ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Original research article

Sonographic follow-up of diaphragm function in COVID-19: an exploratory study

Carlijn Veldman, Wytze S. de Boer, Huib A.M. Kerstjens, Mireille A. Edens, Jan Willem K. van den Berg

Please cite this article as: Veldman C, de Boer WS, Kerstjens HAM, *et al*. Sonographic follow-up of diaphragm function in COVID-19: an exploratory study. *ERJ Open Res* 2023; in press (https://doi.org/10.1183/23120541.00623-2022).

This manuscript has recently been accepted for publication in the *ERJ Open Research*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

Abstract word count: 229
Manuscript word count: 2911

Sonographic follow-up of diaphragm function in COVID-19: an exploratory study

Authors:
Carlijn Veldman, MD.[1]
c.veldman@isala.nl

Wytze S. de Boer, MD.[1]
w.s.de.boer@isala.nl

Huib A.M. Kerstjens, MD PhD.[2]
h.a.m.kerstjens@umcg.nl

Mireille A. Edens, PhD.[3]
m.a.edens@isala.nl

Jan Willem K. van den Berg, MD PhD.[1]
j.w.k.van.den.berg@isala.nl

Affiliation:
[1]Department of Pulmonary Medicine, Isala Hospital, Zwolle, The Netherlands
[2] Department of Pulmonology, University of Groningen, University Medical Center Groningen, And Groningen Research Institute for Asthma and COPD GRIAC, all Groningen
[3] Epidemiology Unit, Department of Innovation and Science, Isala Hospital, Zwolle, The Netherlands

Corresponding author information:
Wytze S. de Boer, MD; w.s.de.boer@isala.nl

CV and WSB contributed equally to this manuscript.

The listed authors declare that there is no conflict of interest.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

An abstract has been presented to the ERS. The manuscript is not submitted elsewhere.

Abstract

Introduction:
Survivors of COVID-19 frequently endure chronic disabilities. We hypothesize that diaphragm function has a long recovery time after COVID-19 hospitalization, and may play a role in post-COVID syndrome. The aim of this study was to assess diaphragm function during COVID-19 hospitalization and during recovery.

Methods:
We conducted a prospective single-center cohort study in 49 patients enrolled, of which 28 completed one-year follow-up. Participants were evaluated for diaphragm function. Diaphragm function was assessed using ultrasound measuring of diaphragm thickening fraction (TF) within 24 hours after admission, after 7 days of admission or at discharge, whichever came first, and three and twelve months after hospital admission.

Results:
Estimated mean TF increased from 0.56 (95% CI 0.46-0.66) on admission to 0.78 (95% CI 0.65-0.89) at discharge or seven days after admission, to 1.05 (95% CI 0.83-1.26) three months after admission to 1.54 (95% CI 1.31-1.76) twelve months after admission. The improvements from admission to discharge, 3 months and 12 months were all significant (linear mixed modeling; $p=0.020$, $p<0.001$, and $p<0.001$, respectively) and the improvement from discharge to three months follow-up was borderline significant ($p<0.1$).

Conclusion:
Diaphragm function was impaired during hospitalization for COVID-19. During recovery in hospital and up to one-year follow-up, diaphragm TF improved, suggesting a long recovery time of the diaphragm. Diaphragm ultrasound may be a valuable modality in the screening and follow-up of (post-)COVID-19 patients for diaphragm dysfunction.

Keywords:
COVID-19, Diaphragm function, Thoracic Ultrasound

Introduction

Since the appearance of COVID-19 several studies have described chronic disabilities after recovery from infection with the SARS-CoV-2 virus. Various unresolved symptoms such as shortness of breath, cough or fatigue have been reported in COVID-19 survivors several months after hospital discharge, sometimes referred to as Long COVID-19 or post-COVID syndrome.(1–3) Although it is not fully understood why these symptoms occur, neuromuscular involvement including the diaphragm has been suggested.(4,5)

The diaphragm is the main inspiratory muscle, and, compared with peripheral muscles, appears to be more affected by critical illness and excessive respiratory drive leading to over-exertion.(6) A post-mortem study by Shi et al. (2020) in COVID-19-ICU patients reported angiotensin-converting enzyme 2 (ACE-2) expression in the diaphragm, providing an entry point for SARS-CoV-2 to infect diaphragm myofibers.(7,8) It also found increased expression of genes involved in fibrosis and histological evidence for the development of fibrosis in the diaphragm of COVID-19-ICU patients, distinctly different from that of control-ICU patients. We hypothesize that diaphragm function has a long recovery time after COVID-19, and may play a role in post-COVID syndrome.

Thoracic ultrasound could provide a more feasible method to evaluate the diaphragm function in recovering COVID-19 patients compared to phrenic nerve conduction studies or needle EMG, and can reliably be repeated over time.(9) Thoracic ultrasound can demonstrate atrophy and impaired contractility of the diaphragm.(10–12) Normal values for diaphragm thickening fraction and muscle thickness have been published and can be used for group comparison.(13,14) Several studies conclude that thoracic ultrasound is superior to fluoroscopy for the diagnosis of diaphragm dysfunction.(15,16) The aim of this study was to follow the diaphragm function during hospitalization and further recovery over one year in patients with COVID-19.

Methods

Study design

We conducted a prospective single-center cohort study in Isala hospital, Zwolle, the Netherlands who were admitted to the nursing ward. All consecutive patients fulfilling inclusion criteria but not exclusion criteria were enrolled January and March 2021, except those admitted <24 hours. Eligible patients were 18 ≥ years of age with COVID-19 pneumonitis, confirmed by polymerase chain reaction (PCR) test for SARS-CoV-2 and compatible chest imaging, and hospital admission primarily due to COVID-19 with hypoxemia. We excluded the following patients: 1) those with pre-existent diseases of the diaphragm or neuromuscular disease, 2) those in which the anticipated sonographic diaphragm measurements were not possible (mechanical ventilation, inability to follow vocal instructions), 3) those not able or unwilling to give written informed consent, 4) those living outside the hospital region. All patients were treated according to the local treatment-protocol. Permission for this study was obtained from the Medical Research and Ethics Committee Isala Clinics (number: 210120) and all patients gave written informed consent.

Primary outcome: diaphragm assessment

Diaphragm function was assessed within 24 hours of enrollment, and repeated after seven days, or at hospital discharge, whichever came first, and three and twelve months after enrollment. The diaphragm was measured using the Sparq Ultrasound System (Philips Healthcare, Andover, MA) with a 12 MHz linear array transducer. Diaphragm thickness was measured with the patient in supine position and the ultrasound probe perpendicular to the diaphragm between the intercostal approaches at the right mid-axillary line around the tenth intercostal space, the zone of apposition (Figure 1).(13,17–19) The same location was used for sonographic follow-up.

