









# Invited Review: The spectrum of neuropathology in COVID-19

S. Al-Sarraj\*† , C. Troakes† , B. Hanley‡, M. Osborn‡, M. P. Richardson§ , M. Hotopf§¶ , E. Bullmore\*\*  and I. P. Everall§ 

\*Department of Clinical Neuropathology, King's College Hospital NHS Foundation Trust, London, UK, †London Neurodegenerative Diseases Brain Bank, Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK, ‡Department of Cellular Pathology, Imperial College Healthcare NHS Trust, London, UK, §The Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK, ¶National Institute of Health Research Biomedical Research Centre at South London and Maudsley NHS Foundation Trust, London, UK and \*\*Department of Psychiatry, University of Cambridge, Cambridge, UK

S. Al-Sarraj, C. Troakes, B. Hanley, M. Osborn, M. P. Richardson, M. Hotopf, E. Bullmore and I. P. Everall (2020) *Neuropathology and Applied Neurobiology*

## Invited Review: The spectrum of neuropathology in COVID-19

There is increasing evidence that patients with Coronavirus disease 19 (COVID-19) present with neurological and psychiatric symptoms. Anosmia, hypogeusia, headache, nausea and altered consciousness are commonly described, although there are emerging clinical reports of more serious and specific conditions such as acute cerebrovascular accident, encephalitis and demyelinating disease. Whether these presentations are directly due to viral invasion of the central nervous system (CNS) or caused by indirect mechanisms has yet to be established. Neuropathological examination of brain tissue at autopsy will be essential to establish the neuro-invasive potential of the SARS-CoV-2 virus but, to date, there have been few detailed

studies. The pathological changes in the brain probably represent a combination of direct cytopathic effects mediated by SARS-CoV-2 replication or indirect effects due to respiratory failure, injurious cytokine reaction, reduced immune response and cerebrovascular accidents induced by viral infection. Further large-scale molecular and cellular investigations are warranted to clarify the neuropathological correlates of the neurological and psychiatric features seen clinically in COVID-19. In this review, we summarize the current reports of neuropathological examination in COVID-19 patients, in addition to our own experience, and discuss their contribution to the understanding of CNS involvement in this disease.

Keywords: Brain, pathology, COVID-19, SARS-CoV-2, cerebrovascular accident (CVA), encephalitis, microglia cells, T lymphocytes

## Introduction

A new infectious respiratory disease caused by a novel virus emerged in Wuhan, China, in December 2019

Correspondence: Safa Al-Sarraj, Department of Clinical Neuropathology, King's College Hospital NHS Foundation Trust, London, UK. E-mail: safa.al-sarraj@kcl.ac.uk

Safa Al-Sarraj and Claire Troakes are joint first author

and subsequently spread worldwide. The virus was identified as a member of the Coronaviridae family and shown to be closely related to severe acute respiratory syndrome coronavirus (SARS-CoV), hence it is now termed SARS-CoV-2 and the disease it causes has been named Coronavirus disease 19 (COVID-19).

The virus possesses a spike (S) glycoprotein that is responsible for host cell attachment and mediating fusion of the host cell membrane and viral membrane

during infection. Both SARS-CoV and SARS-CoV-2 have been shown to engage with the angiotensin converting enzyme 2 (ACE2) receptor releasing the S1 sub-unit and triggering pre- to postfusion conformational transition by employing the cellular serine protease TMPRSS2 for protein priming [1,2].

The abundant expression of ACE2 on endothelial cells and smooth muscle cells in virtually all organs suggests that these viruses, once present in the circulation, can spread easily through the body. Indeed, human neural cells, including neurons, are infectible by SARS-CoV and neuronal expression of ACE2 has been detected in the human CNS [3].

Because the RNA sequence similarity between SARS-CoV-2, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) is as high as 79.5%, the mechanisms of SARS-CoV-2 infection may well be similar to those of SARS-CoV, however, the binding affinity of the SARS-CoV-2 spike protein has been reported to be significantly higher than both SARS-CoV and MERS-CoV [4]. Thus, as SARS-CoV-2 and ACE2 have a stronger binding capacity, this new virus appears to possess more powerful pathogenicity and transmissibility [4].

COVID-19 primarily manifests as a respiratory illness with fever, dry cough, breathlessness and pneumonia in severe cases (which show characteristic radiological changes of bilateral lung opacities) [5]. It is also linked to gastrointestinal symptoms (diarrhoea) and cardiovascular system dysfunction. Additionally, there are increasing reports of both neurological and psychiatric symptoms in patients [6]. Loss of taste and smell are commonly reported (often as an early presentation) and there have been a number of clinical reports of headache, nausea, vomiting, impaired consciousness, encephalitis and acute cerebrovascular disease including stroke, venous sinus thrombosis and intracerebral haemorrhage [5-9].

The expression and distribution of ACE2 suggest that SARS-CoV-2 may cause neurological symptoms through either direct or indirect mechanisms [10,11]. However, the definite clinical and pathological basis of CNS involvement in COVID-19 is still poorly understood and the neuro-invasive potential has yet to be determined.

### **Potential routes and mechanisms of CNS involvement in COVID-19**

The mechanisms of CNS infection in human coronaviruses (HCoV-OC43, HCoV-229E, MERS-CoV) have

not yet been definitively elucidated. However previous studies demonstrated the presence of SARS-CoV in brain samples by immunohistochemistry, electron microscopy and real-time reverse transcription-polymerase chain reaction (RT-PCR) [12,13] and in cerebrospinal fluid (CSF) by polymerase chain reaction (PCR) in the acute phase of patient illness [12]. Studies using experimental animals established that SARS-CoV may enter the brain via the olfactory nerve and initially spread to connected brain regions before proliferating more widely – including to areas such as the brainstem and cardiorespiratory centre in the medulla; the latter may potentially contribute to death. The infection of the olfactory nerve is followed by involvement of regions such as the piriform and infralimbic cortices, basal ganglia and dorsal raphe nuclei in the midbrain [14]. Other regions, including the thalamus and hypothalamus are less consistently positive whereas there is significant neuronal loss in the cingulate and anterior olfactory nuclei; a pattern of distribution suggestive of trans-neuronal spread. The common presentation of altered or loss of smell (hyposmia) in COVID-19 suggests this transmission via the olfactory nerve and its anatomical brain connection pathways may also to be utilized by SARS-CoV-2 [14].

Another interesting commonly reported clinical symptom of COVID-19 is hypogeusia (loss of taste) [7]. The sense of taste is conducted via the facial nerve (from the anterior two-thirds of the tongue) and glossopharyngeal nerve and superior laryngeal branch of the vagus nerve (from the posterior two-thirds of the tongue), the information is passed to the nucleus of the solitary tract in the brainstem and then to the thalamus. Thus, these cranial nerves potentially provide another route for trans-neuronal spread to the brain [2].

The presence of SARS-CoV-2 in the general circulation could provide a haematogenous route of entry to the CNS. The virus might utilize the ACE2 receptors in the endothelial cells followed by subsequent budding of the viral particles from the capillary endothelium, damaging them, gaining access through the blood–brain barrier (BBB) and initiating viral budding through interaction with ACE2 receptors in the neurones. Varga *et al.* claim to demonstrate SARS-CoV-2 particles by electron microscopy within endothelial cells in the blood vessels of *postmortem* tissue from kidney, lung, heart and liver (associated with lymphocytic

endotheliitis) [15]. The infection of endothelial cells causes rupture of the capillaries leading to bleeding or haemorrhagic infarction, which have been recently reported in COVID-19 patients (discussed further below) and can result in a fatal outcome.

