF makes the brain susceptible to autoimmune disease. The CSF is

separated from brain parenchyma by the CSF-brain barriers, which if breached can allow entry of pathogenic threats into the brain parenchyma, potentially with onward preferential movement along perivascular spaces.

The blood-CSF-brain route has previously been suggested to contribute to diseases such as MS [68], NMO [69], neurosarcoidosis [70, 71], neuropsychiatric lupus [72] and NMDAR-antibody encephalitis [73]. We have summarised the evidence that would implicate involvement of the blood-CSF-brain route within an expanded range of neurological diseases (Table 2). Within these conditions, lesions are commonly found in locations adjacent to the CSF and markers of disease, such as antibodies, tumour cells and micro-organisms, detectable within the CSF have greater significance for disease development than markers of disease detectable within the blood. Disruption of the BCSFB and CSF-brain barriers is also apparent in these conditions.

The blood-CSF-brain route contrasts with the view that pathogenic threats cross the BBB from the blood into the parenchyma [27]. In the latter situation, a widespread lesion distribution involving deeper regions of the brain would be expected, given the extensive distribution of cerebral blood vessels [74]. This pattern is seen with parenchymal brain metastases [75] but not in the conditions described in Table 2. Moreover, the lack of parenchymal MRI gadolinium enhancement in NMO [66] and cell-surface antibody mediated autoimmune encephalitis [65] suggest that in these conditions the BBB remains largely intact throughout the disease process. Where parenchymal gadolinium enhancement is seen, such as in MS [76] and primary CNS lymphoma [77], BBB disruption is clearly also important for disease pathogenesis. However, the blood-CSF-brain route may also be important for disease initiation. In support of this, longitudinal studies of NMO have shown leptomeningeal enhancement prior to the development of underlying parenchymal or spinal lesions [78]. Moreover, it has been proposed that there are two phases of inflammation in MS, with major BBB disruption occurring in early disease, while in later disease there is lymphocyte accumulation in the meninges and VRS, without BBB breakdown [79]. The importance of entry route into the brain is exemplified by the different clinical presentation caused by anti-MOG antibodies. In adults, these autoantibodies cause anti-MOG antibody disease, which results in optic nerve, spinal cord, brainstem and cortical grey matter lesions with relative sparing of the deep white matter, in keeping with a blood-CSF-brain route of disease transfer [80] (Table 2). However, the same autoantibodies can also cause acute disseminated encephalomyelitis (ADEM) in children. In ADEM there are a widespread white matter lesions, which exhibit MRI gadolinium enhancement indicative of BBB disruption [81].

This article has focused on and highlighted the potential role of the choroid plexus as an entry route for immune cells, tumour cells and micro-organisms into the CSF. However, the choroid plexus is not the only entry route into the CSF. Another possible route for the exchange of pathology is between the meningeal blood vessels and the CSF. This would be supported by the frequent presence of leptomeningeal enhancement in the conditions described in this paper, suggestive of gadolinium contrast leakage from these vessels. Meningeal blood vessels have also been suggested as an entry route for lymphocytes into the CSF [15]. Pathogenic threats could potentially enter the CSF via this route in addition to the choroid plexus or alternatively pathology within the CSF may secondarily disrupt the leptomeningeal blood vessels causing

their increased permeability. Recent studies have also shown that immune cells reside in larger numbers within the dura mater than previously expected [82, 83]. The dural myeloid cells were shown to move to sites of CNS injury [83] and the dural B lymphocytes were shown to mature into antigen presenting subtypes following neuroinflammation [82]. These findings could suggest an exchange between the dural and CSF, independent of the blood. Another potential route of pathological and immunological entry in to the CSF is via the other circumventricular organs such as the median eminence [84].

Various investigative techniques can be used to assess disruption of the blood-CSF-brain pathway (Table 1). Advances in neuroimaging and in vitro barrier models will allow for improved assessment of blood-CSF-brain pathway disruption. Structural MRI is starting to be used to investigate in vivo changes to the choroid plexuses [85]. Dynamic contrast enhanced MRI has also been employed to assess the flow of contrast from the blood into and throughout the CSF spaces [51]. This could potentially be utilised to assess changes is BCSFB transfer and CSF flow in disease states. Furthermore, ultra-high resolution 7T MRI has the potential to accurately examine small spaces such as the VRS [86]. PET imaging also holds potential for examining changes in brain solute exchange using compartmental modelling, as has already been shown in MS and Alzheimer's disease [87]. In vitro models are valuable for assessing exchange across the brain barriers. While cellular models of the BBB and BCSFB exist, models of the CSF-brain barriers are not yet available.

Many of the conditions described in this paper are associated with considerable long-term morbidity and increased mortality. Identifying and improving the understanding of the blood-CSF-brain route of pathological entry into the brain could lead to more effective treatments. The efficacy of systemic therapies can be limited when disease spread via or to the CSF. For example, treatments to remove autoantibodies including plasma exchange and intravenous immunoglobulin, are less effective in conditions where autoantibodies are intra-thecally synthesised. Examples include NMDAR-antibody encephalitis compared to peripheral autoimmune conditions such as myasthenia gravis [88]. Current untargeted systemic treatments can have severe adverse effects. For example, immune suppression and reconstitution as part of advanced treatments for MS can increase the risk of opportunistic infection and other autoimmune diseases [89]. If patients could be identified as having a primarily CSF based pathology this could allow for more targeted treatment, such as direct drug delivery into the CSF. This already occurs with intrathecal chemotherapy in leukaemia [90] and leptomeningeal carcinomatosis [91], intrathecal antimicrobials in cryptococcal meningitis [92, 93], and has recently been trialled with intrathecal immunosuppression in primary progressive MS [94]. Intrathecal delivery devices already exist for providing analgesic and antispasmodic medications [95]. Other potential directions for future research include the development of treatments that reduce the transfer of pathology across the BCSFB and the CSF-brain barriers.

To conclude, we have outlined a disease model in which the CSF is a principal conduit of disease via an indirect blood-CSF-brain entry route, circumventing the BBB, rather than a passive bystander in the pathogenesis of CNS diseases. We have described the shared characteristics of diseases where the pathogenesis likely involves this route. By highlighting

this pathway of disease, we hope that specific targeted therapies can be developed to treat a group of conditions often associated with significant morbidity.

References

1 Wright BL, Lai JT, Sinclair AJ. Cerebrospinal fluid and lumbar puncture: a practical review. J Neurol 2012; 259: 1530-45

2 Miyan J, Cains S, Larcombe S, Naz N, Jimenez AR, Bueno D, Gato A. Subarachnoid cerebrospinal fluid is essential for normal development of the cerebral cortex. Semin Cell Dev Biol 2020; 102: 28-39

3 Spector R, Robert Snodgrass S, Johanson CE. A balanced view of the cerebrospinal fluid composition and functions: Focus on adult humans. Exp Neurol 2015; 273: 57-68

4 Johanson CE, Duncan JA, 3rd, Klinge PM, Brinker T, Stopa EG, Silverberg GD. Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. Cerebrospinal Fluid Res 2008; 5: 10

5 Bradbury M. Lymphatics and the central nervous system. Trends in Neurosciences 1981; 4: 100-1

6 Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci Transl Med 2012; 4: 147ra11

7 Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, Derecki NC, Castle D, Mandell JW, Lee KS, Harris TH, Kipnis J. Structural and functional features of central nervous system lymphatic vessels. Nature 2015; 523: 337-41

8 Aspelund A, Antila S, Proulx ST, Karlsen TV, Karaman S, Detmar M, Wiig H, Alitalo K. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med 2015; 212: 991-9

9 Abbott NJ, Pizzo ME, Preston JE, Janigro D, Thorne RG. The role of brain barriers in fluid movement in the CNS: is there a 'glymphatic' system? Acta Neuropathol 2018; 135: 387-407

10 Smith AJ, Yao X, Dix JA, Jin BJ, Verkman AS. Test of the 'glymphatic' hypothesis demonstrates diffusive and aquaporin-4-independent solute transport in rodent brain parenchyma. Elife 2017; 6:

11 Szentistványi I, Patlak CS, Ellis RA, Cserr HF. Drainage of interstitial fluid from different regions of rat brain. Am J Physiol 1984; 246: F835-44

