Diaphragm Muscle Weakness Might Explain Exertional Dyspnea Fifteen Months After Hospitalization for COVID-19

Binaya Regmi[1], Janina Friedrich[1], Benedikt Jörn[1], Mehdi Senol[1], Alberto Giannoni[2], Matthias Boentert[3,4], Ayham Daher[1], Michael Dreher[1]*, Jens Spiesshoefer[1,2]*

[1] Department of Pneumology and Intensive Care Medicine, University Hospital RWTH Aachen, Aachen, Germany; [2] Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy;[3] Department of Neurology with Institute for Translational Neurology, University Hospital of Muenster, Muenster, Germany; [4] Department of Medicine, UKM Marienhospital Steinfurt, Steinfurt, Germany

*Senior authors, M.D. and J.S., contributed equally to this work.

Running head: Diaphragm muscle weakness and dyspnea after COVID-19

Authors contributions: JS, JF, BJ, BR and MES collected the data. JS, BR, AG, MB and MD wrote the manuscript and contributed significantly to the study design. The manuscript was revised by all other authors.

Funding: This research received no external funding. Internal funding was provided by the RWTH Aachen Faculty of Medicine (START Grant supporting the junior research group around Dr. Jens Spiesshoefer).

Corresponding author: Dr. Jens Spiesshoefer, Department of Pneumology and Intensive Care Medicine, University Hospital RWTH Aachen, Aachen, Germany; Phone: +49 241 8037036; E-Mail: jspiesshoefer@ukaachen.de

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Descriptor: 8.27

Word count article: 3253 **Word count** abstract: 249

At a Glance Commentary

Current scientific knowledge on the subject: Up to one-third of COVID-19 survivors report some degree of dyspnea that cannot be explained by routine clinical diagnostic measures, including pulmonary function tests and cardiac evaluation. Therefore the pathophysiological basis for the "long COVID" symptoms and how long they persist is not yet fully understood.

What this study adds to the field: This study used state-of-the-art in-depth techniques to determine diaphragm muscle strength in patients 15 months after hospitalization for COVID-19 and its relationship to otherwise unexplained dyspnea on exertion. It is therefore the first study to: (a) demonstrate that diaphragm muscle weakness is present 15 months after hospitalization for COVID-19 independent of initial disease severity (i.e. even in patients who did not require mechanical ventilation); and (b) identify diaphragm muscle weakness as a correlate for persistent dyspnea in patients after COVID-19 in whom lung and cardiac function is normal.

This article is open access and distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives License 4.0 (http://creativecommons.org/licenses/by-nc-nd/4.0/). For commercial usage and reprints please contact Diane Gern (dgern@thoracic.org).

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

ABSTRACT

Rationale: Dyspnea is often a persistent symptom after acute coronavirus disease 2019 (COVID-19), even if cardiac and pulmonary function are normal.

Objective: This study investigated diaphragm muscle strength in patients after COVID-19 and its relationship to unexplained dyspnea on exertion.

Methods: Fifty patients previously hospitalized with COVID-19 (14 female, age 58±12 years, half of whom were treated with mechanical ventilation and half who were treated outside the intensive care unit) were evaluated using pulmonary function testing, 6-minute walk test, echocardiography, twitch transdiaphragmatic pressure following cervical magnetic stimulation of the phrenic nerve roots, and diaphragm ultrasound. Diaphragm function data were compared with values from a healthy control group.

Main results: Moderate or severe dyspnea on exertion was present at 15 months after hospital discharge in approximately two-thirds of patients. No significant pulmonary function or echocardiography abnormalities were detected. Twitch transdiaphragmatic pressure was significantly impaired in post-COVID-19 patients compared with controls, independent of initial disease severity (14±8 vs. 21±3 cmH_2O in mechanically ventilated patients versus controls [p=0.02], and 15±8 vs. 21±3 cmH_2O in non-ventilated patients versus controls [p=0.04]). There was a significant association between twitch transdiaphragmatic pressure and the severity of dyspnea on exertion (p=0.03).

Conclusions: Diaphragm muscle weakness was present 15 months after hospitalization for COVID-19 even in patients who did not require mechanical ventilation, and this weakness was associated with dyspnea on exertion. The current study therefore identifies diaphragm muscle weakness as a correlate for persistent dyspnea in patients after COVID-19 in whom lung and cardiac function are normal.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Keywords: coronavirus; diaphragm muscle strength; pulmonary function; dyspnea

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

INTRODUCTION

It is now more than two years since the beginning of the coronavirus disease 2019 (COVID-19) pandemic.[1] Therefore, a substantial population has recovered from severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection.[2-5] Some of these individuals experience a range of symptoms and abnormalities that may persist for more than several months after recovery from acute illness.[2-4,6] The new terms "long COVID" and "post-COVID syndrome" have been used to describe these findings. However, whether these abnormalities are unique to COVID-19, how long they persist and their underlying pathophysiology are not well understood, especially in patients in whom invasive mechanical ventilation was necessary (inevitable) to manage acute COVID-19. Some patients with COVID-19 treated with invasive mechanical ventilation remain symptomatic after hospital discharge, with dyspnea on exertion being one of the most frequent symptoms even when cardiac and pulmonary function are within normal limits.[6,7]

This raises the question as to what might be causing exertional dyspnea in these patients. The presence of diaphragm weakness in patients who survived COVID-19 has been suggested,[8] but remains unproven by objective measures. This lack of research on diaphragm muscle weakness in COVID-19 survivors is striking for two reasons. First, postmortem studies have documented the extrapulmonary presence of SARS-CoV-2, including immune-mediated skeletal muscle myopathy.[9] Secondly, prolonged hospitalization, especially with mechanical ventilation, may independently predispose to diaphragm atrophy or weakness.[10,11] Our group had previously reported diaphragm dysfunction with impaired volitional diaphragm function and control as a potential determinant of exertional dyspnea after COVID-19 illness in a hypothesis-generating research letter.[12] To further investigate the potential role of diaphragm dysfunction on otherwise unexplained dyspnea, the present study used a multimodal approach utilizing state-of-the-art assessments, including both volitional

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

and non-volitional invasive measures, to determine diaphragm muscle strength in patients previously hospitalized for the management of COVID-19 and its relationship to exertional dyspnea.

METHODS

Study design and participants

This prospective non-interventional study (NCT04854863) included patients who had been hospitalized at RWTH University Hospital Aachen (Aachen, Germany) between February 2020 and April 2021 for the management of COVID-19 and required supplemental oxygen therapy and/or invasive mechanical ventilation. Patients consented to attend one research visit approximately 15 months after discharge. Those with comorbidities that are known to cause dyspnea on exertion (such as treated systolic heart failure, anemia and heart defects including valve disorders, chronic obstructive pulmonary disease or neuromuscular disorders) were excluded (Figure 1).

For comparison of diaphragm muscle strength, post-COVID-19 patients were matched 3:1 with a control group of healthy subjects (recruited prior to the COVID-19 pandemic with identical technical equipment and standardization of the investigations) for age, sex, body mass index and comorbidities.

The study was approved by the local ethics committee and written informed consent was obtained from all patients. The trial was conducted according to Good Clinical Practice and the Principles of the Declaration of Helsinki 2002.

Intensive care unit stay routine follow-up

The severity of acute respiratory distress syndrome on the day of intubation was determined using the “Berlin Definition”.[13] Laboratory parameters, arterial blood gas analysis, and

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

ventilation variables, including the partial pressure of oxygen/fraction of inspired oxygen ratio were extracted from medical records. At follow-up approximately 15 months later, patients underwent pulmonary function testing, electrocardiography, and transthoracic echocardiography. Serum, plasma, and whole blood samples were obtained and analyzed. With support from a trained study team, patients answered clinical questionnaires to determine dyspnea (Medical Research Council dyspnea scale,[14] Borg dyspnea scale[15]) and fatigue (modified Fatigue Severity Index[16]). Whole-body plethysmography (MasterLab, Viasys, Hoechberg, Germany) was performed according to current guidelines[17,18] before and after bronchodilation (diffusion capacity of carbon monoxide was determined after bronchodilation only). Samples for capillary blood gas analysis were taken from the arterialized earlobe of all patients while breathing room air without supplemental oxygen (ABL 800 flex, Radiometer, Copenhagen, Denmark). Borg dyspnea scale scores were determined before and after a 6-minute walk test without supplemental oxygen.[17,18] Patients were then classified into three subgroups based on the severity of reported dyspnea after the 6-minute walk test: mild or no dyspnea (Borg dyspnea scale score 0–1, Medical Research Council dyspnea scale score of I); moderate dyspnea (Borg dyspnea scale score 2–5, Medical Research Council dyspnea scale score of II/III); or severe dyspnea (Borg dyspnea scale score of 6 or more, Medical Research Council dyspnea scale score of IV/V).

