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LETTER TO THE EDITOR

Neuropathologic features of four autopsied COVID-19 patients

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Abstract

Published descriptions of the neuropathological features of COVID-19 patients have been controversial, ranging from only modest or no pathology to severe hypoxic and hemorrhagic phenotypes, thrombotic complications, acute disseminated encephalomyelitis-like changes, and encephalitis and meningitis. Here, we describe the neuropathological findings of four COVID-19-positive patients autopsied at the Helsinki University Hospital during the spring of 2020. While three of the patients (age range 63–90) exhibited merely mild to moderate hypoxia-associated changes, one 38-year-old subject with obesity, diabetes (type 2), Parkinson's disease and a very severe clinical course was found to have severe ischemic injury, abundant microhemorrhages and enlarged perivascular spaces most pronounced in the white matter and deep gray matter. The pattern of ischemic changes suggested a defect in microcirculation. In addition, a few small perivascular white matter lesions, with macrophages engulfing myelin, were found. No signs of encephalitis or meningitis were detected in any of the patients. When conducting RT-PCR and immunohistochemical analyses of brain tissue, we could not demonstrate in any of the patients marked injury or presence of SARS-CoV2 in the olfactory epithelium, olfactory bulbs or brain areas responsible for respiratory control. In conclusion, our small autopsy series demonstrates various hypoxia-associated neuropathological features in COVID-19 patients, but no evidence of neurotropism or meningitis/encephalitis.

INTRODUCTION

Many symptoms of COVID-19, such as stroke, anosmia and dysregulation of breathing are considered to be directly or partly attributable to neuropathological processes (4). Furthermore, severe radiologically verified manifestations, such as acute hemorrhagic necrotizing encephalitis (AHLE) and acute disseminated encephalomyelitis (ADEM) have been reported (5). Here, we present neuropathological findings of a series of autopsies performed at the Helsinki University Hospital April 14 through May 18, 2020, comprising four patients who had tested positive for SARS-CoV2 with RT-PCR prior to death. A full neuropathological examination was performed on two patients, while two had extended neuropathological sampling of at least nine CNS areas including olfactory bulbs/tracts during autopsy. Olfactory

mucosa was sampled from all four and carotid bodies from three patients.

MATERIALS AND METHODS

Autopsies

Clinical autopsies were performed at the Department of Pathology, HUS Diagnostic Center according to Finnish legislation, with consent granted by the next of kin. The modified full autopsies were carried out in an autopsy room appropriate for handling infective decedents, with appropriate personal protective equipment. All autopsies included the complete exploration of the visceral cavity and craniotomy. Modifications included additional virology sampling, extended neuropathological sampling (Cases 1 and 2),

samples of the olfactory mucosa with the cribriform plate and sampling of the carotid bodies where possible (Cases 1–3) (Supporting Information).

Neuropathological examination (Cases 3 and 4)

Full neuropathological examination of the brain was performed on Cases 3 and 4. Each brain was dissected after 15 days of formaldehyde-fixation and at least 20 samples from different brain areas were collected. Hematoxylin-eosin, Luxol fast blue and iron (Prussian blue) stainings were performed using standardized protocols at the Department of Pathology, Helsinki University Hospital.

Immunohistochemistry (IHC) and RT-PCR

IHC was performed at the Department of Pathology, University of Helsinki and Helsinki University Hospital using standard methods (selected samples; antibody information and details for IHC for SARS-CoV-2/2019 are shown in Supporting Information).

RT-PCR was conducted at the Department of Virology, University of Helsinki according to standard protocols. RNA extraction from tissues was carried out by the Trizol method. The primers and probes used have been described by Corman *et al* (1). Cycle threshold (Ct) values above 37 were considered negative.

