The pathology of the brain and the eye in SARS-CoV-2 infected patients: A review

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Abstract:

Background: Patients with SARS-CoV-2 may present or eventually develop central nervous system and ophthalmic signs and symptoms. Varying reports have emerged regarding isolation of viral RNA from these tissue sites, as well as largely autopsy-based histopathologic descriptions of the brain and the eye in patients with CoVID-19.

Evidence acquisition: A primary literature search was performed in literature databases such as PubMed, Google Scholar, and Cochrane Library. Keywords were used alone and in combination including the following: SARS CoV-2, CoVID-19, eye, brain, central nervous system, histopathology, autopsy, ocular pathology, aqueous, tears, vitreous, neuropathology, encephalitis.

Results: The reported ophthalmic pathologic and neuropathologic findings in patients with SARS-CoV-2 are varied and inconclusive regarding the role of direct viral infection versus secondary pathology. The authors own experience with autopsy neuropathology in CoVID-19 patients is also described. There is a particular paucity of data regarding the histopathology of the eye. However, it is likely that the ocular surface is a potential site for inoculation and the tears a source of spread of viral particles.

Conclusions: Additional large post-mortem studies are needed to clarify the role of SARS-CoV in the ophthalmic and neuropathologic manifestations of CoVID-19.
Background

It has been over a year since the novel, highly transmissible severe acute respiratory syndrome coronavirus (SARS-CoV-2) was discovered in Wuhan, China, leading to the disease state termed Coronavirus Disease 2019 (CoVID-19).\textsuperscript{1} Previous severe coronavirus diseases were caused by the severe acute respiratory syndrome coronavirus (SARS-CoV) of 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV), described in 2012.\textsuperscript{1,2} Patients infected with SARS-CoV-2 range from asymptomatic to critically ill with symptoms spanning mild pneumonia to respiratory failure, shock, and multi organ system dysfunction.\textsuperscript{2}

Since its discovery, frequent and varied neurologic manifestations have been associated with SARS-CoV-2 infection, with increasing reports of neurologic and psychiatric symptoms.\textsuperscript{3,4} In addition to the common, early presenting signs of loss of taste and smell, patients with SARS-CoV-2 have developed headaches, nausea, vomiting, changes in consciousness, encephalopathy, and acute cerebrovascular disease.\textsuperscript{3,5-8} It has yet to be determined whether these neurologic manifestations are due to direct invasion of SARS-CoV-2 into the central nervous system (CNS) or an indirect mechanism, and there are several proposed hypotheses. SARS-CoV-2 infection may also present with ophthalmic manifestations in rare cases,\textsuperscript{9} with ocular symptomatology occurring in 5\%\textsuperscript{10} to 32\%\textsuperscript{9} of patients during the course of illness.

In this review, we examine the current literature regarding the mechanism of SARS-CoV-2 infection of and entry into the eye and brain, as well its associated neuro- and ophthalmic pathologic findings. Included are relevant clinical microbiologic, surgical and autopsy pathology studies.
**SARS CoV-2 and the brain Central nervous system infection**

A transcerebral route of SARS-CoV-2 viral entry may occur via the olfactory mucosa and bulb. Increased magnetic resonance imaging (MRI) signal in the olfactory cortex associated with involvement by infection has been documented. A proposed mechanism is that, similar to other coronaviruses, the virus could be internalized in nerve terminals, transported retrogradely and trans-synaptically spread to the CNS. SARS-CoV-2’s main docking receptor is angiotensin 1-converting enzyme 2 (ACE2) and requires transmembrane protease serine 2 (TMPRSS2) for proteolytic processing of the spike protein in order to enter cells. While expression of TMPRSS2 and ACE2 has been detected in the nasal mucosa, these proteins were only present in the sustentacular cells, and not in neurons. Another alternative is that the infection causes inflammation within the olfactory mucosa leading to anosmia. It is also possible that the virus is transported to the CNS by the cerebrospinal fluid (CSF) without breaking the blood brain barrier (BBB). However, there are conflicting reported CSF results. Some studies have identified SARS-CoV-2 RNA in the CSF, but only in modest amounts, while others did not detect viral genomic material in CSF samples using polymerase chain reaction (PCR) based tests.

