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Detection of blood-brain barrier disruption in brains of patients with COVID-19, but no evidence of brain penetration by SARS-CoV-2

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Mounting evidence supports the connection between coronavirus disease 2019 (COVID-19) due to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

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Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. For this type of study formal consent is not required. Tissue was obtained from the following Institutions: University of Washington, University of Glasgow, and University of Pennsylvania. Tissue was acquired at routine diagnostic autopsy, and approval for its use was granted by the respective institutional review boards.

and neurological manifestations, such as cognitive dysfunction (“brain fog”), headache, and neuropsychiatric disorders [2, 6]. Autopsy studies on limited available cases have reported a spectrum of neuropathological changes in COVID-19 patients, in particular neuroinflammation and microvascular injury [3, 5, 11, 14]. However, there have been many caveats to these findings, including difficulty in determining whether these pathologies are a direct consequence of cerebral viral infection, or arise due to systemic complications of COVID-19 such as coagulopathy or ischemia [8, 14], or are associated with comorbidities such as neurodegenerative disease (NDD) [7]. Indeed, data thus far provide little evidence of SARS-CoV-2 in the brain in association with systemic infection [12, 14]. In particular, a recent comprehensive autopsy study of 44 patients died of COVID-19 observed limited evidence of inflammation or direct viral cytopathology in the central nervous system [12]. Accordingly, we performed post-mortem neuropathological studies to identify cerebral SARS-CoV-2 in cases with or without NDD and determine whether there was evidence of direct vascular injury in the form of blood-brain barrier (BBB) disruption.

Consecutive research brain donations fulfilling the requirements for each study group were identified within the archive holdings of the multi-center Collaborative Neuropathology Network Characterizing Outcomes from TBI (CONNECT-TBI) [10] as either: 1) COVID-19 infection (COVID+, identified as patients with a known history of COVID-19 infection and/or confirmed at autopsy with positive SARS-CoV-2 qualitative PCR nasopharyngeal/ oropharyngeal swabs) and history of a known NDD clinical diagnosis (COVID+ NDD+, n=10); 2) COVID+ with no NDD (COVID+ NDD-, n=2); 3) no COVID-19 (COVID-, identified as patients died prior to October 2019) with NDD (COVID- NDD+, n=6), and 4) controls of similar age with no COVID and no NDD (COVID- NDD-, n=5) (Supplemental Table 1, Supplemental Table 2, and Supplemental Materials and Methods, online resource). A standard set of tissue sections was selected for microscopic evaluation, including cingulate gyrus, hippocampus, thalamus, and medulla. Using hybridization chain reaction (HCR) RNA fluorescence in situ hybridization (RNA-FISH), no SARS-CoV-2 viral RNA was detected in any brain region examined across all study groups, including those who were COVID+ (Fig. 1). These findings are consistent with recent RT-PCR and RNA-FISH based studies which, similarly, report no detectable SARS-CoV-2 RNA in brain tissue homogenates or brain sections [3, 5]. While these and our observations might suggest there is no penetration of SARS-CoV-2 into the brain, we still do not rule out the neuroinvasive capacity of SARS-CoV-2 due to the possibility of viral RNA replication clearance at the time of death [16].

The consistent observation of brain microvascular injury in patients with COVID-19 [5] led us to suspect that blood-brain barrier (BBB) disruption might contribute to cerebral consequences of infection. To investigate this, we used immunohistochemistry to examine and evaluate potential extravasation of the serum protein fibrinogen, which does not normally cross the BBB [1]. Immunostaining of fibrinogen in each case was assessed and semi-quantitatively scored in line with published experience [1]. In all cases with COVID-19 infection (COVID+ NDD+ and COVID+ NDD-), we observed evidence of widespread BBB disruption. Specifically, in all regions examined, widespread perivascular and parenchymal fibrinogen staining was present (Fig. 2 and Supplemental Table 1, online resource). In

comparison, in non-infected control cases (COVID-NDD-) there was typically no or at most minimal fibrinogen staining.

Notably, disruption of BBB is a neuropathological feature in many NDDs (Supplemental Fig. 1) and even normal aging [13]. To address this, our study included both aged individuals and those with comorbid NDDs as controls when assessing COVID-19 related neuropathological changes. Nevertheless, the extensive fibrinogen extravasation we observed in context of SARS-CoV-2 infection was in excess of that observed in normal aging, with widespread and substantial fibrinogen extravasation in both brain gray and white matter in our youngest COVID+, aged just 41 years (Fig. 2b). While we observed limited evidence of microthrombi within some small vessels, most of the fibrinogen staining was present in regions without apparent microthrombi. Taken together, our findings suggest a plausible association between COVID-19 infection and BBB disruption. Nonetheless, our relatively small number of cases warrants a more extensive examination to confirm these findings.