RESULTS

The clinical features of the subjects are summarized in Table 1. Clinically, only one patient showed severe neurological symptoms (Table 1, Case 3) and his clinical and neuropathological characteristics will be described in detail below. One patient had ageusia documented in his clinical record without further elaboration on olfaction (Table 1, Case 1) and two were documented to have only minimal respiratory distress and to lack hyperventilation in response to hypoxia (Table 1, Cases 1 and 3). All patients had several previously diagnosed comorbidities of varying severity (Table 1). The information of the blood group was available for three patients, and, interestingly, they were all A RhD+.

Case 3 was a 38-year-old male with obesity (BMI 38), hypertension and diabetes (type 2) associated with retinopathy and polyneuropathy. Seven months before his death, a clinical diagnosis of Parkinson's disease (PD) was made, with an MRI scan negative for vascular degenerative changes at that time. Twenty-three days prior to death, the patient developed fever, sore throat and cough. He had loose stools for a day. The symptoms persisted and he developed gradually worsening shortness of breath. His condition deteriorating, he was taken to the hospital on day 9 after onset of symptoms. On admission, the patient was hypoxemic and in severe respiratory distress. After sedation and intubation, he was transferred to the ICU and later tested positive for SARS-CoV2. Intensive care included antibiotics, dialysis and assisted ventilation, but extracorporeal membrane oxygenation (ECMO)

was not used. At first, consciousness was regained during sedation breaks, but after seven days at the ICU the patient no longer responded. Repeated CT scans failed to show explanatory findings and a neurologist was called to assess the patient. On day 14 at the ICU (day 23 after symptom-onset), the neurologic prognosis was considered poor due to suspected hypoxic or encephalitic injury and the patient deceased after withdrawal of support. A clinical autopsy with neuropathological examination was conducted.

At the neuropathological examination the brain weighed 1768 g. Mild brain swelling, depigmentation of the substantia nigra and locus coeruleus, discoloration of the watershed areas and a few lacunae in inferior putamen (~5 mm in size) were noted. In addition, abundant enlarged perivascular spaces and microhemorrhages were found mostly in the cerebral and cerebellar white matter, in the deep gray matter and the brain stem. These vascular changes were mostly sized <1 mm and their quantity was disproportionate to the patient's age (Figure 1A).

Microscopically, abundant enlarged perivascular spaces with some hemosiderophages were observed, but we also identified a high density of acute microhemorrhages sized <1 mm, with a distribution similar to that noted macroscopically (see above). These changes included scattered T lymphocytes with very few B lymphocytes and some lesions also showed abundant granulocytes and faint positivity in iron staining (Figure 1B,D,E). We only detected minor intravascular deposits of fibrinoid material in some cerebral and subarachnoidal vessels (Figure 1C). No thrombotic material was found in cerebral microvessels. Severe hypoxic-ischemic injury was seen, with pyknotic and eosinophilic neurons in hypoxia-sensitive areas. Many axonal spheroids with strong APP-immunopositivity were detected, most prominent in the midbrain (peduncle), pons (near the surface of the pontine basis) and medulla (in the pyramid). Supratentorially, there were swollen APP-positive axons particularly in the white matter tracts. In the white matter surrounding the vascular lesions, the neuropil was edematous; scattered small, congested vessels and perivascular small nodules of swollen axons with APP positivity (Figure 1F,G), but very few inflammatory cells, were found. While we observed no vascular changes pathognomonic for hypertension or diabetes, some vessels showed subendothelial hemorrhage (Figure 1B,D) and only few vessels exhibited mild inflammation of the vascular wall (not shown). In Luxol fast blue staining, a few perivascular white matter lesions connected with Iba1-positive microglia/macrophage-type cells and macrophages engulfing myelin were observed (Figure 1H,I), with no associated APP-immunopositivity. As expected, we found many alpha-synuclein-positive Lewy bodies and neurites in the substantia nigra, consistent with brainstem-predominant Lewy body disease/PD. It should be noted that no signs of meningitis or encephalitis were seen.

