

Clinical Features and Histopathological Changes of Skeletal Muscle in Patients with COVID-19: Two Case reports

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Case report

Keywords: SARS-CoV-2, COVID-19, skeletal muscle, muscle injury, pathology

Posted Date: July 24th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-45595/v1>

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Abstract

Background

To the best of our knowledge, muscle soreness is a common manifestation for the coronavirus disease-19 (COVID-19) patients, but the mechanism of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) injury to skeletal muscle remains unclear, there has been no publication focused on muscle involvement in COVID-19 patients.

Case presentation

We present the case of two Chinese men with COVID-19, whose common symptoms were fatigue and muscle soreness. They went through different treatments, patient 1, 81-year-old, eventually died of multi-organ failure, and patient 2, 53-year-old, underwent amputation of the mid-lower section of left thigh. Laboratory tests in both patients showed abnormal biochemical parameters associated with skeletal muscle injury. We obtained skeletal muscle samples from these two patients, one from postmortem biopsy of gastrocnemius muscle and the other from a resected left lower limb due to thrombosis. The pathological findings in patient 1 were mainly scattered atrophic muscles, while fiber necrosis and minor inflammation were identified in patient 2, and the mild infiltrations were confirmed by CD68 and LCA staining to be predominantly macrophages and lymphocytes.

Conclusions

We report the clinical and laboratory features together with histopathological findings in skeletal muscle tissues from two COVID-19 cases and speculate that the SARS-CoV-2 may cause skeletal muscle injury. Due to the particularity of individual differences in case reports, the background of chronic neuromuscular disease in patient 1 and a minimal compartment syndrome caused by thrombosis in patient 2 need to be excluded prior to the conclusion that the skeletal muscles have been involved in COVID-19.

Background

Since the coronavirus disease-19 (COVID-19) outbreak in late December 2019, more than 14 million infections have been confirmed around the world by 18 July 2020^[1–2]. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the seventh member of the family of coronaviruses that infect humans. It has four major structural proteins, including the spike surface glycoprotein, small envelope protein, matrix protein, and nucleocapsid protein^[3–5]. The spike proteins of SARS-CoV-2 binds with a metalloproteinase named angiotensin-converting enzyme 2 (ACE2) expressed on the surface of the host cells^[6, 7]. ACE2 is abundantly present in human epithelia and all kinds of smooth muscle cells in all organs studied^[8]. Of note, 11–48% of COVID-19 patients present with muscle ache. However, there has been no publications focused on muscle involvement in the patients^[9–11]. The present study was conducted through one postmortem biopsy and one amputated specimen from a patient who developed

arterial thrombosis during the course of COVID-19 treatment. We tried to elucidate the clinicopathology findings of skeletal muscle in these patients of SARS-CoV-2 infection.

Materials And Methods

The two cases presented here met the clinical diagnostic criteria as provided in the National Health Commission of China Guidelines. Electronic medical records were searched to identify the patients' clinical features and laboratory findings. Demographic data, medical history, chest computed tomographic (CT) scans, duration of illness, and laboratory findings related to our study such as nucleic acid test, complete blood count (CBC), and biochemical parameters of muscle, including Creatine kinase, Creatine kinase–MB, Lactate dehydrogenase were reviewed.

Pathology examination and immunohistochemistry staining

The biopsy specimens were delivered to the surgical pathology lab and processed according to the routine biosafety standards. Hematoxylin and eosin-stained sections were prepared.

Immunohistochemistry staining (IHC) was performed using antibodies against ACE2 (Bioss company, Beijing), LCA, CD20, CD3, CD4, CD8, and CD68 (Agilent Technologies, US). All the antibodies were in prediluted form, and IHC was performed using an automated machine Leica Bond Max.

Real-Time reverse transcription polymerase chain reaction assay for SARS-CoV-2

The RT-PCR assay for SARS-CoV-2 was performed on selected tissue blocks. Formaldehyde fixed and paraffin embedded (FFPE) tissue samples were collected, and 20 series cuts with 4- μ m for each case were put into a centrifugate tube. Extraction of total RNA and real-time reverse transcription polymerase chain reaction (PCR) assay were performed according to the manufacturer's protocol, and target genes and diagnostic criteria were the same as described^[12].

