REVIEW

Evidence of central nervous system infection and neuroinvasive routes, as well as neurological involvement, in the lethality of SARS-CoV-2 infection

Jia-Mei Liu[1] | Bai-Hong Tan[2] | Shuang Wu[1] | Yue Gui[1] | Jia-Le Suo[1] | Yan-Chao Li[1]

[1]Department of Histology and Embryology, College of Basic Medical Sciences, Norman Bethune College of Medicine, Jilin University, Changchun, Jilin, China

[2]Laboratory Teaching Center of Basic Medicine, Norman Bethune Health Science Center of Jilin University, Jilin Province, China

Correspondence
Yan-Chao Li, Department of Histology and Embryology, College of Basic Medical Sciences, Norman Bethune College of Medicine, Jilin University, Changchun, 130021 Jilin, China.
Email: liyanchao@jlu.edu.cn

Abstract
The outbreak of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has become a significant and urgent threat to global health. This review provided strong support for central nervous system (CNS) infection with SARS-CoV-2 and shed light on the neurological mechanism underlying the lethality of SARS-CoV-2 infection. Among the published data, only 1.28% COVID-19 patients who underwent cerebrospinal fluid (CSF) tests were positive for SARS-CoV-2 in CSF. However, this does not mean the absence of CNS infection in most COVID-19 patients because postmortem studies revealed that some patients with CNS infection showed negative results in CSF tests for SARS-CoV-2. Among 20 neuropathological studies reported so far, SARS-CoV-2 was detected in the brain of 58 cases in nine studies, and three studies have provided sufficient details on the CNS infection in COVID-19 patients. Almost all in vitro and in vivo experiments support the neuroinvasive potential of SARS-CoV-2. In infected animals, SARS-CoV-2 was found within neurons in different brain areas with a wide spectrum of neuropathology, consistent with the reported clinical symptoms in COVID-19 patients. Several lines of evidence indicate that SARS-CoV-2 used the hematopoietic route to enter the CNS. But more evidence supports the trans-neuronal hypothesis. SARS-CoV-2 has been found to invade the brain via the olfactory, gustatory, and trigeminal pathways, especially at the early stage of infection. Severe COVID-19 patients with neurological deficits are at a higher risk of mortality, and only the infected animals showing neurological symptoms became dead, suggesting that neurological involvement may be one cause of death.

KEYWORDS
coronavirus, nervous system, neuroinvasion, pathophysiology

1 | INTRODUCTION

Since December 2019, a novel coronavirus (CoV), the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has rapidly spread among human beings and caused a worldwide outbreak of severe pneumonia (COVID-19). Genomic analysis shows that SARS-CoV-2 is in the same betacoronavirus (βCoV) clade as MERS-CoV and SARS-CoV.[1] It is similar to SARS-CoV in genetic sequence and even exploits the same cellular receptor to enter into host cells.[2]

Based upon the clinical and experimental data available for CoVs, we proposed in February 2020 that SARS-CoV-2 may possess a neuroinvasive potential similar to other CoVs.[3,4] Since then, a variety of neurological manifestations have been documented in patients with COVID-19. A considerable number of patients with COVID-19 showed only neurological symptoms at the time of diagnosis,[5-9] which raises the question whether neurological complications were caused by direct SARS-CoV-2 infection in the central nervous system (CNS) or not.

The neurological involvement in COVID-19 mainly falls into three categories: (1) CNS involvement, such as dizziness, headache, impaired consciousness, acute cerebrovascular disease, and epilepsy, (2) peripheral nervous system involvement, including anosmia, hypogeusia, visual impairment, and neuralgia, and (3) skeletal muscle damage.[10]

Many neurological symptoms, such as encephalopathy, stroke, Guillain–Barré syndrome, acute hemorrhagic necrotizing encephalitis, and acute disseminated encephalomyelitis, might be associated with systemic inflammatory response syndrome, sepsis, multiorgan failure, or postinfectious, immune-mediated complications.[11,12] However, some neurological symptoms, such as encephalitis and anosmia–hyposmia, might be caused by direct invasion of the CNS by the virus. It is quite likely that the neurotropism of SARS-CoV-2 leads to the relatively high percentage of neurological involvement.

In some COVID-19 patients, neurological manifestations were supported by abnormal changes of head computed tomography, magnetic resonance imaging, and/or electroencephalography. However, most neuroimaging findings reported only nonspecific imaging patterns, which could not provide a specific diagnosis. Moreover, the complex clinical course and long ICU (intensive care unit) stay of COVID-19 patients also acted as confounding factors. Therefore, a clear cause–effect relationship between SARS-CoV-2 infection and neuroimaging findings is difficult to establish in most cases in the absence of more specific detection.[13]

In this review, we assessed the so far documented evidence for SARS-CoV-2 neuroinvasion, mainly obtained from cerebrospinal fluid (CSF) tests, postmortem, and experimental studies. Confirming the neuroinvasive potential of SARS-CoV-2 and clarifying the underlying mechanism is crucially important for understanding COVID-19 and its potential long-term sequelae.

2 | RETRIEVAL STRATEGIES

An exhaustive search of case reports, cohort studies, series of cases, postmortem studies, animal models, and clinical trials related to the possible neuroinvasion of SARS-CoV-2 was performed through MEDLINE/PubMed and COVID-19-related preprints from medRxiv and bioRxiv from December 1, 2020, to September 14, 2020. In addition, the references of relevant articles were also scanned for additional studies related to SARS-CoV-2 and CNS infection.

The papers on COVID-19 were retrieved by using "novel coronavirus disease 2019 or COVID-19 or severe acute respiratory syndrome coronavirus 2 or SARS-CoV-2 or 2019 novel coronavirus or 2019 nCoV" in Title/Abstract (Strategy 1). To reveal the involvement of the nervous system in COVID-19, the following keywords in Title/Abstract were combined with Strategy 1: "Neurological or nervous system or CNS or PNS or brain or cerebrum or cerebral or cerebellum or cerebellar or thalamus or thalamic or hippocampus or hippocampal or pons or pontes or pontine or brainstem or oblongata or medulla oblongata or spinal cord or cerebrospinal or neuron or nerve or neural or encephalitis or anosmia or hyposmia."

The papers were selected based on their relevance to the question whether SARS-CoV-2 possesses a neuroinvasive potential. The titles and abstracts were first screened, and the full texts were then obtained from the library of Jilin University. Reviews, meta-analyses, opinion, correspondence, perspective, and letters to the editor containing no original data of interest may be cited, but only original contributions were comprehensively analyzed in this study.

3 | RESULTS

3.1 | The evidence of SARS-CoV-2 in CSF

Up to September 14, 2020, a total of 57 case reports/case series have described CSF tests for SARS-CoV-2. Among them, 13 studies reported positive results in 13 of 67 patients, and 43 reported negative results in 951 patients. The positive CSF detection of SARS-CoV-2 was 1.28% among the pooled 1018 cases.

A piece of evidence for SARS-CoV-2 in the CSF was first reported by Moriguchi et al.[8] in Japan on March 8, 2020. In Yamanashi, Japan, a man aged 24 years had visited two separate clinics before a definite diagnosis in the affiliated hospital of Yamanashi University. This patient presented fatigue, fever, and headache, but no abnormal changes in chest X-ray or blood tests. However, on the ninth day after the onset of illness, he became unconscious and developed transient generalized seizures. Brain imaging examinations confirmed him as a case of meningitis/encephalitis associated with SARS-CoV-2 infection. Although polymerase chain reaction (PCR) was negative in the nasopharyngeal swab, SARS-CoV-2 RNA has been demonstrated in the CSF.[8]

A parallel study of concomitant encephalitis was reported for a male patient aged 56 years on March 16, 2020, in Beijing Ditan Hospital, China.[14] Similarly, the PCR test of SARS-CoV-2 was positive in the CNS.

