UNIVERSITA' DEGLI STUDI DI PARMA

DOTTORATO DI RICERCA IN

SCIENZE MEDICHE E CHIRURGICHE TRASLAZIONALI

CICLO XXXVI

The Functional Status of Patients with Post COVID-19: from Resting and Exertional Assessment to Therapeutic Approach

Coordinatore:

Chiar.mo Prof. Nicola Sverzellati

Tutore:

Chiar.mo Prof. Alfredo Antonio Chetta

Dottoranda:

Dott.ssa Annalisa Frizzelli

Anni Accademici 2020/2021 – 2022/2023

Contents

Abbreviations

6MWT	six minutes walking test
ACBT	Active Cycle of Breathing Techniques
ACE	angiotensin converting enzyme
ACE-2	angiotensin converting enzyme II
ARDS	acute respiratory distress syndrome
AT	anaerobic threshold
AUC	area under ROC curve
AX	integrated area of low-frequency reactance
BP	blood pressure
CCI	Charlson Comorbidity Index
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
COSeSco	COVID-19 Sequelae Score
COVID-19	coronavirus disease 2019
CPET	cardiopulmonary exercising test
CRP	C-reactive protein
CT	computed tomography
DB	dysfunctional breathing
DLCO	diffusing capacity of the lung for carbon monoxide
ET	exercise training
ETR	exercise training rehabilitation
FEF_{25-75}	forced mid-expiratory flow between 25 and 75% of forced vital capacity
FEF_{50}	maximum expiratory flow at 50% of forced vital capacity
FEF_{75}	maximum expiratory flow at 75% of forced vital capacity
FET	forced expiratory technique
FEV_1	forced expiratory volume in 1 second
FEV_3	forced expiratory volume in three seconds
FEV_6	forced expiratory volume in six seconds
FVC	forced vital capacity
HRCT	High-resolution Computed Tomography
HRR	heart rate recovery at maximal exercise
HS	healthy subjects
IC	inspiratory capacity
ICU	Intensive Care Unit
IFN-gamma	interferon-gamma
IL-6	interleukin-6
ILD	interstitial lung disease
IMT	inspiratory muscle training
IOS	impulse oscillometry system
IQR	1^{st} and 3^{rd} quartiles
K-BILD	King's Brief Interstitial Lung Disease
KCO	Krogh constant
LC	Long-COVID
LCI	Lung clearance index
LCQ	Leicester Cough Questionnaire
MDT	multidisciplinary team
MERS	Middle East respiratory syndrome

OUES	oxygen uptake efficiency slope
PA	physical activity
PCFS	Post COVID-19 functional status
PEmax	maximum expiratory pressure
$PETCO_2$	pressure of carbon dioxide
PImax	maximum inspiratory pressure
POTS	postural orthostatic tachycardia syndrome
PR	pulmonary rehabilitation
QoL	quality of life
r	Pearson r coefficient
R5	airway resistance at 5Hz
R20	airway resistance at 20Hz
R5-R20	difference between the resistance at 5Hz versus the resistance at 20Hz
RMT	inspiratory/expiratory (respiratory) muscles
ROC	Receiver Operating Characteristic
RR	respiratory rate
SAD	small airway disease
SARS	Severe acute respiratory syndrome
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
SpO_2	peripheral oxyhaemoglobin saturation
SPPB	Short Physical Performance Battery
T_e	expiratory time
T_i	inspiratory time
T_I/T_{TOT}	fractional inspiratory time of the duty cycle
TLC	total lung capacity
TNF-alpha	tumour necrosis factor-alpha
V_1	baseline visit
V_2	visit at follow-up
VA	alveolar volume
VAS	visual analogue scale
VE	minute ventilation
$VE/\dot{V}CO_2$	ventilatory efficiency
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_2$/HR	oxygen pulse
$\dot{V}O_2$/work rate	metabolic efficiency
V_T	tidal volume
V_T/T_I	mean inspiratory flow
Watts	workload
WHO	World Health Organization
X5	Reactance at 5Hz
$\Delta PETCO_2$	difference between $PETCO_2$ peak and $PETCO_2$ rest
ρ	Spearman rho coefficient

Abstract

Introduction

During the first 3 years of the pandemic coronavirus disease 2019 (COVID-19), survivors experienced different clinical recovery and long-term impact on lung, related to residual respiratory symptoms with functional impairment, since in its initial common presentation the disease summarized features of interstitial viral pneumonia.

Clinicians were primarily focused on post-acute lung function assessment to better describe the pathological mechanisms of lung *sequelae* and to manage appropriately post COVID-19 condition. The first part of the research project is aimed to evaluate the lung clinical and functional impact of pulmonary disease.

After describing the common gas exchanges alterations and ventilation inhomogeneity as related to higher lung damage on acute phase, rather than restrictive dysfunctional defects, for which pulmonary function tests appeared insufficient to clearly explain prolonged breathlessness after COVID-19, our research interest has been directed to unexplained long-lasting dyspnoea and fatigue on exertion in Long-COVID (LC) syndrome. In this context, cardiopulmonary exercising test (CPET) could comprehensively and objectively assess the disorders of breathing pattern in dyspnoeic LC population with normal pulmonary function by means of T_I/T_{TOT} (*fractional inspiratory time of the duty cycle*) and V_T/T_I (*mean inspiratory flow*) at rest and during maximal exercise compared to healthy subjects. Finally, in terms of ventilatory abnormalities of CPET deconditioning with higher diaphragm intolerance and lower efficiency, our research aimed to pulmonary rehabilitation (PR) as a means to improve uncomfortable dyspnoea and fatigue due to a higher diaphragm intolerance and lower efficiency by means of Active Cycle of Breathing Techniques (ACBT) combined with a moderate daily physical activity (PA). Moreover, in our research we evaluated if respiratory impairments could be related to small airways dysfunction and could be improved after rehabilitation protocol.

Materials and Methods

Firstly, we analysed the clinical parameters of post COVID-19 outpatient participants from University Hospital of Parma (Italy) that significantly correlated with radiological (HRCT) score in the acute phase, clustering the population study into two significant different groups according to normal/abnormal lung function at follow-up. Subsequently, we identified the independent variable that best significantly discriminated subjects with radiological residual features at follow-up. Finally, we elaborated a predictive score (COSeSco- COVID-19 Sequelae Score) to identify individuals at higher risk of pulmonary sequelae at HRCT at follow-up.

During the second part of our research, by means of CPET, we analysed the pattern of breathing by recording T_I/T_{TOT} and V_T/T_I at baseline and on exercise in a LC population complained of unexplained dyspnoea lasting at least 3 months after SARS-CoV-2 infection, from outpatient clinic of University Hospital of Parma (Italy) and the Hospital of Piacenza, Italy. As control group, we recruited routine outpatient clinic healthy, age, sex and BMI-matched subjects (HS).
Lastly, in the third part of 3-year research project, we assessed the benefits of a 6-week independent home respiratory rehabilitation programme, consisted of an ACBT 10-min session twice a day and at least 6000 steps per day in LC patients from University Hospital of Parma (Italy), who complained of dyspnoea, asthenia and cough at least 24 weeks after acute viral infection. The VAS scale, the mMRC dyspnoea and the Leicester Cough Questionnaire (LCQ), were all administered to the patients at baseline and at follow-up, as well as participants performed spirometry, oscillometry (IOS) and multiple-breath nitrogen washout before and after the rehabilitation.

Results

In the first part of our research, we confirmed that most of 121 post COVID-19 patients showed radiological and lung function changes expressed as diffusing capacity of the lung for carbon monoxide (DLCO) and total lung capacity (TLC) approximately 4 months after acute phase. Moreover, by clustering the study population according to the normal/abnormal values of DLCO they showed specific features. Finally, we elaborated the COSeSco score, which was able to significantly discriminate COVID-19 survivors at higher risk of residual HRCT score >10% in the follow-up.
From the second research study, we found that 42 LC patients were characterised by significantly greater values of T_I/T_{TOT} at rest and at the peak of exercise, and lower values in V_T/T_I at peak, compared to 41 HS. Most LC participants with $T_I/T_{TOT} > 0.38$, as cut-off value, showed lower values in oxygen uptake and in maximal workload, associate with ventilatory inefficiency.
Regarding the third part of research project, in which we analysed data from 33 LC patients, it should be noted that the most statistically findings after the PR concerned dyspnoea by mMRC, health status by VAS scale (0-100) and cough by LCQ (p= 0.0032, p= 0.0416, p=0.0001, respectively). Considering peripheral airway function, R5-R20 (measure of distal airway resistance) (0.074±0.097 vs 0.053±0.071 KPa s l^{-1}), AX index (reactance) (0,62 ± 0,88 vs. 0,53 ± 0,59 KPa s l^{-1}), and LCI (8.51 ± 2 vs. 8.39±1,77), all improved after the rehabilitation course, although not statistically (p= 0,1737, p=0,4243, p=0.9056, respectively). At baseline, about one third of study population is characterised by small airway dysfunction (R5-R20 ≥ 0.07 KPa s l^{-1}) and the average baseline score of R5-R20 at baseline was above normal limits and improved after rehabilitation. Contrasting data are obtained in terms of FEF_{25-75} e FEF_{75} (forced expiratory flow at 25% and 75% of the pulmonary volume and at

25%, respectively) showing a functional worsening, while expiratory dynamic lung volumes (FVC and FEV_3) improved at follow-up.

Among different functional parameters (questionnaires, spirometry, IOS or MBNW) mMRC only correlates with R5-R20 (r= 0.4084, p= 0.0183) and AX (r = 0.3953, p = 0.0228).

Conclusion

We confirmed that resting and exertional lung functional consequences after 3 months are related to the lung acute involvement during COVID-19 and patients with higher lung damage during the acute phase had worse lung diffusing capacity on the follow-up as abnormal gases exchange and ventilation heterogeneity.

An impaired breathing pattern at rest and on exercise may explain long-lasting breathlessness related to deconditioning in LC syndrome with normal spirometry, due to a higher diaphragm intolerance and lower efficiency than the reference controls.

Finally, rehabilitation programme including breathing control exercises and a moderate physical activity can improve dyspnoea, healthy status and cough. While the efficacy of non-pharmacologic options is still being investigated, it is likely that LC individuals will be best served by an integrative multidisciplinary approach.

First part – Lung Functional Assessment in Post COVID-19

1.1 Background of the first study: from COVID-19 with Systemic Manifestations to Post COVID-19 with Functional Sequelae

On 11th March 2020, the World Health Organization (WHO) declared a pandemic for COVID-19 (coronavirus disease 2019), the new disease supported by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1], the seventh new coronavirus featuring a severe acute respiratory syndrome [2]. The disease developed more frequently as interstitial viral pneumonia and most patients had a mild form, 14% of infected individuals presented a severe form and 5% had a critical form [3]. Moreover, a percentage ranging from about 15% to 30% has developed acute respiratory distress syndrome (ARDS) [4].

Remarkably, a relevant part of subject affected by pneumonia presented an atypical form of respiratory distress characterized by loss of lung perfusion regulation and hypoxic vasoconstriction, as documented by a mismatch between relatively preserved lung mechanics and the severity of hypoxemia [5].

Radiologically, COVID-19 typical presentations on chest CT (computed tomography) consisted of parenchymal ground glass opacities with round or "crazy paving" pattern, variably associated with lung consolidations in a peripheral, diffuse or lower lung zone distribution [6].

SARS-CoV-2 uses the angiotensin converting enzyme II (ACE-2), as the cellular receptor with homology to angiotensin converting enzyme (ACE) [7] that degrades angiotensin II to its metabolites, including vasodilators angiotensin- (1-9) and angiotensin- (1-7), with a down-regulation of the renin-angiotensin system. ACE-2 is expressed in various tissues such as the upper and lower respiratory tract, cardiac muscle, and gastrointestinal mucosa [8].

Data from many studies have shown that inflammatory indices such as CRP (C-reactive protein), IL-6 (interleukin-6), IFN-gamma (interferon-gamma), and TNF-alpha (tumour necrosis factor-alpha) increase (9-12). In addition, oxidative stress could play a key role in the development of acute respiratory syndrome [13], since the constant expression of significantly high levels of these cytokines is related to increased viral load, lung damage and impaired function, disease progression and poor outcome [14].

At the beginning of pandemic, the most common symptoms included fever, cough, dyspnoea, myalgia and fatigue, while the frequent reported laboratory abnormalities consisted of lymphopenia and elevated liver transaminases, neutrophilia, thrombocytopenia, decreased haemoglobin, decreased albumin, and renal damage [15].

Studies have shown a significant association between troponin levels and poor outcome in patients with COVID-19 [15], associated with a causative hypothesis of myocarditis, due to a direct viral damage on the cardiac muscle or thrombus growing on ruptured coronary plaque, related to intense inflammatory stress on pre-existent coronary artery disease [17].
Patients may be predisposed to thromboembolic disease, with haemostatic abnormalities, such as elevated levels of fibrinogen, fibrinogen degradation products, D-dimer and prolonged activate partial thromboplastin time [18].

The ACE-2 receptor is also expressed on kidney tubular cells, and viral RNA has been found in renal tissue and urine samples. Inflammatory lung changes could damage the kidneys, as tubular epithelial cells injury could provoke severe lung damage [19].

Surprisingly, SARS-CoV-2 has been detected in the stools of some infected individuals [8]. The virus may enter the enterocytes by means of the ACE-2 receptor and the following lower receptor expression could reduce the uptake of dietary tryptophan, resulting then in an intestinal microbiota change, colitis and diarrhoea [20].

Chain and colleagues proposed that the underpinning mechanisms of liver abnormalities in COVID-19 related to bile duct cells dysfunction may involve a severe inflammatory response to infection, direct cytotoxicity due to viral replication in liver cells, pneumonia-related hypoxia, drugs and a possible relapse of previous liver disease [21].

SARS-CoV-2 may enter the central nervous system (CNS) by the olfactory bulb through retrograde neuronal course, or by the hematogenous system, provoking inflammation and subsequent demyelination, provoking central or peripheral symptoms. Severe neurological disease was characterized by acute cerebrovascular events, impaired consciousness and skeletal muscle injury [22]. Conjunctivitis with impaired anterior ocular surface might be related to the viral infection into ocular or nasopharyngeal tissues, since ACE-2 receptor has been identified in the conjunctiva [23].

Concerning skin involvement, an Italian study confirmed erythematous rash, generalized urticaria and chickenpox-like vescicles as cutaneous manifestations in COVID-19 population [24].