Alternatively, SARS-CoV-2 could breach the BBB to infect the CNS. In addition to being an enzyme, ACE2 is also a functional surface receptor and can be used by SARS-CoV-2 to enter host cells. ACE2 is highly expressed in many cells including endothelium, cardiac, renal, pulmonary and blood cells. It is also involved in the renin-angiotensin-aldosterone system (RAAS). SARS-CoV-2 could therefore cause disease by activating RAAS, especially in patients with other comorbidities such as diabetes mellitus, hypertension and vascular disease. Comorbidities are present in many patients infected with CoVID-19 and the comorbidities alone
could potentially increase the permeability of the BBB.\textsuperscript{11,24} Paniz-Mondolfi \textit{et al.} report a patient with Parkinson’s disease and CoVID-19 in which viral particles were found by electron microscopy in the frontal lobe microvessels and neurons.\textsuperscript{11,25} A study by Kantonen \textit{et al.} of a CoVID-19-positive patient with Parkinson’s disease, obesity, diabetes and hypertension showed hypoxic/ischemic neuronal damage, white matter lesions, microhemorrhages and enlarged perivascular spaces, but no evidence of SARS-CoV-2 in the brain at autopsy.\textsuperscript{11,26} Conversely, Zubair \textit{et al.} demonstrated neurons with entrapped viral particles by electron microscopy.\textsuperscript{27} One proposed mechanism of viral involvement of the CNS is through cellular transport across the endothelium via interacting with ACE2 on the vessels and subsequently neurons and glia.\textsuperscript{16,27} Another mechanism, known as the “Trojan horse mechanism,” is that by infecting leukocytes, viral particles could then cross the BBB.\textsuperscript{16,28} This could be exacerbated by CoVID-19-related systemic inflammation that may increase the permeability of the BBB.\textsuperscript{16,29} A potential last mechanism could be entrance via the median eminence capillaries and tanycytes of the hypothalamus which also express ACE2 and TMPRSS2.\textsuperscript{11,30}

Lastly, another hypothesis of CNS involvement postulates indirect systemic factors as the cause of brain injury. There are reports of autoimmune encephalitis in patients with CoVID-19 where patients have excessive antigen-driven immune responses.\textsuperscript{3,31} Patients with SARS have been shown to produce autoantibodies against the coronavirus spike protein that react with endothelial and epithelial cells, which results in cytotoxicity.\textsuperscript{3,32} These antibodies can then induce immune reactions against endothelial cell antigens in cerebral vessels or neurons, resulting in autoimmune encephalitis and edema.\textsuperscript{3}

Some patients with CoVID-19 develop encephalopathy and encephalitis. While altered mental status is rare overall in hospitalized CoVID-19 patients, the majority of critically ill
patients experience altered mental status.\textsuperscript{6,11,33} It is unknown if these alterations in mental status are due to encephalopathy related to the systemic illness or caused directly by SARS-CoV-2.\textsuperscript{11} A few cases of CoVID-19 patients with encephalitis have been reported.\textsuperscript{11,18-19,34-36} As mentioned above, two of these reports described identification of SARS-CoV-2 in the CSF, albeit in modest amounts.\textsuperscript{11,18-19} Efe et al. discussed a case where the diagnosis of temporal lobe encephalitis was confirmed on biopsy material, showing hypoxic neuronal damage and perivascular lymphocytic infiltrates, however the assessment of SARS-CoV-2 in tissue was not reported.\textsuperscript{11,34}

Cerebrovascular disease, including ischemic strokes, is found in a subset of patients with CoVID-19 (1-3\% of hospitalized patients and up to 6\% of critically ill patients with CoVID-19).\textsuperscript{6,11,37-38} An early report by Oxley et al. documents embolic strokes in young, otherwise healthy patients with CoVID-19.\textsuperscript{11,39} However, later reports demonstrate that patients with embolic strokes are usually older and have numerous other vascular comorbidities.\textsuperscript{11,40} While it is possible that SARS-CoV-2 may contribute to a vascular event, similar to other infections that alter systemic inflammatory conditions and coagulability, it is uncertain whether the strokes are caused directly by the infection or if they are related to the patient’s comorbidities while they happen to be CoVID-19-positive at the time.\textsuperscript{11,41}