Results

Clinical features and laboratory findings

Epidemiological, clinical, radiological characteristics and treatment history were summarized in Figs. 1 and 2, respectively. Generalized clinical findings together with laboratory parameters were shown in Table 1.

Table 1
Clinical and laboratory findings of the patients

	Patient 1	Patient 2
Clinical characteristics		
Age (years)	81	53
Gender	Male	Male
Epidemiological or exposure history	Yes (contact with infected person)	Yes (exposure to relevant environment) #
Family members were affected or not	Yes	No
Basic diseases	Type 2 diabetes; Hypertension	No
Signs and symptoms		
Fever	+	-
Cough	-	+
Fatigue	+	+
Muscle soreness	+	+
Shortness of breath	+	+
Other symptoms	No	Swelling of left lower extremity
Laboratory characteristics		
White blood cell count ($\times 10^9$ cells per L); (normal range 3.5–9.5)	11.89 (↑)	10.76 (↑)
Neutrophil count ($\times 10^9$ cells per L); (normal range 1.8–6.3)	10.99 (↑)	8.70 (↑)
Lymphocyte count ($\times 10^9$ cells per L); (normal range 1.1–3.2)	0.28 (↓)	0.90 (↓)
C-reactive protein (mg/L); (normal range 0.0–10.0)	159.43 (↑)	60.0 (↑)
D-dimer ($\mu\text{g/L}$); (normal range 0–200)	12972 (↑)	914 (↑)

+ = positive. - = negative. ↑ = above normal range. ↓ = below normal range. # Exposure to relevant environment, such as Hankou, the area in Wuhan where the epidemic was first detected. NA, not available; §, in our hospital

	Patient 1	Patient 2
Prothrombin time (s); PT (normal range 9.4–12.5)	14.1 (↑)	20.1 (↑)
Activated partial thromboplastin time (s); APTT (normal range 25.1–36.5)	21.5 (↓)	38.6 (↑)
Creatine kinase (U/L); (normal range < 171)	NA	NA
CK-MB (U/L); (normal range 0–25)	28 (↑)	NA
LDH (U/L); (normal range 125–243)	513 (↑)	NA
IL-6 (ng/L); (normal range < 10)	51.8 (↑)	NA
PCT (ng/L); (normal range < 0.5)	0.92 (↑)	NA
SARS-CoV-2 quantitative RT-PCR	(+)	(–) (Multiple) [§]
CT evidence of pneumonia		
Typical signs of viral infection	Yes	Yes
<i>+ = positive. – = negative. ↑ = above normal range. ↓ = below normal range. # Exposure to relevant environment, such as Hankou, the area in Wuhan where the epidemic was first detected. NA, not available; §, in our hospital</i>		

Patient 1 was an 81-year-old male who was admitted to our hospital with symptoms of fatigue, muscle soreness, and anorexia for two weeks. He had a past medical history of diabetes and hypertension for over 20 years. Initially, he had been exposed to a family member who was found to be infected with COVID-19, and his CT image showed features of viral pneumonia one week later. The diagnosis was confirmed by a positive nucleic acid test for SARS-CoV-2. He was given oral antiviral therapy and quarantined at home. His condition deteriorated, however, and was hospitalized in another hospital for one week before he was transferred to the intensive care unit (ICU) in our hospital. He was given comprehensive treatment, including intravenous antibiotics, assisted oxygenation, and oral medication to manage his hypertension and blood glucose. Corticosteroid was used to control the cytokine storm and stabilize homeostasis. His temperature varied from normal to 37.4°C. However, there was no improvement in his condition. He died of multi-organ failure on Feb 20. The duration of the clinical course was 24 days (Fig. 1).

