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REVIEW ARTICLE

COVID-19 and autopsy findings in the brain

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Abstract

COVID-19 is primarily a respiratory disease, but very soon after the pandemic outbreak it became clear that the infection affects multiple organs, including the brain. Because of the major manifestation of the disease in the lungs and safety concerns about the virus spread during brain removal, our knowledge about changes in the brain was falling behind. The amount of publications describing autopsy findings in the brain, including our own observations, has increased, but frequently the reported data are inconsistent. It seems that most of the changes are attributable to the effects of severe infection, ventilation treatment and prothrombotic systemic effects of SARS-CoV-2 virus. Among the most common changes are brain edema, diffuse ischemic changes and perivascular hemorrhages, but also ischemic and hemorrhagic microinfarcts and infarcts. Other frequent findings are microthrombi, perivascular and intraparenchymal lymphocytic and microglial infiltration and astrogliosis. Specific changes like viral encephalitis and acute disseminated encephalomyelitis-like changes were described only in individual case reports. Finally, there are controversies about whether the SARS-CoV-2 actually infects neurons and glial cells. Changes in gene expression in neural, ependymal, and glial cells are also reported. This review aims to sum up the most common autopsy findings in the brains of COVID-19 patients and provide possible clinical implications of these findings.

Introduction

During the past three years of the pandemic, we have accumulated information about presentations of the COVID-19 disease, including data related to neurological symptoms of SARS-CoV-2 infection. The symptoms vary in severity from loss of smell, taste, and headache to life-threatening conditions like stroke (Hingorani *et al.* 2022). In hospitalized patients are neurological manifestations associated with higher in-hospital mortality. Patients with preexisting neurological disorders are at a higher risk for developing such complications (Chou *et al.* 2021). With the growing number of patients that overcome the infection, the importance of less dramatic, but still debilitating problems is increasing. This refers to the consequences of the central nerve system (CNS) complications of the disease and post-COVID sequelae known as "long COVID.". Morphological findings in CNS contribute to a better understanding of the pathophysiology of neurological symptoms in COVID-19 patients. Neurological symptoms and complications are multifactorial. The underlying mechanisms most likely combine these factors:

- neurotropism of SARS-CoV-2 and direct infection of CNS
- thrombi and microthrombi caused by infection of SARS-CoV-2, systemic inflammatory response with cytokine storm and hypercoagulation state caused by the inflammatory response and anti-phospholipid antibodies production (Zuo *et al.* 2020).
- hypoxia from pulmonary damage in COVID-19 pneumonia, hypoperfusion resulting from thrombi and microthrombi, and cardiovascular complications of COVID-19.
- metabolic derangement
- bleeding caused by anticoagulative treatment indicated in COVID-19 patients
- immune cross-reaction to viral antigens

Macroscopic findings

Most brains of patients deceased with COVID-19 do not have any significant findings at the gross examination (Remmelink *et al.* 2020; Schurink *et al.* 2020; Meinhardt *et al.* 2021; Mukerji & Solomon, 2021). The most common lesions are petechial bleedings and subarachnoid hemorrhages, infarcts ranging from lacunar infarcts, watershed infarcts to large acute/subacute infarcts, and edemas with or without herniation (Jaunmuktane *et al.* 2020; Remmelink *et al.* 2020; Fabbri *et al.* 2021; Meinhardt *et al.* 2021; Mukerji & Solomon, 2021). Atherosclerosis of brain vessels is rarely noted in published reports, though it is probably common. One work includes the evaluation of arteriosclerosis of the basal arteries ranging from mild to severe in 21% of patients. Most of the patients had moderate arteriosclerotic changes (Matschke *et al.* 2020).

Microscopic findings

The most common microscopic changes observed in the brain tissue were edema (Fig. 1), which is very often a non-specific agonal change (Matschke *et al.* 2020), hypoxic changes and ischemic lesions, hemorrhage, infiltration by immune cells, including perivascular, parenchymal, and meningeal infiltration by macrophages, microglia, and lymphocytes, astrogliosis, and microthrombi. Some of these findings are addressed in more detail in the following text.

Hypoxic changes and ischemic lesions

Ischemic injury ranging from mild hypoxic injury to infarcts, is the dominant type of pathological changes in the brain tissue (Jaunmuktane *et al.* 2020; Matschke *et al.* 2020; Mukerji & Solomon, 2021). In patients with global hypoxia resulting from lung damage is not surprising to find diffuse ischemic changes and, in more severe cases, infarctions in watershed areas. However, in patients with SARS-CoV-2 infection, there were also diffuse small ischemic infarcts and territorial infarcts. Ischemic strokes are quite common events in the general population, and risk factors for the fatal course of COVID-19 overlap with risk factors of stroke (Luo *et al.* 2022). The incidence of COVID-related ischemic strokes among hospitalized patients varies considerably in studies, ranging from 0,5% to 5,9% (Finsterer *et al.* 2022; Luo *et al.* 2022). Although, according to Finsterer *et al.* (2022), the overall prevalence and incidence of ischemic stroke did not increase since the pandemic outbreak. Nevertheless, there are also case reports describing non-fatal ischemic strokes related to SARS-CoV-2 infection in otherwise healthy children (Osman *et al.* 2022; Scala *et al.* 2022), and COVID-19-related ischemic stroke has a worse prognosis, with mortality up to 38% (Finsterer *et al.* 2022; Luo *et al.* 2022).

