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Association Between SARS-CoV-2 Infection and Immune-Mediated Myopathy in Patients Who Have Died

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IMPORTANCE Myalgia, increased levels of creatine kinase, and persistent muscle weakness have been reported in patients with COVID-19.

OBJECTIVE To study skeletal muscle and myocardial inflammation in patients with COVID-19 who had died.

DESIGN, SETTING, AND PARTICIPANTS This case-control autopsy series was conducted in a university hospital as a multidisciplinary postmortem investigation. Patients with COVID-19 or other critical illnesses who had died between March 2020 and February 2021 and on whom an autopsy was performed were included. Individuals for whom informed consent to autopsy was available and the postmortem interval was less than 6 days were randomly selected. Individuals who were infected with SARS-CoV-2 per polymerase chain reaction test results and had clinical features suggestive of COVID-19 were compared with individuals with negative SARS-CoV-2 polymerase chain reaction test results and an absence of clinical features suggestive of COVID-19.

MAIN OUTCOMES AND MEASURES Inflammation of skeletal muscle tissue was assessed by quantification of immune cell infiltrates, expression of major histocompatibility complex (MHC) class I and class II antigens on the sarcolemma, and a blinded evaluation on a visual analog scale ranging from absence of pathology to the most pronounced pathology. Inflammation of cardiac muscles was assessed by quantification of immune cell infiltrates.

RESULTS Forty-three patients with COVID-19 (median [interquartile range] age, 72 [16] years; 31 men [72%]) and 11 patients with diseases other than COVID-19 (median [interquartile range] age, 71 [5] years; 7 men [64%]) were included. Skeletal muscle samples from the patients who died with COVID-19 showed a higher overall pathology score (mean [SD], 3.4 [1.8] vs 1.5 [1.0]; 95% CI, 0-3; $P < .001$) and a higher inflammation score (mean [SD], 3.5 [2.1] vs 1.0 [0.6]; 95% CI, 0-4; $P < .001$). Relevant expression of MHC class I antigens on the sarcolemma was present in 23 of 42 specimens from patients with COVID-19 (55%) and upregulation of MHC class II antigens in 7 of 42 specimens from patients with COVID-19 (17%), but neither were found in any of the controls. Increased numbers of natural killer cells (median [interquartile range], 8 [8] vs 3 [4] cells per 10 high-power fields; 95% CI, 1-10 cells per 10 high-power fields; $P < .001$) were found. Skeletal muscles showed more inflammatory features than cardiac muscles, and inflammation was most pronounced in patients with COVID-19 with chronic courses. In some muscle specimens, SARS-CoV-2 RNA was detected by reverse transcription–polymerase chain reaction, but no evidence for a direct viral infection of myofibers was found by immunohistochemistry and electron microscopy.

CONCLUSIONS AND RELEVANCE In this case-control study of patients who had died with and without COVID-19, most individuals with severe COVID-19 showed signs of myositis ranging from mild to severe. Inflammation of skeletal muscles was associated with the duration of illness and was more pronounced than cardiac inflammation. Detection of viral load was low or negative in most skeletal and cardiac muscles and probably attributable to circulating viral RNA rather than genuine infection of myocytes. This suggests that SARS-CoV-2 may be associated with a postinfectious, immune-mediated myopathy.

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Supplemental content

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In 2019, a new coronavirus variant with drastic global impact emerged; it currently sums up to 168 million confirmed cases of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and an estimated 3.5 million deaths from coronavirus disease 2019 (COVID-19).[1] Independent studies suggest that 30% to 60% of patients with SARS-CoV-2 infections experience myalgia.[2-4] Myalgia and increased levels of creatine kinase were found to be more pronounced in patients with critical illness needing intensive care support than individuals who were mildly affected.[5-7] Signs of skeletal muscle injury were associated with a more severe clinical course and higher mortality rates.[7,8] Several case reports describe rhabdomyolysis,[9-14] and magnetic resonance imaging findings have suggested myositis.[15-18] To our knowledge, 3 case reports included biopsies, 2 of which advocated a dermatomyositis-like phenotype.[18-21] Finally, in a large follow-up study, two-thirds of survivors of COVID-19 experienced fatigue or muscle weakness and 2% to 3% myalgia, even 6 months after an infection with SARS-CoV-2.[22] Similar results have been found elsewhere.[23,24]

Myopathy ranging from mild myositis to fatal rhabdomyolysis has been associated with many different viruses.[25-39] However, muscle biopsies are rarely performed in clinically suspected cases of virus-associated myositis. Therefore, until now, to our knowledge, only case reports and small series offer some histopathological insights. Here, we report comprehensive histopathological, virological, immunological, and ultrastructural findings of skeletal muscle samples from individuals who died with severe COVID-19 in comparison with patients with critical illnesses that were not COVID-19.

Key Points

Question Is there a COVID-19–associated myopathy, and is it a viral or postviral phenomenon?

Findings In this case-control autopsy study, 26 of 43 individuals (60%) who had died with a diagnosis of COVID-19 showed signs of muscle inflammation, ranging from mild to severe inflammatory myopathy. Inflammation was more pronounced in patients who were chronically ill and those who had seroconverted to SARS-CoV-2 than those who died after acute or subacute courses of COVID-19 and those who died of other illnesses, and no evidence was found for a direct infection of muscle tissue.

Meaning In this study, SARS-CoV-2 was associated with an immune-mediated myopathy.

Methods

Study Design

Between March 2020 and February 2021, cryopreserved tissue samples of quadriceps and deltoid muscles and cryopreserved or formalin-fixed paraffin-embedded samples of lung and heart tissue samples were obtained from individuals who died with clinical features of COVID-19 (atypical, bilateral pneumonia in chest computed tomography and characteristic laboratory findings). Exclusion criteria were a postmortem interval of more than 6 days and lack of cryopreserved skeletal muscle specimens. In addition, cryopreserved deltoid and/or quadriceps muscle tissues and formalin-fixed paraffin-embedded–preserved heart and lung samples from individuals with nasopharyngeal swabs negative for SARS-CoV-2 and absence of clinical features of COVID-19 infection were used as controls; these were individuals with critical illness who had nonseptic or septic clinical courses and underwent routine autopsy (**Table**; eTable 1 in the Supplement). Clinical records were consulted for age, sex, preexisting medical conditions, onset of clinical symptoms, length of hospitalization, duration of intensive care treatment, laboratory results, therapeutic measures, and complications. In all individuals, a whole-body autopsy was performed at the Departments of Pathology and Neuropathology, Charité–Universitätsmedizin Berlin, Berlin, Germany. The primary cause of death was defined as the condition or injury that initiated the sequence of events leading to death.[40]

This study was approved by the local ethics committees as well as by the Charité–Berlin Institute of Health COVID-19 research board and was in compliance with the Declaration of Helsinki; autopsies were performed on the legal basis of section 1 of the Autopsy Act of the State of Berlin and section 25(4) of the German Infection Protection Act. Informed consent to autopsy was either given by patients themselves, close relatives, or their legal guardians. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines were followed.

