RESEARCH LETTER

Diaphragm Pathology in Critically Ill Patients With COVID-19 and Postmortem Findings From 3 Medical Centers

Extrapulmonary manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are now widely recognized and have important clinical implications.[1,2] To our knowledge, the association of SARS-CoV-2 with the respiratory muscles has not been studied. This is surprising, as the respiratory muscles drive alveolar ventilation and their weakness results in acute respiratory failure. In critically ill patients undergoing ventilation, respiratory muscle weakness prolongs mechanical ventilation and increases mortality.[3] The aim of this study was to investigate the association of severe coronavirus disease 2019 (COVID-19) with the respiratory muscles in critically ill patients and compare the findings with those obtained from non-COVID-19 critically ill patients.

Supplemental content

Methods | Our study focused on the diaphragm, the main muscle of respiration. Consecutive diaphragm muscle specimens were collected during autopsy from the corpses of 26 patients who had been critically ill with COVID-19 in 3 academic medical centers in the Netherlands (referred to as COVID-19–intensive care unit [ICU]) in April and May 2020. As a control group, autopsy diaphragm specimens were collected from corpses of 8 patients who had been critically ill without COVID-19 (referred to as control-ICU). Specimens from the left midcostal diaphragm were used for analyses. Methodological details are described in the eMethods and eTables 2 and 3 in the Supplement. This study was approved by the medical ethical committee at Amsterdam UMC, and written informed consent was provided by the decedents' next of kin. Data were analyzed using SPSS, version 22 (IBM), and visualized with GraphPad Prism, version 7.0 (GraphPad). Statistical significance was set at $P < .05$.

Results | The median age of COVID-19–ICU patients was 71 years (interquartile range, 61-74 years), and 21 (81%) were men. Twenty-four patients (92.3%) received invasive mechanical ventilation for a median of 12 days (interquartile range, 6-25 days). The number of days receiving invasive mechanical ventilation and ICU length of stay were comparable between COVID-19–ICU and control-ICU patients. COVID-19–ICU patients had higher body mass index (calculated as weight in kilograms divided by height in meters squared) and were less likely to be treated with steroids (**Table**). No patients in either group had preexisting neuromuscular disease.

We report angiotensin-converting enzyme 2 (ACE-2) in the diaphragm of COVID-19–ICU and control-ICU patients (**Figure**, A). The ACE-2 predominantly localizes at the myofiber membrane (Figure, A), providing an entry point for SARS-CoV-2 to infect diaphragm myofibers. Evidence for SARS-CoV-2 viral RNA in the diaphragm was found in 4 patients (15.4%; Figure, B). Further analyses, for which we applied RNA in situ hybridization, indicated that viral RNA localized inside diaphragm myofibers (Figure, B). The RNA sequencing analyses showed that 315 genes were upregulated and 281 were downregulated in the diaphragm of COVID-19–ICU patients compared with control-ICU patients. Subsequent analyses of all upregulated and downregulated genes revealed activation of fibrosis pathways (fibroblast growth factor signaling). In line with these findings, epimysial and perimysial fibrosis was more than 2-fold higher in the diaphragms of COVID-19–ICU patients compared with control-ICU patients (Figure, C).

Discussion | In this study, we provide unique evidence for ACE-2 expression in the human diaphragm and SARS-CoV-2 viral infiltration in the diaphragm of a subset of COVID-19–ICU patients. In COVID-19–ICU patients, we report increased expression of genes involved in fibrosis and histological evidence for the development of fibrosis in the diaphragm. This myopathic phenotype was distinctly different from that of control-ICU patients, with comparable duration of mechanical ventilation and ICU length of stay.[4,5] It remains to be established whether diaphragm myopathy is a direct effect of SARS-CoV-2. Only 3 patients in the control-ICU group (37.5%) had viral lung disease, and the association of viral pneumonia with diaphragm muscles is unknown. We hypothesize that severe diaphragm myopathy associated with COVID-19, as described in this study, may lead to diaphragm weakness and might contribute to ventilator weaning failure, per-

Table. Summary of the Demographic and Clinical Characteristics of the Study Patients

Characteristic	COVID-19–ICU (n = 26)	Control-ICU (n = 8)	*P*-value
Age, median (IQR), y	71 (61-74)	66 (64-68)	.44
Sex, No. (%), male	21 (81)	6 (75)	>.99
BMI, mean (SD)	28 (4)	25 (4)	.02
Duration of ICU stay, median (IQR), d	13 (8-25)	12 (9-12)	.35
Duration of IMV, median (IQR), d	12 (6-25)	10 (6-12)	.25
Duration of NMB administration, median (IQR), h	0 (0-100)	84 (0-240)	.45
Systemic steroid administration, No. (%)	11 (44)	7 (88)	.05
Maximum CRP level, median (IQR), mg/dL	33.1 (25.9-39.4)	32.1 (27.6-45.3)	.72

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); COVID-19, coronavirus disease 2019; CRP, C-reactive protein; ICU, intensive care unit; IMV, invasive mechanical ventilation; IQR, interquartile range; NMB, neuromuscular blocking agents.