The third possible route of entry of SARS-CoV-2 into the CNS is via immune cells. The virus enters the body through the respiratory tract and first infects the epithelial cells of the trachea and bronchi and the alveolar cells of the lung, subsequently infecting the resident immune cells which carry the virus to other organs, including the brain [16].

A further mechanism of CNS involvement comes from reports of autoimmune encephalitis in COVID-19 patients [17], a condition which has been hypothesized to be related to a genetic susceptibility that leads to excessive self-response and antigen-driven immune responses [18-20]. In SARS patients, autoantibodies against the coronavirus spike protein were found to react with human epithelial and endothelial cells, and cause cytotoxicity [21,22]. Therefore, similarly, patients with COVID-19 may produce antibodies against SARS-CoV-2 which also attack antigens in human endothelial cells in cerebral vessels or in neurons (through the disrupted BBB), resulting in cerebral oedema and autoimmune encephalitis.

#### Principle mechanisms of SARS-CoV-2 CNS infection:

The virus attaches to cells in the CNS through interaction between the spike (S) glycoprotein and the host ACE2 receptors, which are reported to be present in neurons as well as endothelial cells.

#### Potential modes of CNS involvement by SARS-CoV-2

- (1) Through the olfactory nerve and/or the hypoglossal, facial, glossopharyngeal and vagus cranial nerves with trans-synaptic neuronal spread to other brain regions. Supported by frequent initial presentation of hyposmia and hypogeusia.
- (2) Haematogenous route via endothelial cell infection, utilizing ACE2 receptors in the endothelial cells followed by gaining access through the BBB with viral budding via interaction with ACE2 receptors in the neurons.

- (3) Immune cell route. The virus first infects the epithelial cells of the trachea, bronchi and alveolar cells of the lung. Then it infects the resident immune cells, which carry the virus to other organs including the brain.
- (4) An auto-immune mechanism

#### Clinical evidence of CNS involvement by SARS-CoV-2

SARS-CoV-2 infection presents with common symptoms of fever, cough, fatigue, headache and dyspnoea. In severe cases, patients develop pneumonia, acute respiratory distress syndrome, cardiac symptoms and multi organ failure. Recent evidence suggests that COVID-19 may also involve the central and peripheral nervous systems (PNS). There are increasing clinical reports of symptoms suggesting CNS involvement; such as headache, nausea, vomiting, impaired consciousness and acute cerebrovascular disease including stroke, venous sinus thrombosis and intracerebral haemorrhage [5-7,23].

In a retrospective study of 214 patients with COVID-19 by Mao *et al.*, 36.4% of patients were found to have non-specific neurological manifestations, such as dizziness, headache, nausea and more specific symptoms such as hypogeusia and hyposmia [7]. In severe cases with acute cerebrovascular disease or disturbance of consciousness many patients showed lower lymphocyte levels compared with those without CNS symptoms. The CNS involvement appeared to indicate poor prognosis and death [7].

Wang *et al.* reported probable neurological manifestations such as headache (6.5%), vomiting (3.6%), dizziness (9.4%), nausea (10.1%) and myalgia (34.8%) in their review of clinical manifestations in 138 hospitalized COVID-19 positive patients [5]. Additionally

Chen *et al.* studied 99 SARS-CoV-2 positive patients with common symptoms of fever, cough and shortness of breath in the majority of patients, and described neurological manifestations in a number of them – specifically, nausea and vomiting (1%), confusion (9%), headache (8%) and muscle ache (11%) [23].

More specific neurological presentations of meningo-encephalitis have been described by several authors in different age groups, including in children [24]. A 24-year-old male with meningitis/encephalitis associated

with COVID-19 was described by Moriguchi *et al.* [25]. The patient was admitted with unconsciousness and convulsions after complaining of fever and vomiting but had no neck stiffness. Brain MRI showed hyperintensity along the lateral ventricle and hippocampus. Additionally, Huang *et al.* and Xiang *et al.* published cases in which SARS-CoV-2 RNA was isolated from the CSF, suggesting neuro-virulence of the virus [26,27], however, it is not certain if this indicates replicative/infective potential of the virus.

Another neurological entity reported in COVID-19 patients has been termed acute necrotizing encephalopathy. It is reported by Dixon *et al.* in a 59-year-old female who deteriorated on day 6 after admission. MRI demonstrated brain stem swelling and symmetrical haemorrhagic lesions in the brain stem, amygdalae, putamina and thalamic nuclei [28]. Cases with a similar presentation and characteristic MRI features including symmetric, multifocal lesions with invariable thalamic, brain stem, cerebral white matter and cerebellar multiple necrotic regions were also reported by Mao *et al.* and Poyiadji *et al.* [7,29].

A review of the current literature showed that acute cerebrovascular disease is one of the more common, serious neurological complications seen in COVID-19 populations. In Italian, Dutch and Chinese cohorts of COVID-19 patients admitted with confirmed infection, the rate of ischaemic stroke was 2.5, 3.7 and 5% respectively, despite venous thromboprophylaxis [8,30,31].

Mao and colleagues reported cerebrovascular accidents (CVA) in 5% of their cohort; with five ischaemic and one haemorrhagic stroke [7]; two of these patients were admitted with acute cerebrovascular accident as the initial presentation without prodromal symptoms but were found to have lung lesions and tested positive for COVID-19. The other patients presented with cough, fever and acute cerebrovascular symptoms, the COVID-19 test was negative initially but confirmed positive on repeat a few days later.

CVA resulting in an intracranial bleed is reported by Sharifi-Razavi *et al.* in a 79-year-old SARS-CoV-2 positive male who presented with fever, cough and coarse crepitation in the base of the lung, where the lung CT showed a ground-glass opacity in the left lower lobe [32]. There were no co-morbidities or any known predisposing factors for intra cerebral haemorrhage, such as hypertension or anticoagulant treatment, and results were normal for platelets and prothrombin time.

On the other hand, Helms *et al.* reported acute ischaemic strokes (diagnosed by MRI) in SARS-CoV-2 positive patients with encephalopathy who presented with prominent agitation and confusion and corticospinal tract signs; they suggested that higher D-dimers may be indicative of a higher risk of cerebrovascular disease (D-dimer is a product of fibrin clot degradation and indicates recent or current clot formation) [33].

Interestingly, CVA in COVID-19 is not only confined to older patients or those with cardiovascular co-morbidities, but it also has led to younger patients presenting with ischaemic stroke, including large vessel occlusions [34]. Additionally, COVID-19 patients can develop significant hypoxia leading to decreased cerebral oxygenation and infarcts, particularly in those with pre-existing cerebrovascular disease [35,36].