Furthermore, most patients did not experience recovery from the acute phase, which is typically understood as complete *restitutio ad integrum* of the lung parenchyma [25].
Zhao et al. confirmed that pulmonary radiological and physiological impairments in patients three months after hospital discharge were predictable by high levels of D-dimer upon admission [26]; the authors documented a slight decrease in both forced vital capacity (FVC) and total lung capacity (TLC) indicating a temporary restrictive impairment during the initial 3-month follow-up with a functional recovery reported after 6 months [26].

Huang and colleagues described during early convalescence phase reduced lung diffusion capacity for carbon monoxide (DLCO), lower TLC, and impaired six minutes walking test (6MWT) in severe cases of infection [27]. Similarly, previous acute respiratory viral infections such as SARS (Severe acute respiratory syndrome) and MERS (Middle East respiratory syndrome) provided long-term sequelae [28].

1.2 Aim of the first study [29]

As it is crucial to evaluate lung function impairments for the detection and treatment of respiratory and functional complications caused by COVID-19, we conducted a study to characterise the respiratory profile of a cohort of survivors.
In this context, we aimed to develop a predictive risk assessment score for pulmonary radiological sequelae at High-resolution Computed Tomography (HRCT) in COVID-19 population beyond the acute phase in a large cohort of COVID-19 survivors in clinical follow-up.

1.3 Materials and Methods of the first study

Participants in the study were adults with radiologically and laboratory previous confirmed diagnosis of COVID-19-related pneumonia, who subsequently referred to outpatient clinic follow-up of "Respiratory Disease and Lung Function Unit" at University Hospital of Parma (Italy), from May 2020 to February 2021.

Participants anthropometric features, such as age, gender, smoking habit, comorbidities expressed by Charlson Comorbidity Index (CCI) [30], HRCT score of pneumonia (0–100%) [31], blood laboratory profile, and treatment, were all collected at the time of hospitalisation.
Dyspnoea (assessed with the modified Medical Research Council - mMRC) [32], pulmonary function tests at rest, exercise capacity by means of the 6MWT [32], and HRCT score were considered at a follow-up visit (approximately 4 months after hospital admission).

1.4 Study Design and Statistical Analysis of the first study

With a 2-tailed Pearson correlation, we analysed the parameters that significantly correlated with the hospital HRCT score, and then using the two functional variables with the best correlation, participants could be clustered.
Subsequently, the independent variable that best significantly discriminated subjects with radiological residual features at follow-up was identified. By means of the receiver operating characteristic (ROC) curve to identify specific cut-off, the level of discrimination threshold of functional pulmonary sequelae was then checked for significant variables (dependent variables).
Lastly, these variables were elaborated to define the risk assessment score (COSeSco) to identify individuals at higher risk of pulmonary sequelae at HRCT at follow-up [33]. The statistical analysis was obtained using GraphPad Prism 5 software (San Diego, CA, USA), and the level of statistical significance was $p < 0.05$.

This study was approved by the Hospital Local Ethics Committee (No. 34570, 14th September 2020) and written informed consent was obtained from all participants.

1.5 Results of the first study

It is worth noting that most participants (76 over 121, 63%) had functional abnormality (DLCO and/or TLC <80% pred) at follow-up.
A correlation analysis found that age, BMI, CCI, forced expiratory volume in 1 second (FEV_1), FVC, DLCO, TLC, alveolar volume (VA), 6MWT distance (m), peripheral oxyhaemoglobin saturation (SpO_2) at baseline and as average value during the test, all significantly correlated with the radiological score at hospital admission. DLCO and TLC showed the strongest correlation with the

CT scan score at hospital admission (p= −0.48 and p= −0.39, respectively; both $p < 0.001$) (Table 1) [29].

Table 1: COVID-19 patient characteristics at hospital admission and follow-up [29].

	Patients, *N*	Patients with DLCO and/ or TLC ≥80% at follow-up	Patients with DLCO and/ or TLC <80% at follow-up
Patients, *n* (%)	121 (100)	45 (37)	76 (63)***
Age, years (mean ± SD)	60.43±11.65	54.36±10.16	64.03±11.01***
Sex (M/F)	83/48	29/16	44/32
BMI at hospital admission, kg/m^2 (mean ± SD)	28.95±5.57	27.33±4.55	29.43±5.29*
Smoking habit, %			
Never	51.24	60.00	46.05
Former	39.67	33.33	43.20
Current	9.09	6.67	10.53
CCI at hospital admission (median, minimum, 25–75 percentile, maximum)	2 (0, 1, 4, 9)	1 (0, 0, 3, 5)	3 (0, 2, 5, 9)***
HRCT at hospital admission, score % (mean ± SD)	41.30±23.02	26.21±16.30	49.21±22.15***
Therapy during hospitalization, %			
Systemic corticosteroids	32.29	20.69	37.31
Heparin	75.00	74.63	75.86
Antivirals	67.71	65.52	68.66
Antibiotics	92.71	82.76	98.51**
Oxygen only	62.89	72.41	59.70
Noninvasive ventilation	22.68	10.34	28.36*
Invasive mechanical ventilation	7.22	6.90	7.46
D-dimer during hospitalization, ng/mL (mean ± SD)	1,396±1,824	643±304	1,749±1,117*
Time between post-hospital admission and follow-up, days (mean ± SD)	115±43	111±47	117±41
Time between post-discharge and follow-up, days (mean ± SD)	100±43	105±47	97±42
HRCT at follow-up, score % (mean ± SD)	7.74±12.72	3.51±7.35	11.22±15.04**
mMRC at follow-up (median, minimum, 25–75 percentile, maximum)	1 (0, 0, 1, 3)	1 (0, 0, 1, 2)	1 (0, 0, 1, 3)*
Lung function test at follow-up (mean ± SD)			
FEV_1, % predicted	100.10±19.75	110.77±16.76	93.54±18.64***
FVC, % predicted	103.10±20.11	113.75±17.37	96.62±18.96***
FEV_1/FVC	0.79±0.08	0.80±0.05	0.78±0.10
TLC, % predicted	94.87±15.84	103.70±11.95	89.19±15.48***
DLCO, %	76.84±15.34	92.04±9.19	67.46±9.84***
KCO, %	91.45±16.20	96.90±11.31	88.09±17.85**
VA, %	86.77±15.18	98.11±10.60	79.90±13.32***
6MWT at follow-up (mean ± SD)			
Walking distance, m	404.50±77.11	435.70±75.02	387.10±72.88***
Walking distance, % of predicted	103.90±17.10	108.50±23.70	101.10±10.52
SpO_2 at baseline, % (mean ± SD)	95.69±1.57	95.93±1.25	95.55±1.73
SpO_2 mean, % (mean ± SD)	94.18±2.56	95.11±1.43	93.63±2.91**

6MWT, 6-Minute Walk Test; BMI, body mass index; COVID-19, coronavirus disease 2019; CCI, Charlson Comorbidity Index; DLCO, lung diffusion capacity for carbon monoxide; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HRCT, high-resolution computed tomography; KCO, DLCO/VA; mMRC, modified Medical Research Council; *n*, number; m, meters; SD, standard deviation; SpO_2, peripheral oxygen saturation; TLC, total lung capacity; VA, alveolar volume. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ between patients with and without lung function impairment.

Considering normal/abnormal values (≥/<80%) of DLCO and/or TLC, study population was subdivided into the two groups characterized by significantly different HRCT scores at admission and follow-up, age, BMI, CCI, 6MWT distance, mMRC and D-dimer outcomes.

Among functional variables, further significant differences were found for FEV_1, FVC, KCO (Krogh constant), VA and mean SpO_2 during the 6MWT at follow-up.

The linear analysis of DLCO over the HRCT score at hospital admission and follow-up also reported significant AUC (0.89, 95% CI: 0.85–0.94, and 0.98, 95% CI: 0.97–0.99, respectively, both $p < 0.001$).

Anormal DLCO (<80% pred) significantly categorised the study sample according to the presence or absence of lung sequelae by HRCT score at follow-up approximately 4 months after hospital admission (>10% and ≤10%, respectively) (Fig.1) [29]. On the other hand, a lower TLC (<80% predicted) did not successfully discriminate between participants with and without pulmonary sequelae at follow-up, as assessed by the HRCT score (Fig.2) [29].

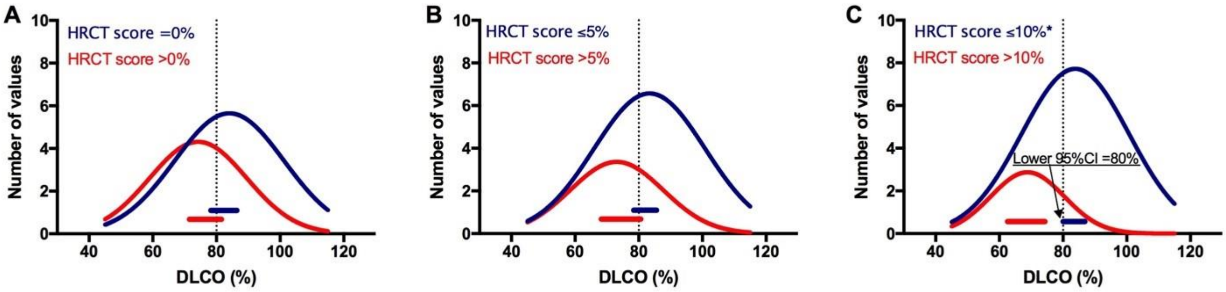


Figure 1. Anormal DLCO (Lung Diffusion Capacity for Carbon monoxide) (<80% predicted) significantly categorised the study sample according to the presence or absence of lung sequelae by HRCT score at follow-up (>10% and ≤10%, respectively) [29].

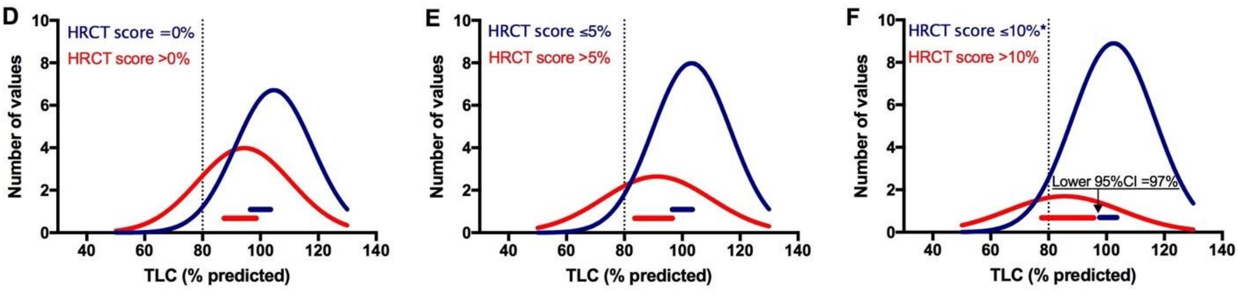


Figure 2. Abnormal TLC (total lung capacity) (<80% predicted) did not successfully discriminate between participants with and without pulmonary sequelae at follow-up, as assessed by the HRCT score [29].

The ROC analysis defined the specific characteristics of COVID-19 individuals with functional sequelae 4 months after admission, such as DLCO <80% (independent variable) and, accordingly, by radiologic sequelae (HRCT score >10%).

Specific cut-off of each dependent variable by ROC analysis were found. We confirmed that these patients were ≥65 years with a BMI ≥25 kg/m^2, or CCI ≥4, or 6MWT (% pred) <100%, or mMRC ≥2, or D-dimer ≥500 ng/mL [29]. Specific scores were then assigned to each variable cut-off according to the level of sensitivity and specificity. Subsequently, the indices were pooled to elaborate the COSeSco model, in which the sum of the scores resulted in COSeSco values from 0 to 15.
COSeSco was significantly ($p < 0.001$) effective by ROC analysis in differentiating subjects in terms of radiologic sequelae (HRCT score >10%) at follow-up. The best model with the greatest overall sensitivity and specificity is that for COSeSco values of 2 [29].

1.6 Discussion of the first study

In this research, clinical and radiological characteristics of a total of 121 participants with a previous COVID-19-related pneumonia were assessed, and it confirmed that most of them (63%), about 4 months after acute phase, showed abnormal diffusing lung capacity and total lung capacity in an overall mean CT score of 11.22%, in line with other reports [27].
Data confirmed that a variable percentage of SARS and MERS survivors (from 20% to 60%) showed persistent function impairments and radiological signs of pulmonary fibrosis [34-36].

In a previous study, Zhao and colleagues reported that survivors affected by respiratory functional impairments after three months from discharge were those with increased inflammation or coagulopathy in the acute phase, but they were not significantly different in age in comparison with patients with abnormal lung function, while survivors characterized by radiological sequelae at the follow-up were significantly older when compared to patients without residual radiological changes [26]. The authors also reported a 16% prevalence of decreased DLCO when lung function assessment was performed three months after discharge from hospital [26]. Different studies described a higher prevalence (between 44% and 56%), performing pulmonary function test during the first month post-infection [27,37,38].
In our research, we further confirmed the significant relationship between age and lung DLCO and HRCT score.

As reported, several different pathogenetic mechanisms underpinned lung injury in COVID-19 such as alveolar and vascular injury, hyaline membrane proliferation, alveolar septal fibrous progression, and pulmonary consolidation. These acute changes all provided a possible risk of lung fibrosis and/or pulmonary hypertension. In this context, it was necessary to conduct a follow-up of the survivors after their discharge [37].

The British Thoracic Society in 2020 recommended monitoring survivors with severe pneumonia through lung function tests 12 weeks after discharge, while for mild/moderate pneumonia patients have their lung function assessed if chest X-rays reveal abnormalities. Detected alterations in lung function with accompanying radiological CT abnormality should prompt referral to a specialist in interstitial lung disease [39].

The detection of abnormal lung function (mainly due to a reduction in the DLCO values) after discharge is reported in most follow-up studies [40,41].

In a systematic review Torres-Castro and co-workers found that impaired diffusion lung capacity, restrictive pattern and obstructive pattern were found in 39%, 15% and 7% of patients, respectively. In severe patients, Torres-Castro and colleagues described a very high prevalence of altered diffusion capacity (66%), especially those with high inflammatory indices [40].

Qin and colleagues conducted a prospective study to detect the main functional and radiological changes during 3-month follow-up. In this research, the authors found a statistically significant difference for diffuse lung capacity among patients with severe pneumonia compared to non-severe pneumonia group. Moreover, another interesting finding was that survivors with decreased DLCO were more likely to have interstitial changes [41].

Regarding exercise tolerance, Huang et al. reported a greater walking distance decline by six-min walk test (in terms of meters and %pred) during the follow-up visits in severe patients compared with non-severe ones. The severe group also showed a higher incidence of DLCO impairment, thus confirming the significant relationship between DLCO and 6MWT [27].