Phrenic nerve stimulation studies

Twitch transdiaphragmatic pressure was recorded and analyzed using balloon catheters (Cooper Surgical, Trumbull, CT, USA) transnasally inserted into the stomach and the distal esophagus as previously described (Figure 2).[19] Magnetic phrenic nerve stimulation was used for invasive measurement of twitch transdiaphragmatic pressure and for phrenic nerve conduction studies as previously described.[19] After transnasal placement of the catheter, few

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Sniff-Manuevers were performed to confirm the position of esophageal and gastral transducers. Posterior cervical magnetic stimulation was performed with the subject in the seated position. Stimuli were delivered using a MagPro Compact™ magnetic stimulator equipped with a 2 Tesla 12 cm C-100 circular coil (MagVenture, Willich, Germany).[19] For posterior cervical magnetic stimulation, the coil was placed at C7 and then moved up towards C6 until the highest reproducible twitch transdiaphragmatic pressure was obtained.[19] At least five stimuli were delivered to achieve the highest possible twitch transdiaphragmatic pressure showing less than 10% variation from the preceding two stimulations.[19] Supramaximality of magnetic stimuli (with 0.1 msec duration each and 2.0 Tesla maximum magnetic field output) was achieved by judging the relationship between stimulation intensity and the amplitude of twitch transdiaphragmatic pressure (Figure 2). To minimize twitch potentiation resulting from

previous voluntary diaphragm activation, there was a resting period of 5 minutes (with no speaking and no maneuvers and only quiet breathing) prior to the stimulation. Between two twitches, a period of at least 30 seconds was ensured. The state of FRC was achieved by requesting the participants to hold their breath following a normal, passive exhalation and demonstrating it. Stimulation at functional residual capacity was determined by visual observation of abdominal movements combined with visualization of pressure curves on a large flat screen to reproducibly generate a state of FRC.[19]

Invasive inspiratory muscle strength measurements

After performing cervical magnetic stimulation, subjects were also instructed to repeatedly perform a maximum sniff maneuver and a maximum Mueller maneuver as measures of volitional diaphragm muscle strength to achieve maximum deflection of the transdiaphragmatic pressure curve.[19] The highest of five consecutive efforts was taken for

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

analysis.[19] Reduced twitch transdiaphragmatic pressure reflecting diaphragm muscle strength impairment was defined as a value below 16 cmH_2O (which has previously been defined as the lower limit of normal[19]).

Twitch superimposition

The diaphragm voluntary activation index reflects the percentage of diaphragmatic muscle mass activated by voluntary effort or the extent of diaphragmatic activation during any given inspiratory effort. This parameter has been proposed for assessment of central drive to the diaphragm.[19] First, maximum twitch transdiaphragmatic pressure at functional residual capacity and maximum voluntary transdiaphragmatic pressure were determined, the latter by encouraging the subject to perform a maximum inspiratory effort against an occluded airway at functional residual capacity. Repetitive increasing stimuli were then deployed during voluntary inspiration (still with the airway occluded). During isovolumetric activation of the diaphragm, twitch interpolation was specifically timed by visual determination of 100% of the individual maximum voluntary transdiaphragmatic pressure (Figure 2).[19]

Invasive expiratory muscle strength measurement

The lower thoracic nerve roots were magnetically stimulated at the tenth vertebra (TH10) with rostrocaudal adjustment of the coil position (by no more than 2 vertebrae) to achieve the highest reproducible twitch gastric pressure.[20] Stimulation intensity was 100% of the maximum magnetic output with no threshold testing because it has been established that, unlike magnetic phrenic nerve stimulation, supramaximal stimulation of the lower thoracic (expiratory) nerves is not possible.[21] Stimulation was performed at functional residual capacity. The lower thoracic nerve roots were stimulated at the tenth vertebrae with a clear

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

instruction to go up and down (by no more than 2 vertebrae) to identify the position where the highest reproducible twitch gastric pressure could be achieved (Figure 2). Subjects were then also instructed to repeatedly perform a maximum cough and a maximum Valsalva maneuver as volitional invasive metrics of expiratory muscle strength. Cough, Valsalva and twitch gastric pressure were recorded using the same technical set-up described above.

Diaphragm ultrasound

Diaphragm ultrasound was performed on the right hemidiaphragm as previously described.[22] Briefly, a portable ultrasound device (LOGIQ S8-XD, GE Healthcare, London, UK) with a 10-MHz linear transducer was used for evaluation of diaphragm thickness in the zone of apposition. The diaphragm thickening ratio (DTR) was calculated as thickness at total lung capacity (TLC) divided by thickness at functional residual capacity (FRC).[22]

Statistical analysis

Statistical analyses were performed using Sigma Plot™ software (Version 13.0, Systat, Erkrath, Germany). The primary endpoint was a reduction in twitch transdiaphragmatic pressure following supramaximal magnetic stimulation of the phrenic nerve roots. Assuming a two-sided significance level of 0.05 (alpha) and 80% power (beta), a sample size of 25 subjects per group was calculated to allow detection of a 25% difference in twitch transdiaphragmatic pressure [20] between (each of the two groups of) post-COVID-19 patients and normal values from the healthy control group that were determined previously.[19]

Data are expressed as mean and standard deviation if normally distributed, or as median and interquartile range. For comparisons between two groups, Fisher's exact t-test or the Mann-Whitney U test was used as appropriate. Comparisons between patient subgroups based on exertional dyspnea severity were performed using one-way ANOVA with Tukey post-hoc

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

test for pairwise comparisons when normal distribution could be assumed. Otherwise, the Kruskal-Wallis test with Bonferroni post-hoc tests was used. In contrast to single t-tests where this was not applicable Bonferroni correction was used for (post-hoc) t-tests performed after (three) patients subgroups had been compared using one way ANOVA. For all analyses, a P value of ≤ 0.05 was considered statistically significant.

RESULTS

Study participants

Of 286 patients with severe COVID-19 who required invasive mechanical ventilation in the intensive care unit or received supplemental oxygen only and were seen for follow-up in the outpatient clinic, fifty fulfilled all inclusion criteria and were reassessed at 15 months after hospital discharge (Figure 1) (Table 1).

Twenty-five patients met the Berlin definition[13] for severe acute respiratory distress syndrome and required invasive mechanical ventilation. The majority of these (84%) also required prone positioning, nearly one-third (32%) received extracorporeal membrane oxygenation therapy, and nearly two-thirds (60%) developed acute renal failure requiring continuous renal replacement therapy (Table 1). The mean duration of hospitalization in these patients was 50±27 days (Table 1). The remaining twenty-five patients were not treated in the intensive care unit but needed supplemental oxygen therapy during hospitalization (Table 1). None of the non-ventilated patients received non-invasive ventilation therapy at any point during their hospital admission. This subgroup had a mean hospital stay of 13±6 days ($p<0.0001$ vs. ventilated patients) (Table 1).

Twenty-seven patients (54%) participated in pulmonary rehabilitation programs after discharge. The rate of participation in pulmonary rehabilitation was higher in ventilated

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

patients (n=22; 88%) compared with non-ventilated patients (n=5; 20%). Except for six patients (12%), most were able to return to their previous daily activities or work.

Dyspnea and fatigue

Outpatient clinic follow-up occurred at approximately 15 months (432±124 days) post-discharge. Regarding patient-reported outcomes, the severity of exertional dyspnea at follow-up was no/mild in fourteen patients (28%), moderate in twenty-four patients (48%) and severe in twelve patients (24%). Severe dyspnea on exertion was reported by eight patients (32%) in the ventilated group and four patients (16%) in the non-ventilated group. Moderate dyspnea was reported by ten ventilated patients (40%) and fourteen non-ventilated patients (56%), while seven patients in each group (28%) reported mild or no dyspnea (Figure 3). Performance on the 6-minute walk test was similarly impaired in both ventilated and non-ventilated post-COVID-19 patients, with no significant between-group difference (Table S1). Both groups also experienced fatigue, although patients from the ventilated group had a significantly greater degree of fatigue than those who did not require ventilation ($p=0.04$) (Table S1). Dyspnea severity was significantly associated with both distances achieved in the 6-minute walk test and fatigue scores (both $p<0.01$) (Table S2). At follow-up, vaccination rate of patients receiving at least two doses was very high (84% vs 88%) in both cohorts.