In a case study published on April 17, Duong et al.[6] were still unable to send CSF specimens for PCR testing through local commercial, government, or academic laboratories by the day of the writeup submission (Day 9 after admission). However, in an update to this article on May 6, 2020, the CSF of this patient was subsequently found to be positive for SARS-CoV-2 on reverse transcription-PCR (RT-PCR).[15] Of note, SARS-CoV-2 infection in this case was entirely confined to the CNS, with no involvement of other organ systems.

On June 3, Färber et al.[16] reported that a young male infant was admitted to the emergency department with apparent acute sepsis. CSF testing initially showed no infection but then SARS-CoV-2 was detected in the lower pharynx and CSF.

On June 16, 2020, Kremer et al.[17] reported a retrospective study of 40 COVID-19 patients. Among 28 patients who underwent CSF examination, only one patient was positive for SARS-CoV-2 RNA in the CSF.

On June 20, Domingues et al.[18] reported a patient with mild respiratory symptoms and neurological manifestations compatible with clinically isolated syndrome. The viral genome of SARS-COV-2 was detected and sequenced in the CSF with 99.74%–100% similarity between the patient virus and worldwide sequences.

On June 26, Westhoff et al.[19] reported a 69-year-old man, who suffered from COVID-19 pneumonia, meningoencephalitis, and nephritis. RT-PCR test was positive for SARS-CoV-2 in the CSF and the biopsy specimens from the kidneys. Of note, the detection of SARS-CoV-2 was negative in blood samples, and therefore a false positive finding caused by perfusion with the infected blood is unlikely.

On July 23, Mardani et al.[20] described a 64-year-old woman with COVID-19-induced respiratory distress whose treatment resulted in a negative nasopharyngeal swab RT-PCR result for SARS-CoV-2. However, after a few weeks, relapse occurred, as indicated by symptoms of acute meningoencephalitis. RT-PCR test for SARS-CoV-2 became positive in her CSF, nasopharyngeal, and tracheal aspiration specimens.

On July 31, Fadakar et al.[21] reported a 47-year-old male who suffered from progressive vertigo and ataxia for 7 days before admission. Neurological examination revealed cerebellar dysfunction, and brain MRI (magnetic resonance imaging) depicted edema of the cerebellar hemisphere associated with leptomeningeal enhancement. CSF analysis showed mild lymphocytic pleocytosis, elevated protein, and lactate dehydrogenase. SARS-CoV-2 RNA was detected in the oropharyngeal/nasopharyngeal and CSF specimens.

On August 8, Helms et al.[22] conducted a bicentric cohort study of 150 COVID-19 patients with acute respiratory distress syndrome between March 3 and May 5, 2020. CSF examination revealed inflammatory disturbances in 18 of 28 patients, and RT-PCR of SARS-CoV-2 was positive in one patient (1 of 28).

On August 11, Cebrián et al.[23] described a 74-year-old woman with gastrointestinal manifestations followed by headache and impaired consciousness. This patient did not develop fever, cough, anosmia, hypogeusia, or respiratory symptoms. Neurological examination revealed impaired consciousness, photophobic appearance, confusion, and incoherent speech. Notably, both nasopharyngeal and CSF qRT-PCR tests were positive for SARS-CoV-2.

On September 8, Virhammar et al.[24] reported a case of COVID-19-related acute necrotizing encephalopathy. SARS-CoV-2 RNA was found in CSF 19 days after symptom onset after testing negative twice. Although monocytes and protein levels in CSF were only marginally increased, this patient never experienced a hyperinflammatory state. Moreover, extremely high concentrations of neurofilament light-chain protein, glial fibrillary acidic protein, and tau, were detected in the CSF.

On September 14, Khodamoradi et al.[25] reported a 49-year-old woman who presented COVID-19 meningitis but without pulmonary involvement or brain MRI changes.[25] However, two CSF tests at the interval of 1 week were positive.

So far, most studies could not detect SARS-CoV-2 in the CSF. For example, Yin et al.[26] reported a COVID-19 patient with neurological symptoms, but without a positive reaction to viral RNA in the CSF. Similarly, Helms et al.[27] reported negative nucleic acid assays for SARS-CoV-2 in all the CSF samples from seven patients.

Of note, on June 11, Destras et al.[28] reported a large retrospective systematic screening of 578 CSF samples, corresponding to 555 patients, received during the outbreak in Lyon from February 1 to May 11, 2020. Only two postmortem CSF samples from two COVID-19 patients were slightly positive for SARS-CoV-2. For one patient, a blood sample was available and was positive for SARS-CoV-2, whereas brain biopsy samples from the two patients were both negative, suggesting contamination of the CSF by blood. These data suggest that SARS-CoV-2 tests in CSF are not relevant in the general population.

Espíndola et al.[29] suggested that the patients with COVID-19 displaying distinct neurological disorders might have extremely low levels of SARS-CoV-2 RNA in the CSF because the immune clearance of viruses from the CSF might be before the neurological manifestations. Thus, the detection of SARS-CoV-2 in CSF may depend on disease severity, the time of sample collection, or the sensitivity of the molecular test used.

Supporting this, Wang et al.[30] report a 68-year-old COVID-19 man with serious neurological damage and mental abnormalities, whose CSF test showed negative result. However, immunoglobulin M and immunoglobulin G (IgG) antibodies against SARS-CoV-2 were 100 times higher in the CSF than in the serum.

Similarly, Lu et al.[31] reported a case of SARS-CoV-2 infection with manic-like symptoms. After a manic-like attack, IgG antibody specific to SARS-CoV-2 was detected in the CSF, but RT-PCR in CSF for SARS-CoV-2 was negative.

In a preprint recently deposited in bioRxiv, Song et al.[32] reported the detection of IgG antibodies specific to the spike protein of SARS-CoV-2 in CSF from a COVID-19 patient with acute encephalopathy. The antibodies were able to block SARS-CoV-2 infection in brain organoids, which highlights the potential of SARS-CoV-2 neuroinvasion and subsequent immune response in the CNS.

Of note, CSF examination is not consistently available, even if in some cases CSF has been obtained.[6,31,33] Although CSF detection of SARS-CoV-2 was unavailable or negative in some cases, concomitant neurological symptoms have been remarkably improved after the SARS-CoV-2 nucleic acid test became negative in the nasopharyngeal swab.[5,26,34]

3.2 | Autopsy evidence for the neuroinvasion of SARS-CoV-2

Up to September 14, 2020, a total of 20 autopsy studies on neuropathology were retrieved, among which CNS detection of SARS-CoV-2 was negative in 12 (of 12) cases from three studies,[36,42,43] and positive

in 58 (of 87) cases (66.7%) from nine studies,[46-54] and unavailable for 109 cases in eight studies.[35,37–41,44,45]

Among these studies, two did not describe neuropathological findings, since autopsy of the brain was not their main focus.[35,36] One study reported acute hemorrhagic necrotizing encephalitis and acute disseminated encephalomyelitis-like changes in a patient who died from complications of COVID-19.[37] In two studies, brain autopsy revealed only vascular injury.[38,39] Another study used ultrasound-guided minimally invasive autopsy, and found reactive gliosis in eight (89%) patients, neuronal satellitosis in five (55.5%), small-vessels disease three (33.3%), and perivascular hemorrhages in one (11.1%) patient.[40] However, none of these studies carried out detection of SARS-CoV-2 in the brain.