Hypoxic changes are commonly seen as hypereosinophilic "red neurons," (Fig. 1) often with scattered necrotic neurons in many brain regions. They might indicate terminal hypoxic-ischemic injury (Reichard *et al.* 2020; Schurink *et al.* 2020; Solomon *et al.* 2020; Bryce *et al.* 2021; Fabbri *et al.* 2021). Solomon *et al.* (2020) observed acute hypoxic injury in the cerebrum and cerebellum in all 18 patients with loss of neurons in the cerebral cortex, hippocampus, and cerebellar Purkinje cell layer, without the presence of thrombi or vasculitis. Generally, in most studies, the hypoxic-ischemic injury is usually mentioned in all, or at least in some cases (Deigendesch *et al.* 2020; Hanley *et al.* 2020; Kantonen *et al.* 2020).

Small, multiple ischemic lesions or infarcts not visible on gross examination were also described (Reichard *et al.* 2020). They were found as focal ischemic necroses (Remmelink *et al.* 2020) (Fig. 1) and hypoxic-ischemic lesions in the brainstem and brain (Meinhardt *et al.* 2021). Bryce *et al.* found ischemic infarctions in 19 out of 58 cases, in 17 cases associated with microthrombi, while in one case, the infarction was restricted to the pituitary gland. The infarctions were predominantly small and diffusely distributed in the neocortex and deep gray matter structures. A large cerebral artery territory infarct was present only in one case. Infarcts in a watershed territory, were present in 2 cases (Bryce *et al.* 2021). In another study, fresh territorial ischemic lesions were detected in 6 out of 43 patients. These appeared to be of thromboembolic origin (Matschke *et al.* 2020). Other authors reported territorial infarcts in 1 out of 5 patients (Hanley *et al.* 2020) and in 1 out of 42 patients (Serrano *et al.* 2022). Combined territorial infarcts and watershed infarcts were found in a patient that had overcome cardiac arrest and ECMO therapy before death (Jaunmuktane *et al.* 2020).

Hemorrhagic lesions

The size of hemorrhage in the brain in COVID-19 patients ranges from perivascular microhemorrhages (Fig. 1) and intraparenchymal and subarachnoid small

