Postmortem Assessment of Olfactory Tissue Degeneration and Microvasculopathy in Patients With COVID-19

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➕ **Multimedia**

➕ **Supplemental content**

IMPORTANCE Loss of smell is an early and common presentation of COVID-19 infection. Although it has been speculated that viral infection of olfactory neurons may be the culprit, it is unclear whether viral infection causes injuries in the olfactory bulb region.

OBJECTIVE To characterize the olfactory pathology associated with COVID-19 infection in a postmortem study.

DESIGN, SETTING, AND PARTICIPANTS This multicenter postmortem cohort study was conducted from April 7, 2020, to September 11, 2021. Deceased patients with COVID-19 and control individuals were included in the cohort. One infant with congenital anomalies was excluded. Olfactory bulb and tract tissue was collected from deceased patients with COVID-19 and appropriate controls. Histopathology, electron microscopy, droplet digital polymerase chain reaction, and immunofluorescence/immunohistochemistry studies were performed. Data analysis was conducted from February 7 to October 19, 2021.

MAIN OUTCOMES AND MEASURES (1) Severity of degeneration, (2) losses of olfactory axons, and (3) severity of microvasculopathy in olfactory tissue.

RESULTS Olfactory tissue from 23 deceased patients with COVID-19 (median [IQR] age, 62 [49-69] years; 14 men [60.9%]) and 14 control individuals (median [IQR] age, 53.5 [33.25-65] years; 7 men [50%]) was included in the analysis. The mean (SD) axon pathology score (range, 1-3) was 1.921 (0.569) in patients with COVID-19 and 1.198 (0.208) in controls ($P < .001$), whereas axon density was 2.973 (0.963) $\times 10^4/mm^2$ in patients with COVID-19 and 3.867 (0.670) $\times 10^4/mm^2$ in controls ($P = .002$). Concomitant endothelial injury of the microvasculature was also noted in olfactory tissue. The mean (SD) microvasculopathy score (range, 1-3) was 1.907 (0.490) in patients with COVID-19 and 1.405 (0.233) in control individuals ($P < .001$). Both the axon and microvascular pathology was worse in patients with COVID-19 with smell alterations than those with intact smell (mean [SD] axon pathology score, 2.260 [0.457] vs 1.63 [0.426]; $P = .002$; mean [SD] microvasculopathy score, 2.154 [0.528] vs 1.694 [0.329]; $P = .02$) but was not associated with clinical severity, timing of infection, or presence of virus.

CONCLUSIONS AND RELEVANCE This study found that COVID-19 infection is associated with axon injuries and microvasculopathy in olfactory tissue. The striking axonal pathology in some cases indicates that olfactory dysfunction in COVID-19 infection may be severe and permanent.

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COVID-19, caused by SARS-CoV-2 infection, has created a health crisis and impacted many lives globally. Patients infected with SARS-CoV-2 reveal a wide spectrum of clinical presentations, ranging from asymptomatic infection to fatal disease. In addition to respiratory illnesses, various nonrespiratory manifestations of COVID-19 have also been reported. One of the most prevalent nonrespiratory symptoms is olfactory dysfunction. Olfactory dysfunction of variable severity, including anosmia, hyposmia, and parosmia, reportedly affect 30% to 70% of patients with COVID-19.[1-6] The prevalence of olfactory dysfunction has prompted US Centers for Disease Control and Prevention to list new loss of smell as a cardinal symptom of COVID-19 on its webpage. Olfactory dysfunction occurs early in the course of infection and has no direct association with disease severity or viral loads.[6-8] In one study, it was recorded as the first presenting symptom among approximately 12% of patients.[9] In most cases, the symptoms spontaneously resolve within 3 to 4 weeks.[6,10] A subset of patients nevertheless developed persistent olfactory impairment up to 12 months postinfection,[4,6,11,12] suggesting that injury to the olfactory system may be severe or permanent.

The mechanism underlying olfactory dysfunction in COVID-19 is currently unknown. Various hypotheses concerning both direct cell injury and secondary inflammation from viral infection of the olfactory pathway have been proposed.[13-16] The most notable theory is SARS-CoV-2 infection of olfactory receptor neurons (ORNs) through nasal mucosa. However, there is conflicting evidence as to whether SARS-CoV-2 is capable of infecting ORNs,[16-19] which appear to lack the expression of key cell entry molecules for SARS-CoV-2.[20,21] While bystander effect from SARS-CoV-2 infection of olfactory epithelium or supporting cells may be sufficient to cause olfactory neuronal dysfunction, recovery of smell sense in about half of the patients is far too rapid and incompatible with direct neuronal damage.[22] Moreover, the lack of bona fide evidence of viral replication or significant inflammation in the central nervous system[16,23,24] further raises doubts about the role of direct viral to olfactory bulb in olfactory dysfunction. To evaluate the association of COVID-19 infection with olfactory bulb, we hereby performed an ultrastructural and histopathological analysis of deceased patients with COVID-19 and age- and disease severity–compatible deceased controls.

Methods

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline for cohort studies. This postmortem study was not considered human subjects research and therefore was exempt from institutional review boards review at University of Maryland Baltimore.