It is unclear why the BBB might be compromised by COVID-19 infection, but neuroinflammation may play a role in promoting this disruption, as BBB disruption and neuroinflammation are commonly observed as comorbidities [5]. With regard to a potential specific mechanism, a previous study suggests that the spike protein attached to brain endothelial cells could further exacerbate the BBB disruption by triggering a pro-inflammatory response [15]. Nevertheless, despite a disrupted BBB, there is no direct evidence of SARS-CoV-2 entry into the brain in infected humans [3, 5], including in our cases. This is in contrast with experimental data showing that the spike protein of SARS-CoV-2 could be absorbed across the BBB in a mouse model [9], possibly through adsorptive-mediated transcytosis of the spike protein involving angiotensin-converting enzyme 2 (ACE2). In addition, treatment with anti-spike or anti-ACE2 antibodies has been shown to reduce the entry of SARS-CoV-2 into the BBB [4].

Overall, we find autopsy evidence of widespread BBB disruption in the brains of individuals with history of COVID-19 infection, but no detectable virus in tissue sections. Conceivably, BBB dysfunction may contribute to the neurological impairment during disease progression and the long-lasting cerebral symptoms in survivors. Nevertheless, we must acknowledge limitations in case numbers and clinical information in this series. More comprehensive studies of COVID-19 related BBB disruption, including neuropathology and advanced imaging studies, are required to explore the contribution of this pathology to immediate and late neurological consequences of COVID-19 outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data and materials availability:

All data are available in the main text and the supplementary materials.

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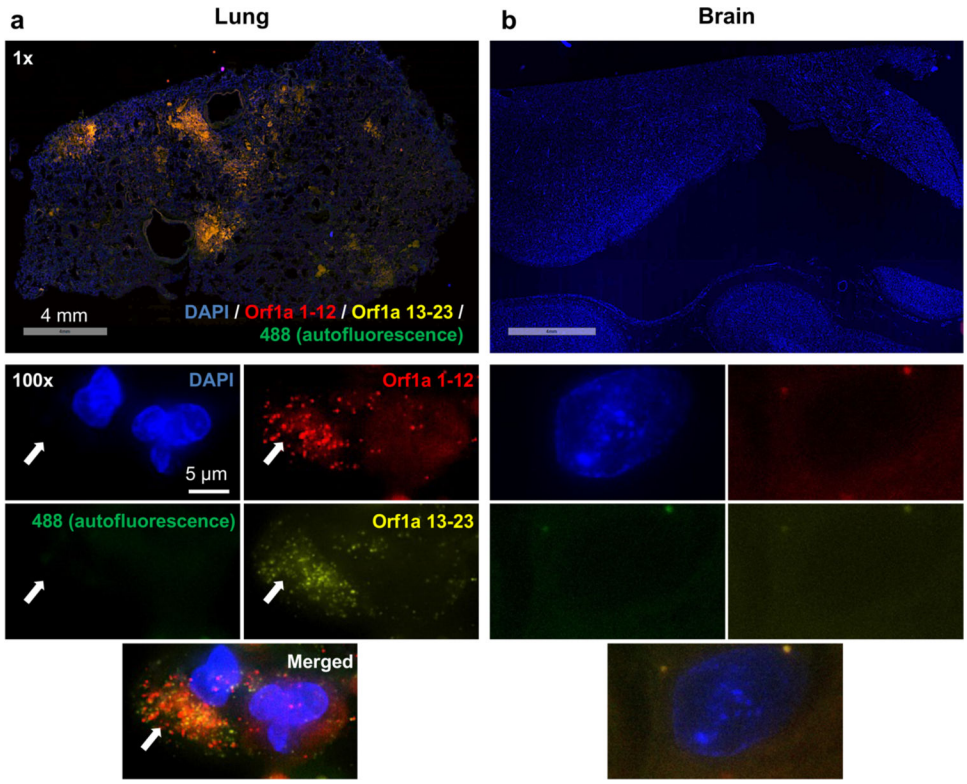

Fig. 1. No SARS-CoV-2 viral RNA detectable in the brain tissue.

