Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Lee M-H, Perl DP, Nair G, et al. Microvascular injury in the brains of patients with Covid-19. N Engl J Med. DOI: 10.1056/NEJMc2033369

Table of Contents

Materials and Methods

Patient Population

For this study, during the period of March to July 2020, brain tissues were collected from 16 autopsy cases at the Office of the Chief Medical Examiner, New York City and 3 cases at the University of Iowa College of Medicine (Table S1). This represents a sample size of convenience. All patients were confirmed to be infected with SARS-CoV-2 by PCR from nasopharyngeal swabs either ante- or post-mortem. Patients ranged in age from 5 to 73 years (median, 50 years), and had survivals from time of first symptoms to death ranging from hours to 2 months. Many had previous medical histories reflecting potential vulnerability, e.g., obesity (n=5), cardiovascular disease/hypertension (n=6), diabetes mellitus (n=6), nursing care residence related to old traumatic brain or other injuries (n=3), drug or alcohol use disorder (n=3), systemic medical disease (lupus erythematosus and mucopolysaccharidosis type III [n=1 each]); many patients had more than one risk factor. Recent symptoms among those receiving antemortem medical care were predominantly respiratory, though some (particularly children) had gastrointestinal complaints. Agitated delirium was the prevailing picture in 2 cases. Collapse and sudden death occurred in a subset of cases. Eight patients were found dead at home or in public settings, with limited known recent medical history, but with sufficient concern on the part of family members to prompt swabbing for SARS-CoV-2 (Table S1)

Postmortem MRI

MR Microscopy (MRM) was performed on smaller tissue slabs using an 11.7 Tesla MRI system (Avance III, Bruker Corporation) equipped with a 12-cm gradient coil (Resonance Research Inc) with maximum gradient strength of 100G/cm^2. The brainstem tissue blocks were moved to custom containers filled with Fomblin and imaged using a quadrature Tx/Rx coil. T_2*-weighted images were acquired (3D gradient-echo sequence, TR=100 ms, TE= about 10 ms, FA=30°, 100-micron isotropic resolution). Total acquisition time depended on the size of the block being imaged and averaged about 50 hours.

Olfactory bulbs were transferred to 6-mm glass test tubes (Fisher Scientific) filled with Fomblin and imaged using a custom-built solenoid transmit-receive coil of 6-mm inner diameter and 10-mm length, with a custom-built transmit/receive switch and preamplifier proximal to the coil T_2*-weighted images were acquired (3D gradient-echo sequence, TR=120 ms, TE= 6 ms, FA=35°, 25-micron isotropic resolution). Total acquisition time depended on the size of the block being imaged and averaged about 25 hours.

Histopathology Work-up and Chromogenic Immunohistochemistry

Immunohistochemical analysis was performed on autopsied brain tissues from 13 of the 19 cases received. Autopsied brains were fixed in formalin, dissected primarily in the coronal plane and selected regions studied. This included the olfactory bulb and tract, cerebral cortex in representative regions of the frontal, temporal, parietal and occipital lobes, basal ganglia, hippocampus, midbrain, cerebellum, pons and medulla. Where *ex vivo* MRI exams showed the presence of abnormalities, additional sampling was directed to those regions. Five-micron thick sections were obtained from the paraffin blocks and were processed as follows. The slides were deparaffinized and rehydrated using xylene and graded ethanol. For general morphologic evaluation, hematoxylin and eosin and luxol fast blue staining were performed. For immunohistochemistry, antigen retrieval was done by Heat-Induced Antigen Retrieval (HIAR) methods in 10 mM citrate buffer at pH 6.0 or 10 mM Tris/EDTA buffer at pH 9.0, or Enzyme-Induced Antigen Retrieval (EIAR) method in proteinase K. Peroxidase blocking was achieved by incubating tissues in 0.3% to 3.0% hydrogen peroxide for 10 minutes at room temperature and protein blocking was done with protein block solution (DAKO) for 30 minutes at room

temperature. Sections were covered with primary antibodies and incubated overnight at room temperature. Antibodies used in immunohistochemistry are listed in Table S2. After washing with 1x TBS containing 0.05% TritonX-100, sections were incubated with PowerVision polymeric horseradish peroxidase (poly-HRP) anti-mouse IgG (Leica Biosystems) or PowerVision poly-HRP anti-rabbit IgG for 2 hours at RT. Antibody binding was developed with 3,3′-diaminobenzidine (DAB; Vector Laboratories). Sections were counterstained with hematoxylin (DAKO). Slides were then coverslipped using EcoMount mounting medium (Biocare Medical). Images were processed using a whole slide scanner (Aperio AT2, Leica Biosystems).

Multiplex Fluorescence Immunohistochemistry

Multiplex fluorescence immunohistochemistry was performed on 5 µm-thick formalin fixed paraffin embedded (FFPE) sections using iterative antibody staining, stripping and re-staining steps to accumulate a total of 14-plex biomarker imaging data of interest. Eight regions from 5 patients were analyzed. Briefly, the sections were first deparaffinized using standard Xylene/Ethanol/Rehydration protocol followed by antigen unmasking using a HIAR method in 10 mM Tris/EDTA buffer at pH 9.0. The sections were then incubated with Human BD Fc Blocking solution (BD Biosciences) to block endogenous Fc receptors, and then incubated in True Black Reagent (Biotium) to quench intrinsic tissue autofluorescence. The sections were then iteratively immunoreacted for 1 hour at room temperature using 1-5 µg/ml cocktail mixtures of antibodies. Application of multiple primary antibodies was followed by washing off excess antibodies in PBS supplemented with 1 mg/ml bovine serum albumin (BSA) and staining the sections for 1 hour at room temperature using a 1 µg/ml cocktail mixture of appropriately cross-adsorbed secondary antibodies (Thermo Fisher, Jackson ImmunoResearch, Li-Cor Biosciences) conjugated to one of the following spectrally compatible fluorophores: Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 594, Alexa Fluor 647, IRDye 680LT, or IRDye 800CW. After washing off excess secondary antibodies, sections were counterstained using 1 µg/ml DAPI (Thermo Fisher) for visualization of cell nuclei. Slides were then coverslipped using Immu-Mount™ medium (Thermo Fisher Scientific) and imaged using a multispectral epifluorescence microscope.