Study Design

Postmortem tissue from brain, lung, and other organs was collected. Pertinent clinical information was reviewed. Data on race and ethnicity were not collected. Information regarding the sense of smell and taste was obtained from the clinical notes for 3 individuals and by interviewing close family members of the deceased for the remaining individuals. Infection severity of each individual with COVID-19 was determined based on the baseline severity categorization from US Food and Drug Administration's COVID-19: Developing Drugs and Biological Products for Treatment or Prevention, Guidelines for Industry.

Setting

Autopsies were performed from April 7, 2020, to September 11, 2021, at the University of Maryland Medical Center, Maryland Office of the Chief Medical Examiner or Orlando Health with consent from next of kin. Data analysis was conducted from February 7 to October 19, 2021.

Participants

Deceased persons with reverse transcription-polymerase chain reaction (PCR)–confirmed COVID-19 and age- and disease severity–compatible controls who tested negative for COVID-19 via PCR were included in the study. One infant with congenital anomalies was excluded owing to the young age and the possibility of olfactory pathology associated with the congenital disorder.

Postmortem COVID-19 Testing

Postmortem specimens, eg, nasopharyngeal, tracheal, or bronchial swab, were collected and submitted for reverse transcription PCR analysis using the cobas SARS-CoV-2 Test on the 6800 system (Roche). Positive results were indicative of presence of SARS-CoV-2 RNA. Nondiagnostic results meant (1) a sample at concentrations near or below the limit of detection of the test, (2) a variant in the target region, (3) infection with some other sarbecovirus (eg, SARS-CoV), or (4) other factors. Postmortem testing for 1 control individual was done by droplet digital PCR of lung tissue.

Tissue Processing

Routine histology was performed on olfactory, brain, and lung tissues. Semithin plastic sections and electron microscopy were

Key Points

Question What are the neuropathologic changes of COVID-19 in the olfactory region?

Findings In this cohort study of 23 deceased patients with COVID-19 and 14 matched controls, more severe axon pathology, axon losses, and microvascular pathology were noted in olfactory tissue from patients with COVID-19 than that from the control individuals. The olfactory pathology was particularly severe in patients with reported smell alterations but were not associated with the clinical severity, timing of infection, or the presence of SARS-CoV-2 in the olfactory tissue.

Meaning In the region of olfactory bulb and olfactory tract, COVID-19 infection was associated with axon pathology and microvasculopathy, particularly in patients with smell alterations; the olfactory pathology did not result from direct viral injury and may be associated with local inflammation.

performed on all olfactory tissues. Droplet digital PCR and immunofluorescence studies were performed on olfactory or orbitofrontal brain tissue for all cases except for 1, for which additional tissue was not available. Immunohistochemical studies were performed on cases with additional tissue available.

Electron Microscopy

Tissue was fixed in formaldehyde, 4%, and glutaraldehyde, 1%, in phosphate buffer and postfixed with osmium tetroxide, 1%, in phosphate buffer. Tissue was then dehydrated with alcohol, cleared with propylene oxide, and embedded in epoxy resin Epon 812. Ultrathin sections from the blocks were stained with uranyl acetate and lead citrate, and semithin sections were stained with toluidine blue.

Scoring of Axon and Microvascular Pathology

All electron microscopy images were taken by one of us (C.-Y.H.) in a blinded manner. Three 1500× to 2500× magnification axon images were scored individually by 3 board-certified neuropathologists (C.-Y.H., H.A., and M.M.) separately, and 5 1500× to 3000× magnification microvasculature images were scored individually by 3 board-certified pathologists (C.-Y.H., C.D., and J.C.P.) separately based on the criteria listed in eTable 2 in the Supplement. All scoring was done in a blinded manner. Mean scores from 3 or 5 images were calculated for each patient from each pathologist, and therefore each patient had 1 axon score and 1 vessel score from each pathologist. The final score used for statistical analyses was a mean of 3 pathologists' scores. An analysis for intraobserver reliability in axon pathology scoring yielded a Fleiss κ of 0.411 (eTable 3 in the Supplement), which was considered moderate agreement. Fleiss κ for microvascular pathology scoring was 0.373, which was considered fair agreement among the pathologists.[25]

Axon Density Analyses

Quantifications of myelinated axons in the lateral olfactory tract on toluidine blue-stained semithin sections were performed by Fiji software using a protocol established by Engelmann et al.[26] Mean axon densities from 3 randomly selected microscopic fields were calculated for each case.

Droplet Digital PCR

Testing was performed in a Clinical Laboratory Improvement Amendments of 1988–certified laboratory using a modification of the Bio-Rad SARS-CoV-2 droplet digital PCR assay (Bio-Rad Laboratories) on formalin-fixed and paraffin-embedded tissue. N1 and N2 targets are 2 unique sequences within the nucleocapsid gene of SARS-CoV-2. Amplification of gene *RPP30* was used as quality control. The limit of detection varies based on the number of droplets generated but was approximately 5 copies of the viral genome.

Immunofluorescence Studies

Formalin-fixed and paraffin-embedded tissue sections were deparaffinized and rehydrated before heat-induced epitope retrieval in Antigen Retriever Citrate Buffer, pH 6.0 (Sigma-Aldrich). The sections were then incubated with the primary antibody mouse anti–SARS-CoV/SARS-CoV-2 spike antibody,

clone 1A9 (GeneTex; 1:500) and the secondary antibody goat antimouse antibody, Alexa Fluor 546 (Thermo Fisher; 1:400). Images were acquired by Carl Zeiss LSM700 confocal microscope. Lung tissue from patients with and without COVID-19 were used as positive and negative controls, respectively.