(**a**) The presence of SARS-CoV-2 viral RNA in the lung tissue, potentially associated with epithelial cell infection, was revealed through hybridization with probes of SARS-CoV-2 Orf1a (coupled with amplifier labeling Alexa Fluor 647 to Orf1a 1–12 and Alexa Fluor 546 to Orf1a 13–23). Amplifier labeling Alexa Fluor 488 green was used as the negative control marking tissue autofluorescence to minimize false positivity. (**b**) In contrast, no SARS-CoV-2 viral RNA was detected in the brain tissue. Scale bars 4 mm for the top low power images, 5 μm for the below high power images.

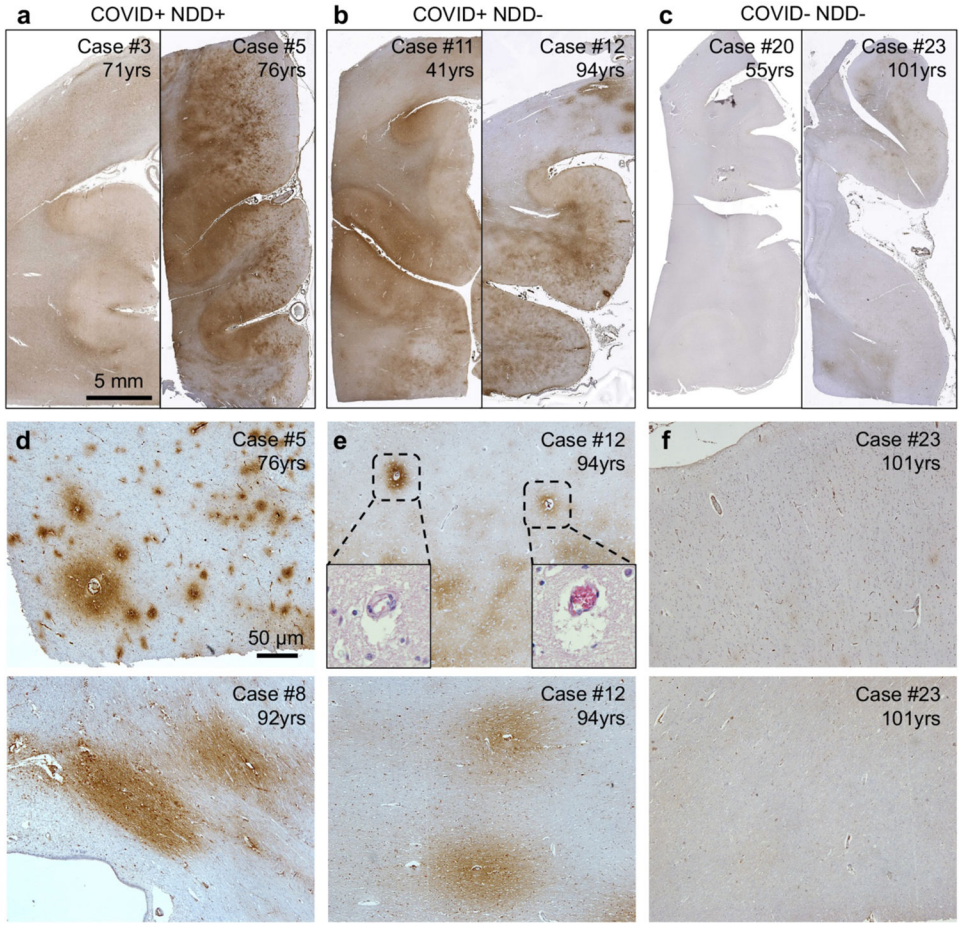

Fig. 2. Representative fibrinogen staining in COVID+ cases and in controls.

(**a**) Moderate (left, score of 2) to extensive (right, score of 3) fibrinogen immunoreactivity in cingulate gyrus was observed in COVID+ NDD+ cases. (**b**) Similarly, widespread, multifocal extensive fibrinogen immunoreactivity (score of 3) was identified in two COVID+ NDD- cases. (**c**) In contrast, absent (left, score of 0) to sparse (right, score of 1) fibrinogen immunoreactivity was observed in COVID- NDD-cases. (**d**) Substantial and widespread microscopic fibrinogen immunoreactivity in cingulate cortex (upper panel) and corpus callosal white matter (lower panel) was identified in COVID+ NDD+ cases. (**e**) In COVID+ NDD- cases, multifocal fibrinogen immunoreactivity was also evident in cingulate cortical layers (upper panel) and associated white matter (lower panel). H&E staining (inset) shows clumps of red blood cells in certain small vessels associated with fibrinogen extravasation. (**f**) Only limited fibrinogen immunoreactivity was observed in COVID- NDD-cases. Scale bars **a-c** 5 mm, **d-f** 50 µm.