Reactive and acute inflammatory microvasculopathy in 36 COVID-19 autopsy brains

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Abstract

Background: Hypercytokinemia, the renin-angiotensin system (RAS), and hypoxia are implicated in brain morbidity in COVID-19. There is little evidence of direct SARS-CoV-2 brain infection, although focal microvascular infection and 'planted' antigens have been suggested.

Methods: Histopathology of the brains from 36 consecutive autopsies of patients who were RT-PCR positive for SARS-CoV-2 was studied. Immunostaining for serum proteins, complement components and virus, as well as viral *in-situ* hybridization, were employed. The Mann-Whitney U test was used to compare activation of complement in brain microvessels in COVID-19 cases with immunostaining findings in pre-pandemic autopsy brains.

Results: Neuropathologic findings in this COVID-19 cohort identified widespread reactive microvasculopathy (ectasia, mural distortion, and intussusceptive arborization) and acute intraluminal neutrophilic endotheliitis in the microcirculation in all 36 cases. Prominent vascular neutrophilic transmural migration was found in several cases where it was best identified in larger microvessels. In 25 cases there was acute microcirculatory perivasculitis. Activation of complement components, which included membrane attack complex, was significantly higher in microvascular walls in the COVID-19 cohort than in pre-pandemic cases.

Conclusions: The literature suggests that in COVID-19 patients reactive microvasculopathy most likely originates from hypoxia, hypercytokinemia, and RAS dysfunction, while direct and indirect virus-induced factors may contribute. Acute endotheliitis, transmural migration, and acute perivasculitis constitute the early phase of type 3 hypersensitivity vasculitis in all of the cohort cases. The presence of activated complement components in microvascular walls in COVID-19 autopsy cases compared to controls is consistent with type 3 hypersensitivity vasculitis. Viral antigen in or 'planted' on microvessels or other antigen-antibody complexes could cause of this type of autoimmune vasculitis proximate to death. Coupling of neurogliovascular units could be compromised, if only temporarily in various brain regions, during the progression (initiation to healing) of these microvascular findings even in the absence of thrombosis or mural dehiscence. However, no specific neurological alteration in this cohort can be attributed directly to specific histopathologic findings.

Background

The SARS-CoV-2 pandemic beginning in 2019 involves injury to the major organs including the central nervous system (CNS). The major classes of CNS lesions in the resulting disease of COVID-19 include infarcts, hemorrhage, encephalopathy, encephalitis, and possible para- and postinfectious immune-related conditions [1–3]. Multifocal immunostaining for SARS-CoV-2 proteins has been reported in brain tissue [3, 4] and there is focal evidence of virions in brain parenchyma and in vascular endothelial cells [5–10]. Neuropathology studies have also revealed mild cellular parenchymal inflammation, focal vascular wall or perivascular chronic inflammation [2, 11], and microcirculatory ectasia [12].

The suggested origin of CNS lesions in COVID-19 include the entrance of SARS-CoV-2 through the olfactory apparatus or infection of vascular endothelial cells, with indirect CNS damage from hypoxia due to pulmonary disease, coagulopathy, or virus-induced dysfunction or imbalance of the renin-angiotensin system (RAS) [1, 3, 13–15]. Autoimmunity may play a role in CNS damage with the development of hypercytokinemia (“cytokine storm”), while it has also been speculated that type 3 hypersensitivity vasculitis might occur in the CNS [1, 3, 4, 16, 17].

This report describes microcirculatory findings that may be relevant to neurological complications of COVID-19. These microcirculatory alterations, including reactive microvasculopathy and acute neutrophilic endotheliitis, were seen in 36 consecutive autopsy brains available for study.

Methods

Case Selection

Following appropriate autopsy room engineering clearance and upgraded equipment acquisition, available brains removed from 36 COVID-19 autopsies were studied. All patients had at least one RT-PCR–positive SARS-CoV-2 test and clinical findings typical of COVID-19 as previously described in our region’s population [18]. Eight non-COVID-19 cases were selected for immunostaining comparison of selected complement components.

Results

COVID-19 case cohort clinical and histopathologic findings

Clinical features of 36 COVID-19 autopsy cases included adults ranging from 32 to 84 years of age, with 17 being male, and with 25 African Americans. Survival after hospital arrival ranged from 30 minutes to 84 days (mean survival 20.4 ± 18.1 days). All patients had comorbidities typical of COVID-19 including hypertension (69.4%), diabetes mellitus (52.8%), obesity (50%), ischemic heart disease (41.7%), chronic pulmonary disease (22.9%), dementia or mental disorder (16.7%), cancer (11.1%), drug abuse (8.3%), and HIV infection (8.3%). Three patients had no past medical history recorded. Respiratory distress or acute respiratory failure at admission were common findings. Many patients reported headache, weakness, fatigue, or loss of smell shortly prior to admission. D-dimer serum levels were taken in 21 cases, including one within normal reference range with others varying from mildly to greatly elevated. Ventilator time varied from acute use only to multiple weeks of use, not always continuously. Steroid therapy was provided in 22 cases, remdesivir was given in 13 cases, and there was administration of tocilizumab in three cases and of convalescent plasma in one case. There were seven deaths between 5 and 9 AM (19.4% of cases, a period accounting for 16.6% of the day) and 12 deaths between 4 and 10 AM (33.3% of cases; 25% of the day). One patient was fully vaccinated against SARS-CoV-2. Two patients had one vaccine dose (Table 1).