Primary outcome parameter was diaphragm thickening fraction (TF) as a quantitative measure of diaphragm strength. TF is defined as a fraction of diaphragm thickness at the end of maximal inspiration (TDinsp) and at the end of maximal expiration (TDexp): TF = $(T_{end\text{-}inspiration}-T_{end\text{-}expiration})/T_{end\text{-}}$

$_{expiration}$.(14,20) The lower limit (LLN) of TF is defined as 0.20 and of TDexp 0.15mm.(18) Measurements of the diaphragm function were performed in duplo by two investigators with training and experience in ultrasound of the diaphragm. The measurement with the optimal inspiration and expiration was chosen. Afterwards, a second observer repeated the measurements, blinded to the outcome of the first observer. In case of discrepancy, consensus was reached by discussion between the two observers.

Figure 1: Ultrasound image of the diaphragm at the zone of apposition.

Secondary outcomes
Secondary outcomes were rate of dyspnea, peripheral muscle strength and quality of life directly after diaphragm assessment during all moments of follow-up. The rate of dyspnea was assessed by using a 0-100mm horizontal visual analogue scale (VAS) for breathlessness, with 0 signifying no breathlessness and 100 signifying the worst possible intensity of breathlessness.(21) Peripheral muscle strength was measured by using hand held dynamometry using the Jamar Hydraulic Hand Dynamometer. Force of strength was measured in kilograms. Measurements were done with both hands and the mean of both measurements was used. Overall quality of life was measured by EuroQol-5-Dimension Level (EQ-5D-5L).(22) The EQ-5D-5L assesses health in five dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression), each of which has five levels of response (no problems, slight problems, moderate problems, severe problems, extreme problems/unable to). The EQ-5D-5L also includes a vertical VAS (EQ-VAS), with participants asked to mark how their health is today on a scale of 0–100. A higher number reflects a better quality of life for both the EQ-5D-5L and the EQ-VAS. The questionnaires were presented last to the patients, to limit possible influence on the sonographer's interpretations. All results of the different tests were withheld from the participant during the course of the study.

Statistical analysis
Statistical analyses were performed using SPSS statistics 27.0 software. Categorical data were presented as n (%), and continuous data were presented as mean (±SD) or median (range), depending on the distribution. For analyses of the primary endpoint, diaphragm function (TF), linear mixed model analysis for repeated TF measurements was performed, using the time point of measurement as a fixed effect. Bonferroni-adjustment was applied. Akaike's Information Criterion (AIC) was used to select the covariance structure. Other data were analyzed using paired samples T-test, Wilcoxon signed rank test or Mann-Whitney U test depending on data distribution and paired or unpaired data. P-values less than 0.05 were considered significant.

Correlations were tested using Pearson or Spearman's correlation coefficient, depending on data distribution. Interrater reliability was analyzed by using the Intraclass Correlation Coefficient (ICC) with a two-way mixed effects model and an absolute agreement definition for single measurements: values less than 0.5, between 0.5-0.75, between 0.75-0.9 and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively, all based on 95% confidence interval of the ICC estimate.(23)

Explorative sample size calculation
No formal sample size calculation was done since this was an explorative study on the novel topic of diaphragm recovery after COVID-19 infection. An explorative sample size calculation was based on estimating the mean diaphragm thickening fraction. The standard deviation was set at 0.5 based on literature (13) and the two-sided 95% confidence interval width was set at 0.5, which results in a minimum sample size of n=18.

Results

Patients

Between January 23th and March 30th 2021, 49 patients with COVID-19 pneumonitis were included shortly after admission. The recruitment and loss to follow-up is depicted in the Consort diagram (Figure 2). None of the patients were diagnosed with pulmonary lung embolism, active heart failure or a secondary bacterial infection during admission. At admission median FiO_2 was 0.33 (range 0.21-0.85). Baseline characteristics are depicted in table 1. 34 patients were assessed by ultrasound seven days after admission, 29 patients after three months of follow-up, and 28 patients after twelve months of follow-up; the reduction in patient population over time did not lead to a clear selection bias (Supplemental Table S1).

Figure 2: Consort flow diagram.

Characteristic	Admission
Number of patients	49
Age in years (median, range)	62 (37-85)
Female (%)	12 (25)
BMI kg/m2 (median, range)	28 (21 - 48)
Use of steroids during admission (%)	49 (100)
Smoking - Never (%) - Current (%) - Former (%)	 19 (39) 1 (2) 29 (59)
Comorbidities - Diabetes mellitus (%) - Hypertension (%) - Cardio-vascular (%) - Chronic lung disease (asthma/COPD) (%) - Malignancy (%)	 9 (18) 23 (47) 5 (10) 7 (14) 2 (4)
Days of prehospital illness (median, range)	8 (1-28)
Days to measuring point (median, range)	9 (3-29)
Duration of hospitalization in days (median, range)	7 (2-54)
Clinical characteristics on admission - PaCO2 (kPa) (median, range) - PaO2 (kPa) (median, range) - Respiratory Rate (breaths/min) (median, range) - PaO2/FiO2 ratio (mmHg) (median, range)	 4,2 (2,8 - 6,0) 7,0 (5,3 - 14,3) 23 (14 - 42) 235 (125 - 289)
Maximal received supplemental oxygen (%) during hospitalization - Low-flow nasal cannula - Venturi mask (FiO2 0.40-0.60) - Non-rebreather mask - High-flow nasal oxygen - Invasive ventilation	 21 (43) 4 (8) 13 (27) 1 (2) 10 (20)

Table 1: Patient characteristics (number or mean unless otherwise specified).

Primary outcome: diaphragm assessment

Estimated means of diaphragm TF were 0.56 (95% CI 0.46-0.66) on the day of admission, 0.78 (95% CI 0.65-0.89) on the day of discharge/seven days after admission, 1.06 (95% CI 0.83-1.26) three months after admission, and 1.54 (95% CI 1.31-1.76) twelve months after admission (Figure 3). TF measurements improved from admission to discharge (95%CI -0.41- -0.014, p= 0.030), discharge vs. three months follow-up (95%CI -0.56-0.02, p=0.085), admission vs. 3 months follow-up (95% CI -1.06-

-0.46, p<0.001) and admission vs. twelve months follow-up, showed (95% CI -0.72- -0.25, p<0.001) (Figure 3, Supplemental Figure S1). Case-by-case analysis showed an improvement of TF in 26 (93%) patients from admission to twelve months follow-up; and a decrease in two (7%) patients. Of these two patients one (-15%) showed improvement between seven days after admission and twelve months follow-up (+96%). Lower TF on admission was associated with higher change in TF from admission to twelve months of follow-up (Supplemental Figure S2). At admission, four patients had a TDexp lower than 0.15 cm and two patients had a TF less than 0.20, signifying that 6/49=14% had abnormally low values at admission. During follow-up at three and twelve months none of the participants had a TF below the lower limit of normal, and six patients (21%) and three patients (11%) had an abnormal TDexp, respectively. No significant difference in TF was found between men and women (p=0.264, p=0.627, p=0.291, p=0.676, respectively at the four respective measurement time points). The Intraclass Correlation Coefficient (ICC) (23) for interrater reliability for TF for all measurements together over time was 0.905 [lower bound 0.869, upper bound 0.931.