Many authors have described the involvement of the spinal cord and peripheral nervous system in COVID-19 patients presenting with Guillain-Barre Syndrome, acute myelitis and encephalomyelitis [29,37-39]. A possible association between CNS focal symptoms compatible with demyelinating disease (in the spinal cord) and SARS-CoV-2 infection was recently reported by Domingues *et al.* [40]. Non-specific muscle-related symptoms were reported by Mao *et al.* and patients showed elevated creatine kinase and lactate dehydrogenase levels compared to those without muscle symptoms [7]. Although it is possible that this could be related to ACE2 in skeletal muscle [41], other causes may be critical illness myopathy; an infection-mediated harmful immune response due to elevated pro-inflammatory cytokines.

Finally, some non-specific psychological problems are also recorded in COVID-19 patients, such as anxiety, depression, insomnia and distress [6,42] and some patients with COVID-19 were found to be mentally confused [23]. Xu *et al.* found that 35% had mild, and 13% had moderate to severe psychological symptoms when they reviewed 89 survivors of COVID-19 [42].

In summary, and according to these current studies, SARS-CoV-2 infection can cause a spectrum of neurological diseases including cerebrovascular accidents, viral encephalitis and meningoencephalitis, providing evidence for viral infection within the CNS [7,25,26]. To date there are relatively few reports of autopsy brain examination to verify the pathology and pathogenesis of these symptoms, which may actually be the result of secondary involvement of the CNS due to respiratory

failure and other complications, and do not necessarily indicate CNS COVID-19 disease. Furthermore, the mechanisms implicated in the neurological symptoms are not clear without closer correlation with both pathology and neuroimaging.

#### Neurological manifestations in COVID-19 patients

- Dizziness, headache, nausea, impaired consciousness (non-specific neurological manifestation)
- Hypogeusia and hyposmia
- Cerebrovascular accident (infarction, intra cerebral bleeding, venous thrombosis)
- Acute necrotizing encephalopathy
- Meningo-encephalitis
- Guillain–Barre syndrome
- Myalgia, elevated creatine kinase and lactate dehydrogenase levels (non-specific muscular disease features)
- Anxiety, depression, insomnia, distress, mental confusion (non-specific psychiatric symptoms)

#### Postmortem studies of COVID-19

Recently, a number of articles have reported *postmortem* examination of COVID-19 patients [43–47]. The pathological features of COVID-19 greatly resemble those seen in SARS and MERS coronavirus infection [12,13,48]. The most common *postmortem* findings are diffuse alveolar damage, acute renal tubular injury, lymphocyte depletion and evidence of micro- or macrothrombosis in many organs. Vasquez-Bonilla *et al.* conducted a systematic meta-analysis of histopathological observations from patients who died with COVID-19 [49]. They concluded that SARS-CoV-2 infection can result in diverse, multi-organ pathology, the most significant being; diffuse alveolar damage, microthrombi, bronchopneumonia, necrotizing bronchiolitis, lymphocytic myocarditis, acute tubular injury and haemophagocytosis and histiocytosis in the lymph nodes and bone marrow. The reported pathology in the brain includes microthrombi, ischaemic necrosis, acute haemorrhagic infarction and oedema.

In a recent publication on autopsy findings in the United Kingdom by Hanley *et al.*, diffuse alveolar

damage was the most consistent lung finding, with thrombotic phenomena in at least one major organ, lymphocyte depletion (particularly T-cells) in haematological organs and haemophagocytosis [50]. There were additional, unexpected findings such as acute pancreatitis, adrenal microinfarction, pericarditis, disseminated mucormycosis, aortic dissection and marantic endocarditis. These findings support the evidence of there being four dominant inter-related pathological processes in severe COVID-19; diffuse alveolar damage, thrombosis, haemophagocytosis and immune cell depletion [50]. Brain examination was also carried out in this cohort by the authors of this review (see below for more details). However, in general, documentation of detailed brain pathology in COVID-19 patients is incomplete.

In their articles detailing *postmortem* findings in patients who died with COVID-19, Xu *et al.* and Fox *et al.* did not examine the brain [46,47]. Barton *et al.* reported no macroscopic abnormalities in the brain but microscopic examination was not undertaken [44] and Paniz-Mondolfi *et al.* reported only electron microscopy of brain tissue, claiming demonstration of viral particles in small vesicles within endothelial cells [51], however, this requires confirmation by further studies. In contrast, Schaller *et al.* report no morphologically detectable pathology and no signs of encephalitis or vasculitis in their cohort of 10 patients [52].

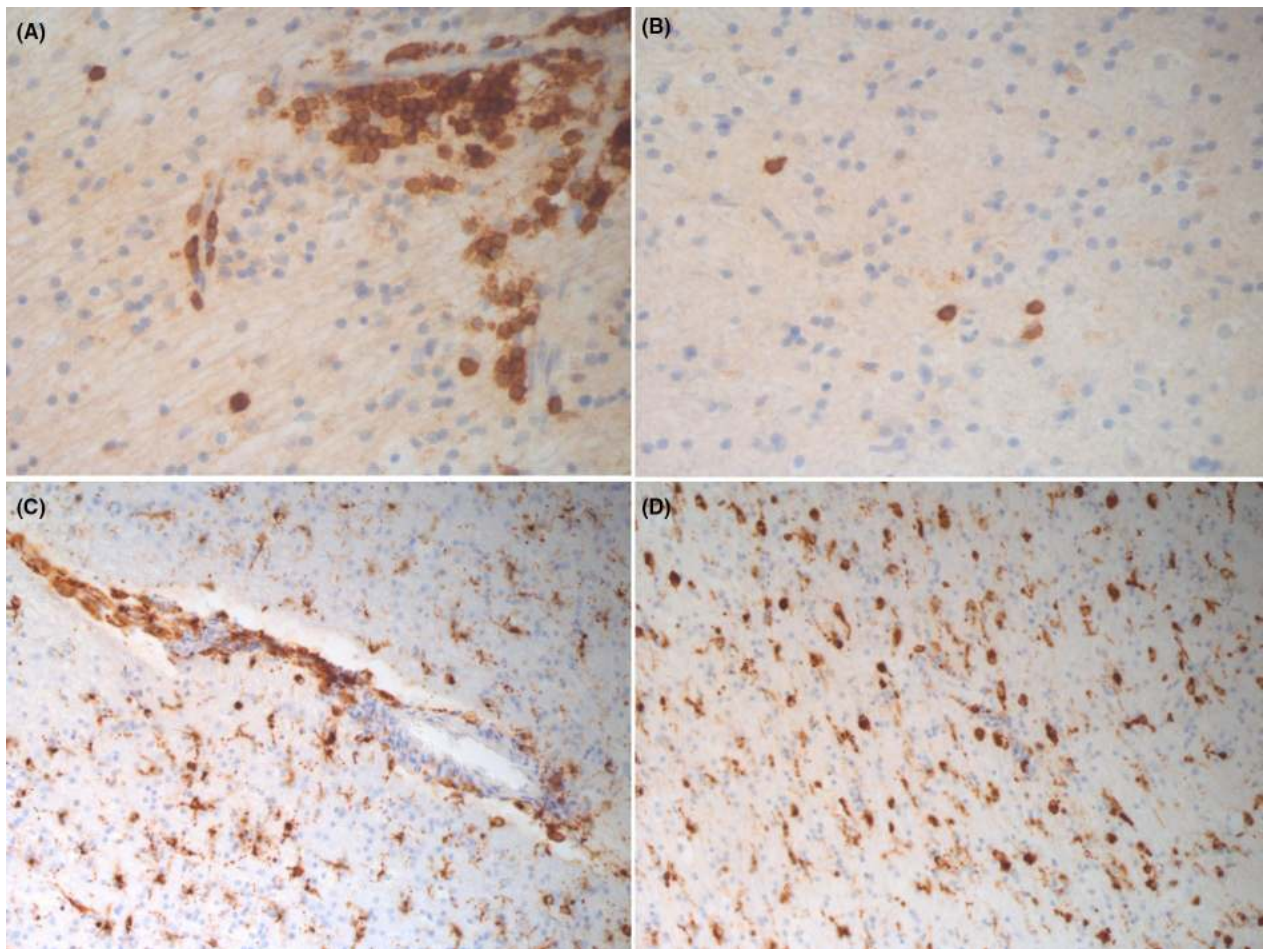
Brains from 18 patients who died after the onset of symptoms of COVID-19 were examined by Solomon *et al.* [36]. They showed ischaemic changes and reactive changes of Alzheimer type II astrocytes but there was no specific pathology such as meningitis or encephalitis. Immunohistochemical staining for SARS-CoV-2 in the above study was negative but the virus was detected at low levels by RT-PCR in five patients, however, there was no correlation with these results and the interval from the onset of symptoms to death.