Table 1
Selected clinical data for 36 COVID-19 autopsy brain cases

Case no.	Age (yr)/sex	Major comorbidities	BMI[a]	D-dimer[b]	Ventilator (da)	COVID-19–related treatment	Survival (da)/time of death
1[c, d]	67/M	DM2	20.1	N/R	Acutely	N/R	0.021/0530
2[c]	61/F	N/R	N/R	N/R	Acutely	N/R	0.035/1316
3	44/M	HIV, HCV, TB, recent head injury rehabilitation	25.1	N/R	Acutely	N/R	0.063/0543
4	84/F	HTN, IHD, arthritis, dementia, chronic venous stasis	23.4	N/R	Acutely	N/R	0.09/1408
5	47/F	HTN, DM	N/R	N/R	Acutely	N/R	0.167/1728
6	72/F	N/R	43	N/R	Acutely	N/R	0.25/1245
7	61/M	HTN, IHD, COPD, ethanol abuse	22	620 DDU	2	DEX, REM	4/0511
8	76/F	HTN, DM, IHD, HIV	25.9	326 DDU	Not intubated	DEX	5/0444
9	49/M	HTN, SAD, BD	34.9	6.9 FEU	1	N/R	5/1404
10[c]	37/M	HTN, HCV, HPV, hepatocellular carcinoma, IV drug use	26	N/R	Not intubated	N/R	5/0744
11	65/M	HTN, DM2, IHD, COPD, pulmonary fibrosis, obesity	32.6	2551 DDU	< 1	DEX, REM	6/1330
12[c]	49/F	HTN, obesity	67.2	N/R	2	DEX, TOC	8/2340
13	61/F	IHD, COPD, emphysema, asthma, rectal carcinoma	22.7	167 DDU	8	DEX	8/0243

Table 1
Selected clinical data for 36 COVID-19 autopsy brain cases (continued)

Case no.	Age (yr)/sex	Major comorbidities	BMI[a]	D-dimer[b]	Ventilator (da)	COVID-19–related treatment	Survival (da)/time of death
14	64/F	HTN, DM2, remote stroke, obesity	34.6	N/R	2	N/R	10/1350
15	63/M	HTN, atrial fibrillation, obesity	46.1	45144 DDU	12	DEX, VAN	12/2025
16[c]	53/F	HTN, DM2, COPD, pulmonary hypertension, emphysema, obesity	47	N/R	Not intubated	TOC	13/1818
17[c]	58/F	HTN, DM, IHD, atrial fibrillation, obesity, CRD	52	N/R	4	N/R	16/1749
18	61/F	HTN, DM2, obesity	51.4	2528 DDU	17	DEX	17/1957
19	68/M	HTN, DM2, CRD, anemia of chronic disease	21.8	2574 DDU	Not intubated	DEX	19/1100
20	62/F	HTN, DM2, IHD, bilateral carotid artery stenosis, HCV, obesity, hypothyroidism	36.8	523 DDU	5	DEX, REM	19/0437
21	69/F	HTN, DM2, IHD, obesity, CRD	44.5	2462 DDU	11	DEX, REM	22/0708
22[e]	56/F	HTN, COPD, asthma, IHD, DM, obesity, CRD, liver disease, blood clotting disorder, DVT	45.5	N/R	Not intubated	DEX, REM	23/0914

Formalin-fixed, paraffin-embedded tissue included 32 to 50 blocks from cerebrum, cerebellum, and brainstem in each COVID-19 cohort case. Blocks included all cerebral and cerebellar lobes and all three brainstem levels. Olfactory bulbs and tracts were taken in 27 cases.

Gross examination of the COVID-19 cohort included brain swelling with cerebellar tonsillar herniation in three cases, non-hemorrhagic cerebral infarcts in eight cases, and mild signs of brain atrophy consistent with age and comorbidities in a few cases. Three cases had significant numbers of petechial hemorrhages scattered in the brain, while several cases had only an occasional petechial hemorrhage. The most significant hemorrhage was in Case 4 where histopathologic findings associated with large hemorrhages included recent cerebral infarcts. Both the hemorrhages and infarcts contained significant infiltrations of polymorphonuclear neutrophils (PMNs). Stains were negative for micro-organisms. One of the cases with cerebellar tonsillar herniations had global hypoxic nerve-cell change. Case 26 had large,

non-hemorrhagic intermediate infarcts in the territory of both middle cerebral arteries. Thrombotic or atherosclerotic obstruction of the common carotid arteries had been identified in that case by clinical imaging. A few scattered microcirculatory paravascular hemorrhages were identified microscopically in many cases. Microthrombi were frequently present in the microcirculation throughout the cohort although in most instances they did not appear to be obstructive. Immunostaining for platelet protein or for fibrin or fibrinogen showed very few microvascular lumina with apparent obstruction. Case15, with hypertension and atrial fibrillation, had relatively few non-microvascular findings, but a non-occlusive organizing basilar artery thromboembolus was present. Thirteen cases (36.1%) had microglial nodules in the brainstem. Isolated neuronophagia was present in the brainstem in six cases (16.7%).

The most numerous brain findings in this cohort, aside from frequent neuronal hypoxic change, were in the microcirculation. The most common microcirculatory mural alterations were types of reactive microvasculopathy. The most common element of reactive microvasculopathy was simple dilation (ectasia) which was present as mild to extensive luminal expansion (Fig. 1A and B). Microcirculatory dilation was frequent in most microscopic sections in all but two cases where it was more sparsely observed. Microthrombi, mostly in dilated microchannels, were found with varying frequency within the cohort. Involved microvessels were sparse in some cases while they tended to be frequent in others, although no quantification was done (Fig. 1A). This finding did not suggest luminal blockage. Many scattered microvessels in most cases were filled with an accumulation of red blood cells and serum protein (Fig. 1B and C) or with white blood cells enmeshed in a loose fibrin network or in a loose platelet-fibrin thromboembolus (Fig. 1D).