Figure 3:

a. Median TF and minimum-maximum range at admission, at discharge or 7 days after admission, three months after admission and twelve months after admission.
b. TF since first sick day in weeks. Data are estimated means with 95% confidence intervals, obtained from linear mixed modeling.
c. Median VAS-breathlessness at admission, day of discharge or seven days after admission, three months after admission and twelve months after admission.
d. Median EQ-VAS at admission, day of discharge or seven days after admission, three months after admission and twelve months after admission.
e. Median measurements of hand dynamometry dominant hand at admission, day of discharge or seven days after admission, three months after admission and twelve months after admission.
f. Median measurements of hand dynamometry non-dominant hand at admission, day of discharge or seven days after admission, three months after admission and twelve months after admission.

Secondary outcomes

Median VAS-breathlessness scores showed a significant improvement between admission and discharge or seven days after admission (p=0.001), as well as between admission and three months follow-up (p<0.001) and between admission and twelve months follow-up (p=0.013). No significant improvement was found between discharge and three months of follow-up (p=0.270), and three and twelve months of follow up (p=0.477) (Figure 3, Supplemental Table S2). No correlation was found between TF and VAS-breathlessness. Mean hand-held dynamometry showed significant improvement between three months follow-up and twelve months follow-up (p=0.004) and between discharge and twelve months follow-up (p=0.013) (Figure 3, Supplemental Table S2).

The EQ-5D-5L scores improved between baseline and 12 months for the dimensions of mobility, self-care, and usual activities (p 0.020, p <0.001, p <0.001, respectively), based on 28 patients who completed follow-up. Anxiety/depression and pain/discomfort showed no change between baseline and 12 months (p 0.816, p 0.308, respectively). Self-rated health scores measured as EQ-VAS improved significantly during recovery (p<0.001) (Figure 3, Supplemental Table S3).

Discussion

We confirmed the presence of diaphragm dysfunction during hospitalization for COVID-19, partially by showing abnormal values in hospital, and certainly by showing marked improvements up to one year. This makes our results compatible with a contribution of diaphragm dysfunction to complaints of Long COVID-19 at least at three months.

Reports of long lasting COVID-19 disease symptoms, so called long-COVID, are quickly rising. A recent systematic review suggested an estimated prevalence of 0.34 in non-hospitalized patients and 0.54 in hospitalized patients.(24) The exact reason why some patients experience long-term symptoms after COVID-19 is uncertain. Patients suffering from long COVID-19 report a wide range of symptoms, but most reported symptoms are fatigue and dyspnea.(25,26) Underlying mechanisms which are suggested among others are long-term biochemical and inflammatory response pathways or hypoxemia secondary to the destruction of capillaries.(27,28) The cause so far of dyspnea has not been coined under one mechanism, and many contributing factors have been suggested, such as altered diffusion capacity, restrictive pattern as well as obstructive pattern.(29) Another mechanism suggested before is diaphragm dysfunction. We confirm that at admission to hospital diaphragm dysfunction is present, though the number of patients with decreases that can be labeled as clearly clinically abnormal is small in our study. The definite improvement in the first seven days of recovery, in hospital, however suggests that the values are below the patients individual normal value, and given the further improvement till one year, their individual normal value is on average at least not reached till one year.

Hand-held dynamometry measures the isometric force of the hand. It could be a good predictor of the overall peripheral muscle strength and has already been used in several studies about lung rehabilitation.(30) Hand-held dynamometry only slightly improved over time in our study, most of which between three and twelve months follow-up, suggesting the diaphragm may be more affected than mean peripheral muscle strength in COVID-19. Several studies have assessed VAS-breathlessness to quantify the severity of breathlessness, with the highest score during admission.(31) Interestingly, in our study, while diaphragm function was still recovering until twelve months of follow-up, VAS-breathlessness showed no significant improvement anymore. Our study suggests that recovering COVID-19 patients mention a poor quality of life, which is already demonstrated by a recent meta-analysis.(22) However, just a limited number of patients mentioned anxiety or depressive feelings, which is in contrast to the suggestion the COVID-19 patients could develop a post-traumatic stress disorder.

Theoretical mechanisms of diaphragm dysfunction in COVID-19 patients are possibly multifold and could include critical illness myopathy, ventilator-induced diaphragm dysfunction, iatrogenic phrenic nerve injury, post-infectious inflammatory neuropathy of the phrenic nerve, and possibly direct neuromuscular involvement of the SARS-CoV-2.(6,8,32–34) Diaphragm injury has been shown in non-ventilated patients after excessive respiratory drive, manifesting as a loss in force-generating capacity and sarcomere disruption on histology.(35) Potential factors inducing excessive respiratory drive include hypoxemia, hypercapnia, stimulation of lung and chest wall receptors, cortical stimuli, and brain stem inflammation.(36–39) Understanding the level of neuromuscular involvement, including the diaphragm, in COVID-19 and chronic disabilities is an active area of research. A post-mortem study in 26 patients reported ACE-2 expression in the diaphragm of COVID-19-ICU and control-ICU patients providing an entry point for COVID-19 to infect diaphragm myofibers, which suggested a possible infectious involvement of the diaphragm.(7,8)

Ultrasound may be a promising and well-tolerated tool for longitudinal assessment in patients with acute or chronic respiratory symptoms, especially post-infection, and is a non-invasive procedure with widespread availability. Unfortunately, it is also a technique known for interobserver variability. To overcome this, we performed measurements in duplo by two independent observers, and our study interrater reliability was excellent.(23) With appropriate protocols and training, ultrasound can be performed by radiology services or even as a hands-on investigation during planned hospital visits. This study followed an algorithm including ultrasound for suspected diaphragm dysfunction which have already been published.(17,20)

The strength of our study next to two observers for diaphragm measurements is the long follow-up time of one year. Our study also has several limitations. Not all patients could be followed until one-year follow-up, among others because of death or admission to the ICU. However, selection bias introduced this way seems to be limited at least for the parameters assessed (Supplementary Table S1e). Furthermore, a relatively small number of women were included. Recent studies suggest distinct values for the LLN of end-expiratory diaphragm thickness in men and women, however TF was similar regardless of gender.(40) Several studies regarding normal values have been published, but large validation studies are lacking. It would have helped if we would have pre-COVID-19 diaphragm function, also to determine whether the one-year values had normalized. Third, no pre-COVID-19 diaphragm function was available due to the study design. Fourth, appropriate patient effort is needed for accurate measurement of thickening fraction, which may have been insufficient in acutely ill patients.(13) Fifth, combined diaphragm function measurements using trans-diaphragmatic pressure measurement, EMG, or both, would have been interesting. However, it was not available in the clinical setting due to the invasive setting of these procedures. Overall, we consider this explorative study provoking, and encourage replication and extension.