Of note, on May 21, 2020, Schaller et al.[41] reported a series of 10 autopsies. They found no signs of encephalitis or CNS vasculitis. Brain remained unaffected in the 10 patients with severe critical COVID-19. In this study, detection for SARS-CoV-2 was not performed.

On August 6, Kantonen et al.[42] reported an autopsy study on two COVID-19 patients. No signs of encephalitis or meningitis were detected in any patients. In one patient, they found severe ischemic injury, abundant microhemorrhages, and enlarged perivascular spaces most pronounced in the white matter and deep gray matter. A few small perivascular white matter lesions, with macrophages engulfing myelin, were also reported. Of note, RT-PCR and immunostaining could not demonstrate in the two patients marked injury or presence of SARS-CoV-2 in the olfactory epithelium, olfactory bulbs, or brainstem.

On September 8, 2020, Jensen et al.[43] reported neuropathological findings in two patients with fatal COVID-19. The first case showed pathological changes consistent with severe multiterritorial cerebral vascular injury. In the second case, however, they found brainstem encephalitis centered on the dorsal medulla and subacute regional infarct involving the cerebellar cortex. In the dorsal medulla, there was a moderate parenchymal infiltrate of T-lymphocytes with neuronophagia, and activated microglia forming microglial nodules. However, in situ hybridization and RT-PCR for SARS-CoV-2 RNA were negative in tissue sampled from the area of pathology.

A total of 14 COVIID patients were included in the three autopsy studies mentioned above. Detection of SARS-CoV-2 by RT-PCR and/ or immunostaining was performed in two studies, but the results did not support the infection of CNS in four COVID-19 patients.[42,43]

On May 13, Bulfamante et al.[44] reported an ultrastructural investigation of postmortem samples from a COVID-19 patient who died from SARS-CoV-2 infection. This patient was admitted with fever, anosmia, dysgeusia, headache, a possible seizure, and acute respiratory failure. Ultrastructural autopsy performed within 3 h of death demonstrated severe and widespread damage in the olfactory nerve, gyrus rectus, and medulla oblongata. The damage involving neurons, glia, nerve axons, and myelin sheath was progressively less severe from the olfactory nerve to the gyrus rectus and to the brainstem.

On June 20, von Weyhern et al.[45] carried out an autopsy study on 6 patients, who died from COVID-19 in April 2020. Surprisingly, this study did not observe conspicuous CNS endotheliitis. In all brain

samples from four cases, wide neuronal cell loss and axon degeneration have been seen in the brainstem, including the dorsal motor nuclei of the vagus nerve, nucleus tractus solitarii, dorsal raphe nuclei, and fasciculus longitudinalis medialis. In addition, localized perivascular and interstitial infiltration of immune cells has also been found in the brain.

In the two studies, specific neuropathological findings have been observed in five COVID-19 patients,[44,45] which could not be attributed to only severe hypoxia observed in critical COVID-19 cases. However, further detection of SARS-CoV-2 was not performed in the brain.

On June 12, 2020, Solomon et al.[46] reported autopsy findings from 18 patients who died from SARS-CoV-2 infection between April 14 and 29, 2020. They found only hypoxic changes, but no encephalitis or other specific brain changes in these patients. qRT-PCR tests in 32 sections from 16 patients, including three sections from the medulla and three sections from the frontal lobes, and olfactory nerves were positive to SARS-CoV-2 nucleocapsid protein.

On August 12, Remmelink et al.[47] carried out a postmortem study on 17 adult patients with COVID-19, who died from respiratory failure or multiple organ failure. They found eight cases with cerebral hemorrhage or hemorrhagic suffusion, three with focal ischemic necrosis, five with edema and/or vascular congestion, and 10 with diffuse or focal spongiosis. SARS-CoV-2 RNA has been found in 9 of 11 cerebral samples.

On August 26, Al-Dalahmah et al.[48] reported an autopsy study of one COVID-19 patient, which was performed 3 h after death. They found cerebellar hemorrhage and acute infarcts in the dorsal pons and medulla, but no evidence of vasculitis. Remarkably, there were microglial nodules and neuronophagia bilaterally in the inferior olives and multifocally in the cerebellar dentate nuclei. PCR tests for SARS-CoV-2 demonstrated the presence of viral transcripts in the nasal epithelium and cerebellar clot, low levels in the olfactory bulb and cerebellum, but no detectable transcripts in the medulla.

In the study reported by Solomon et al.[46] and the study by Al-Dalahmah et al.,[48] the positive PCR results for SARS-CoV-2 in the CNS have not been confirmed in the same brain areas by immunostaining or in situ hybridization. In the study of Remmelink et al.,[47] although SARS-CoV-2 RNA was found in 9 of 11 cerebral samples, autopsy examination did not found evidence of viral encephalitis or vasculitis. These discrepancies raised the question whether the positive PCR tests were false or not.

On April 21, Paniz-Mondolfi et al.[49] reported an ultrastructural finding of SARS-CoV-2 viral particles in the CNS. In this study, a male patient with COVID-19 in the United States of America was admitted to the emergency department because of fever and worsening neurological symptoms. At admission, his blood oxygen saturation had dropped to 94% on room air, despite no abnormal changes in the lung. Although head CT detected no specific alterations, electron microscopic examination of postmortem samples revealed the presence of CoV-like particles in the neurons and capillary endothelial cells in the frontal cortex. Moreover, the presence of SARS-Cov-2 in the brain was confirmed by testing frozen tissue in four RT-PCR assays.

On June 4, Meinhardt et al.[50] carried out an autopsy study on 32 COVID-19 patients for the presence of SARS-CoV-2 infection in the CNS. By precisely investigating anatomically mapping oro- and pharyngeal regions and brains, they not only found CNS infarction due to cerebral thromboembolism, but also demonstrated SARS-CoV-2 neurotropism. RT-qPCR tests showed the highest levels of SARS-CoV-2 copies per cell within the olfactory mucosa sampled directly beneath the cribriform plate (13 of 22). Assessment of subgenomic RNA showed active virus replication in four of 13 SARS-CoV-2 RNA-positive olfactory mucosa samples. In addition, viral load was also demonstrated in the olfactory bulb (3 of 23), trigeminal ganglion (3 of 20), and medulla oblongata (4 of 23). The presence of SARS-CoV-2 in these regions was also supported by immunohistochemistry and electron microscopy.

On July 15, Cantuti-Castelvetri et al.[51] carried out an autopsy study on six COVID-19 patients for the presence of SARS-CoV-2 infection in the olfactory system. Using antibodies against the spike protein of SARS-CoV-2, they detected infection in the olfactory epithelium of five of six COVID-19 patients. Within the brain, the olfactory bulb and tracts displayed immunoreactivity for the spike protein, especially within endothelial cells in small capillaries and medium-sized vessels.

On August 6, Puelles et al.[52] quantified SARS-CoV-2 viral load in autopsy tissue samples from 22 patients who died from COVID-19. The highest levels of SARS-CoV-2 copies per cell were detected in the respiratory tract, and lower levels were detected in the kidneys, liver, heart, brain, and blood. Of 22 patients, eight patients showed positive PCR results for SARS-CoV-2 in the brain. Brain tropism seemed to increase with the number of coexisting conditions. These findings indicate a broad organotropism of SARS-CoV-2.