General autopsy findings included partially resolving diffuse alveolar damage in the lungs, with extensive microthrombosis. Outside the lung vasculature microthrombosis was limited to cervical and mediastinal small veins, without

Table 1. COVID-19 patient characteristics and neuropathological findings with RT-PCR and IHC results.

	Case 1	Case 2	Case 3	Case 4
Sex	Male	Male	Male	Female
Age (years)	63	82	38	90
Symptom-onset to death (days)	25	6	23	26
Death to autopsy (days)	7	6	10	5
Treatment at ICU (days)	2	0	14	0
Medical history	HTN, Gout, CKD with one functional kidney, Smoker	SSS with pacemaker, CAD with MI, PAD, Stroke, DM2, COPD, CRC, CKD	Obesity, HTN, DM2 with retinopathy & polyneuropathy, Recurrent cellulitis, Smoker	HTN, SSS with pacemaker, Asthma/COPD, AD, Osteoporosis, Spinal stenosis, Recurrent lung infections
Blood group	A RhD+	A RhD+	A RhD+	N/A
HLA-type	N/A	N/A	HLA-DRB1	N/A
Neurologic symptoms	Ageusia, delirium during ICU stay	Non reported	Delirium, unconsciousness	Delirium, unconsciousness
Neuropathologic findings	Hypoxic injury (mild), mild perivascular degeneration and scattered inflammatory cells	Hypoxic injury (mild), perivascular degeneration, lacunar infarctions (old)	Severe hypoxic injury, vasculopathy with perivascular hemorrhage and degeneration, white matter lesions, PD	Hypoxic injury, perivascular degeneration, 2 tiny foci with some axonal spheroids, AD (Braak 5, CERAD Moderate), CAA, Limbic predominant DLB
SARS-CoV2 RT-PCR at autopsy (respiratory tract)	+	+	-	+
SARS-CoV2 RT-PCR at autopsy (CNS)	-	-	-	-
SARS-CoV2 IHC (olfactory mucosa)	-	(+; only respiratory epithelium)	-	-
SARS-CoV2 IHC (CNS)	-	-	-	-
SARS-CoV2 IHC (carotid body)	-	-	-	-
Immediate cause of death	Pulmonary embolism	Sudden cardiac death	Neurologic and hemodynamic sequelae of severe COVID-19	Secondary bilateral bacterial pneumonia

Abbreviations: AD = Alzheimer's disease; CAA = cerebral amyloid angiopathy; CAD = coronary artery disease; CKD = chronic kidney disease; CNS = central nervous system; COPD = chronic obstructive pulmonary disease; CRC = colorectal Cancer; d = days; DLB = dementia with Lewy bodies; DM2 = diabetes mellitus type 2; HLA= human leukocyte antigen; HTN = hypertension; ICU = intensive care unit; IHC = immunohistochemistry; MI = myocardial infarction; N/A = not available; PAD = peripheral artery disease; PD = Parkinson's disease; rt-pcr = reverse transcriptase – polymerase chain reaction; SARS-CoV2 = severe acute respiratory syndrome – corona virus 2; SSS = sick sinus syndrome; y = years.

other signs of vasculopathy. Hypoxic-ischemic injury was seen in multiple organs.

Other patients in our series had varying, mild to moderate, end-stage hypoxic-ischemic injuries in brain sections (Table 1). None of our cases showed immunohistochemical or RT-PCR-positivity for SARS-CoV2 in neural tissues or elsewhere outside the respiratory tract (Table 1).