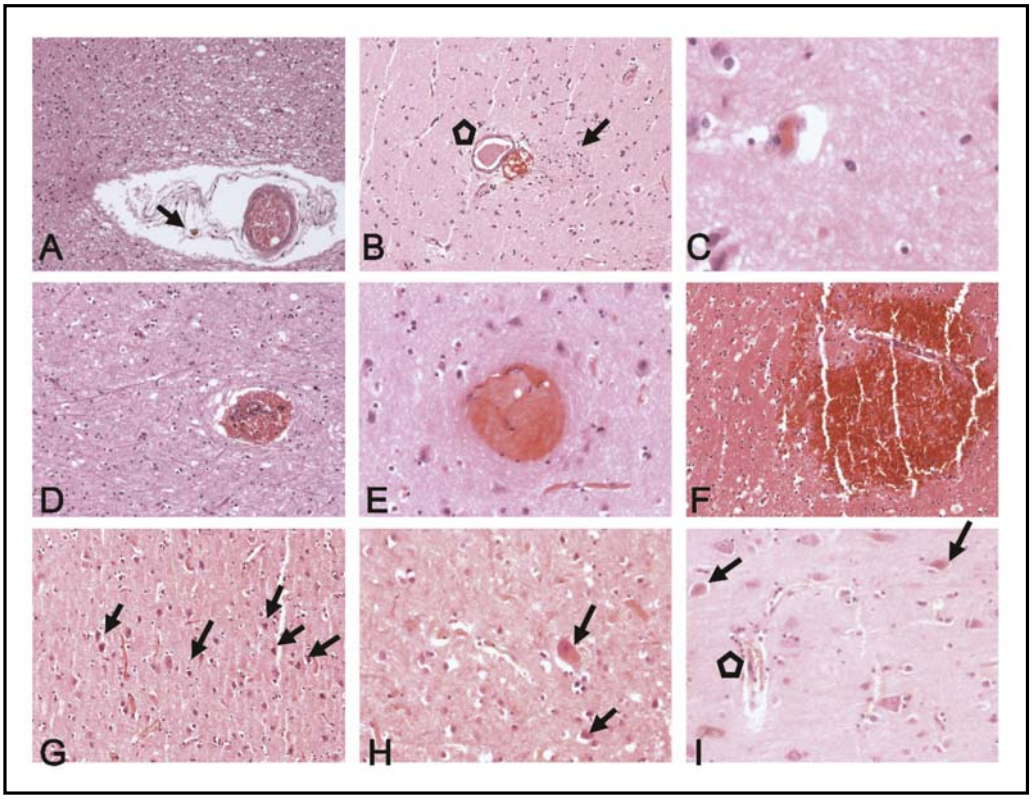

Fig. 1. The most common microscopic findings in the brain in COVID-19 patients. Brain edema with perivascular empty spaces, and extravasation of a few erythrocytes phagocyted by a macrophage (arrow) (A). Microthrombus (pentagon) with ischemia (arrow) in the adjacent tissue (B). Capillary microthrombus might be hard to distinguish from venostasis and hyperemia in hematoxylin and eosin stain (C). Perivascular microhemorrhages (D, E, F). Red neurons (arrow) are the hallmark of diffuse ischemia of the brain (G, H, I). Scarce perivascular lymphocytic and microglial infiltration (pentagon) (I). Hematoxylin and eosin, 100x (A,B,D,F,G,H); 200x (C,E,I).

hemorrhages to massive, fatal lesions. The presence of hemorrhages varies a lot in the literature. Some authors observed no hemorrhages in all of their cases (Deigendesch *et al.* 2020; Matschke *et al.* 2020; Schurink *et al.* 2020) or just a unique appearance of microhemorrhage (Meinhardt *et al.* 2021).

The most common type is perivascular bleeding (Kantonen *et al.* 2020; Santana *et al.* 2021). Hemorrhagic lesions might be diffuse, sized up to 1 cm. They are often found along with diffuse ischemic microinfarcts and large infarcts (Jaunmuktane *et al.* 2020; Reichard *et al.* 2020; Bryce *et al.* 2021). Remmelink *et al.* (2020) observed hemorrhages and hemorrhagic suffusions in 8 out of 11 cases. Massive cerebral hemorrhages are also described (Al-Dalahmah *et al.* 2020; Schurink *et al.* 2020; Bryce *et al.* 2021).

Hemorrhages might be secondary to reperfusion injury in ischemic lesions, which some authors seem not to consider. Santana *et al.* focused on intracranial hemorrhagic and ischemic lesions in CNS in COVID-19 patients. Their study showed either macroscopic or microscopic hemorrhagic/ischemic lesions in 23 out of 44 cases, the most common being perivascular discharge. In 2 cases were petechial bleedings associated with small vessel vasculitis. They found no correlation with anticoagulant, corticosteroid, or antibiotic treatment but increased risk in patients with diabetes (Santana *et al.* 2021).

It is not clear whether these findings are attributable to SARS-CoV-2 infection. One of the mechanisms that affect the findings in the brain in COVID-19 patients is hypoxia requiring ventilation therapy. Microbleedings seen in MRI associated with a critical illness are a well-known phenomenon and were also found in patients with COVID-19 (Fanou *et al.* 2017; Vattoth *et al.* 2020). Similar histopathological findings are documented in COVID-19-negative patients who received extracorporeal membrane oxygenation (ECMO) for acute respiratory distress syndrome, showing macro and microhemorrhages, infarctions, and hypoxic-ischemic brain injury (Khan *et al.* 2021). This was supported by another study demonstrating no significant difference between infarctions and hypoxic-ischemic changes or hemorrhages and microhemorrhages in 42 COVID-19 cases compared to 107 non-COVID controls (Serrano *et al.* 2022).

Thrombi

Most of the studies list microthrombi (Fig. 1) among the most common findings. Bryce *et al.* (2021) found them in 17 out of 58 brains, often associated with microinfarctions. Interestingly, these microthrombi were present in both venous and arterial vessels. Other authors found microthrombi in all of their investigated cases (Deigendesch *et al.* 2020; Fabbri *et al.* 2021) and so presented also several case reports (Jaunmuktane *et al.* 2020; Kirschenbaum *et al.* 2020). In contradiction with the presented data, there are studies reporting t no evidence of small-vessel thromboses or evidence of cerebral bleeding (Matschke *et al.* 2020; Solomon *et al.* 2020).

Evaluation of thrombi is very subjective and frequently performed on small sample size. In most studies, it is done only based on histology with the routine hematoxylin and eosin (HE) stain. Schurink *et al.* analyzed microthrombi, besides other organs, also in 9 brains of COVID-19 patients. They found microthrombi consisting of fibrin, platelets and neutrophils, and neutrophilic plugs in 3 brains. In other organs, neutrophilic plugs were either composed of neutrophils with neutrophilic extracellular traps (NETs) or aggregates of NETs and platelets. In the brain, plugs consisted only of aggregated neutrophils without NETs formation (Schurink *et al.* 2020).