Immunohistochemical Studies

Immunohistochemical stains were performed on formalin-fixed and paraffin-embedded tissue sections using the VENTANA BenchMark automated system (Roche Diagnostics). The primary antibodies used were Phospho-Tau (Ser202, Thr205) (MN1020B; Thermo Fisher), CD3 (2GV6; VENTANA), CD20 (L26; VENTANA), CD68 (LP1; VENTANA), and GFAP (EP672Y; Cell Marque).

Bias

Owing to an 8.5-year age difference between the COVID-19 and control groups, the potential age bias in specific olfactory pathologic features was assessed using multivariable linear regression analysis.

Statistical Methods

Statistical analyses were performed on axon pathology scores, axon densities, and microvascular pathology scores by 2-tailed *t* test or 1-way analysis of variance with Tukey multiple comparison's test. Interobserver variability in axon and microvascular pathology scoring was assessed by Fleiss multirater κ using SPSS statistical software, version 28 (IBM). The associations between COVID-19 diagnosis, age, and specific olfactory pathologic features were assessed using multivariable linear regression analysis performed by SPSS statistical software version 28 (IBM). Two-sided *P* values were statistically significant at .05.

Results

The cohort of our study consisted of 23 patients with COVID-19 whose age ranged from 28 to 93 years at death (median [IQR] age, 62 [49-69] years), and 14 control individuals whose age ranged from 20 to 77 years (median [IQR] age, 53.5 [33.25-65] years). There were 14 men (60.9%) in the COVID-19 group and 7 men (50%) in the control group. Based on clinical history and postmortem testing results, 16 individuals had active SARS-CoV-2 infection at the time of death (**Table**; eTable 1 in the Supplement). Sixteen individuals died of COVID-19 pneumonia or related complications. Six individuals with COVID-19 had significant brain pathology, including ruptured aneurysm, acute intracerebral hemorrhage with intermediate-level Alzheimer disease neuropathologic change, treated brain tumor, diffuse Lewy body disease, global hypoxic-ischemic encephalopathy, and extensive white matter hemorrhage with intravascular thrombi. Among these findings, only the white matter hemorrhage was directly associated with COVID-19. Eight control individuals also had significant brain pathology, including massive cerebral hemorrhagic infarction with intermediate-level Alzheimer disease neuropathologic change, large remote cerebral infarction, cerebellar arteriovenous mal-

Table. Olfactory Bulb/Tract Pathology in Patients With COVID-19

Patient No./sex/ age, y	Smell or taste dysfunction	Interval between symptom onset and death, d	COVID-19 infection severity[a]	Axon Pathology score (range, 1-3)	Density (× 10⁴/mm²)	Vascular pathology score (range, 1-3)	Olfactory tissue ddPCR	IF	PMI, h
Patients with COVID-19									
1/F/30s	Diminished smell	4	Mild	2.78	2.63	2.67	Negative	Negative	74
2/M/60s	Loss of smell	28	Critical	2.67	2.67	1.93	Positive	Positive	14
3/M/50s	Loss of smell	14	Critical	2.56	2.96	2.73	Negative	Negative	20
4/M/60s	Loss of smell	45	Severe	2.56	3.87	1.40	Negative	Negative	162
5/M/50s	Loss of smell and taste	21	Severe	2.33	3.31[b]	2.40	Negative	Negative	26
6/M/20s	Loss of smell and taste	10	Mild	2.22	4.66	2.13	Negative	Negative	68
7/F/60s	Diminished smell	18	Severe	2.11	2.76	2.53	Negative	Negative	15
8/M/40s	Diminished smell	18	Moderate	1.67	3.73[b]	1.27	Negative	Negative	52
9/M/70s	Diminished smell	52	Severe	1.44	2.86	2.33	Negative	Negative	46
10/F/80s	None	110	Mild	2.67	2.84	2.07	Negative	Negative	27
11/F/60s	None	9	Critical	2.11	2.62	1.53	Negative	Negative	192
12/M/40s	None	10	Moderate	1.89	3.94	1.60	Negative	Negative	120
13/M/60s	None	22	Critical	1.67	2.96	2.20	Negative	Negative	59
14/F/40s	None	6	Critical	1.56	3.78	1.60	Positive	Positive	50
15/M/60s	None	35	Critical	1.56	4.28	1.13	Negative	Negative	40
16/M/90s	None	17	Severe	1.56	0.96	1.27	Negative	Negative	18
17/M/60s	None	72	Severe	1.44	3.04	1.47	Negative	Negative	125
18/F/80s	None	8	Mild	1.44	1.33	2.07	Positive	Negative	27
19/M/60s	None	27	Critical	1.33	1.49	1.73	NA	NA	192
20/F/50s	None	37	Critical	1.22	1.54	1.93	Negative	Negative	17
21/F/70s	None	27	Severe	1.11	3.37	1.73	Negative	Negative	39
22/F/30s	Unknown	Unknown	Unknown	3.00	2.48	2.67	Negative	Negative	27
23/M/30s	Unknown	NA	No symptoms	1.44	4.15	1.47	Negative	Negative	61
Control individuals									
1/F/50s	None	NA	NA	1.67	3.96	1.13	NR	NR	116
2/M/60s	None	NA	NA	1.44	2.42	1.20	NR	NR	16
3/F/50s	None	NA	NA	1.44	3.57	1.33	NR	NR	16
4/M/70s	None	NA	NA	1.33	3.86	1.73	NR	NR	83
5/F/20s	None	NA	NA	1.22	3.80	1.53	NR	NR	80
6/M/30s	None	NA	NA	1.22	5.60	1.27	NR	NR	4
7/M/20s	None	NA	NA	1.17	4.15	1.47	NR	NR	25
8/M/60s	None	NA	NA	1.17	3.56	1.87	NR	NR	14
9/F/40s	None	NA	NA	1.11	4.01	1.47	NR	NR	27
10/F/20s	None	NA	NA	1.00	4.01	1.40	NR	NR	32
11/M/40s	None	NA	NA	1.00	4.90	1.00	NR	NR	4
12/M/50s	None	NA	NA	1.00	3.94	1.40	NR	NR	15
13/F/60s	None	NA	NA	1.00	3.99	1.27	NR	NR	16
14/F/70s	None	NA	NA	1.00	2.91	1.60	NR	NR	30