SI conversion factor: To convert CRP to milligrams per liter, multiply by 10.

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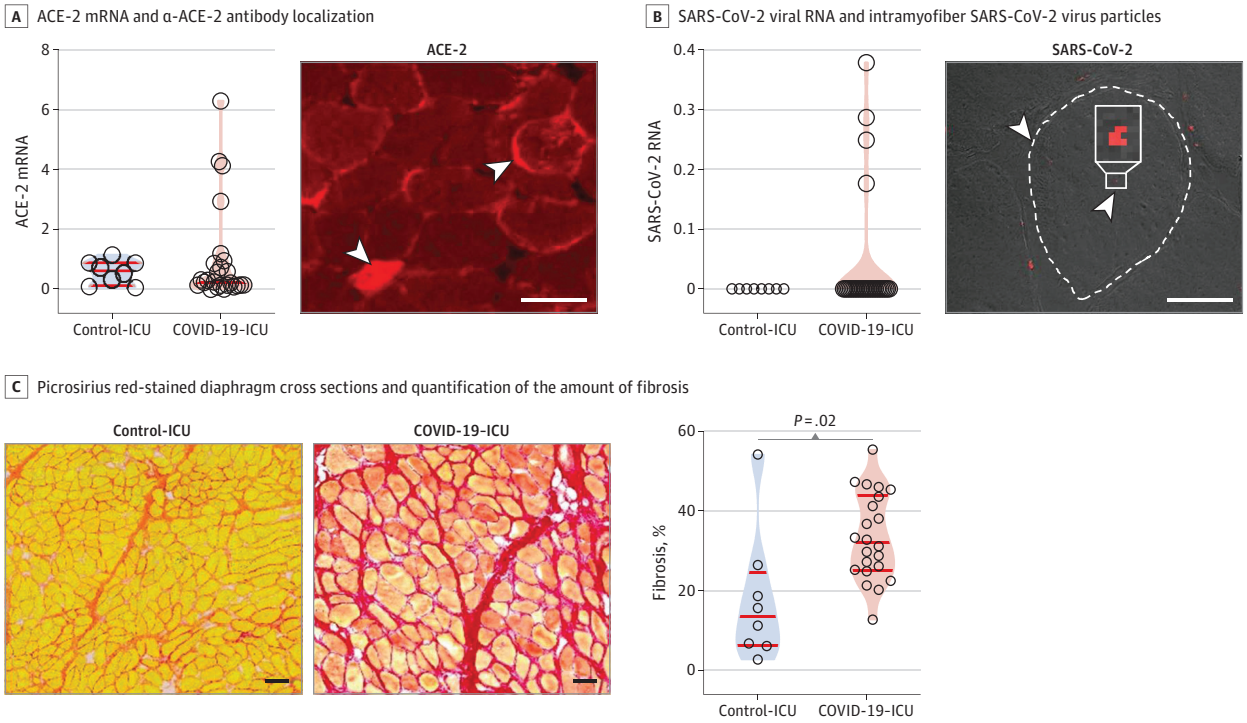
Figure. Angiotensin-Converting Enzyme 2 (ACE-2), SARS-CoV-2, and Fibrosis in the Diaphragms of Patients With COVID-19



A, Left panel: ACE-2 mRNA in diaphragm specimens determined by quantitative polymerase chain reaction (qPCR) and normalized to housekeeping gene *TBP*. Right panel: α-ACE-2 antibody localization with fluoresceine microscopy on diaphragm cross-sections; the arrowheads show membrane and cytosolic localization (bar = 50 µm). B, Left panel: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral RNA, determined by qPCR and normalized to housekeeping gene *TBP*, is detected in the diaphragm of 4 coronavirus disease 2019 (COVID-19)–intensive care unit (ICU) patients (patients 7, 9, 33, and 36). Right panel: in situ hybridization using RNAscope on patient #7 shows intramyofiber SARS-CoV-2 virus particles (red dots, indicated with arrowheads); a myofiber edge is highlighted with dashed line (bar = 30 µm). C, Left panels: representative images of picrosirius red-stained diaphragm cross-sections to highlight fibrosis; patients #22 and 3 are shown (bar = 100 µm). Right panel: quantification of the amount of fibrosis.

sistent dyspnea, and fatigue in patients with COVID-19 who survive their ICU stay.[6]

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