Skeletal Muscle and Peripheral Nerve Histopathology in COVID-19

Author(s):
Joome Suh, M.D.1, 2; Shibani S. Mukerji, M.D., Ph.D.2, 3; Sarah I. Collens, B.S.3; Robert F. Padera, Jr., M.D., Ph.D.2, 4; Geraldine S. Pinkus, M.D.2, 4; Anthony A. Amato, M.D.1, 2; Isaac H. Solomon, M.D., Ph.D.2, 4

Equal Author Contributions:
Dr. Amato and Dr. Solomon contributed equally to this work.

Neurology® Published Ahead of Print articles have been peer reviewed and accepted for publication. This manuscript will be published in its final form after copyediting, page composition, and review of proofs. Errors that could affect the content may be corrected during these processes.
Corresponding Author:
Isaac H. Solomon
ihsolomon@bwh.harvard.edu

Affiliation Information for All Authors: 1. Department of Neurology, Brigham and Women's Hospital, Boston, MA; 2. Harvard Medical School, Boston, MA; 3. Department of Neurology, Massachusetts General Hospital, Boston, MA; 4. Department of Pathology, Brigham and Women's Hospital, Boston, MA

Contributions:
Joome Suh: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Shibani S. Mukerji: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Sarah I. Collens: Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data
Robert F. Padera, Jr.: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Geraldine S. Pinkus: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Anthony A. Amato: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data
Isaac H. Solomon: Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data

Number of characters in title: 63

Abstract Word count: 250

Word count of main text: 3644

References: 38

Figures: 3

Tables: 2

Supplemental: IRB protocol and approval for documentation of waived consent


Acknowledgements: We would like to thank our patients and their families, and the technical expertise provided by the Brigham Women's Hospital autopsy staff (Michelle Siciliano, Jacob Plaisted, John Grzyb) and the staff of the histology, immunohistochemistry, and neuropathology laboratories (Alyson Campbell, Mark Buchanan, Mei Zheng, Sebastian Valentin, Karen Bryan).

Study Funding: The authors report no targeted funding

Disclosures: J. Suh reports no disclosures relevant to the manuscript; S.S. Mukerji reports no disclosures relevant to the manuscript; S.I. Collens reports no disclosures relevant to the manuscript; R.F. Padera, Jr. reports no disclosures relevant to the manuscript; G.S. Pinkus reports no disclosures relevant to the manuscript; A.A. Amato served on medical advisory boards for Alexion, Sarepta, CSL Behring, Strongbridge Pharma, Argenx, Ra Pharmaceuticals, Orphazyme, and is a neurology consultant for Johnson & Johnson (SARS-CoV-2 vaccine study); I.H. Solomon reports no disclosures relevant to the manuscript
Abstract

Objective
To explore the spectrum of skeletal muscle and nerve pathology of patients who died following SARS-CoV-2 infection and assess for direct viral invasion of these tissues.

Methods
Psoas muscle and femoral nerve sampled from 35 consecutive autopsies of patients who died following SARS-CoV-2 infection and 10 SARS-CoV-2-negative controls were examined under light microscopy. Clinical and laboratory data were obtained by chart review.

Results
In SARS-CoV-2-positive patients, mean age at death was 67.8 years (range 43-96 years) and the duration of symptom onset to death ranged from 1-49 days. Four patients had neuromuscular symptoms. Peak creatine kinase was elevated in 74% (mean 959 U/L, range 29-8413 U/L). Muscle showed type 2 atrophy in 32 patients, necrotizing myopathy in 9, and myositis in 7. Neuritis was seen in 9. Major histocompatibility complex-1 (MHC-1) expression was observed in all cases of necrotizing myopathy and myositis and 8 additional patients. Abnormal expression of myxovirus resistance protein A (MxA) was present on capillaries in muscle in 9 patients and in nerve in 7. SARS-CoV-2 immunohistochemistry was negative in muscle and nerve in all patients. In the 10 controls, muscle showed type 2 atrophy in all patients, necrotic muscle fibers in 1, MHC-1 expression in non-necrotic/non-regenerating fibers in 3, MxA expression on capillaries in 2, and inflammatory cells in none, and nerves showed no inflammatory cells or MxA expression.

Conclusions
Muscle and nerve tissue demonstrated inflammatory/immune-mediated damage likely related to release of cytokines. There was no evidence of direct SARS-CoV-2 invasion of these tissues.

**Classification of Evidence**

This study provides class IV evidence that muscle and nerve biopsies document a variety of pathological changes in patients dying with COVID-19.