Virological and Serological Testing

Unfixed and cryopreserved or native tissue samples were used for detection and quantification of SARS-CoV-2 RNA by quantitative reverse transcription–polymerase chain reaction (RT-qPCR) in skeletal muscle, heart, and lung samples, as described previously.[41,42] Only samples with at least 2 positive results were considered positive. Oligonucleotides targeting the leader transcriptional regulatory sequence and a region within the single-guide RNA encoding the SARS-CoV-2 *E* gene were used to detect single-guide RNA, as described previously.[42,43]

We performed anti-SARS-CoV-2 IgG enzyme-linked immunosorbent assays in serum samples using a commercially available kit (Anti-SARS-IgG Kit [Euroimmun]), with an optical density ratio of 1.1 as the threshold for positivity. Antinuclear antibody assays, myositis-specific autoantibodies (anti-nuclear matrix protein-2 [anti-NXP2], anti-transcriptional intermediary factor 1γ [anti-TIF1γ], anti-melanoma differentiation-associated gene 5 [anti-MDA5], anti-signal recognition particle [anti-SRP], anti-Mi2, anti-isoleucyl-transfer RNA [tRNA] synthetase [anti-OJ], anti-glycyl-tRNA synthetase [anti-EJ], anti-threonyl-tRNA synthetase [anti-PL7], anti-alanyl-tRNA synthetase [anti-PL12], antihistidyl tRNA synthetase [anti-Jo1], and anti-small ubiquitin-like modifier-1 activating enzyme [anti-SAE]), and myositis-associated autoantibodies (anti-Ku, anti-PM75, anti-PM100, and anti-Ro52) were conducted in serum samples according to the manufacturer's instructions (ANA-Mosaik 1A EuroPattern and EuroLine,

Table. Patient Characteristics[a,b]

Characteristic	Patients, No. (%)				
	With COVID-19				
	Total	Acute	Subacute	Chronic	Without COVID-19
Patient characteristics					
Total	43	13	16	14	11
Age, median (IQR), y	72 (16)	79 (12)	69 (18)	70 (9)	71 (5)
<35	4 (9)	0	2 (13)	2 (14)	0
35-59	3 (7)	0	2 (13)	1 (7)	0
60-69	10 (23)	2 (16)	4 (25)	4 (29)	5 (45)
70-79	17 (40)	5 (38)	5 (31)	7 (50)	6 (55)
>80	9 (21)	6 (46)	3 (19)	0	0
Sex					
Female	12 (28)	3 (23)	7 (44)	2 (14)	4 (36)
Male	31 (72)	10 (77)	9 (56)	12 (85)	7 (64)
BMI					
Patients with data, No.	42	8	15	14	11
Median (IQR)	28.5 (8)	25.5 (8)	28.0 (13)	30.5 (6)	33.7 (11)
Postmortem interval, median (IQR), h[c]	45 (38)	44 (23)	48 (51)	45 (21)	42 (70)
Duration of illness, median (IQR), d[d]	24 (23)	10 (6)	24 (9)	51 (35)	21 (12)
Days in hospital, median (IQR) [No. of patients with data]	20 (25) [42]	7 (9) [12]	18 (9) [16]	43 (34) [14]	17 (14) [11]
Days in intensive care unit, No. (%) [No. of patients with data]	15 (26) [42]	4 (5) [12]	15 (9) [16]	40 (20) [14]	8 (12) [11]
Complications					
Acute respiratory distress syndrome	37 (86)	10 (77)	14 (88)	13 (93)	4 (36)
Acute kidney failure	31 (72)	7 (54)	11 (69)	13 (93)	9 (82)
Acute liver failure	14 (33)	3 (23)	5 (31)	6 (43)	4 (36)
Bacterial pneumonia	22 (51)	4 (31)	9 (56)	9 (64)	6 (55)
Sepsis or septic shock	24 (56)	2 (15)	10 (63)	12 (86)	7 (64)
Treatments, No. (%)					
Invasive ventilation	28 (65)	3 (23)	11 (67)	14 (100)	9 (82)
Kidney replacement therapy	21 (49)	3 (23)	8 (50)	10 (71)	9 (82)
Extracorporeal membrane oxygenation	16 (37)	1 (8)	5 (31)	10 (71)	3 (27)
Catecholamines	29 (67)	3 (23)	12 (75)	14 (100)	9 (82)
Corticosteroids, No. (%) [No. of patients with data]	20 (49) [41]	6 (46) [11]	10 (63) [16]	4 (29) [14]	1 (9) [11]
Intravenous immunoglobin, No. (%) [No. of patients with data]	3 (7) [41]	0 [11]	0 [16]	3 (22) [14]	1 (9) [11]
No. (%) [No. of patients with data]	2 (5) [41]	0 [11]	1 (6) [16]	1 (7) [14]	1 (9) [11]
Laboratory values[a,b,e]					
Creatine kinase values, U/L					
First, median (IQR) [No. of patients with data]	206 (365) [38]	153 (382) [8]	233 (376) [16]	210.5 (262) [14]	97 (103) [10]
Last, median (IQR) [No. of patients with data]	164 (392) [38]	315 (273) [8]	171 (609) [16]	58 (206) [14]	175 (567) [10]
Highest, median (IQR) [No. of patients with data]	600 (748) [38]	359 (583) [9]	694 (706) [16]	808 (1374) [14]	485 (885) [11]
Creatine kinase-MB fraction values, U/L					
First, median (IQR) [No. of patients with data]	23 (24) [31]	18 (32) [6]	22 (6) [15]	32 (25) [13]	36 (23) [9]
Last, median (IQR) [No. of patients with data]	24 (26) [31]	29 (24) [6]	26 (25) [15]	17.7 (27) [13]	35 (50) [9]
Highest, median (IQR) [No. of patients with data]	41 (29) [39]	35 (73) [7]	41 (20) [15]	42 (23) [14]	55 (269) [10]

(continued)

Table. Patient Characteristics[a,b] (continued)