Clinical follow-up

At follow-up, no significant abnormalities were identified based on pulmonary function tests, capillary blood gas analysis or transthoracic echocardiography (Table S1-S2). White blood cells, platelets and creatine kinase varied significantly across subgroups based on dyspnea severity, but all values were within normal limits (Table S2). There were no associations

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

between the findings of pulmonary function testing, capillary blood gas analysis, or echocardiography dyspnea severity (Table S2).

Diaphragm muscle strength

Twitch diaphragmatic pressure following posterior cervical magnetic stimulation was significantly lower in post-COVID-19 patients than in healthy controls (p=0.02), irrespective of whether patients had been ventilated or not (Figure 3, Table 3). There was a significant correlation between twitch diaphragmatic pressure and the severity of dyspnea on exertion (p=0.03) (Figure 3, Table 2).

Volitional measurements of diaphragmatic pressure were not reduced in patients compared with controls and did not differ significantly between ventilated and non-ventilated patients (Table 3). Significant group differences were seen in the diaphragm voluntary activation index between the ventilated/non-ventilated post-COVID-19 patients compared with healthy controls (p-ANOVA=0.04, Table 3).

Diaphragm ultrasound

Among the ultrasound parameters, only DTR was significantly reduced in both ventilated and non-ventilated COVID-19 cohorts compared with healthy controls (Table 3). No significant differences were seen in any ultrasound values between subgroups based on dyspnea severity (Table 2). We did not observe any diaphragm atrophy using ultrasound measurements in our cohort (Table 2 and 3). However, diaphragm dysfunction was found using ultrasound measurements in 80% of our patients, and the DTR was below the lower limit of normal (2.2 as previously reported[22]) in 40 patients.

Expiratory muscle strength

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

There were no significant differences between twitch gastric pressure following magnetic stimulation of the abdominal muscles and twitch gastric pressure induced by a maximum voluntary cough (Table 2 and 3).

DISCUSSION

This study showed that patients previously hospitalized for COVID-19 have diaphragm weakness 15 months after discharge, irrespective of whether or not acute care included mechanical ventilation. The study findings also indicate that diaphragm weakness is associated with the occurrence of exertional dyspnea. Therefore, diaphragm weakness might explain exertional dyspnea reported by patients with long COVID in the absence of other pulmonary or cardiac function abnormalities.

Our findings support previous studies that did not find any significant pulmonary function test abnormalities in patients who survived moderate-severe COVID-19 disease.[3-5] However, it is not surprising that standard pulmonary function tests do not detect changes in diaphragm muscle strength. Polkey and colleagues showed that in-depth techniques of respiratory musculature assessment increase the accuracy of diagnosing diaphragm weakness by up to 40%.[23] Utilizing gold standard, invasive techniques, the present study showed that diaphragm muscle weakness was present 15 months after COVID-19. The reduction in twitch transdiaphragmatic pressure in COVID-19 patients found in the present study is likely to be clinically relevant because it is similar to reductions reported in neuromuscular disease patients with severe dyspnea.[24,25]

Furthermore, we have shown that diaphragm muscle weakness was related to exertional dyspnea and therefore, diaphragm muscle weakness is a potential pathophysiological correlate of dyspnea on exertion in patients previously hospitalized for COVID-19. However, a direct causal relationship cannot be directly proven and a symptom as complex in its

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

pathophysiology as exertional dyspnea may still show a multifactorial origin. Nevertheless, a direct effect of COVID-19 on the diaphragm seems plausible, especially in light of data from post-mortem autopsy studies that showed potential direct viral infiltration or associated immunomodulatory changes of the diaphragm with development of fibrosis in patients who died after infection with SARS-CoV-2.[26] Therefore, the present study extends these findings by showing a clinical impact of COVID-19 on the human diaphragm on a long-term basis. We noted diaphragm dysfunction in patients who were and those who were not mechanically ventilated during their index hospitalization. This is relevant because there is the potential for critical illness-induced diaphragm dysfunction in mechanically ventilated patients.[27] Critical illness-induced diaphragm dysfunction has been shown to be very common in the first week of invasive mechanical ventilation.[10,28] However, our follow-up took place over a much longer timeframe (15 months post-discharge), and it is not clear whether critical illness-induced diaphragm dysfunction would persist for more than 12 months. Nevertheless, critical illness-induced diaphragm dysfunction might be a potential confounding factor because invasive measurement of transdiaphragmatic pressure ("twitch" transdiaphragmatic pressure) following magnetic stimulation of the phrenic nerve roots in mechanically ventilated patients showed impaired contractility of the diaphragm compared with healthy individuals.[28,29] In addition, our finding of diaphragm weakness in post-COVID-19 patients who had not been mechanically ventilated suggests a virus-specific pathogenetic mechanism rather than a ventilator-specific mechanism for diaphragm dysfunction. While no significant differences in DLCO and DLCO/VA were seen between the dyspnea subgroups, a downward trend was seen (especially in DLCO) paralleling dyspnea severity. These differences might represent persisting pulmonary vascular or interstitial damage that may at least partly contribute to dyspnea symptoms, as indicated in recent studies.[30, 31] However, in contrast to the present

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

findings on diaphragm muscle weakness, impairment in DLCO did not translate into persistent dyspnea in the present study.[30,31]

The main factor limiting 6MWD was dyspnea but most patients also reported a degree of fatigue, which might have contributed to a further reduction in the 6MWD achieved. In addition, the role of peripheral muscle strength should not be underestimated, even if this was only reported by a small proportion of patients (5/50; 10%) during the 6MWT.

The growing number of individuals infected with SARS-CoV-2 worldwide, and therefore the proportion of previously infected patients who experience persistent symptoms (so-called "long COVID"), is an emerging public health issue. Often, persistent symptoms cannot be linked to a specific pathophysiological correlate, making targeted management extremely difficult. Therefore, the identification of a possible underlying mechanism for exertional dyspnea at 15 months after recovery from COVID-19 is clinically relevant. Firstly, it may be reassuring for patients to have a possible explanation for their persistent symptom (dyspnea) after COVID-19. Secondly, respiratory muscle training has been shown to be effective in other groups of patients with diaphragm muscle weakness, and therefore represents a potential therapeutic intervention in this setting.[32,33]

Our study has some limitations. Firstly, we selected a specific patient population that did not have any underlying cardiac or pulmonary disease that could act as a potential confounder and explain the perceived dyspnea. On the other hand, the control group did not have respiratory failure and serious illness at the same time period. Secondly, the ventilated patient cohort included a subset of patients who underwent ECMO, all of whom were treated with paralytics and six received steroids. This is a potential confounder because it can lead to critical illness neuropathy and myopathy. Additional factors that could contribute to diaphragm weakness include phrenic nerve neuropathy due to immune-mechanisms, pre-existing risk factors, use of antiviral drugs, or bedding in the ICU. Furthermore, this

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

observational study does not address whether the changes observed in diaphragmatic muscle strength are specifically attributable to COVID-19 rather than more general post-infection myopathy following an acute lung injury. Specifically designed studies with control subjects who have survived non-COVID pneumonia are required to gain further insights into the pathophysiology. However, it also remains clear that regardless of the specificity of COVID-19, the extent of diaphragm muscle weakness and its clear association with otherwise unexplained persistent dyspnea is a significant finding, particularly because the large number of post-COVID patients worldwide are likely to impose a considerable burden on modern healthcare systems.

CONCLUSION

This study demonstrated a pathophysiological mechanism, namely diaphragm muscle weakness, underlying otherwise unexplained exertional dyspnea in patients previously hospitalized for COVID-19. Additional research is needed to determine whether specific interventions targeting diaphragm muscle weakness, such as inspiratory muscle training, could be an effective intervention to address exertional dyspnea in patients with long COVID.