On August 20, Hanley et al.[53] carried out a postmortem study on 10 COVID-19 patients between March 1 and April 30, 2020.[53] In addition to ischemic changes in the cortex and white matter, moderate to intense microglial activation was the most prominent pathological feature in the CNS (5 [100%] of 5 patients). Of note, viral load quantified by use of qRT-PCR targeting the viral E gene and the viral polymerase gene showed positive results in four of five patients. Tests for subgenomic viral RNA transcripts showed positive results in one of five patients.

On September 9, Freij et al.[54] reported a 5-year-old girl who died from CNS co-infection with SARS-CoV-2 and tuberculosis. Tests for SARS-CoV-2 RNA were negative in CSF, but positive in the biopsy tissue from the cerebellum.

Among the six studies, PCR, immunochemical, and ultrastructural techniques have been used to detect whether SARS-CoV-2 was present in the brain. In three studies, the presence of SARS-CoV-2 in the brain was confirmed at least by two different methods.[49-51] These studies have provided strong evidence for the CNS infection in COVID-19 patients.

Taken together, autopsies of the brain have provided distinctly different pathologies in COVID-19 patients. This is probably due to the fact that, although the patients with COVID-19 who underwent neuropathological analysis all showed signs of CNS involvement, they had different neurological features and probably different neurological diseases. Even so, autopsy evidence has emerged supporting the direct invasion of SARS-CoV-2 in the CNS, at least in some COVID-19 patients.

3.3 | Experimental evidence for the neuroinvasion of SARS-CoV-2

Up to September 14, we retrieved a total of 13 experimental articles relevant to SARS-CoV-2 neuroinvasion, including four in vitro and nine in vivo studies. Among them, one in vitro study reported only the effects of SARS-CoV-2 infection on the blood–brain barrier (BBB), whereas the other experiments all supported the neuroinvasion of SARS-CoV-2.

On June 26, 2020, Bullen et al.[55] reported a human-induced pluripotent stem cell (iPSC)-derived BrainSphere model of SARS-CoV-2 infection. After incubation of BrainSpheres with SARS-CoV-2 for 6 h, a fraction of neural cells were infected, and the replication of the virus became evident 72 h postinfection. Under the electron microscope, virus particles were found in the neuronal cell body extending into apparent neurite structures.

Thereafter, Ramani et al.[56] reported that SARS-CoV-2 could enter three-dimensional human brain organoids within 2 days of exposure, where it preferably targeted the neurons. SARS-CoV-2 infection induced altered distribution of Tau from axons to soma, hyperphosphorylation, and apparent neuronal death.

Song et al.[32] analyzed the neuroinvasive potential of SARS-CoV-2 in the hiPSC-derived forebrain-specific human neural progenitor cells. Similar to the findings reported by Bullen et al.,[55] they observed infection of neuronal cells in 9-week-old organoids as early as 24 h postinfection, with significantly increased number of SARS-CoV-2 positive cells at 96 h. The majority of SARS-CoV-2 infected cells were localized within MAP2-positive cellular fields of mature neurons. Electron microscopic observations showed that the virus could utilize host cell machinery to replicate in the neurons. In addition, SARS-CoV-2 infection has been found in radial glia and neuronal progenitor cells.

To clarify the pathophysiology of COVID-19, several animal models for SARS-CoV-2 infection have been developed. In Golden Syrian hamsters, SARS-CoV-2 RNA was detected in many extrapulmonary tissues, such as the liver, heart, spleen, kidneys, brain, and salivary glands.[57] In rhesus macaques, viral replication was observed in the gut, bladder, heart, skeletal muscles, and spinal cord.[58] These findings are consistent with the extrapulmonary manifestations in COVID-19 patients.

SARS-CoV-2 hACE2 transgenic mouse model has also been successfully developed by several research groups, respectively.[59,60] In a study reported by Sun et al.,[59] robust viral RNA replication of SARS-CoV-2 was found in the lungs, trachea, and brain tissues in the infected mice after intranasal inoculation. Furthermore, immunostaining of brain sections demonstrated robust viral spike protein expression in neurons, astrocytes, and microglial cells.[59]

In a study reported by Jiang et al.,[60] four of 14 infected mice showed noticeable respiratory distress and neurological symptoms from 2 days after infection. SARS-CoV-2 genomic sequences have been isolated from the lung and brain tissues in these mice.

Similarly, Rathnasinghe et al.[61] reported that B6 K18-hACE2 mice intranasally infected with SARS-CoV-2 showed high viral titers in the lung at Day 2 postinfection, and some animals had the virus in the brain. By Day 5 postinfection viral titers in the lung were reduced, whereas titers in the brain had increased.

Using K18-hACE2 mice, Zheng et al.[62] found that the predominant target organs were the lung at early time points, and variably, the brain at later time points.[62] In some, but not all animals, the brain tissue titers gradually increased from Day 2 to 6 postinfection. Immunostaining against the viral N at Day 6 postinfection revealed extensive staining in several brain regions, including olfactory bulb, cerebral cortex, caudate/putamen, thalamus, hypothalamus, and ventral striatum. In addition, the area postrema and hypoglossal nucleus were also infected.

Golden et al.[63] evaluated the pathogenesis of SARS-CoV-2 in mice expressing the human ACE2 gene under the control of the keratin 18 promotor. Brain infection was not observed in the majority of animals examined on Day 3 but was prevalent in mice necropsied on Days 5–11. Evidence of SARS-CoV-2 was found throughout the brain, including the thalamus, hypothalamus, amygdala, cerebral cortex, medulla, pons, and midbrain. Viral spike protein was detected in NeuN-positive cells, indicating viral infection of neurons. Viral antigen was absent in GFAP positive cells, suggesting this virus does not productively infect astrocytes. In the thalamus/hypothalamus, vasculitis was the most common lesion characterized by endothelial hypertrophy and increases in mononuclear leukocytes within the vessel wall and/or filling the perivascular space. However, cells within the vessel walls and perivascular spaces were negative for viral genomic RNA.

Moreover, two experimental papers about SARS-CoV-2 infection were deposited in bioRxiv in August 2020. By using transgenic mice expressing K18-hACE2, Yinda et al.[64] reported that SARS-COV-2 could enter the cerebral cortex and hippocampus after intranasal inoculation. In these regions, SARS-CoV-2 antigen was present in neurons and glial cells along the soma and axons of infected neurons.

By using deer mice, whose ACE2 receptor shares 17 of the 20 critical residues for SARS-CoV-2 binding, Fagre et al.[65] demonstrated robust virus replication in respiratory, digestive, and nervous systems after intranasal inoculation. Of interest, the glomerular layer of main olfactory bulb became spongiotic and immunoreactive at Day 3 postinoculation, and SARS-CoV-2 antigen was detected in the cytoplasm of mitral and microglial cells. Less-severe glial reactions and immunoreactivity were also found within the brainstem, where the presence of SARS-CoV-2 was found multifocally at the level of lateral sulcus nucleus, optic chiasm, hypothalamus, thalamic parabrachial nucleus, and ventral posteromedial nucleus culminating in the gustatory cortex. In addition, this study shows that SARS-CoV-2 might invade the brain via retrograde axonal transmission along gustatory, olfactory, and trigeminal pathways at the early stage of infection.

Taken together, in vitro studies show that SARS-CoV-2 can not only enter and replicate in neuronal cells but also infect many other cells in nerve tissue, including radial glia and neuronal progenitor cells.[32,55,56] In addition, the in vitro findings have provided initial insights into the potential neurotoxic effect of SARS-CoV-2.