In our research study, we confirmed that individuals with lower DLCO (<80% pred) at a follow-up had a high likelihood of having a walking distance <100%pred [29].

In addition, we described that this group of patients had a significant reduction in the KCO value (CO transfer coefficient), indicating damage to the alveolar-capillary barrier.

Surprisingly, in our study, TLC was not significantly effective in differentiating participants with and without pulmonary sequelae at follow-up based on radiological score. It can be assumed that pneumonia induced by SARS-CoV-2 infection, is more likely to lead a change in ga exchange and a tendency towards ventilatory inhomogeneity, as expressed by a reduction in VA, than by a restrictive functional defect (TLC <80%pred) [29].

1.7 Conclusion of the first study

The risk score presented a significant accuracy to predict the detection of functional and radiological sequelae in hospitalised COVID-19-infected individuals. The 6 dependent variables - that characterise patients with the functional sequelae required to calculate the risk of developing critical illness - are easily accessible.

However, this research has some limitations, such as the absence of previous baseline pulmonary function and lack of a control group, that it needs to be validated in an external population.

In addition, we could not stratify the risk of predicted sequelae into low, intermediate, and high risk.

Finally, further multicentre studies could confirm our results, since it is a single centre [29].

1.8 Conclusion in Progress for the Next Step

- Resting and exertional lung functional consequences after 3 months are related to the lung acute involvement during COVID-19.

- Patients with higher lung damage on admission had worse diffusing lung capacity on the follow-up as «abnormal gases exchange» and «ventilation heterogeneity»

- Focusing on «Long-Covid Syndrome» by Cardiopulmonary exercising test in order to describe *ventilation abnormalities* regardless of spirometry.

Second part: Breathing Pattern by Cardiopulmonary Exercising Test in Long-COVID Population with Unexplained Dyspnoea

2.1 Background: Long-COVID Syndrome

The impact of COVID-19 so far has been unprecedented and the long-term signs and symptoms had a further social devastating effect.
Some evidence showed that a variable spectrum of symptoms could remain after the recovery of the acute infectious phase in who have had COVID-19, and this condition is known as Long-COVID (LC).

The National Institute for Health and Care Excellence (NICE) define Long-COVID as:

- Acute COVID-19: signs and symptoms of COVID-19 for up to 4 weeks.
- Ongoing symptomatic COVID-19: signs and symptoms of COVID-19 from 4 to 12 weeks.
- Post-COVID-19 syndrome: signs and symptoms that develop during or after an infection consistent with COVID-19, continue for more than 12 weeks and are not explained by an alternative diagnosis.

In addition to the clinical case definitions, the term "Long-COVID" is used to describe signs and symptoms that continue or develop after acute COVID-19. It includes both ongoing symptomatic COVID-19 (from four to 12 weeks) and post-COVID-19 syndrome (more than 12 weeks) [42].

The Italian National Institutes of Health (ISS- Istituto Superiore di Sanità), in line with NICE, defines "Long-COVID" as the persistence of certain symptoms beyond four weeks after SARS-CoV-2 infection despite a negative diagnostic test [43].

The Center for Disease Control and Prevention (CDC) rather considers the term "Post-COVID", indicating the presence of symptoms four weeks after acute infection [44].

More recently, the World Health Organization (WHO) defined "Long-COVID" (or "post-covid 19 syndrome") as a condition characterized by residual or "de novo" symptoms over three months from probable or confirmed SARS-CoV-2 infection, lasting for at least two months and for which an alternative cause is excluded. Common symptoms include fatigue, shortness of breath, cognitive dysfunction but also others which generally have an impact on everyday functioning. A different definition may be applicable for children [45].

The prevalence of patients with at least one or more residual or new symptoms after recovery within three months after the acute infection varies from 43% to 62%, regardless of disease severity or the need for hospitalisation. In a recent meta-analysis, involving 194 studies conducted on patients with LC, fatigue and breathlessness are two of the leading reported symptoms [46].

This syndrome gained widespread attention following a report published in BMJOpinion on 5 May 2020 in which a professor of infectious diseases, Paul Garner, shared his experience of a seven-week “rollercoaster of ill health” after COVID-19 [47]. Garner also appeared in a feature [48] that had been read over 1 million times by 10 August [49]. Garner’s report spread internationally, gathering a wider patient community around what he called the "long tail" of Covid. Garner reported patients took his account to medical appointments to provide evidence of the realness of their symptoms [50].

The patient-made term *long-COVID* was then made popular following the rise in the use of #LongCovid on Twitter. This, plus the growing number of peer reviewed articles published since, has highlighted a post-COVID-19 syndrome that can last for many weeks after the acute infection. Long-COVID is now recognized term in scientific literature.

Also, epidemiology of this condition is not clear due to different timing of follow-up, variable survivor population, and variance of self-reporting symptoms.
Many studies have confirmed variable incidence rates for LC considering follow-up examination times after the acute phase, 32.6% at two months [51], 87% at two months [52], and 96% at three months [53].

2.1.1: Sex-related Differences in Long-COVID Syndrome [54]

In one of our studies, that was conducted in “Respiratory Disease and Lung Function Unit” at University Hospital of Parma (Italy) in 2021, we focused on male and female symptoms in a LC population. 223 patients were totally enrolled after a median 23-week follow-up. Overall dyspnoea persisted as the most common symptom, although less frequently than in the acute phase of infection, together with asthenia, palpitations and disturbances in the sleep-wake rhythm.

Considering sex differences, women were more symptomatic than men complaining of dyspnoea, weakness, chest pain, palpitations, and sleep disturbance. On the other side, male patients improved significantly in breathlessness and thoracic pain, without differences in fatigue and palpitations. Conversely, female subjects did not improve in dyspnoea and chest pain, worsening in fatigue and palpitations. Both groups showed a significant improvement in cough and a relevant worsening in terms of sleep disturbance. As for myalgia, this improved significantly in the female participants, while the male survivors described a trend towards improvement.

These results are in agreement with previous studies that confirmed that women had more intense symptoms in the acute phase, while the clinical course and prognosis of the disease were more severe in male individuals [55,56].

Similarly, in our study [54] male group presented a more extensive radiological lung impairment, more inflammatory and D-dimer levels, and a greater need for oxygen supplementation. Drugs did not differ significantly in the two groups, which mainly consisted of antibiotics, hydroxychloroquine, and a prophylactic dose of heparin; male individuals were treated with antivirals slightly more frequently, but they received less steroids [54].

Many reports suggested that differences between female and male groups could be related to sex-linked biological and genetic factors (sex hormones and sex chromosomes) and to gender.

High-risk behaviours or jobs, smoking habit or alcohol consumption, and comorbidities, may be all implicated in gender-related COVID-19 vulnerability. It has been hypothesised that the presence of the double X chromosome and differences in sex hormone profile, oestrogen and testosterone, would protect women, especially in youth, from developing a severe form of the disease [57-60].

Moreover, women showed a stronger innate and adaptive response of the immune system and this could explain their better outcome of COVID-19 during the acute phase [57,61,62]. Women generally develop greater humoral and cell-mediated immune responses to antigenic stimulation, infection, and vaccination; this was confirmed also in SARS-CoV-2 infection.

However, no clear evidence explained why women were more likely to experience long-lasting COVID-19 symptoms.

Both a persistent inflammation or an autoimmune response could lead to chronic infection, possibly triggered by residual virus or other quiescent viruses [63].

The literature suggests that sex hormone profiles and differences in the functioning of the innate and adaptive immune systems could make women more sensitive to pain [64,65].

In addition, due to gender-related social factors, men are more common considered the strong gender of masculinity, whereas women are thought to have a lower pain threshold and less endurance.

2.1.2: Pathophysiological Mechanisms in Long-COVID Syndrome

In 2021 Lopez-Leon and colleagues published a systematic review and a meta-analysis in which totally 55 long-term "effects" of COVID-19 were described two weeks following acute infection including signs, symptoms, and laboratory parameters. Fatigue (58%) was confirmed the most common symptoms of long and acute disease following by headache (44%), attention disturbances (27%), hair loss (25%), and dyspnoea (24%). The study focused on neuropsychiatric symptoms, whose cause could be related to direct infection effect, cerebrovascular disease due to state of hypercoagulability and hypoxia, drugs, and social aspects [66] (Fig.3).

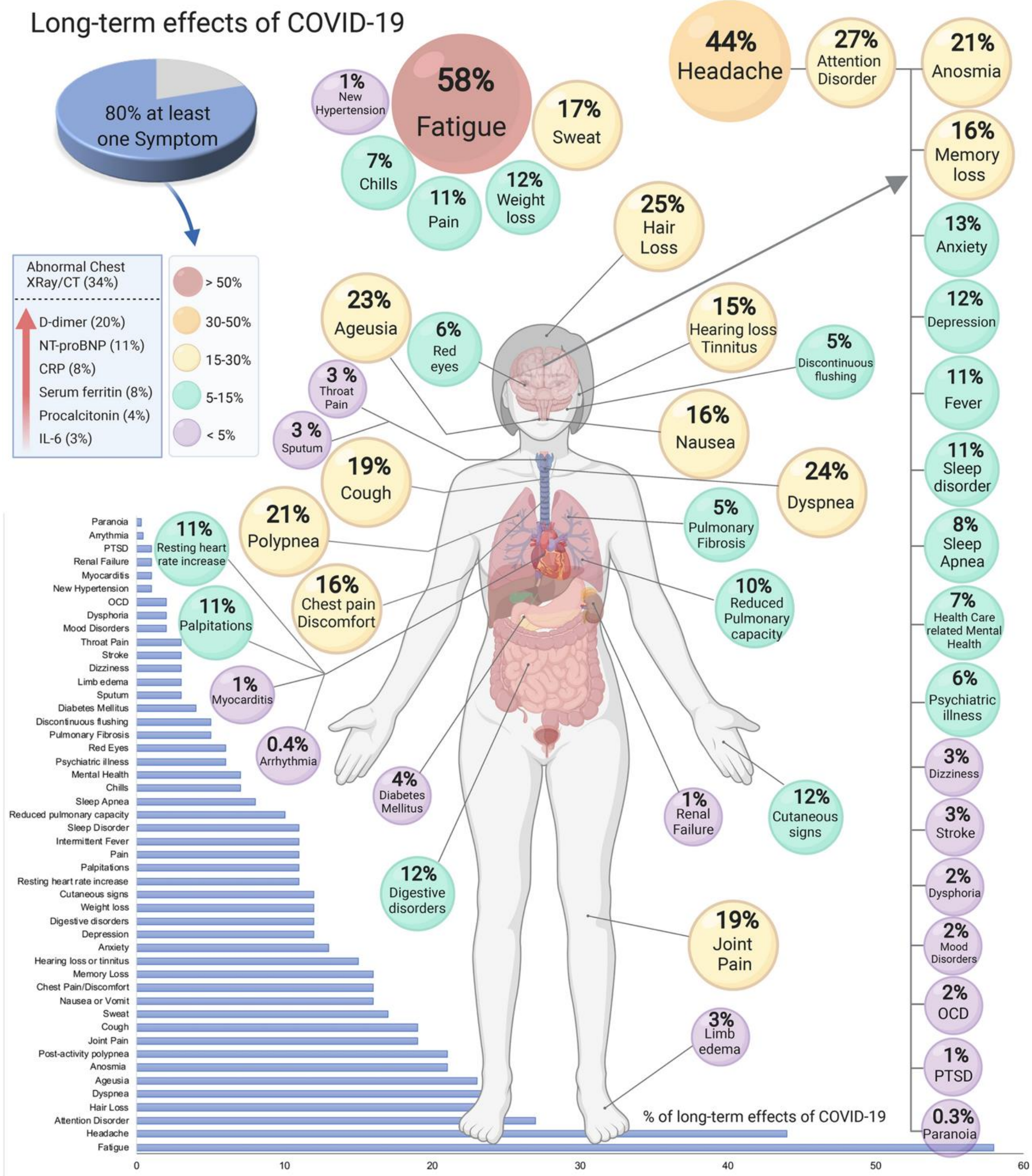


Figure 3. Long-term "effects" of coronavirus disease 2019 (COVID-19). CRP C-reactive protein, CT computed tomography, IL-6 Interleukin-6, NT-proBNP (NT)-pro hormone BNP, OCD Obsessive Compulsive Disorder, PTSD Post-traumatic stress disorder [66].

Regarding pathophysiological mechanisms underpinning LC syndrome, the cited systematic review and meta-analysis considered lung chronic inflammation due to cytokines and reactive oxygen species (ROS).

Also, endothelial damage could provide the activation of fibroblasts and deposition of collagen and fibronectin resulting in fibrotic features.

Finally, the hyperinflammatory and hypercoagulability could be related to endothelial impairment, complement and platelet activation, and platelet-leukocyte interactions, all lead to impairment of coagulant pathways and hypoxemia.

In the CNS, the prolonged and persistent immune response induces glial cells to chronically damage neurons. This state of hypercoagulation increases risk of thrombotic events in the brain. On the other hand, blood-brain barrier injury could induce pathological permeability, allowing blood-derived substances and leukocytes to infiltrate the brain tissue.
Autonomic dysfunction could be the effect of chronic inflammation in the brainstem. It is described that the neurological condition of LC is linked to cognitive dysfunction.

Cardiac sequelae could be secondary to chronic inflammation of cardiomyocytes, myositis and cardiomyocytes death. Dysfunctional autonomic nervous system can explain postural orthostatic tachycardia syndrome (POTS), that includes an increase in heart rate >30 bpm on standing, without a drop in blood pressure (BP), accompanied by breathlessness, palpitations and dizziness on standing (orthostasis).
Persistent cardiac inflammation and cellular injury induce fibroblasts to release extracellular matrix molecules and collagen. Fibrotic features are associated with an increase in cardiac fibromyoblasts, while damage to desmosomal proteins results in reduced cell-to-cell adhesion.
The occurrence of dysautonomia and brain fog suggests neurological contributions to LC, and fatigue, one of the most common symptoms, could also be impacted by neurological dysfunction.
Inflammation is likely to be critical in the pathogenesis of post COVID-19 sequelae. Individuals with LC present elevated inflammatory markers for several months [67].

Concerning the symptom of fatigue, other different mechanisms have been described to better understand the origin.
Chronic inflammation in the brain, as well as the neuromuscular junctions, may result in long term fatigue.
In skeletal muscle, sarcolemma injury and muscle fibre atrophy could be considered, as might a number of psychological and social factors [66].
Regardless the specific molecular trigger, neuromuscular mechanisms might contribute to symptomatic fatigue, given that most common symptoms of post-COVID fatigue relate to physical and cognitive activity, both of which are based on neural circuitry [68].