Acknowledgments: We gratefully thank all the volunteers and patients with COVID-19 whose cooperation made this study possible. We gratefully acknowledge the help of Mrs Merite Emrulai in analyzing patient-related data. English language editing assistance was provided by Nicola Ryan, an independent medical writer. We also wish to thank Dr. Gerold Kierstein (AD Instruments, Oxford, UK) for his help in performing an analysis of twitch transdiaphragmatic pressure gradients following cervical stimulation of the phrenic nerve roots.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Declaration of interests: The authors state that they have no conflicts of interest to declare. There was no external study funder, and therefore no external parties had any role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. Internal funding was provided by the RWTH Aachen Faculty of Medicine (START Grant supporting the junior research group around Dr. Jens Spiesshoefer). This funding did not influence the design of the study; the data collection, analyses, or interpretation of data; the writing of the manuscript, or the decision to publish the results.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

REFERENCES

1. Du Y, Tu L, Zhu P, et al. Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan. A Retrospective Observational Study. Am J Respir Crit Care Med 2020;201:1372-9.

2. Group P-CC. Clinical characteristics with inflammation profiling of long COVID and association with 1-year recovery following hospitalisation in the UK: a prospective observational study. Lancet Respir Med 2022;10:761-75.

3. Spruit MA, Holland AE, Singh SJ, Tonia T, Wilson KC, Troosters T. COVID-19: Interim Guidance on Rehabilitation in the Hospital and Post-Hospital Phase from a European Respiratory Society and American Thoracic Society-coordinated International Task Force. Eur Respir J 2020;56:2002197.

4. Sudre CH, Murray B, Varsavsky T, et al. Attributes and predictors of long COVID. Nat Med 2021;27:626-31.

5. Jamil S, Mark N, Carlos G, Cruz CSD, Gross JE, Pasnick S. Diagnosis and Management of COVID-19 Disease. Am J Respir Crit Care Med 2020;201:P19-P20.

6. Daher A, Balfanz P, Cornelissen C, et al. Follow up of patients with severe coronavirus disease 2019 (COVID-19): Pulmonary and extrapulmonary disease sequelae. Respir Med 2020;174:106197.

7. Daher A, Cornelissen C, Hartmann NU, et al. Six Months Follow-Up of Patients with Invasive Mechanical Ventilation due to COVID-19 Related ARDS. Int J Environ Res Public Health 2021;18:5851.

8. Hennigs JK, Huwe M, Hennigs A, et al. Respiratory muscle dysfunction in long-COVID patients. Infection 2022;50:1391-7.

9. Aschman T, Schneider J, Greuel S, et al. Association Between SARS-CoV-2 Infection and Immune-Mediated Myopathy in Patients Who Have Died. JAMA Neurol 2021;78:948-60.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

10. Goligher EC, Dres M, Fan E, et al. Mechanical Ventilation-induced Diaphragm Atrophy Strongly Impacts Clinical Outcomes. Am J Respir Crit Care Med 2018;197:204-13.

11. Hermans G, Agten A, Testelmans D, Decramer M, Gayan-Ramirez G. Increased duration of mechanical ventilation is associated with decreased diaphragmatic force: a prospective observational study. Crit Care 2010;14:R127.

12. Spiesshoefer J, Friedrich J, Regmi B, et al. Diaphragm dysfunction as a potential determinant of dyspnea on exertion in patients 1 year after COVID-19-related ARDS. Respir Res 2022;23:187.

13. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin Definition. Jama 2012;307:2526-33.

14. Fletcher CM, Elmes PC, Fairbairn AS, Wood CH. The significance of respiratory symptoms and the diagnosis of chronic bronchitis in a working population. Br Med J 1959;2:257-66.

15. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc 1982;14:377-81.

16. Fisk JD, Pontefract A, Ritvo PG, Archibald CJ, Murray TJ. The impact of fatigue on patients with multiple sclerosis. Can J Neurol Sci 1994;21:9-14.

17. Laveneziana P, Albuquerque A, Aliverti A, et al. ERS statement on respiratory muscle testing at rest and during exercise. Eur Respir J 2019;53:1801214.

18. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40:1324-43.

19. Spiesshoefer J, Henke C, Herkenrath S, et al. Transdiapragmatic pressure and contractile properties of the diaphragm following magnetic stimulation. Respir Physiol Neurobiol 2019;266:47-53.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

20. Spiesshoefer J, Henke C, Kabitz HJ, et al. Respiratory Muscle and Lung Function in Lung Allograft Recipients: Association with Exercise Intolerance. Respiration 2020;99:398-408.

21. Kyroussis D, Polkey MI, Mills GH, Hughes PD, Moxham J, Green M. Simulation of cough in man by magnetic stimulation of the thoracic nerve roots. Am J Respir Crit Care Med 1997;156:1696-9.

22. Spiesshoefer J, Herkenrath S, Henke C, et al. Evaluation of Respiratory Muscle Strength and Diaphragm Ultrasound: Normative Values, Theoretical Considerations, and Practical Recommendations. Respiration 2020;99:369-81.

23. Steier J, Kaul S, Seymour J, et al. The value of multiple tests of respiratory muscle strength. Thorax 2007;62:975-80.

24. Henke C, Spiesshoefer J, Kabitz HJ, et al. Respiratory muscle weakness in facioscapulohumeral muscular dystrophy. Muscle Nerve 2019;60:679-86.

25. Spiesshoefer J, Henke C, Kabitz HJ, et al. The nature of respiratory muscle weakness in patients with late-onset Pompe disease. Neuromuscul Disord 2019;29:618-27.

26. Shi Z, de Vries HJ, Vlaar APJ, et al. Diaphragm Pathology in Critically Ill Patients With COVID-19 and Postmortem Findings From 3 Medical Centers. JAMA Intern Med 2021;181:122-4.

27. Dres M, Goligher EC, Heunks LMA, Brochard LJ. Critical illness-associated diaphragm weakness. Intensive Care Med 2017;43:1441-52.

28. Sklar MC, Dres M, Fan E, et al. Association of Low Baseline Diaphragm Muscle Mass With Prolonged Mechanical Ventilation and Mortality Among Critically Ill Adults. JAMA Netw Open 2020;3:e1921520.

29. Watson AC, Hughes PD, Louise Harris M, et al. Measurement of twitch transdiaphragmatic, esophageal, and endotracheal tube pressure with bilateral anterolateral

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

magnetic phrenic nerve stimulation in patients in the intensive care unit. Crit Care Med 2001;29:1325-31.

30. Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet 2021;397:220-32.

31. Wu X, Liu X, Zhou Y, et al. 3-month, 6-month, 9-month, and 12-month respiratory outcomes in patients following COVID-19-related hospitalisation: a prospective study. Lancet Respir Med 2021;9:747-54.

32. Gosselink R, De Vos J, van den Heuvel SP, Segers J, Decramer M, Kwakkel G. Impact of inspiratory muscle training in patients with COPD: what is the evidence? Eur Respir J 2011;37:416-25.

33. Martin AD, Smith BK, Davenport PD, et al. Inspiratory muscle strength training improves weaning outcome in failure to wean patients: a randomized trial. Crit Care 2011;15:R84.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Table 1 Baseline characteristics, medical history and characteristics during intensive care unit stay for the overall study population, and in patient subgroups based on the requirement for invasive mechanical ventilation during hospitalization

	Patients previously hospitalized with COVID-19			
	Total (n=50)	**Ventilated (n=25)**	**Non-ventilated (n=25)**	**Controls (n=8)**
Male sex – no. (%)	36 (72)	19 (76)	17 (68)	5 (60%)
Age – yr	58.06±12.43	58.75±8.62	57.36±15.50	57.11±10.47
Post-discharge time – months	14.80±7.33	16.23±4.35	14.63±4.16	-
Height – cm	175±10	176±8	174±11	178±8
Weight – kg	88.76±17.84	95.20±18.49	82.32±14.89	78.33±7.50
BMI – kg/m^2	28.88±5.03	30.63±5.73	27.14±3.52	24.84±1.71
Total MFIS score	31.04±19.72	36.60±21.61	25.48±16.20	-
Comorbidities – n (%)				
COPD	0 (0)	0 (0)	0 (0)	-
Bronchial asthma	4 (8)	2 (8)	2 (8)	-
Hypertension	28 (56)	12 (48)	16 (64)	-
Systolic heart failure	0 (0)	0 (0)	0 (0)	-
Atrial fibrillation	2 (4)	1 (4)	1 (4)	-
Chronic kidney disease	6 (12)	3 (12)	3 (12)	-
Diabetes mellitus	9 (18)	4 (16)	5 (20)	-

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

In-hospital period				
Length of stay – days	31.72±26.97	50.08±27.35	13.36±5.84	-
Oxygen supplementation – no. (%)	50 (100)	25 (100)	25 (100)	-
Characteristics during ICU stay				
P/F ratio at (ICU) admission		135.32±45.17	247.68±37.26	-
Mean P/F ratio halfway through total ventilation duration		217.44±57.92	n/a	-
Duration of ventilation – days		36.44±22.30	-	-
Duration of ICU stay – days		36.60±30.07	-	-
Patients on ECMO – no. (%)		8 (32)	-	-
Duration of ECMO – days§		15 (10.50–24.50)	-	-
Prone positioning – no. (%)		21 (84)	-	-
Continuous NMB >6 h at any point – n (%)		13 (52)	-	-
Duration of NMB – days		10.08±8.15	-	-
Catecholamine therapy – no. (%)		24 (96)	-	-
Duration of catecholamine therapy – days§		16 (4.50–34.00)	-	-
CRRT – no. (%)		15 (60)	-	-

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Antibiotic therapy – no. (%)	24 (96)	4 (16)	-

Plus-minus values are means ±SD.