Patient 2 was admitted to an outside hospital with main manifestations of fatigue, anorexia, muscle soreness for more than a month, and swelling of left lower limb for half a month (Fig. 2). On January 11, the patient presented with manifestations of muscle soreness throughout the body, poor appetite, asthenia with unknown cause, and denied discomforts such as fever, nausea, and vomiting. On January 21, CT report from the outside hospital showed patchy ground-glass opacity in bilateral lobes, and dense subpleural lesions with an uneven density and unclear margin. Repeated CT examination as reported on January 25 indicated patchy ground-glass opacity in bilateral lobes and more slightly thickened bilateral

pulmonary lesions as compared with the CT report of January 21. CT report of January 28 showed patchy ground-glass opacity in bilateral lobes, and part of the lesion was absorbed compared with the previous CT reports. Novel Coronavirus RNA test was negative on January 28. Antiviral medication was administered (details not specified). Swelling of left lower limbs occurred during the treatment in the outside hospital. B-ultrasonography in the outpatient department of the other hospital indicated arterial thrombosis of the left lower extremity accompanied by deep vein thrombosis in the same extremity. The patient was treated with oral anticoagulation (rivaroxaban + clopidogrel). On February 13, due to aggravation of symptoms, the patient was again taken to the outpatient department of that outside hospital and was given oral medication. The patient complained of a cough and had hyperpigmentation and edema of the left lower limb, but did not have dyspnea and shortness of breath. He was transferred to our hospital on Feb 18. Chest CT examination showed ground-glass shadow, and consolidation could still be seen in the subpleural area of the left and right lower lobes of the lung. Air bronchograms were noticed in the left lower lobe lesion of the lung on Feb 22. Apart from the Chest CT scan images showing bilateral pneumonia with different severity extent over time, he also had popliteal arterial thrombosis in the left lower limb (Fig. 3). Multiple nucleic acid tests for SARS-CoV-2 were negative as a part of pre-surgical tests and preparations. Amputation of the mid-lower section of the thigh was done on Feb 24 in our hospital. The patient recovered well and was discharged on March 10.

From Table 1, laboratory tests showed both patients had abnormalities in their blood, including elevated white blood cell (WBC) count and neutrophil count, and reduced lymphocyte count. Increased creatine kinase–MB (CK-MB) and lactate dehydrogenase (LDH) in patient 1 suggested he had muscle injury, while these parameters were unavailable for patient 2. However, these parameters can also cross-reflect the function of the heart to some extent. Biomarkers of inflammation such as procalcitonin (PCT), interleukins 6 (IL-6), and C-reactive protein (CRP) were elevated in one or both of the patients. Coagulation measures, including D-dimer, activated partial thromboplastin time (APTT), and prothrombin time (PT) were above normal range.

Muscle pathology and ancillary test findings

A review of the section from the gastrocnemius muscle biopsy of patient 1 revealed minor chronic changes (Fig. 4). At the low power view, irregular and atrophic muscle fibers intermingled with normal-shaped stripes and resembled broken marbles (Fig. 4A, B, C). Infiltration of inflammatory cells was minimal, which was confirmed by the scattered positive staining of LCA and CD68. Focal fibrosis and vascular congestion could be identified. Interestingly, ACE2 highlighted the irregular and atrophic fibers while showed a combined membrane and cytoplasmic staining pattern in the normal internal fibers (Fig. 4D).

Multiple specimens were obtained from the left lower limb of patient 2, including artery, vein, nerve, skin, synovial tissue, muscles, and cartilage. Pathological findings showed arterial thrombosis and exfoliation of endothelial cells in the vein. Hyperkeratosis of the residual epidermis, acantholysis of the spinous layer, and formation of vesicles in the epidermis were also observed. Microthrombus was seen in superficial small blood vessels of the dermis. Necrotic vasculitis and thrombosis in the subcutaneous adipose tissue

with a mucoid change of the local stroma were noticed. No inflammatory cell infiltration and structural change were found in the submitted nerve, synovial tissue, and cartilage specimens.

As for the skeletal muscle, changes were mild (Fig. 5). Nuclear chains and centrally nucleated fibers were scattered (Fig. 5A, B). There seemed to be fiber necrosis and minor inflammation (Fig. 5C, D). The infiltrations were predominantly macrophages and lymphocytes, which was also confirmed with CD68 and LCA staining.

RT-PCR assay for SARS-CoV-2 in muscle tissues from both case 1 and 2 are negative (data not shown).

Discussion

To our knowledge, the clinicopathologic findings of the muscle reported here represent the first in COVID-19. Isolated reports for postmortem biopsies or the most recent complete autopsies of SARS-CoV-2 positive individuals covered gross findings in the musculoskeletal system, but lacked a detailed description of muscle pathology ^[13].