In conclusion, impaired diaphragm function during hospitalization for COVID-19 improved during long-term follow-up, even between three and twelve months of follow-up, suggesting a long recovery time of the diaphragm. Diaphragm ultrasound may be a valuable modality in the screening and follow-up of (post-)COVID-19 patients for diaphragm dysfunction.

Author contributions

WSB and CV designed the study in consultation with JWKB. The study was performed by WSB and CV. ME and HAMK helped with the interpretation of the statistics. WSB and CV wrote the manuscript supervised by ME, HAMK, and JWKB. All authors approved the final manuscript.

Acknowledgements

We thank C.A. Sturm for her creative contributions regarding the figures.

Conflict of interest

None declared.

Funding

None

References

1. Mandal S, Barnett J, Brill SE, Brown JS, Denneny EK, Hare SS, et al. 'Long-COVID': a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. Thorax [Internet]. 2021 Apr;76(4):396–8. Available from: https://thorax.bmj.com/lookup/doi/10.1136/thoraxjnl-2020-215818
2. Carfì A, Bernabei R, Landi F. Persistent Symptoms in Patients After Acute COVID-19. JAMA [Internet]. 2020 Aug 11;324(6):603. Available from: https://jamanetwork.com/journals/jama/fullarticle/2768351
3. Rubin R. As Their Numbers Grow, COVID-19 "Long Haulers" Stump Experts. JAMA [Internet]. 2020 Oct 13;324(14):1381. Available from: https://jamanetwork.com/journals/jama/fullarticle/2771111
4. Farr E, Wolfe AR, Deshmukh S, Rydberg L, Soriano R, Walter JM, et al. Diaphragm dysfunction in severe COVID-19 as determined by neuromuscular ultrasound. Ann Clin Transl Neurol [Internet]. 2021 Aug 11;8(8):1745–9. Available from: https://onlinelibrary.wiley.com/doi/10.1002/acn3.51416
5. Satıcı C, Aydın Ş, Tuna L, Köybaşı G, Koşar F. Electromyographic and sonographic assessment of diaphragm dysfunction in patients who recovered from the COVID-19 pneumonia. Tuberk Toraks [Internet]. 2021 Sep 23;69(3):425–8. Available from: http://www.tuberktoraks.org/managete/fu_folder/2021-03/425-428 Celal Satici.pdf
6. Brosnahan SB, Jonkman AH, Kugler MC, Munger JS, Kaufman DA. Covid-19 and respiratory system disorders current knowledge, future clinical and translational research questions. Arterioscler Thromb Vasc Biol. 2020;(November):2586–97.
7. Beyerstedt S, Casaro EB, Rangel ÉB. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. Eur J Clin Microbiol Infect Dis [Internet]. 2021 May 3;40(5):905–19. Available from: https://link.springer.com/10.1007/s10096-020-04138-6
8. Shi Z, de Vries HJ, Vlaar APJ, van der Hoeven J, Boon RA, Heunks LMA, et al. Diaphragm Pathology in Critically Ill Patients With COVID-19 and Postmortem Findings From 3 Medical Centers. JAMA Intern Med [Internet]. 2021 Jan 1;181(1):122. Available from: https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2773060
9. Cappellini I, Picciafuochi F, Bartolucci M, Matteini S, Virgili G, Adembri C. Evaluation of diaphragm thickening by diaphragm ultrasonography: a reproducibility and a repeatability study. J Ultrasound [Internet]. 2021 Dec 1;24(4):411–6. Available from: https://link.springer.com/10.1007/s40477-020-00462-x
10. Cohn D, Benditt JO, Eveloff S, McCool FD. Diaphragm thickening during inspiration. J Appl Physiol [Internet]. 1997 Jul 1;83(1):291–6. Available from: https://www.physiology.org/doi/10.1152/jappl.1997.83.1.291
11. Gottesman E, McCool FD. Ultrasound evaluation of the paralyzed diaphragm. Am J Respir Crit Care Med [Internet]. 1997 May;155(5):1570–4. Available from: http://www.atsjournals.org/doi/abs/10.1164/ajrccm.155.5.9154859
12. Summerhill EM, El-Sameed YA, Glidden TJ, McCool FD. Monitoring Recovery From Diaphragm Paralysis With Ultrasound. Chest [Internet]. 2008 Mar;133(3):737–43. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0012369215513342
13. Boon AJ, Harper CJ, Ghahfarokhi LS, Strommen JA, Watson JC, Sorenson EJ. Two-dimensional ultrasound imaging of the diaphragm: Quantitative values in normal subjects. Muscle Nerve [Internet]. 2013 Jun;47(6):884–9. Available from: https://onlinelibrary.wiley.com/doi/10.1002/mus.23702
14. Laveneziana P, Albuquerque A, Aliverti A, Babb T, Barreiro E, Dres M, et al. ERS statement on respiratory muscle testing at rest and during exercise. Eur Respir J [Internet]. 2019 Jun;53(6):1801214. Available from: http://erj.ersjournals.com/lookup/doi/10.1183/13993003.01214-2018
15. Miller SG, Brook MM, Tacy TA. Reliability of two-dimensional echocardiography in the