The results obtained from animal experiments are generally consistent with each other in terms of neuronal infection, but there are some discrepancies in whether glial cells are infected, which may be due to the different animal sources or virus strains they used. Animal experiments have demonstrated that SARS-CoV-2 does possess a neuroinvasive potential similar to its counterpart, SARS-CoV.[57-60,64,65] Importantly, the in vivo findings show that CNS involvement may contribute to the lethality of SARS-CoV-2 infection.

4 | DISCUSSION

In this review, we have assessed the so far documented evidence for SARS-CoV-2 neuroinvasion, which points out a nonnegligible involvement of CNS in the pathophysiology of COVID-19. These data do not only end the debate about the neuroinvasive potential of SARS-CoV-2 but has also begun to unravel the mechanisms underlying the lethality of SARS-CoV-2 infection.

So far, the presence of SARS-CoV-2 in CSF was reported in at least 13 COVID-19 patients. However, more than 1000 COVID-19 patients who underwent CSF tests showed negative results. The positive detection of SARS-CoV-2 in CSF was only 1.28% among the pooled cases.

Several postmortem studies showed that despite the presence of SARS-CoV-2 in the brain parenchyma of some patients, RT-PCR tests for SARS-CoV-2 were negative in their CSF.[49,54] This indicates that negative outcomes of PCR tests in CSF do not mean the absence of SARS-CoV-2 in the CNS. Therefore, CSF test is not a reliable method to reveal whether the CNS is infected or not.

Not all postmortem studies on neuropathology reported the presence of SARS-CoV-2 in the brain. Among the published autopsy studies, brain detection of SARS-CoV-2 was positive in 58 (of 87) cases in nine studies, but only three have provided sufficient details on the CNS infection in 19 decreased patients with COVID-19.[49-52]

Most autopsies provided insufficient details, which may reflect the challenge of studying such patients. As a matter of fact, complete brain removal was difficult or even not allowed in some studies.[40,47] As neurotropic viruses infect only specific brain areas related to their entry routes, incomplete or random sampling is not suitable for the study of CNS infection.

On the other side, almost all in vitro and in vivo experiments support the neuroinvasive potential of SARS-CoV-2. In culture conditions, SARS-CoV-2 predominantly targeted and replicated in neuronal cells,[32,55,56] and induced altered distribution of Tau from axons to soma, hyperphosphorylation, and apparent neuronal death.[56]

As compared to postmortem studies, animal experiments have provided detailed information on the neuroinvasive potential of SARS-CoV-2,[59-61,63-65] which revealed that the predominant target organs were the lung of infected animals at early time points, and variably, the brain at later time points.[62,63] The presence of the virus in CNS was found within neurons in different brain areas with a wide spectrum of neuropathology, well consistent with the reported clinical symptoms in COVID-19 patients.[65] Not all infected animals showed neurological symptoms or CNS infection,[60,61] which may partially interpret negative detection of SARS-CoV-2 in some COVID-19 patients

As many other animal models, the experimental results from animal models of SARS-CoV-2 infection cannot be simply generalized to human beings. In COVID-19 patients, different ages, underlying comorbidities, complex clinical course, and long ICU stay may all act as confounding factors. Although the patients with COVID-19 all showed signs of CNS involvement, they had different neurological features and probably different neurological diseases.

Nevertheless, the experimental results from animal models are generally consistent with those reported in some postmortem studies on COVID-19 patients. These findings not only provide undisputable evidence for SARS-CoV-2 neuroinvasion but also shed light on the neurological mechanism of COVID-19.

4.1 | More evidence supports the trans-neuronal hypothesis

SARS-CoV-2 can affect the CNS through direct routes— haematogenous and trans-neuronal pathways and also by indirect mechanisms, which include cytokine dysregulation, peripheral immune cell transmigration, neuroinflammation, postinfectious autoimmunity, hypercoagulability, and so forth.[66]

As pointed out by some authors,[67] the finding of viral genome in CSF does not always indicate that SARS-CoV-2 can invade the brain via hematopoietic or trans-neuronal pathway. In the nasal epithelium, between the olfactory neurons and olfactory ensheathing cells, which surround the olfactory neurons, there exists a direct channel connecting the nasal cavity and the CSF surrounding the olfactory bulbs. This channel has been suggested as a trans-cribrial route for the SARS-CoV-2 invasion.[68] In addition, the presence of SARS-CoV-2 in the CSF may be due to the anatomical connection between the CNS lymphatic system and peripheral lymphatic vessels.[67] These speculations are theoretically possible, but supporting evidence is still lacking.

Cantuti-Castelvetri et al.[51] found that SARS-CoV-2 could infect endothelial cells in small capillaries and medium-sized vessels in the olfactory bulb and tracts. Moreover, SARS-CoV-2 viral particles have been ultrastructurally demonstrated in capillary endothelial cells in the frontal cortex.[49] These findings provide evidence supporting that SARS-CoV-2 may enter the CNS via the hematopoietic route.

Buzhdygan et al.[69] found that SARS-CoV-2 spike protein could trigger a proinflammatory response on brain endothelial cells that

may contribute to an altered state of BBB function. In an in vivo study, Perrin et al.[70] found that serum levels of the astroglial marker, S100B protein, were increased at the time of cytokine release syndrome in COVID-19 patient, reflecting an increased permeability of the BBB. These findings indicate that SARS-CoV-2 may also invade the CNS by impaired BBB.

More evidence from clinical, autopsy, and animal studies support the trans-neuronal hypothesis. SARS-CoV-2 viral antigen has been demonstrated in the olfactory bulb and tracts,[51] and the damage in the olfactory pathway was progressively less severe from peripherally to centrally.[44] Moreover, MRI examinations of COVID-19 patients revealed obvious structural changes in the olfactory pathway, including olfactory nerve, olfactory bulb, and olfactory cortex, indicating that SARS-CoV-2 might enter the CNS via an olfactory bulb-mediated trans-neuronal route.[31,71,72]

The trans-neuronal hypothesis is also supported by animal experiments, where immunostaining against SARS-CoV-2 revealed extensive staining in many secondary or tertiary brain regions connected with the olfactory bulb.[62,65] In addition, the results from animal experiments show that SARS-CoV-2 might invade the brain retrogradely along gustatory and trigeminal pathways at the early stage of infection.[65]

4.2 | Neuroinvasion is associated with the lethality of SARS-CoV-2 infection

So far, the contribution of neurological involvement to mortality is still ill-defined in the infection with SARS-CoV-2. It is generally accepted that old age, underlying comorbidities, complex clinical course, and long ICU stay all contribute to clinical deterioration and a higher mortality rate.

To reveal the risk factors of deaths caused by SARS-CoV-2 infection, Zou et al.[73] analyzed clinical data of 121 COVID-19 patients from January 30, 2019 to March 23, 2020. They found that severe cases and death of COVID-19 were associated with older age, comorbidities, organ dysfunction, lymphopenia, high cytokines, and weak immune responses.

von Weyhern et al.[45] carried out an autopsy study on six patients who died from COVID-19 in April 2020. They found that patients older than 65 years with multiple comorbidities died from cardiorespiratory failure, whereas the younger died either from massive intracranial hemorrhage or pulmonary embolism.

Of interest, the patients with COVID-19 requiring ICU admission due to neurological issues, or those in ICU who manifested neurological deficits, are at a higher risk of mortality.[74] Moreover, COVID-19 patients who have recovered from their respiratory symptoms were found to be potentially at higher risk for long-term residual neuropsychiatric and neurocognitive conditions, including depression, obsessive-compulsive disorder, psychosis, Parkinson's disease, and Alzheimer's disease.[75]

In an animal study reported by Jiang et al.,[60] four of 14 infected mice showed noticeable respiratory distress and neurological

symptoms from 2 days after infection. Only the mice showing neurological symptoms became dead, suggesting that neurological involvement may be one cause of death.