Multiplex Fluorescence Image Acquisition

Images were acquired from whole specimen sections using the Axio Imager.Z2 slide scanning fluorescence microscope (Zeiss) equipped with a 20X/0.8 Plan-Apochromat (Phase-2) non-immersion objective (Zeiss), a high resolution ORCA-Flash4.0 sCMOS digital camera (Hamamatsu), a 200W X-Cite 200DC broad band lamp source (Excelitas Technologies), and 8 customized filter sets (Semrock) optimized to detect the following fluorophores: DAPI, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 594, Alexa Fluor 647, IRDye 680LT and IRDye 800CW. Image tiles (600 x 600 µm viewing area) were individually captured at 0.325 micron/pixel spatial resolution, and the tiles seamlessly stitched into whole specimen images using the ZEN 2 image acquisition and analysis software program (Zeiss), with an appropriate color table having been applied to each image channel to either match its emission spectrum or to set a distinguishing color balance. Pseudo-colored stitched images were then exported to Adobe Photoshop, spatially aligned at a pixel level of resolution, and overlaid as individual layers to create multicolored merged composites.

Detection of SARS-CoV-2 in brain tissue by PCR

Frozen autopsy brain tissue was homogenized in RNA lysis buffer with Precellys 24 Tissue Homogenizer. RNA was purified using Qiagen RNeasy Plus Mini Kit. About 1 ug of RNA was reverse transcribed (RT) using Invitrogen SuperScript™ III First-Strand Synthesis SuperMix. 2

µl of RT product was subjected to real time PCR using Applied BioSystems SYBR Green Real-Time PCR Master Mix and homemade PCR primer sets for SARS-Cov-2 detection.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were used as internal control. PCR was also performed using SARS-CoV-2 (2019-nCoV) CDC RUO Primers and Probes. In this assay, Applied Biosystems TaqMan™ Universal PCR Master Mix was used following CDC recommended protocol. Primer and probe sequences are provided in Table S3.

Sections of formalin fixed paraffin embedded brain tissue that were adjacent to sections showing evidence of perivascular and parenchymal infiltrates were used for detection of SARS-CoV-2. RNA was extracted from 10 µm thick sections mounted on glass slide, using Qiagen RNeasy FFPE Kit. SARS-CoV-2 RNA was detected using SARS-CoV-2 (2019-nCoV) CDC RUO Primers and Probes and Promega GoTaq® Probe 1- Step RT-qPCR System. In all SARS-CoV-2 PCR assays, genomic SARS-CoV-2 RNA from BEI Resources (item# NR-52285) was used as a positive control. The limit of detection of the assay is 5 copies of RNA transcripts per PCR reaction. In each of the PCR assays a sample was considered negative if both N1 and N2 were not detected within 40 cycles while internal control RNase P had a cycle threshold value of less than 40.

RNA sequencing
Sequencing libraries were prepared from total RNA from 8 samples using TruSeq Stranded Total RNA Library Prep Kit (Illumina). Sequencing was performed on Illumina HiSeq 2500. Low-quality bases were trimmed from the 3' end of reads and 3' adapter was trimmed using FASTQ/A Clipper with default settings (Hannon lab). Reads shorter than 35 bp were excluded from analysis. Sequencing reads were aligned to an index with the human reference (HG19) and SARS-CoV-2 (NC_045512) using GSNAP (Version 2013-10-28) (Wu and Nacu, 2010) using the following parameters: Mismatches ≤ [(read length+2)/12-2]; Mapping score ≥ 20; Soft-clipping on (-trim-mismatch-score=-3).

RNAscope
RNAscope *in situ* hybridization was performed according to the manufacturer's instructions (Advanced Cell Diagnostics, Hayward, CA). In brief, FFPE section slides were dried for one hour at 60°C, deparaffinized in fresh xylene and fresh 100% ETOH. RNAscope® Hydrogen Peroxide was added to the sections and incubate for 10 min at room temperature. For purposes of antigen retrieval, the slides were boiled in RNAscope® 1X Target Retrieval Reagent for 15 minutes and then incubated in RNAscope® Protease Plus for 30 min at 40°C. After washing, the slides were covered with drops of a probe V-nCoV2019-S (cat# 848561) and incubated for two hours at 40°C. This probe was designed to be specific for SARS-CoV-2, targeting the S gene encoding the spike protein. After hybridizing with the probe, the signals were amplified sequentially with amplifiers and labeled with a label probe using the 2.5 HD Detection Kit (as per manufacture's procedure), at 40°C or room temperature. To reveal the signal, slides were incubated in a red working solution for 10 minutes at room temperature. Slides were then counterstained with 50% hematoxylin and mounted with EcoMount mounting medium. Images were captured using a whole-slide scanner (Aperio AT2).