A second type of reactive microvasculopathy, frequent in all but Cases 11 and 29, consisted of mural distortion in vessels that were mildly to greatly dilated. This second reactive alteration was found in almost every microscopic section of cerebrum and brainstem as well as in many cerebellar sections in 34 of the cohort cases. It was distinguished minimally by irregular mural distortion that often included one or more ampoule-like segments or waists (Fig. 1E). These microcirculatory walls frequently took on a serpiginous or rhythmical appearance formed by adjacent mural waists, including in an occasional capillary (Fig. 1F). With an oblique or cross section, this type of mural distortion with many waists took on a round-tooth, crenated, or starburst appearance (Fig. 2A). Thirty cases (83.3%) had at least a few thin-walled microcirculatory channels with a sinuous, frond-, or tuft-like mural deformation. Channels with this pronounced distortion of their wall were most numerous in cases with longer survival (Fig. 2B–E). In all but one case, variable numbers of microcirculatory channels had adventitial collagenosis or more compact hyaline sclerosis. Dilated or distorted capillaries and microvessels included channels with collagenosis and, in the case of microvessels, with more compact adventitial collagen (Fig. 2D and F).

The third type of reactive microvasculopathy was intussusceptive arborization (IA). IA appeared on cross-section as two or more microcirculatory lumina with a shared wall (Fig. 3A) or as clustered, dilated capillaries. In longitudinal section, IA appeared as crowded capillaries that were mildly twisted (‘looping’) in relation to one another (Fig. 3B). Infrequently-encountered intraluminal (intussusceptive) projections of endothelial cells extended toward the opposite wall (Fig. 3C). These extensions formed cellular

transluminal partitions, or pillars. All cases had some IA present and it was extensively distributed in most cases, being identified at least focally in most microscopic sections. Enlargement of IA complexes appeared as ‘mini-glomeruloid’ formations (Fig. 3D). At least a few ‘mini-glomeruloid’ formations were identified in 14 cases (38.9%).

All cases had many atrophic or regressed (‘string’) capillaries appearing as tubes with a narrow or collapsed and usually empty lumen. Some similar but lengthy microcirculatory channels may have been pre-capillary arteriolar shunts or postcapillary venules rather than capillaries (Fig. 3E). In most cases ‘ghost’ capillaries with no mural nuclei or luminal contents were identified, and these were occasionally frequent at least focally (Fig. 3F).

All 36 cases had acute neutrophilic endotheliitis with a variable amount and distribution within the cohort from frontal lobe to medulla. This finding included only a few vessels in three cases. Acute endotheliitis was recognized in the brain’s microcirculation by the presence of intraluminal PMNs with karyorrhexis (nuclear fragmentation, often including ‘nuclear dust’ formation; also termed “leukocytoclasis”). Affected microcirculatory channels generally had a thin wall, including some large microvessels (Fig. 4) (Table 2).

Table 2
Sites of acute endotheliitis and acute perivasculitis in 36 COVID-19 autopsy brain cases

Case no.	Acute endotheliitis	Acute perivasculitis
1	L thalamus, L internal capsule	None
2	L frontal pole, parietal lobes, R lat temporal lobe, R insula, L calcarine cortex, L med hypothalamus, R internal capsule, R lenticular nuc, cblr inferior vermis, rostral pons, mid-level pons	Mid-level pons
3	Cblr inferior vermis, R midbrain tegmentum, L pontine tegmentum, bulbar nuc of the spinal tract of nerve V, bulbar nuc cuneatus	None
4	Caudal L midbrain, rostral R basis pontis	Rostral L midbrain tegmentum
5	R parietal lobe, med and lat temporal lobes, R insula, L calcarine cortex, R and L corpus striatum, R thalamus, cblr superior vermis and right lat lobe, rostral L pons	None
6	Med occipital lobe, putamen, thalami, anterior perforated substance (basal forebrain olfactory area), cblr lat lobes, midbrain and pontine tegmentum levels (including locus coeruleus), basis pontis levels, mid-level medulla	R temporal fusiform gyrus
7	L frontal pole, R hippocampus, L temporal periventricular white matter (adjacent to caudate nuc tail), cblr R lat lobe	None
8	R frontal pole, L temporal entorhinal cortex, R lat temporal lobe, R parietal lobe, calcarine cortex, R thalamus, R putamen, internal capsule, midbrain tectum, caudal midbrain, rostral basis pontis, bulbar reticular formation	R substantia nigra, caudal midbrain tegmentum, R mid-level basis pontis
9	R lat temporal lobe, R internal capsule, cblr white matter, R pontine tegmentum, L pontine locus coeruleus, basis pontis levels	Cblr L lat lobe

Table 2
Sites of acute endotheliitis and acute perivasculitis in 36 COVID-19 autopsy brain cases (continued)

Case no.	Acute endotheliitis	Acute perivasculitis
10	L frontal pole, parietal lobes, L med temporal lobe, R and L insula, L corpus striatum, thalami, lenticular nuclei, cblr vermis and lat lobes, rostral L midbrain, mid-level and caudal midbrain, rostral pons, mid-level pons, caudal pons, mid-level medulla	R lat temporal lobe, R insula, L lenticular nuc, mid-level midbrain, pontine levels, bulbar levels
11	Frontal, parietal, temporal, insular and calcarine cortex; R hippocampus; thalami; caudate nuclei; internal capsules; L external and extreme capsules; cblr vermis and lat lobes; middle cblr peduncle; midbrain; pontine tegmentum and basis pontis; nuc of tractus solitarius; nuclei of cranial nn V and XII; inferior vestibular nuclei; inferior olivary nuc; olivocerebellar tracts; pyramid	L globus pallidus interna
12	Parietal lobes, R and L insula, R and L corpus striatum, lenticular nuclei, thalami, internal capsules, rostral R midbrain, mid-level midbrain	L frontal pole, L insula, L thalamus, mid-level L midbrain
13	R frontal pole; med and lat temporal lobes; R and L insula; L calcarine cortex; R and L corpus striatum; lenticular nuclei; thalami; cblr vermis and lateral lobes; rostral, mid-level and caudal midbrain; rostral, mid-level and caudal pons; rostral and mid-level medulla	Rostral pons
14	L frontal pole, R calcarine cortex, L internal capsule, R cblr lat lobe, mid-level midbrain, mid-level pons	None
15	Frontal poles, parietal lobe, R temporal entorhinal cortex, L lat temporal lobe, occipital lobes, R putamen, thalami, L basal forebrain, R cblr lat lobe, rostral R midbrain, caudal midbrain, basis pontis levels	Rostral basis pontis
16	Internal capsules, R putamen, R thalamus	L putamen
17	L parietal lobe, R corpus striatum, R calcarine cortex, R internal capsule	None