Characteristic	Patients, No. (%)				
	With COVID-19				
	Total	Acute	Subacute	Chronic	Without COVID-19
Troponin values, ng/mL					
First, median (IQR) [No. of patients with data]	0.040 (0.070) [30]	0.074 (0.149) [8]	0.043 (0.044) [11]	0.035 (0.068) [11]	0.065 (0.105) [10]
Last, median (IQR) [No. of patients with data]	0.069 (0.149) [30]	0.108 (0.832) [8]	0.052 (0.054) [11]	0.106 (0.116) [11]	0.113 (0.236) [10]
Highest, median (IQR) [No. of patients with data]	0.103 (0.152) [32]	0.127 (0.920) [8]	0.078 (0.063) [12]	0.138 (0.107) [12]	0.118 (0.257) [10]
Ferritin values, ng/mL					
First, median (IQR) [No. of patients with data]	1377 (2083) [36]	1418 (3011) [8]	1161 (549) [14]	1998 (3120) [14]	ND
Last, median (IQR) [No. of patients with data]	2779 (7537) [36]	1843 (8080) [8]	2048 (3272) [14]	6436 (12 422) [14]	ND
Highest, median (IQR) [No. of patients with data]	3995 (10 691) [36]	1852 (7845) [8]	2466 (3656) [14]	10 958 (36 849) [14]	ND
Interleukin 6 values, pg/L					
First, median (IQR) [No. of patients with data]	152 (570) [30]	146 (9443) [5]	174 (567) [13]	139 (357) [12]	ND
Last, median (IQR) [No. of patients with data]	282 (988) [30]	362 (9534) [5]	282 (993) [13]	424 (778) [12]	ND
Highest, median (IQR) [No. of patients with data]	926 (5926) [33]	362 (3745) [7]	892 (2998) [14]	1894 (8377) [13]	ND
No. of positive SARS-CoV-2 polymerase chain reaction test results, median (IQR)	2 (5)	1 (1)	3 (3.5)	5 (5.5)	0[f]

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); IQR, interquartile range; ND, no data.

SI conversion factors: To convert creatine kinase to μkat/L, multiply by 0.0167; ferritin to μg/L, multiply by 1.0; troponin to μg/L, multiply by 1.0.

[a] Numbers were rounded, and percentages may not add to 100 because of rounding.

[b] In cases in which data were not available for all patients, numbers of patients with data are indicated.

[c] Defined as the number of hours between time of death and start of autopsy.

[d] Defined as the delay between the first symptoms that led to hospitalization and death.

[e] First, last, and highest values available per patient and hospitalization. When only 1 value was available, this one was classified as the highest.

[f] All control patients were tested at least once, with a median (interquartile range) of 2 (3) nasopharyngeal swabs per patient.

Autoimmune Inflammatory Myopathies 16 Ag immunoassay [Euroimmun]).

Histology and Immunohistochemistry

Specimens from quadriceps and deltoid muscles were snap frozen and stored at −80 °C until further workup. Samples of lung and heart tissues were either fixed in formalin or cryopreserved. Stains on cryopreserved samples were performed on 7-μm cryomicrotome sections, and stains on formalin-fixed paraffin-embedded tissue were performed on 4-μm sections. Routine histological staining (hematoxylin-eosin, Gömöri trichrome, and periodic acid–Schiff) were performed according to standard procedures. Immunohistochemical staining was performed on a Benchmark XT autostainer (Ventana Medical Systems), as described previously.[44] Immunohistochemical detection of SARS-CoV-2 was performed as described previously.[42]

Semiquantitative Scores

Semiquantitative scores were generated for major histocompatibility complex class I (human leukocyte antigen [HLA]-ABC), class II (HLA-DR), CD45, CD68, CD8, and NKp46. Ten random fields of vision were viewed independently by 2 experienced morphologists (W.S. and T.A.) at ×400 magnification with an Olympus BX50 microscope (Ocular WH10X-H/22; high-power field). Positive staining results with MHC class I (DAKO; clone W6/32, 1:100) and MHC class II (DAKO; M0775, 1:100) were defined as a clear upregulation on the sarcolemma; capillaries and arterioles were used as internal positive controls. For quantification of endomysial immune cell

populations, antibodies against CD45 (DAKO; clone UCHL1, 1:100), CD68 (DAKO; clone EBM11, 1:100), CD8 (DAKO; clone C8/144B, 1:100), and NKp46 (R&D Systems; clone MAB1850, 1:100) were used. Positively stained cells were counted and summed up for 10 high-power fields of vision.

Modified Visual Analog Scale Score

We defined 3 categories of muscle pathology (inflammation, necrosis, and degeneration) and applied a modified visual analog scale score inspired by Wedderburn et al.[45] The overall pathology score was the global visual analog scale score, taking into account all 3 categories. All specimens were viewed in a blinded manner and graded on a severity scale from 0 (no pathology) to 10 (most pronounced pathology) by 2 observers independently (W.S. and T.A.).

Electron Microscopy

Electron microscopy was performed on quadriceps muscle specimens from individuals with light microscopic signs of myositis, as described previously.[42] At least 100 capillaries were assessed per sample, and images were interpreted by 2 neuropathologists (W.S. and H.H.G.) with long-standing experience in ultrastructural analysis.

Statistical Analysis

Statistical analyses were performed and graphs created with GraphPad Prism 9 (GraphPad Software). Heat map visualizations were created with Orange Data Mining software version 3.28.0 (University of Ljubljana)[46] and tables with Excel 2016 (Microsoft). Data are presented as counts and percentages, means (SDs), or medians (interquartile ranges [IQRs]). We performed normality testing in GraphPad Prism to decide on median vs mean data calculations. The Mann-Whitney *U* test was applied for comparison of 2 groups. No data points were excluded, and values were considered significant at $P < .05$.

Results

Patients Characteristics

Forty-three individuals with COVID-19 and 11 patients without COVID-19 were included. Of the 43 with COVID-19, 42 had a direct detection of SARS-CoV-2 RNA by nucleic acid amplification tests from the upper respiratory tract registered during the patient's life. In 1 case with clinical features suggestive of COVID-19 and serological proof of infection with SARS-CoV-2, no lifetime RT-qPCR data were available, but high viral loads were detected in the lower respiratory tract after death. In 3 individuals with COVID-19, only 1 of either quadriceps or deltoid muscle samples was collected. In the full cohort, detailed patient histories of 2 patients referred from other hospitals could not be retrieved; also, an insufficient quantity of tissue was preserved for analysis for 1 patient. The median age was 72 (IQR, 16; range, 26-92) years in the COVID-19 cohort and 71 (IQR, 5; range, 65-76) years in the control cohort. Most deceased individuals in both groups were men (31 of 43 with COVID-19 and 7 of 11 without COVID-19). The median duration of illness (DOI), defined as the number of days between the onset of symptoms that led to hospitalization or the first positive RT-qPCR test results and the time of death, was 24 (range, 5-181) days in the COVID-19 cohort and 21 (range, 1-68) days in the control group. We determined 3 subgroups within the COVID-19 cohort: those with acute cases, with DOIs less than 15 days; subacute cases, with DOIs between 15 and 30 days; and chronic cases, with DOIs more than 30 days (Table).