Cerebral venous thromboses (CVT) also manifests in COVID-19 patients with higher mortality. Even though most of the cases in the literature were clinical cases without an autopsy, there are some autopsy case reports of patients with CVT related to COVID-19 (Tomassini *et al.* 2022). However, this is often a clinically and pathologically overlooked complication that can mimic other acute neurological conditions. The microscopic picture is non-specific. The brain shows edema with congestion and perivascular hemorrhagic foci (Scala *et al.* 2022).

Infiltration by immune cells

Increased perivascular and intraparenchymal infiltration by common leukocyte antigen (CD45) positive cells is another prominent change in the brain tissue of COVID-19 patients (Mukerji & Solomon, 2021). Accumulation of cells around blood vessels is a non-specific reaction to virtually any irritation of the CNS (Fig. 1). Since there is no lymphatic tissue in the brain, the perivascular region is the place where immune cells intermingle in the CNS (Ballester & Fuller, 2022). Typical for viral encephalitis is abundant perivascular macrophage and lymphocytic infiltrate and microglial nodules composed of small clusters of microglia. Sometimes they are gathered around dying neurons together with lymphocytes in a process called neuronophagia. These dying neurons are not hypoxic, but they preserve their purple cytoplasm. In more severe encephalitis, the activated microglial infiltration is more diffuse (Kleinschmidt-DeMasters *et al.* 2018; Ballester & Fuller, 2022). Microglial activation is typical for viral encephalitis but not specific since it was also found in COVID-19-negative patients, particularly with severe inflammation states and sepsis (Deigendesch *et al.* 2020; Frank, 2020; Matschke *et al.* 2020; Schurink *et al.* 2020; Mukerji & Solomon, 2021).

Perivascular and intraparenchymal white blood cell infiltration

Infiltration of activated CD68+ microglia in the brain is a hallmark of tissue destruction and is always present around infarctions (Jaunmuktane *et al.* 2020; Reichard *et al.* 2020). Microglial activation is a common finding in the brains of SARS-CoV-2-positive patients and might be present even without prominent infiltration by other inflammatory cells (Hanley *et al.* 2020). Small microglial clusters or nodules were found in COVID-19 autopsy cases (Meinhardt *et al.* 2021; Yang *et al.* 2021; Serrano *et al.* 2022). These cells in the perivascular and parenchymal location in COVID-19 patients express CD68 while retaining the microglia core marker TMEM119 (Matschke *et al.* 2020).

Yang *et al.* found increased infiltration of microglia in the brain parenchyma forming microglial nodules, as well as perivascular infiltration of macrophages and peripheral T lymphocytes. Microglial cells associated with COVID-19 show expression overlaps with marker genes of Alzheimer's-disease-associated microglia. However, activated microglia found in COVID-19 shows also activation of other genes associated with neuroinflammation. Therefore, the microglial subpopulation enriched in patients with COVID-19 probably represents a distinct microglial state (Yang *et al.* 2021).

Scattered lymphocytic infiltrate may be present in the parenchyma (Bryce *et al.* 2021), but more often, it is found around blood vessels or both (Hanley *et al.* 2020; Kirschenbaum *et al.* 2020; Matschke *et al.* 2020; Reichard *et al.* 2020; Schurink *et al.* 2020; Solomon *et al.* 2020; Fabbri *et al.* 2021). Schurink *et al.* (2020) detected extensive inflammation in all examined regions of the brains of 9 autopsied patients, the most striking findings being present in the medulla oblongata and the olfactory bulb. There was a clustering of microglia and CD3+ T lymphocytes in the perivascular regions and in the parenchyma aggregated in small nodules. Interestingly, in most of the cases, there was a predominance of CD4+ T lymphocytes except of a few cases in which CD8+ T lymphocytes were more numerous. Also, Matschke *et al.* (2020) observed that at least part of the perivascular and parenchymal T cells were CD8-positive cytotoxic T lymphocytes. They were present mostly in the perivascular regions but also in clusters with microglia, mainly in the brain stem, and less in the frontal cortex and basal ganglia.

Meningeal infiltration

Infiltration of leptomeninges often accompanies viral encephalitis. The pathological changes might be very subtle. A high degree of clinical suspicion and extensive sampling is crucial for diagnosing (Kleinschmidt-DeMasters *et al.* 2018).

Leptomeningeal infiltration in COVID-19 can be only focal and scarce (Hanley *et al.* 2020; Jaunmuktane *et al.* 2020; Solomon *et al.* 2020). Infiltration of meninges by cytotoxic T lymphocytes together with macrophages seems to be a common finding, but it probably represents a non-specific meningeal reaction and not viral meningitis (Matschke *et al.* 2020).