12 Eide PK, Ringstad G. Delayed clearance of cerebrospinal fluid tracer from entorhinal cortex in idiopathic normal pressure hydrocephalus: A glymphatic magnetic resonance imaging study. J Cereb Blood Flow Metab 2018: 271678X18760974

13 Carare RO, Bernardes-Silva M, Newman TA, Page AM, Nicoll JA, Perry VH, Weller RO. Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. Neuropathol Appl Neurobiol 2008; 34: 131-44

14 Albargothy NJ, Johnston DA, MacGregor-Sharp M, Weller RO, Verma A, Hawkes CA, Carare RO. Convective influx/glymphatic system: tracers injected into the CSF enter and leave the brain along separate periarterial basement membrane pathways. Acta Neuropathol 2018:

15 Engelhardt B, Carare RO, Bechmann I, Flügel A, Laman JD, Weller RO. Vascular, glial, and lymphatic immune gateways of the central nervous system. Acta Neuropathol 2016; 132: 317-38

16 Ma Q, Decker Y, Müller A, Ineichen BV, Proulx ST. Clearance of cerebrospinal fluid from the sacral spine through lymphatic vessels. J Exp Med 2019; 216: 2492-502

17 Wang L, Zhang Y, Zhao Y, Marshall C, Wu T, Xiao M. Deep cervical lymph node ligation aggravates AD-like pathology of APP/PS1 mice. Brain Pathol 2019; 29: 176-92
18 Laman JD, Weller RO. Drainage of cells and soluble antigen from the CNS to regional lymph nodes. J Neuroimmune Pharmacol 2013; 8: 840-56
19 Engelhardt B, Vajkoczy P, Weller RO. The movers and shapers in immune privilege of the CNS. Nat Immunol 2017; 18: 123-31
20 Spector R. Nutrient transport systems in brain: 40 years of progress. J Neurochem 2009; 111: 315-20
21 Hladky SB, Barrand MA. Elimination of substances from the brain parenchyma: efflux via perivascular pathways and via the blood-brain barrier. Fluids Barriers CNS 2018; 15: 30
22 Wardlaw JM, Benveniste H, Nedergaard M, Zlokovic BV, Mestre H, Lee H, Doubal FN, Brown R, Ramirez J, MacIntosh BJ, Tannenbaum A, Ballerini L, Rungta RL, Boido D, Sweeney M, Montagne A, Charpak S, Joutel A, Smith KJ, Black SE, Disease cftFLTNoEotRotPSiCSV. Perivascular spaces in the brain: anatomy, physiology and pathology. Nat Rev Neurol 2020; 16: 137-53
23 Bhattacharya A, Kaushik DK, Lozinski BM, Yong VW. Beyond barrier functions: Roles of pericytes in homeostasis and regulation of neuroinflammation. J Neurosci Res 2020; 98: 2390-405
24 Zheng Z, Chopp M, Chen J. Multifaceted roles of pericytes in central nervous system homeostasis and disease. J Cereb Blood Flow Metab 2020; 40: 1381-401
25 Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. Neurobiol Dis 2010; 37: 13-25
26 Horng S, Therattil A, Moyon S, Gordon A, Kim K, Argaw AT, Hara Y, Mariani JN, Sawai S, Flodby P, Crandall ED, Borok Z, Sofroniew MV, Chapouly C, John GR. Astrocytic tight junctions control inflammatory CNS lesion pathogenesis. In J Clin Invest: 3136-51
27 Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. Nat Med 2013; 19: 1584-96
28 Cserr HF. Physiology of the choroid plexus. Physiol Rev 1971; 51: 273-311
29 Pollay M. The function and structure of the cerebrospinal fluid outflow system. Cerebrospinal Fluid Res 2010; 7: 9
30 Proulx ST. Cerebrospinal fluid outflow: a review of the historical and contemporary evidence for arachnoid villi, perineural routes, and dural lymphatics. Cell Mol Life Sci 2021; 78: 2429-57
31 Zhang ET, Inman CB, Weller RO. Interrelationships of the pia mater and the perivascular (Virchow-Robin) spaces in the human cerebrum. J Anat 1990; 170: 111-23
32 Bakker ENTP, Naessens DMP, VanBavel E. Paravascular spaces: entry to or exit from the brain? Exp Physiol 2019; 104: 1013-7
33 Weller RO, Sharp MM, Christodoulides M, Carare RO, Møllgård K. The meninges as barriers and facilitators for the movement of fluid, cells and pathogens related to the rodent and human CNS. Acta Neuropathol 2018; 135: 363-85
34 Achiron A, Faibel M. Sandlike appearance of Virchow-Robin spaces in early multiple sclerosis: a novel neuroradiologic marker. AJNR Am J Neuroradiol 2002; 23: 376-80
35 Heier LA, Bauer CJ, Schwartz L, Zimmerman RD, Morgello S, Deck MD. Large Virchow-Robin spaces: MR-clinical correlation. AJNR Am J Neuroradiol 1989; 10: 929-36
36 Feldman RE, Rutland JW, Fields MC, Marcuse LV, Pawha PS, Delman BN, Balchandani P. Quantification of perivascular spaces at 7T: A potential MRI biomarker for epilepsy. Seizure 2018; 54: 11-8
37 Bouvy WH, Biessels GJ, Kuijf HJ, Kappelle LJ, Luijten PR, Zwanenburg JJ. Visualization of perivascular spaces and perforating arteries with 7 T magnetic resonance imaging. Invest Radiol 2014; 49: 307-13

38 Francis F, Ballerini L, Wardlaw JM. Perivascular spaces and their associations with risk factors, clinical disorders and neuroimaging features: A systematic review and meta-analysis. Int J Stroke 2019; 14: 359-71
39 Wuerfel J, Haertle M, Waiczies H, Tysiak E, Bechmann I, Wernecke KD, Zipp F, Paul F. Perivascular spaces--MRI marker of inflammatory activity in the brain? Brain 2008; 131: 2332-40
40 Cohen D, Rijnink EC, Nabuurs RJ, Steup-Beekman GM, Versluis MJ, Emmer BJ, Zandbergen M, van Buchem MA, Allaart CF, Wolterbeek R, Bruijn JA, van Duinen SG, Huizinga TW, Bajema IM. Brain histopathology in patients with systemic lupus erythematosus: identification of lesions associated with clinical neuropsychiatric lupus syndromes and the role of complement. Rheumatology (Oxford) 2017; 56: 77-86
41 Sarnat HB. Ependymal reactions to injury. A review. J Neuropathol Exp Neurol 1995; 54: 1-15
42 Dooling EC, Chi JG, Gilles FH. Ependymal changes in the human fetal brain. Ann Neurol 1977; 1: 535-41
43 Felten DL, Harrigan P, Burnett BT, Cummings JP. Fourth ventricular tanycytes: a possible relationship with monoaminergic nuclei. Brain Res Bull 1981; 6: 427-36
44 Gould SJ, Howard S, Papadaki L. The development of ependyma in the human fetal brain: an immunohistological and electron microscopic study. Brain Res Dev Brain Res 1990; 55: 255-67
45 Vígh B, Manzano e Silva MJ, Frank CL, Vincze C, Czirok SJ, Szabó A, Lukáts A, Szél A. The system of cerebrospinal fluid-contacting neurons. Its supposed role in the nonsynaptic signal transmission of the brain. Histol Histopathol 2004; 19: 607-28
46 Duvernoy HM, Risold PY. The circumventricular organs: an atlas of comparative anatomy and vascularization. Brain Res Rev 2007; 56: 119-47
47 Jiménez AJ, Domínguez-Pinos MD, Guerra MM, Fernández-Llebrez P, Pérez-Fígares JM. Structure and function of the ependymal barrier and diseases associated with ependyma disruption. In Tissue Barriers. 2014
48 Del Bigio MR. The ependyma: a protective barrier between brain and cerebrospinal fluid. Glia 1995; 14: 1-13
49 Moore GR, Laule C, Leung E, Pavlova V, Morgan BP, Esiri MM. Complement and Humoral Adaptive Immunity in the Human Choroid Plexus: Roles for Stromal Concretions, Basement Membranes, and Epithelium. J Neuropathol Exp Neurol 2016; 75: 415-28
50 Hladky SB, Barrand MA. Fluid and ion transfer across the blood-brain and blood-cerebrospinal fluid barriers; a comparative account of mechanisms and roles. Fluids Barriers CNS 2016; 13: 19
51 Deike-Hofmann K, Reuter J, Haase R, Paech D, Gnirs R, Bickelhaupt S, Forsting M, Heußel CP, Schlemmer HP, Radbruch A. Glymphatic Pathway of Gadolinium-Based Contrast Agents Through the Brain: Overlooked and Misinterpreted. Invest Radiol 2019; 54: 229-37
52 Vladic A, Klarica M, Bulat M. Dynamics of distribution of 3H-inulin between the cerebrospinal fluid compartments. Brain Res 2009; 1248: 127-35
53 Felgenhauer K. Protein size and cerebrospinal fluid composition. Klin Wochenschr 1974; 52: 1158-64
54 Engelhardt B, Ransohoff RM. The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. Trends Immunol 2005; 26: 485-95
55 Petito CK, Adkins B. Choroid plexus selectively accumulates T-lymphocytes in normal controls and after peripheral immune activation. J Neuroimmunol 2005; 162: 19-27
56 Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, Uccelli A, Lanzavecchia A, Engelhardt B, Sallusto F. C-C chemokine receptor 6-regulated entry of TH-

