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Neurological infection with SARS-CoV-2 — the story so far

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As the COVID-19 pandemic developed and neurological manifestations were reported, concern grew that SARS-CoV-2 might directly invade neuronal cells. However, research throughout the year to address this concern has revealed a different story with inflammatory processes at its centre.

Historically, the great epidemic causes of neurological disease, such as Japanese encephalitis and polio viruses, have directly infected the brain and spinal cord, causing widespread inflammation and destruction. As the pandemic of COVID-19 developed and increasing numbers of patients with neurological disease were reported, the question was, would SARS-CoV-2 behave in a similar way?

Initial reports of patients with COVID-19 who exhibited clinical evidence of brain inflammation (from imaging or lumbar puncture) suggested that SARS-CoV-2 encephalitis can occur[(1)]**,** though the rarity with which the virus was found in the cerebrospinal fluid (CSF), implied that immune-mediated damage is more important than viral replication in neurons. As the numbers of case reports and small series grew, it became clear that anosmia, encephalopathy and stroke were the predominant neurological syndromes associated with COVID-19(1).

Anosmia and associated dysgeusia are common among people infected with SARS-CoV-2, and often occur in the absence of other symptoms. Herpes simplex virus type-1 infects the olfactory bulb and subsequently the brain, leading to encephalitis; animal models have shown that the same is true for some coronaviruses, including SARS-CoV-1, the cause of severe acute respiratory syndrome (SARS). Consequently, concerns were raised initially that olfactory infection with SARS-CoV-2 might lead to CNS disease. However, an elegant study published in July 2020 indicated that SARS-CoV-2 infects the supporting cells in the olfactory epithelium rather than the sensory neurons(2).

In this study, single-cell RNA sequencing gene expression analysis of human nasal biopsy samples showed that the supporting cells, particularly sustentacular and horizontal basal cells, express angiotensin converting enzyme 2 (ACE2) receptors and cell surface transmembrane serine protease 2 (TMPRSS2), both of which are critical for viral entry,

whereas olfactory sensory neurons do not. Immunohistochemical staining confirmed ACE2 protein expression in these same cells. Equivalent observations were made in the mouse, where the deeper olfactory bulb tissue could also be examined. Here, ACE2 receptors were found in vascular cells, predominantly pericytes, and immune cells of the macrophage/monocyte lineage, but not in neurons(2).

SARS-CoV-2 infection of these supporting cells could lead to anosmia via several mechanisms. First, the supporting cells in the olfactory epithelium are responsible for local water and ion balance, and damage to them can influence neuronal signalling from the olfactory sensory neurons to the brain. Second, infection of these cells and pericytes in the olfactory bulb could perturb neuronal signalling through local inflammation with cytokine release. Third, vascular damage and hypoperfusion in the olfactory bulb could contribute to impaired function. Finally, any of these changes could indirectly trigger death of the olfactory sensory neurons. Imaging studies in patients with COVID-19 and anosmia have revealed hyperintensity and swelling of the olfactory bulb, consistent with inflammation, which subsequently resolved, just as the symptoms do in most patients.

Though anosmia is the most common neurological symptom in people with mild disease, alterations in higher mental function are more important among patients who are hospitalized with COVID-19. Terms such as encephalopathy and delirium have been used to describe these changes; different specialities have preferred different terms, which makes it challenging to compare data.

In one study conducted in France, 118 (84%) of 140 consecutive patients admitted to intensive care units (ICUs) with COVID-19 developed delirium with a combination of acute disturbances in attention, awareness and cognition; 88 (69%) had corticospinal tract signs(3). Delirium can occur in any patient in ICU, especially if staff are wearing personal protective equipment and loved ones are excluded. However, accumulating data suggest we are seeing more than expected on this basis and that these symptoms could be characteristic of SARS-CoV-2 infection, especially given that delirium, encephalopathy and other neuropsychiatric manifestations are also seen in patients with milder respiratory and systemic COVID-19, who are not in ICU.