Choroid plexus infiltration

Increased choroid plexus infiltration by CD68-positive cells was also reported. Interestingly, these cells show upregulation in the antiviral defense gene IFITM3 but without clear evidence of SARS-CoV-2 infection (Yang *et al.* 2021).

Astrogliosis

Gliosis is another non-specific reaction to injury of the brain and spinal cord tissues (Ballester & Fuller, 2022). Matschke *et al.* (2020) observed astrogliosis in all of their 43 cases, whereas 86% had astrogliosis in all regions but predominantly in the brain stem and the cerebellum with little involvement of the frontal lobe. On the other hand, there was a case with reactive astrogliosis associated with hemorrhages and ischemic infarcts in hemispheres with no microglial nodules in the brainstem, spinal cord, or deep grey nuclei (Reichard *et al.* 2020). Schurink *et al.* (2020) found astrogliosis associated with perivascular and intraparenchymal leukocytic infiltrates but without signs of infarctions or hemorrhages in all of their 9 cases. Another study described no astrogliosis, but in 8 out of 18 cases observed Alzheimer's type II astrocytosis or pathological features of Alzheimer's disease, with features of Lewy body disease in two of them (Solomon *et al.* 2020).

Astrocytes in SARS-CoV-2 infection might also undergo functional changes that affect the CNS. Yang *et al.* found that there is a subpopulation of astrocytes marked by inflammation and astrogliosis genes with dysregulation of genes that support neurotransmission and synaptic organization. When it comes to neurons, they observed the downregulation of synaptic genes that mediate neurotransmission and upregulation in proximal VIP inhibitory neurons (Yang *et al.* 2021).

Olfactory bulb and olfactory nerves

The olfactory bulb was often studied for the presence of pathological changes as a possible way of SARS-CoV-2 entrance into the brain. In a study focused on the olfactory epithelium in 2 cases, there were leukocytic infiltrates, predominantly CD3+ T lymphocytes, but also CD20+ B lymphocytes in the lamina propria and focal atrophy of the mucosa. Nerve fibers showed infiltration by CD68+ macrophages and CD4+ and CD8+ T lymphocytes. The olfactory tracts showed infiltration by a few isolated CD45-positive cells (Kirschenbaum *et al.* 2020). Astrogliosis and microgliosis with minor T lymphocytic infiltrate were also reported in the olfactory bulb and in chiasma (Matschke *et al.* 2020; Schurink *et al.* 2020). In another study, no abnormality in olfactory bulbs or tracts in 18 COVID-19 patients was detected. However, in these cases, no special stain or IHC was done to evaluate these changes (Solomon *et al.* 2020). In one study focusing on gene expression changes, the amount of microglia in the olfactory bulb did not differ from controls, but there were significant differences in the gene expression profiles (Serrano *et al.* 2022).

Demyelination, acute disseminated encephalomyelitis (ADEM), and acute hemorrhagic leukoencephalitis (AHL)

The morphological hallmark of ADEM is the perivascular demyelination that, unlike in multiple sclerosis, does not extend beyond vessels to a confluent patch and might be associated with hemorrhage. It is considered a postinfectious and postvaccination autoimmune condition caused mainly by influenza and varicella-zoster virus (Ballester & Fuller, 2022). AHL is probably part of a continuum of the disease. Histologically it is similar to ADEM with angiitis and perivenous destructive foci with fibrin leakage, neutrophils, and axonal injury (Kleinschmidt-DeMasters *et al.* 2018).

A case with ADEM-like and AHL-like lesions was described, but the lesions did not fully meet the criteria. Reichard *et al.* identified CD68+ macrophages at the periphery of hemorrhagic lesions and perivascular ADEM-like lesions with scattered CD3+ T-lymphocytes around blood vessels (Reichard *et al.* 2020). Similar features were observed by Bryce *et al.* (2021) and by Kantonen *et al.* (2020) in patients with acute endocarditis and in a middle-aged male with diabetes type 2, obesity, and Parkinson's disease.

SARS-CoV-2 encephalitis

Most of the cases of SARS-CoV-2 encephalitis are only clinically diagnosed. However, there are few autopsy cases of viral encephalitis documented in the literature (von Weyhern *et al.* 2020; Jensen *et al.* 2021). Serrano *et al.* (2022) observed 1 case out of 42 COVID-19 patients with acute hemorrhagic encephalitis. The patient had multiple acute hemorrhages, fibrinoid vascular necrosis in the pons, and neuropil infiltration by B and T lymphocytes and macrophages with SARS-CoV-2 positivity of the brain tissue by RT-PCR. There were no viral inclusions. Interesting is a case of fatal co-infection with tuberculosis and SARS-CoV-2 in a 5-year-old girl. SARS-CoV-2 was detected by RT-PCR from brain biopsy, but the histopathology picture was typical for tuberculosis (Freij *et al.* 2020). Diagnosis of SARS-CoV-2 encephalitis should be made with caution and with a clinical correlation, since it is rare, and a lot of features of viral encephalitis overlap with non-specific changes.