Direct microcirculatory damage from acute endotheliitis was not necessarily found. However, 15 cases (41.7%) had at least one dehiscent capillary (or possibly larger disrupted thin-walled microvessel), with or without limited perivascular hemorrhage. In most of the cohort cases, there were a few scattered microcirculatory vessels with a suggestion of poorly-formed platelet-fibrin thrombi accompanied by PMNs and a few mononuclear cells, and karyorrhectic PMNs were in a few of these vessels (Fig. 4C). Intraluminal eosinophils were found occasionally. Fibrinoid necrosis and intimal fibrous thickening were not seen in the microcirculation.

Transmural PMN migration that resulted in acute perivasculitis involved at least one microvessel in 25 cases (69.4%) (Fig. 5A–E) (Table 2). Karyorrhectic PMNs were often identified among the perivascular PMNs of acute perivasculitis (Fig. 5C–E). Mural transmigration of PMNs from the lumen to the

perivascular space were apparent in a few dilated, thin-walled microvessels (Fig. 4D). Four cases had a few subarachnoidal arterioles in which transmigration was more prominently seen (Fig. 5F). In two of these cases, arteriolar transmigrating PMNs were immunostained for myeloperoxidase.

Overall, a paucity of apparently obstructive thromboemboli was found and there was no pattern to their location. Two microcirculatory channels with thromboemboli on routine hematoxylin and eosin stain were of note because of their location in and near the nucleus of the tractus solitarius. In this instance, the thromboemboli were accompanied by prominent reactive astrocytosis (Fig. 6A).

In 18 cases (50%), a few capillaries, scattered in the brain including in the brainstem, had large, hyperchromatic mural or luminal cell nuclei. In most of these cases, the dark nuclei were greatly elongated with inhomogeneous dense to more open or fenestrated-appearing regions (Fig. 6B and C), but as a group the morphology was not entirely uniform. Some of the densely-stained, elongated nuclei may have been large, hyperchromatic nuclei of hypoxic vessel walls or pillar cells forming IA.

Most cohort cases had mild perivascular chronic inflammatory cellular infiltration that may have represented immunosurveillance. Increasingly prominent yet not marked focal perivascular cuffing appeared to represent chronic perivasculitis in at least four or five cases.

Immunostaining and *in-situ* hybridization for SARS-CoV-2

SARS-CoV-2 immunostaining using antibodies from several sources for antigen identification resulted in negative findings. *In-situ* hybridization was also negative for viral localization in the brain.

Immunostaining of activated complement components

Immunostaining for complement components C3d, C4d, and C5b-9 (membrane attack complex) was performed on 15 cerebral and/or brainstem sections, selected for acute inflammatory microcirculatory findings, from six COVID-19 cases. Five cases were female. COVID-19 patient comorbidities in this group included hypertension (83.3%), obesity (66.7%), ischemic heart disease (66.6%), chronic pulmonary disease (33.3%), diabetes mellitus (33.3%), psychiatric disorder (33.3%), and cancer (16.7%).

Control cases included 16 sections (four cerebral, four brainstem) from eight cases with death prior to December, 2019. Control case comorbidities included hypertension (75%), obesity (62.5%), diabetes mellitus (50%), ischemic heart disease (25%), psychiatric disorder (12.5%), cancer (12.5%), and alcohol abuse (12.5%). Four cases were female. The age range was 53 to 86 years.

Formalin-fixed, paraffin-embedded sections immunostained for complement components were evaluated to determine the number of microcirculatory channel walls positive either focally or diffusely for any one of the three separate complement component stains from each brain tissue block. Complement component staining subtotals were combined for each tissue section as an indication of complement activation *in-situ* in that tissue block. Staining was zero to 40 microcirculatory channel walls in COVID-19 brain tissue sections (Fig. 6D–F). Complement component immunostaining was mostly zero in controls,

with the oldest control patient (with hypertension and cancer) having 14 positive microcirculatory vessels in one section. C4d was more commonly heavily positive than C3d or C5b-9. Some microvascular walls, particularly with C4d complement component activation, appeared to have staining in separated cellular layers (Fig. 7A and B). The combined totals comparing all positive microcirculatory walls in COVID-19 cases with the positive channels in controls were subjected to the Mann-Whitney U-Test (z-ratio, 3.4; $p < 0.01$).

Karyorrhectic PMNs in the COVID-19 cohort sections used for immunostaining were not positively-stained in any microcirculatory channels, where flowing blood was likely to have exchanged luminal cells prior to death. However, two of the cases had acute perivasculitis in which C4d and C5b-9 were present in association with PMNs in affected perivascular spaces (Fig. 7C–F).