Data from analysis of CSF, autopsy samples and imaging data are beginning to elucidate disease mechanisms that could underlie these cognitive disturbances. Typically, pleocytosis is not seen in CSF of individuals with encephalopathic COVID-19, but protein levels can be elevated with matched oligoclonal bands. Elevated plasma and CSF levels of cytokines, glial fibrillary acidic protein and neurofilament light chain in COVID-19 are thought to reflect a proinflammatory systemic and brain response that involves microglial activation and subsequent neuronal damage(4,5). Further evidence for inflammatory mechanisms comes from imaging findings in the French ICU study, which showed meningeal enhancement and diffuse white matter abnormalities as well as microhaemorrhages(3).

In addition to inflammatory changes, coagulopathy and vascular endothelial dysfunction, which cause large vessel strokes in patients with COVID-19, can also lead to small vessel occlusions and microhaemorrhages, which could contribute to more subtle neurological and neuropsychiatric presentations, as suggested by clinical and imaging studies(6). However,

the most definitive data on the underlying mechanisms come from autopsy series. One such study conducted in Germany included 43 individuals, most of whom died in ICUs, general wards or nursing homes from pneumonia or sepsis associated with COVID-19(7). Six of these individuals had acute ischaemic brain lesions. Activation of astrocytes was widespread across many brain areas, whereas activation of microglia was confined to the brainstem and cerebellum. Cytotoxic T cells were also seen in the brainstem and in the meninges of many patients. RNA detection and immunohistochemistry showed that SARS-CoV-2 was widely distributed throughout the brain, especially the brainstem. However, no correlation was seen between the location of the virus and inflammation, or indeed between PCR detection and immunohistochemical staining of the virus. Without double immunostaining, it is hard to be certain which cell types were infected in the brain, but results from an electron microscopy study indicates that infection of vascular endothelial cells is more likely than infection of neurons(8). However in an autopsy study of 33 patients, there was PCR and immunohistochemical evidence of SARS-CoV-2 in cells thought to be olfactory sensory neurons, and in anatomically connected regions of the brain(9).
The observation that some patients with encephalopathic changes respond to corticosteroids, appears to underscore the importance of immune-mediated mechanisms rather than direct viral effects(10).

In summary, if the retina is said to be the window on the brain, then for understanding SARS-CoV-2, the nose has perhaps been the front door. For just as SARS-CoV-2 causes disturbance of smell without infecting olfactory sensory neurons, the evidence to date suggests that the disturbance of higher mental function occurs predominantly without infection of CNS neurons. Although the virus can get into the brain, it seems to predominantly infect vascular and immune cells rather than neurons. Local inflammation upregulates astrocytes and microglia, which perhaps compound the effects of pro-inflammatory cytokines in the circulation caused by severe systemic disease. Microvascular infarcts and haemorrhages, which are part of the systemic coagulopathy and vasculopathy of COVID-19, are probably also critical elements in the development of encephalopathy, delirium and other neurological and neuropsychiatric manifestations of SARS-CoV-2 infection.

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Competing interests
The author declares no competing interests

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Key advances

- Anosmia, encephalopathy and stroke are the most common neurological syndromes associated with SARS-CoV-2 infection[1], though many others have been reported.
- Analysis of human biopsy samples suggests that anosmia results predominantly from SARS-CoV-2 infection of non-neuronal cells in the olfactory epithelium and olfactory bulb[2], leading to local inflammation and neuronal malfunction.
- A high proportion of patients admitted to intensive care units with COVID-19 develop delirium, and evidence suggests that this is caused by microvascular [this word is essential here] and inflammatory mechanisms[3].
- Autopsy data show activation of astrocytes and microglia in COVID-19, particularly in the brainstem, where there is also infiltration of cytotoxic T cells[7,9].

- SARS-CoV-2 can be detected in the brain with PCR and immunohistochemistry, but the evidence to date suggests it is mostly in vascular and immune cells rather than directly infecting neurons[7,9].

