Brain ischemic injury in COVID-19-infected patients: a series of 10 post-mortem cases

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To the Editor:
The coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 symptoms are not limited to the respiratory tract, but complications have been described involving other organs including brain.

At present, data on SARS-CoV-2 neuropathological features are limited (4, 5, 8, 10) and most frequently focused on cases presenting neurological symptoms.

We describe the CNS neuropathological findings detected in 10 individuals who died of SARS-CoV-2-related respiratory failure (lung histopathologic features of eight cases were reported by Damiani et al (2) in absence of specific neurological symptoms. SARS-CoV-2 RNA was searched by real-time PCR analysis in formalin-fixed, paraffin-embedded (FFPE) specimens. Detailed materials and methods, clinical data (Table 1) and neuropathological results (Table 2) are reported in the Supplementary files.

SARS-CoV-2 RNA was present in the olfactory nerve and brain tissue of one (of 10 tested) patients (case 1). In this patient olfactory bulb neurons, olfactory tract and brain tissue did not show any specific histological change suggestive of direct viral damage (Figure 1A). The SARS-CoV-2-RNA positive case presented several comorbidities, had the shortest disease course (death occurred 6 days only after the symptoms onset) and showed viral involvement of kidney, liver and heart in addition to brain and lungs, thus suggesting hematogenous spread.

On macroscopic examination, all cases presented an edematous brain surface with widened gyri, flattened surface, narrowed sulci and meningeal congestion. Brain weight ranged from 1300 to 1870 g. (mean 1560 g.). In two cases, bilateral uncal herniation was identified (cases 5, 6). Areas of cerebral infarction were present in three cases (cases 1, 2, 3). Meninges were grossly congested: purulent accumulation on the leptomeningeal vault was observed in case 8, whereas focal subarachnoid haemorrhage was identified in case 9.

On histology, all cases presented intraparenchymal intravascular microthrombi (Figure 1B) with focal microscopic (usually 1–2 mm in size) cortical or deep-seated (located in the basal ganglia and through the brainstem) recent infarcts (Figure 1C). Small blood vessels ectasia, perivascular edema, perivascular micro-hemorrhages and scattered hemosiderin-laden macrophages were also noticed (Figure 1D,E). Necrotic blood vessels or perivascular inflammation were not identified. Immunohistochemical analysis (CD20, CD3, CD4, CD8 and CD68) did not highlight lymphocytic or macrophage accumulation. Only case 4 showed a mild perivascular T-lymphocytic infiltration CD3+ (Figure 1F) more evident in the leptomeninges. Luxol fast blue and immunohistochemical staining for neurofilaments demonstrated only a slight perivascular myelin reduction with no clear evidence of axonal injury. Activation of microglia and astrocytes was noticed mainly in the brainstem (Figure 1G). No microglial nodules or evidence of neuronophagia were present.

Intravascular microthrombi and multiple infarcts are in keeping with the hypercoagulable state of SARS-CoV-2-infected patients (1) leading to large and small vessels thrombosis. Our data, together with previously published data, indicate that most likely the same pathogenetic events may occur in CNS SARS-CoV-2-related injuries.

Ischemic red neurons were present through the hippocampal CA1 region, the parahippocampal region (case 10) and the cerebellar Purkinje cells, consistent with global ischemic injury. Also the brainstem showed, in addition to microthrombi and ischemic damage, reactive gliosis and microglial activation most likely due to preterminal hypoxic–ischemic injury. These data, consistent with those of Jensen et al (4), Kantonen et al (5) and Solomon et al (8), suggest that the hypoxic-ischemic general condition, related to the respiratory failure, may indeed be worsened by the consequent brainstem damage appearing as a final event (6).