Discussion

The 36 autopsy cases of COVID-19 include adults from close to middle age to elderly, there is almost equal gender representation, and presentation is often with hypoxia and frequently with the diagnosis of 2019 novel coronavirus-infected pneumonia. Clinical course varies considerably in length and complexity. Most patients are hypertensive with adventitial sclerosis as evidence of microvascular wall injury, over half have diabetes mellitus, half are obese, many have chronic heart and/or pulmonary disease, and a few have a history of cancer or other comorbidities. These are the findings in most COVID-19 patients in this age range [1, 3, 18]. African Americans account for over half of the cohort cases, but given the small sample size no effect of race is inferred.

Brain microcirculatory system and microvasculopathy

Normal CNS blood vessels 40–400 μm in diameter generally are referred to as microvessels, and when including capillaries the term microcirculation is used [19]. In normal physiological and in pathological conditions, capillaries and microvessels readily dilate and the microcirculation may require further enhancement of its normal physiological remodelling. This can include segmental capillary atrophy (pruning of regressed capillaries) and the formation of IA [20, 21]. Regressed capillaries that appear during physiological remodelling have been described as ‘strings’ or ‘empty sleeves’ [22]. The CNS microcirculation is below direct detection by magnetic resonance imaging (MRI), although through specific imaging methods the occurrence of brain microvasculopathy in COVID-19 patients has been suggested [23].

Both reactive and acute inflammatory microcirculatory alterations are present in all 36 COVID-19 cohort cases. Principal conditions speculated to underlie microcirculatory injury from COVID-19 are hypoxia [1, 10, 13, 24], hypercytokinemia, and RAS dysfunction [1, 3, 21]. Autoimmunity associated with hypercytokinemia or expressed as type 3 hypersensitivity vasculitis in the CNS has been postulated as a cause of vascular damage in COVID-19 [1, 3, 16, 17]. Predisposing conditions causing chronic vascular-wall injury (*e.g.*, hypertension, diabetes mellitus, chronic hypoxia), as found in this cohort, might leave microcirculatory channels prone to autoimmune damage [17].

Significant direct SARS-CoV-2 infection of the CNS remains an open question. In one immunostaining study, SARS-CoV-2 spike and membrane proteins co-localize in the brain with vascular endothelial staining and with caspase 3, suggesting microcirculatory endothelial-cell viral attachment and perhaps infection with apoptosis. Other organs in that study have similar microcirculatory findings, while the additional step of investigating co-localization of viral RNA in extracranial blood vessels is negative. The conclusion, which may apply to the CNS microcirculation, is that endocytosed pseudovirions formed only of SARS-CoV-2 protein may be associated with vascular endothelial cells [4].

The reactive microvasculopathy in the COVID-19 cohort is of note for its frequency and morphology. Microcirculatory channels with waists, those with starburst profiles, and others with a sinuous, frond-, or tuft-like appearance most likely have a similar origin following mural injury. The IA component is well known in hypoxic brain and in brain tumors [20, 25], and IA has been found in COVID-19 pulmonary tissue more often than in controls [26]. While not being disease-specific, the combination in most of our cohort cases of widespread dilated, distorted, thin-walled microcirculatory channels and of frequent IA in so many microscopic sections is unusual.

Ampoule-like waists similar to those in COVID-19 reactive microvasculopathy have been reported in ataxia-telangiectasia (A-T) in the brain [27, 28], in experimental episodic brain arteriolar network occlusion [29], and in an ischemia-perfusion model in which pericyte contraction causes persistent waists that last even into a return to normoxia [30]. More complex frond-like microvessels, somewhat similar to those in COVID-19, have been described in A-T [31]. The A-T mutation prevents proper microcirculatory remodelling after physiological stress because of the absence of anti-angiogenic mediation that would have been the required brake on the growth and permeability phase of normal microcirculatory healing [32, 33].

The morphology of microcirculatory mural irregularities in the brain in COVID-19, particularly sinuous, frond-, or tuft-like channels, suggests the possibility of stalled microcirculatory remodelling following mural injury when comparing these changes to findings in A-T [31] and to models of experimental hypoxia [20]. These animal models of microcirculatory stress directly address stalled healing.

In general, microcirculatory injury causes hypoxia. In the milieu promoted by microcirculatory injuries, endothelial cells are sensitive to pro-angiogenic stimuli that cause endothelial cells to lose contact with pericytes, which makes endothelial cells prone to apoptosis. This results in segmental regression (atrophy, ‘string’, ‘ghost’, or ‘empty sleeve’ capillaries). In the healing phase (remodelling, pruning), there is competition between pro-angiogenic stimuli and anti-angiogenic mediators such as cytokines. Anti-angiogenic mediation predominates in this phase, allowing vascular regression and healing even in the continued presence of growth stimulation. The inability of pro-angiogenic stimuli to overpower anti-angiogenic mediators avoids a constant proliferative and permeability phase during normal microcirculatory restitution. However, during severe hypoxic stress, including conditions in which IA might develop, the microcirculatory remodelling phase can stall because competing mediators are out of physiological balance [20].

Stalled microcirculatory healing in the brain during severe hypoxia might be exacerbated by hypercytokinemia in COVID-19. The result may be the development of sinuous and other distorted microvascular walls that are reminiscent of A-T microvascular fronds. Furthermore, hypercytokinemia might be one factor in COVID-19 that presumably could stall brain microcirculatory healing even when hypoxia is not severe by upsetting the physiological balance during remodelling mediation.

IA formation appears to be more straightforward as a response to chronic hypoxia. However, IA in the preponderance of sections within the cohort is very uncommon if not unique in a viral disease. 'Mini-glomeruloid' microvascular formations resulting from pronounced IA further suggest an effect induced by hypoxia [20, 25]. However, in COVID-19 it is possible that additional aberrant capillary growth mediation might be involved including perhaps influences of hypercytokinemia or perhaps RAS activity.