17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol 2009; 10: 514-23
57 Strazielle N, Creidy R, Malcus C, Boucraut J, Ghersi-Egea JF. T-Lymphocytes Traffic into the Brain across the Blood-CSF Barrier: Evidence Using a Reconstituted Choroid Plexus Epithelium. PLoS One 2016; 11: e0150945
58 Mezey É, Szalayova I, Hogden CT, Brady A, Dósa Á, Sótonyi P, Palkovits M. An immunohistochemical study of lymphatic elements in the human brain. Proc Natl Acad Sci U S A 2021; 118:
59 Magdoom KN, Brown A, Rey J, Mareci TH, King MA, Sarntinoranont M. MRI of Whole Rat Brain Perivascular Network Reveals Role for Ventricles in Brain Waste Clearance. Sci Rep 2019; 9: 11480
60 Pizzo ME, Wolak DJ, Kumar NN, Brunette E, Brunnquell CL, Hannocks MJ, Abbott NJ, Meyerand ME, Sorokin L, Stanimirovic DB, Thorne RG. Intrathecal antibody distribution in the rat brain: surface diffusion, perivascular transport and osmotic enhancement of delivery. J Physiol 2018; 596: 445-75
61 Gardner C, Magliozzi R, Durrenberger PF, Howell OW, Rundle J, Reynolds R. Cortical grey matter demyelination can be induced by elevated pro-inflammatory cytokines in the subarachnoid space of MOG-immunized rats. Brain 2013; 136: 3596-608
62 Magliozzi R, Howell OW, Durrenberger P, Aricò E, James R, Cruciani C, Reeves C, Roncaroli F, Nicholas R, Reynolds R. Meningeal inflammation changes the balance of TNF signalling in cortical grey matter in multiple sclerosis. J Neuroinflammation 2019; 16: 259
63 Schwerk C, Tenenbaum T, Kim KS, Schroten H. The choroid plexus-a multi-role player during infectious diseases of the CNS. Front Cell Neurosci 2015; 9: 80
64 Gaetani L, Blennow K, Calabresi P, Di Filippo M, Parnetti L, Zetterberg H. Neurofilament light chain as a biomarker in neurological disorders. J Neurol Neurosurg Psychiatry 2019; 90: 870-81
65 Leypoldt F, Armangue T, Dalmau J. Autoimmune encephalopathies. Ann N Y Acad Sci 2015; 1338: 94-114
66 Kim HJ, Paul F, Lana-Peixoto MA, Tenembaum S, Asgari N, Palace J, Klawiter EC, Sato DK, de Seze J, Wuerfel J, Banwell BL, Villoslada P, Saiz A, Fujihara K, Kim SH. MRI characteristics of neuromyelitis optica spectrum disorder: an international update. Neurology 2015; 84: 1165-73
67 Sarbu N, Alobeidi F, Toledano P, Espinosa G, Giles I, Rahman A, Yousry T, Capurro S, Jager R, Cervera R, Bargallo N. Brain abnormalities in newly diagnosed neuropsychiatric lupus: systematic MRI approach and correlation with clinical and laboratory data in a large multicenter cohort. Autoimmun Rev 2015; 14: 153-9
68 Dawson JW. The Histology of Disseminated Sclerosis Edinb Med J 1916; 17: 229-41
69 Asgari N, Berg CT, Mørch MT, Khorooshi R, Owens T. Cerebrospinal fluid aquaporin-4-immunoglobulin G disrupts blood brain barrier. Ann Clin Transl Neurol 2015; 2: 857-63
70 Herring AB, Urich H. Sarcoidosis of the central nervous system. J Neurol Sci 1969; 9: 405-22
71 Mirfakhraee M, Crofford MJ, Guinto FC, Jr., Nauta HJ, Weedn VW. Virchow-Robin space: a path of spread in neurosarcoidosis. Radiology 1986; 158: 715-20
72 Gelb S, Stock AD, Anzi S, Putterman C, Ben-Zvi A. Mechanisms of neuropsychiatric lupus: The relative roles of the blood-cerebrospinal fluid barrier versus blood-brain barrier. J Autoimmun 2018; 91: 34-44
73 Dalmau J. NMDA receptor encephalitis and other antibody-mediated disorders of the synapse: The 2016 Cotzias Lecture. In Neurology. 2016: 2471-82
74 Duvernoy HM, Delon S, Vannson JL. Cortical blood vessels of the human brain. Brain Res Bull 1981; 7: 519-79

75 Hwang TL, Close TP, Grego JM, Brannon WL, Gonzales F. Predilection of brain metastasis in gray and white matter junction and vascular border zones. Cancer 1996; 77: 1551-5
76 Runge VM, Schoerner W, Niendorf HP, Laniado M, Koehler D, Claussen C, Felix R, James AE, Jr. Initial clinical evaluation of gadolinium DTPA for contrast-enhanced magnetic resonance imaging. Magn Reson Imaging 1985; 3: 27-35
77 Kuker W, Nagele T, Korfel A, Heckl S, Thiel E, Bamberg M, Weller M, Herrlinger U. Primary central nervous system lymphomas (PCNSL): MRI features at presentation in 100 patients. J Neurooncol 2005; 72: 169-77
78 Asgari N, Flanagan EP, Fujihara K, Kim HJ, Skejoe HP, Wuerfel J, Kuroda H, Kim SH, Maillart E, Marignier R, Pittock SJ, Paul F, Weinshenker BG. Disruption of the leptomeningeal blood barrier in neuromyelitis optica spectrum disorder. In Neurol Neuroimmunol Neuroinflamm. 2017
79 Lassmann H. Pathogenic Mechanisms Associated With Different Clinical Courses of Multiple Sclerosis. Front Immunol 2018; 9: 3116
80 Juryńczyk M, Jacob A, Fujihara K, Palace J. Myelin oligodendrocyte glycoprotein (MOG) antibody-associated disease: practical considerations. 2019:
81 Baumann M, Sahin K, Lechner C, Hennes EM, Schanda K, Mader S, Karenfort M, Selch C, Hausler M, Eisenkolbl A, Salandin M, Gruber-Sedlmayr U, Blaschek A, Kraus V, Leiz S, Finsterwalder J, Gotwald T, Kuchukhidze G, Berger T, Reindl M, Rostasy K. Clinical and neuroradiological differences of paediatric acute disseminating encephalomyelitis with and without antibodies to the myelin oligodendrocyte glycoprotein. J Neurol Neurosurg Psychiatry 2015; 86: 265-72
82 Schafflick D, Wolbert J, Heming M, Thomas C, Hartlehnert M, Börsch AL, Ricci A, Martín-Salamanca S, Li X, Lu IN, Pawlak M, Minnerup J, Strecker JK, Seidenbecher T, Meuth SG, Hidalgo A, Liesz A, Wiendl H, Meyer Zu Horste G. Single-cell profiling of CNS border compartment leukocytes reveals that B cells and their progenitors reside in non-diseased meninges. Nat Neurosci 2021:
83 Cugurra A, Mamuladze T, Rustenhoven J, Dykstra T, Beroshvili G, Greenberg ZJ, Baker W, Papadopoulos Z, Drieu A, Blackburn S, Kanamori M, Brioschi S, Herz J, Schuettpelz LG, Colonna M, Smirnov I, Kipnis J. Skull and vertebral bone marrow are myeloid cell reservoirs for the meninges and CNS parenchyma. Science 2021:
84 Broadwell RD, Sofroniew MV. Serum proteins bypass the blood-brain fluid barriers for extracellular entry to the central nervous system. Exp Neurol 1993; 120: 245-63
85 Tadayon E, Pascual-Leone A, Press D, Santarnecchi E. Choroid plexus volume is associated with levels of CSF proteins: relevance for Alzheimer's and Parkinson's disease. Neurobiol Aging 2020; 89: 108-17
86 Cai K, Tain R, Das S, Damen FC, Sui Y, Valyi-Nagy T, Elliott MA, Zhou XJ. The Feasibility of Quantitative MRI of Perivascular Spaces at 7T. J Neurosci Methods 2015; 256: 151-6
87 Schubert JJ, Veronese M, Marchitelli L, Bodini B, Tonietto M, Stankoff B, Brooks DJ, Bertoldo A, Edison P, Turkheimer F. Dynamic 11C-PiB PET shows cerebrospinal fluid flow alterations in Alzheimer's disease and multiple sclerosis. Journal of Nuclear Medicine 2019: jnumed. 118.223834
88 Dalmau J, Graus F. Antibody-Mediated Encephalitis. N Engl J Med 2018; 378: 840-51
89 Giovannoni G. Disease-modifying treatments for early and advanced multiple sclerosis: a new treatment paradigm. Curr Opin Neurol 2018; 31: 233-43
90 Marz M, Meyer S, Erb U, Georgikou C, Horstmann MA, Hetjens S, Weiss C, Fallier-Becker P, Vandenhaute E, Ishikawa H, Schroten H, Durken M, Karremann M. Pediatric acute