Abbreviations: ddPCR, droplet digital polymerase chain reaction; F, female; IF, immunofluorescence; M, male; NA, not applicable; NR, not reported; PMI, postmortem interval.

[a] COVID-19 infection severity was determined based on the baseline severity categorization from the US Food and Drug Administration's COVID-19: Developing Drugs and Biological Products for Treatment or Prevention, Guidelines for Industry.
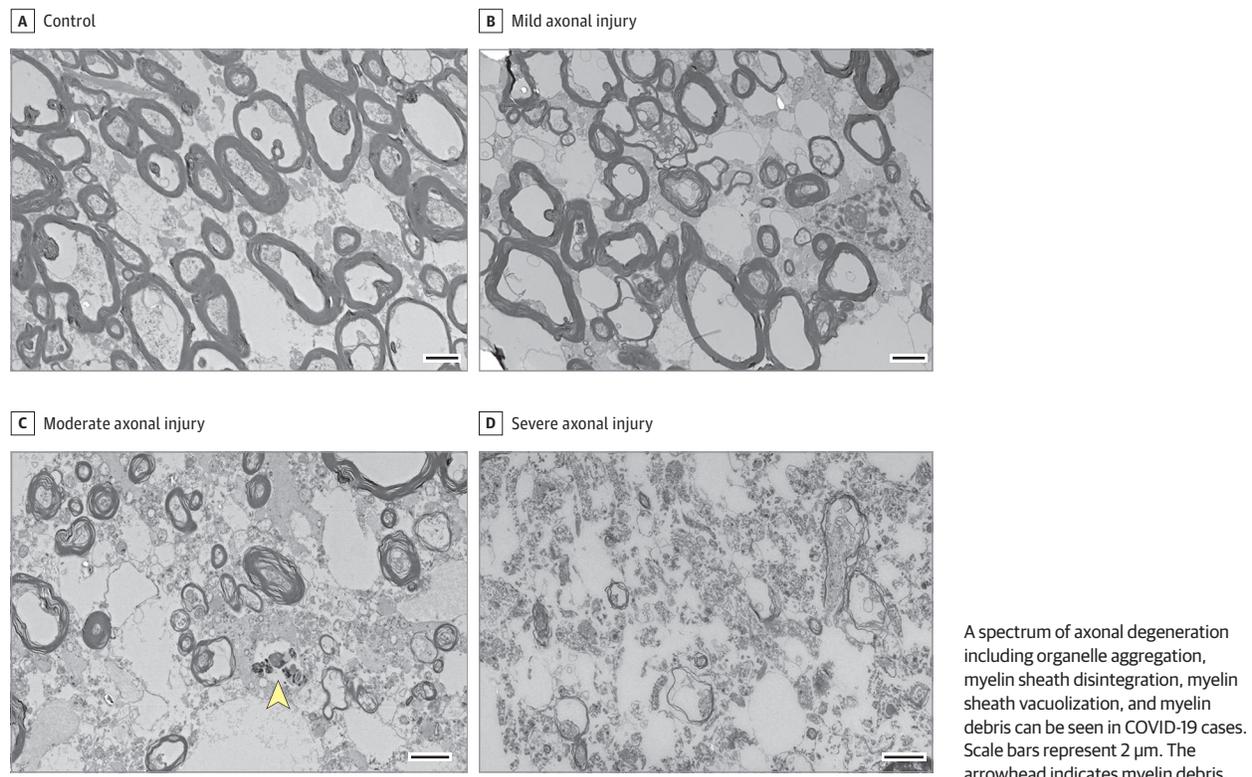
[b] Patchy axonal losses.

formation with hemorrhage, meningitis, frontotemporal lobar degeneration with TDP-43 inclusions, hepatic encephalopathy, and hypoxic-ischemic encephalopathy (eTable 1 in the Supplement).