Lastly, post-infectious syndromes associated with CoVID-19 have been increasingly documented in recent months. As discussed above, infection with SARS-CoV-2 can cause a dysregulated immune response. This immune system alteration may cause delayed and extended effects on the peripheral and central nervous systems. There are reports of immune-mediated responses that resemble various post-infectious inflammatory conditions. For example, Parsons et al. report a neuroradiologic case of acute disseminated encephalomyelitis (ADEM) and Poyiadji et al. report a neuroradiologic case of acute hemorrhagic necrotizing
Toscano et al. report several cases of Guillain-Barre syndrome in patients with recent CoVID-19, but with no detectable SARS-CoV-2 in CSF.\textsuperscript{11,21} The persistence of or development of new symptoms late in the course of a CoVID-19 infection has been described as “long-CoVID” or “CoVID long haulers.”\textsuperscript{16,44} These patients have symptoms >28 days after their CoVID-19 diagnosis and their symptoms vary in severity and frequency, but many describe fatigue, “brain fog,” breathlessness, dysrhythmias and pain which last for months after they are infectious.\textsuperscript{16,44-45} This syndrome is reminiscent of the post-viral illness named myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) which likely is due to long-term alterations caused by the immune response to an initial viral infection.\textsuperscript{16,46}

Neuropathologic autopsy findings in COVID-19 patients

Review of the literature for postmortem findings in patients with CoVID-19 is mostly focused on general autopsy findings, with descriptions of brain findings occurring only later on in the pandemic. This was largely due to limited access to cases and concerns regarding the safety of the autopsy staff.\textsuperscript{16} As more reports have appeared in the literature, the neuropathologic findings have varied from hypoxic/ischemic changes, edema, perivascular chronic inflammation, microthrombi, ischemic necrosis and acute hemorrhagic infarctions.\textsuperscript{3}

Many reports have demonstrated hypoxic/ischemic brain injury with neuronal damage mostly in the brain regions that are vulnerable to hypoxia (neocortex, hippocampus and cerebellum),\textsuperscript{3,26,34,47-54} which is likely associated with respiratory insufficiency in the context of diffuse alveolar damage and multiorgan failure, resulting in reduced blood flow and oxygen to the brain.\textsuperscript{3,49} In addition to ischemic changes, Solomon et al. also demonstrated reactive Alzheimer type II astrocytes in their case series of 18 patients.\textsuperscript{49}
Microvascular and macrovascular injury has also been reported in many cases. Both large and small infarcts including multiple small subcortical infarcts, areas of ischemic necrosis and microscopic hemorrhages have been reported, particularly in patients with pre-existing vascular disease.\(^3\)\(^{48,50-51,53-57}\) Al-Sarraj et al. reported a case of hemorrhagic infarction with microthrombi with coexisting mucormycosis, thought to be a result of the patient’s decreased immune response.\(^3\) Al-Dalahmah et al. described a case of cerebellar hemorrhage and acute infarcts in a 73-year-old man with CoVID-19.\(^56\) Matschke et al. examined 43 patients with CoVID-19 and found territorial ischemic lesions in six patients (43%) and 37 patients with astrogliosis in all evaluated regions (86%).\(^54\)