lymphoblastic leukemia-Conquering the CNS across the choroid plexus. Leuk Res 2018; 71: 47-54
91 Bonig L, Mohn N, Ahlbrecht J, Wurster U, Raab P, Puppe W, Suhs KW, Stangel M, Skripuletz T, Schwenkenbecher P. Leptomeningeal Metastasis: The Role of Cerebrospinal Fluid Diagnostics. Front Neurol 2019; 10: 839
92 Xie H, Luo P, Li Z, Li R, Sun H, Wu D. Continuous intrathecal administration of liposomal amphotericin B for treatment of refractory Cryptococcus neoformans encephalitis: A case report. In Exp Ther Med. 2017: 780-4
93 Saag MS, Graybill RJ, Larsen RA, Pappas PG, Perfect JR, Powderly WG, Sobel JD, Dismukes WE. Practice guidelines for the management of cryptococcal disease. Infectious Diseases Society of America. Clin Infect Dis 2000; 30: 710-8
94 Bhargava P, Wicken C, Smith MD, Strowd RE, Cortese I, Reich DS, Calabresi PA, Mowry EM. Trial of intrathecal rituximab in progressive multiple sclerosis patients with evidence of leptomeningeal contrast enhancement. Mult Scler Relat Disord 2019; 30: 136-40
95 Bottros MM, Christo PJ. Current perspectives on intrathecal drug delivery. In J Pain Res. 2014: 615-26
96 Andersson M, Alvarez-Cermeno J, Bernardi G, Cogato I, Fredman P, Frederiksen J, Fredrikson S, Gallo P, Grimaldi LM, Gronning M, et al. Cerebrospinal fluid in the diagnosis of multiple sclerosis: a consensus report. J Neurol Neurosurg Psychiatry 1994; 57: 897-902
97 Reiber H. Flow rate of cerebrospinal fluid (CSF)--a concept common to normal blood-CSF barrier function and to dysfunction in neurological diseases. J Neurol Sci 1994; 122: 189-203
98 Mayringer I, Timeltaler B, Deisenhammer F. Correlation between the IgG index, oligoclonal bands in CSF, and the diagnosis of demyelinating diseases. Eur J Neurol 2005; 12: 527-30
99 Rudie JD, Rauschecker AM, Nabavizadeh SA, Mohan S. Neuroimaging of Dilated Perivascular Spaces: From Benign and Pathologic Causes to Mimics. J Neuroimaging 2018; 28: 139-49
100 Smirniotopoulos JG, Murphy FM, Rushing EJ, Rees JH, Schroeder JW. Patterns of contrast enhancement in the brain and meninges. Radiographics 2007; 27: 525-51
101 Lisanti CJ, Asbach P, Bradley WG, Jr. The ependymal "Dot-Dash" sign: an MR imaging finding of early multiple sclerosis. AJNR Am J Neuroradiol 2005; 26: 2033-6
102 Nishihara H, Soldati S, Mossu A, Rosito M, Rudolph H, Muller WA, Latorre D, Sallusto F, Sospedra M, Martin R, Ishikawa H, Tenenbaum T, Schroten H, Gosselet F, Engelhardt B. Human CD4(+) T cell subsets differ in their abilities to cross endothelial and epithelial brain barriers in vitro. Fluids Barriers CNS 2020; 17: 3
103 Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, de Seze J, Fujihara K, Greenberg B, Jacob A, Jarius S, Lana-Peixoto M, Levy M, Simon JH, Tenembaum S, Traboulsee AL, Waters P, Wellik KE, Weinshenker BG. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology 2015; 85: 177-89
104 Pittock SJ, Weinshenker BG, Lucchinetti CF, Wingerchuk DM, Corboy JR, Lennon VA. Neuromyelitis optica brain lesions localized at sites of high aquaporin 4 expression. Arch Neurol 2006; 63: 964-8
105 Guo Y, Weigand SD, Popescu BF, Lennon VA, Parisi JE, Pittock SJ, Parks NE, Clardy SL, Howe CL, Lucchinetti CF. Pathogenic implications of cerebrospinal fluid barrier pathology in neuromyelitis optica. Acta Neuropathol 2017; 133: 597-612
106 Barnett Y, Sutton IJ, Ghadiri M, Masters L, Zivadinov R, Barnett MH. Conventional and advanced imaging in neuromyelitis optica. AJNR Am J Neuroradiol 2014; 35: 1458-66
107 Jarius S, Paul F, Franciotta D, Ruprecht K, Ringelstein M, Bergamaschi R, Rommer P, Kleiter I, Stich O, Reuss R, Rauer S, Zettl UK, Wandinger KP, Melms A, Aktas O,