Patient 1 in the present study had underlying diseases of hypertension and diabetes prior to the infection, which is likely a risk factor for his death. The other patient had no past medical history. Clinically, these two patients exhibited fatigue, anorexia, and muscle pain, which are common manifestations in COVID-19. Patient 2 developed limb thrombosis during the clinical course. A recent review suggests biomarkers of inflammation, cardiac and muscle injury, and coagulation measures are significantly elevated in patients with severe and fatal COVID-19 ^[14]. Blood biochemical parameters in the present study are similar to those reported in literature^[9, 11, 14], showing typical complete blood cell profile seen in severe or critical ill cases such as elevated WBC count and lymphocytopenia. The parameters related to skeletal muscle injury was abnormal in one patient and not available in the other patient. The elongation of coagulation time may have contributed to the thrombosis in patient 2. Interestingly, a retrospective study has revealed that coagulopathy is a common abnormality in patients with COVID-19 who was treated with prophylactic anticoagulation or therapeutic anticoagulation therapy^[15].

ACE2 protein was identified to localize in various human organs including lung, gastrointestinal tract, immune system and so on, so it is understandable that the common clinical manifestations involve respiratory and gastrointestinal symptoms such as fever, cough, dyspnea, sore throat, rhinorrhea, chest pain, diarrhea, nausea and vomiting ^[8, 9]. However, to date, the expression of ACE2 in skeletal muscle is not clear. From the present study, we identified a combined membrane and cytoplasmic staining pattern in normal fibers. There are some changes in morphology and biochemical parameters, and the overall muscle pathological findings are underwhelming. No significant acute histological changes or inflammation were identified in the two muscle cases examined, so there is not enough evidence to attribute it directly to an active process. The pathological findings in patient 1 were mainly scattered atrophic muscles, but it might be a preexisting underlying condition before the infection, as the patient was over 80 years old. Atrophic muscles may represent fiber degeneration, and focal fibrosis suggests

this patient possibly had a background of chronic neuromuscular disease. Changes in the muscles could not rule out the possibility of secondary or related to underlying diseases such as denervation due to the sample limitation and lack of control samples. The pathological findings in patient 2 could be due to a minimal compartment syndrome caused by thrombosis because the specimens were from the same limb.

The differential diagnoses may include, but not limited to, denervation, myopathy, or myositis resulted from other causes and drug-induced side effects. Usually, severe or critically ill cases have underlying diseases such as hypertension and diabetes, and would be given a lot of medications such as antibiotics, antivirals, and corticosteroids along with medications for the underlying diseases. So, it is impossible to rule out medication reactions in various organs, including muscles.

Conclusions

we reported the clinical and laboratory features together with histopathological findings in two COVID-19 cases presenting with the symptom of muscle ache and abnormal biochemical parameters related to skeletal muscle injury. Findings from this study may help to gain insight into the changes in skeletal muscles. Frozen muscle specimens, more case numbers with internal control and different phases of COVID-19, and electron microscopic examinations are needed to further the study of the muscle involvement in COVID-19.

Abbreviations

COVID-19

coronavirus disease-19

SARS-CoV-2

severe acute respiratory syndrome coronavirus 2

ACE2

angiotensin-converting enzyme 2

CT

computed tomographic

CBC

complete blood count

IHC

immunohistochemistry staining

FFPE

formaldehyde fixed and paraffin embedded

PCR

polymerase chain reaction

ICU

intensive care unit

WBC

white blood cell

CK-MB

creatine kinase–MB

LDH

lactate dehydrogenase

PCT

procalcitonin

IL-6

interleukins 6

CRP

C-reactive protein

APTT

activated partial thromboplastin time

PT

prothrombin time

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Board of Zhongnan Hospital of Wuhan University (No. 2020015).

Consent for publication

Informed consent was obtained from the patient for the publication of this case report and any accompanying images.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

None.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LMY performed imaging analysis, followed up the cases continuously and drafted the manuscript. DP collated clinical data, drew a schematic diagram of disease progression and participated in manuscript writing. PZY participated in the clinical treatment of patients. CYX was involved in the collection of pathological samples. Pathological analysis of patient samples was performed by FW. PAS participated in the design of the study, clinical treatment of patients, and clinical data collection. All authors read and approved the final manuscript.