Role of SARS-CoV-2 in the brain

The attempts to detect SARS-CoV-2 in the central nervous system showed inconsistent results (Matschke *et al.* 2020; Mukerji & Solomon, 2021; Yang *et al.* 2021).

In one study, SARS-CoV-2 was detected only in the olfactory bulb in 4 out of 7 cases but not in any other brain region (Deigendesch *et al.* 2020). In other studies, the olfactory nerve and brain tissue tested positive by qRT-PCR in 1 out of 10 cases (Fabbri *et al.* 2021) and in 4 out of 20 cases with the highest viral load in the olfactory bulb (Serrano *et al.* 2022). Other authors found no virus by IHC or RT-PCR (Kantonen *et al.* 2020; Schurink *et al.* 2020). Paniz-Mondolfi *et al.* (2020) reported a case with RT-PCR that confirmed SARS-CoV-2 positive brain with electron microscopy visible viral particles in endothelial cells and neural cell bodies of the frontal lobe. However, the interpretation of findings, including IHC and EM results, varies significantly in different studies. Krasemann *et al.* attempted to establish standards for IHC and ultrastructural analyses. These attempts could improve the reproducibility and validity of COVID-19 autopsy studies (Krasemann *et al.* 2022).

Recently, a replicable virus was detected in the brain, even without histologic signs of virus infection. Also, SARS-CoV-2 was present in the brains of patients who tested positive but died for different reasons (Steiner *et al.* 2016). Matschke *et al.* (2020), in their study of the brains of 43 COVID-19 patients, found that SARS-CoV-2 was detected by qRT-PCR or IHC in 53%. The presence in the CNS was not associated with the severity of neuropathological changes (Matschke *et al.* 2020; Maiese *et al.* 2021; Mukerji & Solomon, 2021).

There is increasing evidence that SARS-CoV-2 infiltrates CNS. However, it is not clear to what extent the presence of the virus in the CNS contributes to the pathological findings, besides a few cases of confirmed SARS-CoV-2 encephalitis. Ischemic and hemorrhagic lesions, as well as reactive astrogliosis and microgliosis, do not seem to be specific to SARS-CoV-2 infection of the brain. The majority of the changes are most likely caused by a severe systemic inflammatory response and hypercoagulation state in COVID-19 patients (Maiese *et al.* 2021).

To investigate ADEM/AHL-like pathology in patients with COVID-19, Deigendesch *et al.* (2020) examined the brains of 7 SARS-CoV-2-positive patients and compared the findings with controls with non-septic and septic clinical courses. They found activation of microglia in the brainstem, medulla oblongata, and olfactory bulb in all SARS-CoV-2 positive patients, no different from septic controls but significantly less pronounced in non-septic controls. Also, sparse perivascular and leptomeningeal infiltrates of CD3+ lymphocytes present in some COVID-19 brains were similar to controls with sepsis. Even though they did not find any ADEM-like changes in their small cohort, the comparison with controls provides insight into the origin of microscopic findings in the brains of COVID-19 patients. Serrano *et al.* (2022) found microglial nodules in 2/42 COVID-19 cases which were not present in controls. Also, significant perivascular mononuclear cuffing was found only in the case with encephalitis. However, sparse to occasional moderate perivascular cuffing was found almost in all cases, controls including.

Future perspectives

To better understand changes caused specifically by the virus, we need to use samples with low delay to the autopsy, standardized extensive sampling, and adequate negative controls. Another important aspect in the interpretation of the findings is clinical data, especially comorbidities, symptoms, ventilation therapy, and drugs that might have modified the picture, like dexamethasone, anticoagulants, etc. Besides standard stains and IHC, usage of more sensitive and especially highly specific methods for detecting the virus directly in the concrete cell type in the nerve tissue are to be applied.

With the increasing number of patients suffering from neurological symptoms after recovery from COVID-19, referred to as "long COVID" the importance of finding the underlying mechanisms increases. At present, there is not enough data about the pathological changes in the brains of patients suffering from post-COVID-19 sequelae before death, and current studies are not designed to answer this question. Even though autopsy findings may shed light on the mechanisms of CNS involvement in COVID-19, the pathophysiology of "long COVID-19" especially in patients with only mild symptoms of COVID-19, remains to be elucidated. Among the possible culprits are the microthrombi, which may cause microinfarcts too small for detection by MRI, and functional changes related to COVID-19-modified expression profiles of genes in the brain tissue (Bryce *et al.* 2021; Yang *et al.* 2021; Serrano *et al.* 2022).

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REFERENCES

1 Al-Dalahmah O, Thakur KT, Nordvig AS, Prust ML, Roth W, Lignelli A, et al. (2020). Neuronophagia and microglial nodules in a SARS-CoV-2 patient with cerebellar hemorrhage. *Acta Neuropathol Commun.* **8**(1): 147.