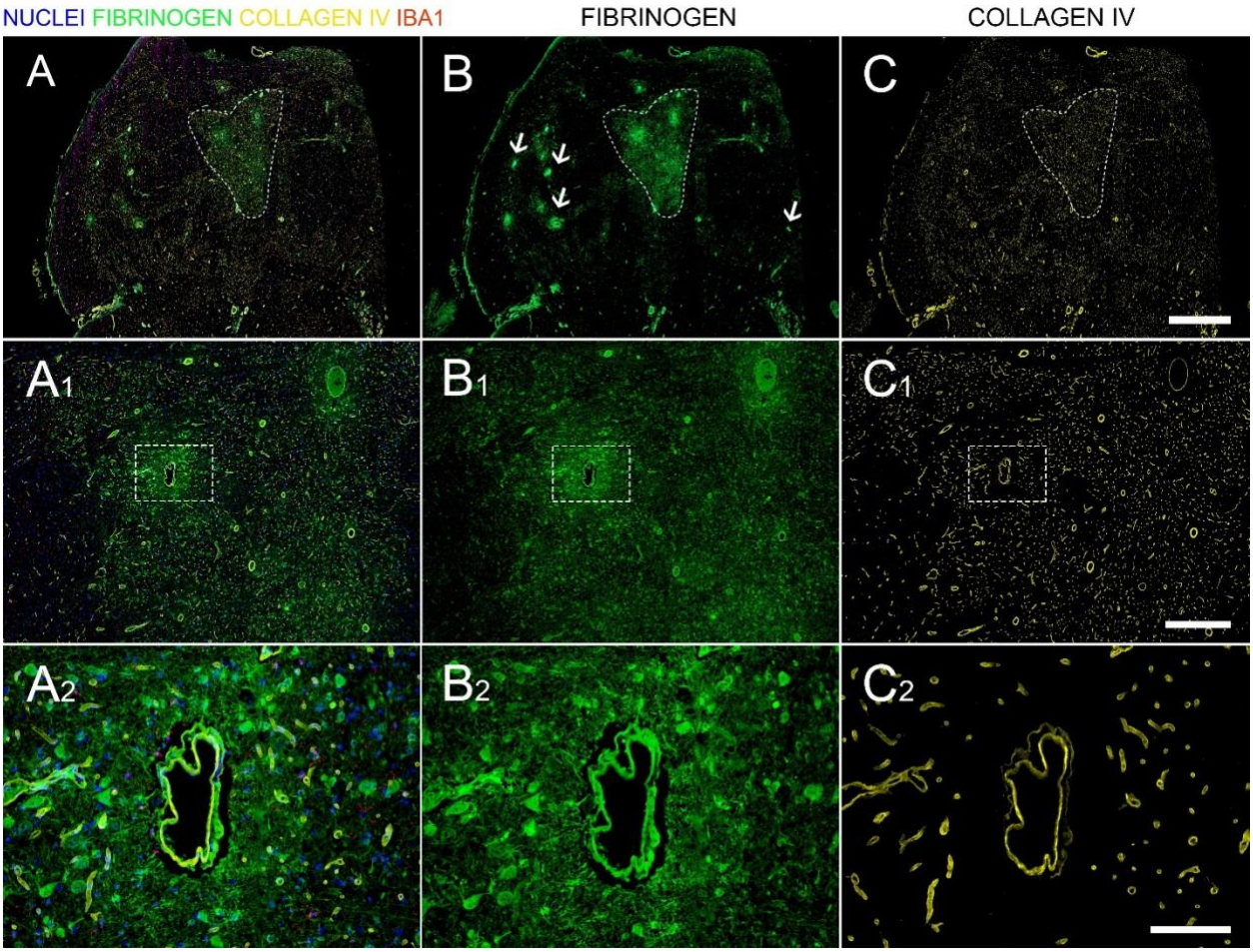


Figure S1. Microvascular pathology in the pons of case IA#1. (A-C) Multiplex fluorescence immunostaining was used to identify fibrinogen (green), collagen IV for blood vessels (yellow), IBA for microglia (red). DAPI (blue) was used to stain the nuclei. (A) Merged image. (B) A large area of fibrinogen leakage shown by a dashed border and multiple small areas (arrows). (C) shows compromised blood vessels in several areas of fibrinogen leakage. A1, B1 and C1 are enlarged images of the area defined by the dashed border in A, B and C. A2, B2 and C2 are enlarged images of the area defined by the dashed box in A1, B1 and C1. Scale bars (C) 5 mm, (C1) 1 mm, (C2) 250 µm.