Acknowledgements

We thank Dr. Negar Khonlou of the University of California, Los Angeles for her expert consultation on the histopathology of the muscle.

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Figures

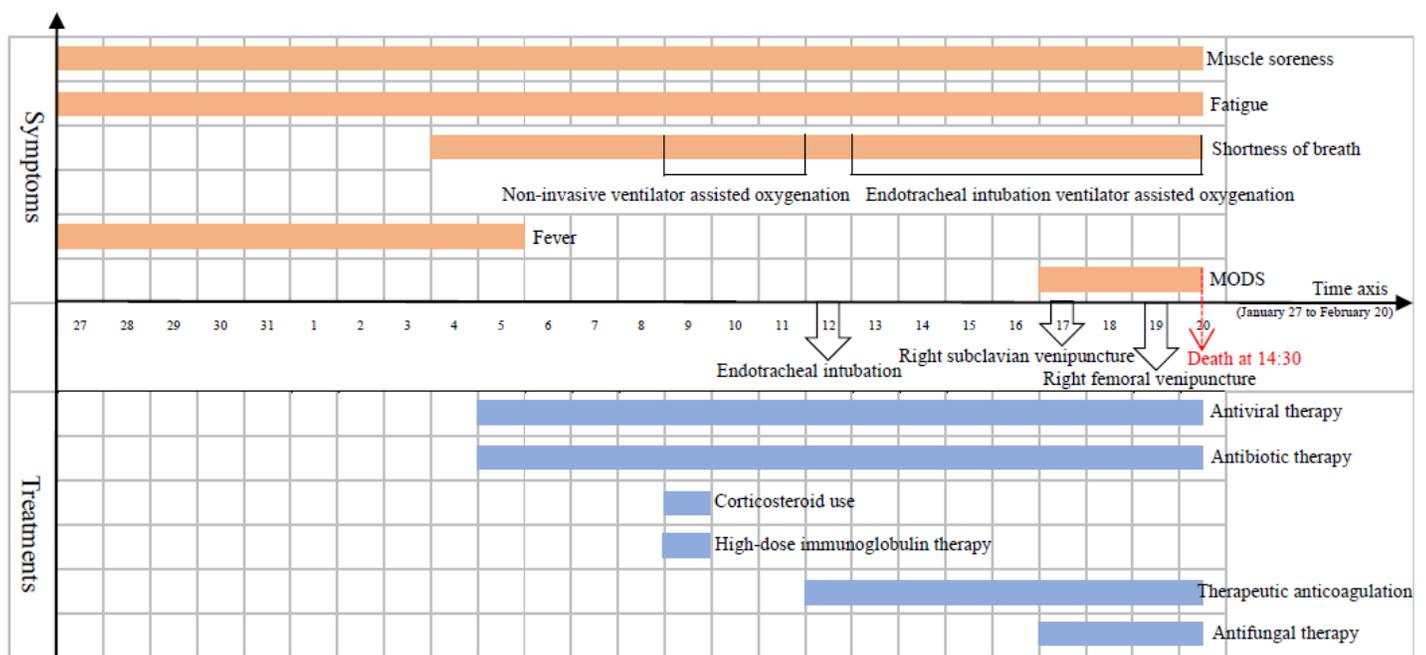


Figure 1

Timeline of disease course of patient 1.