**Introduction**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is associated with myalgia or fatigue in 11-70% of individuals, and elevated creatine kinase (CK) elevation in 9-33%.\textsuperscript{1-7} Rhabdomyolysis and myositis have been reported,\textsuperscript{8-12} but only a few studies included muscle biopsies,\textsuperscript{13-15} and it is unclear whether muscle damage is the result of viral infection of muscle, toxic effect of cytokines, or another mechanism. Additionally, Guillain-Barre syndrome (GBS) and variants have been described, but studies reporting nerve histopathology are lacking.\textsuperscript{1,16-21} We report histopathologic findings in skeletal muscle and peripheral nerve from 35 consecutive autopsies performed on patients with COVID-19 who died between April 5, 2020 and June 13, 2020.

**Methods**

*Patient Cohort*

All patients with SARS-CoV-2 infection who died between April 5, 2020 and June 13, 2020 and subsequently underwent autopsy at Brigham and Women’s Hospital were included in this study. Informed consent for autopsy was obtained from next of kin or healthcare proxy of the deceased. Thirty-three patients were diagnosed by positive pre- or peri-mortem reverse transcriptase polymerase chain reaction (RT-PCR) of nasopharyngeal swabs and two patients were diagnosed by
the presence of SARS-CoV-2 IgM or IgG antibodies (patients 25 and 35). Additionally, ten patients who were negative for SARS-CoV-2 but were critically ill and died during the COVID-19 pandemic were included as negative controls. Patient demographics, clinical data, and laboratory data were extracted from the electronic medical record when available.

*Standard Protocol Approvals, Registrations, and Patient Consents*

This study was approved by the Mass General Brigham Human Research Committee on an excess tissue waived consent protocol.

*Muscle and Nerve Pathology*

Autopsies were performed in a negative pressure isolation room by personnel equipped with powered air-purifying or N95 respirators. Samples of psoas muscle and femoral nerve were collected for each patient, and tissue was fixed in 10% formalin prior to standard processing and paraffin embedding. Five micron thick sections of psoas muscle were stained with 1) hematoxylin and eosin, 2) Masson’s trichrome (Agilent Artisan AR173), 3) anti-myosin (skeletal, fast) antibody (Sigma M4276; 1:10,000 dilution), 4) anti-LCA/CD45 antibody (Dako M0701; 1:600 dilution), 5) anti-SARS-CoV nucleocapsid antibody (Novus Biologicals NB100-56576; 1:500 dilution), 6) anti-HLA Class 1 ABC/major histocompatitibility-1 (MHC-1) antibody (Abcam ab70328; 1:15,000 dilution; Leica BOND III immunostainer, antigen retrieval with ER2, and Leica Bond Polymer Refine Detection), and 7) anti-human myxovirus resistance protein 1 (MxA) antibody (Millipore M143, 1:50 dilution; heat induced epitope retrieval, horseradish peroxidase-conjugated polymeric goat anti-rabbit immunoglobulin antibody, and DAB chromogen). Femoral nerve sections were stained with 1) hematoxylin and eosin, 2) Masson’s trichrome, 3) anti-LCA/CD45 antibody, 4) anti-SARS-CoV nucleocapsid antibody, and 5) anti-MxA antibody. Muscle and nerve sections from cases with increased CD45-positive immune infiltrates were additionally stained with 1) anti-CD4 antibody.
(Cell-Marque EP204, 1:100 dilution), 2) anti-CD8 antibody (Dako CD8/144D, 1:200 dilution), 3) anti-CD20 antibody (Dako L26, 1:250 dilution), and 4) anti-CD68 antibody (Dako PG-M1, 1:200 dilution). Slides were reviewed independently by a board-certified neuropathologist (I.H.S.) and a neurologist board-certified in neuromuscular medicine and clinical neuromuscular pathology (A.A.A.).

Statistics

Categorical variables are presented as n (%). Continuous variables are summarized as mean (range). Statistical comparisons were not performed due to small sample size in each group.

Data availability

Additional data (supplementary tables e-1 and e-2) are available from Dryad (https://doi.org/10.5061/dryad.wwpzgmsjj). Fully anonymized data will be shared by request from any qualified investigator.

Classification of Evidence

The primary research question of this study was to evaluate the effects of SARS-CoV-2 infection on skeletal muscle and peripheral nerve in patients who died with COVID-19, confirmed by nasopharyngeal swab RT-PCR or serology. This study provides class IV evidence that muscle and nerve tissue exhibit inflammatory/immune-mediated damage likely related to release of cytokines, in the absence of direct SARS-CoV-2 invasion of these tissues.