All but 1 of the patients examined had concurrent medical conditions, but none was diagnosed with a primary myopathy during their lives. In 36 of 43 patients with COVID-19 (84%), COVID-19 was the primary underlying cause of death (eTable 1 in the Supplement).

All but 5 patients in the COVID-19 group (38 of 43) and all but 1 in the control cohort (10 of 11) were admitted to an intensive care unit. The median time in intensive care was 14.5 (range, 0-173) days in the COVID-19 and 8.0 (range, 0-43) days in the control group.

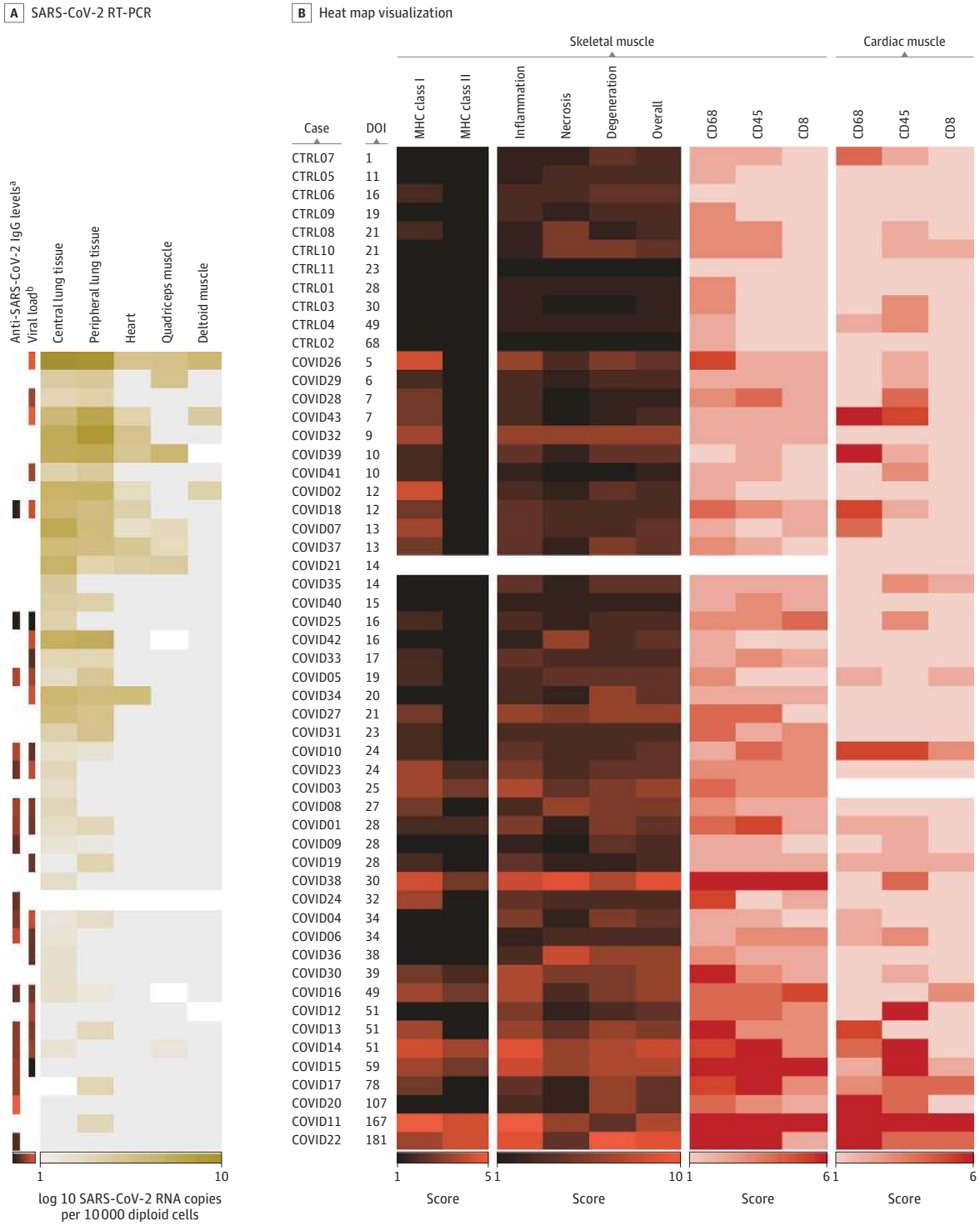
Detailed information on administered therapies was available for 41 patients with COVID-19. Twenty of these (49%) received corticosteroids, 3 (7%) received intravenous immunoglobulins, 2 (5%) received anakinra, and 1 (2%) received ruxolitinib. In the control group, 1 patient received intravenous immunoglobulins, dexamethasone, cyclophosphamide, and bortezomib as treatment for multiple myeloma.

The median of the first measured value of creatine kinase was significantly higher in the COVID-19 cohort (206 U/L [to convert to microkatal per liter, multiply by 0.0167]; range, 16-6367 U/L; 95% CI, 132-191 U/L; vs 97 U/L; range, 18-819 U/L; 95% CI, 18-254 U/L; $P = .045$). In total, 14 patients (33%) with COVID-19 and 2 control patients (18%) showed creatine kinase levels greater than 1000 U/L. Patients with chronic COVID-19 showed higher maximum ferritin levels (10 958 [95% CI, 3589-66 866] ng/mL) than patients with acute courses (1852 [95% CI, 102-50 974] ng/mL; $P = .04$) or subacute courses (2466 [95% CI, 1015-7674] ng/mL; $P = .004$) (Table). Procalcitonin, C-reactive protein, and leukocyte counts as nonspecific markers of systemic inflammation were comparable between groups (eTable 1 in the Supplement).