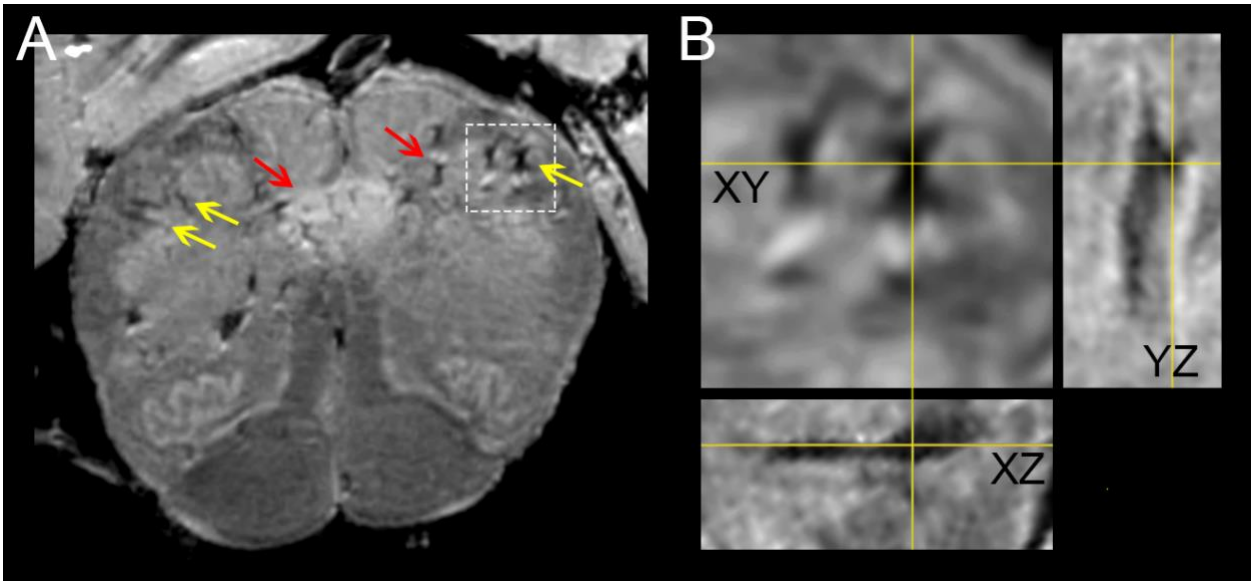
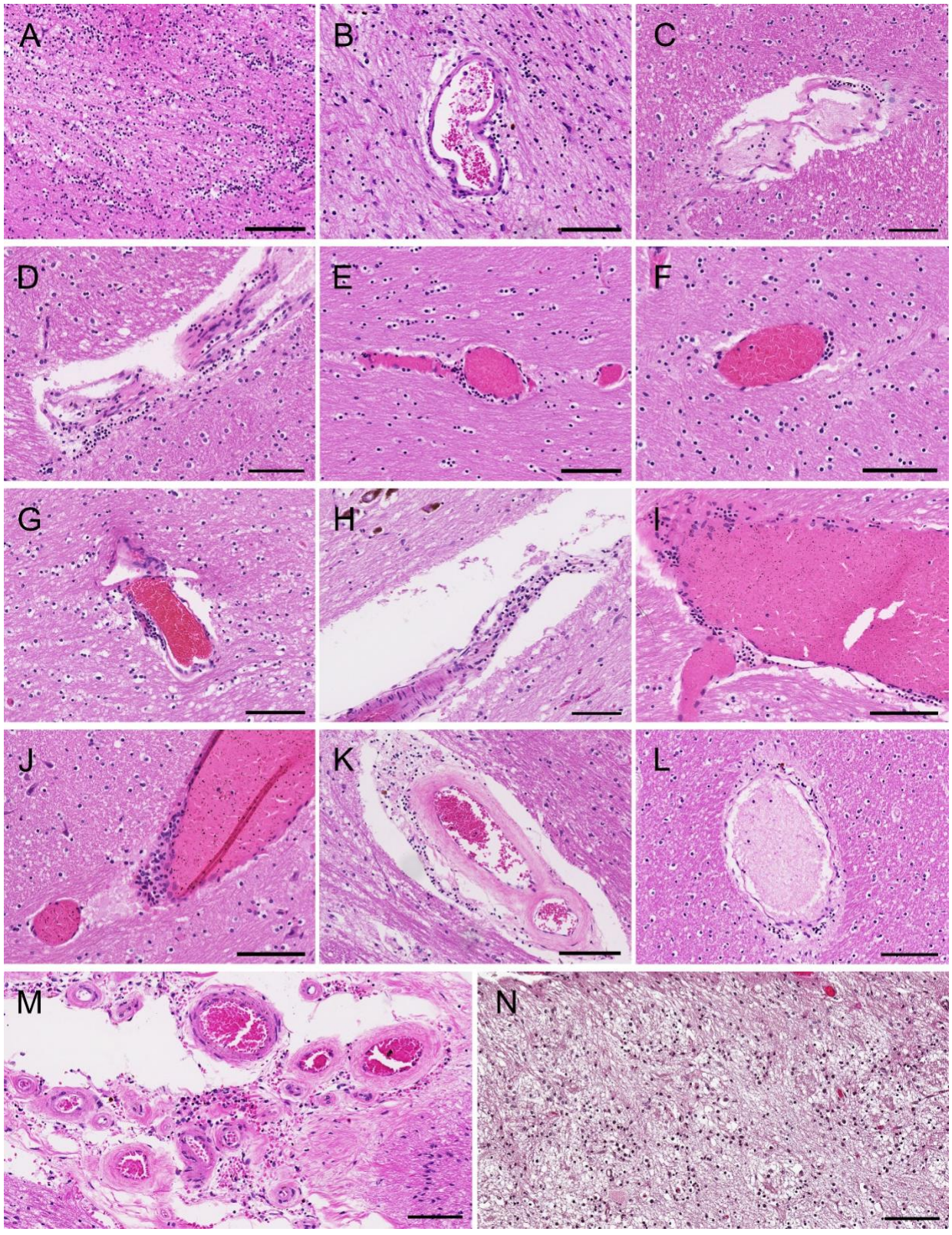