More specific brain pathology was reported by Reichard *et al.* in a 71-year-old male previously diagnosed with ischaemic heart disease who developed postoperative complications including pneumonia and who was subsequently proven to be positive for SARS-CoV-2 [53]. The patient died after more than 2 weeks of hospitalization and the *postmortem* gross examination of the brain showed widespread recent ischaemia and small foci of intraparenchymal bleeding associated with damage to the white matter resulting in axonal injury,

demyelination and macrophage infiltration. The authors comment on the resemblance of the pathology to that of acute disseminated encephalomyelitis (ADEM) although it is not typical and also raises the differential diagnosis of the cause being vascular in origin; possibly caused by microthromboembolic events related to COVID-19. These appearances are similar to those reported by Jaunmuktane *et al.*, Bryce *et al.*, and Remmelink *et al.* who also described cases with large and small infarcts, multiple small subcortical infarcts, foci of ischaemic necrosis and small microbleeds [54-56].

The authors of this review examined the brain from eight cases obtained as the first sequentially available consented autopsies from individuals who had died of COVID-19-related complications [50], and not selected according to a specific clinical presentation. They

observed pathological features of recent ischaemia in six of the brains, which was most likely a result of reduced blood and oxygen supply to the brain, secondary to diffuse alveolar lung damage and multi-organ failure, and in keeping with the previous report from Solomon *et al.*[36]. More importantly, there was noticeable activation of microglial cells and a few perivascular T lymphocytes in all cases (Figure 1). It is likely that these findings are secondary to severe systemic infection with multi-organ failure and being on a ventilator, and this is further supported by the failure to detect viral RNA or protein in a subset of four cases examined by immunohistochemistry, *in situ* hybridization (ISH) and reverse transcriptase-PCR. However, one cannot exclude the possibility of low-grade viral infection below the sensitivity of the conducted techniques,

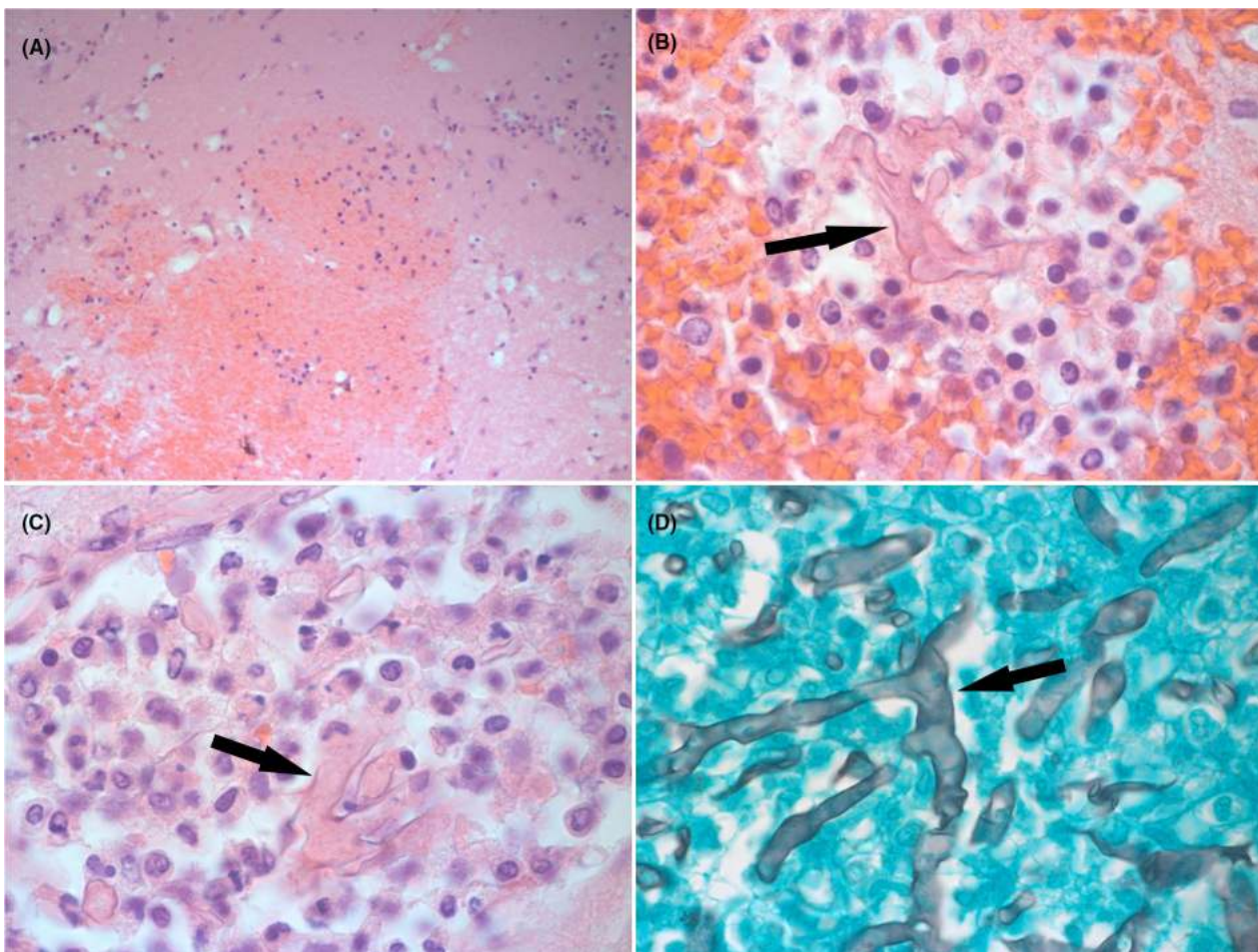


**Figure 1.** (A) Perivascular T lymphocyte infiltration in the frontal lobe (CD3), (B) occasional T lymphocytes in the white matter of the frontal lobe (CD3), (C) activated microglial cells in the white matter and perivascular spaces in the frontal lobe (CD68), (D) activated microglial cells in the white matter of the frontal lobe (CD68), (A, B x640, C, D x400).

particularly in those cases which show more than occasional perivascular T lymphocytes, or intense activation of microglial cells [55]. This view is supported by experimental work from Netland *et al.* who suggested that a minimal inflammatory cell process was observed in experimental mice infected with SARS-CoV even when infection was widespread, which raises the possibility of neurotoxicity associated with minimal cellular infiltration [14]. A previous report suggests a mechanism of endothelial cell infection and lymphocytic endotheliitis related to SARS-CoV-2 infection, which was associated with accumulation of lymphocytes around blood vessels as well as apoptotic bodies [15]. While electron microscopy was not performed in the authors' cohort, the

light microscopic examination did not detect changes in endothelial cells and the T lymphocytes were clearly in perivascular spaces with no infiltration into the blood vessel walls or endothelial cells.