Results

Clinical Features

Patient demographics, neuromuscular symptoms, and pertinent home and inpatient medications that may affect muscle or peripheral nerve histopathology are presented in Table 1 (details for individual
patients are provided in supplemental table e-1). Twelve of 35 patients (34%) were women and 23 (66%) were men. Mean age at death was 67.8 years (range 43-96 years). Time from symptom onset to death ranged from 1 to 49 days and time from positive SARS-CoV-2 RT-PCR test to death was less than one day to 44 days. Four patients complained of myalgia or weakness in arms and legs. Diabetes and connective tissue disease were present in 17 and 3 patients, respectively. Of 26 patients with known pre-mortem home medications, 11 patients were on statins, two were on corticosteroids (patients 1 and 6), one (patient 25) was on imatinib for gastrointestinal stromal tumor, one (patient 27) was on colchicine, and one (patient 32) was on pembrolizumab for squamous cell lung cancer. Five patients received tocilizumab, 5 received remdesivir, and 4 or 5 received hydroxychloroquine (one patient was in a placebo-controlled trial) during hospitalization for COVID-19. One patient received dexamethasone (patient 6) but not specifically for COVID-19. Characteristics of the 10 COVID-19-negative control patients are also shown in table 1 and supplemental table e-1. There were no major differences compared to the COVID-19-positive cohort except for a lower prevalence of diabetes in the control group. Three patients in the control group (patients C1, C3, and C5) had neuromuscular complaints of generalized weakness.

Laboratory Data

Twenty-seven of 35 patients with COVID-19 had CK values available from the hospitalization prior to death (supplemental table e-2). Of these, 20 patients (74%) had elevated peak CK with a mean of 959 U/L and range 29-8413 U/L (normal: 39-308 U/L for males and 26-192 U/L for females). Nineteen patients had repeat CKs, 9 of which were still elevated prior to death. Sixteen of the 20 patients also had elevated peak high-sensitivity (HS) troponin T levels, of whom 3 had evidence of acute cardiac injury on pathologic examination (patients 1, 24, and 30). Peak white blood cell (WBC) count was elevated in 20/27 (74%) patients with a mean of 18.9 K/uL and range 1.7-64.9
Peak C-reactive protein was elevated in all 25 patients measured, with mean 154 mg/L and range 6 to >300 mg/L (normal: 0-3 mg/L). In the control patients, peak CK was only available in 2 patients (patients C9 and C10) and were elevated at 1746 and 1152 U/L, respectively. Peak WBC count was elevated in all 8 patients with available WBC counts, with a mean of 22.1 K/uL and range 11.8-43.3.

Muscle Histopathology

Microscopic examination of muscle (figure 1, table 2) showed type 2 fiber atrophy in 32 of 35 patients with COVID-19, a necrotizing myopathy in 9 (no inflammatory cells aside from myophagocytosis of necrotic fibers), and myositis in 7 (defined by perivascular and endomysial inflammatory cell infiltrates). In patients with myositis, CD68-positive, CD4-positive, and/or CD8-positive histiocytes and T cells were observed more frequently than CD20-positive B cells. Diffuse or multifocal MHC-1 immunostaining of non-necrotic/non-regenerating muscle fibers was evident in all 16 patients with myositis or necrotizing myopathy and in 8 additional patients. One patient (patient 35) exhibited MHC-1 staining predominantly in perifascicular muscle fibers, a finding often seen in dermatomyositis; however, there was no abnormal MxA expression or documentation of clinical features suggestive of dermatomyositis. Abnormal MxA immunostaining was observed in 4/9 patients with necrotizing myopathy, 3/7 with myositis, and 2 without either. Of these 9 patients, MxA was only observed in the capillaries in 8, and in both myocytes and capillaries in 1 patient. SARS-CoV-2 nucleocapsid immunohistochemistry (IHC) was negative in all 35 cases.

In the 10 control patients, all of whom had multiple medical comorbidities, type 2 atrophy was observed in all patients. One patient (patient C4) had rare necrotic muscle fibers that expressed MHC-1 and MxA; this patient did not have a history of statin use, use of drugs with potential myotoxicity, or cancer. Myositis was not observed in any patients. MHC-1 immunostaining of non-
necrotic/non-regenerating fibers was seen in 3 patients (patients C1, C5, and C8), and MxA immunostaining was seen in 1 patient (patient C8) in addition to patient C4 mentioned above. None of these patients had a documented history of myopathy or connective tissue disease.

