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LETTER TO THE EDITOR

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

Viscardo P. Fabbri[1,2]; Maria P. Foschini[1,2,*]; Tiziana Lazzarotto[3,4]; Liliana Gabrielli[4]; Giovanna Cenacchi[1,5]; Carmine Gallo[2]; Raffaele Aspide[6]; Guido Frascaroli[7]; Pietro Cortelli[1,8]; Mattia Riefolo[3,5]; Caterina Giannini[1,9]; Antonietta D'Errico[3,5]

[1] Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, 40139, Italy.
[2] Unit of Anatomic Pathology, Department of Oncology, Bellaria Hospital, Via Altura 3, Bologna, 40139, Italy.
[3] Department of Experimental, Diagnostic and Specialty Medicine, Azienda Ospedaliero-Universitaria di Bologna, Via Albertoni 15, Bologna, 40138, Italy.
[4] Operative Unit of Clinical Microbiology, Azienda Ospedaliero-Universitaria di Bologna, Via Albertoni 15, Bologna, 40138, Italy.
[5] Unit of Pathology, Azienda Ospedaliero-Universitaria di Bologna, Via Albertoni 15, Bologna, 40138, Italy.
[6] Anesthesia and Neurointensive Care Unit, IRCCS Institute of Neurological Science of Bologna, via Altura 3, Bologna, 40139, Italy.
[7] Intensive Unit Care, Azienda Ospedaliero-Universitaria di Bologna, Via Albertoni 15, Bologna, 40138, Italy.
[8] IRCCS Institute of Neurological Science of Bologna, Bellaria Hospital, Via Altura 3, Bologna, 40139, Italy.
[9] Division of Anatomic Pathology, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, USA.

Corresponding author

Maria P. Foschini, MD, Department of Biomedical and Neuromotor Sciences, University of Bologna, Section of Anatomic Pathology at Bellaria Hospital, Via Altura 3, Bologna 40139, Italy. (Email: mariapia.foschini@unibo.it)

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).

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*.

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

Table 2. Summary of neuropathological findings. Abbreviations: OT = olfactory tract and bulb, B = brain, L = lungs.

	Brain weight (g)	Macroscopic findings	Histological findings	OT CoV-2	B CoV-2	L CoV-2
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	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 infraction 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 • Left pyramidal tract atrophy	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

Table 2. (Continued)

	Brain weight (g)	Macroscopic findings	Histological findings	OT CoV-2	B CoV-2	L CoV-2
Case 7	1650	• Oedema • Meningeal congestion	• Global hypoxic-ischemic injury • Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages • Endovascular microthrombi • Recent microscopic infarcts • Sparse microglial activation, especially in *medulla oblongata*	No	No	Yes
Case 8	1300	• Oedema • Meningeal congestion • Meningeal purulent accumulation	• Global hypoxic-ischemic injury • Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages • Endovascular microthrombi • Recent microscopic infarcts • Microglial activation, especially in *medulla oblongata* • Initial feature of Acute purulent meningitis	No	No	Yes
Case 9	1490	• Oedema • Meningeal congestion with focal blood extravasation • Atherosclerosis	• Global hypoxic-ischemic injury • Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages • Endovascular microthrombi • Focal leptomeningeal haemorrhage • Recent parieto-occipital lobe infarction and microscopic infarcts. • Spinal cord Schwannoma	No (brain and spinal cord)	No	Yes
Case 10	1350	• Oedema • Meningeal congestion	• Global hypoxic-ischemic injury • Small vessels ectasia, variable perivascular oedema, perivascular micro-haemorrhages • Endovascular microthrombi • Recent microscopic infarcts, the largest in the para-hippocampal region	No (brain and spinal cord)	No	Yes