Virological Analysis

Testing for SARS-CoV-2 via RT-qPCR was positive in at least 1 of 2 different lung samples in 38 of 43 patients with COVID-19 (88%), heart tissue of 10 of 42 patients (23%), quadriceps muscle tissue of 7 of 41 patients (16%), and deltoid muscle tissue of 2 of 42 patients (5%). Subgenomic viral RNA was detected in samples from 20 patients (lung, 17; heart, 2; quadriceps muscle, 2; deltoid muscle, 1). Seroconversion had occurred in 17 of the 18 patients for which anti-SARS-CoV-2 antibodies were measured during their lives (**Figure 1**A). Viral load in lungs inversely correlated with the DOI (central lung specimens, log 10: Spearman *r*, −0.86 [95% CI, −0.93 to −0.75]; $P < .001$) and was higher in patients treated with corticosteroids (central lung specimens [log 10] of patients not treated with corticosteroids: n = 20; median [IQR], 2 [2.5] log 10 SARS-CoV-2 RNA copies per 10 000 diploid cells; vs those treated with corticosteroids: n = 18; median [IQR], 3 [4] log 10 SARS-CoV-2 RNA copies per 10 000 diploid cells; 95% CI, 1-6; $P < .01$) (Figure 1A; eFigure 3 in the Supplement).

Figure 1. Summary of Virological and Histopathological Findings



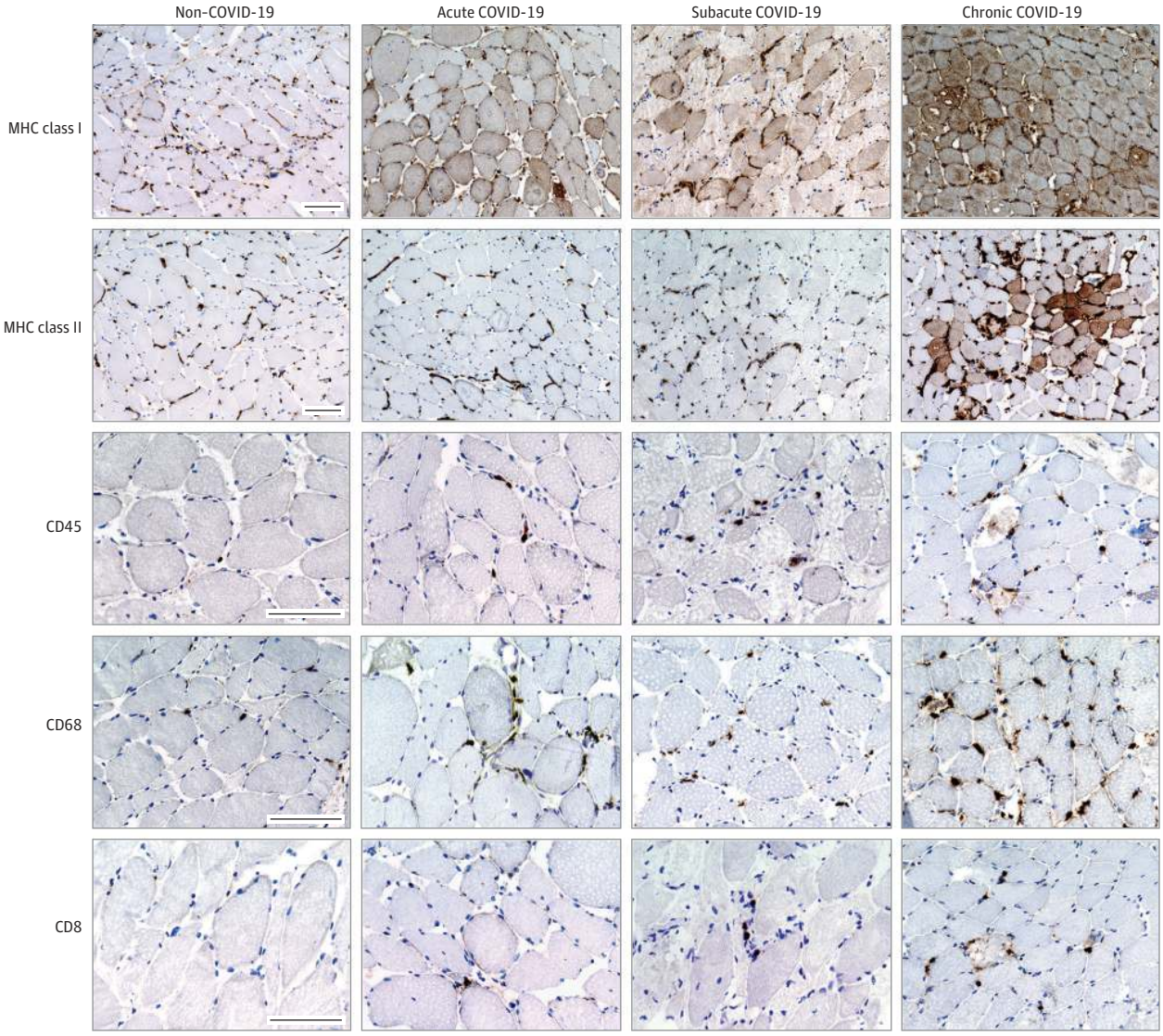
A, Quantification of SARS-CoV-2 RNA by reverse transcription–polymerase chain reaction (RT-PCR) tests of tissues in patients with COVID-19.

[a] Serum anti-Sars-CoV-2 IgG levels (n = 17; black indicates a negative result; red, an optical density gradient).

[b] Viral load from nasopharyngeal swabs within the 5 days prior to death by SARS-CoV-2 RNA RT-PCR testing (in copy numbers per microliter; n = 23; black indicates a negative result; red, a viral load gradient).

B, Heat map visualization of histopathological analysis of skeletal and heart muscle specimens. Scores are described in the methods section, absolute cell counts are shown in eTable 2 in the Supplement, and statistical analysis can be found in eFigure 2 in the Supplement. White rectangles indicate missing data. COVID followed by a number indicates a patient with COVID-19; CTRL followed by a number, a control patient; DOI, duration of illness; MHC, major histocompatibility complex.