Routine histopathology was examined but did not reveal obvious abnormalities in olfactory tissue from individuals with COVID-19 (eFigure 1 in the Supplement). There was also no obvious increase in lymphocytic infiltrates, microglia, or reactive astrocytes in COVID-19 olfactory tissue (eTable 4 in the Supplement). Conversely, semithin section and ultrastructural analyses by electron microscopy demonstrated a spectrum of axon pathology ranging from mild changes to severe

Figure 1. Electron Micrographs Demonstrate a Spectrum of Olfactory Axonal Injuries in Patients With COVID-19

A spectrum of axonal degeneration including organelle aggregation, myelin sheath disintegration, myelin sheath vacuolization, and myelin debris can be seen in COVID-19 cases. Scale bars represent 2 μm. The arrowhead indicates myelin debris.

axonal injury and axonal losses (**Figure 1**, **Figure 2**, and **Table**). Axon pathology was further scored by 3 board-certified neuropathologists based on the criteria listed in eTable 2 in the Supplement. Compared with controls, individuals with COVID-19 showed significantly worse olfactory axonal damage (**Figure 3** and **Table**). The mean (SD) axon pathology score (range, 1-3) was 1.921 (0.569) in patients with COVID-19 and 1.198 (0.208) in controls (95% CI, 0.444-1.002; $P < .001$). The mean (SD) axon density in lateral olfactory tract was 2.973 (0.963) $\times 10^4/mm^2$ in patients with COVID-19 and 3.867 (0.670) $\times 10^4/mm^2$ in controls (95% CI, 0.347-1.440; $P = .002$), indicating a 23% loss of olfactory axons. Overall, individuals with reported smell alterations had significantly more severe olfactory axon pathology than cases with intact smell (Figure 3). The mean (SD) axon pathology score was 2.26 (0.457) in patients with COVID-19 with smell alterations and 1.63 (0.426) in patients with intact smell (95% CI, 0.229-1.031; $P = .002$). However, there was no significant difference in axon densities between patients with COVID-19 with smell alterations (mean [SD], 3.272 [0.689] $\times 10^4/mm^2$) and those with intact smell (mean [SD], 2.693 [1.097] $\times 10^4/mm^2$; 95% CI, −0.336 to 1.496; $P = .28$).

In addition to axon pathology, individuals with COVID-19 were remarkable for endothelial injury of the microvasculature, an immune-mediated process seen primarily in the heart and lung.[27,28] Ultrastructural findings, including endothelial cytoplasmic swelling, vacuolization, structural damage of mitochondria, and subendothelial edema, were frequently observed in patients with COVID-19 (**Figure 4**). The mean (SD) mi-

crovasculopathy score (range, 1-3) was 1.907 (0.490) in patients with COVID-19 and 1.405 (0.233) in control individuals (95% CI, 0.259-0.745; $P < .001$). Similar to axon pathology, patients with COVID-19 with smell alterations also had a worse microvascular pathology score (mean [SD], 2.154 [0.528]) than those with intact smell (mean [SD], 1.694 [0.329]; 95% CI, 0.071-0.850; $P = .02$). Notably, mild microvascular injury was present in control individuals, suggesting that some degree of endothelial abnormalities may be an end-of-life change rather than a pathological condition. In comparison, moderate to severe endothelial injuries were noted particularly in patients with COVID-19 with abnormal sense of smell and therefore may be clinically significant.

Of note, olfactory axon and microvascular pathology do not appear to be associated with clinical severity or timing of the infection. In addition, SARS-CoV-2 was only detected in olfactory tissue from 3 patients by droplet digital PCR or immunofluorescence (Table), suggesting that olfactory pathology was not caused by direct viral injury.

Because advancing age has been previously associated with neuropathologic changes, eg, tau deposits, multivariable linear regression was conducted to test whether the association of COVID-19 with axon and vascular pathology was independent of age. After controlling for age, COVID-19 diagnosis remained associated with increased axonal pathology score (β, 0.774; 95% CI, 0.439-1.110; $P < .001$), reduced axonal density (β, −0.661; 95% CI, −1.209 to −0.114; $P = .02$), and increased vascular pathology score (β, 0.522; 95% CI, 0.222-0.822; $P = .001$). Nevertheless, age was associated with decreasing

Figure 2. Axon Losses Ranging From Mild to Severe Are Observed in Toluidine Blue-Stained Semithin Sections of the Lateral Olfactory Tract From Patients With COVID-19