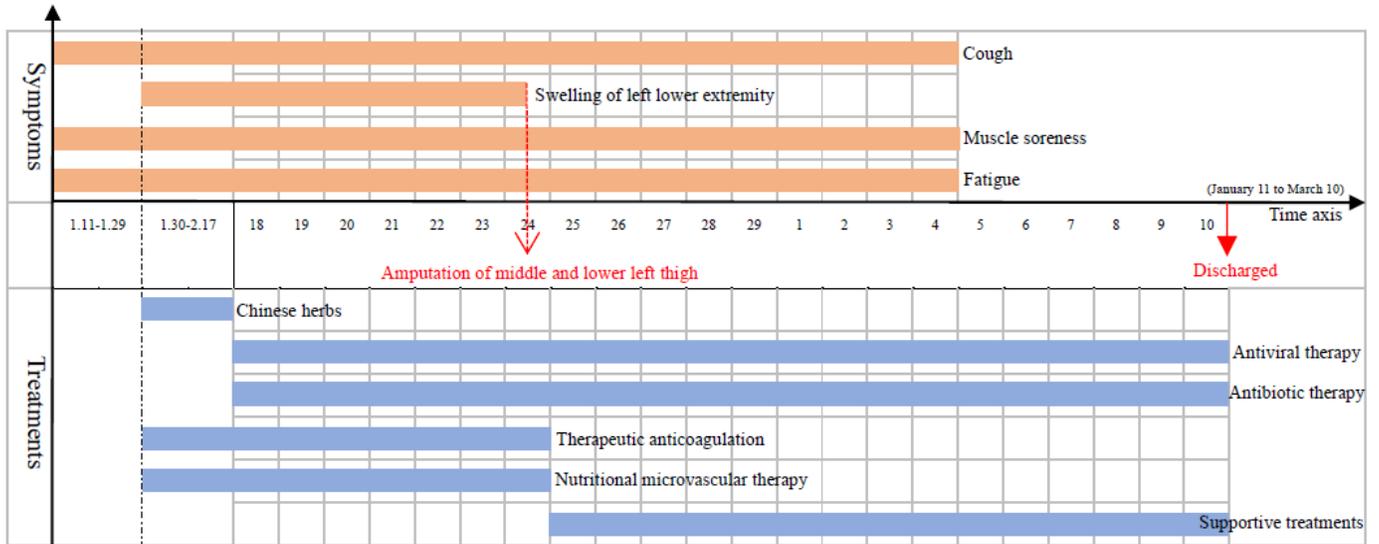


Figure 2

Timeline of disease course of patient 2.