Reports of an inflammatory cell component have varied. Netland et al. demonstrated a mild inflammatory process in experimental mice infected with SARS-CoV, suggesting neurotoxicity with minimal inflammatory cell infiltration.\(^3,58\) Other studies have also shown a minimal inflammatory T-cell response with microglial activation and occasional microglial nodules, but no overt encephalitis or vasculitis.\(^49,53,55,59-60\) All eight cases evaluated in the series reported by Al-Sarraj et al. showed few perivascular T-cells and activation of microglial cells.\(^3\) Lee et al. also describe microvascular injury associated with minimal perivascular inflammation, but with perivascular and perineuronal microglia and macrophages, suggestive of neuronophagia within the substantia nigra, dorsal motor nucleus of the vagus, medullary pre-Bötzinger complex and olfactory bulb, as well as hypertrophic astrocytes.\(^60\) Al-Dalahmah et al. also described microglial nodules and neuronophagia involving the inferior olives and cerebellar dentate nuclei.\(^56\) Activated microglia and infiltrating T-cells were present mostly in the brainstem and cerebellum with leptomeningeal T-cells in 34 of the 43 patients in Matschke et al.’s study.\(^54\) Nauen et al. additionally report luminal megakaryocytes in cortical capillaries present in 5
cases.\textsuperscript{52} A case reported by Stoyanov \textit{et al.} described an acute necrotizing encephalitis with a necrotizing olfactory bulbitis.\textsuperscript{61} Pan-encephalitis, meningitis and neuronal cell damage involving the brainstem was reported by von Weyhern \textit{et al.}, however these results have been disputed.\textsuperscript{62-64}

Reports of CNS involvement by SARS-CoV-2 genetic material have been heterogenous. A subset of studies document the presence of SARS-CoV-2 genetic material in brain tissue detected by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), immunostaining or electron microscopy (as described above)\textsuperscript{3,25,27,47,53} For example, Matschke \textit{et al.} reportedly detected SARS-CoV-2 by qRT-PCR or immunostaining in the brains of 21 of their 43 patients (53%).\textsuperscript{54} However, many other studies did not detect SARS-CoV-2 genetic material in their examined central and peripheral nervous system samples.\textsuperscript{3,11,16,26,49,59}

As mentioned above, Reichard \textit{et al.} describe one patient with hemorrhagic white matter lesions present throughout the cerebrum with associated axonal injury and macrophage infiltration, in a perivascular ADEM-like appearance. There were also microscopic areas of white matter necrosis and axonal injury and rare organizing microscopic infarcts within the cortex.\textsuperscript{48} This is an example of a post-infectious syndrome that was demonstrated at autopsy.

The authors of this review have evaluated brains from 20 patients with CoVID-19 at autopsy. Ages at death ranged from 21-91 years, with 16 males and 4 females. The cohort included 11 white, 6 Hispanic or Black and 3 patients of unknown ethnicity. Seven cases had associated neuropsychiatric diagnosis (4/20: dementia; 2/20: schizophrenia; 1/20: bipolar disorder), and seven patients suffered from acute encephalopathy in the context of their disease.

Neuropathologic examination revealed cerebrovascular disease in most cases, arteriolar sclerosis specifically was the most frequent finding: mild in 4/20, moderate in 9/20, moderate to
Severe in 3/20. Acute hypoxic ischemic injury was seen in 13/20 patients. Nine cases had subacute/remote infarctions and/or hemorrhages, three associated with cerebral amyloid angiopathy. Two cases showed acute microscopic ischemic lesions, and petechial hemorrhages were present in two cases (one case overlapped with acute ischemic lesions) (Figure 1 A & B). Periventricular inactive demyelinating lesions of uncertain origin were present in two cases. In addition to routine Luxol hematoxylin & eosin (LH&E) and hematoxylin & eosin (H&E) sections, twelve cases were examined in detail with immunohistochemistry for CD3, CD68, CD163 and in-situ hybridization (ISH) for CoVID-19/SARS-CoV-2 and compared with five age-matched control patients who died from pneumonia. Sections of the cerebellum, frontal and temporal lobes were compared as they were the most involved by inflammatory infiltrates. Mild-to-moderate perivascular and leptomeningeal CD68/CD163-positive macrophages and activated microglia with a white matter predominance were present in all cases (Figure 1C). All cases also showed sparse to moderate perivascular and leptomeningeal CD3-positive T cells (Figure 2D). Interestingly, the age-matched controls showed a similar degree and distribution of inflammatory activation. All cases were negative for SARS-CoV-2 genomic material by ISH. Our findings are similar to those described in other reports and favor an indirect systemic inflammatory reaction and/or cytokine response with potential associated microvascular injury as the underlying mechanism for cerebral involvement in CoVID-19 infection.
SARS CoV-2 and the eye