A Control

B Mild axon loss

C Moderate axon loss

D Severe axon loss

Scale bars represent 10 μm.
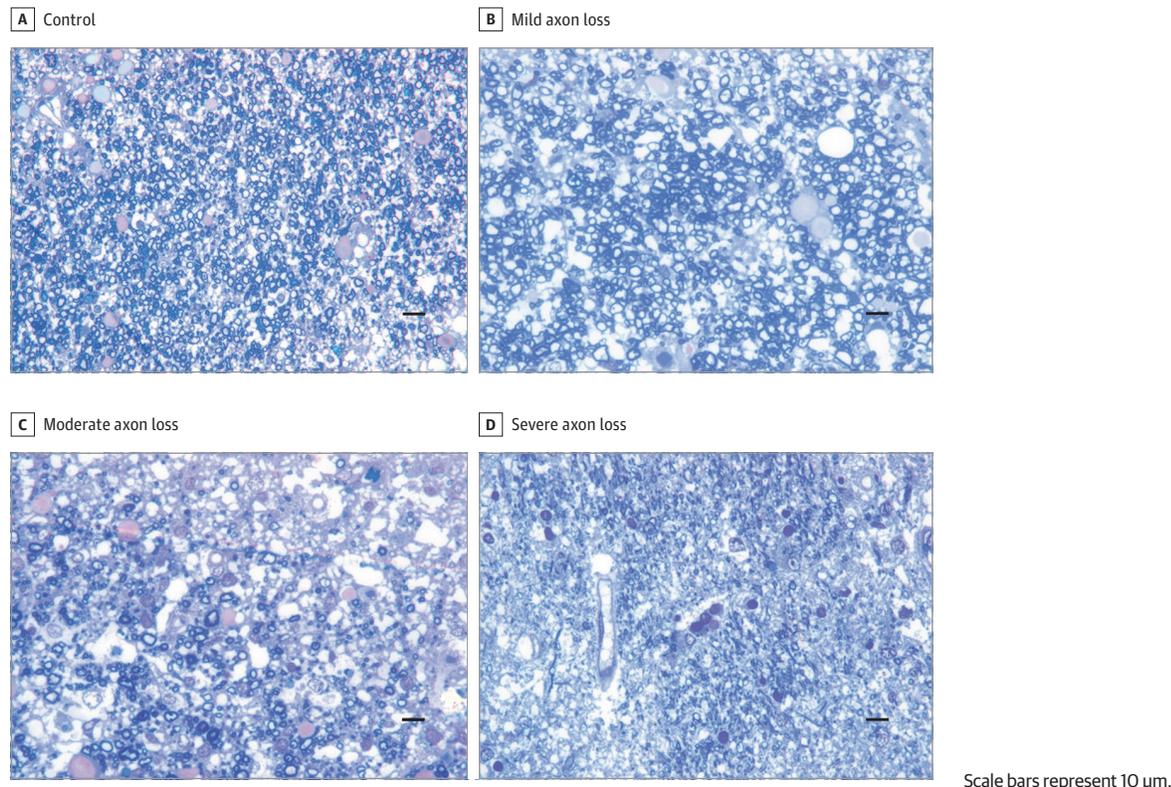
axon densities independent of COVID-19 status (β, −0.028; 95% CI, −0.043 to −0.013; *P* < .001; eFigure 2 in the Supplement).

To further address whether age-associated tau pathology contributed to olfactory axon pathology, phospho-tau immunohistochemical stain was performed on olfactory tissue from both patients with COVID-19 and control individuals. In addition, neuropathologic evaluation for Alzheimer disease was performed on patients with COVID-19 and control individuals older than 40 years (eTable 5 in the Supplement). The COVID-19 group with smell alterations had 2 patients with low-level Alzheimer disease neuropathologic change; the COVID-19 group with intact smell had 1 patient with intermediate-level and 3 patients with low-level change; and the control group had 1 individual with intermediate-level and 2 individuals with low-level change. All 3 groups had 2 patients with Braak stage higher than II. In terms of tau deposits in olfactory tissue, the COVID-19 group with smell alterations had 1 patient with moderate neurofibrillary tangles (NFTs), and the COVID-19 group with intact smell had 2 patients with frequent and 1 patient with moderate NFTs. The frequency of NFTs in olfactory tissue roughly associated with the Braak staging. Although the control group did not have any individual with moderate or frequent NFTs in olfactory tissue, this might be because the 2 control individuals with higher Braak staging had no sufficient olfactory tissue for assessment. Overall, patients with COVID-19 did not have more severe age-related tau pathology in brain or olfactory tissue than control individuals.

This finding further supported the results from the multivariable analyses that the difference in olfactory pathology between patients with COVID-19 and controls could not simply be accounted for by the age difference.

It was noteworthy that some cases in this study had a prolonged postmortem interval. While the autolytic process associated with prolonged postmortem interval may cause changes in ultrastructures of axons and vessels, patients with longer postmortem interval did not appear to have worse axon or microvascular pathology within each group (Table).

Discussion

The olfactory pathway begins with ORNs in the nasal mucosa. ORNs extend their axons to the olfactory bulb to form synapses with mitral cells and tufted cells, the second-order olfactory neurons in the olfactory bulb. These neurons then project their axons through the lateral olfactory tract into the olfactory cortex. Olfactory dysfunction can occur if any of the components in the olfactory pathway gets injured.

Olfactory dysfunction is most commonly associated with viral infection of the upper respiratory tract.[29,30] The role of viral injuries in postviral olfactory dysfunction remains unclear; some studies reveal near-complete degeneration of the ORNs in nasal mucosa, whereas others show unremarkable histology.[29,31] A study from Meinhardt and colleagues[16] examined the nasal mucosa from patients with