2 Ballester LY, Fuller GN (2022). Central Nervous System. In Longacre TA, editors. Mills and Sternberg's Diagnostic Surgical Pathology. Philadelphia: Wolters Kluywer. 432–445.

3 Bryce C, Grimes Z, Pujadas E, Ahuja S, Beasley MB, Albrecht R, et al. (2021). Pathophysiology of SARS-CoV-2: the Mount Sinai COVID-19 autopsy experience. *Mod Pathol.* **34**(8): 1456–1467.

4 Deigendesch N, Sironi L, Kutza M, Wischnewski S, Fuchs V, Hench J, et al. (2020). Correlates of critical illness-related encephalopathy predominate postmortem COVID-19 neuropathology. *Acta Neuropathol.* **140**(4): 583–586.
5 Fabbri VP, Foschini MP, Lazzarotto T, Gabrielli L, Cenacchi G, Gallo C, et al. (2021). Brain ischemic injury in COVID-19-infected patients: a series of 10 post-mortem cases. *Brain Pathol.* **31**(1): 205–210.
6 Fanou EM, Coutinho JM, Shannon P, Kiehl TR, Levi MM, Wilcox ME, et al. (2017). Critical Illness-Associated Cerebral Microbleeds. *Stroke.* **48**(4): 1085–1087.
7 Finsterer J, Scorza FA, Scorza CA, Fiorini AC (2022). Ischemic stroke in 455 COVID-19 patients. *Clinics (Sao Paulo).* **77**: 100012.
8 Frank S (2020). Catch me if you can: SARS-CoV-2 detection in brains of deceased patients with COVID-19. *Lancet Neurol.* **19**(11): 883–884.
9 Freij BJ, Gebara BM, Tariq R, Wang AM, Gibson J, El-Wiher N, et al. (2020). Fatal central nervous system co-infection with SARS-CoV-2 and tuberculosis in a healthy child. *BMC Pediatr.* **20**(1): 429.
10 Hanley B, Naresh KN, Roufosse C, Nicholson AG, Weir J, Cooke GS, et al. (2020). Histopathological findings and viral tropism in UK patients with severe fatal COVID-19: a post-mortem study. *Lancet Microbe.* **1**(6): e245–e253.
11 Hingorani KS, Bhadola S, Cervantes-Arslanian AM (2022). COVID-19 and the brain. *Trends Cardiovasc Med.* **32**(6): 323–330.
12 Chou SH, Beghi E, Helbok R, Moro E, Sampson J, Altamirano V, et al. (2021). Global Incidence of Neurological Manifestations Among Patients Hospitalized With COVID-19-A Report for the GCS-NeuroCOVID Consortium and the ENERGY Consortium. *JAMA Netw Open.* **4**(5): e2112131.
13 Jaunmuktane Z, Mahadeva U, Green A, Sekhawat V, Barrett NA, Childs L, et al. (2020). Microvascular injury and hypoxic damage: emerging neuropathological signatures in COVID-19. *Acta Neuropathol.* **140**(3): 397–400.
14 Jensen MP, Le Quesne J, Officer-Jones L, Teodosio A, Thaventhiran J, Ficken C, et al. (2021). Neuropathological findings in two patients with fatal COVID-19. *Neuropathol Appl Neurobiol.* **47**(1): 17–25.
15 Kantonen J, Mahzabin S, Mayranpaa MI, Tynninen O, Paetau A, Andersson N, et al. (2020). Neuropathologic features of four autopsied COVID-19 patients. *Brain Pathol.* **30**(6): 1012–1016.
16 Khan IR, Gu Y, George BP, Malone L, Conway KS, Francois F, et al. (2021). Brain Histopathology of Adult Decedents After Extracorporeal Membrane Oxygenation. *Neurology.* **96**(9): e1278–e1289.
17 Kirschenbaum D, Imbach LL, Ulrich S, Rushing EJ, Keller E, Reimann RR, et al. (2020). Inflammatory olfactory neuropathy in two patients with COVID-19. *Lancet.* **396**(10245): 166.
18 Kleinschmidt-DeMasters BK, Beckham JD, Tyler KL (2018). Infections and Inflammatory Disorders. In Perry A, Brat DJ, editors. Practical Surgical Neuropathology: A Diagnostic Approach. Philadelphia: Elsevier. 558–568.
19 Krasemann S, Dittmayer C, von Stillfried S, Meinhardt J, Heinrich F, Hartmann K, et al. (2022). Assessing and improving the validity of COVID-19 autopsy studies - A multicentre approach to establish essential standards for immunohistochemical and ultrastructural analyses. *EBioMedicine.* **83**: 104193.
20 Luo W, Liu X, Bao K, Huang C (2022). Ischemic stroke associated with COVID-19: a systematic review and meta-analysis. *J Neurol.* **269**(4): 1731–1740.
21 Maiese A, Manetti AC, Bosetti C, Del Duca F, La Russa R, Frati P, et al. (2021). SARS-CoV-2 and the brain: A review of the current knowledge on neuropathology in COVID-19. *Brain Pathol.* **31**(6): e13013.