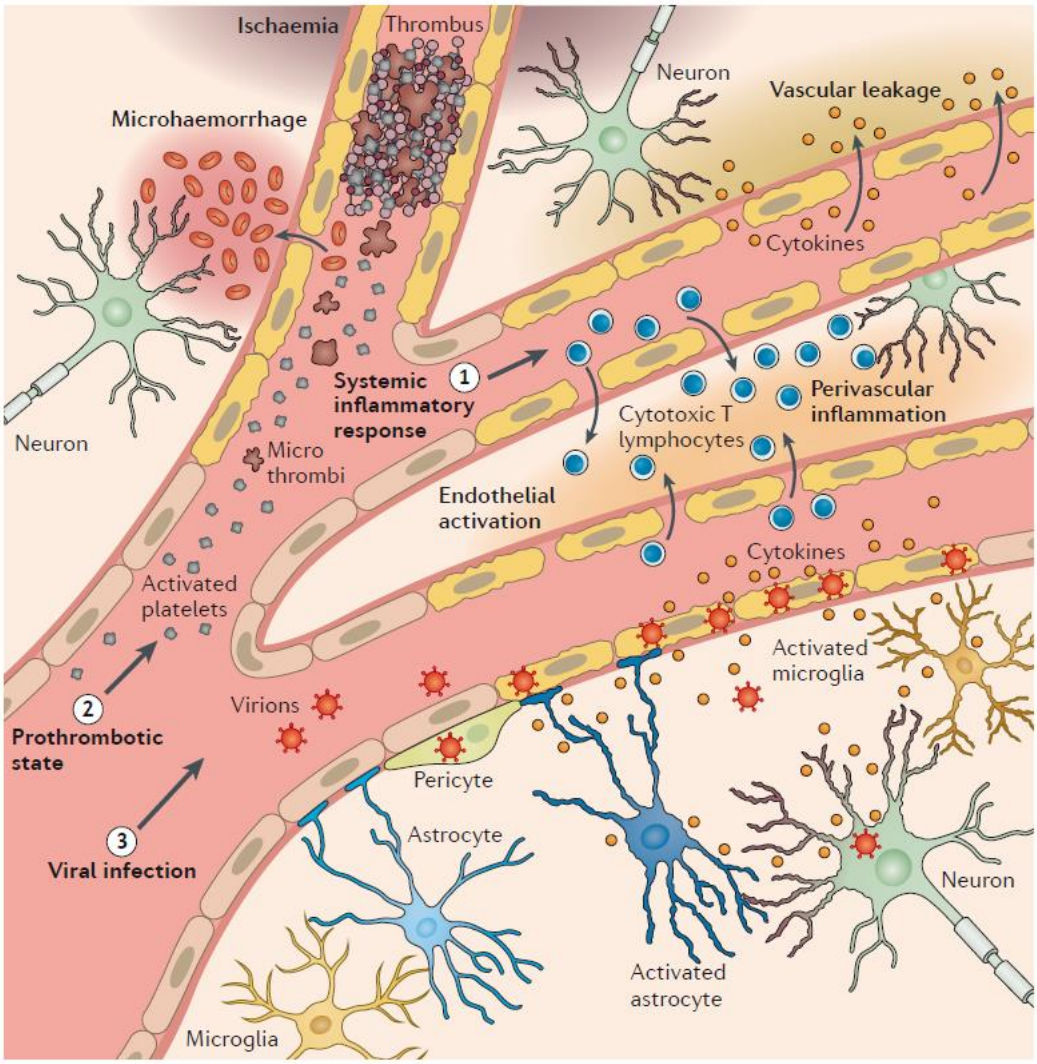
Figure legend:

Current understanding of the predominant disease mechanism causing CNS Covid-19 disease
1- A systemic inflammatory disease causes activation of the vascular endothelium, a proinflammatory cytokine response with vascular leakage, and inflammatory cells crossing into the brain
2- A prothrombotic state with activated platelets and microthrombi leads to thrombus formation, ischaemia and associated microhaemorrhage
3. Virus enters the vascular endothelium and associated cells such as pericytes leading to activation of microglia and astrocytes, with virus occasionally entering neurons.

Or shortened figure legend:

Current understanding of the predominant disease mechanism causing CNS Covid-19 disease, showing the importance of a systemic inflammatory response, prothrombotic state and direct viral invasion

Fig 1


Ischaemia
Thrombus
Neuron
Vascular leakage
Microhaemorrhage
Cytokines
Systemic inflammatory response
1
Perivascular inflammation
Cytotoxic T lymphocytes
Neuron
Micro thrombi
Endothelial activation
Cytokines
Activated platelets
Activated microglia
Virions
2
Prothrombotic state
Pericyte
Astrocyte
3
Viral infection
Neuron
Activated astrocyte
Microglia