Figure 2. Inflammatory Changes in Skeletal Muscles of Individuals Critically Ill With Diseases Other than COVID-19 Compared With Patients With COVID-19



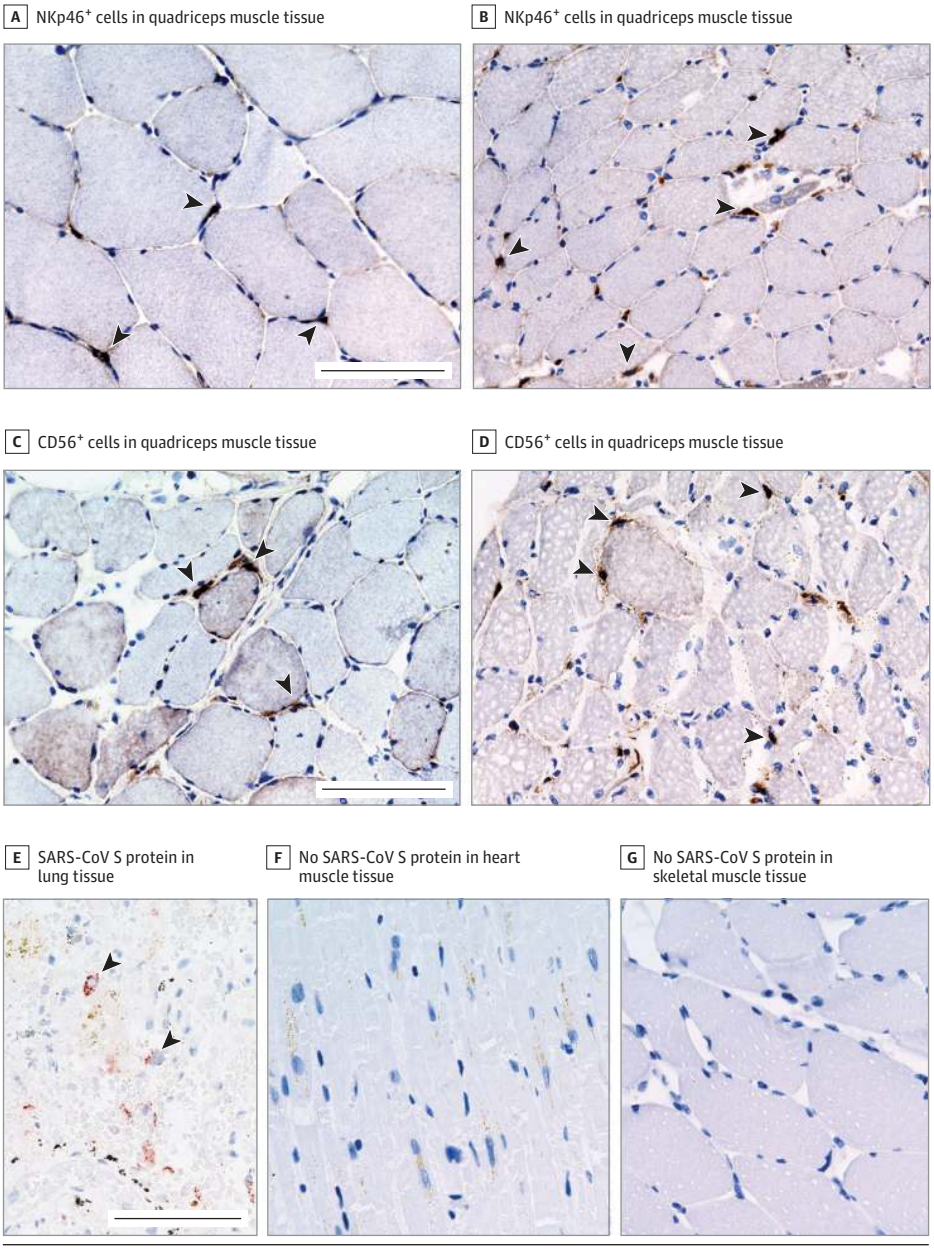
Representative micrographs of postmortem samples from quadriceps muscles from a patient without COVID-19 (CTRL07), compared with samples from patients with acute (COVID37), subacute (COVID23), and chronic (COVID14) COVID-19 disease courses. Major histocompatibility complex (MHC) class I and MHC class II: original magnification ×200; CD45, CD68, and CD8: original magnification ×400; scale bars: 100 µm.

Histology and Immunohistochemistry of Skeletal Muscles

Patients with COVID-19 showed a higher mean (SD) overall pathology score (3.4 [1.8] vs 1.5 [1.0]; 95% CI, 0-3; $P < .001$). Among control patients, the highest score for inflammation was 2, while in the COVID-19 group, 26 of 42 cases (62%) showed a score higher than 2 (mean [SD], 3.5 [2.1] vs 1.0 [0.6]; 95% CI, 0-4; $P < .001$). Signs of degenerating muscle fibers were more frequently encountered in the COVID-19 cohort (mean [SD], 3.4 [1.8] vs 1.5 [1.4]; 95% CI, 0-4; $P < .005$). Clinically significant expression of MHC class I (HLA-ABC) antigens on the sarcolemma (defined as a score >1 of 6) could be found in 23 of 42 patients with COVID-19 (55%) but none of the controls. Upregulation of MHC class II (HLA-DR) was found in 7 of 42 patients with COVID-19 (17%), all of whom had a DOI longer than 21 days, and in none of the control samples (Figure 1; **Figure 2**; eTable 2 in the Supplement). In 3 individuals with COVID-19, MHC class I and MHC class II expression on myofibers showed unequivocal perifascicular staining patterns (eFigure 1 in the Supplement).

Five individuals in the COVID-19 cohort (12%) showed marked infiltration by CD45-positive leukocytes and CD8-positive T cells (score >4 of 6). For all other biopsy samples, infiltrations of CD45-positive leukocytes and CD8-positive T cells were moderate to mild but significantly higher than in control samples (CD45: median [IQR], 28 [20] vs 13 [11] cells per 10 high-power fields; 95% CI, 7-33 cells per 10 high-power fields; $P < .001$; CD8: median [IQR], 7.5 [6.5] vs 2 [2]

Figure 3. Immunohistochemistry Showing Natural Killer Cells and No SARS-CoV-2 Spike Protein Detection



Representative micrographs of postmortem samples from quadriceps muscle (A-D), lung (E and F), and heart (G) tissue of patients with COVID-19. A, NKp46-positive cells (arrowheads) in a patient with an acute course (the patient coded as COVID43); B, NKp46-positive cells (arrowheads) in a patient with a subacute course (COVID38); C, CD56-positive cells (arrowheads) in a patient with an acute case (COVID37); D, CD56-positive cells (arrowheads) in a patient with a chronic case (COVID14); E, SARS-CoV S protein detection (arrowheads) in lung tissue of a patient with an acute case (COVID26); F, no SARS-CoV S protein detection in heart muscle tissue of a patient with an acute case (COVID34) with positive real-time quantitative polymerase chain reaction results; G, no SARS-CoV S protein detection in skeletal muscle tissue of a patient with an acute case (COVID29) with positive real-time quantitative polymerase chain reaction results. Original magnification ×400; scale bar: 100 µm.

cells per 10 high-power fields; 95% CI, 1-9 cells per 10 high-power fields; $P < .001$; Figure 1 and 2; eTable 2 and eFigure 2 in the Supplement). We found NKp46-positive and CD56-positive natural killer cells in close contact with myofibers, and their numbers were increased compared with control samples (median [IQR], 8 [8] vs 3 [4] cells per 10 high-power fields; 95% CI, 1-10 cells per 10 high-power fields; $P < .001$; **Figure 3**; eTable 2 and eFigure 2 in the Supplement).