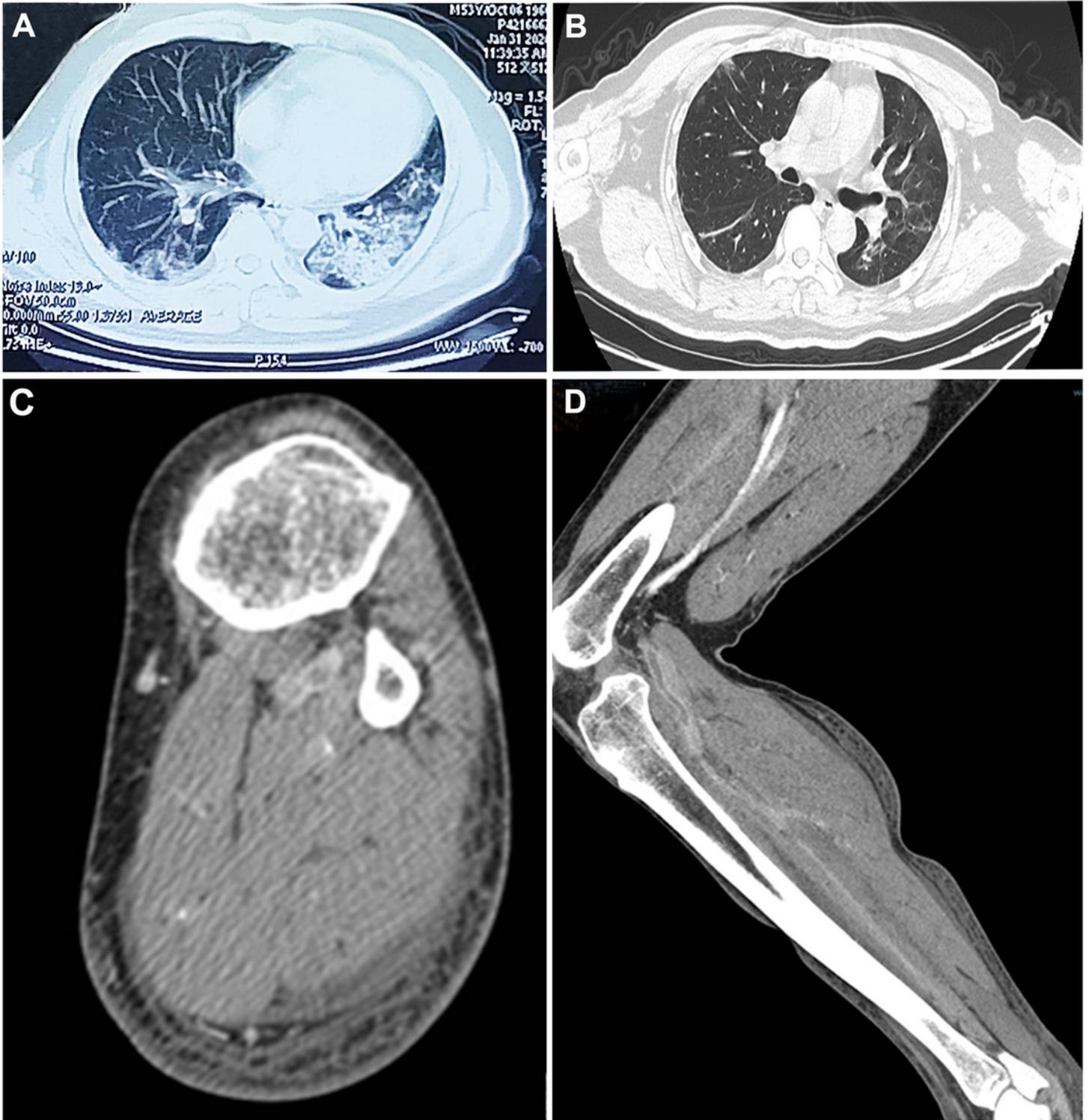


Figure 3

Radiographical images of chest CT scan and left leg of patient 2. Chest CT examination showing ground-glass shadow and consolidation in the subpleural area of the left and right lower lobes of the lung, air bronchogram in the left lower lobe on Jan 31 and Feb 22 (A, B). B-ultrasonography displaying the location and length of thrombosis in the popliteal artery of the left lower extremity on Feb 20 (C, D).