Regarding vaccination against coronavirus, Taquet et al. in 2022 found that the risk of developing LC was not significantly different between those with and without COVID-19 vaccination. Vaccination did not reduce risk of anxiety/depression, headache, abdominal complains, chest/throat discomfort, abnormal breathing and cognitive symptoms. However, certain symptoms, notably fatigue and myalgia, were less common in the vaccinated population [69] (Fig.4).

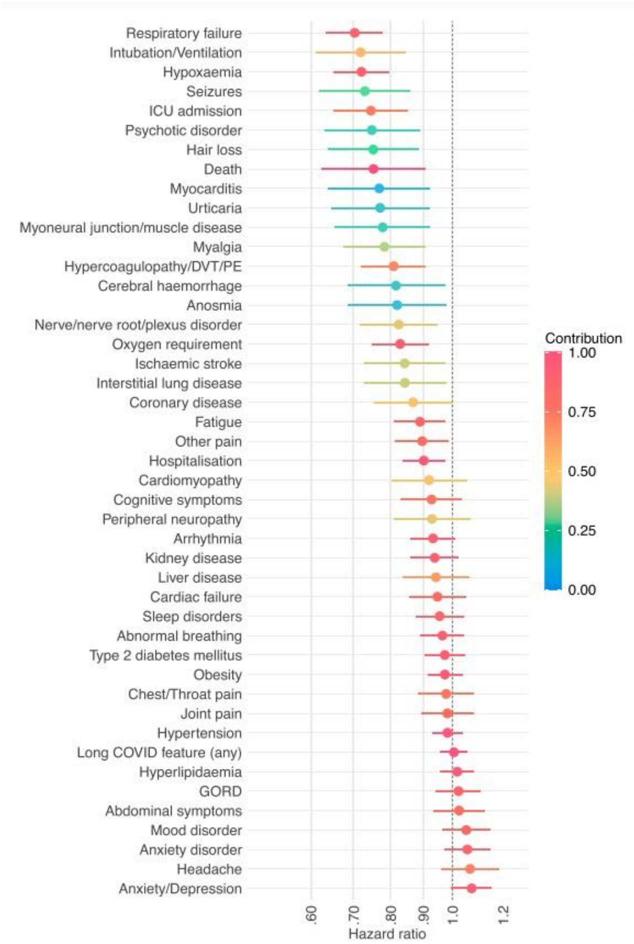


Figure 4. Hazard ratios for the outcome within 6 months of infection with SARS-CoV-2 between individuals vaccinated vs. unvaccinated against coronavirus-19. HR < 1 indicate outcomes less common among vaccinated individuals [69].

2.1.3: Pathophysiological Mechanisms of Breathing Pattern in Long-COVID Syndrome

The most common complaints during Long-COVID (from here on LC) are represented by dyspnoea and fatigue on exertion [66].

In this context, the cardiopulmonary exercise test (CPET) is a valuable tool to understand physiological limitations to exercise performance and to assess to the different physiological processes that become dysfunctional in LC.

The origin of breathlessness and poor exercise performance in LC patients has been investigated but contrasting findings were obtained mostly because of the heterogeneity and small sample size of survivors, variety of procedures and methodology.

Persistence of dyspnoea in patients that experienced COVID-19 pneumonia or milder forms of infection seems not to be related to the degree of disease severity or the residual impairment in lung function, which often corresponds to a mild and moderate reduction in diffusing capacity of the lung for carbon monoxide and in total lung capacity.

In one of the studies focusing on mechanisms underpinning long-term dyspnoea and exercise performance using CPET, Steinbeis and colleagues reported that breathlessness (measured by mMRC score) was negatively correlated with overall exercise tolerance and oxygen uptake, and with ventilatory efficiency and breathing equivalents. Surprisingly, breathing equivalents were similar among patients regardless dyspnoea. These findings indicate that impairment of ventilatory mechanics due to neural or muscular causes could play a more relevant role for the respiratory sequelae than lung parenchymal damage [70].

Unexplained breathlessness is one of the main indications to perform CPET and several studies in LC patients with unexplained dyspnoea have published. In this context, CPET was used as a cornerstone to understand physiologically the degree and origin of any limitation, recognising that most post COVID-19 symptoms could be worsened by exertion.

The first research studies focused on a CPET profile of deconditioning, because of an acute inflammatory process, prolonged bed rest, post-traumatic syndrome and depression.
Rinaldo and colleagues studied a group of LC patients three months after hospital discharge [71]. The researchers apparently found no evidence of ventilatory limitation, though 55% of the subjects had a reduced peak oxygen consumption ($\dot{V}O_2$) in a maximal exercise protocol. The authors concluded that the combination of an early anaerobic threshold (AT), blunted oxygen pulse ($\dot{V}O_2$/HR) and reduced metabolic efficiency ($\dot{V}O_2$/work rate) was considered as a pattern of deconditioning. However, deconditioning is an inaccurate diagnosis in which direct measure of cardiac output (for oxygen deliver) or peripheral oxygen uptake (for peripheral muscle pathology) are missing. In common practice, deconditioning suggests a passive process of muscle inefficiency, often secondary to decreased use (e.g., immobility, illness).

Skjørten and colleagues described that reduced peak oxygen uptake obtained by CPET correlated with disease severity, suggesting a peripheral muscle system limitation. In this study, the authors also found impaired ventilatory efficiency, a combination of raised respiratory rate and dead space ventilation, and increased chemosensitivity in LC patients [72].

In 2021 Motijunaite and collaborators conducted a prospective single-centre study of 114 patients at about three months after acute infection [73]. They were interested in pulmonary, cardiac and functional capacity by means of CPET to better understand the discrepancy between dyspnoea and lung function.
Cardiovascular inefficiency was considered in the presence of left ventricular systolic or diastolic dysfunction, and/or reduced peak oxygen uptake (<85% of predicted value), and/or reduced oxygen pulse, chronotropic insufficiency or reduced peak circulatory power.

Ventilatory limitation was described with a significant reduction of pulmonary function tests at rest (i.e., FEV_1 <70 % pred and/or DLCO <70% pred); breathing reserve <30%; or hypoxaemia (SpO2 ⩽88%) by exertion.

Peripheral cause of disability was considered if there was a decreased oxygen uptake at peak with AT < 40% of predicted, without specific ventilatory or cardiac limitation.

Physiological features of inadequate hyperventilation by exertion consist of higher $VE/\dot{V}CO_2$ slope (>40), increased ventilatory equivalents, as well as higher VE measured at AT in absence of clear pulmonary or cardiac limitation.

The authors found a higher prevalence of exercise intolerance due to peripheral deconditioning when related to the study by Rinaldo et al. [71], although one third of the participants showed a pattern of inadequate exercise hyperventilation (as confirmed by increased $VE/\dot{V}CO_2$ slope) [73]. This condition could be explained by an abnormal central ventilatory control following a respiratory infection and resulting in respiratory alkalosis.

For clinical practice, the identification of hyperventilation syndrome is an essential step to be able to resort to respiratory physiotherapy with an expert therapist, although sometimes spontaneous recovery can be described.

Subsequent research focused on dysfunctional breathing (DB) as abnormal and irregular ventilatory pattern on exertion and it was recognized in a variable range from 29% [74] to 63% [75,76] of the LC patients. Importantly, concerning ventilatory response, these findings reported relevant percentages of LC individuals affected by DB during incremental maximal exercise, with and without hyperventilation [74-76] (Fig. 5, Fig. 6 and Fig. 7).

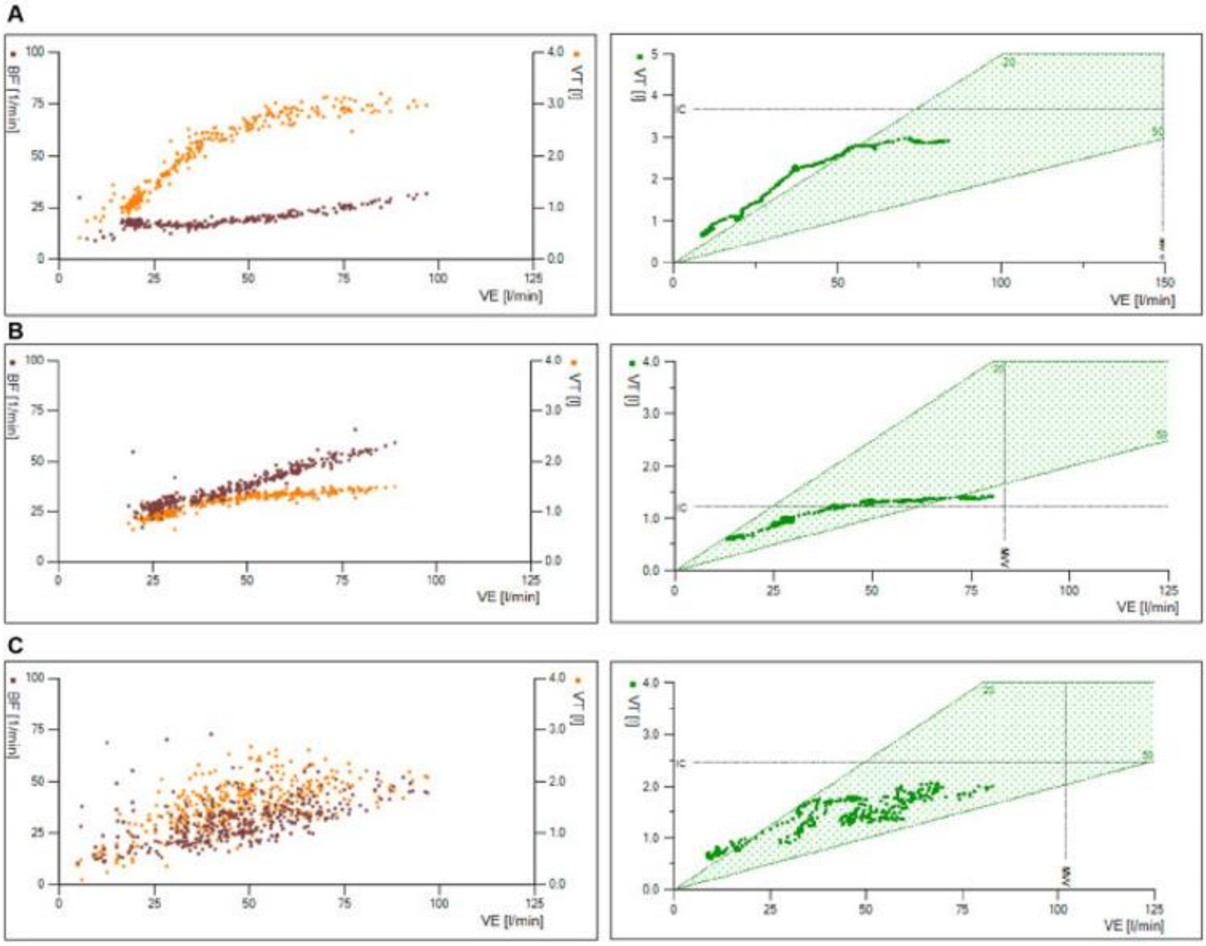


Figure 5. (A) Normal subject. (B) Respiratory limitation showing a regular, but limited increase of tidal volume with high breathing frequency. (C) Dysfunctional breathing with an erratic pattern [74].

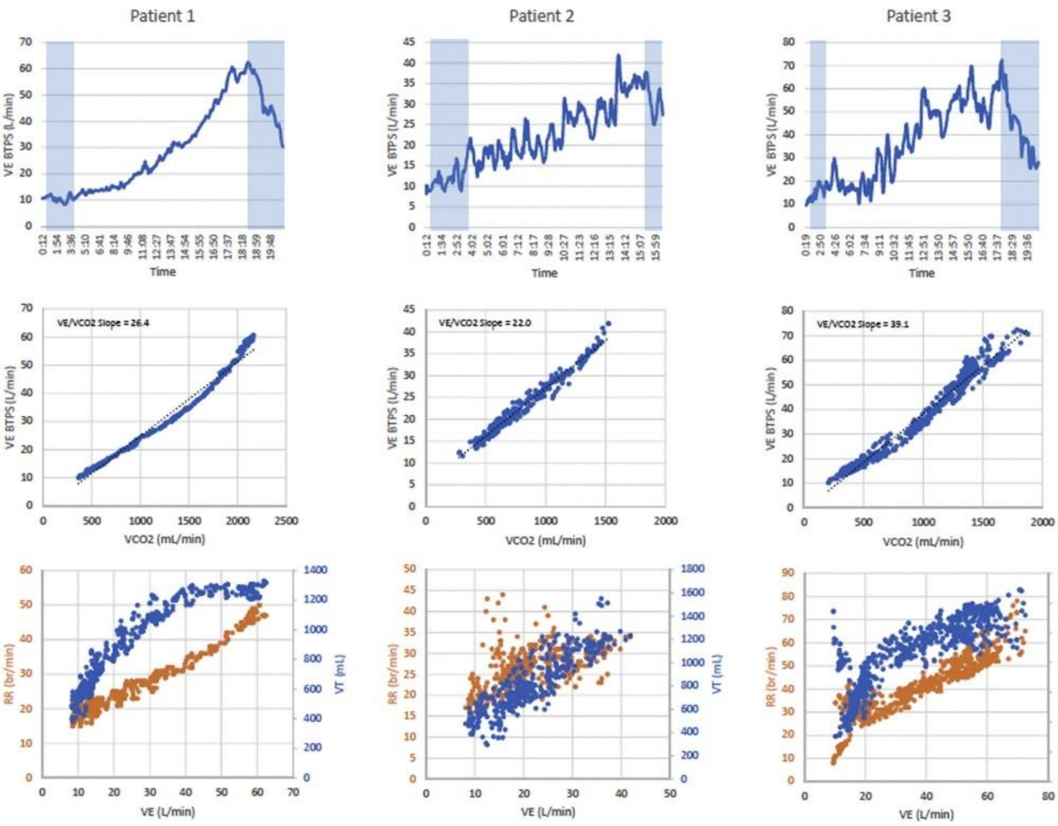


Figure 6. Patient #1 Shows Normal Ventilatory Pattern. Patients #2 and #3 show examples of the dysfunctional breathing [75].