§Median (interquartile range) values for non-normally distributed data.

BMI body mass index, COPD chronic obstructive pulmonary disease, CRRT continuous renal replacement therapy, ECMO extracorporeal membrane oxygenation, ICU intensive care unit, MFIS modified fatigue impact scale, NMB neuromuscular blockade, P/F partial pressure of oxygen/fraction of inspired oxygen.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Table 2. Diaphragm muscle strength at 15 months after hospitalization for COVID-19 in patient subgroups based on the severity of dyspnea on exertion at 12-month follow-up

	Exertional dyspnea severity			
	None/mild (n=14; 28%)	**Moderate (n=24; 48%)**	**Severe (n=12; 24%)**	**P Value§**
Age – yr	55.36±11.08	55.43±11.78	66.46±12.30	0.60 [x]
Male sex – no. (%)				
BMI – kg/m²	27.73±2.74	29.33±6.23	29.34±4.50	0.61
mMRC dyspnea scale score	1.00±0.00	2.04±0.75	3.58±1.08	**<0.001*** [x] [†]
Non-volitional invasive RMS				
CMS twPDI – cmH_2O	17.73±8.16	14.51±8.07	9.53±6.52	**0.03** [†]
CMS twPes – cmH_2O	–9.94±5.38	–6.39±4.27	–6.34±6.19	0.09
CMS twPgas – cmH_2O	7.79±5.49	7.90±5.84	3.20±2.57	**0.03** [x]
CMS MRR normalized – cmH_2O/msec	–8.65±3.28	–10.85±6.81	–12.52±8.82	0.33
CMS MCR normalized – cmH_2O/msec	21.89±6.98	21.54±8.33	21.06±11.78	0.97
CMS t½ – msec	72.14±38.06	74.17±44.81	57.78±37.97	0.59
CMS time to peak – msec	67.64±37.93	68.17±46.46	51.61±25.45	0.61
twPgas TH10 – cmH_2O	27.46±17.16	21.53±18.85	17.99±15.70	0.40
Volitional invasive RMS				
Sniff PDI – cmH_2O	91.86±29.11	79.34±26.86	63.25±24.06	**0.03** [†]
Sniff Pes – cmH_2O	–65.69±26.25	–65.88±24.13	–49.74±19.29	0.13
Mueller PDI – cmH_2O	83.96±43.12	71.11±40.74	50.31±28.08	0.10
Mueller Pes – cmH_2O	–46.81±21.32	–58.34±33.78	–41.03±20.20	0.19
Valsalva Pgas – cmH_2O	222.44±70.89	144.66±71.69	115.65±78.26	**<0.001** [*] [†]
Cough Pgas – cmH_2O	183.86±60.73	153.69±74.48	121.22±69.60	0.09
Neural control				
DVAI – %	53.07±29.99	52.21±34.02	48.58±30.59	0.93
Diaphragm ultrasound				

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Amplitude TB – cm	1.70±0.50	1.80±0.39	1.93±0.76	0.55
Velocity TB – cm/sec	1.73±0.96	1.59±0.50	1.73±0.93	0.81
Sniff velocity – cm/sec	7.95±2.04	7.24±2.02	6.97±3.78	0.61
Thickness at FRC – cm	0.23±0.06	0.18±0.04	0.20±0.06	0.06
Thickness at TLC – cm	0.42±0.12	0.38±0.09	0.38±0.12	0.44
DTR	1.88±0.26	2.10±0.36	1.86±0.33	0.06

Plus-minus values are means ±SD.

§Analysis of variance.

Significance differences ($p<0.05$) within paired t-tests between each groups, if ANOVA is significantly different ($p<0.05$). * indicates significant difference between mild and moderate; x between moderate and severe and †between mild and severe dyspnea groups respectively. $p<0.05$ is highlighted bold.

CMS cervical magnetic stimulation (of the phrenic nerve roots), DTR diaphragm thickening ratio, DVAI diaphragm voluntary activation index, FRC functional residual capacity, MCR maximum contraction rate, mMRC Modified Medical Research Council, MRR maximum relaxation rate, PDI transdiaphragmatic pressure, Pes esophageal pressure, Pgas gastric pressure, RMS respiratory muscle strength, twPDI twitch transdiaphragmatic pressure, twPes twitch esophageal pressure, twPgas twitch gastric pressure, twPgas TH10 twitch gastric pressure (in response to magnetic stimulation of the expiratory nerve roots); TB tidal breathing; FRC forced vital capacity; TLC total lung capacity; DTR diaphragm thickening ratio.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Table 3. Diaphragm muscle strength in control subjects and at 15 months after hospitalization in ventilated or non-ventilated patients with COVID-19

	Controls (n=8)	**COVID-19 (ventilated) (n=25)**	**COVID-19 (non-ventilated) (n=25)**	**P Value§**
Age – yr	57.11±10.46	57.36±15.50	58.75±8.62	0.90
Male sex – no. (%)	6 (67)	19 (76)	17 (68)	
BMI – kg/m²	24.84±1.71	30.63±5.73	27.14±3.52	**0.002** θ ∇
Non-volitional invasive RMS				
CMS twPDI – cmH_2O	20.92±3.32	13.65±8.37	14.79±8.09	**0.05**
CMS twPes – cmH_2O	–12.19±3.87	–6.59±5.06	–7.37±5.24	**0.02** ∇
CMS twPgas – cmH_2O	8.67±2.95	6.84±6.04	6.63±4.89	0.59
CMS MRR normalized – cmH_2O/msec	–9.73 (–12.66, –6.49)	–9.44 (–11.97, –7.24)	–10.07 (–14.01, –5.73)	0.55
CMS MCR normalized – cmH_2O/msec	20.73±7.73	22.42±10.16	20.73±7.73	0.34
CMS t½ – msec	110.00 (90.00–195.00)	75.00 (50.00–100.00)	50.00 (30.00–80.00)	**0.001** ∇ #
CMS time to peak – msec	100.00 (75.00–140.00)	60.00 (50.00–75.00)	60.00 (35.50–75.75)	**0.002** #
twPgas TH10 – cmH_2O	30.72 (18.33–55.82)	17.38 (6.55–24.12)	23.51 (11.23–32.10)	0.20

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Volitional invasive RMS				
Sniff PDI – cmH_2O	91.80±17.68	75.59±29.38	82.38±27.37	0.29
Sniff Pes – cmH_2O	–65.00±17.27	–58.69±24.48	–65.22±24.04	0.58
Mueller PDI – cmH_2O	87.18±37.53	62.04±36.22	77.39±42.82	0.19
Mueller Pes – cmH_2O	–35.12±32.68	–43.91±23.89	–58.00±31.09	0.08
Valsalva Pgas – cmH_2O	111.24 (84.32–153.79)	128.45 (88.12–171.43)	195.04 (108.81–247.15)	0.62
Cough Pgas – cmH_2O	116.35 (94.19–157.82)	127.79 (90.82–177.69)	173.49 (133.41–219.39)	0.10
Neural control				
DVAI – %	68.69±21.65	45.34±27.44	61.10±36.97	**0.04**
Diaphragm ultrasound				
Amplitude TB - cm	1.52±0.75	1.69±0.60	1.92±0.47	0.16
Velocity TB – cm/sec	1.14±0.52	1.36±0.50	1.96±0.89	**0.002** $^{\theta\ \#}$
Sniff Velocity – cm/sec	8.11±3.05	6.75±3.11	8.01±1.86	0.19
Thickness at FRC - cm	0.19±0.06	0.19±0.04	0.21±0.07	0.66
Thickness at TLC - cm	0.61±0.06	0.38±0.100	0.40±0.12	**<0.001** $^{\nabla\ \#}$
DTR	3.39±1.32	1.96±0.42	2.01±0.27	**<0.001** $^{\nabla\ \#}$

Plus-minus values are means ±SD.