assessment of clinically significant abnormal hemidiaphragm motion in pediatric cardiothoracic patients: Comparison with fluoroscopy*. Pediatr Crit Care Med [Internet]. 2006 Sep;7(5):441–4. Available from: http://journals.lww.com/00130478-200609000-00006
16. Houston JG, Morris AD, Howie CA, Reid JL, McMillan N. Technical report: Quantitative assessment of diaphragmatic movement — A reproducible method using ultrasound. Clin Radiol [Internet]. 1992 Dec;46(6):405–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0009926005806889
17. Boon AJ, Sekiguchi H, Harper CJ, Strommen JA, Ghahfarokhi LS, Watson JC, et al. Sensitivity and specificity of diagnostic ultrasound in the diagnosis of phrenic neuropathy. Neurology [Internet]. 2014 Sep 30;83(14):1264–70. Available from: http://www.neurology.org/cgi/doi/10.1212/WNL.0000000000000841
18. Patel Z, Franz CK, Bharat A, Walter JM, Wolfe LF, Koralnik IJ, et al. Diaphragm and Phrenic Nerve Ultrasound in <scp>COVID</scp> -19 Patients and Beyond. J Ultrasound Med [Internet]. 2022 Feb 27;41(2):285–99. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jum.15706
19. Boussuges A, Rives S, Finance J, Brégeon F. Assessment of diaphragmatic function by ultrasonography: Current approach and perspectives. World J Clin Cases [Internet]. 2020 Jun 26;8(12):2408–24. Available from: https://www.wjgnet.com/2307-8960/full/v8/i12/2408.htm
20. Vivier E, Mekontso Dessap A, Dimassi S, Vargas F, Lyazidi A, Thille AW, et al. Diaphragm ultrasonography to estimate the work of breathing during non-invasive ventilation. Intensive Care Med [Internet]. 2012 May 5;38(5):796–803. Available from: http://link.springer.com/10.1007/s00134-012-2547-7
21. Currow D, Louw S, McCloud P, Fazekas B, Plummer J, McDonald CF, et al. Regular, sustained-release morphine for chronic breathlessness: a multicentre, double-blind, randomised, placebo-controlled trial. Thorax [Internet]. 2020 Jan;75(1):50–6. Available from: https://thorax.bmj.com/lookup/doi/10.1136/thoraxjnl-2019-213681
22. Malik P, Patel K, Pinto C, Jaiswal R, Tirupathi R, Pillai S, et al. Post-acute COVID-19 syndrome (PCS) and health-related quality of life (HRQoL)—A systematic review and meta-analysis. J Med Virol [Internet]. 2022 Jan 7;94(1):253–62. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jmv.27309
23. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. J Chiropr Med [Internet]. 2016 Jun;15(2):155–63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1556370716000158
24. Chen C, Haupert SR, Zimmermann L, Shi X, Fritsche LG, Mukherjee B. Global Prevalence of Post-Coronavirus Disease 2019 (COVID-19) Condition or Long COVID: A Meta-Analysis and Systematic Review. J Infect Dis [Internet]. 2022 Apr 16; Available from: https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiac136/6569364
25. Cabrera Martimbianco AL, Pacheco RL, Bagattini ÂM, Riera R. Frequency, signs and symptoms, and criteria adopted for long COVID-19: A systematic review. Int J Clin Pract [Internet]. 2021 Oct 2;75(10). Available from: https://onlinelibrary.wiley.com/doi/10.1111/ijcp.14357
26. Michelen M, Manoharan L, Elkheir N, Cheng V, Dagens A, Hastie C, et al. Characterising long COVID: a living systematic review. BMJ Glob Heal [Internet]. 2021 Sep 27;6(9):e005427. Available from: https://gh.bmj.com/lookup/doi/10.1136/bmjgh-2021-005427
27. Østergaard L. SARS CoV-2 related microvascular damage and symptoms during and after COVID-19: Consequences of capillary transit-time changes, tissue hypoxia and inflammation. Physiol Rep [Internet]. 2021 Feb;9(3). Available from: https://onlinelibrary.wiley.com/doi/10.14814/phy2.14726
28. Doykov I, Hällqvist J, Gilmour KC, Grandjean L, Mills K, Heywood WE. 'The long tail of Covid-19' - The detection of a prolonged inflammatory response after a SARS-CoV-2 infection in asymptomatic and mildly affected patients. F1000Research [Internet]. 2021 Jan 8;9:1349. Available from: https://f1000research.com/articles/9-1349/v2
29. Torres-Castro R, Vasconcello-Castillo L, Alsina-Restoy X, Solis-Navarro L, Burgos F, Puppo H, et

al. Respiratory function in patients post-infection by COVID-19: a systematic review and meta-analysis. Pulmonology [Internet]. 2021 Jul;27(4):328–37. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2531043720302452
30. O'Shea SD, Taylor NF, Paratz JD. Measuring Muscle Strength for People With Chronic Obstructive Pulmonary Disease: Retest Reliability of Hand-Held Dynamometry. Arch Phys Med Rehabil [Internet]. 2007 Jan;88(1):32–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0003999306013700
31. Lombardi F, Calabrese A, Iovene B, Pierandrei C, Lerede M, Varone F, et al. Residual respiratory impairment after COVID-19 pneumonia. BMC Pulm Med [Internet]. 2021 Dec 17;21(1):241. Available from: https://bmcpulmmed.biomedcentral.com/articles/10.1186/s12890-021-01594-4
32. McCool FD, Manzoor K, Minami T. Disorders of the Diaphragm. Clin Chest Med [Internet]. 2018 Jun;39(2):345–60. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0272523118300121
33. Koralnik IJ, Tyler KL. <scp>COVID</scp> -19: A Global Threat to the Nervous System. Ann Neurol [Internet]. 2020 Jul 23;88(1):1–11. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ana.25807
34. Keyhanian K, Umeton RP, Mohit B, Davoudi V, Hajighasemi F, Ghasemi M. SARS-CoV-2 and nervous system: From pathogenesis to clinical manifestation. J Neuroimmunol [Internet]. 2021 Jan;350:577436. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165572820306974
35. OROZCO-LEVI M, LLORETA J, MINGUELLA J, SERRANO S, BROQUETAS JM, GEA J. Injury of the Human Diaphragm Associated with Exertion and Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med [Internet]. 2001 Nov;164(9):1734–9. Available from: http://www.atsjournals.org/doi/abs/10.1164/ajrccm.164.9.2011150
36. Liu X, Liu X, Xu Y, Xu Z, Huang Y, Chen S, et al. Ventilatory Ratio in Hypercapnic Mechanically Ventilated Patients with COVID-19–associated Acute Respiratory Distress Syndrome. Am J Respir Crit Care Med [Internet]. 2020 May 15;201(10):1297–9. Available from: https://www.atsjournals.org/doi/10.1164/rccm.202002-0373LE
37. Ziehr DR, Alladina J, Petri CR, Maley JH, Moskowitz A, Medoff BD, et al. Respiratory Pathophysiology of Mechanically Ventilated Patients with COVID-19: A Cohort Study. Am J Respir Crit Care Med [Internet]. 2020 Jun 15;201(12):1560–4. Available from: https://www.atsjournals.org/doi/10.1164/rccm.202004-1163LE
38. Hooijman PE, Beishuizen A, Witt CC, de Waard MC, Girbes ARJ, Spoelstra-de Man AME, et al. Diaphragm Muscle Fiber Weakness and Ubiquitin–Proteasome Activation in Critically Ill Patients. Am J Respir Crit Care Med [Internet]. 2015 May 15;191(10):1126–38. Available from: http://www.atsjournals.org/doi/10.1164/rccm.201412-2214OC
39. Marini JJ, Gattinoni L. Management of COVID-19 Respiratory Distress. JAMA [Internet]. 2020 Jun 9;323(22):2329. Available from: https://jamanetwork.com/journals/jama/fullarticle/2765302
40. Boussuges A, Rives S, Finance J, Chaumet G, Vallée N, Risso J-J, et al. Ultrasound Assessment of Diaphragm Thickness and Thickening: Reference Values and Limits of Normality When in a Seated Position. Front Med [Internet]. 2021 Oct 27;8. Available from: https://www.frontiersin.org/articles/10.3389/fmed.2021.742703/full