Figure S2. Microvascular pathology in the medulla of case IA#3. MRI of the medulla from case IA#3 was performed on a 11.7T scanner at 100 microns isotropic resolution. (A) Arrows indicate punctate and linear hypointense (yellow arrows) and hyperintense (red arrows) signals. (B) Orthogonal slice view reconstructed at the level of the hypointensity (dashed box, A). The area defined by the dashed box in A is displayed as three view planes, as indicated by the cross lines (XY plane, YZ plane and XZ plane) to analyze from different directions. This view shows a prominent blood vessel accompanied by a punctate region of hypointensity, possibly microbleeding.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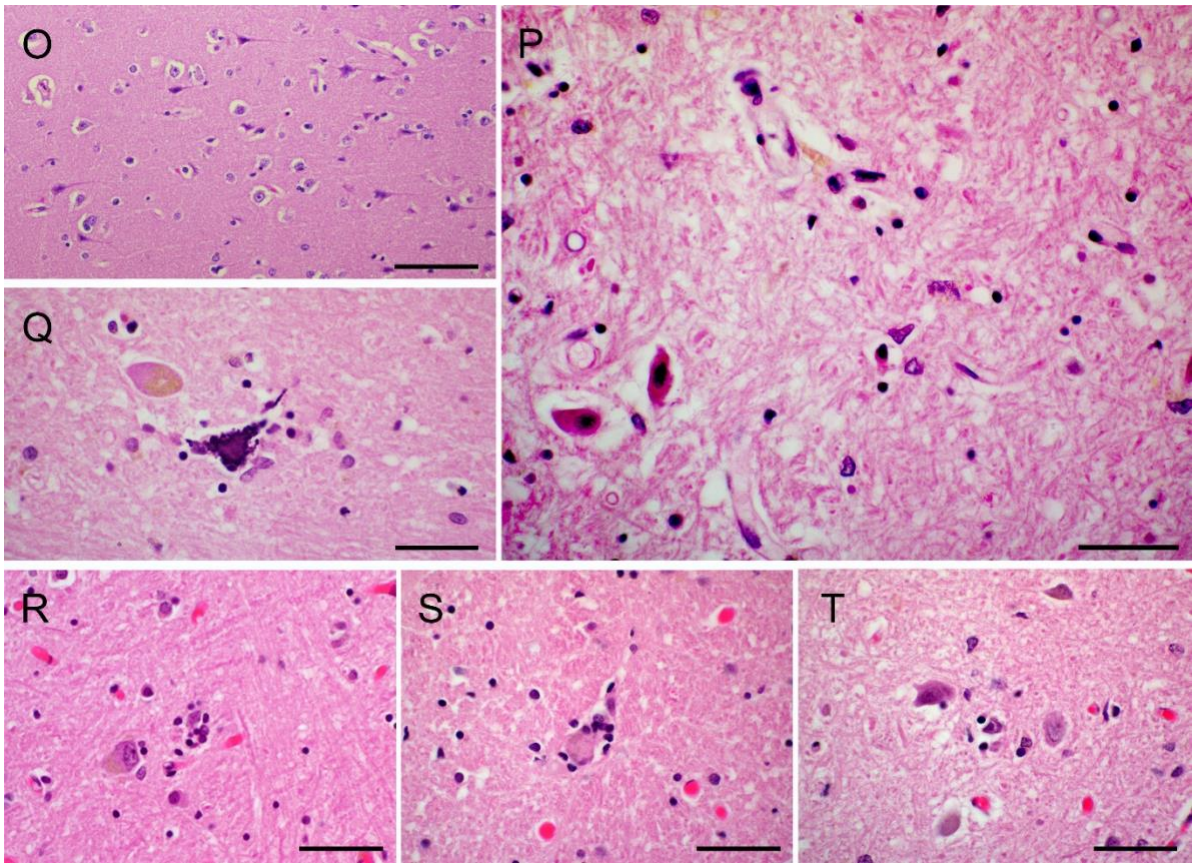

Figure S3. Microscopic H&E section with pathology. Hematoxylin-eosin stains were performed in brain tissues of patients with COVID-19. Diffusely scattered mononuclear cell infiltration in the brain tissues, prominently in the perivascular area, was found in the olfactory tract of NY#12 (A), cortex of IA#1 (B), subcortical white matter of IA#1 (C,D) and NY#6 (E-G), midbrain of NY#7 (H), pons of NY#1 (I) and NY#5 (J), medulla of IA#1 (K) and cerebellar white matter of 1A#1 (L). Moderate inflammatory cell infiltrates were present in the meninges of IA#1 (M) and olfactory bulb of NY#14 (N). Evidence of acute hypoxia in the form of eosinophilic degeneration of neurons in susceptible region was shown in the layer 5 of neocortex of IA#1 (O). Microglial activation and neuronal loss were evident in the dmX of NY#2 (P). Microglial nodules containing neurons undergoing neuronophagia were also seen in the thalamus of NY#5 (Q), and in the pons (R, S) and dmX (T) of NY#4. dmX, dorsal motor nucleus of the vagus. Scale bars (A-O) 100 μm, (P-T) 50 μm