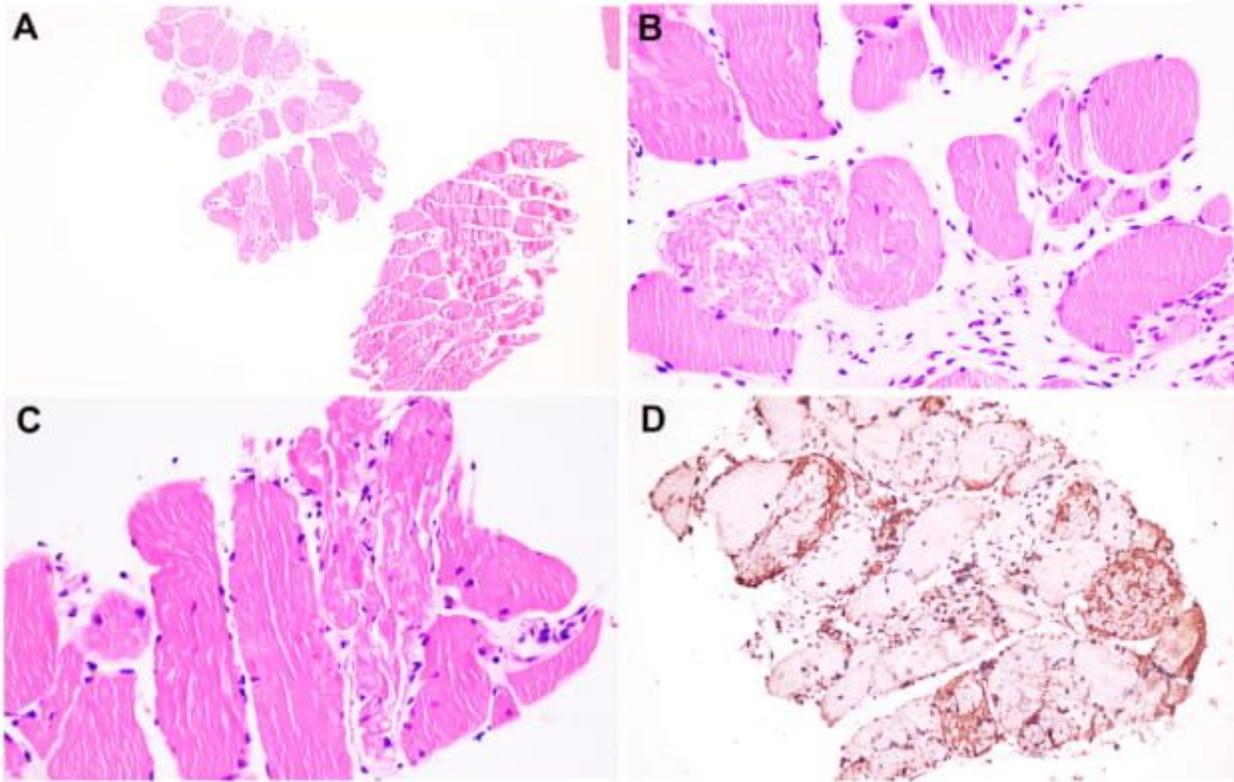


Figure 4

Histologic changes in the muscle from patient 1. At the low power of view, irregular and atrophic muscle fibers interspersed among normal-shaped stripes (A, B, C). ACE2 highlighting the irregular and atrophic fibers (D).

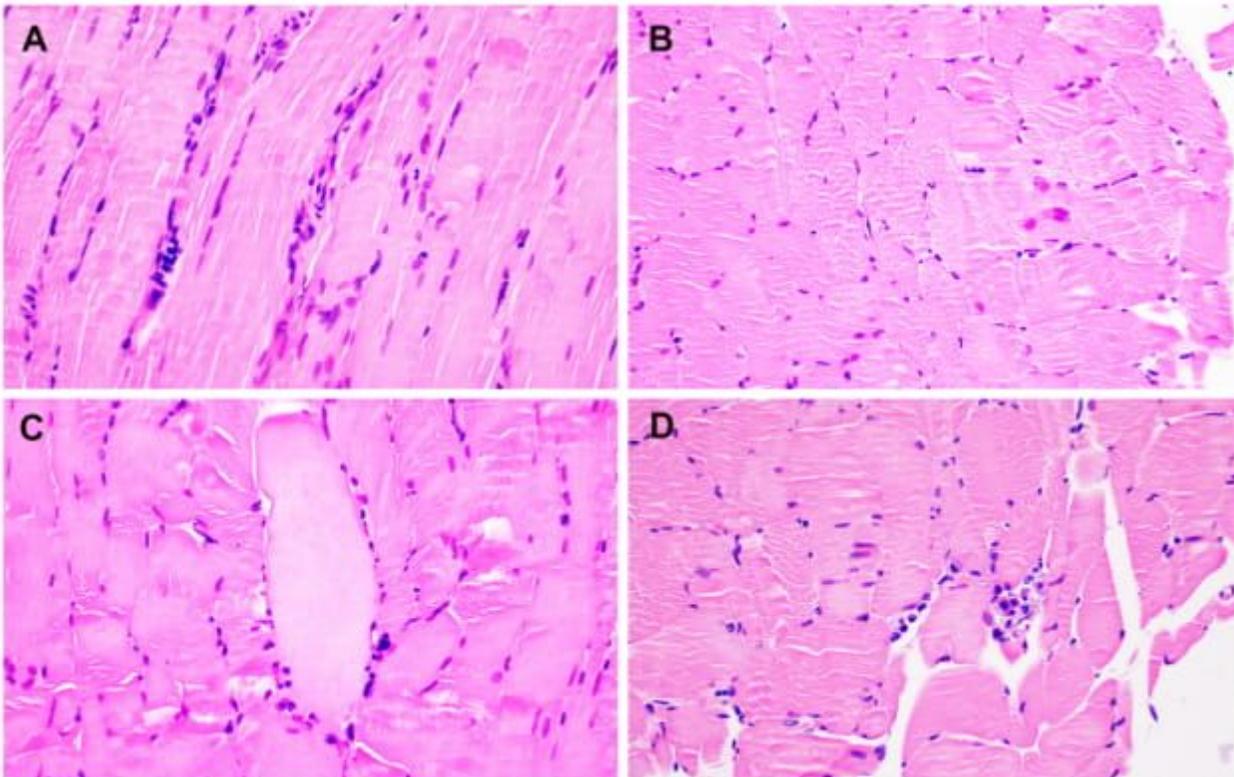


Figure 5

Pathological findings in the muscle from patient 2. Nuclear chains (A) and scattered centrally-nucleated fibers (B), fiber necrosis (C) and minor inflammation (D).