Potential associations between histopathologic findings in muscle and medical history were reviewed for COVID-19-positive patients (figure 2). Three of the 9 patients with necrotizing myopathy took statins pre-mortem (patients 4, 10, and 17), which was similar to the proportion of patients without necrotizing myopathy that took statins (8/26 patients). However, medication history was not known in 5/9 with necrotizing myopathy and in 4/26 without. Nevertheless, myotoxicity related to statins and other medications would not be expected to show MHC-1 expression in non-regenerating, non-necrotic muscle fibers or MxA expression in capillaries. Two of the 7 patients with myositis (patients 1 and 25) and one of the 28 patients without myositis (patient 20) had an underlying connective tissue disease. One patient with myositis (patient 32) received 2 cycles of an immune checkpoint inhibitor (pembrolizumab) in the 2 months preceding death. However, none of these patients had a documented history of myopathy associated with these conditions or medication use.

Mean time from onset of COVID-19 symptoms to death was 12.8 days in patients with necrotizing myopathy, 17.1 days in patients with myositis, and 18.1 days in those with neither finding. Statistical comparisons were not performed due to small sample size in each group. Peak WBC count (available in 27 patients) was elevated in 5/7 patients (71%) with necrotizing myopathy, 4/5 patients (80%) with myositis, and 11/15 patients (73%) without either finding. Peak CK was elevated in 10/12 patients (83%) with myositis or necrotizing myopathy and lab results. Peak CK was also elevated in 10/15 patients (67%) without these histopathologic findings, 5 of whom were MHC-1 positive. Associations are best seen in Figure 2.
Nerve Histopathology

Microscopic examination of nerve showed neuritis in 9 patients (figure 3, table 2), of whom 4 also had myositis (patients 24, 25, 32, 35). Perivascular inflammatory cells were observed in 6 patients, endoneurial infiltrates in 1, and both perivascular and endoneurial inflammatory cells in 2. CD68-positive histiocytes were most abundantly observed in all cases, but were sometimes co-predominant with CD8-positive, or less often, CD4-positive T cells. MxA immunostaining was observed in 7/35 (20%) of cases in the capillaries, only one of which had neuritis. SARS-CoV-2 IHC was negative in all 35 cases. Neither inflammatory cell infiltrates nor abnormal MxA expression were observed in the control cases.

Review of medical history for conditions associated with neuritis revealed a history of diabetes in 4/9 patients (44%) with neuritis (patients 3, 15, 23, 24), and in 13/26 (50%) without neuritis (table 1, figure 2). History of connective tissue disease was present in 2 patients with neuritis (patients 20, 25) and in one without. One patient with neuritis received pembrolizumab (patient 32); this patient also had myositis as mentioned above. Of 35 patients, only two (patients 6, 23) had a history of polyneuropathy pre-dating SARS-CoV-2 infection. One (patient 6) had a history of diabetes and received chemotherapy (including vincristine) for acute lymphoblastic leukemia but had no inflammation on nerve examination. The other (patient 23) had diabetes and neuritis on histopathology. Mean time from onset of COVID-19 symptoms to death was 13.4 days in patients with neuritis, and 17.6 days in patients without. Peak WBC count was elevated in all 6 patients with neuritis and 14 additional patients (of 27 patients with available laboratory values).

COVID-19 Therapies

Twelve patients received tocilizumab, hydroxychloroquine, and/or remdesivir during hospitalization for COVID-19. While formal statistical analyses were not performed, use of these medications did
not appear to be associated with specific histopathologic features in muscle or nerve. Myositis was seen in 1 patient who took tocilizumab (patient 1), and one patient who took tocilizumab plus remdesivir (patient 12). Necrotizing myopathy was seen in one patient who took hydroxychloroquine (patient 4), and 1 patient who took hydroxychloroquine plus tocilizumab (patient 10). Neuritis was seen in one patient who took tocilizumab (patient 26).

Neuromuscular Symptoms

Documentation of neuromuscular symptoms or exam during hospitalization for SARS-CoV-2 infection was lacking for most patients. Nonspecific fatigue was not included as a neuromuscular symptom in this study. Four patients had myalgia or subjective weakness affecting arms and legs (patients 4, 10, 14, 16). These 4 patients had type 2 fiber atrophy, necrotic myocytes and MHC-1 immunostaining on non-necrotic/non-regenerating muscle fibers. Peak CK levels were elevated at 488-2806 U/L. Nerve histopathology was normal in these 4 patients.