Table S1. Patients characteristics

# Case	Age/ Sex	PMI (hrs)	Past Medical Hx	Recent Hx	Survival from first signs /symptoms	Gross pathology on inspection
IA#1	73/M	38	HTN, obesity (BMI 33.4)	Sinus congestion, fever, cough pneumonia, ARDS, Oxygen mask then on ventilator, renal failure, small pericardial effusion, ventricular arrythmia, terminal event: pulmonary embolism. Ante-mortem swab+	16 days	**Severe pulmonary congestion with multifocal hemorrhages, pleural effusion**, renal cortical pallor, fibrosis and calcification on epicardial surface
IA#2	39/M	36	Methamphetamine use disorder, HTN, cardiac myopathy and kidney disease	Cough, dyspnea, pulmonary emboli with cardiac, renal and respiratory failure. Ante-mortem swab PCR+	2 days	Cardiac hypertrophy, pulmonary and renal infarcts
IA#3	64/F	20	Coronary artery disease, HTN, DM, urinary bladder squamous cell carcinoma	Acute heart failure complicated by COVID, recent cystectomy. Ante-mortem swab PCR+	Days to weeks (presumed)	**Bronchopneumonia, emphysema**
NY#1	50/M	33	DM, sciatica, back pain	Cough 3-5 days, found dead in bed at home, PM swab +	5 days	**Acute DAD**, HASCVD, fatty liver, nephrosclerosis
NY#2	63/M	70	DM, HTN	Flu-like symptoms 7 days, found dead at home, PM swab +	1 week	Suggestive of **acute lung injury** (autolysis), HASCVD
NY#3	39/M	34	Drug use disorder	Unknown, found dead in subway, PM swab +	Hours to days (presumed)	**Acute lung injury**, toxicology + fentanyl, heroin, alcohol, fatty liver, LVH
NY#4	58/F	29	Obesity, asthma, DM, schizophrenia, had hospitalization with intubation for asthma for 1 month, discharged 1 month earlier	Unknown, found dead at home, PM swab +	Days to weeks (presumed)	**Acute lung injury**, fatty liver, diabetic kidney disease

NY#5	58/F	30	Obesity	GI symptoms few days, found dead at home in bed with foaming at mouth (rectal temperature 102 °F), No respiratory symptoms. PM swab +	Days	**Acute DAD and fibrin thrombi**, LVH, nephrosclerosis
NY#6	24/M	13	Healthy, no known illnesses	Flu-like symptoms for 3 weeks Sudden death (collapsed while standing), PM swab +	3 weeks	**Acute lung injury**
NY#7	55/M	105	HTN, DM, HLD, obesity, depression	Fever, cough body aches for one week, agitated delirium, subdued by force, hospitalized 6 days, ARDS, put on ventilator, hospital swab and PM swab +	2 weeks	**Acute DAD with fibrin thrombi**, fatty liver disease and fibrosis, GSW to limbs
NY#8	54/M	50	Old TBI from assault 3 years prior, post-traumatic seizures, DM, in nursing home	Fever, respiratory symptoms, admitted for 4 days. Discharged to rehab center on oxygen. Died 2 days later. Ante-mortem swab +	7-10 days	Aspiration pneumonia
NY#9	5/M	15	Speech delay	Cough, nasal congestion, fever, abdominal pain, diarrhea (MIS-C), on ECMO, swab +	Days	**Acute lung injury**, hyper-sensitivity myocarditis, coronary arteritis
NY#10	63/M	368	Old TBI from assault 21 years prior, s/p craniotomy, post-traumatic seizures, DM, HLD, in nursing home	No reported recent symptoms, PM swab +	Hours to days (presumed)	**Acute DAD with fibrin thrombi**, HASCVD, hepatic cirrhosis
NY#11	37M	35	SLE, HTN, alcohol use disorder, COVID-19 6 weeks prior, back to work when swab -, 2 weeks prior TN	No recent reported symptoms, sudden death at home, PM swab +	Days to weeks (presumed)	**Acute lung injury**, LVH

NY#12	69/M	54	HTN, HLD, CAD, s/p aortic dissection repair 6 years prior, with paraplegia, COVID-19 pneumonia over prior 2 months	Found dead in nursing home. Televisit 2 days prior, no complaints. Prior nursing home swab PCR+	2 months	**Acute lung injury**, acute and chronic pyelonephritis, acute pancreatitis with microabscesses, splenomegaly with splenitis
NY#13	10/M	27	Obesity	Fever, sudden collapse, resuscitation with hours survival, putative MIS-C	Hours to days	**Acute lung injury, hypertrophic cardiomyopathy, superficial perivascular dermatitis**
NY#14	57/M	26	Unknown	Unwitnessed fall down a flight of stairs. Found unresponsive and pulseless with multiple fractures, “Ground glass” opacities on chest Xray in ER, PM swab +	Hours to days (presumed)	**Acute lung injury**
NY#15	19/M	28	Previously healthy, father had COVID-19 1 month prior	Acute body pain in middle of night, sudden death (found in ventricular fibrillation and then systole), PM swab +	Hours to days (presumed)	**Acute lung injury**, splenomegaly
NY#16	13/F	52	Mucopolysaccharidosis III, intestinal bezoar, s/p surgeries	GI symptoms, complications, on ECMO, putative MIS-C	Days	**Acute lung injury**, peritonitis, **conjunctivitis**

All deaths occurred between March and July 2020.
PMI=postmortem interval (time between death and placement of brain tissue in fixative; may include long intervals of refrigerated storage); CAD=coronary artery disease; DAD=diffuse alveolar damage; DM=diabetes mellitus; ECMO=extracorporeal membrane oxygenation; ER=emergency room; GI=gastrointestinal; GSW=gunshot wound; HASCVD=hypertensive and atherosclerotic cardiovascular disease; HLD=hyperlipidemia; HTN=hypertension; Hx=History; LVH=left ventricular hypertrophy; MIS-C=multisystem inflammatory syndrome in children; NP=neuropathology; PM=postmortem; SAH=subarachnoid hemorrhage; SDH=subdural hemorrhage; SLE=Systemic lupus erythematosus; TBI=traumatic brain injury; Bold-faced type=findings considered related to COVID-19; Underlining=deaths that were sudden or unexpected.