Ocular surface: conjunctiva, cornea and tears

Early in the CoVID-19 pandemic, ophthalmologists in the Hubei province of China noted external ocular manifestations of some patients being treated for SARS-CoV-2 infection, including conjunctivitis with conjunctiva hyperemia, edema (chemosis) and increased tearing, in as many as 30% of patients. This led to hypotheses about the conjunctiva as a site of direct inoculation for infection as well as the potential for tears as a reservoir for the virus and source of viral spread. The literature that emerged providing evidence of SARS-CoV-2 in conjunctival secretions is mixed. During the initial phases of the outbreak, researchers began testing conjunctival samples from confirmed CoVID 19-infected patients, likely spurred by the early detection of SARS-CoV in the tears of some patients in the early 2000’s. SARS-CoV-2 RNA has now been identified in conjunctival swab samples and rarely in the tears of patients with varying systemic disease severity and in those with or without ocular signs and symptoms. This is an important finding that suggests asymptomatic patients may shed the virus in the absence of visible ocular abnormalities. In some studies, severely to critically ill patients were more likely to have conjunctivitis. However, there was a low, and similar, prevalence of positive RT-PCR results from conjunctival swabs in patients with ocular symptoms and a known positive nasopharyngeal swab and those without symptoms (around 16% in both instances). Overall, given the difference in detection rates from nasopharyngeal and conjunctival swabs, viral load may be low in conjunctival secretions.

ACE2, the key cell surface receptor that binds the SARS-CoV 2 spike protein for inoculation of the virus into human cells and TMPRSS2, a cell surface protease that facilitates viral entry (discussed above in more detail), have been shown to be expressed on the ocular
surface in conjunctival surgical specimens and enucleated autopsy globes. Through both Western blot analysis of corneal epithelium and immunohistochemical localization in the anterior segment, Zhou et al. noted that ACE2 is localized on the ocular surface including within the epithelium of the conjunctiva and cornea, particularly within the limbal epithelial cells and the superficial squamous cells of the conjunctiva. TMPRSS2 also showed immunohistochemical localization throughout all the epithelial layers of the cornea and conjunctiva.

Given the frequent use of corneal tissue from deceased donors for transplantation, small autopsy studies have also interrogated corneal discs or buttons from deceased patients with known SARS-CoV-2 infection for the presence of viral RNA. Genomic RNA was found in over half of cases, and in these cases RNA was also found in conjunctival swabs and occasionally in aqueous or vitreous humor samples. In this study, half of all patients had detectable RNA in their conjunctival swab specimens (with or without detection in the cornea), a much a higher rate than found in specimens take from live patients. Corneal histology in this study was unremarkable, and immunohistochemistry did not detect staining for the SARS-CoV-2 spike protein.

**Posterior pole**

**Retina/vitreous**

Although the primary ocular manifestation of CoVID-19 appears to be conjunctivitis, the ophthalmic literature also reports conflicting data on the presence of viral RNA in ocular fluids as well as clinical and pathologic data regarding the posterior pole findings. Three of 14 deceased CoVID-19 patients, in a study by Casagrande et al., showed PCR evidence of SARS-CoV-2 RNA isolated from retinal biopsies obtained at autopsy. However, direct evidence of
tissue localization (through immunohistochemistry or in situ hybridization) was not
demonstrated or studied and retinal histopathology was not described.\textsuperscript{77}

Clinically, retinal, and particularly retinovascular changes have been noted in SARS
CoV-2 positive patients\textsuperscript{78,79} In a study examining retinal findings in hospitalized patients with
severe CoVID-19, Pereira \textit{et al.} found that 10 out of 18 patients had retinal abnormalities on
dilated eye exam including flame shaped hemorrhages in 22.2\%, cotton wool spots in 16.7\%, and
retinal sectoral pallor in one patient suggestive of retinal ischemia.\textsuperscript{79} In a similar study of 12
patients with CoVID-19, Marinho \textit{et al.} noted that all patients had hyper-reflective lesions at the
level of ganglion cell and inner plexiform layers more prominent in the papillomacular bundle
bilaterally on optical coherence tomography (OCT). Subtle cotton wool spots and micro-
hemorrhages were also noted in this cohort.\textsuperscript{78} In 2 concordant studies, researchers identified
cotton wool spots,\textsuperscript{80-81} retinal hemorrhages, and venous dilation as significant findings in the
eyes of CoVID-19 patients.\textsuperscript{80} Given that these findings are diagnostically non-specific and often
found in elderly patients with common chronic systemic conditions such as hypertension and
diabetes mellitus, it is unclear if these retinal findings are indicative of a thrombotic complication
due to the systemic effects of CoVID-19.\textsuperscript{82,83} Additionally, other cohorts of severely ill patients
with CoVID-19 showed no fundus abnormalities.\textsuperscript{76}