Discussion

The pathophysiology of SARS-CoV-2-associated myopathy is poorly understood. The possibility of skeletal muscle infection by the virus has been considered as muscle expresses angiotensin converting enzyme 2 (ACE2), which is a cell surface receptor used by SARS-CoV-1 and SARS-CoV-2 for host cell entry. Negative SARS-CoV-2 IHC in muscle in our study argues against this hypothesis. However, in one study that examined the diaphragm muscle obtained from 26 consecutive autopsies of critically ill COVID-19-infected patients who died, SARS-CoV-2 RNA was found in the muscle in 4 cases (15.4%). In situ hybridization localized the RNA to inside the sarcolemma. This discrepancy with our study findings may be explained by differences in methods to detect the virus or examination of different muscles.
Twenty-four of 35 patients in our study had evidence of an inflammatory or immune-mediated myopathy with necrotic fibers, inflammatory cell infiltrates, or MHC-1 immunostaining of non-necrotic/non-regenerating muscle fibers. Our observations suggest that muscle damage occurs secondary to an inflammatory response, including damage from cytokines.

To date, literature on skeletal muscle histopathology in COVID-19 is sparse. One study reported muscle biopsy findings in 3 patients infected with SARS-CoV-2 who were clinically suspected of having critical illness myopathy. Biopsies revealed scattered necrotic and regenerating fibers in one patient, and rare atrophic and regenerative fibers in two others. No biopsies stained positive for MHC-1 or membrane attack complex (C5b9). In these patients, the histopathologic findings likely reflected the clinically suspected critical illness myopathy rather than COVID-19-associated myopathy.

Myositis was reported in a 58 year-old patient with SARS-CoV-2 infection with facial weakness, nasal dysarthria, and dysphagia. Muscle biopsy showed perivascular and endomysial inflammation and MHC-1 expression. The patient had a dermatomyositis-specific autoantibody detected in the serum. Viral invasion of muscle was not seen on electron microscopy. Another autopsy series reported “myositis” in 2 of 10 autopsies, but it is unclear how “myositis” was defined. A figure showed necrotic fibers undergoing myophagocytosis in one patient. We recently reported a patient with marked weakness and elevated CK (up to 30,000 U/L), who had overexpression of MHC-1 and MxA on perifascicular muscle fibers and capillaries, suggestive of a type-1 interferonopathy. One patient in our autopsy series (patient 35) also had MHC-1 expression on perifascicular muscle fibers but without MxA expression.

How do our findings compare to myopathy associated with other coronavirus infections? In a small post-mortem series of patients who died from SARS-CoV-1 infection, myofiber atrophy and
necrosis were also the most common histopathologic findings in skeletal muscle.\textsuperscript{26} MHC-1 and MxA staining were not performed. Findings were thought to reflect critical illness myopathy or specific changes of SARS-CoV-1-associated myopathy. Another series found vasculitis in muscle.\textsuperscript{27} The virus was not detected in muscle with methods of viral culture, electron microscopy, immunohistochemistry, or \textit{in situ} hybridization.\textsuperscript{26, 28} One post-mortem case report on a patient with cutaneous T-cell lymphoma and Middle East respiratory syndrome coronavirus (MERS-CoV) showed necrotic fibers and an inflammatory infiltrate comprised of CD68-positive histiocytes and mixed CD4-positive and CD8-positive T-cells.\textsuperscript{29} Electron microscopy identified viral-like particles in macrophages infiltrating muscle but not in muscle fibers.

In nerve biopsies, we found perivascular and endoneurial inflammatory cell infiltrates (neuritis) in nine patients. History of diabetes was present in 4 patients, connective tissue disease in 2 and immune checkpoint inhibitor use in 1, conditions in which neuritis can be seen.\textsuperscript{30, 31} None had signs or symptoms of GBS. We cannot exclude these conditions as potential etiologies of the observed neuritis (e.g., diabetic lumbosacral radiculoplexus neuropathy or diabetic amyotrophy), though we think these are unlikely to be coincidental occurrence in the patients with inflammatory cell infiltrates in their nerves and MxA expression on capillaries, which would not be seen in these disorders. SARS-CoV-2 was not found in our nerve biopsies by immunohistochemistry, suggesting that the virus does not infect peripheral nerve.

In contrast to our findings, a central nervous system focused post-mortem series reported SARS-CoV-2 immunostaining in cranial nerves (glossopharyngeal and vagal nerves), albeit only in 2 of 40 patients, raising the possibility that viral infection of peripheral nerve may occur.\textsuperscript{32} In that study, SARS-CoV-2 immunostaining was found in undefined cells within the medulla from which these
cranial nerves originate. Contiguous spread of infection from the medulla to these cranial nerves is conceivable.