Kristoferitsch W, Wildemann B. Cerebrospinal fluid findings in aquaporin-4 antibody positive neuromyelitis optica: results from 211 lumbar punctures. J Neurol Sci 2011; 306: 82-90
108 Dujmovic I, Mader S, Schanda K, Deisenhammer F, Stojsavljevic N, Kostic J, Berger T, Drulovic J, Reindl M. Temporal dynamics of cerebrospinal fluid anti-aquaporin-4 antibodies in patients with neuromyelitis optica spectrum disorders. J Neuroimmunol 2011; 234: 124-30
109 Kim SM, Waters P, Vincent A, Go MJ, Park KS, Sung JJ, Lee KW. Cerebrospinal fluid/serum gradient of IgG is associated with disability at acute attacks of neuromyelitis optica. J Neurol 2011; 258: 2176-80
110 Marignier R, Ruiz A, Cavagna S, Nicole A, Watrin C, Touret M, Parrot S, Malleret G, Peyron C, Benetollo C, Auvergnon N, Vukusic S, Giraudon P. Neuromyelitis optica study model based on chronic infusion of autoantibodies in rat cerebrospinal fluid. J Neuroinflammation 2016; 13: 111
111 Nishiyama S, Ito T, Misu T, Takahashi T, Kikuchi A, Suzuki N, Jin K, Aoki M, Fujihara K, Itoyama Y. A case of NMO seropositive for aquaporin-4 antibody more than 10 years before onset. Neurology 2009; 72: 1960-1
112 Jarius S, Franciotta D, Paul F, Ruprecht K, Bergamaschi R, Rommer PS, Reuss R, Probst C, Kristoferitsch W, Wandinger KP, Wildemann B. Cerebrospinal fluid antibodies to aquaporin-4 in neuromyelitis optica and related disorders: frequency, origin, and diagnostic relevance. J Neuroinflammation 2010; 7: 52
113 Klawiter EC, Alvarez E, 3rd, Xu J, Paciorkowski AR, Zhu L, Parks BJ, Cross AH, Naismith RT. NMO-IgG detected in CSF in seronegative neuromyelitis optica. Neurology 2009; 72: 1101-3
114 Saji E, Arakawa M, Yanagawa K, Toyoshima Y, Yokoseki A, Okamoto K, Otsuki M, Akazawa K, Kakita A, Takahashi H, Nishizawa M, Kawachi I. Cognitive impairment and cortical degeneration in neuromyelitis optica. Ann Neurol 2013; 73: 65-76
115 Misu T, Takano R, Fujihara K, Takahashi T, Sato S, Itoyama Y. Marked increase in cerebrospinal fluid glial fibrillar acidic protein in neuromyelitis optica: an astrocytic damage marker. J Neurol Neurosurg Psychiatry 2009; 80: 575-7
116 Takano R, Misu T, Takahashi T, Sato S, Fujihara K, Itoyama Y. Astrocytic damage is far more severe than demyelination in NMO: a clinical CSF biomarker study. Neurology 2010; 75: 208-16
117 Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, Pache F, Stich O, Beume LA, Hummert MW, Ringelstein M, Trebst C, Winkelmann A, Schwarz A, Buttmann M, Zimmermann H, Kuchling J, Franciotta D, Capobianco M, Siebert E, Lukas C, Korporal-Kuhnke M, Haas J, Fechner K, Brandt AU, Schanda K, Aktas O, Paul F, Reindl M, Wildemann B. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: Epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. J Neuroinflammation 2016; 13: 280
118 Jarius S, Ruprecht K, Kleiter I, Borisow N, Asgari N, Pitarokoili K, Pache F, Stich O, Beume LA, Hummert MW, Trebst C, Ringelstein M, Aktas O, Winkelmann A, Buttmann M, Schwarz A, Zimmermann H, Brandt AU, Franciotta D, Capobianco M, Kuchling J, Haas J, Korporal-Kuhnke M, Lillevang ST, Fechner K, Schanda K, Paul F, Wildemann B, Reindl M. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 1: Frequency, syndrome specificity, influence of disease activity, long-term course, association with AQP4-IgG, and origin. J Neuroinflammation 2016; 13: 279
119 Mariotto S, Gajofatto A, Batzu L, Delogu R, Sechi G, Leoni S, Pirastru MI, Bonetti B, Zanoni M, Alberti D, Schanda K, Monaco S, Reindl M, Ferrari S. Relevance of antibodies to myelin oligodendrocyte glycoprotein in CSF of seronegative cases. Neurology 2019; 93: e1867-e72

120 Fang B, McKeon A, Hinson SR, Kryzer TJ, Pittock SJ, Aksamit AJ, Lennon VA. Autoimmune Glial Fibrillary Acidic Protein Astrocytopathy: A Novel Meningoencephalomyelitis. JAMA Neurol 2016; 73: 1297-307
121 Flanagan EP, Hinson SR, Lennon VA, Fang B, Aksamit AJ, Morris PP, Basal E, Honorat JA, Alfugham NB, Linnoila JJ, Weinshenker BG, Pittock SJ, McKeon A. Glial fibrillary acidic protein immunoglobulin G as biomarker of autoimmune astrocytopathy: Analysis of 102 patients. Ann Neurol 2017; 81: 298-309
122 Long Y, Liang J, Xu H, Huang Q, Yang J, Gao C, Qiu W, Lin S, Chen X. Autoimmune glial fibrillary acidic protein astrocytopathy in Chinese patients: a retrospective study. Eur J Neurol 2018; 25: 477-83
123 Kimura A, Takemura M, Yamamoto Y, Hayashi Y, Saito K, Shimohata T. Cytokines and biological markers in autoimmune GFAP astrocytopathy: The potential role for pathogenesis and therapeutic implications. J Neuroimmunol 2019; 334: 576999
124 Trip SA, Miller DH. Imaging in multiple sclerosis. J Neurol Neurosurg Psychiatry 2005; 76 Suppl 3: iii11-iii8
125 Liebsch R, Kornhuber ME, Dietl D, Grafin von Einsiedel H, Conrad B. Blood-CSF barrier integrity in multiple sclerosis. Acta Neurol Scand 1996; 94: 404-10
126 Rodriguez-Lorenzo S, Ferreira Francisco DM, Vos R, van Het Hof B, Rijnsburger M, Schroten H, Ishikawa H, Beaino W, Bruggmann R, Kooij G, de Vries HE. Altered secretory and neuroprotective function of the choroid plexus in progressive multiple sclerosis. Acta Neuropathol Commun 2020; 8: 35
127 Jarius S, Konig FB, Metz I, Ruprecht K, Paul F, Bruck W, Wildemann B. Pattern II and pattern III MS are entities distinct from pattern I MS: evidence from cerebrospinal fluid analysis. J Neuroinflammation 2017; 14: 171
128 Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, Reynolds R, Aloisi F. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 2007; 130: 1089-104
129 Magliozzi R, Howell OW, Reeves C, Roncaroli F, Nicholas R, Serafini B, Aloisi F, Reynolds R. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Ann Neurol 2010; 68: 477-93
130 Howell OW, Reeves CA, Nicholas R, Carassiti D, Radotra B, Gentleman SM, Serafini B, Aloisi F, Roncaroli F, Magliozzi R, Reynolds R. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain 2011; 134: 2755-71
131 Howell OW, Schulz-Trieglaff EK, Carassiti D, Gentleman SM, Nicholas R, Roncaroli F, Reynolds R. Extensive grey matter pathology in the cerebellum in multiple sclerosis is linked to inflammation in the subarachnoid space. Neuropathol Appl Neurobiol 2015; 41: 798-813
132 Irani SR, Gelfand JM, Al-Diwani A, Vincent A. Cell-surface central nervous system autoantibodies: Clinical relevance and emerging paradigms. Ann Neurol 2014; 76: 168-84
133 Gresa-Arribas N, Titulaer MJ, Torrents A, Aguilar E, McCracken L, Leypoldt F, Gleichman AJ, Balice-Gordon R, Rosenfeld MR, Lynch D, Graus F, Dalmau J. Antibody titres at diagnosis and during follow-up of anti-NMDA receptor encephalitis: a retrospective study. Lancet Neurol 2014; 13: 167-77
134 Gadoth A, Zekeridou A, Klein CJ, Thoreson CJ, Majed M, Dubey D, Flanagan EP, McKeon A, Jenkins SM, Lennon VA, Pittock SJ. Elevated LGI1-IgG CSF index predicts worse neurological outcome. Ann Clin Transl Neurol 2018; 5: 646-50
135 Lai M, Hughes EG, Peng X, Zhou L, Gleichman AJ, Shu H, Mata S, Kremens D, Vitaliani R, Geschwind MD, Bataller L, Kalb RG, Davis R, Graus F, Lynch DR, Balice-Gordon R, Dalmau J. AMPA receptor antibodies in limbic encephalitis alter synaptic receptor location. Ann Neurol 2009; 65: 424-34