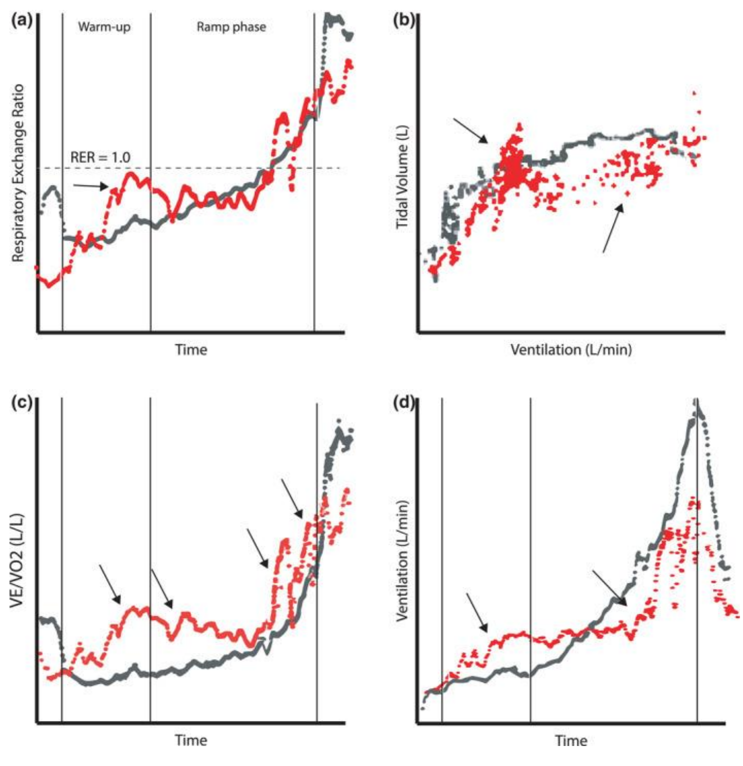


Figure 7. Ventilatory data in relation to the four criteria used to determine presence of an abnormal breathing pattern during exercise from two post-COVID patients with normal (grey) and abnormal (red) breathing pattern [76].

Dyspnoea could be the result of dysfunctional breathing (DB), that is considered a group of respiratory disorders characterised by chronic changes in the respiratory pattern as a physiological response to disease but also as pathological response to the absence or in excess of physiological respiratory or cardiac disease. A *gold-standard* diagnostic method is missing, so the exact prevalence of the clinical condition is unknown and patient selection criteria in different studies can play an important role. The identification of DB is currently based on the exclusion of organic disease [77].

DB, as a neural breathing disorder, could be considered the result of an abnormal breathing drive developing a respiratory discomfort without a particular cardiopulmonary disease.
It is noteworthy that the diagnosis of DB is based on the visual analysis of the plots showing the relationships between tidal volume (V_T), respiratory rate (RR) and minute ventilation (VE); therefore, the identification of DB is subjective and not be reproducible. Analysis of tracking of respiratory flow, frequency and volumes during tidal breathing can give useful information.

Among different breathing disorders, hyperventilation syndrome is commonly defined by the rapid RR, and V_T similar to inspiratory capacity (IC); in erratic breathing pattern, the patient is unable to coordinate a maximal expiratory and inspiratory manoeuvre; in the thoracic dominant breathing, the large volume breaths occur with minimal inspiratory reserve capacity; forced abdominal expiratory pattern is characterised by tidal breathing occurring at low volumes, with minimal expiratory reserve volume; and finally, thoraco-abdominal asynchrony demonstrates asynchrony during quiet tidal breathing [77].

A very simple and well-known analysis of the breathing pattern could be performed with the ratio of the inspiratory time (T_I) - during the tidal volume (V_T) -, to the total breath duration (T_I/T_{TOT}) whereas the inspiratory flow could be measured by the ratio of V_T to T_I.
In this context, T_I/T_{TOT} represents the *duty cycle* of the respiratory system, whereas V_T/T_I has been widely defined as a measure of *respiratory drive* [78] (Fig. 8).

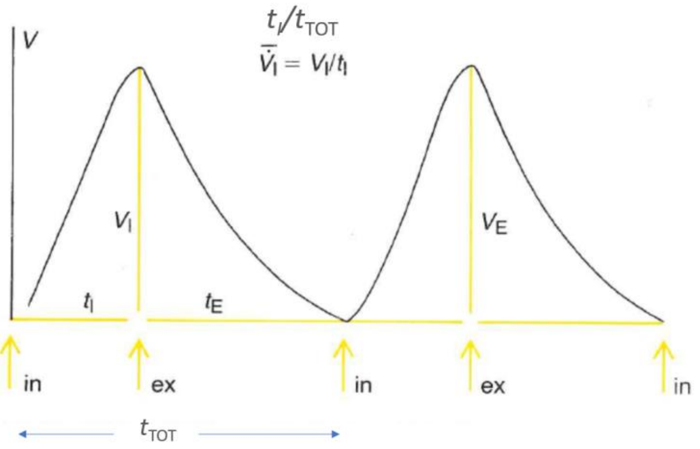
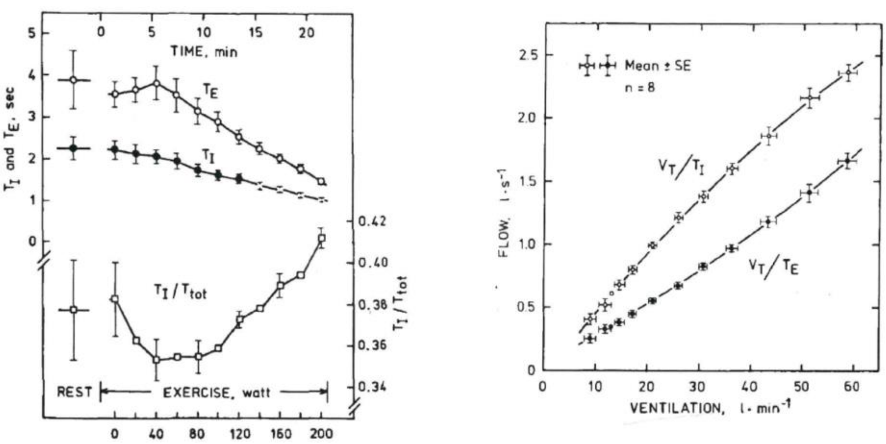


Figure 8. Expiratory (t_E) and inspiratory (t_I) durations and the ratio of inspiratory to ratio of inspiratory to total breath duration (T_I/T_{TOT}). Inspiratory flow could be measured by V_T/T_I.

T_I/T_{TOT} has been termed also *fractional inspiratory time* and when it is prolonged it predisposes to respiratory muscle fatigue and it is of equal importance to the tension developed by the muscle, as a determinant of diaphragmatic fatigue [79].

Up to now, assessment of BP on effort in a very small health study population has been published in a report of Lind et al. During incremental-load exercise, T_I/T_{TOT} rises with rising minute ventilation [80].



(A) (B)

Figure 9. (A): Expiratory (T_E) and inspiratory (T_I) durations and the ratio of inspiratory to ratio of inspiratory to total breath duration (T_I/T_{TOT}) at rest and during incremental-load exercise. (B): Relations between mean inspiratory flow (V_T/T_I), mean expiratory flow (V_T/T_E), and ventilation at rest and during incremental-load exercise [80].

2.2 Aim of the second study [81]

The primary aim of our study was to analyse pathophysiological mechanisms of BP underpinning unexplained dyspnoea in LC patients, by means of T_I/T_{TOT} and V_T/T_I at rest and during maximal exercise by means of CPET.

Moreover, to understand the differences of breathing pattern between LC sample study and healthy subjects, we analysed physiological response to maximal incremental-load exercise in a large cohort of LC individuals with normal pulmonary function but suffering from unexplained dyspnoea and in a control group. In this context, T_I/T_{TOT} and V_T/T_I of all the participants were evaluated, using a standardised CPET method, and then we considered the different ventilatory profiles.

2.3 Materials and Methods of the second study

We conducted a prospectively cross-sectional study enrolling subjects affected previously by SARS-CoV-2 infection that was confirmed by RT- PCR from nasopharyngeal swabs.
Study population referred for CPET at the "Respiratory Disease and Lung Function Unit" (University Hospital of Parma, Italy), and at the Cardiac Unit ("G. da Saliceto" Hospital of Piacenza, Italy) for unexplained dyspnoea lasting at least three months after SARS-CoV-2 infection.
We excluded patients performing impaired pulmonary function or affected by known cardiopulmonary illness.
We also recruited routine outpatient clinic never smoker, healthy, age-, sex- and BMI-matched participants, as healthy subjects (HS).

The study was approved by the Local Ethics Committee of North Emilia (approval number: 131, dated 18 March 2022).

2.3.1 Pulmonary Function and Cardiopulmonary Exercising Test

All participants in the study performed pulmonary function tests, following the international recommendations [82] and by means of a flow-sensing spirometer (Vmax 22 and 6200, Sensor

Medics, Yorba Linda, CA, USA). Dynamic lung volumes (FVC and FEV_1) were obtained and expressed as percentage of the predicted values [83].

All subjects with normal spirometry then underwent CPET as a standardised procedure [84].

Study population sit on an electromagnetically braked cycle ergometer (Corival PB, Lobe Bv, Groningen, The Netherlands; Cosmed, Rome, Italy). The maximal exercise protocol consisted of an initial 3-min time of rest, followed by 3-min unloaded cycling warm-up time, and then by incremental of 5–20 Watts/min protocol, based on anthropometry, in order to achieve an exercise time in between 8 and 12 minutes.
Participants were asked to maintain a pedalling frequency of 60 rotations/min monitored by a digital display, while they were monitored by a 12-lead electrocardiogram (Welch Allyn CardioPerfect, Delft, The Netherlands) and by a pulse oximeter (Pulse Oximeter 8600, Nonin Medical Inc., MPLS, MN, USA; Cosmed, Rome, Italy). In the same time, blood pressure was measured every 2 minutes. Exercise was stopped according to the standardised criteria [84]. Predicted values were calculated according to equations by Wasserman et al. [85].

Main breath-by-breath parameters were recorded during the maximal exam (CPX/D; Med Graphics, St. Paul, MN, USA; Quark CPET, Cosmed, Rome, Italy), such as $\dot{V}O_2$ (mL/kg/min), $\dot{V}CO_2$ (mL/kg/min), V_T (L), RR (bpm) and minute ventilation (VE) (L/min) [85].

Ventilatory tolerance on exercise was also assessed in terms of breathing reserve (BR, %). This parameter was calculated by the formula 1- (peak ventilation/maximum voluntary ventilation, MVV) * 100, where MVV was obtained by FEV_1 * 40.
Ventilation response during exercise was expressed as a linear regression function by plotting VE against $\dot{V}CO_2$ obtained every 10 seconds [84]. Then, the slope was determined from the VE/$\dot{V}CO_2$ regression line.
The end-tidal pressure of carbon dioxide ($PETCO_2$) in mmHg was obtained as mean of $PETCO_2$ during the 3 minutes at the rest phase and during the last 20 seconds of the maximal exam and it was also recorded as $\Delta PETCO_2$ (difference between $PETCO_2$ peak and $PETCO_2$ rest).
The pattern of breathing was analysed by recording T_I/T_{TOT} and V_T/T_I for the duration of the exam (rest time and exercise period).

Regarding metabolic capacity, peak workload (Watts) and peak $\dot{V}O_2$ (mL/kg/min) were measured as the mean value of Watts and $\dot{V}O_2$ during the last 20 seconds of the test.

AT was expressed as absolute value of $\dot{V}O_2$ in mL/kg/min and it was defined by both V-slope and ventilatory equivalents methods ("dual method approach") [84].

The cardiovascular performance on exertion was considered by oxygen pulse (O_2Pulse, expressed as $\dot{V}O_2$/HR) and oxygen uptake efficiency slope (OUES) i.e., the relation between oxygen uptake and ventilation [86]. Also, heart rate recovery at maximal exercise (HRR) in bpm was recorded [87].

Dyspnoea related to maximal incremental exercise was obtained at the end of the exam by means of a visual analogue scale (VAS) scored 0-100, which consisted of a 100 mm-horizontal line with "none" at the left end of the scale and "very severe" placed at the right side. Dyspnoea VAS assessment was then divided by the maximum workload (in mm/watt).

2.4 Statistical Analysis of the second study

Due to the explorative nature of the study no formal sample size calculation was performed.
The normal distribution of investigated variables was evaluated by means of Kolmogorov–Smirnov Goodness-of-Fit test.
Continuous variables characterized by normal distribution were reported as means ± standard deviation (SD), whereas continuous variables characterized by non-normal distribution were reported as medians and 1st and 3rd quartiles (IQR). The number of participants and percentage were reported as categorical variables.
Concerning the correlation analysis, the Pearson (r) correlation coefficient will be used for linear or normally distributed variables; the Spearman (ρ) correlation coefficient will be used for not linear or not normally distributed variables.
Comparisons between variables were determined by unpaired *t-test* or by Chi-square test, when appropriate.

The T_I/T_{TOT} cut-off value of 0.38, the median T_I/T_{TOT} value at the peak of exercise in the control group, was chosen a posteriori.

Appropriate curve-fitting models were identified to analyse during exercise: T_I/T_{TOT}, [Y = (Y0-Plateau) * exp(-K * X) + Plateau, where Y0 is the Y value when X is zero, plateau is the Y value at infinite values, and K is the rate constant expressed in reciprocal of the X axis] and V_T/T_I [Y = Y

Intercept + Slope * X, where Y Intercept is the Y value where the line intersects the Y axis, and slope is the slope of the line, expressed in Y units divided by X units].

A $p < 0.05$ was taken as significant. Statistical analysis and diagrams were obtained by Prism 8 (©2018 GraphPad Software, La Jolla, CA, USA).

2.5 Results of the second study

Totally, 42 LC participants and 40 HS controls were enrolled. They aged between 22 and 66 years in LC population, and between 26 and 79 years in HS sample.
Concerning lung function, we found that among LC group, spirometric parameters were normal, although FEV_1 values were statistically lower than for HS controls (Table 2).

Table 2. Subjects' characteristics and variables. Values are expressed as mean ± SD. Bold values represent statistical significance [81].