It is possible that viral invasion of muscle and nerve occurred at an earlier stage in the illness, and that active viral infection resolved by the time of death, although 22 patients (63%) had detectable SARS-CoV-2 in the lower respiratory tract by IHC suggesting ongoing infection in other tissues. Viral RNA may be cleared from muscle and nerve tissue due to efficient type I interferon response (e.g., including MxA expression) or other mechanisms, but not be cleared from higher burdened organs like the lungs.

Notably, MxA expression was observed in endothelial cells in 9/35 muscle and 7/35 nerve biopsies in our autopsy series, which is likely the result of the host response to SARS-CoV-2 infection. MxA is a type-1 interferon-inducible protein that is normally expressed in response to viral infections and prevents viral replication in the host. However, overexpression of type-1 interferons can be toxic, and abnormal expression of MxA in various tissues is seen in type-1 interferonopathies including dermatomyositis, systemic lupus erythematosus and idiopathic pernio (chilblains). As mentioned, we previously reported a patient with COVID-19-associated myopathy who had overexpression of MxA on perifascicular muscle fibers and capillaries, as typical of dermatomyositis and suggestive of a type-1 interferonopathy.\textsuperscript{15} Perniosis with MxA expression in endothelial cells and surrounding dermal and epidermal tissues has been reported in children and young adults late in the course of mild confirmed or presumed COVID-19 infection.\textsuperscript{33, 34} It is speculated that efficient induction of type-1 interferons and activation of the innate immune system quickly eradicate the virus and result in a mild infection but may cause collateral tissue damage. In critically ill patients, similar acral manifestations can occur due to severe thrombotic retiform purpura, in which abnormal MxA expression is not seen.\textsuperscript{34} Such cases could represent an insufficient type-1 interferon response to the
virus. These studies suggest that the type-I interferon response is protective in viral infection, but overexpression may be toxic to certain tissues. Peripheral nerve and skeletal muscle appear to be bystander victims of the host response and cytokine dysregulation. We suspect that an exaggerated type-I interferon response might be involved in some cases of COVID-19 associated myopathy and neuropathy. However, the lack of consistent expression in all cases of necrotizing myopathy, myositis, neuritis, and expression in some cases even without these features indicate that other cytokines may be involved in muscle and nerve damage. Abnormal serum levels of several cytokines have been detected in patients with SARS-CoV-2 such as type-1 and gamma interferons, interleukin(IL)-1, IL-6, and tumor necrosis factor (TNF)-alpha, among others.\cite{2,15,35,36} We do not think the histopathologic findings in this study simply reflect nonspecific changes in muscle and nerve of patients with severe illness as muscle and nerve biopsies obtained from autopsies of 10 control patients only revealed necrotizing myopathy in 1 patient and no cases with myositis or neuritis.

With regards to laboratory data, we noted that the proportion of SARS-CoV-2-positive patients in our study with elevated CK was higher (74%) than has been reported in other studies (9-33%).\cite{1-7} This is likely explained by the fact that we used peak CK rather than admission CK levels, and our cohort was comprised of patients with more severe COVID-19. Higher CK levels are associated with poorer outcomes.\cite{37} It is possible that cardiac injury contributed to the elevated CK, as 16 of the 20 patients with elevated peak CK levels had elevated peak high-sensitivity (HS) troponin T levels. However, only 3 of these patients had evidence of acute cardiac injury on pathologic examination. Troponin T may not be specific for cardiac damage and can be elevated in patients with myopathy without cardiac injury,\cite{38} concordant with our clinical experience.
There are limitations to this study. First, we did not perform targeted histopathologic examinations of clinically symptomatic muscle and nerve. We do not know whether psoas muscle and femoral nerve were clinically affected. Clinical information was obtained retrospectively, and documentation of neuromuscular symptoms and exams was limited. These were extremely ill patients, who ended up on ventilators sedated. Three died in the ER, and 5 more within two days of admission. The focus on the evaluations of these patients prior to intubation was stabilization. Second, due to laboratory biosafety concerns, specimens were entirely fixed in formalin for paraffin-embedded sections, and frozen tissue, which is routinely used to assess muscle histopathology, was not available, nor were plastic sections and electron microscopy for muscle and nerve. Additionally, since this is a post-mortem cases series of patients who ultimately succumbed to the virus, our results may not reflect the full spectrum of histopathologic findings in patients with various degrees of illness severity. Our findings may be skewed to those patients with the most severe infections. Lastly, as mentioned, viral RNA may have been cleared from muscle and nerve tissue prior to death, possibly due to a robust type I interferon response.