136 Kreye J, Wenke NK, Chayka M, Leubner J, Murugan R, Maier N, Jurek B, Ly LT, Brandl D, Rost BR, Stumpf A, Schulz P, Radbruch H, Hauser AE, Pache F, Meisel A, Harms L, Paul F, Dirnagl U, Garner C, Schmitz D, Wardemann H, Pruss H. Human cerebrospinal fluid monoclonal N-methyl-D-aspartate receptor autoantibodies are sufficient for encephalitis pathogenesis. Brain 2016; 139: 2641-52
137 Abreu MR, Jakosky A, Folgerini M, Brenol JC, Xavier RM, Kapczinsky F. Neuropsychiatric systemic lupus erythematosus: correlation of brain MR imaging, CT, and SPECT. Clin Imaging 2005; 29: 215-21
138 McLean BN, Miller D, Thompson EJ. Oligoclonal banding of IgG in CSF, blood-brain barrier function, and MRI findings in patients with sarcoidosis, systemic lupus erythematosus, and Behcet's disease involving the nervous system. J Neurol Neurosurg Psychiatry 1995; 58: 548-54
139 Atkins CJ, Kondon JJ, Quismorio FP, Friou GJ. The choroid plexus in systemic lupus erythematosus. Ann Intern Med 1972; 76: 65-72
140 Gershwin ME, Hyman LR, Steinberg AD. The choroid plexus in CNS involvement of systemic lupus erythematosus. J Pediatr 1975; 87: 588-90
141 Stock AD, Der E, Gelb S, Huang M, Weidenheim K, Ben-Zvi A, Putterman C. Tertiary lymphoid structures in the choroid plexus in neuropsychiatric lupus. JCI Insight 2019; 4:
142 Jeltsch-David H, Muller S. Neuropsychiatric systemic lupus erythematosus: pathogenesis and biomarkers. Nat Rev Neurol 2014; 10: 579-96
143 Wengert O, Rothenfusser-Korber E, Vollrath B, Bohner G, Scheibe F, Otto C, Hofmann J, Angstwurm K, Ruprecht K. Neurosarcoidosis: correlation of cerebrospinal fluid findings with diffuse leptomeningeal gadolinium enhancement on MRI and clinical disease activity. J Neurol Sci 2013; 335: 124-30
144 Williams DW, 3rd, Elster AD, Kramer SI. Neurosarcoidosis: gadolinium-enhanced MR imaging. J Comput Assist Tomogr 1990; 14: 704-7
145 Klock C, Cerski M, Goldani LZ. Histopathological aspects of neurocryptococcosis in HIV-infected patients: autopsy report of 45 patients. Int J Surg Pathol 2009; 17: 444-8
146 Hammoud DA, Mahdi E, Panackal AA, Wakim P, Sheikh V, Sereti I, Bielakova B, Bennett JE, Williamson PR. Choroid Plexitis and Ependymitis by Magnetic Resonance Imaging are Biomarkers of Neuronal Damage and Inflammation in HIV-negative Cryptococcal Meningoencephalitis. In Sci Rep. 2017
147 Skripuletz T, Schwenkenbecher P, Pars K, Stoll M, Conzen J, Bolat S, Pul R, Vonberg RP, Sedlacek L, Wurster U, Stangel M, Trebst C. Importance of Follow-Up Cerebrospinal Fluid Analysis in Cryptococcal Meningoencephalitis. Dis Markers 2014; 2014:
148 Duarte SBL, Oshima MM, Mesquita J, do Nascimento FBP, de Azevedo PC, Reis F. Magnetic resonance imaging findings in central nervous system cryptococcosis: comparison between immunocompetent and immunocompromised patients. Radiol Bras 2017; 50: 359-65
149 Vandenhaute E, Stump-Guthier C, Lasierra Losada M, Tenenbaum T, Rudolph H, Ishikawa H, Schwerk C, Schroten H, Durken M, Marz M, Karremann M. The choroid plexus may be an underestimated site of tumor invasion to the brain: an in vitro study using neuroblastoma cell lines. Cancer Cell Int 2015; 15: 102
150 Iguchi Y, Mano K, Goto Y, Nakano T, Nomura F, Shimokata T, Iwamizu-Watanabe S, Hashizume Y. Miliary brain metastases from adenocarcinoma of the lung: MR imaging findings with clinical and post-mortem histopathologic correlation. Neuroradiology 2007; 49: 35-9
151 Schlegel U, Schmidt-Wolf IG, Deckert M. Primary CNS lymphoma: clinical presentation, pathological classification, molecular pathogenesis and treatment. J Neurol Sci 2000; 181: 1-12

152 Hottenrott T, Schorb E, Fritsch K, Dersch R, Berger B, Huzly D, Rauer S, Tebartz van Elst L, Endres D, Stich O. The MRZ reaction and a quantitative intrathecal IgG synthesis may be helpful to differentiate between primary central nervous system lymphoma and multiple sclerosis. J Neurol 2018; 265: 1106-14
153 Scott BJ, Douglas VC, Tihan T, Rubenstein JL, Josephson SA. A Systematic Approach to the Diagnosis of Suspected Central Nervous System Lymphoma. JAMA Neurol 2013; 70: 311-9
154 Batchelor T, Loeffler JS. Primary CNS lymphoma. J Clin Oncol 2006; 24: 1281-8
155 Koeller KK, Smirniotopoulos JG, Jones RV. Primary central nervous system lymphoma: radiologic-pathologic correlation. Radiographics 1997; 17: 1497-526

Table 1. Investigations to evaluate disruption of the blood-CSF-brain pathway. GFAP - Glial fibrillary acidic protein; NFL – Neurofilament light chains.

Investigation	Description
Pathological assessment	Post-mortem and brain biopsy specimens can undergo pathological assessment to assess for disease infiltration, disruption of normal anatomy and for localising lesions, in relation to the brain barriers.
Qalb	The CSF/serum ratio of albumin. Albumin is produced in the liver and is the most abundant plasma protein. It enters the CSF via the BCSFB. Qalb therefore increases with BCSFB dysfunction [96] but may also be seen in situations of reduced CSF flow and albumin clearance [97].
Raised CSF parenchymal proteins	High levels of parenchymal proteins (e.g., GFAP and NFL) in the CSF, especially if there is not a similar rise in the serum, is suggestive of CSF-brain barrier disruption.
Intrathecal antibody synthesis	Antibodies produced in the CSF by lymphocytes can be detected using two different techniques. Firstly, the IgG-Index where an increased CSF/serum ratio of antibodies titres, accounting for Qalb, suggests intrathecal antibody synthesis. The second, more sensitive technique, is to detect oligoclonal protein bands using protein electrophoresis to determine the presence of specific antibodies in the CSF that are not present in the serum [98]
MRI	Parenchymal lesion gadolinium contrast enhancement is suggestive of BBB breakdown [76]. Distinctive linear contrast enhancement is suggestive of pathological accumulation along the perivascular spaces or in the VRS [99]. Leptomeningeal enhancement follows accumulation of contrast at the pial surface following contrast leak from leptomeningeal blood vessels [100] and suggests inflammation at the pial surface [78]. The ‘dot-dash’ sign is seen in damage to the ependymal layer [101].
Barrier models	The ability of cells and proteins to cross the BBB and BCSFB can be tested in vitro using specialised cell models [102].
Passive transfer into animal models	If the passive transfer of human CSF pathology, such as antibodies, into the CSF of animals causes a similar disease phenotype this supports an essential role of the CSF pathology, especially if this does not occur with transfer into the serum.

Table 2. The characteristics of conditions in which a blood-CSF-brain indirect route of pathological spread is suspected. NMO – Neuromyelitis optica; AQP4 – Aquaporin-4; MOG – myelin oligodendrocyte glycoprotein.

ANTI-MOG ANTIBODY DISEASE	**SIMILAR TO NMO - OPTIC NERVE, SPINAL CORD AND BRAINSTEM BUT GREATER CORTICAL GREY MATTER INVOLVEMENT. RELATIVE SPARING OF THE DEEP WHITE MATTER [80]**	**RAISED QALB [117]**	**CASES OF ANTI-MOG ANTIBO DETECTABLE IN CSF BUT NO SERUM [118, 119]**
GFAP ASTROCYTOPATHY	Leptomeninges, periventricular, cortical grey matter, spinal cord, retina, cerebellum and hippocampus [120-122]	Unknown	CSF anti-GFAP antibodies have bet diagnostic sensitivity than serum an [120]
MULTIPLE SCLEROSIS	Widespread with predominance of periventricular 'Dawson's fingers' lesions [124]	Raised Qalb, during clinical relapse [125] Choroid plexus involvement [56, 102, 126] Lymphocytes transfer into the CSF across choroid plexus in animal models [56] Reduced PET tracer influx into the ventricles [87]	Intrathecal antibody synthesis usual present [127] CSF lymphocyte proliferation in se progressive disease [128]

