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

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Word count: 3889
Figures: 4
Tables: 2

Acknowledgements

We would like to thank Federico Baratto for his support with the figures.

Author contributions
This article was conceived by Oliver Cousins, who produced the first draft of the manuscript. All authors commented previous versions of the manuscript and critically revised the work. All authors read and approved the final manuscript.

**Conflict-of-interest statement**

No conflict of interest has been declared by the authors.

**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, 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

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). Solute movement 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 cribiform 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]. Tanyctyes 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 ³H-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], neurosarcoïdosis [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 into 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 in BCFSB 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 BCFSB 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 BCFSB 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.

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

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Pathological assessment</td>
<td>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.</td>
</tr>
<tr>
<td>Qalb</td>
<td>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].</td>
</tr>
<tr>
<td>Raised CSF parenchymal proteins</td>
<td>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.</td>
</tr>
<tr>
<td>Intrathecal antibody synthesis</td>
<td>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].</td>
</tr>
<tr>
<td>MRI</td>
<td>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].</td>
</tr>
<tr>
<td>Barrier models</td>
<td>The ability of cells and proteins to cross the BBB and BCSFB can be tested in vitro using specialised cell models [102].</td>
</tr>
<tr>
<td>Passive transfer into animal models</td>
<td>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.</td>
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</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>ANTI-MOG ANTIBODY DISEASE</th>
<th>SIMILAR TO NMO - OPTIC NERVE, SPINAL CORD AND BRAINSTEM BUT GREATER CORTICAL GREY MATTER INVOLVEMENT. RELATIVE SPARING OF THE DEEP WHITE MATTER [80]</th>
<th>RAISED QALB [117]</th>
<th>CASES OF 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]</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFAP ASTROCYTOPATHY</td>
<td>Leptomeninges, periventricular, cortical grey matter, spinal cord, retina, cerebellum and hippocampus [120-122]</td>
<td>Unknown</td>
<td>CSF anti-GFAP antibodies have better diagnostic sensitivity than serum antibodies [120]</td>
</tr>
<tr>
<td>MULTIPLE SCLEROSIS</td>
<td>Widespread with predominance of periventricular ‘Dawson’s fingers’ lesions [124]</td>
<td>Raised Qalb, during clinical relapse [125]</td>
<td>Intrathecal antibody synthesis usually present [127]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Choroid plexus involvement [56, 102, 126]</td>
<td>CSF lymphocyte proliferation in secondary progressive disease [128]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lymphocytes transfer into the CSF across choroid plexus in animal models [56]</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced PET tracer influx into the ventricles [87]</td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>Description</td>
<td>Intrathecal Antibodies Synthesis</td>
<td>CSF Antibody Levels and Disease Severity</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
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<td>------------------------------------------</td>
</tr>
<tr>
<td><strong>CELL-SURFACE ANTIBODY MEDIATED AUTOIMMUNE ENCEPHALITIS</strong></td>
<td>Specific for antibody e.g. hippocampus in NMDAR-antibody encephalitis [132]</td>
<td>Unknown</td>
<td>Intrathecal antibodies synthesis is predominant with NMDAR-antibody encephalitis and CSF antibodies are diagnostically sensitive than serum antibodies [73, 133]</td>
</tr>
<tr>
<td><strong>NEUROPSYCHIATRIC LUPUS</strong></td>
<td>Periventricular and brainstem lesions are common [137]</td>
<td>Raised Qalb [138]</td>
<td>CSF antibody titres correlate better with disease severity compared with serum levels [133]</td>
</tr>
<tr>
<td></td>
<td>Additional subcortical infarctions relating to microthrombi accumulation [40]</td>
<td>Choroid plexus involvement [72, 139-141]</td>
<td>In an animal model of NMDAR-antibody encephalitis is produced following transfer of human NMDAR-antibodies into CSF [73, 136]</td>
</tr>
<tr>
<td><strong>NEUROSARCOIDOSIS</strong></td>
<td>Leptomeninges, periventricular, perivascular, VRS, optic nerve, brainstem [70]</td>
<td>Raised Qalb, especially during active disease [143]</td>
<td>Reactive intrathecal antibody synthesis is associated with better prognosis [144]</td>
</tr>
<tr>
<td></td>
<td>Choroid plexus involvement [70]</td>
<td></td>
<td>Organism grown from CSF culture [146]</td>
</tr>
<tr>
<td><strong>CRYPTOCOCCAL INFECTION</strong></td>
<td>Leptomeninges, VRS and basal ganglia [145]</td>
<td>Choroid plexus infiltration by organism [146]</td>
<td>Raised Qalb is a poor prognostic factor [147]</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>LEPTOMENINGEAL CARCINOMATOSIS</th>
<th>Leptomeninges [91]</th>
<th>Raised Qalb [91]</th>
<th>Reactive intrathecal antibody synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transfer of malignant cells across in vitro BCSFB [90, 149]</td>
<td>Tumour cells detectable in CSF [91]</td>
<td></td>
</tr>
<tr>
<td>PRIMARY CNS LYMPHOMA</td>
<td>Periventricular ‘butterfly’ lesions, leptomeninges, basal ganglia, cerebellum [151]</td>
<td>Raised Qalb [152]</td>
<td>Tumour cells detected by flow cytometry and a minority have reactive intrathecal antibody synthesis [153]</td>
</tr>
<tr>
<td></td>
<td>Choroid plexus involvement [77]</td>
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</tbody>
</table>
**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-glial 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. Tanyocytes 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).