Acute neutrophilic endotheliitis involves various organs in COVID-19, but it has not been demonstrated in the CNS in this disease [11, 17]. In our cohort, acute endotheliitis is found in two of the three major brain regions in half of the cases, including in the brainstem in all but one case. Acute endotheliitis is an autoimmune vasculitis that is the early phase of type 3 hypersensitivity vasculitis. In acute neutrophilic endotheliitis, karyorrhexis is seen as a tuft-like, beaded, or 'nuclear dust' PMN nuclear fragmentation within microcirculatory channels, as found throughout our case cohort. PMN activation in this phase of autoimmune vasculitis is the main vascular damage effector along with hypercytokinemia [15, 34]. Many karyorrhectic intraluminal PMNs mark the microcirculation affected by acute endotheliitis in our cohort. This finding is beyond the occasional karyorrhectic PMNs found within the CNS microcirculation in some autopsy cases, particularly in patients with hypertension where the intermediate fibrinoid necrosis stage of type 3 hypersensitivity vasculitis may develop [34–36]. It is also of note that karyorrhectic PMNs in autoimmune vasculitis precede mononuclear cell recruitment in small-vessel vasculitis, and that these vasculitides can include eosinophils [34], as identified in our COVID-19 cohort.

Dehiscent microcirculatory channels might be consistent with type 3 hypersensitivity-induced small-vessel necrosis [34, 37]. However, dehiscent channels may also form in a different manner in this cohort such as following hypercytokinemia, RAS dysfunction, and microcirculatory remodelling, as well as following thromboembolism [38] and possibly the effects of viral capsid proteins or direct infection. Dehiscent microcirculatory channels might also develop after stalled healing of capillaries when pericytes are lost and endothelial cells undergo apoptosis.

Neutrophilic extracellular traps (NETs), although proposed as a possible mechanism for cerebrovascular injury [39], have yet to be demonstrated in the CNS in COVID-19. NET morphology involves extracellular chromatic material with a different morphology than apoptosis with pyknosis or nuclear fragmentation [40].

Findings in this cohort proximate to death consistent with the early phase of type 3 hypersensitivity vasculitis, in addition to acute endotheliitis, include PMN mural transmigration which leads to acute perivasculitis [34, 36, 41, 42]. Acute perivasculitis is present in 72.2% of our cohort cases. Fibrinoid necrosis and intimal fibrosis that occur in subsequent stages of type 3 hypersensitivity vasculitis are not

identified. The early neutrophilic phase may occur days to weeks after circulating immune complexes form in response to a foreign antigen or through molecular mimicry (often to a viral infection). The immune complexes can deposit on a tissue site, which is typically a vascular wall in a viral infection. In some instances, the circulating antibody will complex with a ‘planted’ antigen [15, 17]. The possibility of circulating and ‘planted’ SARS-CoV-2 protein in brain microcirculatory channels requires further scrutiny.

An interesting ‘planted’ antigen scenario has been suggested for SARS-CoV-2 infections. Sequence analysis demonstrates that some human chaperone proteins might be able to participate in molecular mimicry with SARS-CoV-2 because of shared amino acid sequences. It has been postulated that chaperones might become localized in vascular endothelial-cell plasma membrane following signalling from shear and metabolic stress such as that related to risk factors for hypertension and diabetes mellitus [43].

Specific inhibitors of anaphylaxis might provide effective prophylactic treatment for terminal complement component generation in type 3 hypersensitivity vasculitis [17]. Treatment for acute endotheliitis is likely to include anti-inflammatory and immune-modulating drugs and also inhaled nitric oxide to induce vasodilation and for its anticoagulant and direct antiviral activity [44]. Dexamethasone treatment during at least part of the disease course had no appreciable effect in our cohort.

Other potential CNS effects due to microvasculopathy in COVID-19

Cardiopulmonary pacing regions in the brain include the major integrating nucleus of the tractus solitarius and its many connections, such as the hypothalamus, the sensorimotor cortex, and the insular cortex [45]. All of these brain regions exhibit microcirculatory injury in this COVID-19 cohort, including both reactive microvasculopathy and less frequently acute endotheliitis. From a viewpoint centered on COVID-19 microvasculopathies, a variety of functional microcirculatory problems might arise in any brain area from any of the aforementioned origins in COVID-19 to result in local neuronal dysfunction reflected as isolated, multifocal, fleeting, recurring, minor, or devastating CNS or peripheral nervous system change.

Vascular endothelial cells, microvascular-wall pericytes, perivascular astrocytes, resident CNS microglia, and neurons form neurogliovascular units (NVUs) in the CNS. Neuronal activity induces this cellular complex, which is coupled together in each NVU, to cooperate in the regulation of blood flow in support of uninterrupted neuronal transmission. Microcirculatory remodelling following infarcts, hemorrhage, and viral infections is accompanied by NVU uncoupling during which activation of pericytes, astrocytes, and resident microglia occurs, including the production of pro-inflammatory cytokines. NVU uncoupling decreases the client neuron’s energy availability, thereby decreasing neuronal activity. Reactive, thromboembolized, or acutely inflamed CNS microcirculatory channels thus provide a negative effect on neuronal activity by denying the metabolic support of episodically-required blood flow [30, 45]. Functional MRI performed on COVID-19 patients has shown early results regarding brain regions with neuronal activity/vascular flow mismatches that would be the expected finding in brain circulatory lesions [46, 47].

Hypoxia sufficient enough to induce a sustained contraction of pericytes results in ampoule-like waists in the microcirculation until after NVU recoupling begins [30, 48]. Therefore, the waists in dilated microvessels in the COVID-19 cohort might serve as signs of recent or continuing NVU uncoupling. This appears to apply to Case 1 with only minutes of survival upon hospital arrival as well as to the remainder of the cohort with survival up to 84 days.