One of the examined cases showed haemorrhagic infarction associated with mucormycosis and microthrombi (Figure 2). Mucormycosis is a relatively uncommon fungal infection suggesting that this individual had reduced immunity, possibly secondary to COVID-19. Lower lymphocyte counts in those with CNS symptoms with COVID-19 and immune deficiency pathogenesis has been reported. There were also microthrombi in small blood vessels around and within the infarcted brain tissue which could have developed

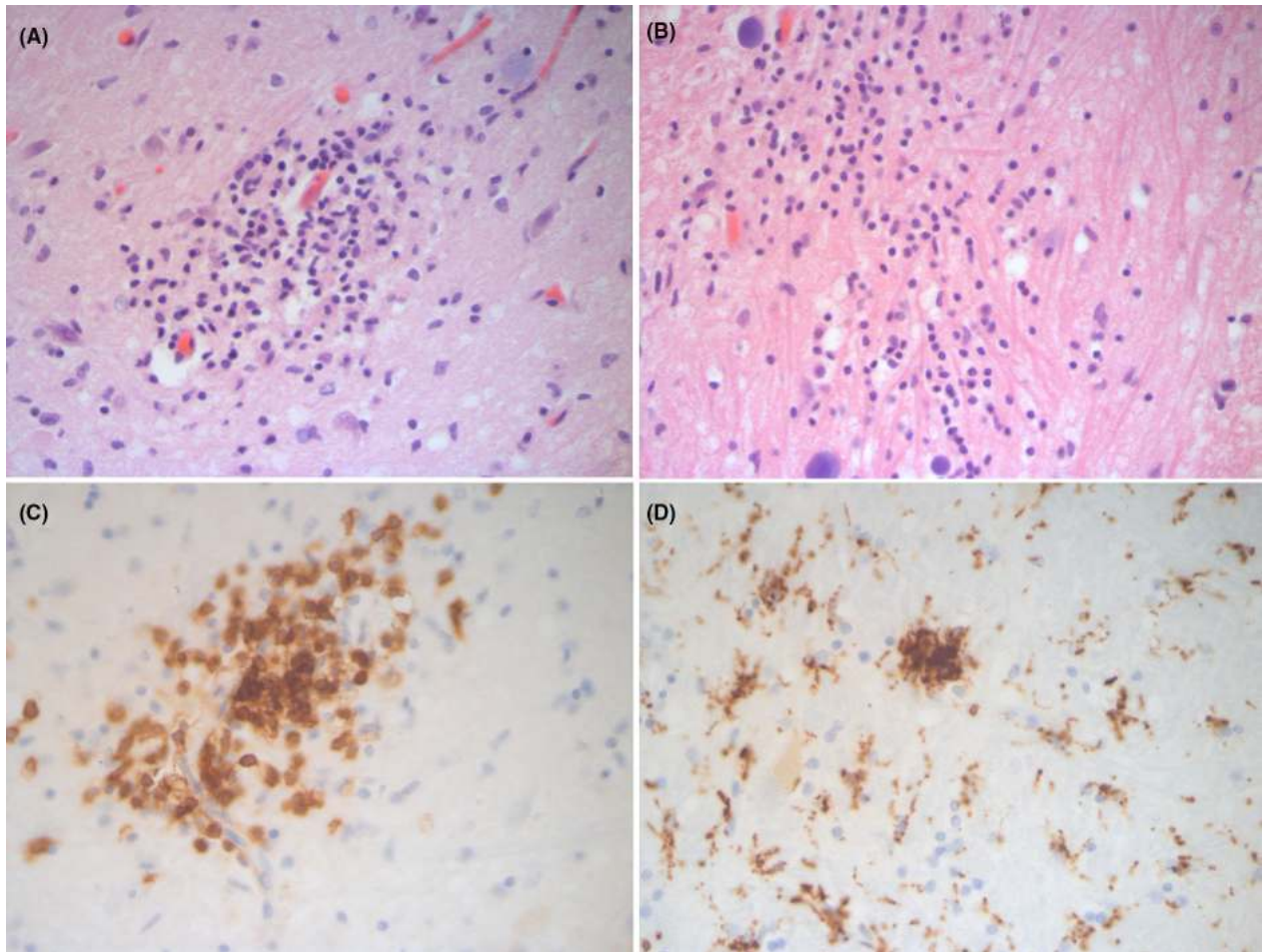


**Figure 2.** (A) Haemorrhagic infarction demonstrating necrotic areas and areas of recent haemorrhages associated with focal macrophage infiltration and mild proliferation of capillaries (haematoxylin and eosin stain) x100, (B) and (C) higher power images of necrotic and haemorrhagic areas in A showing macrophage infiltration and mucormycosis (arrows) (haematoxylin and eosin stain) x630. (D) mucormycosis (arrow) showing broad and non-septate hyphae (Grocott silver stain) x630.

secondary to the infarction and are consistent with the observation that cerebrovascular events are one of the common complications of COVID-19.

Additionally, within this study of eight cases, a case of encephalitis was reported, which is likely a more specific neuropathological feature of COVID-19. The brain showed intense and localized T lymphocyte infiltration and activation of microglial cells to the medulla, without necrosis, oedema, demyelination or meningitis (Figure 3). There was no inflammation in the rest of the sampled brain regions. Clinically, there was no evidence of encephalitis apart from dysphagia, which may be a non-specific indicator of brain stem involvement. The close proximity of the inflammatory foci to the reticular formation and cardiorespiratory centre in the medulla suggest that brain stem COVID-

19 encephalitis might have disrupted the function of the cardiorespiratory centre. This case was one of four brains subjected to extensive investigation for SARS-CoV-2 infection by immunohistochemistry, ISH and RT-PCR, all of which were negative. However, as discussed above, the possibility of direct infection by SARS-CoV-2 cannot be completely excluded due to the potential lack of sensitivity in using formalin fixed paraffin-embedded tissue, other technical reasons, or even that the virus is no longer present in the tissue despite inflammation remaining. The identification of active and replicative/infective SARS-CoV-2 RNA in the brain is still not definite [36,54,55]. Indeed, in viral encephalitis involving the most prevalent viruses known to reach the brain (mainly herpes viruses, arboviruses and enteroviruses), the viral antigen can



**Figure 3.** (A) and (B) focal heavy infiltration of the parenchyma of the medulla by inflammatory cells typical of what is called a microglial nodule but with no necrosis x400. (C) The inflammatory cells are T lymphocytes in the same spot as A (CD3) and (D) intense microglial cell activation in the same spot as B (CD68) x400.



only be detected in 3–30 out of 100 000 infected persons [57]. The other differential diagnoses such as opportunistic (non SARS-CoV-2) viral infection (due to reduced immunity)[12] or autoimmune encephalitis, have also to be considered.