Figure 3. Olfactory Axon Pathology, Axon Losses, and Microvasculopathy Are Significantly Worse in Patients With COVID-19 Than Control Individuals
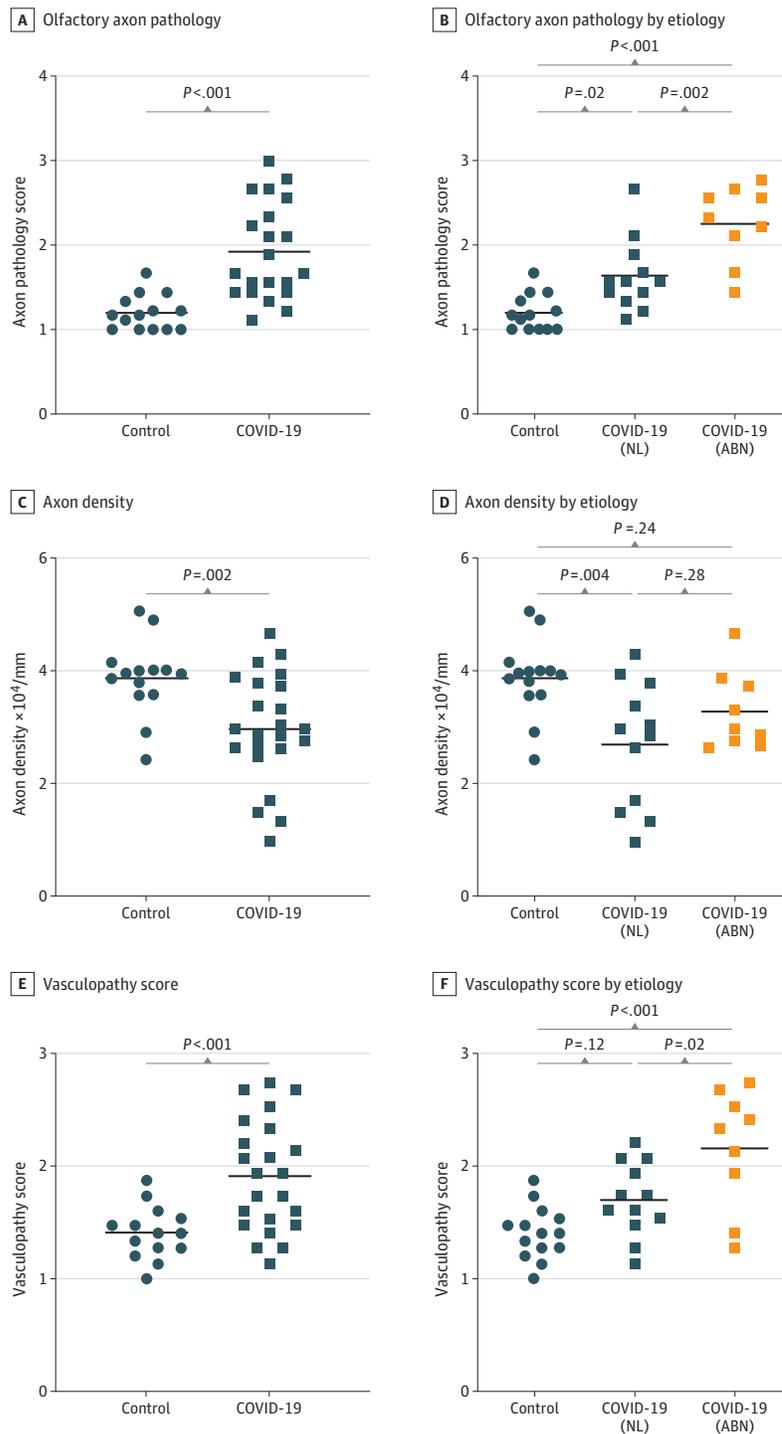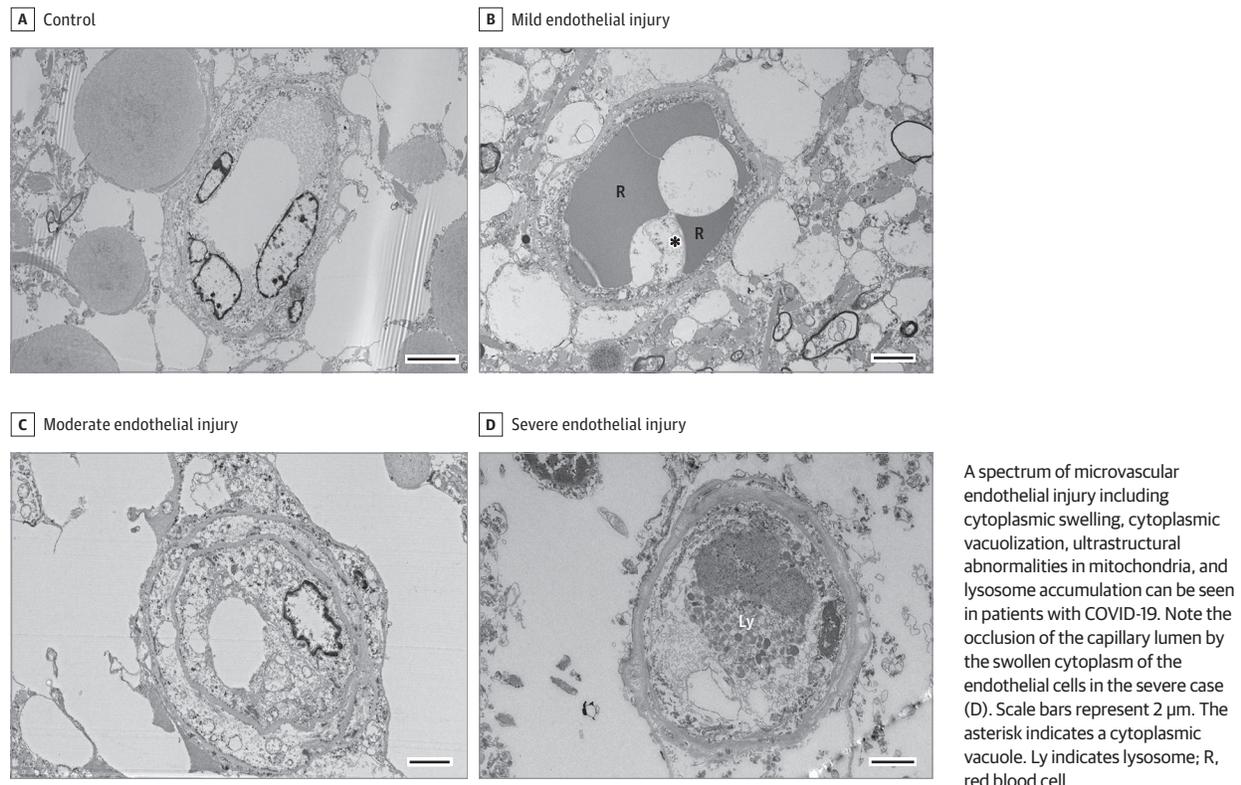