§Analysis of variance.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Significance differences ($p<0.05$) within paired t-tests between each groups, if ANOVA is significantly different ($p<0.05$). θ indicates significant difference between ventilated and non-ventilated; ∇ between ventilated and control and # between non-ventilated and control groups respectively. $p<0.05$ is highlighted bold.

CMS cervical magnetic stimulation (of the phrenic nerve roots), DVAI diaphragm voluntary activation index, MCR maximum contraction rate, MRR maximum relaxation rate, PDI transdiaphragmatic pressure, Pes esophageal pressure, Pgas gastric pressure, RMS respiratory muscle strength, twPDI twitch transdiaphragmatic pressure, twPes twitch esophageal pressure, twPgas twitch gastric pressure, twPgas TH10 twitch gastric pressure (in response to magnetic stimulation of the expiratory nerve roots), TB tidal breathing; FRC forced vital capacity; TLC total lung capacity; DTR diaphragm thickening ratio.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

FIGURE LEGENDS

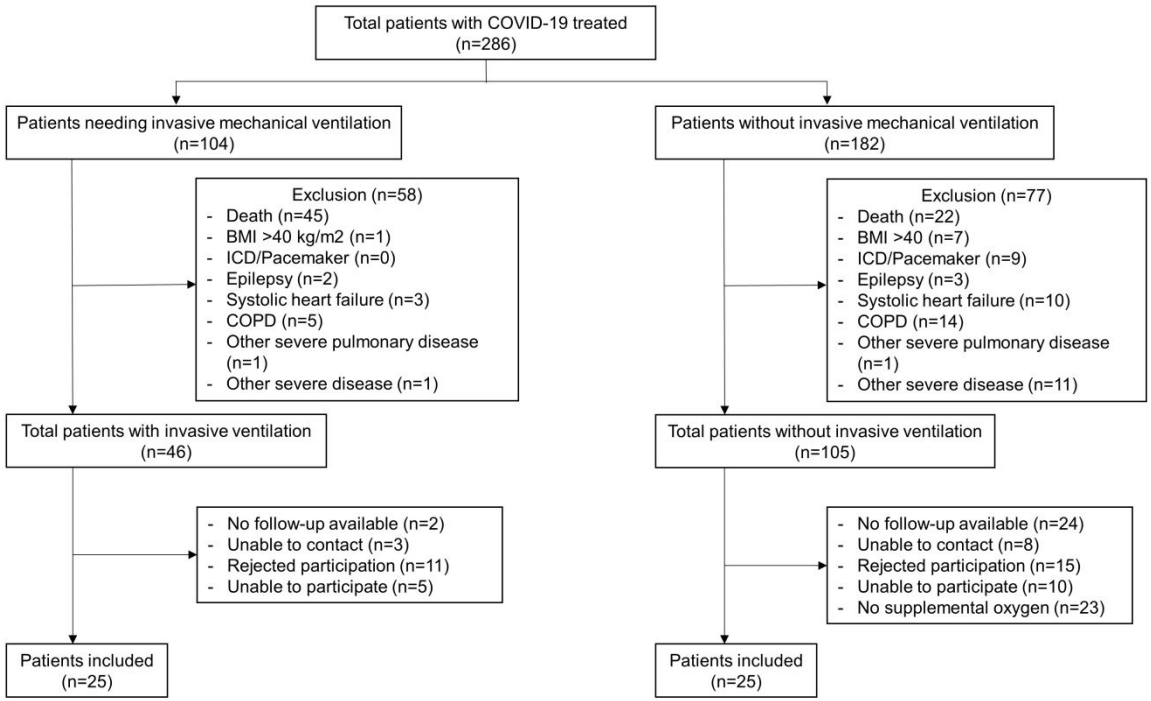

Figure 1. CONSORT diagram. BMI, body mass index; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease identified in 2019; ICD, implantable cardioverter/defibrillator.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

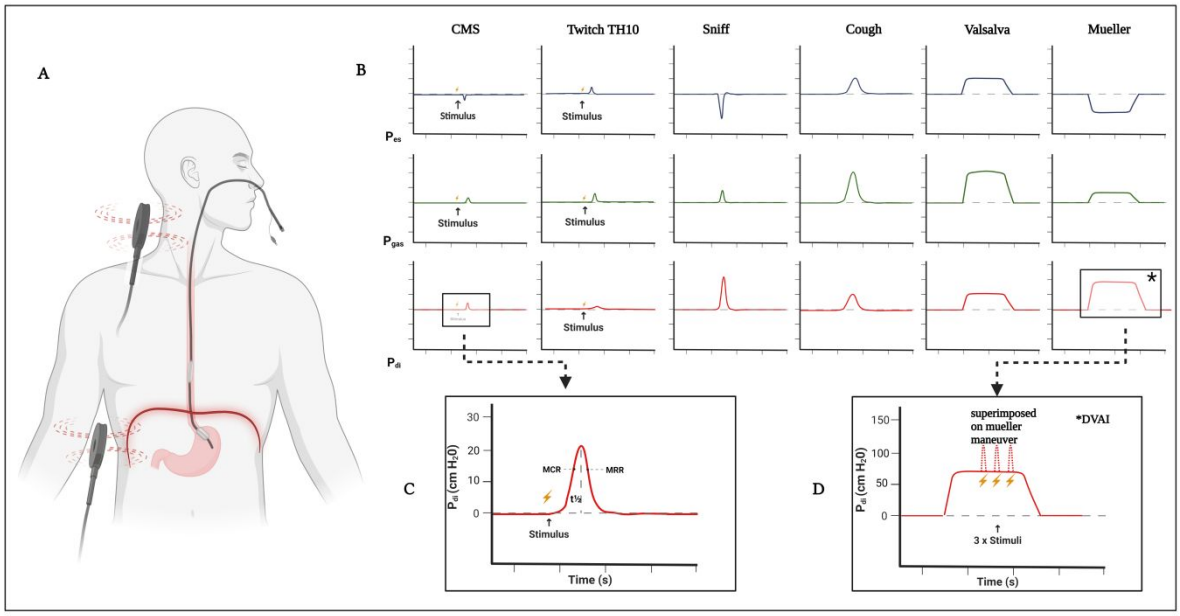

Figure 2. Experimental setup. **A**: Subject in the respiratory physiology lab with transnasal placement of double-balloon catheter measuring pressure from esophageal and gastral sensors for the calculation of transdiaphragmatic pressure; magnetic coil placement for delivery of cervical magnetic stimulation (CMS) and TH10 is shown. **B**: Curves during different voluntary and non-voluntary maneuvers. Readings from esophageal (Pes), gastric (Pgas) pressure sensors and calculated diaphragmatic pressure (Pdi) are shown. **C**: Representative twitch pressure recording following a CMS and further in-depth analysis of a twitch curve; pressure amplitude, duration of the pressure deflection, maximum rate of contraction (MRC), maximum rate of relaxation (MRR), and half relaxation time (t½) were analyzed. MRC is defined as the positive peak of the pressure derivative as a function of time (i.e. the steepest slope of the inclining twitch diaphragmatic pressure [twPDI] curve), and reflects the maximum velocity of diaphragm contraction. MRR is defined as the negative peak of the pressure derivative over time and measures the initial part of the pressure decay, reflecting maximum velocity of muscle relaxation. Both MRC and MRR were adjusted for twPDI.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Finally, t½ was defined as the time taken for twPDI amplitude to decrease by 50% from the maximum. **D:** CMS twitches superimposed on voluntary contraction and voluntary transdiaphragmatic pressure; performed on Mueller maneuver (negative esophageal pressure and positive gastric pressure). The diaphragm voluntary activation index (DVAI) reflects the percentage of diaphragmatic muscle mass activated by voluntary effort or the extent of diaphragmatic activation during any given inspiratory effort.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