In addition to the case reported here, the authors of this review have diagnosed two more cases of brain stem encephalitis in COVID-19 positive patients (consents for publication are not available at the time of review) showing similar features to the above-mentioned case with localized inflammation involving the medulla only.

#### Summary of the main systemic postmortem findings of COVID-19

- (1) Acute interstitial pneumonia, diffuse alveolar damage with endothelial and epithelial injury, hyaline membranes, fibrin deposition, reactive pneumocytes, mild lymphocytic and histiocytic intra-alveolar inflammation
- (2) Pulmonary and systemic microthrombi consistent with induced hypercoagulable state
- (3) Depletion of white pulp of the spleen associated with lymphopenia
- (4) Haemophagocytosis and histiocytosis in lymph nodes and bone marrow
- (5) Acute tubular injury
- (6) Focal lymphocytic myocarditis and Pericarditis
- (7) Pancreatitis

In summary, the neuropathological changes reported in various studies to date may be caused by direct cytopathic effects of SARS-CoV-2 replication in the brain or indirectly by a systemic toxic reaction due to failure of the respiratory system and/or by the harmful immune response and cytokine reaction induced by viral infection. Clarification of the SARS-CoV-2 brain pathology and the presence of viral RNA and/or proteins is essential in order to understand the aetiology of the now well-described clinical neurological features reported in COVID-19. This will require a large-scale clinical and molecular neuropathological cohort network study similar to that which occurred 30 years ago in response to the HIV/AIDS epidemic [58].

#### Articles of autopsy assessment including CNS examination

Total neuropathologically examined autopsy brains (to the date of this report) - 81 brains

Barton et al., 2020: Brain from one patient

- Macroscopic abnormalities in the brain but there was no microscopic examination.

Paniz-Mondolfi et al., 2020: Brain from one patient

- Electron microscopy from brain tissue demonstrating viral particles in small vesicles of endothelial cells.

Solomon et al., 2020: Brains from 18 patients

- Ischaemic changes and reactive changes of Alzheimer's type II astrocytes
- Immunohistochemical stains for SARS-CoV-2 negative but virus was detected by PCR at low levels in five patients

Reichard et al., 2020: Brain from one patient

- Widespread recent ischaemia
- Multiple foci of small pin point haemorrhages associated with axonal damage, demyelination and macrophage infiltration.
- Subcortical white matter pallor with myelin loss, perivascular cellular infiltrates as macrophages

Hanley and Al-Sarraj, 2020: Brains from eight patients

- Activation of microglial cells and perivascular T lymphocyte infiltration in all cases
- Recent ischaemia in four cases
- One brain showed haemorrhagic infarction associated with mucormycosis infection
- One brain showed features of brain stem encephalitis
- IHC, ISH and RT-PCR are negative for SARS-CoV-2

Remmelink et al., 2020: Brains from 17 patients

- Cerebral haemorrhage
- Ischaemic necrosis
- Oedema

Jaunmuktane et al., 2020: Brains from 2 patients

- Multiple large infarcts
- Subcortical white matter microvascular lesions, including microhaemorrhages.

Schaller et al., 2020: Brains from 10 patients

- No morphologically detectable pathology, no signs of encephalitis or vasculitis.
- RT-PCR for SARS-CoV2 was negative in the CSF

Bryce et al., 2020: Brains from 23 patients

- Large and small infarcts
- Microthrombi
- Ischaemia
- Micro haemorrhages
- Focal parenchymal infiltrate of T lymphocytes detected in two cases

## Conclusion

Despite the numerous clinical reports of neurological and psychiatric symptoms in COVID-19, it remains difficult to ascertain how the different neurological features relate to overall pathology; more specifically whether the results are directly or indirectly due to viral infection or secondary to hypoxia, sepsis, cytokine reaction and multi-organ failure. The evidence of a causal relationship between the SARS-CoV-2 virus and autopsy brain findings remains equivocal.

According to the available clinical and limited pathological data, the possible CNS pathology includes:

- (1) Ischaemic/hypoxic and toxic encephalopathy, causing brain dysfunction due to a combination of metabolic disorders, and reduction of oxygen supply to the brain due to pneumonia during the process of acute infection (infection with CoV, especially SARS-CoV-2, has been widely reported to cause cytokine storm syndromes) The clinical symptoms may include headache, dysphoria, mental disorder, delirium, disorientation, loss of consciousness and coma. This appears to be the most common pathology in COVID-19 patients with a clinical manifestation of neurological symptoms.

- (2) Acute cerebrovascular accident (CVA) This includes haemorrhagic and ischaemic infarction, intracerebral haemorrhage and acute necrotizing encephalopathy. SARS-CoV-2 causes a global inflammatory response and a hypercoagulable state evidenced by increased D-dimers, prolonged prothrombin time and disseminated intravascular coagulation which may render these patients prone to acute cerebrovascular events [59]. It also infects and damages the endothelial cells via binding of the spike (S) protein of the virus to the angiotensin converting enzyme 2 (ACE2) receptors. Therefore, patients with severe COVID-19 may be at risk of thrombogenesis due to both biochemical hypercoagulable states and direct vascular endothelial injury.

- (3) Viral encephalitis and meningitis Although viral encephalitis is expected to be one of the common neurological manifestations, the current published data indicate it is in fact a rare complication, to date there are few clinically diagnosed cases, but they are not verified by neuropathological studies. There appear to be difficulties in diagnosing viral CNS infection, mainly due to variation in the symptoms throughout the disease process and that viral encephalitis may be difficult to distinguish from a non-viral encephalopathy or from an encephalopathy associated with a systemic viral infection occurring outside the CNS.

- (4) Acute myelitis and Guillain–Barre syndrome There is some clinical evidence that this is one of the complications of COVID-19 but there has not yet been any pathological confirmation.

- (5) Opportunistic infection There is good clinical and pathological evidence of lymphopenia and reduced numbers of T lymphocytes in COVID-19, which may predispose vulnerable patients to opportunistic infection particularly if this is combined with steroid treatment. However, further clinical and pathological evidence is required to confirm increased vulnerability of COVID-19 patients to opportunistic infection.

- (6) There is evidence of demyelinating lesions caused by SARS-CoV and even linked to subsequent multiple sclerosis during the previous SARS pandemic, but the pathological evidence is not strong More closely linked clinical and pathological studies are needed in this field.

**Expected COVID-19 pathology in the CNS**

- (1) Ischaemic/hypoxic encephalopathy
- (2) Increase activated microglial cells and variable perivascular T lymphocyte infiltration
- (3) Cerebrovascular disease
  - Haemorrhagic infarction
  - Ischaemic infarction
  - Intracerebral haemorrhage
  - Acute multiple necrotizing encephalopathy
- (4) Opportunistic infection
- (5) Encephalitis/meningitis
- (6) Acute myelitis
- (7) Guillain–Barre syndrome
- (8) Demyelinating diseases

**Future work**

More detailed work involving a large number of autopsy brain examinations from COVID-19 patients, combined with close correlation with neurological manifestation, neuroimaging, general autopsy findings and isolation of SARS-CoV-2 from brain tissue and cerebrospinal fluid is required to clarify the role of CNS involvement in disease process and mortality.