Table S2. List of antibodies used for immunohistochemical studies

Target	Antigen	Host (Ig class)	Clone	Source	Cat. No.
Pan-T cells	CD3	Rabbit (IgG)	2GV6	Ventana-Roche	790-4341
Helper-inducer T-cells	CD4	Rabbit (IgG)	SP35	Ventana-Roche	790-4423
Cytotoxic T-cells	CD8	Rabbit (IgG)	SP57	Ventana-Roche	790-4460
B lymphocytes	CD20	Mouse (IgG2a)	L26	Ventana-Roche	760-2531
Macrophages	CD68	Mouse (IgG1)	KP1	Thermo Fisher Scientific	14-0688-82
Microglia	IBA1	Rabbit (IgG)	Polyclonal	Wako Chemicals	019-19741
Reactive astrocytes	GFAP	Rabbit (IgG)	Polyclonal	Dako	Z0334
Neurofilaments	NF-H	Mouse (IgM)	SMI37	Biolegend	SMI-37R
Amyloid precursor protein	APP	Rabbit (IgG)	Polyclonal	Abcam	ab2072
Vascular niche integrity	Fibrinogen	Sheep (IgG)	Polyclonal	Millipore Sigma	AB7144F
	Collagen IV	Rabbit (IgG)	Polyclonal	Abcam	Ab6586
SARS-CoV viral protein	SARS-CoV Spike	Rabbit (IgG)	Polyclonal	Sino Biological	40150-T62-COV2
SARS-CoV viral protein	SARS-CoV Nucleocapsid	Mouse (IgG1)	05	Sino Biological	40143-MM05
SARS-CoV viral protein	SARS-CoV Spike	Rabbit (IgG)	Monoclonal	antibodies-online	ABIN6952495
SARS-CoV viral protein	SARS-CoV Nucleocapsid	Mouse (IgG1)	3861	antibodies-online	ABIN6952433
SARS-CoV viral protein	SARS-CoV Envelope	Rabbit (IgG)	Polyclonal	antibodies-online	ABIN1031551

Table S3. Primer and probe sequences

Primer name	Sequence
ORF-1a	Forward: CAAAGGGAGGTAGGTTTGTACTT
	Reverse: CAAGGTGGTTCCAGTTCTGTAT
ORF-1b	Forward: TCCTTGGAATGTAGTGCGTATAA
	Reverse: CAAAGCCATGTGCCCATAAG
Spike	Forward: ACAGGCACAGGTGTTCTTAC
	Reverse: GATCACGGACAGCATCAGTAG
GAPDH	Forward: TGCACCACCAACTGCTTAGC
	Reverse: GGCATGGACTGTGGTCATGAG
2019-nCoV_N1	Forward: GAC CCC AAA ATC AGC GAA AT
	Reverse: TCT GGT TAC TGC CAG TTG AAT CTG
	Probe: FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1
2019-nCoV_N2	Forward: TTA CAA ACA TTG GCC GCA AA
	Reverse: GCG CGA CAT TCC GAA GAA
	Probe: FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1
RNase P (RP)	Forward: AGA TTT GGA CCT GCG AGC G
	Reverse: GAG CGG CTG TCT CCA CAA GT
	Probe: FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1

Table S4. Neuropathological findings in the postmortem brain

# Case	Histopathological findings (H&E)	Immunohistochemical findings (Analyzed brain regions)	MRI findings (Analyzed brain regions)
IA#1	Perivascular infiltrates, eosinophilic degenerating neurons in the cortex and hippocampus, perivascular pallor in the cerebellar white matter	(Olfactory bulb, Cortex, hippocampus, Pons, Medulla, Cerebellum, Choroid plexus) CD68 +++ infiltrates in the perivascular areas, widespread in the parenchyma; Lymphocytes +++ in the perivascular regions / parenchyma, GFAP +++ proliferation, appearing swollen in the deep WM; IBA1 +++ widespread infiltrates in the white matter, Fibrinogen leakage	(Olfactory bulb, Cortex Pons, Medulla, Cerebellum) Focal hyper- and hypointense signals
IA#2	Perivascular infiltrates, acutely hypoxic-ischemic neurons, hypertensive vasculopathy	(Olfactory bulb, Medulla) Gliosis of the olfactory bulb; Microglial activation in the olfactory bulb and medulla	Not analyzed
IA#3	None	Not analyzed	(Whole brainstem) Focal hyperintense signals
NY#1	Scattered acutely ischemic neurons	(Pons) CD68 +++ glial nodule; Lymphocytes + in the perivascular regions; GFAP +++	(Pons) Focal hyperintense signals
NY#2	Microglial prominence in medullary tegmentum, sparse leptomeningeal and parenchymal perivascular lymphocytes; acute neuronal ischemia, neuronal loss and gliosis, hippocampus (CA1 and subiculum)	Not analyzed	Not analyzed
NY#3	Acute neuronal ischemia, focal gliosis of hippocampus	Not analyzed	(Pons) Focal hyperintense signals