In severe hypoxia, these pericyte-mediated microvascular contractions trap red cells [30, 48]. This microcirculatory finding is mimicked during temporary arteriolar network occlusion in animals wherein the microcirculation is distorted by ampoule-like waists and there is red cell and serum protein accumulation in non-flowing microvessels until functional shunting of retrograde flow begins [29]. The dilated microvessels filled by red cells and serum protein in our COVID-19 cohort might only be signs of a terminal event. However, it is possible that such functional shunting to provide oxygen and energy to deprived microvascular beds occurs in COVID-19 cases considering the extent of the reactive microvasculopathy. This set of microcirculatory alterations could be an additional burden on the maintenance of neuronal activity that would likely already be burdened by hypoxia, hypercytokinemia, microthrombi, thromboembolism, acute endotheliitis, and possibly pseudovirion endocytosis or a viral infection. A further effect postulated to “stall” capillary blood flow in COVID-19 patients is the presence of increased mononuclear cells that are part of the inflammatory response [24]. Together, these concomitant microcirculatory stress factors might underlie some of the reported neurological symptoms in COVID-19 [1, 3, 24].

A further factor that could induce microcirculatory stress has been the observation of possible megakaryocytes in cerebral capillaries in COVID-19 [49]. There is a similar finding in 18 of our cohort cases (50%). It should be noted, however, that large, hyperchromatic capillary mural nuclei, occasionally with a syncytial appearance, can be found in CNS and skeletal muscle endothelial cell infections caused by some bacteria and viruses [50, 51]. Enlarged but often clear capillary mural nuclei are reported in animal models of cerebral hypoxia [52]. Intussusceptive extension of endothelial cells which have large dark nuclei early in IA have a similar appearance [26], as seen in our cohort. It is likely that these scattered, single-capillary findings in tissue sections are not of a single cause, and further study is warranted.

Finally, note is taken of the possibility of a central hypoventilation syndrome which has been suggested to occur in COVID-19 involving the brainstem’s central cardiopulmonary pacing network [53]. In this syndrome, there is failure of the switch from automatic to voluntary breathing around daybreak, and thus respiratory effort ceases as normal pacing fails from a variety of causes [54]. Failure of the switch from automatic to voluntary breathing remains a question that is not reliably supported in our small case cohort.

Conclusion

The major finding in the 36 COVID-19 cases is the widespread presence of reactive and acute inflammatory CNS microvasculopathy proximate to death. Lack of new neurologic findings following admission, except for acute changes most likely caused by hypoxia associated with pulmonary infection with SARS-CoV-2 or exacerbation of known cardiopulmonary disease, leads to no direct clinical correlation with histopathologic findings. The literature suggests that in COVID-19 patients reactive microvasculopathy most likely originates from hypoxia, hypercytokinemia, and RAS dysfunction, while direct and indirect virus-induced factors may contribute. In all of our cohort cases, acute endotheliitis, transmural migration, and acute perivasculitis with complement component activation to membrane attack complexes constitute the early phase of type 3 hypersensitivity vasculitis. Viral antigen in or ‘planted’ on microvessels or other antigen-antibody complexes are well known factors that could be operative in this cohort in the development of this early allergic microvasculitis. This finding, along with exaggerated forms of reactive microvasculopathy, may interfere, even if only temporarily, with microvascular functioning that supports the brain’s neuronal activity.

Abbreviations

A-T
Ataxia-telangiectasia
CNS
Central nervous system
HIV
Human immunodeficiency virus
IA
Intussusceptive arborization
MRI
Magnetic resonance imaging
NETs
Neutrophilic extracellular traps
NVUs
Neurogliovascular units
PMNs
Polymorphonuclear neutrophils
RAS
Renin-angiotensin system

Declarations

Ethics approval

These studies were determined to be exempt by the University IRB.

Consent for publication

Not applicable.

Availability of data and materials

The datasets created and analyzed during the current study, other than protected health information, are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no completing interests.

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Authors' Contributions

GLL provided or assisted in special autopsy equipment procurement and hospital autopsy engineering safety clearance for the autopsy suite. All authors participated in performance of postmortem examinations. FDSL and MSS collected most clinical information, with all other authors contributing. RHR, GLL, and RSVH conceived the project, and RHR, GLL, SEF, and RSVH prepared, reviewed and edited the first draft. RHR collected and analyzed immunostaining results with input from GLL and RSVH. RHR provided photomicrographs and performed statistical analysis. All authors read and approved the final manuscript.

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Figures

Figure 1

Microcirculatory ectasia and mural distortion. **A** Mildly dilated microcirculatory channel in an olfactory tract contains multiple microthrombi and serum protein (Case 15). **B** Greatly dilated, thin-walled microvessel in thalamus with red cell accumulation and serum protein (Case 28). **C** Dilated microcirculatory channel with red cell accumulation and serum protein has a thin, serpiginous wall (Case 1). **D** Dilated, thin-walled microcirculatory channel in substantia nigra of rostral midbrain with microthrombi, fibrin-platelet thrombus, and nuclear fragmentation (arrow) in several PMNs (Case 33).