**Acknowledgements**

The authors would first like to acknowledge the valuable contribution and generous gift of tissue donation by the deceased and their families. We are grateful to the mortuary staff at Westminster for facilitating post-mortem examination. We thank Dr Clayton A. Wiley MD/PhD from the Department of Pathology, University of Pittsburgh, who performed *in situ* hybridization studies on brain and lung sections. We thank Professor Ammar Al-Chalabi and colleagues in the Neurology Department of King's College Hospital for initial discussions regarding the clinical presentation. We also thank Dr Istvan Bodi, Dr Andrew King and Dr Zita Reisz for reviewing the histology sections and Ms Shalmai Jones for her technical assistance in producing the slides and immunostaining, and Dr Ross Laxton and Dr Malur Sudhanva for performing RT-PCR. This paper represents independent research funded by

the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. Tissue samples were provided by the Imperial College Healthcare NHS Trust Tissue Bank (funded by the NIHR Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London) and by the London Neurodegenerative Diseases Brain Bank at King's College London (supported by the Medical Research Council UK and as part of the Brains for Dementia Research project - jointly funded by Alzheimer's Society and Alzheimer's Research UK).

**Author contributions**

SAS and CT led on the literature review and drafted the manuscript. BH and MO carried out *postmortem* autopsy and sample dissection. SAS carried out neuropathological examination. CT conducted immunohistochemical staining, ISH hybridization and the administration of the project. IE and MH provided funding. BH, MO, EB, MR, IE, MH contributed with discussion of clinical presentations and analysis of data. All authors contributed to the critical review and editing of the manuscript.

**Ethical approval**

Human samples used in this research project were obtained from the Imperial College Healthcare Tissue Bank (ICHTB). ICHTB is approved by Wales REC3 to release human material for research (17/WA/0161), and the samples for this project (R20012) were issued from sub-collection reference number MED\_MO\_20\_011. The London Neurodegenerative Diseases Brain Bank is approved as a research tissue bank by Wales REC 3 (18/WA/0206).

**Conflict of interest**

The authors report no conflict of interest.

**Data availability statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References

- 1 Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003; **426**(6965): 450–4
- 2 Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; **395**: 565–74
- 3 Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett.* 2002; **532**(1–2): 107–10
- 4 Zou L, Ruan F, Huang M, Liang L, Huang H, Hong Z, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med.* 2020; **382**: 1177–9
- 5 Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical Characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*; **323**: 1061
- 6 Varatharaj A, Thomas N, Ellul MA, Davies NWS, Pollak TA, Tenorio EL, et al. Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. *Lancet Psychiatry* 2020
- 7 Mao L, Wang M, Chen S, He Q, Chang J, Hong C, et al. Neurological Manifestations of Hospitalized Patients with COVID-19 in Wuhan, China: a retrospective case series study. *JAMA Neurol* 2020; **77**
- 8 Li Y, Wang M, Zhou Y, Chang J, Xian Y, Mao L, et al. Acute cerebrovascular disease following COVID-19: a single center, retrospective, observational study. *Stroke Vasc Neurol* 2020
- 9 Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497–506
- 10 Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631–7
- 11 Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020; **181**: 271–80.e8
- 12 Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, et al. Multiple organ infection and the pathogenesis of SARS. *J Exp Med* 2005; **202**: 415–24
- 13 Ding Y, Wang H, Shen H, Li Z, Geng J, Han H, et al. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. *J Pathol* 2003; **200**: 282–9
- 14 Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. *J Virol* 2008; **82**: 7264–7275
- 15 Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet* 2020; **395**: 1417–8
- 16 Guo Y, Korteweg C, McNutt MA, Gu J. Pathogenetic mechanisms of severe acute respiratory syndrome. *Virus Res* 2008; **133**: 4–12
- 17 Dubey D, Pittcock SJ, Kelly CR, McKeon A, Lopez-Chiriboga AS, Lennon VA, et al. Autoimmune encephalitis epidemiology and a comparison to infectious encephalitis. *Ann Neurol* 2018; **83**: 166–77
- 18 Skopouli FN, Katsiogiannis S. How stress contributes to autoimmunity—lessons from Sjogren's syndrome. *FEBS Lett* 2018; **592**: 5–14
- 19 Karagianni P, Alexopoulos H, Sourdi A, Papadimitriou D, Dimitrakopoulos AN, Moutsopoulos HM. West Nile Virus infection triggering autoimmune encephalitis: Pathophysiological and therapeutic implications. *Clin Immunol* 2019; **207**: 97–9
- 20 Fung SY, Yuen KS, Ye ZW, Chan CP, Jin DY. A tug-of-war between severe acute respiratory syndrome coronavirus 2 and host antiviral defence: lessons from other pathogenic viruses. *Emerg Microbes Infect* 2020; **9**: 558–70
- 21 Lin YS, Lin CF, Fang YT, Kuo YM, Liao PC, Yeh TM, et al. Antibody to severe acute respiratory syndrome (SARS)-associated coronavirus spike protein domain 2 cross-reacts with lung epithelial cells and causes cytotoxicity. *Clin Exp Immunol* 2005; **141**: 500–8
- 22 Yang YH, Huang YH, Chuang YH, Peng CM, Wang LC, Lin YT, et al. Autoantibodies against human epithelial cells and endothelial cells after severe acute respiratory syndrome (SARS)-associated coronavirus infection. *J Med Virol* 2005; **77**: 1–7
- 23 Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol* 2020; **5**: 811
- 24 McAbee GN, Brosgol Y, Pavlakis S, Agha R, Gaffoor M. Encephalitis Associated with COVID-19 Infection in an 11-Year-Old Child. *Pediatr Neurol* 2020; **109**: 94
- 25 Moriguchi T, Harii N, Goto J, Harada D, Sugawara H, Takamino J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis* 2020; **94**: 55–8
- 26 Xiang P, Xu XM, Gao LL, Wang HZ, Xiong HF, Li RH. First case of 2019 novel coronavirus disease with encephalitis. *ChinaXiv.* 2020
- 27 Huang YH, Jiang D, Huang JT. SARS-CoV-2 detected in cerebrospinal fluid by PCR in a Case of COVID-19 Encephalitis. *Brain Behav Immun* 2020
- 28 Dixon L, Varley J, Gontsarova A, Mallon D, Tona F, Muir D, et al. COVID-19-related acute necrotizing