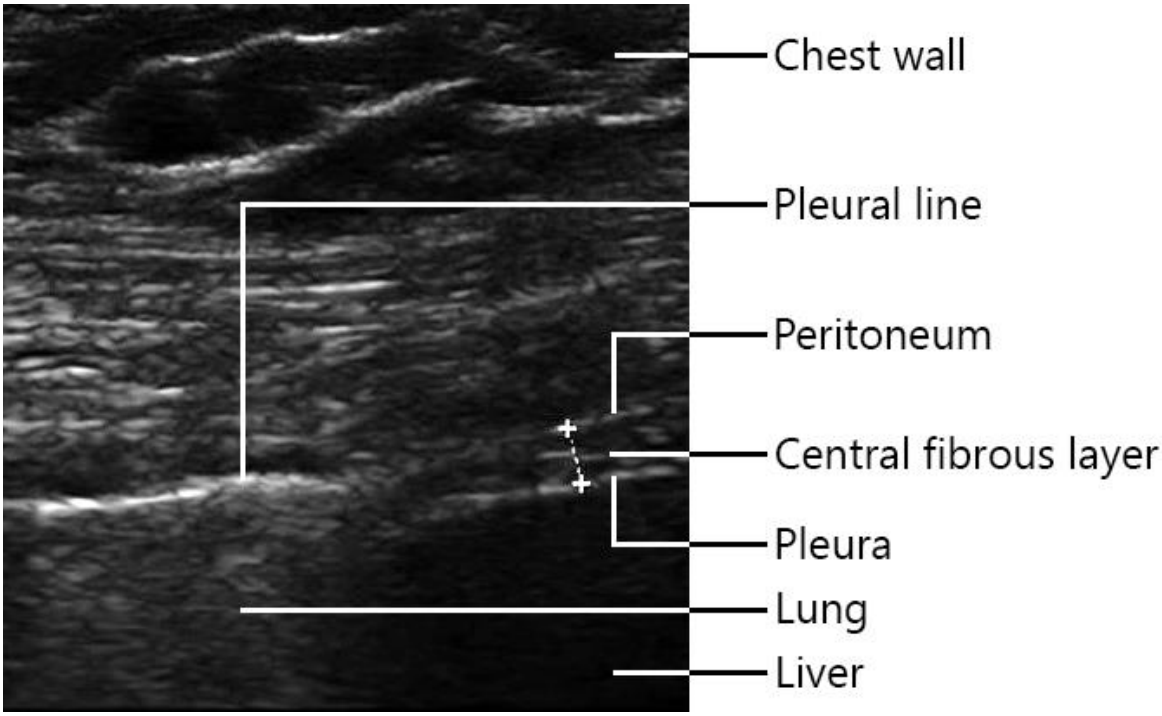

Figure 1: Ultrasound image of the diaphragm at the zone of apposition.

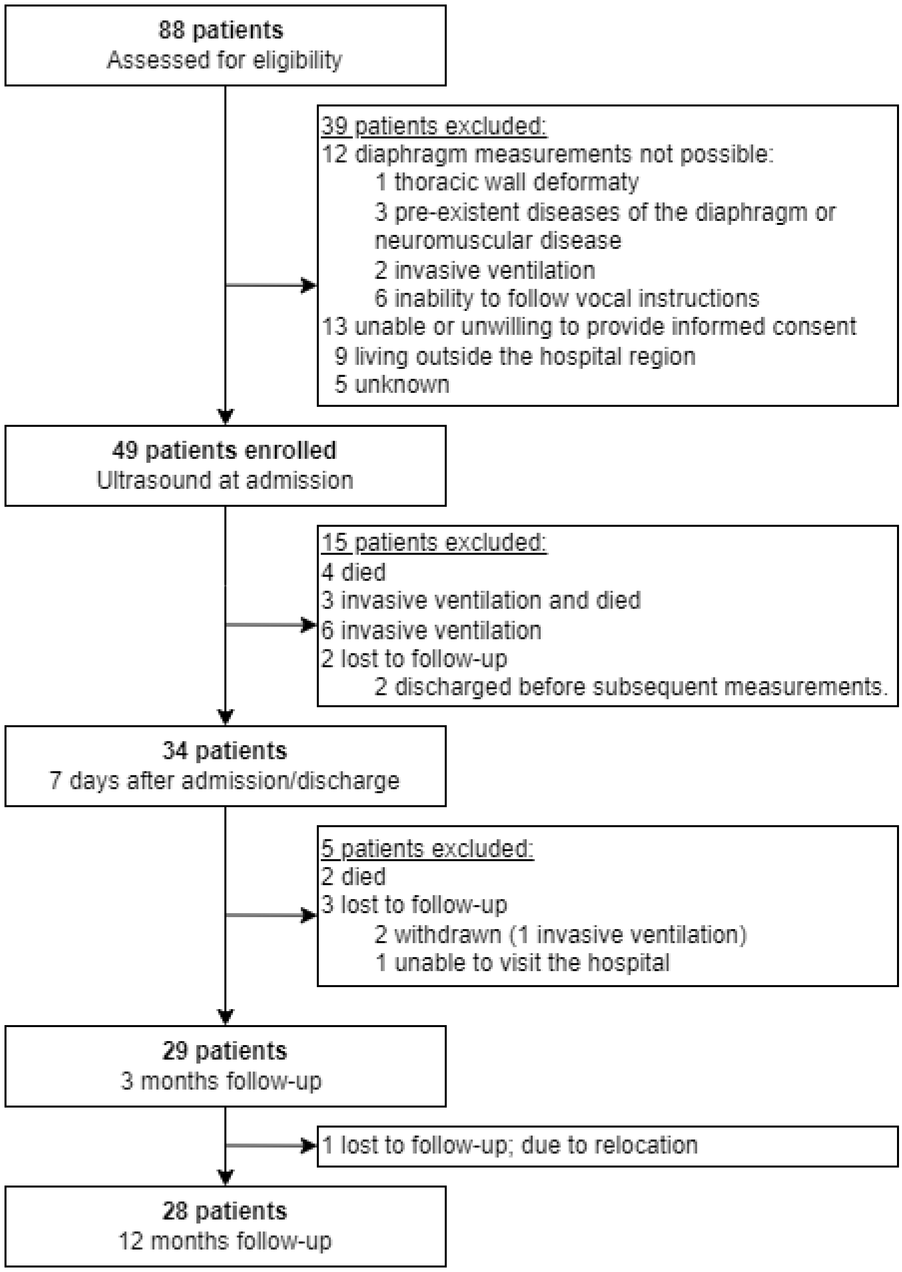

Figure 2: Consort flow diagram.