Bacterial superinfection was histologically suspected in two cases (cases 8, 9): leptomeningeal thrombi composed of dense fibrin with neutrophils were detected (Figure 1H, I).
| Case | Age  | Gender | Symptoms duration before death (days) | PM interval (hours) | Associated pathologies | Symptoms | Other |
|------|------|--------|--------------------------------------|-------------------|-----------------------|----------|-------|
| 1    | 51   | M      | 6                                    | 31                | • Ictus cerebri (2006) • Hypertension • Glaucoma • Kidney failure • Drug abuser | • Dyspnea • Fever | • Dialysis • C. glabrata |
| 2    | 64   | M      | 16                                   | 72                | • Obesity • Hypertension • Dyspnea • Fever | • Dyspnea | • Dialysis |
| 3    | 70   | F      | 13                                   | 38                | • Ictus cerebri (2015) • Obesity • Smoker | • Dyspnea | • Fever |
| 4    | 62   | M      | 14                                   | 36                | • Obesity • Hypertension • Obesity | • Dyspnea | • Fever |
| 5    | 44   | M      | 26                                   | 29                | • Diabetes type I • Obesity • Hypertension | • Dyspnea | • ECMO • E. coli |
| 6    | 64   | F      | 25                                   | 50                | • Obesity • Crohn disease • Obesity • Hypertension | • Dyspnea | • C. glabrata |
| 7    | 52   | M      | 16                                   | 55                | • Hypertension | • Dyspnea | • P. aeruginosa • S. aureus • C. albicans • S. capitis |
| 8    | 66   | M      | 35                                   | 25                | • Hypertension • Dyslipidemia | • Fever | • During hospitalization, pneumonia SARS-CoV2 related • MRSA • PNX • S. aureus |
| 9    | 74   | M      | 26                                   | 24                | • Ischemic cardiomyopathy (previous acute myocardial infarction) • OB • Atrial fibrillation • Diabetes type II • Hypothyroidism | • Urinary retention (pelvic mass) • Fever • Syncope • Previous pneumonia (February) | |
| 10   | 62   | F      | 17                                   | 37                | • Hypothyroidism | • Fever • Muscular weakness | |

Table 1. clinical data. Abbreviations: M = male; F = female; ECMO = Extra-Corporeal Membrane Oxygenation; BAL = Bronchoalveolar Lavage; PNX = pneumothorax; OB = obstructive bronchitis; MRSA = Methicillin-resistant *Staphylococcus aureus*. 
Table 2. Summary of neuropathological findings. Abbreviations: OT = olfactory tract and bulb, B = brain, L = lungs.

| Case 1 | 1480 | Oedema | Left frontal lobe infarction | Meningeal congestion | Atherosclerosis | Global hypoxic-ischemic injury | Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages | Endovascular microthrombi | Microglial activation, especially in medulla oblongata | Glial scar consistent with previous frontal lobe infarct | Recent microscopic cortical infarcts | Right pyramidal tract atrophy | Recent microscopic cortical infarcts | Left pyramidal tract atrophy | Moderate meningeal chronic lymphocytic infiltration (composed of T-lymphocytes, CD3 and CD4 positive) | Yes | Yes | Yes |
| Case 2 | 1670 | Oedema | Right parietal lobe infarction | Meningeal congestion | Global hypoxic-ischemic injury | Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages | Endovascular microthrombi | Microglial activation, especially in medulla oblongata | Recent cerebral parietal infarction and microscopic cortical infarcts | No | No | Yes |
| Case 3 | 1320 | Oedema | Right frontal lobe infarction | Meningeal congestion | Global hypoxic-ischemic injury | Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages | Endovascular microthrombi | Sparse microglial activation, especially in medulla oblongata | Ancient frontal lobe infarction (glial scar) and recent microscopic infarcts | No | No | Yes |
| Case 4 | 1870 | Oedema | Meningeal congestion | Global hypoxic-ischemic injury | Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages | Endovascular microthrombi | Recent microscopic infarcts | Moderate meningeal chronic lymphocytic infiltration (composed of T-lymphocytes, CD3 and CD4 positive) | No | No | Yes |
| Case 5 | 1870 | Oedema | Uncal herniation | Meningeal congestion | Global hypoxic-ischemic injury | Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages | Endovascular microthrombi | Recent microscopic infarcts | No | No | Yes |
| Case 6 | 1350 | Oedema | Uncal herniation | Meningeal congestion | Global hypoxic-ischemic injury | Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages | Endovascular microthrombi | Recent microscopic infarcts | No | No | Yes |
| Case   | Brain weight (g) | Macroscopic findings | Histological findings                                                                                      | OT CoV-2 | B CoV-2 | L CoV-2 |
|--------|------------------|----------------------|-----------------------------------------------------------------------------------------------------------|----------|---------|---------|
| 7      | 1650             | Oedema               | • Global hypoxic-ischemic injury<br>• Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages<br>• Endovascular microthrombi<br>• Recent microscopic infarcts<br>• Sparse microglial activation, especially in medulla oblongata | No       | No      | Yes     |
| 8      | 1300             | Oedema<br>Meningeal congestion<br>Meningeal purulent accumulation | • Global hypoxic-ischemic injury<br>• Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages<br>• Endovascular microthrombi<br>• Recent microscopic infarcts<br>• Microglial activation, especially in medulla oblongata<br>• Initial feature of Acute purulent meningitis | No       | No      | Yes     |
| 9      | 1490             | Oedema<br>Meningeal congestion with focal blood extravasation<br>Atherosclerosis | • Global hypoxic-ischemic injury<br>• Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages<br>• Endovascular microthrombi<br>• Focal leptomeningeal haemorrhage<br>• Recent parieto-occipital lobe infarction and microscopic infarcts<br>• Spinal cord Schwannoma | No (brain and spinal cord) | No      | Yes     |
| 10     | 1350             | Oedema<br>Meningeal congestion | • Global hypoxic-ischemic injury<br>• Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages<br>• Endovascular microthrombi<br>• Recent microscopic infarcts, the largest in the para-hippocampal region | No (brain and spinal cord) | No      | Yes     |
In these patients, *Pseudomonas aeruginosa, Candida albicans, Staphylococcus capitis, Staphylococcus aureus* and *Methicillin-resistant Staphylococcus aureus* (MRSA) were, respectively, isolated in bronchoalveolar lavage fluid and from blood cultures.