E Dilated, thin-walled microcirculatory channel in cerebellar inferior vermis near a deep nucleus has a serpiginous shape with a prominent “waist” forming an ampoule-like feature (arrow) (Case 6). **F** Serial “waists” form a rhythmic or serpiginous capillary wall in insular cortex that has hypoxic nerve-cell change (Case 28). Scale bars: 20 mm in A, C, D and F; 50 mm in E; 100 mm in B

Figure 2

Microvasculopathy with excessive mural distortion. **A** This starburst-shaped, thin-walled microvessel with multiple “waists” is in frontal pole subcortical white matter (Case 8). **B** More than serpiginous, this microvascular channel in deep cerebellar white matter has a sinuous form (Case 36). **C** Sinuous, frond- or tuft-like mural deformation of microvessel in medial temporal entorhinal cortex (Case 23). **D** Severe mural distortion with irregular adventitial collagenosis of a microvessel in midbrain tegmentum near substantia nigra (Case 14). **E** Tuft-like mural deformation of microvessel in pulvinar of thalamus (Case 36). **F** Dilated microvessel with somewhat compact adventitial collagenosis in periventricular calcarine white matter (Case 13). Scale bars: 50 mm in A, C and F; 10 mm in B and E; 20 mm in D

Figure 3

Intussusceptive arborization and ‘string’ and ‘ghost’ capillaries. **A** Two capillary channels with a shared wall represent capillary ‘looping’ or intussusceptive arborization (IA) in frontal pole neocortex. Note adjacent pyramidal neuron that might have been in the neurogliovascular unit with the capillary complex (Case 7). **B** Horizontal section of IA in the right insular neocortex (Case 25). **C** Dilated capillary in cerebellar folium has almost starburst mural distortion and a forming pillar of an endothelial tip cell (arrow) and stalk cell appearing to stretch toward the established inner mural cell at lower left (Case 15). **D** IA with multiplication of mature pillars in a ‘mini-glomeruloid’ formation in frontal pole neocortex (Case 1). **E** ‘String’ vessel that may be a lengthy white matter capillary in mid-level of medulla adjacent to inferior olivary nucleus (Case 30). **F** Frontal pole neocortex, with hypoxic nerve-cell change, contains at least four ‘ghost’ capillaries (Case 12). Scale bars: 10 mm in A, C, and D; 20 mm in B and F; 50 mm in E

Figure 4

Acute endotheliitis. **A** In midbrain tegmentum, a thin-walled microcirculatory channel has many karyorrhectic PMNs including some fragmenting into dot-like nuclear dust (Case 10). **B** Similar finding as in **A** is seen here in subarachnoidal microvessels by the cerebellar superior vermis (Case 10). **C** Small microcirculatory channel with mural collagenosis in medial temporal subependymal white matter has karyorrhectic PMNs and mononuclear cells (Case 11). **D** In lateral temporal white matter, a dilated thin-walled microvessel is filled with PMNs, many with karyorrhexis, and mononuclear cells. There is scattered 'nuclear dust' (black arrow) and a few karyorrhectic PMNs appear to be transmigrating into fibrous adventitia (white arrow) (Case 11). **E** Mixture of karyorrhectic PMNs, some 'nuclear dust', and many mononuclear cells in very dilated microvessel in internal capsule near hypothalamus (Case 23). **F** Pyknotic and karyorrhectic PMNs arrayed along luminal border of microvessel in lateral hypothalamus (Case 34). Scale bars: 10 mm in A–C, E and F; 20 mm in D

Figure 5

Acute perivasculitis and mural PMN transmigration. **A** Dilated, thin-walled microvessel with serpiginous profile (arrows) is surrounded by perivascular hemorrhage containing PMNs in rostral pontine tegmentum (Case 25). **B** Higher magnification of perivascular hemorrhage in **A** includes many PMNs indicating acute perivasculitis (Case 25). **C** Cerebellar folial white matter microvessel with collagenosis and perivascular hemorrhage with PMNs (Case 31). **D** Temporal fusiform gyrus white matter microvessel has perivascular hemorrhage with PMNs, some appearing to be karyorrhectic (Case 31). **E** Many PMNs ringing microvessel in mid-level basis pontis are karyorrhectic, a feature that can be prominent in acute perivasculitis, also known as leukocytoclastic vasculitis (Case 10). **F** Subarachnoidal arteriolar wall with transmigrating PMNs (Case 31). Scale bars: 50 mm in A; 20 mm in B and C; 10 mm in D–F

Figure 6

Thromboembolism, hyperchromatic capillary nuclei, and complement component activation **A** Thromboembolized microcirculatory channel at edge of nucleus of the tractus solitarius (NTS; arrow) and similar blood vessel near the nucleus. There is prominent reactive gliosis in and near the NTS (Case 36).

B Large, irregularly hyperchromatic nucleus (or nuclei) in capillary in the minimal parietal boundary zone may be mural or intraluminal (Case 7). **C** Left thalamic capillary contains irregularly hyperchromic nucleus or nuclei (Case 16). **D** Dilated microcirculatory wall in rostral basis pontis is focally positive with immunostaining for C3d (Case 25). **E** Microcirculatory wall shown in **D** is seen here heavily positive for C4d. C5b-9 immunostaining was negative (Case 25). **F** Microcirculatory wall in crus cerebri of midbrain is focally positive for C5b-9 (Case 4). Scale bars: 20 mm in A; 10 mm in B–F

Figure 7

Complement component activation. **A** and **B** show rostral basis pontis microcirculatory channels with immunostaining positive for C4d (Case 25). **C** C5b-9 immunostaining in perivascular glia limitans (arrow) at edge of perivascular hemorrhage in periaqueductal central gray matter of midbrain (Case 4).

D C4b positive immunostain associated with PMNs, in perivascular hemorrhage shown in **C**, indicates acute perivasculitis with complement component activation (Case 4). **E** C5b-9 positive stain associated with PMNs in perivascular hemorrhage shown in **C** and **D** indicates membrane attack complex formation in acute perivasculitis (Case 4). **F** C5b-9 immunostaining in acute perivasculitis in rostral pontine tegmentum (Case 25). Scale bars: 10 mm in A, B, D–F; 20 mm in C