A, C, and E, Comparisons between individuals with COVID-19 and control individuals (*t* test). Individuals with COVID-19 are further divided into smell normal (NL) and smell abnormal (ABN) groups for the comparisons in B, D, and F (1-way analysis of variance).

COVID-19 and demonstrated the capability of SARS-CoV-2 to infect ORNs. Another study from Khan and colleagues[19] showed that nonneuronal sustentacular cells in the nasal mucosa were in fact the main target for SARS-CoV-2. While the pathologic effect of COVID-19 infection on nasal mucosa may be sufficient to cause olfactory dysfunction, it fails to explain the persistent anosmia experienced by some patients[4,6,11,12] since cell damage in nasal mucosa is largely repairable.

To our knowledge, this cohort study was the first to examine the ultrastructural changes of olfactory bulb and olfactory tract in COVID-19 infection. Previous studies of

Figure 4. Electron Micrographs of Olfactory Microvasculature From Patients With COVID-19 Demonstrate a Spectrum of Endothelial Injuries

A spectrum of microvascular endothelial injury including cytoplasmic swelling, cytoplasmic vacuolization, ultrastructural abnormalities in mitochondria, and lysosome accumulation can be seen in patients with COVID-19. Note the occlusion of the capillary lumen by the swollen cytoplasm of the endothelial cells in the severe case (D). Scale bars represent 2 µm. The asterisk indicates a cytoplasmic vacuole. Ly indicates lysosome; R, red blood cell.

olfactory bulb have been limited to conventional histologic examination without providing appropriate controls or information regarding patients' smell function.[23,32] Results from this study not only confirmed at an ultrastructural level the previously reported radiological abnormalities in olfactory bulb[33,34] but also demonstrated how the pathologic findings were associated with smell alterations in patients with COVID-19. Consistent with findings reported by other groups,[16,19] this study also did not find evidence of viral infection in olfactory bulb from most patients with COVID-19. Therefore, the axon and microvascular pathology in olfactory bulb and olfactory tract were most likely not caused by direct viral injury.

Endothelial cell injury and dysfunction is a common phenomenon in COVID-19 infection, particularly in the heart and lung.[27,28] It is thought to be caused by excessive cytokine release from immune cells, respiratory epithelial cells, and alveolar epithelial cells and has been shown to correlate with COVID-19 severity.[28] However, this study did not find a strong association between olfactory endothelial injury and disease severity, suggesting that local inflammation in the upper respiratory tract may be sufficient to cause endothelial and axonal damage in the olfactory pathway. Schwabenland and colleagues[35] performed deep spatial profiling and found a mild increase in lymphocytes and microglia in olfactory bulb and brain stem from patients with COVID-19. Although we did not see an obvious overall increase in inflammatory cells in our cohort, it remained possible that focal or perivascular infiltrates may have a role in axonal or microvascular injuries associated with COVID-19 infection.

Limitations

This study did not examine the pathologic association of COVID-19 with nasal mucosa, which contains primary olfactory neurons and their supporting cells. Therefore, how pathologic changes in nasal mucosa may contribute to smell alterations in COVID-19 infection could not be assessed in this cohort.

In this study, information regarding smell function was obtained by subjective self-report or reporting from patients' family members. Objective olfactory function assessment would be considered medically unnecessary for patients with serious illness and was impossible for patients receiving mechanical ventilation. While subjective self-report may not accurately reflect olfactory function, the issue appears to be underreporting rather than overreporting of olfactory dysfunction.[36]

Conclusions

The results of this postmortem cohort study demonstrated that COVID-19 infection could cause axon injuries and microvasculopathy in olfactory tissue. Overall, patients with smell alterations had more severe olfactory pathology than those with intact smell. While most individuals in our

study demonstrated mild to moderate findings, the severity of pathology in some COVID-19 cases indicates that the olfactory system damage and dysfunction can be permanent.

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