Song et al.[32] further investigated the consequence of CNS involvement in SARS-CoV-2 infection by using transgenic mice overexpressing hACE2. They found that the mice expressing hACE2 in the brains showed significant weight loss and death after either intranasal or intraventricular inoculation, even at the extremely low challenge virus dose. In contrast, the mice expressing hACE2 in the lungs showed signs of lung pathology after intranasal inoculation, but no weight loss or death. The study highlights the possible lethal consequence of SARS-CoV-2 neuroinvasion in COVID-19 patients.

Among the COVID-19 patients with dyspnea, more than half needed intensive care.[76,77] Many critical patients failed early attempts at weaning from invasive mechanical ventilation so that the time of ICU stay appears to be long.[78,79] This is surprising as most of them had recovered from pneumonia. Further clinical evaluation of these patients indicated an involvement of the brainstem and especially of the respiratory center.[80]

Consistent with this, at least three autopsy studies have described brainstem abnormalities after SARS-CoV-2 infection.[43-45] Brainstem infection with SARS-CoV-2 was also reported in an animal experiment, where viral antigen was present multifocally at the level of lateral sulcus nucleus, optic chiasm, hypothalamus, thalamic parabrachial nucleus, and ventral posteromedial nucleus culminating in the gustatory cortex.[65]

Once in the CNS, SARS-CoV-2 will enter and replicate in neurons, leading to cell death. Infection of some brain areas, especially the brainstem, may lead to cardiorespiratory dysfunction and even death. The invasion of viruses and the death of neurons will subsequently activate astrocytes and microglia, inducing inflammatory reaction, which, together with intense cytokine release during florid cytokine storm, may make BBB more permeable, thereby facilitating the entry of SARS-CoV-2 into the CNS.

To summarize, this review has assessed the so far documented evidence for the neuroinvasive potential of SARS-CoV-2. These data have provided strong support for the infection of CNS and shed light on the association of neurological involvement with the lethality of SARS-CoV-2 infection. Awareness of the neuroinvasive potential of SARS-CoV-2 has important guiding significance for the prevention, treatment, and prognosis of SARS-CoV-2 infection.

ACKNOWLEDGMENT

DATA AVAILABILITY STATEMENT
The authors state that all the data presented in the manuscript are available on MEDLINE/PubMed, WebOfScience, and COVID-19-related preprints from medRxiv and bioRxiv.

ORCID
Yan-Chao Li http://orcid.org/0000-0002-2884-9829

REFERENCES

1. Yu JW, Wang L, Bao LD. Exploring the active compounds of traditional Mongolian medicine in intervention of novel coronavirus (COVID-19) based on molecular docking method. *J Funct Foods*. 2020;71:104016. https://doi.org/10.1016/j.jff.2020.104016

2. Ceccarelli M, Berretta M, Venanzi Rullo E, Nunnari G, Cacopardo B. Differences and similarities between Severe Acute Respiratory Syndrome (SARS)-CoronaVirus (CoV) and SARS-CoV-2. Would a rose by another name smell as sweet? *Eur Rev Med Pharmacol Sci*. 2020;24:2781-2783.

3. Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J Med Virol*. 2020;92:552-555. https://doi.org/10.1002/jmv.25728

4. Li YC, Bai WZ, Hashikawa T. Response to commentary on "The neuroinvasive potential of SARS-CoV-2 may play a role in the respiratory failure of COVID-19 patients". *J Med Virol*. 2020;92: 707-709. https://doi.org/10.1002/jmv.25824

5. Chacón-Aguilar R, Osorio-Cámara JM, Sanjurjo-Jimenez I, González-González C, López-Carnero J, Pérez-Moneo B. COVID-19: fever syndrome and neurological symptoms in a neonate. *An Pediatr (Engl Ed)*. 2020;92(6):373-374. https://doi.org/10.1016/j.anpede.2020.04.001

6. Duong L, Xu P, Liu A. Meningoencephalitis without respiratory failure in a young female patient with COVID-19 infection in Downtown Los Angeles, early April 2020. *Brain Behav Immun*. 2020; 87:33. https://doi.org/10.1016/j.bbi.2020.04.024

7. Gutiérrez-Ortiz C, Méndez-Guerrero A, Rodrigo-Rey S, et al. Miller Fisher syndrome and polyneuritis cranialis in COVID-19. *Neurology*. 2020;95(5):e601-e605. https://doi.org/10.1212/WNL.0000000000009619

8. Moriguchi T, Harii N, Goto J, et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. *Int J Infect Dis*. 2020; 94:55-58. https://doi.org/10.1016/j.ijid.2020.03.062

9. Wang HY, Li XL, Yan ZR, Sun XP, Han J, Zhang BW. Potential neurological symptoms of COVID-19. *Ther Adv Neurol Disord*. 2020;13: 1756286420917830. https://doi.org/10.1177/1756286420917830

10. Khalili N, Haseli S, Bahrami-Motlagh H, et al. Neurologic Involvement in COVID-19: radiologists' perspective. *Acad Radiol*. 2020; 27(7):1051-1053. https://doi.org/10.1016/j.acra.2020.04.035

11. Ellul MA, Benjamin L, Singh B, et al. Neurological associations of COVID-19. *Lancet Neurol*. 2020;19(9):767-783. https://doi.org/10.1016/S1474-4422(20)30221-0

12. Pezzini A, Padovani A. Lifting the mask on neurological manifestations of COVID-19. *Nat Rev Neurol*. 2020:1-9. https://doi.org/10.1038/s41582-020-0398-3

13. Kandemirli SG, Dogan L, Sarikaya ZT, et al. Brain MRI findings in patients in the intensive care unit with COVID-19 infection. *Radiology*. 2020;297:201697-E235. https://doi.org/10.1148/radiol.2020201697

14. Xiang P, Xu XM, Gao LL, et al. First case of 2019 novel coronavirus disease with encephalitis. *ChinaXiv*. 2020 T202003.00015. http://www.xinhuanet.com/english/2020-03/05/c_138846783.htm

15. 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;87:149. https://doi.org/10.1016/j.bbi.2020.05.012

16. Färber K, Stäbler P, Getzinger T, Uhlig T. 10 Wochen alter Säugling mit Sepsisverdacht und SARS-CoV-2-Nachweis in Liquor und Rachen [Suspected sepsis in a 10-week-old infant and SARS-CoV-2 detection in cerebrospinal fluid and pharynx]. *Monatsschr Kinderheilkd*. 2020:1-4. German. https://doi.org/10.1007/s00112-020-00942-8

17. Kremer S, Lersy F, de Sèze J, et al. Brain MRI findings in severe COVID-19: a retrospective observational study [published online ahead of print June 16, 2020]. *Radiology*. 2020:202222. https://doi.org/10.1148/radiol.2020202222

18. Domingues RB, Mendes-Correa MC, de Moura Leite FBV, et al. First case of SARS-COV-2 sequencing in cerebrospinal fluid of a patient with suspected demyelinating disease. *J Neurol.* 2020:1-3. https://doi.org/10.1007/s00415-020-09996-w

19. Westhoff TH, Seibert FS, Bauer F, et al. Allograft infiltration and meningoencephalitis by SARS-CoV-2 in a pancreas-kidney transplant recipient [published online ahead of print July 26, 2020]. *Am J Transplant.* 2020:ajt.16223. https://doi.org/10.1111/ajt.16223