CELL-SURFACE ANTIBODY MEDIATED AUTOIMMUNE ENCEPHALITIS	Specific for antibody e.g. hippocampus in NMDAR-antibody encephalitis [132]	Unknown	Intrathecal antibodies synthesis is predominant with NMDAR-antibod encephalitis and CSF antibodies are diagnostically sensitive than serum antibodies [73, 133] Intrathecal antibodies synthesis can occur in LGI1 and AMPAR-antibod encephalitis and is associated with v clinical outcome [134, 135] CSF NMDAR-antibody levels corre better with disease severity than ser levels [133] An animal model of NMDAR-antib encephalitis is produced following t of human NMDAR-antibodies into [73, 136]
NEUROPSYCHIATRIC LUPUS	Periventricular and brainstem lesions are common [137] Additional subcortical infarctions relating to microthrombi accumulation [40]	Raised Qalb [138] Choroid plexus involvement [72, 139-141]	Intrathecal antibody synthesis occur CSF antibody titres correlate better disease severity compared with seru
NEUROSARCOIDOSIS	Leptomeninges, periventricular, perivascular, VRS, optic nerve, brainstem [70]	Raised Qalb, especially during active disease [143] Choroid plexus involvement [70]	Intrathecal antibody synthesis occur
CRYPTOCOCCAL INFECTION	Leptomeninges, VRS and basal ganglia [145]	Choroid plexus infiltration by organism [146] Raised Qalb is a poor prognostic factor [147]	Reactive intrathecal antibody synthe associated with better prognosis [14 Organism grown from CSF culture

LEPTOMENINGEAL CARCINOMATOSIS	Leptomeninges [91]	Raised Qalb [91] Transfer of malignant cells across in vitro BCSFB [90, 149]	Reactive intrathecal antibody synth Tumour cells detectable in CSF [91
PRIMARY CNS LYMPHOMA	Periventricular 'butterfly' lesions, leptomeninges, basal ganglia, cerebellum [151]	Raised Qalb [152] Choroid plexus involvement [77]	Tumour cells detected by flow cyto and a minority have reactive intrath antibody synthesis [153]

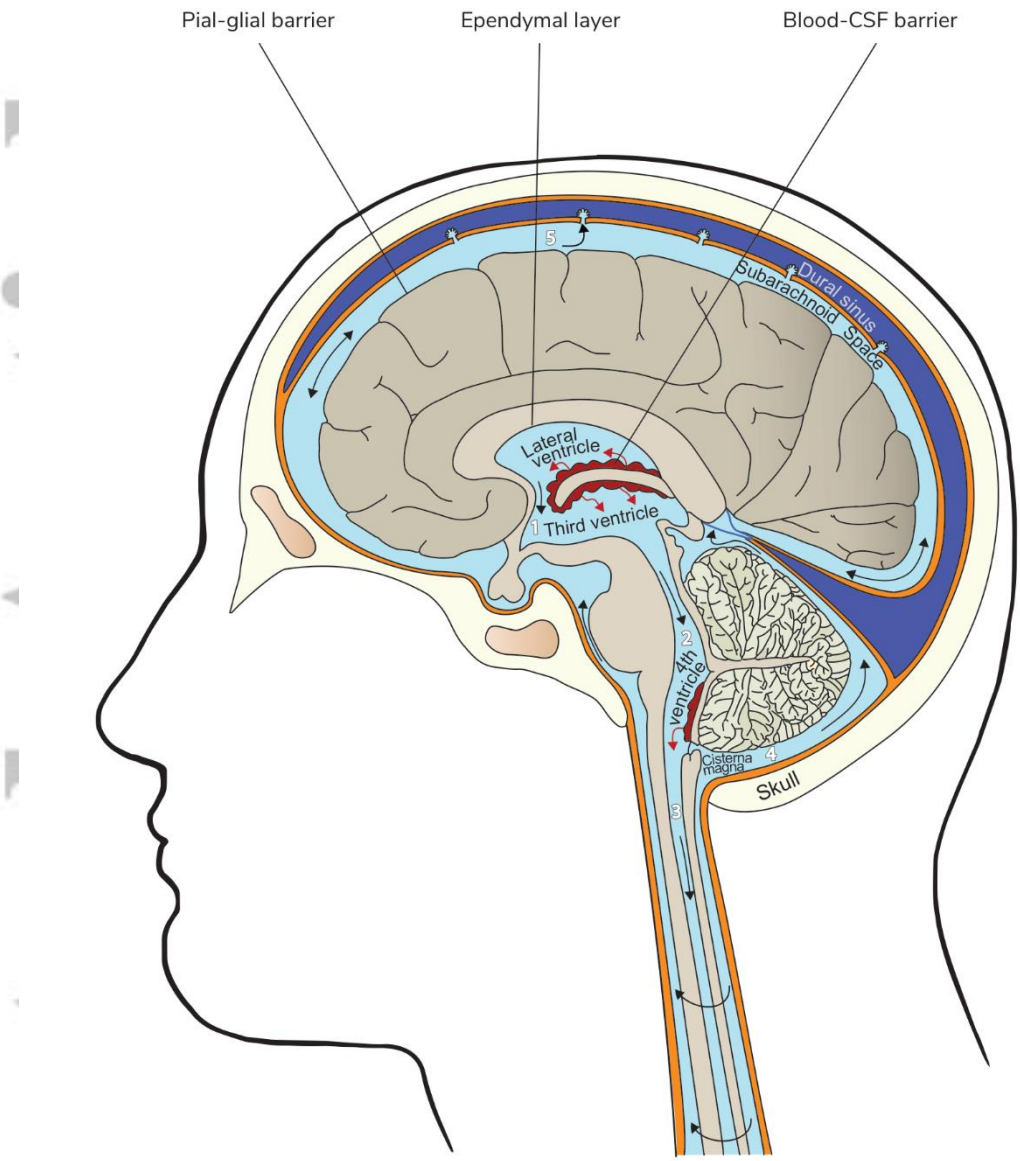

Fig. 1 The classic model of CSF dynamics. Ventricular CSF is produced from the blood via the choroid plexuses in the walls of each of the four ventricles (red arrows). CSF flows (black arrows) from the lateral into the third ventricle (1), then flows into the fourth ventricle (2) via the cerebral aqueduct. From the fourth ventricle CSF flow is either into the central canal of the spine (3) or into the subarachnoid space at the cisterna magna (4). CSF flows within the subarachnoid space and is cleared via the arachnoid villi into the dural venous sinuses (5). The location of the brain barriers discussed in this review are also shown (black circles).

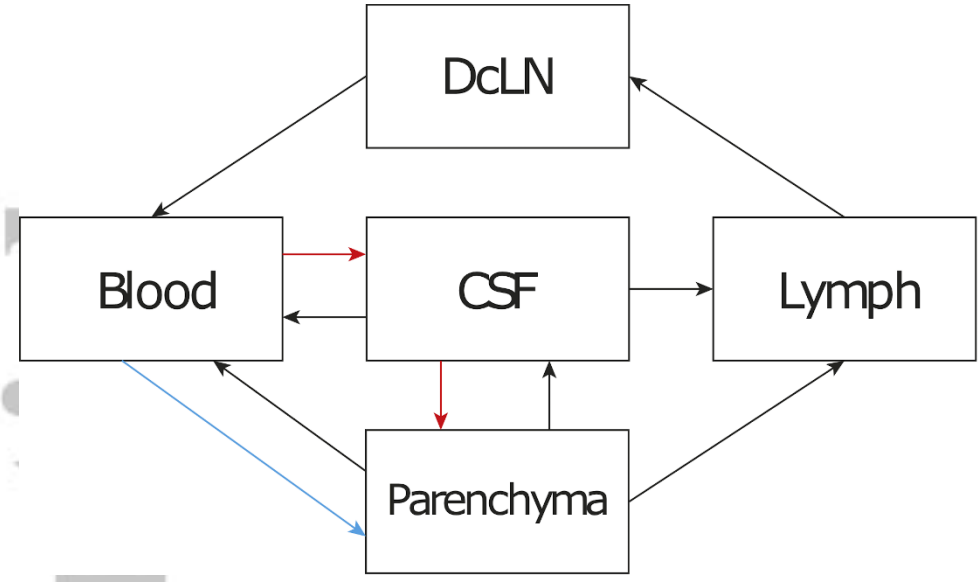

Fig. 2 Simplified model of brain solute exchange. The direct pathway into the parenchyma is across the blood-brain-barrier (blue arrow). The indirect pathway requires movement of solutes firstly from the blood into the CSF and then into the parenchyma (red arrows). Drainage from the parenchyma (black arrows) can be directly back into the blood, into the CSF or via blood vessel walls into the dural/cervical lymphatics. Solutes in the CSF can re-enter the blood at the choroid plexus or enter the lymph. The lymph flows into the deep cervical lymph nodes (DcLN) and finally on to the blood.