- encephalopathy with brain stem involvement in a patient with aplastic anemia. *Neurol Neuroimmunol Neuroinflamm* 2020; 7
- 29 Poyiadji N, Shahin G, Noujaim D, Stone M, Patel S, Griffith B. COVID-19-associated acute hemorrhagic necrotizing encephalopathy: CT and MRI features. *Radiology* 2020; 201187
- 30 Lodigiani C, Iapichino G, Carenzo L, Cecconi M, Ferrazzi P, Sebastian T, et al. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. *Thromb Res* 2020; 191: 9–14
- 31 Klok FA, Kruip M, van der Meer NJM, Arbous MS, Gommers D, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. *Thromb Res*. 2020; 191: 145–7
- 32 Sharifi-Razavi A, Karimi N, Rouhani N. COVID-19 and intracerebral haemorrhage: causative or coincidental? *New Microbes New Infect* 2020; 35: 100669
- 33 Helms J, Kremer S, Merdji H, Clere-Jehl R, Schenck M, Kummerlen C, et al. Neurologic features in severe SARS-CoV-2 Infection. *N Engl J Med* 2020; 382: 2268–70
- 34 Oxley TJ, Mocco J, Majidi S, Kellner CP, Shoirah H, Singh IP, et al. Large-vessel stroke as a presenting feature of Covid-19 in the Young. *N Engl J Med* 2020; 382: e60
- 35 Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. *Nature* 2020; 579: 265–9
- 36 Solomon IH, Normandin E, Bhattacharyya S, Mukerji SS, Keller K, Ali AS, et al. Neuropathological Features of Covid-19. *N Engl J Med*. 2020
- 37 Zhao H, Shen D, Zhou H, Liu J, Chen S. Guillain-Barre syndrome associated with SARS-CoV-2 infection: causality or coincidence? *Lancet Neurol* 2020; 19: 383–4
- 38 Sedaghat Z, Karimi N. Guillain Barre syndrome associated with COVID-19 infection: a case report. *J Clin Neurosci* 2020; 76: 233–5
- 39 Toscano G, Palmerini F, Ravaglia S, Ruiz L, Invernizzi P, Cuzzoni MG, et al. Guillain-barre syndrome associated with SARS-CoV-2. *N Engl J Med* 2020; 382: 2574–6
- 40 Domingues RB, Mendes-Correa MC, de Moura Leite FBV, Sabino EC, Salarini DZ, Claro I, et al. First case of SARS-COV-2 sequencing in cerebrospinal fluid of a patient with suspected demyelinating disease. *J Neurol* 2020
- 41 Cabello-Verrugio C, Morales MG, Rivera JC, Cabrera D, Simon F. Renin-angiotensin system: an old player with novel functions in skeletal muscle. *Med Res Rev* 2015; 35: 437–63
- 42 Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *BMJ* 2020; 368: m606
- 43 Copin MC, Parmentier E, Duburcq T, Poissy J, Mathieu D, Lille C-I, et al. Time to consider histologic pattern of lung injury to treat critically ill patients with COVID-19 infection. *Intensive Care Med* 2020
- 44 Barton LM, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. COVID-19 Autopsies, Oklahoma, USA. *Am J Clin Pathol* 2020; 153: 725–33
- 45 Sonzogni A, Previtali G, Seghezzi M, Grazia Alessio M, Gianatti A, Licini L, et al. Liver histopathology in severe COVID 19 respiratory failure is suggestive of vascular alterations. *Liver International*. 2020;40(9):2110–2116. <http://dx.doi.org/10.1111/liv.14601>.
- 46 Fox SE, Akmatbekov A, Harbert JL, Li G, Quincy Brown J, Vander Heide RS. Pulmonary and cardiac pathology in African American patients with COVID-19: an autopsy series from New Orleans. *Lancet Respir Med* 2020
- 47 Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020; 8: 420–2
- 48 van den Brand JM, Smits SL, Haagmans BL. Pathogenesis of Middle East respiratory syndrome coronavirus. *J Pathol* 2015; 235: 175–84
- 49 Vasquez-Bonilla WO, Orozco R, Argueta V, Sierra M, Zambrano LI, Munoz-Lara F, et al. A review of the main histopathological findings in the Coronavirus Disease 2019 (COVID-19). *Hum Pathol* 2020
- 50 Hanley B, Naresh KN, Candice Roufosse C, Nicholson AG, Weir J, Cooke GS, et al. Clinicopathological Correlation in fatal severe COVID-19: diffuse alveolar damage, thrombosis, haemophagocytosis and T-lymphocyte depletion. *Lancet Microbe*.
- 2020.**
- 51 Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednický J, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J Med Virol* 2020; 92: 699–702
- 52 Schaller T, Hirschbühl K, Burkhardt K, Braun G, Treppel M, Markl B, et al. Postmortem examination of patients With COVID-19. *JAMA* 2020
- 53 Reichard RR, Kashani KB, Boire NA, Constantopoulos E, Guo Y, Lucchinetti CF. Neuropathology of COVID-19: a spectrum of vascular and acute disseminated encephalomyelitis (ADEM)-like pathology. *Acta Neuropathol* 2020; 140: 1–6
- 54 Jaunmuktane Z, Mahadeva U, Green A, Sekhawat V, Barrett NA, Childs L, et al. Microvascular injury and hypoxic damage: emerging neuropathological

- signatures in COVID-19. *Acta Neuropathol* 2020; **140**: 397–400
- 55 Bryce C, Grimes Z, Pujades E, Ahuja S, Beasley MB, Albrecht R, et al. Pathophysiology of SARS-CoV-2: targeting of endothelial cells renders a complex disease with thrombotic microangiopathy and aberrant immune response. The Mount Sinai COVID-19 autopsy experience. *medRxiv*. 2020
- 56 Rummelink M, De Mendonca R, D'Haene N, De Clercq S, Verocq C, Lebrun L, et al. Unspecific post-mortem findings despite multiorgan viral spread in COVID-19 patients. *Crit Care* 2020; **24**: 495
- 57 Desforges M, Le Coupanec A, Dubeau P, Bourgouin A, Lajoie L, Dube M, et al. Human coronaviruses and other respiratory viruses: underestimated opportunistic pathogens of the central nervous system? *Viruses* 2019; **12**
- 58 Davies J, Everall IP, Lantos PL. The contemporary AIDS database and brain bank—lessons from the past. *J Neural Transm Suppl* 1993; **39**: 77–85
- 59 Gonzalez-Pinto T, Luna-Rodriguez A, Moreno-Estebanez A, Agirre-Beitia G, Rodriguez-Antiguedad A, Ruiz-Lopez M. Emergency room neurology in times of COVID-19: malignant ischaemic stroke and SARS-CoV-2 infection. *Eur J Neurol* 2020

Received 14 July 2020

Accepted after revision 3 September 2020

Published online Article Accepted on 16 September 2020



Minerva Access is the Institutional Repository of The University of Melbourne

**Author/s:**

Al-Sarraj, S;Troakes, C;Hanley, B;Osborn, M;Richardson, MP;Hotopf, M;Bullmore, E;Everall, IP

**Title:**

Invited Review: The spectrum of neuropathology in COVID-19

**Date:**

2020-10-20

**Citation:**

Al-Sarraj, S., Troakes, C., Hanley, B., Osborn, M., Richardson, M. P., Hotopf, M., Bullmore, E. & Everall, I. P. (2020). Invited Review: The spectrum of neuropathology in COVID-19. *NEUROPATHOLOGY AND APPLIED NEUROBIOLOGY*, 47 (1), pp.3-16. <https://doi.org/10.1111/nan.12667>.

**Persistent Link:**

<http://hdl.handle.net/11343/252622>

**License:**

[cc-by](#)