Variables	Healthy Controls (No. 40)	Long COVID Patients (No. 42)	*p*
Age (years)	47 ± 11	49 ± 12	0.494
Sex (F/M)	22/18	23/19	0.983
BMI (Kg/m^2)	25 ± 4	26 ± 3	0.121
FVC (% pred)	108 ± 15	101 ± 17	0.066
FEV_1 (% pred)	106 ± 12	99 ± 15	**0.025**
FEV_1/FVC (%)	82 ± 6	81 ± 6	0.255
VO_2 peak (mL/kg/min)	31 ± 10	23 ± 8	**0.001**
VO_2 peak (% pred)	105 ± 27	84 ± 21	**0.001**
Workload (Watts)	181 ± 65	123 ± 43	**0.001**
Workload (% pred)	117 ± 36	82 ± 22	**0.001**
AT (mL/kg/min)	21 ± 10	16 ± 8	**0.030**
O_2 Pulse rest (mL/bpm)	4.9 ± 2.4	4.1 ± 1.3	0.069
O_2 Pulse peak (mL/bpm)	14.6 ± 4.5	11.7 ± 3.6	**0.002**
OUES (mL/min)	2288 ± 687	1688 ± 686	**0.001**
HR rest (bpm)	75 ± 14	85 ± 15	**0.002**
HR peak (bpm)	152 ± 19	147 ± 15	0.217
HR peak (% pred)	88 ± 9	86 ± 8	0.377
HR recovery (bpm)	25 ± 9	20 ± 10	**0.009**
BR (%)	50 ± 11	51 ± 14	0.768
VE peak (L/min)	72 ± 26	59 ± 21	**0.023**
Vt rest (L)	0.74 ± 0.3	0.74 ± 0.3	0.994
Vt peak (L)	2.37 ± 0.8	1.97 ± 0.5	**0.007**
RR rest (bpm)	14 ±5	14 ± 6	0.770
RR peak (bpm)	31 ± 7	31 ± 9	0.847
$PETCO_2$ rest (mmHg)	33 ± 6	34 ± 4	0.319
$PETCO_2$ peak (mmHg)	41 ± 5	41 ± 5	0.921
Δ $PETCO_2$ (mmHg)	8 ± 6	7 ± 5	0.468
VE/VCO_2 Slope (L)	26 ± 4	30 ± 4	**0.001**
T_I/T_{Tot} rest	0.29 ± 0.09	0.36 ± 0.09	**0.001**
T_I/T_{Tot} peak	0.39 ± 0.05	0.42 ± 0.06	**0.034**
V_T/T_I rest (mL/s)	685 ± 397	605 ± 262	0.286
V_T/T_I peak (mL/s)	3155 ± 1101	2560 ± 850	**0.008**
VAS dyspnoea (mm/watts)	0.48 ± 0.19	0.64 ± 0.22	**0.022**

All participants performed CPET with a good compliance and no complications appeared.

We noted that the average time between SARS-CoV-2 infection and CPET was 12 months.

In our findings we described that LC sample presented a worse metabolic and cardiovascular performance on exercise, as confirmed by lower values of $\dot{V}O_2$ at the peak and at anaerobic threshold, worse maximal workload, lower oxygen pulse values at the peak, and decreased OUES values, as well as greater values in VAS dyspnoea scale, as compared to HS group (Table 2).

Analysing ventilation tolerance on exertion in LC group, they showed significantly higher $VE/\dot{V}CO_2$ slope than HS controls, but not in terms of in BR or $PETCO_2$ values (Table 2).

Considering breathing pattern, when compared to HS sample, LC group did not differ in terms of RR both at rest and at the peak of exercise, but they performed lower V_T at the peak.
LC patients also were characterised by significantly greater values of T_I/T_{TOT} at rest and at the peak of exercise (Table 2) (Fig. 10), and lower values in V_T/T_I at peak (Table 2) (Fig. 11).

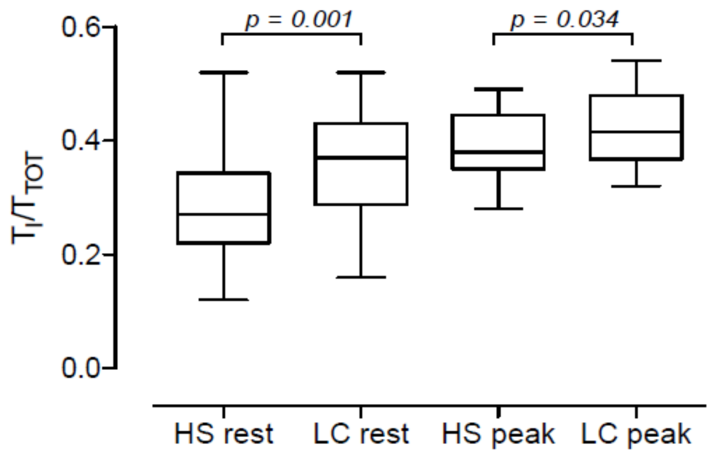


Figure 10. Mean, SD and range values of T_I/T_{TOT} at rest and at peak of exercise in 40 healthy subjects and 42 Long-COVID patients [81].

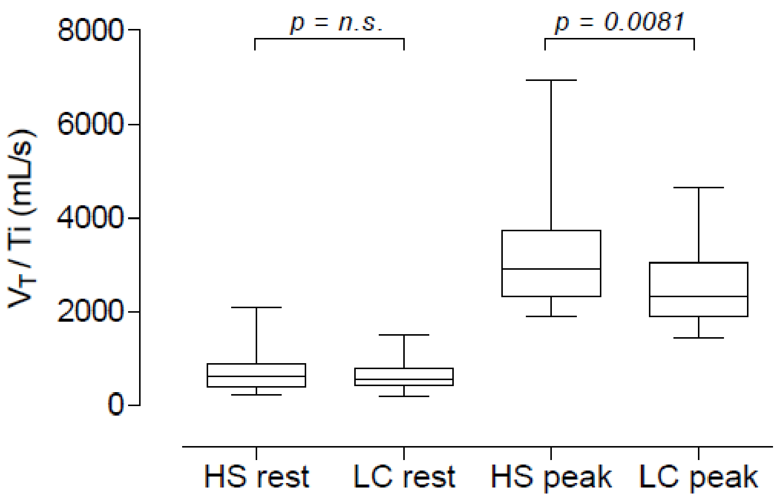


Figure 11. Mean, SD and range values of V_T/T_I at rest and at peak of exercise in 40 healthy subjects and 42 Long-COVID patients [81].

The best-fit curves of the data points on exercise of T_I/T_{TOT} and V_T/T_I plotted against VE (expressed as % peak) were significantly different between LC group and controls (Fig. 12).

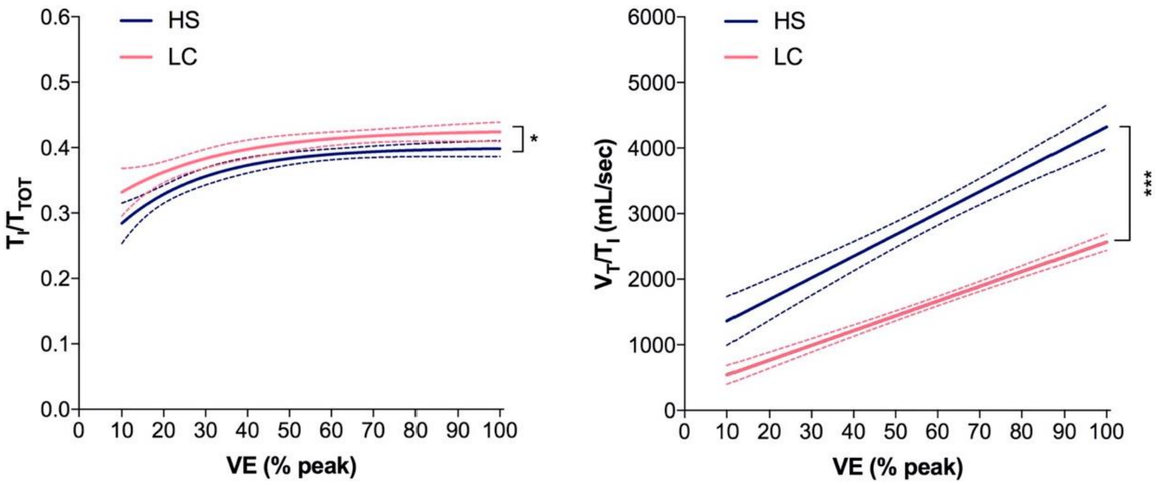


*Figure 12. Best fitting curves of data points of T_I/T_{TOT} (left panel) and V_T/T_I (right panel) and 95% confidence bands during exercise in 42 Long-COVID patients and 40 healthy subjects. * $p < 0.05$; *** $p < 0.001$ [81].*

Moreover, LC participants performed T_I/T_{TOT} at peak significantly related to $\Delta PETCO_2$ (p= 0.009) (Fig.13).

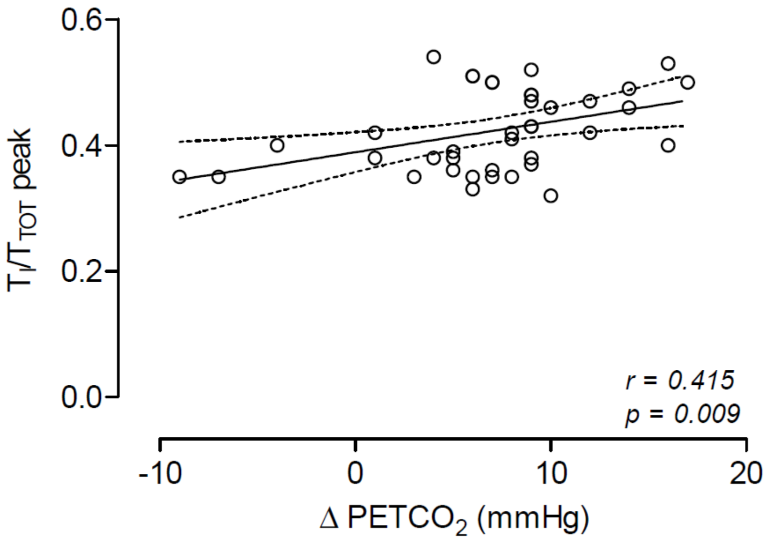


Figure 13. Relationship between T_I/T_{TOT} values at peak of exercise and $\Delta PETCO_2$ values in 42 LC patients [81].

We also analysed the sample of LC patients by means of T_I/T_{TOT} cut-off value of 0.38 and found that 29 patients out of 42 LC participants presented $T_I/T_{TOT} > 0.38$. They showed lower values in $\dot{V}O_2$ peak (21 ± 5 mL/kg/min vs. 27 ± 10 mL/kg/min; p = 0.011) (Fig. 14 – upper panel) and in maximal workload (114 ± 28 watts vs. 144 ± 60 watts; p = 0.035), and higher values in $VE/\dot{V}CO_2$ slope (31 ± 4 L vs. 28 ± 3 L; p = 0.036) (Fig. 14 – lower panel), when compared to the other 13 subjects with $T_I/T_{TOT} \leq 0.38$.

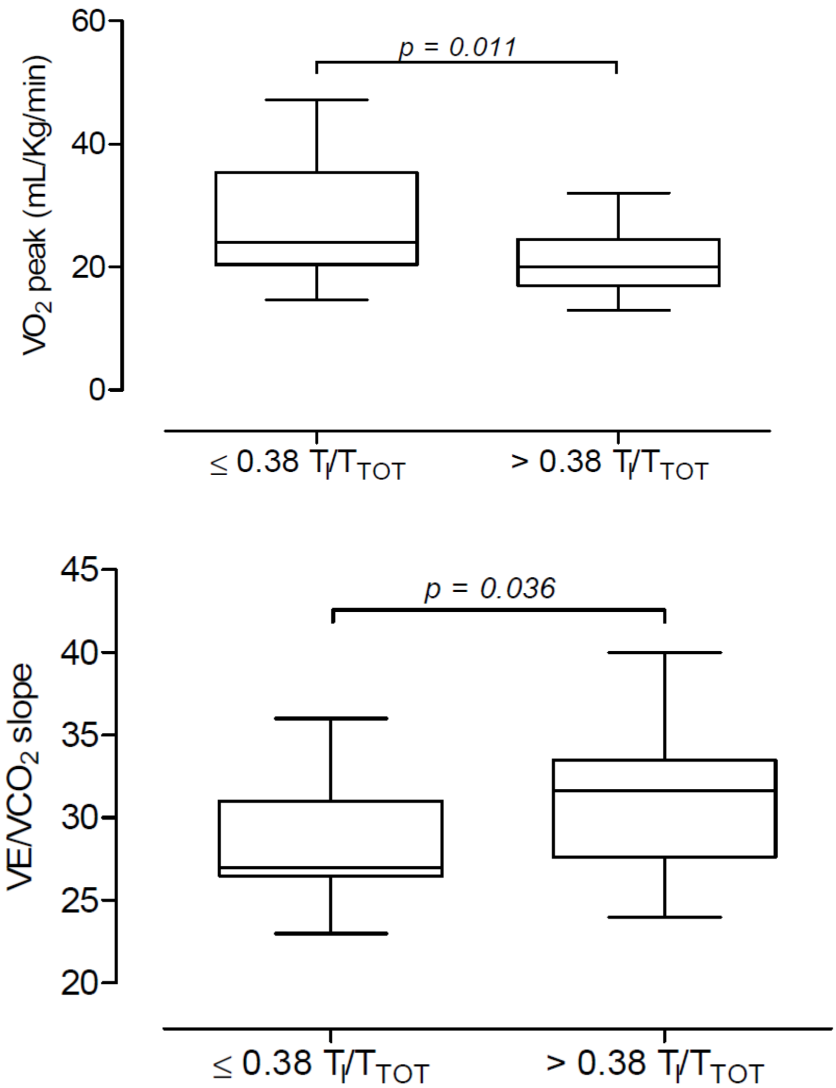


Figure 14. Mean, SD and range values of $\dot{V}O_2$ peak (upper panel) and of VE/$\dot{V}CO_2$ slope (lower panel) in 13 LC with $T_I/T_{TOT} \leq 0.38$ and in 29 Long-COVID patients with $T_I/T_{TOT} > 0.38$ peak of exercise. In the current study, LC had higher values in VE/$\dot{V}CO_2$ slope, as compared to controls, but they did not differ in terms of $PETCO_2$, thereby showing ventilatory inefficiency without hyperventilation [81].

2.6 Discussion of the second study

In this research study, we found that LC sample showed an increase in mean V_T/T_I (as inspiratory flow values) that during exercise, but lower than that in control group. So, we can conclude that LC group developed a lower minute ventilation at peak exercise, as compared to HS controls.

Our results are in line with previous findings by Lind and Hesser [80] who firstly analysed breathing pattern using T_I/T_{TOT} and V_T/T_I on maximal exercise in a very small sample of male healthy individuals.

Therefore, as found in LC population, higher values of T_I/T_{TOT} provide respiratory muscle fatigue as the tension developed by the muscle as a determinant of the fatigue of the diaphragm [79].