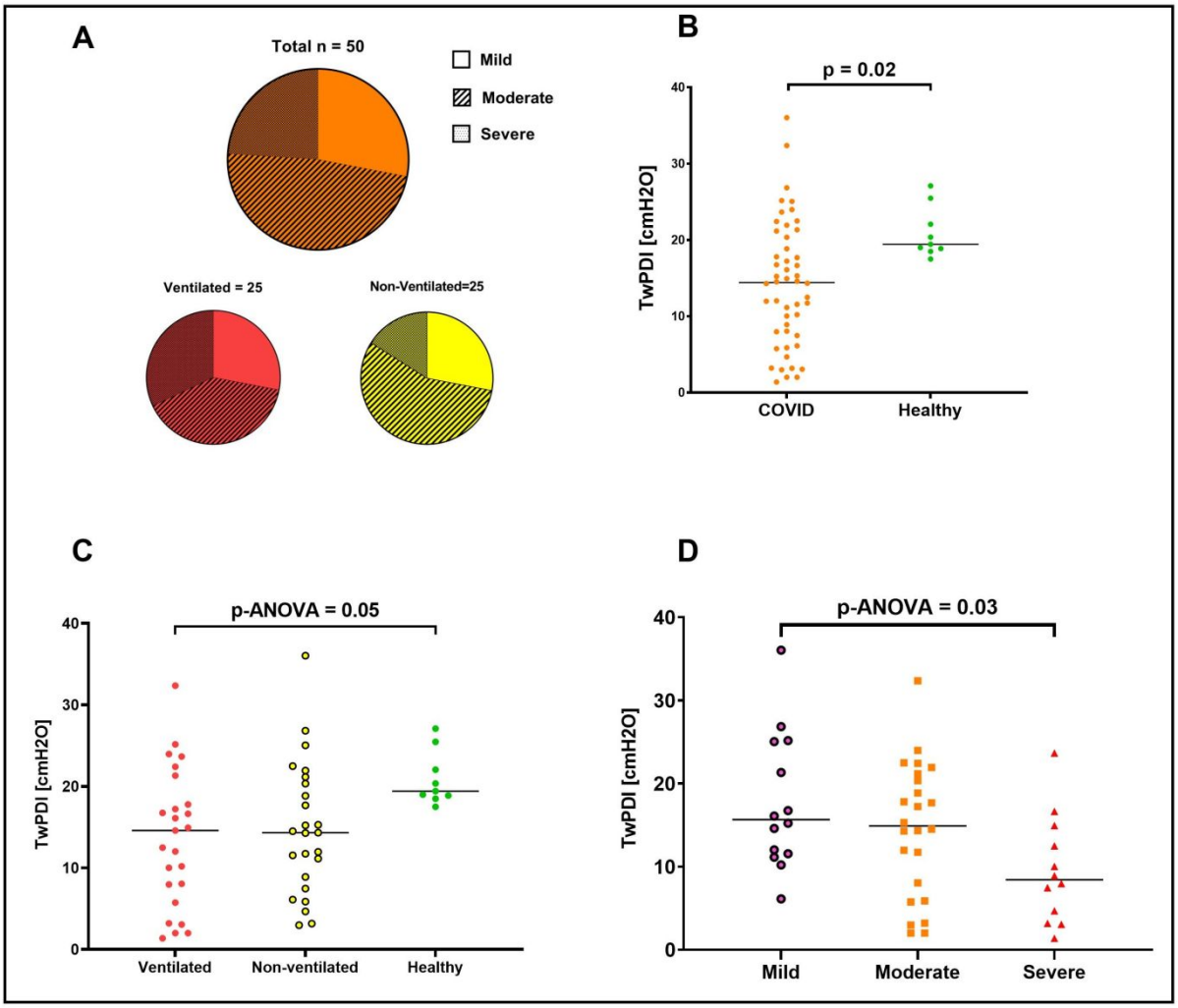

Figure 3. The impact of diaphragm muscle weakness on exertional dyspnea at fifteen months after hospitalization for COVID-19.

A: Proportion of patients with different levels of exertional dyspnea severity in the total cohort and in subgroups who did or did not undergo invasive mechanical ventilation. **B**: Differences in twitch diaphragmatic pressure (twPDI) following posterior cervical magnetic stimulation (CMS) in patients previously hospitalized for COVID compared with healthy controls**. C**: Differences in twPDI following posterior CMS in patient subgroups who did or did not undergo invasive mechanical ventilation and in healthy controls. **D**: Differences in twPDI following posterior CMS in patient subgroups based on the severity of exertional dyspnea.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Diaphragm Muscle Weakness Might Explain Exertional Dyspnea Fifteen Months After Hospitalization for COVID-19

Binaya Regmi, Janina Friedrich, Benedikt Jörn, Mehdi Senol, Alberto Giannoni, Matthias Boentert, Ayham Daher, Michael Dreher, Jens Spiesshoefer

ONLINE DATA SUPPLEMENT

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Table of Contents

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Supplementary Tables

Table S1. Pulmonary function tests, 6-minute walk test, echocardiography and laboratory findings at 12-month follow-up for the overall study population, and in patient subgroups based on the requirement for invasive mechanical ventilation during hospitalization

	Patients previously hospitalized with COVID-19				
	Total (n=50)	**Ventilated (n=25)**	**Non-ventilated (n=25)**	**P value**	**Normal values, if applicable**
Pulmonary function parameters and capillary blood gases					
TLC – % predicted	102.6±17.09	102.73±15.37	101.67±18.79	0.84	
Vital capacity – % predicted	95.48±15.58	97.52±15.17	93.69±16.04	0.42	
RV/TLC – % predicted	111.91±21.26	107.83±17.26	115.48±24.02	0.34	
FEV_1 – % predicted	90.52±15.66	94.73±15.29	86.83±14.91	0.09	
FEV_1/FVC – %	79.37±7.88	80.59±7.49	78.31±8.21	0.34	
DLCO - % predicted	69.77±15.93	67.21±16.15	71.79±15.80	0.35	
DLCO/VA – % predicted	81.90±20.74	80.45±24.69	83.05±16.73	0.62	
PaO_2 – mmHg	70.29±13.57	71.88±17.99	69.02±8.77	0.28	
$PaCO_2$ – mmHg	36.94±4.38	37.60±4.78	36.39±4.04	0.37	

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

pH	7.43±0.04	7.42±0.05	7.44±0.04	0.06	
Base excess – mmol/L	0.65±2.08	-0.03±2.08	1.24±1.97	0.07	
6-minute walk test					
Distance – m	478.78±86.21	469.44±98.28	486.41±76.47	0.54	
Fatigue					
MFIS score	31.04±19.72	36.60±21.61	25.48±16.20	**0.04**	
Echocardiography					
LVEF - %	53.98±2.01	53.71±2.10	54.21±1.89	0.42	
LVEDD – mm	48.14±9.04	49.30±4.75	47.17±11.16	0.21	
IVSD – mm	10.39±2.24	10.30±1.72	10.46±2.61	0.75	
Left atrial area – cm^2	18.67±5.60	19.16±3.13	15.83±5.02	0.57	
TAPSE ≥18 mm – no. (%)	50 (100)	25 (100)	25 (100)	1.0	
Right atrial area – cm^2	16.29±4.97	16.89±5.00	15.83±5.02	0.36	
Hematology					
White blood cells – 1/nL	6.93±2.32	7.15±2.22	6.73±2.44	0.54	4-10
Hemoglobin – g/dL	13.79±1.64	14.07±1.69	13.54±1.58	0.29	11.2-15.7
Platelets – 1/nL	237.73±61.02	239.14±52.89	236.50±68.47	0.89	150-400
Lymphocytes – %	25.52±9.26	27.14±9.23	24.18±9.09	0.28	19-52
Coagulation					

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

D–dimer – ng/mL§	335.00 (243.00–514.00)	326.50 (166.00–560.75)	335.00 (250.00–479.00)	0.63	<600
Clinical chemistry					
AST – U/L§	22.00 (17.25–26.75)	22.50 (8.75)	21.50 (17.50–26.25)	0.65	<50
ALT – U/L§	21.00 (16.25–30.00)	21.50 (16.25–33.75)	21.00 (16.25–28.00)	0.34	<35
Gamma-GT – U/L§	24.50 (17.00–45.75)	26.00 (17.00–53.00)	23.00 (16.00–41.00)	0.43	<60
Lactate dehydrogenase – U/L	215.28±69.56	196.95±37.41	227.79±85.11	0.13	<250
Creatine kinase – U/L	116.17±66.62	108.11±51.92	123.14±77.37	0.46	<190
hs-Troponin T – pg/mL§	8.00 (6.00–17.00)	9.00 (4.00–17.00)	8.00 (6.00–18.25)	0.44	<14
NT-proBNP – pg/mL§	90.70 (47.38–236.80)	79.00 (48.35–218.30)	102.00 (32.80–243.40)	0.24	<486
Creatinine – mg/dL§	1.10 (0.80–1.32)	1.10 (0.84–1.57)	0.95 (0.77–1.21)	0.11	<1.0
C-reactive protein – mg/L§	1.80 (1.15–4.85)	1.30 (0.60–2.20)	2.35 (1.68–9.13)	0.95	<5
Procalcitonin – ng/mL	0.13±0.03	0.07±0.04	0.06±0.03	0.80	<0.5
Cytokines					
sIL-2 receptor – U/mL§	466.00 (384.00–627.00)	472.00 (392.00–668.50)	428 (341.00–626.50)	0.50	158-623
IL-6 – pg/mL§	3.10 (1.50–4.56)	1.91 (1.50–3.77)	3.57 (2.09–5.04)	0.26	<7

Plus-minus values are means ±SD.