Infective meningoencephalitis has been well-documented as a complication during SARS-CoV-2 infection (7). In the remaining cases, leptomeningeal vascular congestions was seen. The leptomeningeal vascular alterations detected in the present cases, are consistent with the findings described by Helms et al who detected, on Magnetic Resonance Imaging, leptomeningeal spaces enhancement in 8/13 patients and bilateral frontal hypoperfusion in 11 patients (3). Furthermore, in Helms et al series, three asymptomatic patients presented small acute or subacute ischemic strokes (3).

![Figure 1. A: SARS-CoV-2 positive olfactory tract did not show any specific pathological features suggestive of viral damage (case 1). B: Microthrombi were seen in small intraperenchymal vessels located in the brain stem of SARS-CoV-2 positive case (case 1). C: Cortical microthrombi were seen in small intraperenchymal vessels located in the brain stem of SARS-CoV-2 positive and negative cases (case 9). F: Very rare perivascular lymphocytes were present (case 4, medulla oblongata). Almost all lymphocytes were CD3+ (inset). G: Medulla oblongata showed diffuse GFAP positivity both in SARS-CoV-2 positive and negative cases (case 2). H: Gross examination of case 8, showing purulent accumulation on the leptomeningeal vault. I: in case 8, leptomeningeal vessels were enlarged and filled with septic thrombi, mainly composed of granulocytes](Colour figure can be viewed at wileyonlinelibrary.com)
The present study has some limitations, including the small sample size and the absence of pre-mortem specific neurologic symptoms. In addition, autopsies were not consecutive, but performed on cases that experienced an unexpectedly fatal course. Therefore, data shown here may not reflect the pathologic involvement of all SARS-CoV-2-infected patients. Nevertheless, in spite of these limitations, this study supports the hypothesis formulated by Romoli et al (9) that SARS-CoV-2-related brain injury maybe the consequence of several pathogenetic mechanisms in addition to direct viral damage. Furthermore, brain lesions were present even in the absence of specific neurologic symptoms. Therefore, it is possible that brain involvement is an underestimated feature in SARS-CoV-2-infected patients.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

All the data supporting the findings of this study (histologic specimens, clinical data) are available from the corresponding author on request. All the data that have been cited in this paper are openly available in PubMed® at https://pubmed.ncbi.nlm.nih.gov/.

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