22 Matschke J, Lutgehetmann M, Hagel C, Sperhake JP, Schroder AS, Edler C, et al. (2020). Neuropathology of patients with COVID-19 in Germany: a post-mortem case series. *Lancet Neurol.* **19**(11): 919–929.
23 Meinhardt J, Radke J, Dittmayer C, Franz J, Thomas C, Mothes R, et al. (2021). Olfactory transmucosal SARS-CoV-2 invasion as a port of central nervous system entry in individuals with COVID-19. *Nat Neurosci.* **24**(2): 168–175.
24 Mukerji SS, Solomon IH (2021). What can we learn from brain autopsies in COVID-19? *Neurosci Lett.* **742**: 135528.
25 Osman RS, Dawood SS, Thawer SP, Mandania SM, Amirali MH, Bulimba MN, et al. (2022). SARS-CoV-2 precipitating a stroke in a child? A case report from Tanzania. *Pan Afr Med J.* **42**: 33.
26 Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednicky J, et al. (2020). Central nervous system involvement by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *J Med Virol.* **92**(7): 699–702.
27 Reichard RR, Kashani KB, Boire NA, Constantopoulos E, Guo Y, Lucchinetti CF (2020). Neuropathology of COVID-19: a spectrum of vascular and acute disseminated encephalomyelitis (ADEM)-like pathology. *Acta Neuropathol.* **140**(1): 1–6.
28 Remmelink M, De Mendonca R, D'Haene N, De Clercq S, Verocq C, Lebrun L, et al. (2020). Unspecific post-mortem findings despite multiorgan viral spread in COVID-19 patients. *Crit Care.* **24**(1): 495.
29 Santana MF, Frank CHM, Almeida TVR, Jeronimo CMP, de Araujo Pinto RA, Martins YF, et al. (2021). Hemorrhagic and thrombotic manifestations in the central nervous system in COVID-19: A large observational study in the Brazilian Amazon with a complete autopsy series. *PLoS One.* **16**(9): e0255950.
30 Scala MR, Spennato P, Cicala D, Piccolo V, Varone A, Cinalli G (2022). Malignant cerebral infarction associated with COVID-19 in a child. *Childs Nerv Syst.* **38**(2): 441–445.
31 Serrano GE, Walker JE, Tremblay C, Piras IS, Huentelman MJ, Belden CM, et al. (2022). SARS-CoV-2 Brain Regional Detection, Histopathology, Gene Expression, and Immunomodulatory Changes in Decedents with COVID-19. *J Neuropathol Exp Neurol.* **81**(9): 666–695.
32 Schurink B, Roos E, Radonic T, Barbe E, Bouman CSC, de Boer HH, et al. (2020). Viral presence and immunopathology in patients with lethal COVID-19: a prospective autopsy cohort study. *Lancet Microbe.* **1**(7): e290–e299.
33 Solomon IH, Normandin E, Bhattacharyya S, Mukerji SS, Keller K, Ali AS, et al. (2020). Neuropathological Features of Covid-19. *N Engl J Med.* **383**(10): 989–992.
34 Steiner N, Balez R, Karunaweera N, Lind JM, Munch G, Ooi L (2016). Neuroprotection of Neuro2a cells and the cytokine suppressive and anti-inflammatory mode of action of resveratrol in activated RAW264.7 macrophages and C8-B4 microglia. *Neurochem Int.* **95**: 46–54.
35 Tomassini L, Paolini D, Petrasso PEY, Manta AM, Piersanti V, Straccamore M, et al. (2022). What about cerebral venous sinus thrombosis? A series of three autopsy cases. *Leg Med (Tokyo).* **56**: 102052.
36 Vattoth S, Abdelhady M, Alsoub H, Own A, Elsotouhy A (2020). Critical illness-associated cerebral microbleeds in COVID-19. *Neuroradiol J.* **33**(5): 374–376.
37 von Weyhern CH, Kaufmann I, Neff F, Kremer M (2020). Early evidence of pronounced brain involvement in fatal COVID-19 outcomes. *Lancet.* **395**(10241): e109.
38 Yang Y, Hu X, Xiong L, Fu P, Feng W, Li W, et al. (2021). Clinical characteristics of hospitalized mild/moderate COVID-19 patients with a prolonged negative conversion time of SARS-CoV-2 nucleic acid detection. *BMC Infect Dis.* **21**(1): 141.
39 Zuo Y, Estes SK, Ali RA, Gandhi AA, Yalavarthi S, Shi H, et al. (2020). Prothrombotic autoantibodies in serum from patients hospitalized with COVID-19. *Sci Transl Med.* **12**(570).