§Median (interquartile range) values for non-normally distributed data. $p<0.05$ is highlighted bold.

ALT = alanine transaminase, AST aspartate transaminase, DLCO diffusing capacity for carbon monoxide, FEV_1 forced expiratory volume in 1 second, FVC forced vital capacity, Gamma-GT gamma-glutamyltransferase, hs-Troponin-T high sensitive troponin-T, IL-6 interleukin-6, IVSD interventricular septal end diastole, LVEF left

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

ventricular ejection fraction, LVEDD left ventricular end-diastolic diameter, MFIS modified fatigue impact scale, NT-proBNP N-terminal pro B-type natriuretic peptide, $PaCO_2$ partial pressure of carbon dioxide, PaO_2 partial pressure of oxygen, RV residual volume, sIL2 soluble interleukin-2 receptor, TAPSE tricuspid annular plane systolic excursion, TLC total lung capacity, VA alveolar volume.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Table S2. Pulmonary function tests, 6-minute walk test, echocardiography and laboratory findings at 12-month follow-up in patient subgroups

	Exertional dyspnea severity				
	None/mild (n=14; 28%)	**Moderate (n=24; 48%)**	**Severe (n=12; 24%)**	**P Value (ANOVA)**	**Normal Values, if applicable**
Pulmonary function parameters and capillary blood gases					
TLC – % predicted	106.83±19.34	100.75±14.83	98.38±19.02	0.45	
Vital capacity – % predicted	99.66±16.40	96.14±12.84	87.34±18.96	0.18	
RV/TLC – % predicted	108.81±16.29	109.00±14.17	123.88±36.40	0.17	
FEV_1 – % predicted	97.24±16.53	89.78±14.82	81.86±12.79±	0.07	
FEV_1/FVC – %	80.23±6.73	78.71±8.53	79.07±8.64	0.85	
DLCO - % predicted	76.76±14.43	68.60±15.97	59.44±24.59	0.06	
DLCO/VA – % predicted	81.23±17.57	82.27±20.41	71.81±27.90	0.18	
PaO_2 – mmHg	69.51±20.61	69.93±7.88	72.25±9.81	0.67	
$PaCO_2$ – mmHg	39.99±3.60	37.51±2.87	37.96±2.42	0.07	
pH	7.42±0.03	7.44±0.04	7.44±0.06	0.31	

based on the severity of dyspnea on exertion at 12-month follow-up

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Base excess – mmol/L	1.21±1.52	0.58±1.98	–0.01±3.10	0.53	
6-minute walk test					
Distance – m	521.50±55.54	483.31±92.01	390.43±39.87	**0.003** x †	
Fatigue					
MFIS score	11.93±6.86	31.96±15.85	51.50±15.09	**<0.001*** x †	
Echocardiography					
LVEF - 50%	54.64±1.29	54.10±2.00	52.91±2.23	0.09	
LVEDD – mm	49.86±6.74	46.62±11.25	50.00±3.16	0.21	
IVSD – mm	10.64±1.50	10.19±2.59	10.44±2.30	0.73	
Left atrial area – cm^2	17.46±5.34	18.00±6.07	17.33±3.94	0.20	
TAPSE ≥18 mm – no. (%)	14 (100)	14 (100)	14(100)	1.0	
Right atrial area – cm^2	17.46±4.03	15.67±5.06	16.00±5.67	0.38	
Hematology					
White blood cells – 1/nL	5.74±1.46	6.94±2.16	8.55±2.80	**0.01**†	4-10
Hemoglobin – g/dL	14.31±2.06	13.75±1.42	13.12±1.27	0.21	11.2-15.7
Platelets – 1/nL	203.93±60.02	265.81±59.90	226.10±35.49	**0.007***	150-400
Lymphocytes – %	28.42±10.76	24.47±8.42	24.10±9.76	0.38	19-52
Coagulation					
D–dimer – ng/mL§	247.00 (151.00–434.00)	354.50 (249.50–530.50)	418.50 (269.25–740.75)	0.45	<600

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

Clinical chemistry					
AST – U/L§	22.00 (16.75–27.00)	22.00 (17.50–26.50)	22.00 (15.50–44.50)	0.77	<50
ALT – U/L§	20.50 (15.75–24.75)	21.00 (16.50–36.00) -	21.00 (17.50–36.00)	0.52	<35
Gamma-GT – U/L§	19.50 (16.00–28.75)	25.00 (15.25–46.75)	43.00 (21.50–92.00)	0.55	<60
Lactate dehydrogenase – U/L	225.50±78.49	211.48±71.16	207.38±52.92	0.80	<250
Creatine kinase – U/L	134.00 (99.00–192.50)	99.00 (68.25–122.25)	74.50 (59.00–97.50)	**0.02***	<190
hs-Troponin T – pg/mL§	8.50 (3.75–20.75)	7.50 (5.25–13.00)	9.00 (8.00–35.50)	0.21	<14
NT-proBNP – pg/mL§	59.20 (26.75–281.20)	102.00 (43.55–169.50)	91.70 (56.95–716.50)	0.42	<486
Creatinine – mg/dL§	0.93 (0.79–1.27)	1.10 (0.81–1.20)	1.50 (0.80–2.79)	0.12	<1.0
C-reactive protein – mg/L§	1.15 (1.00–2.08)	2.10 (1.63–8.18)	2.70 (0.90–11.45)	0.21	<5
Procalcitonin – ng/mL	0.03 (0.02–0.06)	0.04 (0.03–0.05)	0.05 (0.04–0.08)	0.89	<0.5
Cytokines					
sIL-2 receptor – U/mL§	405.00 (378.50–613.75)	428.00 (341.00–537.00)	501.00 (454.50–852.25)	0.42	158-623
IL-6 – pg/mL§	2.46 (1.50–3.77)	3.49 (1.50–6.39)	3.37 (1.78 –8.72)	0.21	<7

Plus-minus values are means ±SD.

§Median (interquartile range) values for non-normally distributed data.

ANOVA, Analysis of variance.

Significance differences ($p<0.05$) within paired t-tests between each groups, if ANOVA is significantly different ($p<0.05$). * indicates significant difference between mild and moderate; x between moderate and severe and †between mild and severe dyspnea groups respectively. $p<0.05$ is highlighted bold.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society

ALT = alanine transaminase, AST aspartate transaminase, DLCO diffusing capacity for carbon monoxide, FEV_1 forced expiratory volume in 1 second, FVC forced vital capacity, Gamma-GT gamma-glutamyltransferase, hs-Troponin-T high sensitive troponin-T, IL-6 interleukin-6, IVSD interventricular septal end diastole, LVEF left ventricular ejection fraction, LVEDD left ventricular end-diastolic diameter, MFIS modified fatigue impact scale, NT-proBNP N-terminal pro B-type natriuretic peptide, $PaCO_2$ partial pressure of carbon dioxide, PaO_2 partial pressure of oxygen, RV residual volume, sIL2 soluble interleukin-2 receptor, TAPSE tricuspid annular plane systolic excursion, TLC total lung capacity, VA alveolar volume.

AJRCCM Articles in Press. Published January 03, 2023 as 10.1164/rccm.202206-1243OC
Copyright © 2023 by the American Thoracic Society