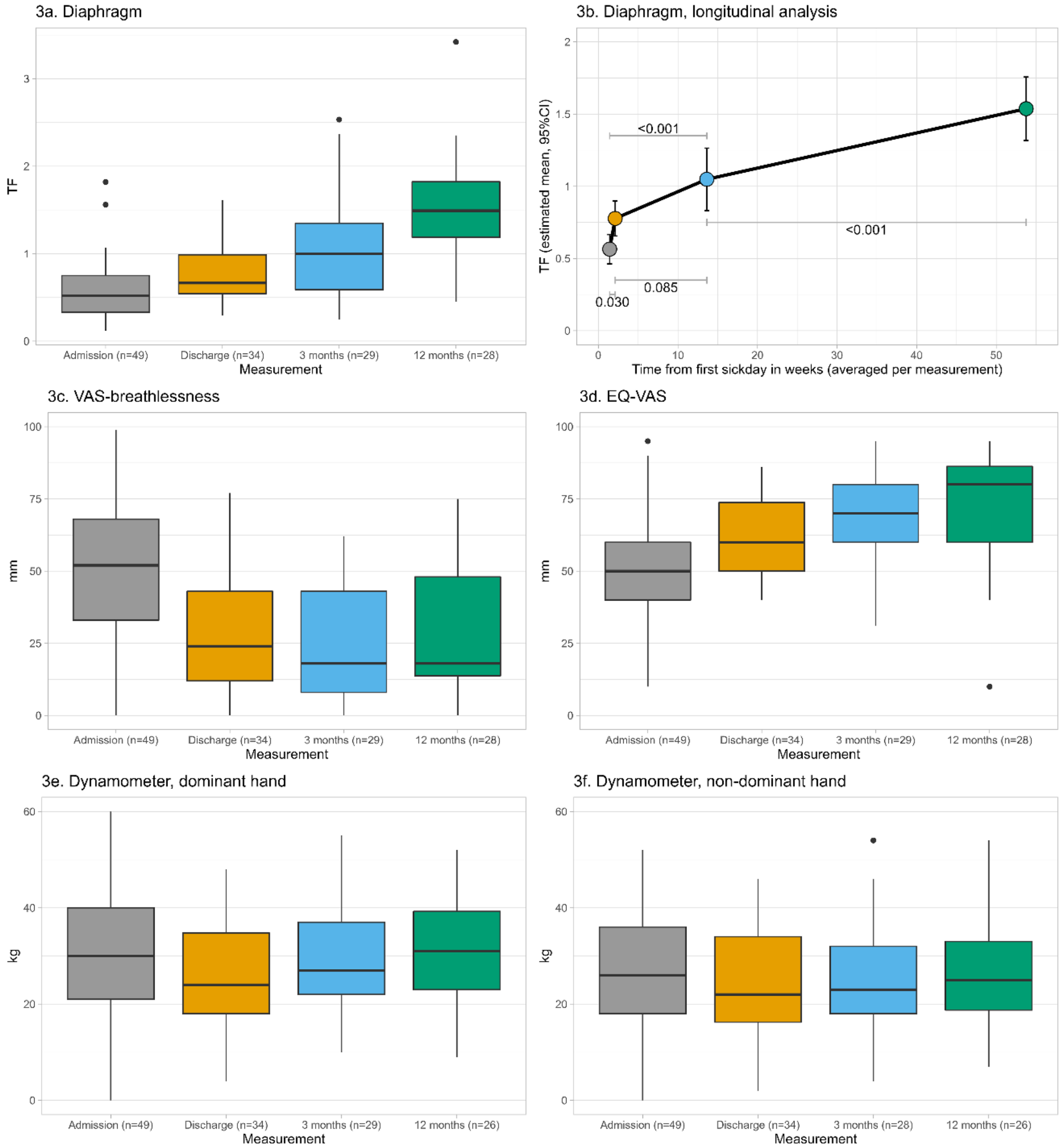

Figure 3: a. Median TF and minimum-maximum range at admission, at discharge or 7 days after admission, three months after admission and twelve months after admission.

b. TF since first sick day in weeks. Data are estimated means with 95% confidence intervals, obtained from linear mixed modeling.

c. Median VAS-breathlessness at admission, day of discharge or seven days after admission, three months after admission and twelve months after admission.

d. Median EQ-VAS at admission, day of discharge or seven days after admission, three months after admission and twelve months after admission.

e. Median measurements of hand dynamometry dominant hand at admission, day of discharge or seven days after admission, three months after admission and twelve months after admission.

f. Median measurements of hand dynamometry non-dominant hand at admission, day of discharge or seven days after admission, three months after admission and twelve months after admission.

Supplemental File

Table S1. Patient characteristics over time.

Characteristic	**Admission**	**Discharge**	**3 months**	**12 months**
Number of patients	49	34	29	28
Median age in years (range)	62 (37-85)	61.5 (43-85)	61 (43-85)	61,5 (43-85)
Female (%)	12 (25)	12 (35)	10 (35)	9 (32)
Median BMI kg/m2 (range)	28 (21 - 48)	27 (21-48)	27 (21-48)	27 (21-38)
Use of steroids during admission (%)	49 (100)	34 (100)	29 (100)	28 (100)
Smoking - Never (%) - Current (%) - Former (%)	 19 (39) 1 (2) 29 (59)	 15 (44) 1 (3) 18 (53)	 14 (48) 1 (3) 14 (48)	 13 (46) 1 (4) 14 (50)
Comorbidities - Diabetes mellitus (%) - Hypertension (%) - Cardio-vascular (%) - Chronic lung disease (asthma/COPD) (%) - Malignancy (%)	 9 (18) 23 (47) 5 (10) 7 (14) 2 (4)	 8 (24) 17 (50) 5 (15) 6 (18) 2 (6)	 6 (21) 15 (52) 3 (9) 4 (14) 1 (3)	 6 (21) 15 (54) 3 (11) 4 (14) 1 (4)
Days of prehospital illness (median, range)	8 (1-28)	8 (1-28)	9 (1-28)	9,5(1-28)
Days to measuring point (median, range)	9 (3-29)	13,5 (8 - 32)	94 (77-117)	377 (339 - 434)
Duration of hospitalization in days (median, range)	7 (2-54)	6 (2 - 37)	5 (2 - 37)	5 (2 - 37)
Clinical characteristics on admission - PaCO2 (kPa) (median, range) - PaO2 (kPa) (median, range) - Respiratory Rate (breaths/min) (median, range) - PaO2/FiO2 ratio (mmHg) (median, range)	 4,2 (2,8 - 6,0) 7,0 (7,0 - 14,3) 23 (14 - 42) 235 (125 - 289)	 4,3 (2,8 - 6,0) 7,0 (5,6 - 9,3) 22 (14 - 33) 246 (145 - 289)	 4,3 (2,8 - 5,9) 7,2 (6,3 - 9,1) 22 (14 - 33) 246 (145 - 289)	 4,3 (2,8 - 5,9) 7,2 (6,3 - 9,1) 22 (14 - 33) 246 (145 - 289)
Maximal received supplemental oxygen during hospitalization (%) - Low-flow nasal cannula	 21 (43)	 19 (56)	 17 (59)	 17 (61)

- Venturi mask (FiO2 0.40-0.60)	4 (8)	4 (12)	4 (14)	4 (14)
- Non-rebreather mask	13 (27)	9 (26)	8 (28)	7 (25)
- High-flow nasal oxygen	1 (2)	1 (3)	0 (0)	0 (0)
- Invasive ventilation	10 (20)	1 (3)	0 (0)	0 (0)

Table S2. Secondary outcomes.