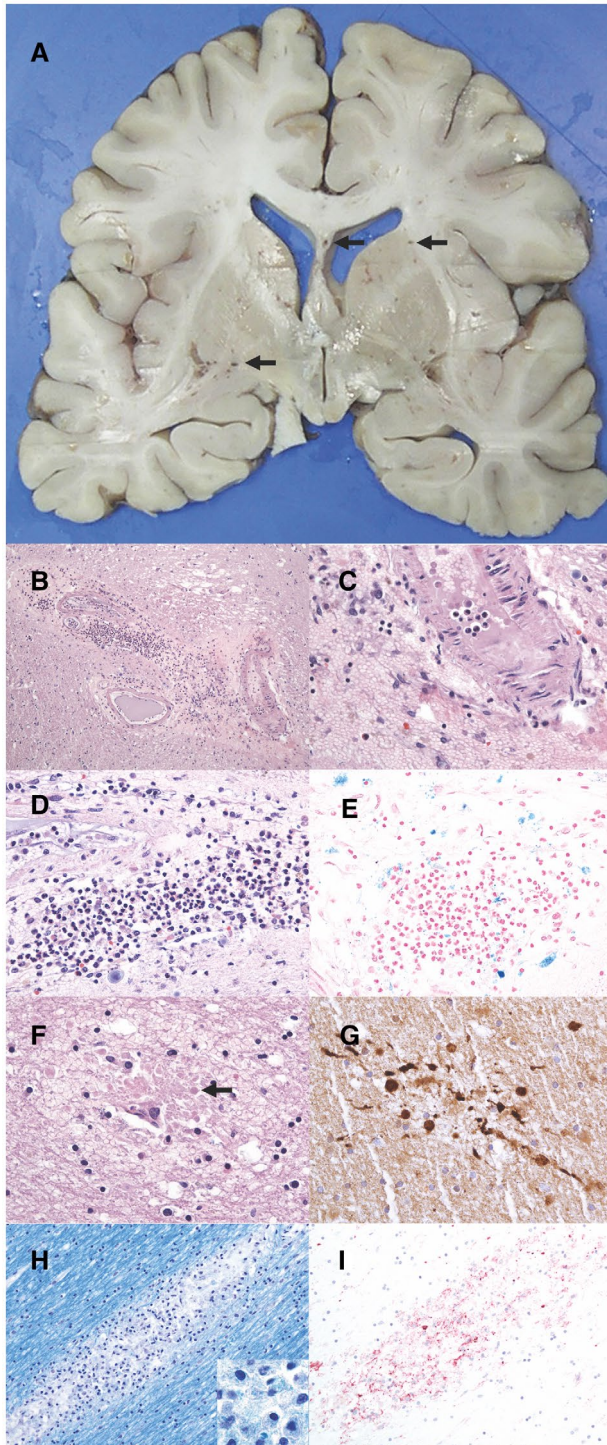


Figure 1. A. Coronal section of cerebral hemispheres exhibiting abundant enlarged perivascular spaces and microhemorrhages in the white matter and deep gray matter (arrows). In inferior putamen (both sides) lacunae are seen. B. Microscopic section from the internal capsule region shows abundant perivascular inflammation and perivascular hemorrhage, but very few inflammatory cells in the surrounding brain areas (H&E, 40× magnification). C–E. Higher resolution images from the region exhibited in B (H&E, 400× magnification). In (C), a small amount of fibrinoid material is seen in the lumen of the vessel. D. Abundant granulocytes are detected in this region in addition to perivascular hemorrhage. E. Iron staining of the same location as in (D) shows faint positivity. F. A small focus of perivascular hemorrhage, with axonal edema and spheroids (arrow) (H&E, 400× magnification), highlighted by IHC for APP in (G) (400× magnification). H. Luxol fast blue staining from the white matter of the cingular gyrus shows loss of myelin and microglial reaction (200× magnification). Macrophages engulfing myelin are shown in insert (1000× magnification). H&E staining of this section exhibited a vessel in this location (see Supporting Information). I. Iba-1 IHC staining of the same location highlights the microglial cells (200× magnification).

DISCUSSION

Thus far, published data on the neuropathological changes associated with COVID-19 have been scarce and controversial, and neuropathological descriptions have ranged from only modest or no pathology to severe hemorrhagic and hypoxic phenotypes, thrombotic complications, ADEM-like changes and encephalitis and meningitis (6–8,10). Many COVID-19 patients have comorbidities, suffer from hypoxia and have been treated in ICU for varying times, which all complicate interpretation of the findings. However, precise investigation and description of neuropathological COVID-19-associated changes will significantly contribute to understanding of the neuropathologic processes occurring in the disease progression.