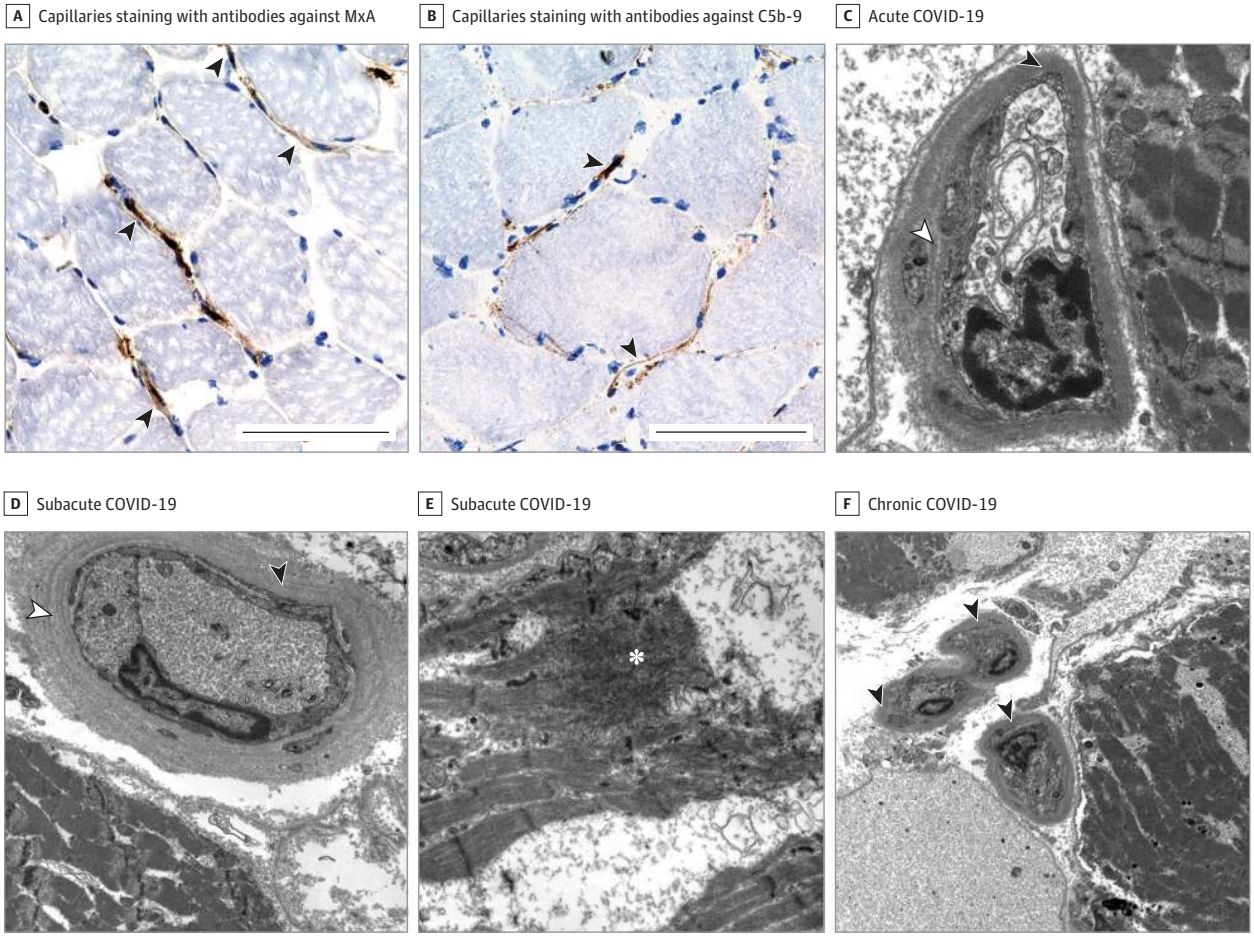
In 8 of 43 specimens (18%), we found notable capillary expression of human myxovirus resistance protein 1 (MxA). This finding was not present in control samples (**Figure 4**; eTable 2 in the Supplement).

A subset of COVID-19 samples displayed signs of small and medium vessel angiitis, with perivascular infiltrates of CD45-positive leukocytes, CD8-positive T cells, and CD68-positive macrophages but without transmural vessel infiltration (eFigure 1 in the Supplement). Necrotic fibers and capillary expression of C5b-9 were similarly found in specimens from patients with and without COVID-19. Of note, immunohistochemistry testing against SARS-CoV-2 spike protein did not yield positive results in skeletal muscle specimens that were positive by RT-qPCR (Figure 3G).

Histology and Immunohistochemistry of Heart Muscles

Numbers of myocardial CD68-positive macrophages were higher in patients with COVID-19 than control patients (median [IQR], 12 [25] vs 7 [10] macrophages; 95% CI, 1-20 macrophages; $P = .02$). Numbers of CD45-positive leukocytes

Figure 4. Immunohistochemical and Ultrastructural Analysis of Capillary Pathology



Representative light microscopic (A and B) and transmission electron microscopy images (C-F) of quadriceps specimens from patients who died with COVID-19. A, Capillary staining with antibodies against human myxovirus resistance protein 1 in a patient with an acute case (the patient coded as COVID29). B, Capillary staining with antibodies against C5b-9 in a patient with a subacute case (COVID05); original magnification, ×400; scale bar: 100 µm. C-F, Ultrastructural analyses showing basement membrane thickening (C-D and F; black arrowheads), basement membrane duplications (C-D; white arrowheads), sometimes by several layers (D, asterisks), and cytoplasmic bodies (E, asterisk). C, Image from a patient with an acute case (COVID07); original magnification: ×12 000. D, Image from a patient with a subacute case (COVID25); original magnification ×7000. E, Image from a patient with a subacute case (COVID38), original magnification ×12 000. F, Image from a patient with a chronic case (COVID14); original magnification ×3000.

and CD8-positive T cells were not significantly increased (Figure 1; eTable 2 in the Supplement). Numbers of myocardial CD45-positive leukocytes moderately correlated with numbers of CD45-positive leukocytes in skeletal muscle (Spearman *r,* 0.66; Pearson *r*, 0.71; $P < .001$; eFigure 3 in the Supplement). Immunohistochemistry against SARS-CoV-2 spike protein did not yield positive results in cardiac muscle specimens that were positive by RT-qPCR (Figure 3F).