V_T/T_I has been termed mean inspiratory flow rate and it is considered as a measure of respiratory drive, since it was found to be related to indices of neural respiratory centre output, such as $P_{0.1}$ (airway occlusion pressure at 100 ms) and the ventilatory response to hypercapnia [88].

Altogether, our findings suggest that LC survivors present a breathing pattern with a higher diaphragm intolerance and lower efficiency than the reference controls. Most of the work of breathing is performed by the diaphragm. After respiratory illness, diaphragmatic performance may be impaired and the accessory muscles of the respiratory system could be recruited more strongly [89], resulting in an abnormal breathing pattern and dyspnoea, especially when mechanical ventilation is required or in conditions of general physical deconditioning.

Limitations might be considered when interpreting the results of our research.
First of all, we had no data on breathing patterns before SARS-CoV-2 infection and no comparison before and after infection.
Secondly, we used $PETCO_2$ to estimate indirectly $PaCO_2$ to exclude hyperventilation syndrome, without arterial blood gases due to non-invasive nature of the study.

On the other hand, we believe the selection of age-, sex- and BMI-matched controls as a strength of the work.
In addition, breathing patterns were investigated using an objective and standardised approach based on T_I/T_{TOT} and V_T/T_I measurements.

2.7 Conclusion of the second study

In conclusion, we found impairments in resting and exercise breathing patterns and a CPET profile of deconditioning in individuals with previous SARS-CoV-2 infection who subsequently complained of prolonged unexplained dyspnoea.
To improve this uncomfortable condition, pulmonary rehabilitation (PR), including breathing control techniques, may be useful.

Third part: Effects of Pulmonary Rehabilitation and Physical Activity on Long-COVID Population

3.1 Background: the Importance of Pulmonary Rehabilitation

During the first three years of the pandemic COVID-19, the global mortality rate has reduced from 101600 deaths weekly in January 2021 to 6500 deaths weekly in March 2023. The number of hospitalizations has also declined. Globally, as 12 October 2023, there have been 771.1191.203 confirmed cases of COVID-19, including more than 6 million dead, reported to WHO. As of 5 October 2023, a total 13.516.809 vaccine doses have been administrated [1].

The effective vaccination reduced severity of acute disease [90], together with the use of acute therapies like dexamethasone, interleukin-6 (IL-6) inhibitors, anti-viral drugs and baricitinib [91].

In the first quarter of 2020, approximately 1 in 6 immune-naïve patients hospitalised with COVID-19 developed severe illness requiring ICU (Intensive Care Unit) admission, most of whom required ventilation (non-invasive or invasive mechanical ventilation) [92].
The risk of severe COVID-19 in immune-naïve patients is less clear due to increasing exposure to SARS-CoV-2, and identifying a truly immune-naïve population is difficult.

Subjects recovered from the acute phase of COVID-19 can develop prolonged or persistent impairments in almost all body systems associated with a variable burden of signs and symptoms (as generally known Long-COVID syndrome, LC). The precise prevalence of the condition is unknown and varies depending on the method of measurement, the cohort examined, the virus variant and vaccination status.

The complexity of the clinical disorders makes rehabilitation difficult to target and requires the definition of specific protocols.
In this context, international guidelines on managing LC are not specific for rehabilitation and to date, the effectiveness of breathing exercises and retraining after acute COVID-19 has not been established. It had been reported that post-COVID patients could have an impaired physical condition when they were discharged home, even after early mobilization [93].

Despite clinical indications of pulmonary rehabilitation (PR) have been proposed by international guidelines and recommendations, the feasibility and the effects of rehabilitation programs in patients recovering from COVID-19 remained to be defined in the first periods of pandemic [94-96].

In 2020 as international scientific societies and clinicians elaborated recommendations about the acute phase of COVID-19 [94-96], further approaches including pulmonary rehabilitation have been recommended also for post-acute phase of the disease [97-100].

In this regard, rehabilitation represents an important treatment option for survivors after COVID-19, especially for those who have been hospitalised. It can improve functional capacity, reduce deconditioning after prolonged immobilisation in the ICU, facilitate return to work, and improve quality of life (QoL) [101].

As COVID-19 is a multi-systemic disease, rehabilitation requires a multidisciplinary team (MDT) rehabilitation. Whenever possible it should be started in the ICU. Progressive rehabilitation programmes are best started within the first thirty days (post-acute phase), as recommended by the National Institute for Health and Care Excellence (NICE), to have the greatest impact on recovery [102].

In 2020 long-term clinical consequences of COVID-19 were unknown, but evidence from previous coronavirus outbreaks demonstrated impaired pulmonary and physical condition, reduced QoL and emotional distress.
Worse lung function was associated with damage caused by both mechanical ventilation and pathophysiology of viral response [103].
Moreover, prolonged immobility and use of sedatives have been related to respiratory and peripheral muscle weakness. All these factors contribute to worsening functional capacity and QoL [104].

A new consensus statement has been developed by multidisciplinary experts (rehabilitation, sports and exercise medicine, rheumatology, psychiatry, psychology, pain medicine, and general practice) working at the Defence Medical Rehabilitation Centre, Stanford Hall, UK.
These recommendations have been obtained by evidence-based consensus to guide medical care and rehabilitation, based on emerging evidence from COVID-19 survivors and knowledge gained from previous coronavirus epidemics [105].

This consensus statement provides a general framework that sums up the evidence and likely requirements for multidisciplinary rehabilitation after COVID-19 disease for a target population of active individuals, including military personnel and athletes.

Here the definition of rehabilitation:

"Rehabilitation is patient-centred and tailored to individual patient needs; any rehabilitation programme should take into account comorbidities that may affect a patient's progress or ability to partake in a programme" [106].

Regarding pulmonary rehabilitation (PR), it has been promoted to provide comprehensive care and improve the functional status of patients with respiratory disease [107], as it can be considered as "*a multidisciplinary intervention based on personalised evaluation and treatment which includes, but is not limited to, exercise training, education, and behavioural modification designed to improve the physical and psychological condition of people with respiratory disease"*.

PR includes complementary practises, such as optimising medical management, prescribing exercise, educational and psychosocial programmes, behavioural modification strategies and occupational therapy.
PR has been confirmed to be able to improve symptoms, functional exercise capacity, and QoL in patients affected by respiratory disease, even in case of progressive lung damage. These effects are achieved by treating the secondary morbidity causing the impairment and improving function rather than the respiratory disease itself [108].

Most of programmes internationally including exercise training (ET), that plays a key role in PR [109] and it is based on the general principles of exercise physiology: duration, intensity, frequency, specificity and reversibility [108].

Most evidence on PR in 2020 was reported in older populations with chronic obstructive pulmonary disease (COPD) or younger groups with asthma, however there was evidence of PR in pneumonia disease [110,111] interstitial lung disease (ILD) and SARS [112] as well as emerging reports of PR being used in the early stages of COVID-19 [113].

With a focus on COVID-19 during the acute phase, most physical and functional concerns were peripheral muscle dysfunction (due to deconditioning and decreased lean body mass, ICU neuropathy, fatigue and hypoxaemia), respiratory muscle impairment (dysfunctional breathing pattern, and exercise-induced laryngeal obstruction), cardiac injury and deconditioning, and psychosocial factors (anxiety, depression, guilt, sleep disturbance and addiction).

Several patients infected at the beginning of the pandemic were referred to ambulatory respiratory rehabilitation with the aim of improving functional impairment and/or persisting dyspnoea [99].

Jiandani and colleagues with a preliminary retrospective study in 2020 explored the physiotherapy practices that could be implemented in COVID-19 survivors in the ICU in terms of mobilization and oxygen need. Most common programs following appropriate measures of infection containment consisted of therapeutic positioning, early mobilization and breathing exercises with promising effects in early patient ambulation and discharge [114].

One of the first studies to confirm that PR was possible and effective in patients recovering from COVID-19, including those requiring ventilation or oxygen supplementation, was a real-life retrospective multicentric research by Zampogna and co-workers. 140 patients admitted to an inpatient PR programme confirmed improvements in Short Physical Performance Battery (SPPB, primary outcome) and in Barthel Index (BI), as well in 6MWD [115].

This pulmonary rehabilitation program was according to the Italian Position Paper [95].

In 2020 in another retrospective analysis of 137 hospitalized patients with COVID-19-induced pneumonia, Lv et al. found that the frequency of pulmonary dysfunction in short-term (two weeks) after discharge was relatively high, manifesting as restrictive dysfunction and small airway injury. In fact, focusing on the severe group of patients, 88.9% of them had mean forced inspiratory volume (IVC) < 80% pred, and 55.6% of patients had an FVC <80% pred.

Analysing the small airway function, 55.6%, 40.7%, and 25.9% of individuals had maximum expiratory flow rate at 25%, 50% and 75% of the vital capacity (MEF_{25}, MEF_{50}, and MEF_{75}) values <70%, respectively.

On the other hand, in non-severe sample 79.1% of patients had an IVC < 80% pred, and 16.4% of subjects had an FVC <80% pred. The mean MEF_{25}, MEF_{50}, and MEF_{75} <70% values were 57.3%, 30%, and 13.6% respectively. These preliminary findings focused on pulmonary function and suggested rehabilitation treatment if required [116].

Then, in 2021, Lopes and colleagues focused on small airway dysfunction by means of impulse oscillometry (IOS) at 2nd and 5th months after acute phase. They demonstrated that in COVID-19

survivors who complained of respiratory symptoms, impaired small airway function was the most frequent abnormality.
During this 3-month follow-up, a significant improvement in respiratory symptoms (cough and/or dyspnoea) was described. In the comparison between the 2^{nd} and 5^{th} months post COVID-19, an abnormality on IOS was observed in almost 90% of patients with persistent respiratory symptoms, with this frequency decreasing to approximately 70% in the 5^{th} month. Regarding spirometry, the authors observed that it was abnormal in approximately 44% and 34% of patients at 2^{nd} and 5^{th} months, respectively, but the difference was not significant [117].

To describe the characteristics of real-life patients referred with LC and their evolution during rehabilitation, Bouteleux et al. reported that 64% of their cohort were middle-aged with no significant comorbidities and mild severity at presentation. The prevalence of hyperventilation syndrome was striking [118]. On the other hand, according to data heterogeneity in rehabilitation, programme duration can be variable in this population.

Consistent with previous reports in a similar population [119], data suggested that lung function was almost preserved in terms of spirometry. Data from Sonnweber and colleagues supported that impaired lung function is poorly described by spirometry alone, as diffusing lung capacity is most impaired parameter in COVID-19 survivors. However, these authors also described a favourable evolution of lung function at 60 and 100 days, demonstrating persistence of dyspnoea more than four months after infection. This suggests a lack of correlation between respiratory function and symptoms.

Bouteleux and co-workers recognized the lack of diagnostic method for hyperventilation syndrome and that a standardised CPET could have more accurately prevented the detection of other aetiologies of DB; however, hyperventilation syndrome improved in study population between first and second assessments. The authors reminded the screening for hyperventilation syndrome should be included in the evaluation of patients referred to rehabilitation with persistent dyspnoea related to COVID-19, once all other causes have been excluded [118].
Long-lasting clinical and functional consequences qualifying patients for outpatient rehabilitation after SARS-CoV-2 infection may occur unexpectedly also in patients with mild initial manifestations of the disease. In this context, pulmonary function tests appeared to be insufficient to clearly explain prolonged breathlessness after COVID-19.

It could be important screening of hyperventilation syndrome in COVID-19 outpatients referred for rehabilitation in relation to their dyspnoea. This component as well as the associated functional impairment, may improve with early referral to rehabilitation.

ERS (European Respiratory Society) - ATS (American Thoracic Society) Task Force guidance strongly recommended identifying rehabilitation needs in COVID-19 patients, whether or not they required hospitalisation [120].

PR is a feasible, safe and effective therapeutic option in COVID-19 patients regardless of disease severity. Gloeckl et al. conducted research in 2021 to prospectively consider the efficacy, feasibility and safety of PR in COVID-19 patients, comparing PR outcomes between patients with a mild/moderate versus severe/critical disease [121].

Patients participated in a 3-week multidisciplinary inpatient PR. The PR programme was based on the PR protocol lung fibrosis. Participants performed 6MWT on admission and at the moment of discharge from PR and 30 meters was considered a minimal important difference, as 6-min walking test was the primary outcome of this study [122].
An endurance shuttle walk test (ESWT) was performed at 85% of the maximum walking speed derived from an incremental shuttle walk test [122].
The author found that upon admission to PR, patients with severe/critical COVID-19 had significantly lower exercise performance (6MWD 344 m versus 509 m; $p<0.001$) and worse lung function (FVC 75.1% versus 80.0%; $p<0.004$) compared to patients with mild/moderate COVID-19.
At PR discharge, patients in both groups improved significantly exercise performance and lung function. PR was well-tolerated and uneventful.

In 2022 a randomized controlled trial considered the role of the inspiratory muscle training (IMT) on recovery post COVID-19 as an important home-based rehabilitation strategy and the study confirmed a positive effect [123]. 281 adults about 9 months after infection were randomised 4:1 to an 8-week IMT or to an “usual care” control arm.
IMT uses airflow-restricted breathing to challenge the respiratory muscles, inducing a hypertrophic response similar to that seen in the peripheral musculature following programme of resistance training [124], and can be performed independently at home by a handheld inspiratory flow resistive device. Intervention participants were asked to perform three unsupervised IMT sessions per week for 8 weeks. Before each session, participants performed a maximal inspiratory effort from residual volume to determine sustained maximal inspiratory pressure.

QoL and breathlessness questionnaires (King's Brief Interstitial Lung Disease, K-BILD, and Transition Dyspnoea Index, TDI), respiratory muscle strength, and fitness (Chester Step Test) were all assessed pre- and post-intervention.
The main findings were no difference between groups in K-BILD total score post-intervention but IMT induced clinically meaningful improvements in the K-BILD domains for breathlessness and chest symptoms, as well as improvements in breathlessness according to TDI, as IMT also improved respiratory muscle strength and estimated aerobic fitness.

In another study Nopp and co-workers [125] considered the effect of a multi-professional and individualized rehabilitation in LC patients according to the Austrian guidelines for outpatient pulmonary rehabilitation [126].
At baseline, participants were evaluated in terms of functional tolerance, lung function, symptoms, and QoL. Over a period of 6 weeks, they underwent individual endurance, strength, and inspiratory muscle training, 3 times weekly for 3–4 hours each session, under a supervision of physicians, physiotherapists, and sports scientists. Individual patient education, psychosocial counselling by a psychologist, nutritional counselling by a dietician, and smoking cessation sessions were an essential part of the programme. In brief, a 6-week outpatient PR significantly improved exercise capacity in terms of 6MWD, and also dyspnoea, fatigue, and QoL. Particularly, the authors described an increase in pulmonary function (FEV_1 and DLCO) and inspiratory muscle strength, with a lowering of one grade on the post COVID-19 functional status (PCFS) scale [127].