NY#4	Microglial prominence and focal encephalitis (neuronophagia) of insula, basal nuclei, pons, medulla; Incidental capillary telangiectasia in pons and basal ganglia	(Midbrain) CD68 +++ widespread, focal aggregate; Lymphocytes ++ in the parenchyma; GFAP +++; IBA1 +++ widespread, perineuronal aggregation,	(Midbrain) Focal hyperintense signals
NY#5	Focal mineralization of thalamic neurons	(Basal ganglia, Rostral pons) CD68 ++ perivascular infiltrates, widespread; Lymphocytes + perivascular/parenchymal cell infiltrates; GFAP ++ perivascular activation; IBA1 +++ widespread, Fibrinogen leakage	(Basal ganglia, Pons) Focal hyperintense signals
NY#6	Sparse perivascular parenchymal and leptomeningeal lymphocytes and microglial nodules in medulla	(Cortex, Pons, Choroid plexus) CD68 +++ glial nodules in pons; Lymphocytes +++ perivascular/parenchymal infiltrates; GFAP +++; IBA1 +++ perineuronal expression, glial nodule, Fibrinogen leakage	(Pons) Focal hyperintense signals (Spinal cord) No signal alterations
NY#7	Acute neuronal ischemia and microglial reaction, hippocampus and temporal neocortex	(Midbrain) CD68 +++ widespread, focal, perivascular aggregate	(Midbrain) Focal hyperintense signals
NY#8	Subdural neomembranes, old contusions, slight perivascular lymphocytes in pons	(Pons, Choroid plexus) CD68 ++ widespread; Lymphocytes + perivascular/parenchymal infiltrates; GFAP +++	(Pons) No signal alterations
NY#9	Right middle cerebral artery infarct (s/p ECMO), left middle cerebral artery inflammation	Not analyzed	Not analyzed
NY#10	Edema and astrocytosis in cortex	(Cortex) CD68 and GFAP +++ due to old TBI, not COVID related	(Cortex) No signal alterations
NY#11	Sparse perivascular lymphocytes	(Cortex) CD68 + perivascular infiltrates	Not analyzed

NY#12	Acute SAH	(Olfactory tract) CD68 +++ infiltrates; Lymphocytes +++ perivascular/parenchymal infiltrates; GFAP +++; IBA1 +++	(Olfactory tract) No signal alterations
NY#13	Sparse perivascular lymphocytes in medulla	(Cortex) CD68 + perivascular infiltrates	Not analyzed
NY#14	Acute SDH, SAD, and contusions	(Olfactory bulb) CD68 +++ infiltrates; Lymphocytes +++ parenchymal infiltrates	(Olfactory bulb) Focal hyperintense signals
NY#15	Not analyzed	Not analyzed	(Olfactory bulb) No signal alterations
NY#16	Sparse perivascular lymphocytes and microglial nodules in brainstem, neuronal storage of mucopolysaccharides, gliosis and neuronal loss in thalamus and hypothalamus	(Olfactory bulb) No abnormalities seen	Not analyzed

For abbreviations, see Table S1. Semi-quantitation of immunostained cells: += slight; ++=moderate; +++=numerous

In summary, of the 19 cases, vascular pathology as demonstrated by MRI, multiplex fluorescent imaging and/or H&E was found in 11 cases, perivascular infiltrates were present in 13 cases, and acute ischemic hypoxic neurons on H&E staining were seen in 6 cases. Changes suggestive of neuronophagia with microglial activation were present in 5 cases. In 2 cases none of these pathological findings were seen.

Table S4A: Brain regions studied by MRI and immunohistochemistry

No.	# Case	Brain region
1	IA#1	Olfactory bulb (MRI/IHC/MFI), Cortex and basal ganglia (MRI/IHC), Brainstem and cerebellum (MRI/IHC/MFI)
2	IA#2	Olfactory bulb (IHC), Brainstem (IHC)
3	IA#3	Brainstem (MRI)
4	NY#1	Brainstem (MRI/IHC)
5	NY#2	ND
6	NY#3	Brainstem (MRI)
7	NY#4	Brainstem (MRI/IHC)
8	NY#5	Cortex and Basal ganglia (MRI/IHC), Brainstem (MRI/IHC/MFI)
9	NY#6	Cortex (IHC), Brainstem (MRI/IHC/MFI), Thoracic spinal cord (MRI)
10	NY#7	Brainstem (MRI/IHC)
11	NY#8	Cortex (MRI), Brainstem (MRI/IHC)
12	NY#9	ND
13	NY#10	Cortex (MRI/IHC)
14	NY#11	Cortex (IHC)
15	NY#12	Olfactory tract (MRI/IHC)
16	NY#13	Cortex (IHC)
17	NY#14	Olfactory bulb (MRI/IHC/MFI)
18	NY#15	Olfactory bulb (MRI)
19	NY#16	Olfactory bulb (MFI)

ND = not done; MRI = magnetic resonance imaging; IHC = chromogenic immunohistochemistry; MFI = multiplex fluorescent imaging

Table S5. Test results of SARS-CoV-2 RNA

Case #	Brain region	PCR on frozen tissue	PCR on fixed tissue	RNA seq on frozen tissue	RNA scope
IA#1	olfactory bulb	Negative	ND	Negative	Negative
	frontal cortex	Negative	Negative	Negative	Negative
	pons	Negative	Negative	Negative	Negative
	medulla	ND	Negative	ND	Negative
	cerebellum	ND	Negative	ND	Negative
IA#2	olfactory bulb	Negative	ND	Negative	ND
	frontal cortex	Negative	ND	Negative	ND
	pons	Negative	ND	Negative	ND
IA#3	frontal cortex	Negative	ND	Negative	ND
	pons 1	Negative	ND	ND	ND
	pons 2	Negative	ND	ND	ND
	pons 3	Negative	ND	ND	ND
NY#2	pons	ND	ND	ND	Negative
NY#3	midbrain	ND	ND	ND	Negative
NY#4	basal ganglia	ND	ND	ND	Negative
	pons	ND	Negative	ND	Negative
NY#6	cortex	ND	ND	ND	Negative
	pons	ND	Negative	ND	Negative
NY#7	midbrain	ND	ND	ND	Negative
NY#9	cortex	ND	Negative	ND	ND
NY#11	olfactory bulb	ND	Negative	ND	Negative
NY#12	olfactory tract	ND	Negative	ND	Negative
NY#14	Carotid body	ND	ND	ND	Negative

ND = not done

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