20. Mardani M, Nadji SA, Sarhangipor KA, Sharifi-Razavi A, Baziboroun M. COVID-19 infection recurrence presenting with meningoencephalitis. 29. *New Microbes New Infect.* 2020;37:100732. https://doi.org/10.1016/j.nmni.2020.100732

21. Fadakar N, Ghaemmaghami S, Masoompour SM, et al. A first case of acute cerebellitis associated with coronavirus disease (COVID-19): a case report and literature review[published online ahead of print July 30, 2020]. *Cerebellum.* 2020;31:1-4. https://doi.org/10.1007/s12311-020-01177-9

22. Helms J, Kremer S, Merdji H, et al. Neurologic Features in Severe SARS-CoV-2 Infection. *N Engl J Med.* 2020;382(23):2268-2270. https://doi.org/10.1056/NEJMc2008597

23. Cebrián J, Gonzalez-Martinez A, García-Blanco MJ, et al. Headache and impaired consciousness level associated with SARS-CoV-2 in CSF: a case report. *Neurology.* 2020;95(6):266-268. https://doi.org/10.1212/WNL.0000000000010213

24. Virhammar J, Kumlien E, Fällmar D, et al. Acute necrotizing encephalopathy with SARS-CoV-2 RNA confirmed in cerebrospinal fluid. *Neurology.* 2020;95:445-449. https://doi.org/10.1212/WNL.0000000000010250

25. Khodamoradi Z, Hosseini SA, Gholampoor Saadi MH, Mehrabi Z, Sasani MR, Yaghoubi S. COVID-19 meningitis without pulmonary involvement with positive cerebrospinal fluid PCR. *Eur J Neurol.* 2020:ene.14536. https://doi.org/10.1111/ene.14536

26. Yin R, Feng W, Wang T, et al. Concomitant neurological symptoms observed in a patient diagnosed with coronavirus disease 2019. *J Med Virol.* 2020;92:1782-1784. https://doi.org/10.1002/jmv.25888

27. Helms J, Kremer S, Merdji H, et al. Neurologic features in severe SARS-CoV-2 infection. *N Engl J Med.* 2020;382:2268-2270. https://doi.org/10.1056/NEJMc2008597

28. Destras G, Bal A, Escuret V, Morfin F, Lina B, Josset L. Systematic SARS-CoV-2 screening in cerebrospinal fluid during the COVID-19 pandemic. *Lancet Microbe.* 2020;1(4):e149. https://doi.org/10.1016/S2666-5247(20)30066-5

29. Espíndola OM, Siqueira M, Soares CN, et al. Patients with COVID-19 and neurological manifestations show undetectable SARS-CoV-2 RNA levels in the cerebrospinal fluid. *Int J Infect Dis.* 2020;96:567-569. https://doi.org/10.1016/j.ijid.2020.05.123

30. Wang M, Li T, Qiao F, Wang L, Li C, Gong Y. Coronavirus disease 2019 associated with aggressive neurological and mental abnormalities confirmed based on cerebrospinal fluid antibodies: a case report. *Medicine (Baltimore).* 2020;99(36):e21428. https://doi.org/10.1097/MD.0000000000021428

31. Lu S, Wei N, Jiang J, et al. First report of manic-like symptoms in a COVID-19 patient with no previous history of a psychiatric disorder. *J Affect Disord.* 2020;277:337-340. https://doi.org/10.1016/j.jad.2020.08.031

32. Song E, Zhang C, Israelow B, et al. Neuroinvasive potential of SARS-CoV-2 revealed in a human brain organoid model. *bioRxiv.* 2020;6(25):169946. https://doi.org/10.1101/2020.06.25.169946

33. Senel M, Abu-Rumeileh S, Michel D, et al. Miller-Fisher syndrome after COVID-19: neurochemical markers as an early sign of nervous system involvement [published online ahead of print August 11, 2020]. *Eur J Neurol.* https://doi.org/10.1111/ene.14473

34. Ye M, Ren Y, Lv T. Encephalitis as a clinical manifestation of COVID-19. *Brain Behav Immun.* 2020;88:945-946. https://doi.org/10.1016/j.bbi.2020.04.017

35. Edler C, Schröder AS, Aepfelbacher M, et al. Dying with SARS-CoV-2 infection-an autopsy study of the first consecutive 80 cases in Hamburg, Germany [published correction appears in Int J Legal Med. 2020;134(5):1977]. *Int J Legal Med.* 2020;134(4):1275-1284. https://doi.org/10.1007/s00414-020-02317-w

36. Skok K, Stelzl E, Trauner M, Kessler HH, Lax SF. Post-mortem viral dynamics and tropism in COVID-19 patients in correlation with organ damage. *Virchows Arch.* 2020:1-11. https://doi.org/10.1007/s00428-0;

37. 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):1-6. https://doi.org/10.1007/s00401-020-02166-2

38. Conklin J, Frosch MP, Mukerji S, et al. Cerebral Microvascular Injury in Severe COVI-19 [published online ahead of print July 24, 2020]. *medRxiv.* https://doi.org/10.1101/2020.07.21.20159376

39. Bradley BT, Maioli H, Johnston R, et al. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: a case series [published correction appears in Lancet. 2020;396(10247):312]. *Lancet.* 2020;396(10247):320-332. https://doi.org/10.1016/S0140-6736(20)31305-2

40. Duarte-Neto AN, Monteiro RAA, Silva LFF, et al. Pulmonary and systemic involvement of COVID-19 assessed by ultrasound-guided minimally invasive autopsy. *Histopathology.* 2020;77:186-197. https://doi.org/10.1111/his.14160

41. Schaller T, Hirschbühl K, Burkhardt K, et al. Postmortem examination of patients with COVID-19. *JAMA.* 2020;323:2518. https://doi.org/10.1001/jama.2020.8907

42. Kantonen J, Mahzabin S, Mäyränpää MI, et al. Neuropathologic features of four autopsied COVID-19 patients [published online ahead of print, August 6, 2020]. *Brain Pathol.* https://doi.org/10.1111/bpa.12889

43. Jensen MP, Le Quesne J, Officer-Jones L, et al. Neuropathological findings in two patients with fatal COVID-19 [published online ahead of print, September 8, 2020]. *Neuropathol Appl Neurobiol.* https://doi.org/10.1111/nan.12662

44. Bulfamante G, Chiumello D, Canevini MP, et al. First ultrastructural autoptic findings of SARS-Cov-2 in olfactory pathways and brainstem. *Minerva Anestesiol.* 2020;86(6):678-679. https://doi.org/10.23736/S0375-9393.20.14772-2

45. von Weyhern CH, Kaufmann I, Neff F, Kremer M. Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. *Lancet.* 2020;395(10241):e109. https://doi.org/10.1016/S0140-6736(20)31282-4

46. Solomon IH, Normandin E, Bhattacharyya S, et al. Neuropathological features of Covid-19. *N Engl J Med.* 2020;383(10):989-992.