Our observations suggest that SARS-CoV-2 is frequently associated with inflammatory cell infiltrates and MxA expression in endothelial cells in both muscle and nerve, as well as necrosis of muscle fibers and abnormal MHC-1 expression in muscle. Although we did not measure cytokine levels in blood, the histopathological abnormalities seen in our patients suggest that these findings may be secondary to the storm of cytokine release rather than direct viral infection of these tissues. Further studies are needed to better understand the pathogenic mechanisms of myopathy and neuropathy associated with SARS-CoV-2.
### Appendix 1. Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joome Suh, M.D.</td>
<td>Brigham and Women's Hospital, Boston, MA</td>
<td>Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data</td>
</tr>
<tr>
<td>Shibani S. Mukerji, M.D., Ph.D.</td>
<td>Massachusetts General Hospital, Boston, MA</td>
<td>Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data</td>
</tr>
<tr>
<td>Sarah I. Collens, B.S.</td>
<td>Massachusetts General Hospital, Boston, MA</td>
<td>Drafting/revision of the manuscript for content, including medical writing for content; Analysis or interpretation of data</td>
</tr>
<tr>
<td>Robert F. Padera, Jr., M.D., Ph.D.</td>
<td>Brigham and Women's Hospital, Boston, MA</td>
<td>Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data</td>
</tr>
<tr>
<td>Geraldine S. Pinkus, M.D.</td>
<td>Brigham and Women's Hospital, Boston, MA</td>
<td>Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data</td>
</tr>
<tr>
<td>Anthony A. Amato, M.D.</td>
<td>Brigham and Women's Hospital, Boston, MA</td>
<td>Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data</td>
</tr>
<tr>
<td>Isaac H. Solomon, M.D., Ph.D.</td>
<td>Brigham and Women's Hospital, Boston, MA</td>
<td>Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Study concept or design; Analysis or interpretation of data</td>
</tr>
</tbody>
</table>
References


### Table 1. Demographics and Clinical History in COVID-19 Decedents and COVID-19-Negative Controls

<table>
<thead>
<tr>
<th></th>
<th>COVID-19-positive patients (n=35)</th>
<th>COVID-19-negative controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age at death</strong></td>
<td>67.8 (43-96)</td>
<td>71.3 (54-84)</td>
</tr>
<tr>
<td><strong>Female sex</strong></td>
<td>12 (34.2%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td><strong>PMH (n, %)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>connective tissue disease</td>
<td>3 (8.6%)</td>
<td>0</td>
</tr>
<tr>
<td>diabetes</td>
<td>17 (48.6%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>CKD</td>
<td>7 (20%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>polyneuropathy</td>
<td>2 (5.7%)</td>
<td>0</td>
</tr>
<tr>
<td>cancer</td>
<td>6 (17.1%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td><strong>Home medications</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>statin</td>
<td>11/26 (42.3%)</td>
<td>4/9 (44.4%)</td>
</tr>
<tr>
<td>steroid</td>
<td>2/26 (7.7%)</td>
<td>2/9 (22.2%)</td>
</tr>
<tr>
<td>colchicine</td>
<td>1/26 (3.8%)</td>
<td>0</td>
</tr>
<tr>
<td>chemotherapy</td>
<td>1/26 (3.8%)</td>
<td>1/9 (11.1%)</td>
</tr>
<tr>
<td>immune checkpoint inhibitor</td>
<td>1/26 (3.8%)</td>
<td>1/9 (11.1%)</td>
</tr>
<tr>
<td><strong>Inpatient medications for COVID-19</strong> (n, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>remdesivir</td>
<td>5 (14.3%)</td>
<td>N/A</td>
</tr>
<tr>
<td>tocilizumab</td>
<td>5 (14.3%)</td>
<td>N/A</td>
</tr>
<tr>
<td>hydroxychloroquine</td>
<td>4 or 5 (11.4 or 14.3%)*</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Neuromuscular symptoms</strong> (n, %)</td>
<td>4/35 (11.4%)</td>
<td>3/10 (30%)</td>
</tr>
</tbody>
</table>
\(^\text{denominator denotes total number of patients for whom pre-mortem home medications were known}