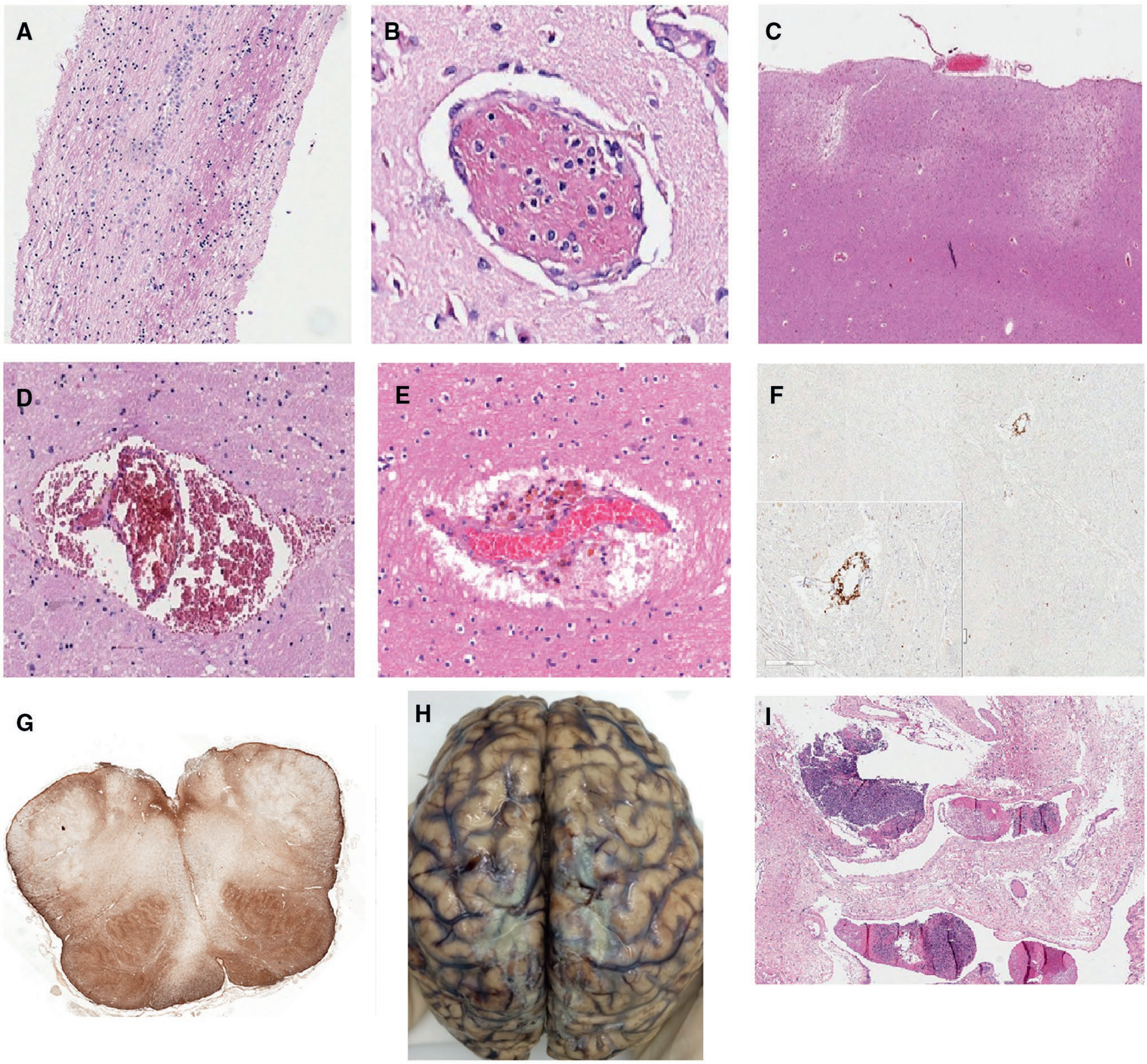

Figure 1. A: SARS-CoV-2 positive olfactory tract did not show any specific pathological features suggestive of viral damage (*case 1*). B: Microthrombi were seen in small intraperenchymal vessels located in the brain stem of SARS-CoV-2 positive case (*case 1*). C: Cortical microscopic ischemic areas in occipital cortex (*case 4*). D, E: Micro-haemorrhages and rare haemosiderin laden macrophages 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]

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).

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 neurological 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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