Finally, a report describing the MRI findings within the globes of 127 CoVID-19 patients
revealed 9 patients with one or more macular nodules which appeared hyperintense on FLAIR
sequence.\textsuperscript{10} The nature of these nodules is uncertain, but given that in other intracranial sites this
type of signal may represent intracerebral artery stenoocclusion, these lesions may be related to
focal ischemia within the retina.\textsuperscript{84-85}
Choroid

The histopathology of eyes from patients with CoVID-19 has best been demonstrated in an autopsy study from Switzerland comparing 10 enucleated post-mortem eyes from 5 infected patients with control eyes. The anterior segments and retina were histologically normal for age, with occasional mild conjunctival inflammation. However, the choriocapillaris showed congestion and endothelial swelling with focal apoptosis, not seen in control eyes.

Immunohistochemical analysis of these autopsy globes revealed fibrin microthrombi in the choriocapillaris and larger choroidal vessels of 8 eyes from 4 patients. This finding alone is non-specific and may be caused by sepsis or serious infection; however, no microthrombi were detected in the eyes of control patients who died from conditions such as biliary sepsis and bronchopneumonia. Apoptotic changes and cleaved caspase 3 expression (an apoptosis executor) were also found in endothelial and inflammatory cells infiltrating the choriocapillaris of eyes from SARS-CoV-2 infected patients. Additionally, ACE-2 receptor expression was demonstrated in endothelial cells of the choroid, in addition to the previously described expression in the conjunctival and corneal epithelium. Histologic evidence of the more severe ocular complications that have been reported in animal models of SARS-CoV including retinitis and optic neuritis were not seen.
Conclusion

The documented neuropathologic findings in patients with SARS-CoV-2 are heterogeneous. Some reports support a direct cytopathic effect of SARS-CoV-2 viral material within the central nervous system, while others point towards a systemic cytokine reaction and/or dysregulated immune response caused by the systemic viral infection. Others highlight potential endothelial and vascular injury as the main process. More post-mortem neuropathologic studies with large patient cohorts are needed to verify the neuropathologic changes and presence or absence of SARS-CoV-2 genetic material within the central and peripheral nervous system.

The limited literature regarding the ophthalmic manifestations of SARS CoV-2 suggests the virus may be shed in low levels through tears or conjunctival secretions with the cornea and conjunctival epithelia as potential portals for viral entry. The intraocular pathology including choroidal microthrombi as well as the clinical retinal findings, like in the brain, may be secondary to vascular endothelial changes, as direct evidence of viral RNA within the neural retina and uvea has not been convincingly demonstrated.
References


and occasionally detectable intraocular SARS-CoV-2 RNA in five fatal COVID-19 cases.


Figure legend

Figure 1. Pathology of ischemic lesions in a patient who died from severe COVID-19.

A) Microscopic analysis of cerebellum at 200x magnification (LH&E). Representative focus of microscopic ischemic lesion with significant loss, but not absence, of myelin. B) Microscopic analysis of splenium white matter at 100x magnification (LH&E) showing a microscopic hemorrhagic (arrow) and ischemic lesion (asterisk). C) Microscopic analysis of cerebellum at 200x magnification. CD68 immunohistochemistry stain demonstrating aggregates of macrophages associated with the ischemic lesion. D) Microscopic analysis of cerebellum at 200x magnification. CD3 immunohistochemistry stain showing only rare CD3-positive T cells, consistent with no significant inflammation associated with the lesion.