Our small autopsy series covered cases at different stages of disease, with varying comorbidities and of various ages. Most (3/4) of our patients (age range 63–90) exhibited only modest hypoxia-related neuropathological changes, according with some previous reports (7,8). However, one under middle-aged patient (38 years old), with obesity, diabetes (type 2) and PD showed abundant perivascular hemorrhages and a few foci of white matter lesions reminiscent of one previous case report (6). In contrast to it, the hemorrhages observed were smaller (mostly <1 mm in diameter), there were no neocortical infarcts and the white matter lesions appeared less pronounced. In addition, another recent report described petechial hemorrhages in severe forms of COVID-19 (10).

All of the CNS samples in our study tested by RT-PCR were found negative for SARS-CoV2. We could neither demonstrate marked injury nor presence of SARS-CoV2 by IHC in the brain areas responsible for respiratory control or the carotid bodies. In all patients' specimens, the olfactory neuroepithelial cells appeared intact and none colocalized with SARS-CoV2 immunohistochemistry (Supporting Information). Thus, we could not provide evidence for neurotropism having affected the CNS or more specifically being associated with anosmia/ageusia or

respiratory dysregulation. Furthermore, contrary to a recent report (10) and according with some other studies (7,8), we did not detect any signs of meningitis or encephalitis in any brain areas. Viral antigen could only be detected by IHC in the respiratory epithelium of Case 2 with the shortest illness (6 days) in contrast to the other patients with longer illness (23–26 days). The blood group A RhD+ was overrepresented in our case series, reminiscent of a recent study of SARS-CoV2 pneumonia (3).

The ischemic changes seen in Case 3 were severe and their pattern suggested a defect in microcirculation. Patients with obesity and associated vasculopathies, such as our Case 3, have been found prone to develop severe forms of the COVID-19 disease. In their pathogenesis, some reports have ascribed particular importance to endothelial dysfunction/endotheliitis (2,9). Whether the changes we detected were caused by possible comorbid small vessel disease, hypoxia or their combination, and how these changes may be linked to a SARS-CoV2 infection, cannot be determined on the basis of these scanty cases.

In conclusion, our small autopsy series demonstrates various hypoxia-associated neuropathological features, but no evidence for COVID-19 infection involving neurotropism or encephalitis. Comprehensive neuropathological studies among COVID-19 patients are warranted.

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DECLARATIONS

The autopsies were conducted according to Finnish legislation at the Department of Pathology, HUS Diagnostic Center, Helsinki. Consent was given by the next-of-kin. The study is part of the Clin COVID project approved by the Ethics committee of the Helsinki University Hospital and HUS Research Center.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site:

Figure S1. **A.** Subarachnoidal vessels showing some intravascular fibrinoid material (H&E, 200× magnification). **B.** Vessel exhibited in Fig. 1H-I (H&E, 200× magnification). **C.** Spheroids stained with IHC for APP (200× magnification). **D.** Nasal epithelium (Case 1) showing a thickened basement membrane as a sign for chronic rhinitis. **E.** Carotid body (Case 2) showing no apparent pathology (H&E, 200× magnification). **F.** Carotid body (Case 2) negative for SARS-CoV2 using IHC for viral Spike-protein (200× magnification). **G.** Olfactory epithelium (Case 2) showing neuroepithelial cells with IHC for PGP9.5 (200× magnification). **H.** Olfactory epithelium from the same location as (G) negative for SARS-CoV2 using IHC for viral Spike-protein (200× magnification). **I.** Respiratory epithelium (Case 2) negative for neuroepithelial cells (IHC for PGP9.5, 200× magnification). **J.** Respiratory epithelium from the same location as I positive for SARS-CoV2 (IHC for Spike-protein, 200× magnification).

Table S1. Antibody information.