*one patient was enrolled in hydroxychloroquine vs. placebo trial

CKD = chronic kidney disease

N/A = not applicable
Table 2. Muscle and Nerve Histopathology

<table>
<thead>
<tr>
<th></th>
<th>COVID-19-positive patients (n=35)</th>
<th>COVID-19-negative controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Psoas muscle histopathology (n, %)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2 atrophy</td>
<td>32 (91.4%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>Necrotic fibers w/o inflammation</td>
<td>9 (25.7%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Inflammation +/- necrotic fibers</td>
<td>7 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>MHC-1 IHC*</td>
<td>24 (68.6%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>MxA IHC (of capillaries or myocytes)</td>
<td>9 (25.7%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>SARS-CoV-2 IHC</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Femoral nerve histopathology (n, %)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>9 (25.7%)</td>
<td>0</td>
</tr>
<tr>
<td>MxA IHC (of capillaries)</td>
<td>7 (20%)</td>
<td>0</td>
</tr>
<tr>
<td>SARS-CoV-2 IHC</td>
<td>0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*In non-necrotic, non-regenerating fibers

MHC-1 = Major histocompatibility complex 1

MxA = Human myxovirus resistance protein 1

IHC = immunohistochemistry

N/A = not applicable
**Figure Legends**

**Figure 1. Psoas Muscle Histopathology.** Histological findings in psoas muscle include perivascular and endomysial inflammation (A), comprised of mixed CD4 (B), CD8 (C), CD20 (D), and CD68 (E) immune infiltrates, as well as occasional necrotic fibers (arrows) (F). SARS-CoV-2 immunohistochemistry (IHC) was negative in skeletal muscle for all patients (G). Positive control for SARS-CoV-2 IHC shows sarcoplasmic staining in cardiac myocytes (H), MHC-1 IHC reveals variable sarcolemmal and sarcoplasmic staining patterns including (I) diffuse and (J) perifascicular distributions (arrows). MxA IHC highlights rare necrotic fibers (K) and scattered capillary cell walls (arrows) (L). Panels A-E and I are from patient 1, panels F and K are from patient 2, panel H is from patient 6, panels G and L are from patient 25, and panel J is from patient 35. Sections A and F were stained with hematoxylin and eosin, B with CD4 IHC, C with CD8 IHC, D with CD20 IHC, E with CD68 IHC, G and H with SARS-CoV-2 nucleocapsid IHC, I and J with MHC-1 IHC, and K and L with MxA IHC. Images from I and J taken with 20x objective, A-H with 40x objective, and K and L with 60x objective.
Figure 2. Clinical Characteristics, Laboratory Findings, and Muscle and Nerve Histopathology. Heat map showing clinical findings from 35 COVID-positive patients (patient numbers 1-35) and 10 COVID-negative control patients (patient numbers C1-C10) including age, sex, relevant past medical history, statin use, neuromuscular symptoms during hospitalization (A), and laboratory values for peak creatine kinase and white blood cell count (B). Heat map showing major histopathologic findings in skeletal muscle and peripheral nerve (C-D), including presence of necrotic fibers, inflammation assayed by anti-LCA/CD45 immunohistochemistry (IHC), MHC-1 IHC, MxA IHC and SARS-CoV-2 nucleocapsid protein IHC (right). White boxes indicate Suh 24 data not available. Abbreviations: CK, creatine kinase; IHC, immunohistochemistry; MHC-1, Major Histocompatibility Complex-1; MxA, human myxovirus resistance protein 1; NM, neuromuscular; PMH, past medical history; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WBC, white blood cell.
Figure 3. Femoral Nerve Histopathology. Histological findings include perivascular and endoneurial inflammation (A), in the absence of demyelination (B), comprised of mixed CD4 (C), CD8 (D), CD20 (E), and CD68 (F) immune cell infiltrates. SARS-CoV-2 IHC was negative in all cases (G). MxA IHC highlights scattered capillary cell walls (arrows) (H). Panel H is from patient 19, panels B and G are from patient 20, panel A is from patient 25, and panels C-F are from patient 26. Section from A stained with hematoxylin and eosin, B with Masson’s Trichrome, C with CD4 IHC, D with CD8 IHC, E with CD20 IHC, F with CD68 IHC, G with SARS-CoV-2 nucleocapsid IHC, and H with MxA IHC. Image A taken with 20x objective, images B-G with 40x objective, and image H with 60x objective.
Skeletal Muscle and Peripheral Nerve Histopathology in COVID-19
Neurology published online June 7, 2021
DOI 10.1212/WNL.0000000000012344

This information is current as of June 7, 2021