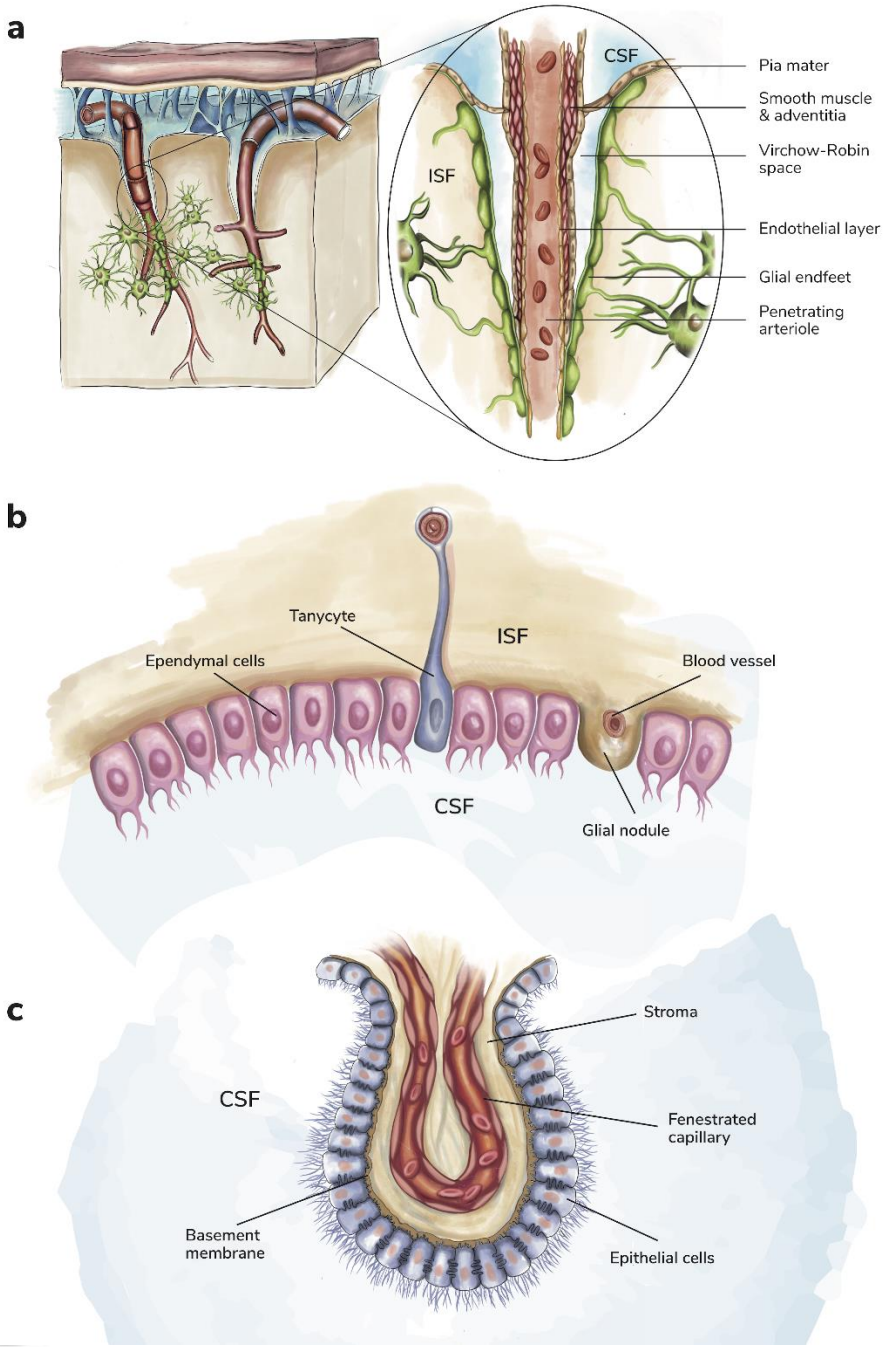

Fig. 3 Barriers to brain solute exchange **(a)** Penetrating arteriole at the cortical surface. The blood vessel is covered with endothelium, smooth muscle cells and adventitia. The CSF is separated from the ISF by the pial-glia barrier, which is comprised of the pia mater and glial end-feet. The pia mater extends to cover the arteriole in the subarachnoid space. The Virchow-Robin space (VRS) surrounds the penetrating arteriole. The VRS and ISF are separated by the pial-glial barrier. **(b)** The choroid plexus is a collection of outpouchings into the ventricular CSF. These are highly vascular structures which contain fenestrated capillaries surrounded by an extracellular fluid called the stroma. The ciliated choroid plexus epithelial cells are connected by tight junctions and sit on a basement membrane, this forms the blood-CSF-barrier. **(c)** The ependymal layer separating the ventricular CSF and the ISF. This is mainly

comprised of ciliated ependymal cells. Tanycytes are also present and connect to both the CSF and blood vessels. Glial nodules, which can contain blood vessels, form in areas of ependymal damage.

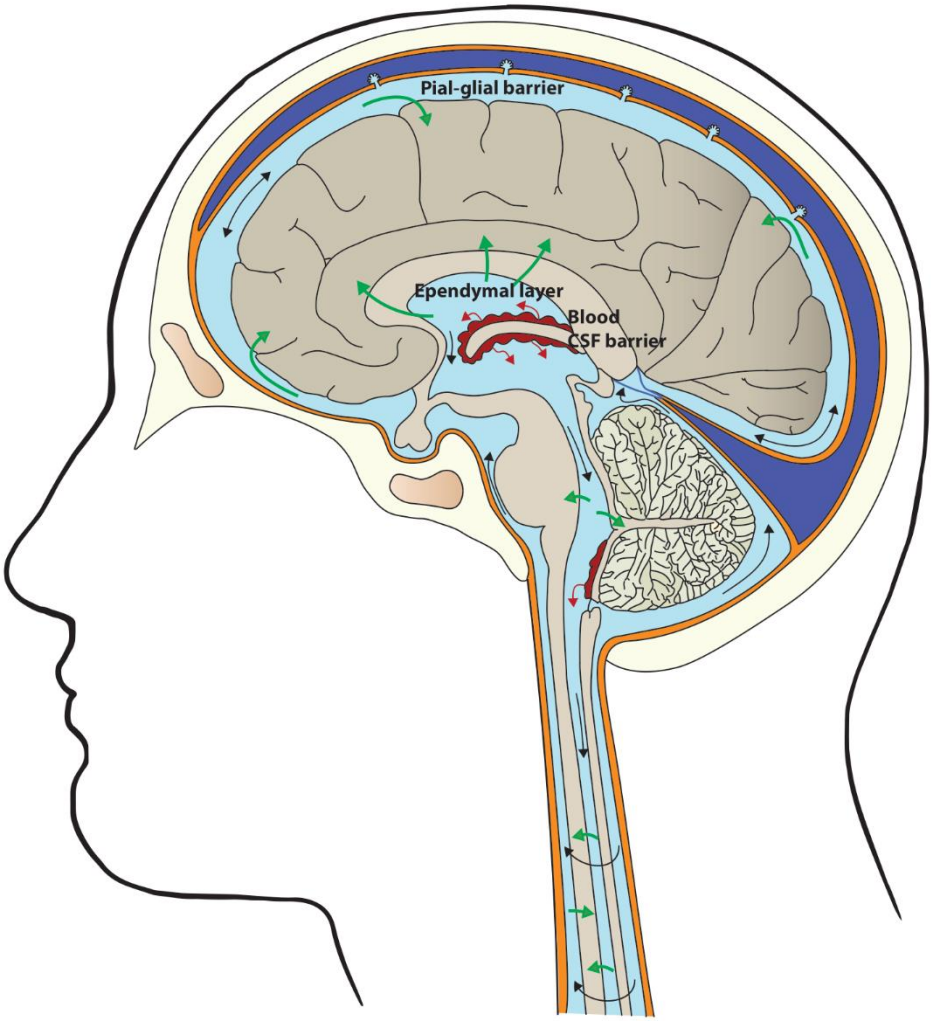

Fig. 4 The blood-CSF-brain indirect route of pathology influx into the brain. Micro-organisms, tumour cells and immune cells/antibodies can enter the CSF via the choroid plexus, (dark red) where they cross the blood-CSF barrier. Rapid transport throughout the CSF spaces occurs (black arrows). Entry into parenchyma occurs across the ependymal layer and the pial-glial barrier (green arrows).

Graphical Abstract