47. Remmelink M, de Mendonç A R, D'Haene N, et al. Unspecific post-mortem findings despite multiorgan viral spread in COVID-19 patients. *Crit Care.* 2020;24(1):495. https://doi.org/10.1186/s13054-020-03218-5

48. Al-Dalahmah O, Thakur KT, Nordvig AS, et al. Neuronophagia and microglial nodules in a SARS-CoV-2 patient with cerebellar hemorrhage. *Acta Neuropathol Commun.* 2020;8(1):147. https://doi.org/10.1186/s40478-020-01024-2

49. Paniz-Mondolfi A, Bryce C, Grimes Z, et al. Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J Med Virol.* 2020;92(7):699-702. https://doi.org/10.1002/jmv.25915

50. Meinhardt J, Radke J, Dittmayer C, et al. Olfactory transmucosal SARS-CoV-2 invasion as port of central nervous system entry in COVID-19 patients. *bioRxiv.* https://doi.org/10.1101/2020.06.04.135012

51. Cantuti-Castelvetri L, Ojha R, Pedro LD, et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and provides a possible pathway into

the central nervous system. *bioRxiv*. 2020. https://doi.org/10.1101/2020.06.07.137802

52. Puelles VG, Lütgehetmann M, Lindenmeyer MT, et al. Multiorgan and renal tropism of SARS-CoV-2. *N Engl J Med*. 2020;383(6):590-592. https://doi.org/10.1056/NEJMc2011400

53. Hanley B, Naresh KN, Roufosse C, et al. Histopathological findings and viral tropism in UK patients with severe fatal COVID-19: a post-mortem study. *Lancet Microbe*. 2020. https://doi.org/10.1016/S2666-5247(20)30115-4

54. Freij BJ, Gebara BM, Tariq R, et al. Fatal central nervous system co-infection with SARS-CoV-2 and tuberculosis in a healthy child. *BMC Pediatr*. 2020;20(1):429. https://doi.org/10.1186/s12887-020-02308-1

55. Bullen CK, Hogberg HT, Bahadirli-Talbott A, et al. Infectability of human BrainSphere neurons suggests neurotropism of SARS-CoV-2 [published online ahead of print June 26, 2020]. *ALTEX*. https://doi.org/10.14573/altex.2006111

56. Ramani A, Müller L, N Ostermann P, et al. SARS-CoV-2 targets neurons of 3D human brain organoids [published online ahead of print September 2, 2020]. *EMBO J*. https://doi.org/10.15252/embj.2020106230

57. Chan JFW, Zhang AJ, Yuan S, et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility [published online ahead of print March 26, 2020]. *Clin Infect Dis*. https://doi.org/10.1093/cid/ciaa325

58. Bao L, Deng W, Gao H, et al. Reinfection could not occur in SARS-CoV-2 infected rhesus macaques. *bioRxiv*. 2020. https://doi.org/10.1101/2020.03.13.990226v2

59. Sun SH, Chen Q, Gu HJ, et al. A mouse model of SARS-CoV-2 infection and pathogenesis. *Cell Host Microbe*. 2020;28(1):124-133. https://doi.org/10.1016/j.chom.2020.05.020

60. Jiang RD, Liu MQ, Chen Y, et al. Pathogenesis of SARS-CoV-2 in transgenic mice expressing human angiotensin-converting enzyme 2. *Cell*. 2020;182(1):50-58. https://doi.org/10.1016/j.cell.2020.05.027

61. Rathnasinghe R, Strohmeier S, Amanat F, et al. Comparison of transgenic and adenovirus hACE2 mouse models for SARS-CoV-2 infection [published online ahead of print July 6, 2020]. *bioRxiv*. https://doi.org/10.1101/2020.07.06.190066

62. Zheng J, Roy Wong LY, Li K, et al. K18-hACE2 mice for studies of COVID-19 treatments and pathogenesis including anosmia [published online ahead of print August 10, 2020]. *bioRxiv*. https://doi.org/10.1101/2020.08.07.242073

63. Golden JW, Cline CR, Zeng X, et al. Human angiotensin-converting enzyme 2 transgenic mice infected with SARS-CoV-2 develop severe and fatal respiratory disease. *JCI Insight*. 2020:142032. https://doi.org/10.1172/jci.insight.142032

64. Yinda CK, Port JR, Bushmaker T, et al. K18-hACE2 mice develop respiratory disease resembling severe COVID-19 [published online ahead of print August 11, 2020]. *bioRxiv*. https://doi.org/10.1101/2020.08.11.246314

65. Fagre A, Lewis J, Eckley M, et al. SARS-CoV-2 infection, neuropathogenesis and transmission among deer mice: implications for reverse zoonosis to New World rodents [published online ahead of print August 7, 2020]. *bioRxiv*. https://doi.org/10.1101/2020.08.07.241810

66. Mehta S, Bhandari S, Mehta S. Brain autopsies in fatal COVID-19 and postulated pathophysiology: more puzzling than a Rubik's cube [published online ahead of print September 9, 2020]. *J Clin Pathol*. https://doi.org/10.1136/jclinpath-2020-206967

67. Pouga L. Encephalitic syndrome and anosmia in COVID-19: do these clinical presentations really reflect SARS-CoV-2 neurotropism? A theory based on the review of 25 COVID-19 cases [published online ahead of print July 16, 2020]. *J Med Virol*. https://doi.org/10.1002/jmv.26309

68. Baig AM, Sanders EC. Potential neuroinvasive pathways of SARS-CoV-2: Deciphering the spectrum of neurological deficit seen in Coronavirus Disease-2019 (COVID-19). *J Med Virol*. 2020;92:1845-1857. https://doi.org/10.1002/jmv.26105

69. Buzhdygan TP, de Ore BJ, Baldwin-Leclair A, et al. The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in vitro models of the human blood-brain barrier [published online ahead of print June 15, 2020]. *bioRxiv*. https://doi.org/10.1101/2020.06.15.150912

70. Perrin P, Collongues N, Baloglu S, et al. Cytokine release syndrome-associated encephalopathy in patients with COVID-19 [published online ahead of print August 27, 2020]. *Eur J Neurol*. https://doi.org/10.1111/ene.14491

71. Li CW, Syue LS, Tsai YS, et al. Anosmia and olfactory tract neuropathy in a case of COVID-19 [published online ahead of print June 20, 2020]. *J Microbiol Immunol Infect*. S1684-1182. https://doi.org/10.1016/j.jmii.2020.05.017

72. Lin E, Lantos JE, Strauss SB. Brain imaging of patients with COVID-19: findings at an academic institution during the height of the outbreak in New York City [published online ahead of print August 20, 2020]. *Am J Neuroradiol*. https://doi.org/10.3174/ajnr.A6793

73. Zou L, Dai L, Zhang Y, et al. Clinical characteristics and risk factors for disease severity and death in patients with Coronavirus Disease 2019 in Wuhan, China. *Front Med (Lausanne)*. 2020;7:532. https://doi.org/10.3389/fmed.2020.00532

74. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with Coronavirus Disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med*. 2020;180(7):1-11. https://doi.org/10.1001/jamainternmed.2020.0994

75. Nath A. Neurologic complications of coronavirus infections. *Neurology*. 2020;94:809-810. https://doi.org/10.1212/WNL.0000000000009455

76. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507-513. https://doi.org/10.1016/S0140-6736(20)30211-7

77. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061-1069. https://doi.org/10.1001/jama.2020.1585

78. Grasselli G, Zangrillo A, Zanella A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. *JAMA*. 2020;323:1574-1581. https://doi.org/10.1001/jama.2020.5394

79. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China [published correction appears in Lancet, 2020]. *Lancet*. 2020;395:497-506. https://doi.org/10.1016/S0140-6736(20)30183-5

80. Manganelli F, Vargas M, Iovino A, Iacovazzo C, Santoro L, Servillo G. Brainstem involvement and respiratory failure in COVID-19. *Neurol Sci*. 2020;41(7):1663-1665. https://doi.org/10.1007/s10072-020-04487-2