Secondary outcome	Admission vs. discharge	Discharge vs. 3 month follow-up	3 months follow-up vs. 12 months follow-up	Admission vs. 3 months follow-up	Admission vs. 12 months follow-up
VAS breathlessness (median)	52 (0-99) vs. 24 (0-77) **p=0.001**	24 (0-77) vs. 18 (0-62) p=0.270	18 (0-62) vs. 18 (0-75) p=0.477	52 (0-99) vs. 18 (0-62) **P<0.001**	52 (0-99) vs. 18 (0-75) **p=0.013**
Hand held dynamometry (mean in kg)	28 (±13) vs. 25 (±11) p=0.736	25 (±11) vs. 27 (±12) p=0.508	27 (±12) vs. 30 (±13) **p=0.004**	28 (±13) vs. 27 (±12) p=0.489	28 (±13) vs. 30 (±13) **p=0.025**

Table S3. EQ-5D-5L results.

Dimension	Admission n (%)	Discharge n (%)	3 months follow-up n (%)	12 months follow-up n (%)	Admission vs. Discharge *P* value	Admission vs. 3 months follow-up *P* value	Admission vs. 12 months follow-up *P* value
Mobility							
No problems	8(16,7)	12 (35,3)	15(51,7)	16(57,1)	0,120	0,005	0,020
Slight problems	18(37,5)	10(29,4)	8(27,6)	5(17,9)			
Moderate problems	13(27,1)	5(14,7)	5(17,2)	3(10,7)			
Severe problems	7(14,6)	2(14,7)	1(3,4)	4(14,3)			
Unable	2(4,2)	5(14,7)	0(0)	0(0)			
Self-care							
No problems	12(24,5)	12(35,3)	26(89,7)	27(96,4)	0,102	<0,001	<0.001
Slight problems	11(46,9)	12(35,3)	3(10,3)	1(3,6)			
Moderate problems	16(32,7)	8(23,5)	0(0)	0(0)			
Severe problems	6(12,2)	0(0)	0(0)	0(0)			
Unable	4(8,2)	2(5,9)	0(0)	0(0)			
Usual activities							
No problems	8(16,3)	7(20,6)	12(41,4)	13(46,4)	0,024	<0,001	<0,001
Slight problems	4(24,5)	6(17,6)	6(20,7)	7(25,0)			
Moderate problems	9(18,4)	10(29,4)	11(37,9)	6(21,4)			
Severe problems	6(12,2)	1(2,9)	0(0)	2(7,1)			
Unable	22(44,9)	10(29,4)	0(0)	0(0)			
Pain/discomfort							
No problems	21(42,9)	19(55,9)	17(58,6)	10(35,7)	0,005	0,030	0,816

Slight problems	9(18,4)	10(29,4)	6(20,7)	10(35,7)			
Moderate problems	17(34,7)	5(14,7)	6(20,7)	7(25,0)			
Severe problems	2(4,1)	0(0)	0(0)	1(3,6)			
Extreme problems	0(0)	0(0)	0(0)	0(0)			
Anxiety/depression							
No problems	30(61,2)	25(73,5)	23(79,3)	21(75,0)	0,193	0,088	0,308
Slight problems	7(14,3)	6(17,6)	5(17,2)	5(17,9)			
Moderate problems	8(16,3)	1(2,9)	1(3,4)	2(7,1)			
Severe problems	2(4,1)	1(2,9)	0(0)	0(0)			
Extreme	1(2,0)	1(2,9)	0(0)	0(0)			
EQ VAS	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)			
	49,0(17,7)	61,5(13,5)	70,9(13,3)	72,5(19,8)	0.001	<0,001	<0,001

Figure S1. Linear mixed model analysis of estimated means with changes in TF from baseline (grey) in percentage at discharge or 7 days after admission (yellow), three months after admission (blue) and twelve months after admission (green).

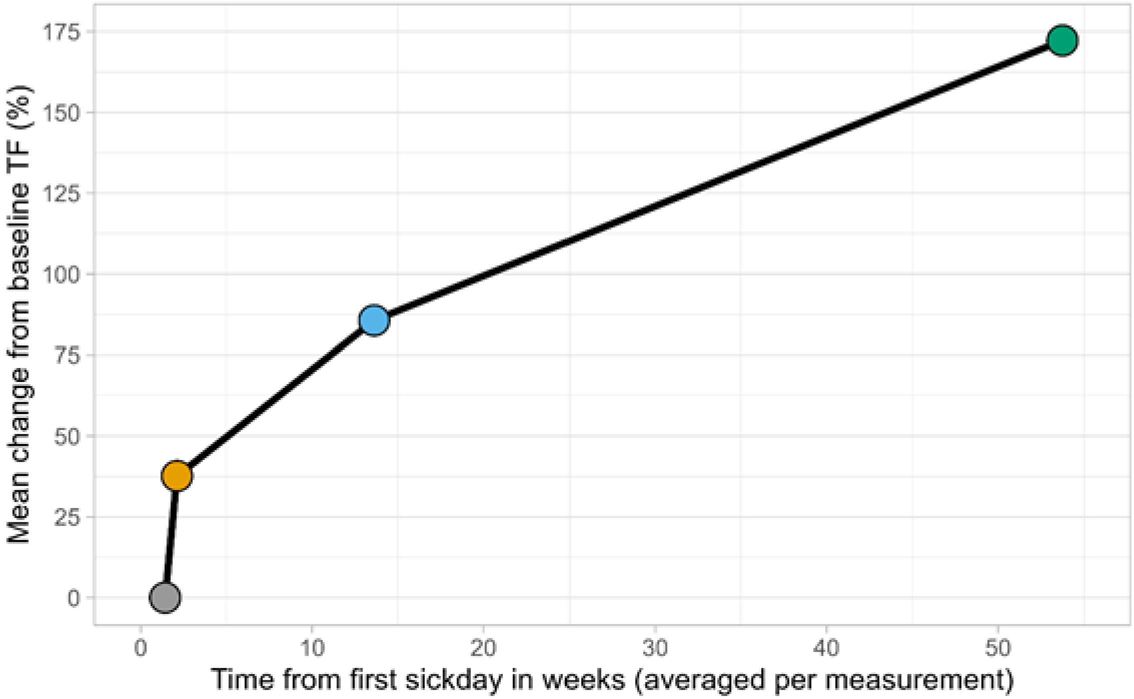

Figure S2. Association between baseline TF (on admission) and change in TF from admission to twelve months of follow-up with curve fitting based on geometric smoothing method.