Ultrastructural Analysis

Tubuloreticular inclusions as evidence of endothelial damage mediated by type I interferon were not found in any of the 5 analyzed samples with light microscopic signs of myositis. However, capillaries showed basement membrane thickening and duplications, sometimes by several layers. Pericyte processes were prominent around some capillaries and debris with whorled figures was found in others, indicating a regenerative process after a primary injury. Endothelial cells were markedly thicker in many cases and harbored increased numbers of organelles with frequent swollen mitochondria and a granular appearance of cytoplasm with numerous ribosomes, indicating ongoing regenerative processes. Obstruction or acute thrombosis of small vessels was not observed. At many occasions, prominent cytoplasmic bodies as nonspecific indicators of severe muscle injury were identified (Figure 4).

Autoantibodies

In 10 patients with COVID-19, antinuclear antibody assays were performed during their lives, 2 of which showed a positive titer of 1:320. For 7 patients with COVID-19 and manifest myositis, serum samples were available for autoantibody profiling. None of the patients had relevant titers

of myositis-specific autoantibodies, but in samples from 1 young woman, Ro52 autoantibodies were present at low levels (eTable 3 in the Supplement).

Discussion

In this case-control study of patients who died with and without COVID-19, we found that most patients who died with severe COVID-19 displayed signs of myositis on a spectrum ranging from mild to severe inflammation. Significant upregulation of MHC class I antigens in the early phase of the disease and concomitant upregulation of MHC class II antigens on myofibers in later stages indicate involvement of skeletal muscle in the immune response against SARS-CoV-2. Inflammation of skeletal muscle correlated with DOI and inflammation of heart muscle. Overall, inflammatory changes were more pronounced in skeletal muscles than cardiac muscle, which is in line with findings suggesting myocarditis only in a subset of patients.[47-49] In our study, some patients showed capillary expression of MxA, indicating a type I interferon signature and a perifascicular expression of MHC antigens, reminiscent of dermatomyositis.[50-52] However, we did not find MxA expression on the sarcolemma in any case, nor did we find tubuloreticular inclusions in capillaries of 5 selected patients, including 1 with capillary MxA expression and 2 with perifascicular MHC expression. Ultrastructural analysis revealed capillary alterations, suggesting ongoing remodeling processes. An important role for the vasculature niche has been described early in the pandemic.[53-55]

We identified natural killer cells in proximity to myofibers in many specimens and hypothesize that they may play a role in the pathogenesis of COVID-19–associated myositis. Natural killer cells are known first-line effector and regulator cells in viral disease, and their role in other types of myositis has been described.[56]

In an autopsy case series from the 2003 outbreak of SARS-CoV-1, the authors found necrotic myofibers in 4 of 8 patients.[57] We found that necrotic myofibers and capillary complement deposition were not confined to muscles of patients with COVID-19. Therefore, we conclude that those findings are not specific but rather consequences of sepsis and/or critical illness.[58] With the heterogeneous course of COVID-19, with sometimes long stays in intensive care and often septic states, appropriate control groups are mandatory.[59] Our control group was comparable with the COVID-19 cohort with regard to invasive treatments, clinical and laboratory indicators of sepsis, and general systemic inflammation. Length of hospitalization and intensive care unit stays were longer in the cohort with COVID-19, as seen elsewhere.[60]

Immunohistochemical staining with antibodies against SARS-CoV-2 spike protein did not yield positive results, and no overt viral particles were found by electron microscopy.[61] Because increased blood levels of SARS-CoV-2 RNA were reported in patients with critical illness,[62] it is conceivable that positivity by RT-qPCR is attributable to circulating genomic viral RNA rather than genuine infection of myocytes. It has been shown that SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) in conjunction with transmembrane serine protease 2 and/or cathepsin L to enter host cells,[63,64] and ACE2 seems to be expressed in human cardiomyocytes but not skeletal muscle.[65] A recent study did not find ACE2-transmembrane serine protease 2 and ACE2-cathepsin L coexpression in skeletal muscle tissue.[66] This, together with our findings that show significant inflammation mostly in individuals with subacute and chronic courses after seroconversion, argues for an immune-mediated myositis rather than a direct viral infection of myofibers.

Our study included patients from the first pandemic wave who did not receive corticosteroids. Interestingly, when compared with individuals affected more recently, who were treated with corticosteroids, we did not find a significant reduction of inflammation but found higher viral loads in respiratory tissues, which to our knowledge had not been described before now (eFigure 3 in the Supplement).

In 7 patients who died with COVID-19 and clear signs of myositis, we could obtain cryopreserved serum samples and performed autoimmune diagnostic tests by analyzing known myositis-specific and myositis-associated autoantibodies. One sample showed weakly positive results for anti-Ro52 testing, which has been associated with different types of myositis.[67-69] Two of 10 patients with COVID-19 for whom an antinuclear antibody assay was performed during their lives showed a positive result, which is in line with other findings.[70-72]

Limitations

Morphological autopsy studies may be biased by autolytic changes. We used controls with comparable postmortem intervals to circumvent this.

Our cohort of patients with COVID-19 included only those with severe disease courses with fatal outcomes, which limits extrapolation to patients with mild SARS-Cov-2 infections. In addition, data on clinical correlates (myalgia, weakness) prior to death were scarce.

We included control specimens from patients with critical illness admitted to our intensive care unit who were SARS-CoV-2 negative and did not show clinical features of COVID-19. However, because this control cohort did not show evidence for other viral infections (eg, influenza), we cannot exclude that the findings in the COVID-19 cohort reflect general features of severe viral infection rather than effects specific to SARS-CoV-2 alone.

Because of the longer duration of intensive care treatment of the COVID-19 cohort compared with the control group, we cannot exclude that critical illness myopathy and intensive care unit–acquired weakness affected the morphology of skeletal muscle more in these patients than controls. However, the signs of myositis we observed are not typical features of critical illness myopathy.[73-76]

The same holds true for iatrogenic effects of myotoxic drugs. Prolonged treatment with corticosteroids may cause myopathic changes, and propofol has been associated with necrotizing myopathy but not with myositis.[77-80]

Conclusions

In this case-control study of patients who had died with and without COVID-19, most individuals with severe COVID-19 showed signs of myositis ranging from mild to severe. SARS-CoV-2 may be associated with a postinfectious myositis in patients with severe illness. Whether these findings can be extrapolated to milder disease courses and potentially explain chronic muscle fatigue syndromes as described in post-acute COVID-19 syndromes and whether autoimmune mechanisms are involved will need to be addressed in future studies.

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