The following study, published in 2022, was the first to investigate benefits of a cardiopulmonary rehabilitation, based on continuous moderate-intensity aerobic and resistance training in 26 post COVID-19 patients [128]. Cardiopulmonary rehabilitation course improved maximal exercise tolerance ($\dot{V}O_2$) and reduced VE/$\dot{V}CO_2$ slope. The authors also found a significant improvement in submaximal exercise tolerance in the 6MWT, in QoL, and a simultaneous reduction of fatigue after rehabilitation [128].
Aerobic training was prescribed between 60% and 80% of $\dot{V}O_2$ peak by means CPET. Exercise was performed on a 40-minute treadmill: 5-minute warm-up, 30-minute conditioning, and 5-minute cool-down [129]. Patients who were candidates for rehabilitation, had limiting sequelae from 15 days after hospital discharge or persistent symptomatic COVID-19 up to two months.
Resistance training consisted of exercises for upper (triceps, biceps, and shoulder abductors) and lower (quadriceps, hip abductors, and sural triceps) muscles. The load of the exercises was 60% of the one-repetition maximum. The load was increased every six sessions. The exercises were performed twice weekly in three sets of 8 to 12 repetitions.

After the rehabilitation course, the number of patients with ventilatory disorders and respiratory muscle weakness reduced. Tolerance to maximal exercise improved with a significant increase of 18.62% in $\dot{V}O_2$ peak and 29.05% in $T\dot{V}O_2$ peak - time to peak oxygen consumption. $VE/\dot{V}CO_2$ slope also reduced 5.21%, while submaximal exercise tolerance increased 70.57 m. Finally, mean $\dot{V}O_2$ peak was below 20 ml/kg/min in the initial assessment, then it increased 3.56 ml/kg/min [129].

Subsequently, between January and June 2022, a different cross-sectional study was conducted six months after the COVID-19 diagnosis, enrolling a group of 54 young COVID-19 survivors and 46 healthy young individuals [130]. The study focused on PCFS scale [127], maximum inspiratory pressure (PImax), maximum expiratory pressure (PEmax), and peripheral muscle strength by dynamometer, lung function by spirometry, dyspnoea and fatigue by modified Borg scale, and physical activity levels. The long-term respiratory and lower extremity muscle weakness compromised physical activity and a lower upper extremity muscle strength was present in young subjects with mild COVID-19 and normal lung function. They also complained of prolonged symptoms, such as fatigue (79.6%), exertional dyspnoea (50%), and cough (20.4%). The effects of respiratory, peripheral muscle training, and physical activity counselling should be investigated in patients after mild COVID-19, even in the long term [130].

The effects of a home-based respiratory muscle training for long-term post COVID-19 symptoms were investigated in another trial [131]. The training program consisted of inspiratory (IMT) or inspiratory/expiratory (respiratory) muscles (RMT). The researches also assessed the effects of these interventions on respiratory muscle function, physical and lung function, as well as on the psychological state. Totally, 88 patients complaining of fatigue and dyspnoea were randomly (1:1 ratio) assigned to IMT, IMT sham, RMT or RMT sham groups. The sham groups were given a device with no resistance.

Participants undertook a 40-munite training daily, split into two sessions, 6 times per week, over 8 weeks. The training load, regardless of whether participants performed IMT or RMT (real or sham), was individually tailored and it increased according to the same distribution schedule for both IMT and RMT.

The findings confirmed that only the RMT group showed a large and clinically important increase in QoL, while IMT and RMT interventions improved respiratory muscle function and lower limb muscle strength [131].

In 2023 the efficacy of a 3-month exercise training rehabilitation (ETR) - 2 x 60-minute sessions of ETR weekly for a total of 10 weeks - protocol on dyspnoea in LC patients with a previous CARDS (COVID ARDS) was confirmed in a multicentre randomised trial [132].
For the continuous endurance exercise, participants started at 60-70% of their maximal peak exercise capacity on a cycle ergometer. All subjects performed lower limbs, upper limb and trunk muscular strength training during each session.
Subjects in the control group received standard protocol based on low-to-moderate intensity aerobic exercise on exercise bicycle, ergometer or treadmill, strength-training for limbs and trunk muscles as well as stretching, balance exercises, electrostimulation and respiratory therapy.
A 90-day ERT improved dyspnoea compared to standard protocol, and also the physical dimension of QoL. For COVID-19 survivors with breathlessness, high-intensity exercise training may be the most suitable rehabilitation strategy [132].

A randomised controlled trial was conducted in 2023 to evaluate home-based exercise training tailored to survivors with sever/critical COVID-19. The protocol consisted of 60-80 minute/session, 3 times weekly. After conclusion of the course, the 16-week home-based exercise training confirmed the benefits in physical domains in QoL, functional capacity and prolonged symptoms in patients with previous severe/critical COVID-19, when compared to standard of care [133].

The US Physical Activity Guidelines for Americans 2nd edition in 2018 established a goal of 3 hours/ week of moderate-intensity aerobic physical activity to maximise health benefits [134].

In a retrospective observational study, Sallis et al. confirmed that patients affected by COVID-19 who were consistently inactive in the previous 24 months had a worse prognosis (hospitalisation and death) when compared to patients who were more physically active [135].

However, many previous studies have demonstrated numerous strong benefits from physical activity, especially among those who suffered from a variety of chronic diseases [136-139].

Tamburlani et al. conducted a systematic review in 2023, in which they assessed the efficacy of PR protocols in the post-acute phase of COVID-19 to improve respiratory function and physical performance, symptoms and QoL, and the severity of lung complications [140]. They found that many studies about rehabilitation had low methodological quality and significant differences due to the lack of control groups, which is necessary to better understand the long-terms effects. The studies analysed two key macro-areas: lung function and functional capacity during exercise and almost all of the them utilized various scales and tools to evaluate these areas.

The different types of training of PR considered in this systematic review included breathing, aerobic, fitness, strength, and neuropsychological aspects. Overall, in this combination PR revealed itself to be capable of improving pulmonary and muscular function, and the workout capacity and muscle strenght, fatigue status, anxiety and depression.

As Greenhalgh and colleagues considered in 2020 about management of post-acute COVID-19 in primary care, the "breathing control" technique is aimed at normalising breathing patterns and increasing the respiratory muscles efficiency (including the diaphragm), thereby reducing energy use, airway irritation, fatigue, and breathlessness [141].

It is possible that the physiological effects of Active Cycle of Breathing Techniques (ACBT) may differ slightly across different patient populations, depending on the degree of sputum production, stage of disease, and whether the patient is medically stable, or in an exacerbated state. Airway clearance techniques such as the ACBT have been shown to result in favourable outcomes in people with a wide range of lung disease, including non-CF (cystic fibrosis) bronchiectasis, [142] cystic fibrosis [143] and COPD [144].

3.2 Aim of the third study

As we found in our previous findings [81] by means of CPET, LC population could be characterised by an impaired breathing pattern with a higher diaphragm intolerance and lower efficiency than the reference controls, as confirmed by higher values of T_I/T_{TOT} and lower values of V_T/T_I on exercise [103].

As known, most of the work of breathing is performed by the diaphragm, as it is responsible for about 80% of the work of breathing; so, after illness, diaphragmatic function may be reduced and accessory respiratory neck and shoulders muscles may be more recruited [89], resulting in an abnormal breathing pattern and dyspnoea, especially when mechanical ventilation is required or in conditions of general physical deconditioning. This leads to shallow breathing, higher fatigue, breathlessness and increased energy expenditure. The tidal volume could be obtained mainly from the action of the external intercostal muscles rather than the diaphragm, generating a less efficient inspiratory cycle.

Our objectives could be considered as follows:

- to assess if ACBT and moderate physical activity (PA) can improve Long-COVID related symptoms;
- to assess if respiratory impairments are related to small airways dysfunction and if it could be improved by a pulmonary rehabilitation protocol.

In this context, we focused our last research to demonstrate if respiratory rehabilitation ACBT combined with a moderate daily PA may improve dyspnoea and fatigue in LC survivors.

3.3 Materials and Methods of the third study

The study is ongoing at "Respiratory Disease and Lung Function Unit" at University Hospital of Parma (Italy) from March 2022 until September 2023. It is a single-centre, observational, longitudinal, retrospective/prospective study.

Participants were included in the study if they were referred to the Post-acute COVID-19 outpatient clinic of University Hospital of Parma and met the following inclusion criteria: adults of either sex with a diagnosis of LC, defined as complaining of at least one symptom between dyspnoea, asthenia and cough 24 weeks after acute SARS-CoV-2 infection, and patients who signed the informed consent form.

Exclusion criteria included patients affected by other chronic respiratory diseases (i.e., asthma, pulmonary fibrosis, bronchiectasis, sarcoidosis, interstitial lung disease, pulmonary hypertension) and active smokers. Subjects who were unable to meet the criteria for acceptability and repeatability of functional testing were excluded from enrolment. This study is named "*Effetto della riabilitazione respiratoria e dell'attività fisica sui sintomi del long COVID. Studio pilota*" Protocol no. 361/2022/OSS/UNIPR ("Effect of respiratory rehabilitation and physical activity on long COVID symptoms. Pilot study") and it started after the approval of Local Ethics Committee of North Emilia (protocol no. 28421, date 06/07/2021).

3.3.1 Description of Treatment: Rehabilitation Programme

Enrolled patients were assigned to a 6-week independent home respiratory rehabilitation programme, including:

- An ACBT session to be performed twice a day, for 10 minutes per cycle;
- moderate PA consisting of at least 6000 steps per day.

Clinical and functional data were collected over a 6-week period at 2 different visits:

- baseline visit (V_1);
- follow-up visit after 6 weeks of respiratory rehabilitation (V_2).

ACBT exercises consist of breath control, deep breathing and puffing, performed in cycles until chest felt clear.

ACBT and the forced expiratory technique (FET) are commonly used to promote airway clearance in people with chronic lung disease associated with excessive mucus production. Abnormal secretion can predispose the airways to infection and inflammation, potentially leading to airway obstruction and sputum plugging. Treatments that aim to clear secretions may decrease the frequency of infections, therefore preventing further airway damage and the following worsening of lung condition [145,146].

The FET is based on one/two manoeuvres of forced expirations (huffs), followed by relaxed breath control [147], and it plays an essential role in the ACBT, along with chest expansion exercises and breath control periods [148].

A typical ACBT cycle is a simple breathing technique that involves seven-phase active cycle represented by alternating: breath control, three or four chest expansions, two-minute of breath control again, forced expiration and huff. It is possible to vary number and frequency of each component, but it is essential that all of these occur and that they are separated by breath control. It is thought that by altering thoracic pressures and airway dynamics, the forced expiratory manoeuvres (low- and high- volume huffing) promote secretion movement [149,150].

Below are the summarising methods of each of the phases that make up the cycle:

1. Breath control: start by breathing calmly with minimal effort for two minutes;
2. Chest expansion: inhale slowly and deeply, possibly through the nostrils; then exhale gently, as if breathing a sigh. Repeat the expansion four or five times;
3. Breath control: breathe calmy for two minutes;
4. Forced exhalation: exhale deeply and blow out the air quickly and forcefully. Repeat forced exhalation three times;
5. Huffing: exhale through open mouth and throat. Repeat three times;
6. Breath control: breathe calmly for two minutes;
7. Chest expansion: five times.

Following the protocol study, participants repeated ACBT twice daily. Each session lasted ten minutes, for a total of 20 mins per day. Specific information about ACBT was provided in the leaflet given to the patients [150] (Fig. 15).

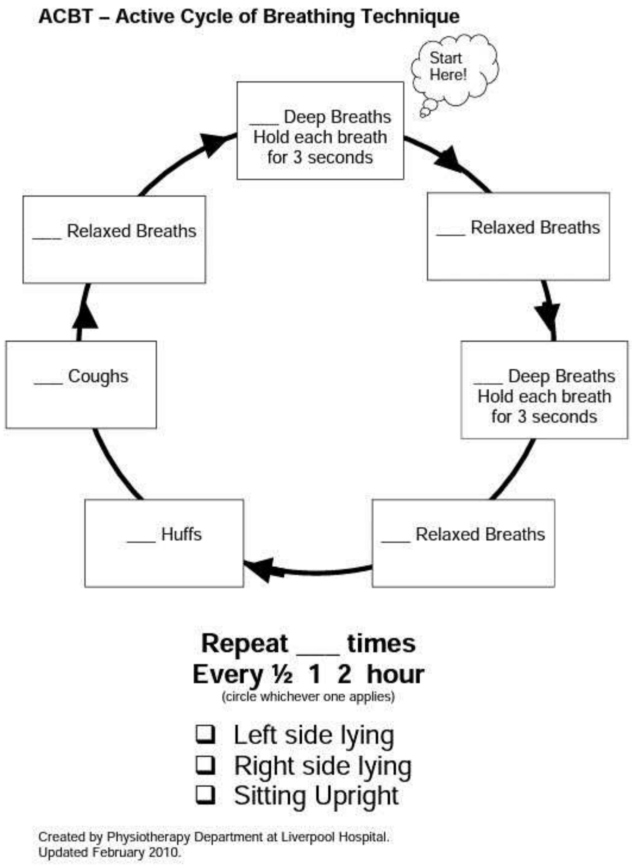


Figure 15. Leaflet handed out to patients with explanation of individual ACBT sequences (Phsysiotherapy Department at Liverpool Hospital. Updated February 2010) [150].

Breathing control has been reported to prevent bronchospasm and oxygen desaturation, whereas chest expansion exercises help loosen and clear secretions and improve collateral ventilation.

The "breathing control" technique is designed to normalise breathing patterns and increase the efficiency of the respiratory muscles (including the diaphragm) resulting in less energy expended, less airway irritation, reduced fatigue, and improved breathlessness.

The patient should sit in a supported position and inhale and exhale slowly, preferably inhaling through the nose and exhaling through the mouth, while relaxing the chest and shoulders and lifting the abdomen. The aim should be to achieve an inspiration/expiration ratio of 1:2, using this technique frequently throughout the day, in five- or ten-minute sessions (or longer if beneficial). For different breathing strategies - such as diaphragmatic breathing, slow deep breathing, puckered lip breathing, yoga techniques, Buteyko - specialist advice is needed to be personalised for each patient [141,151] (Fig. 16).

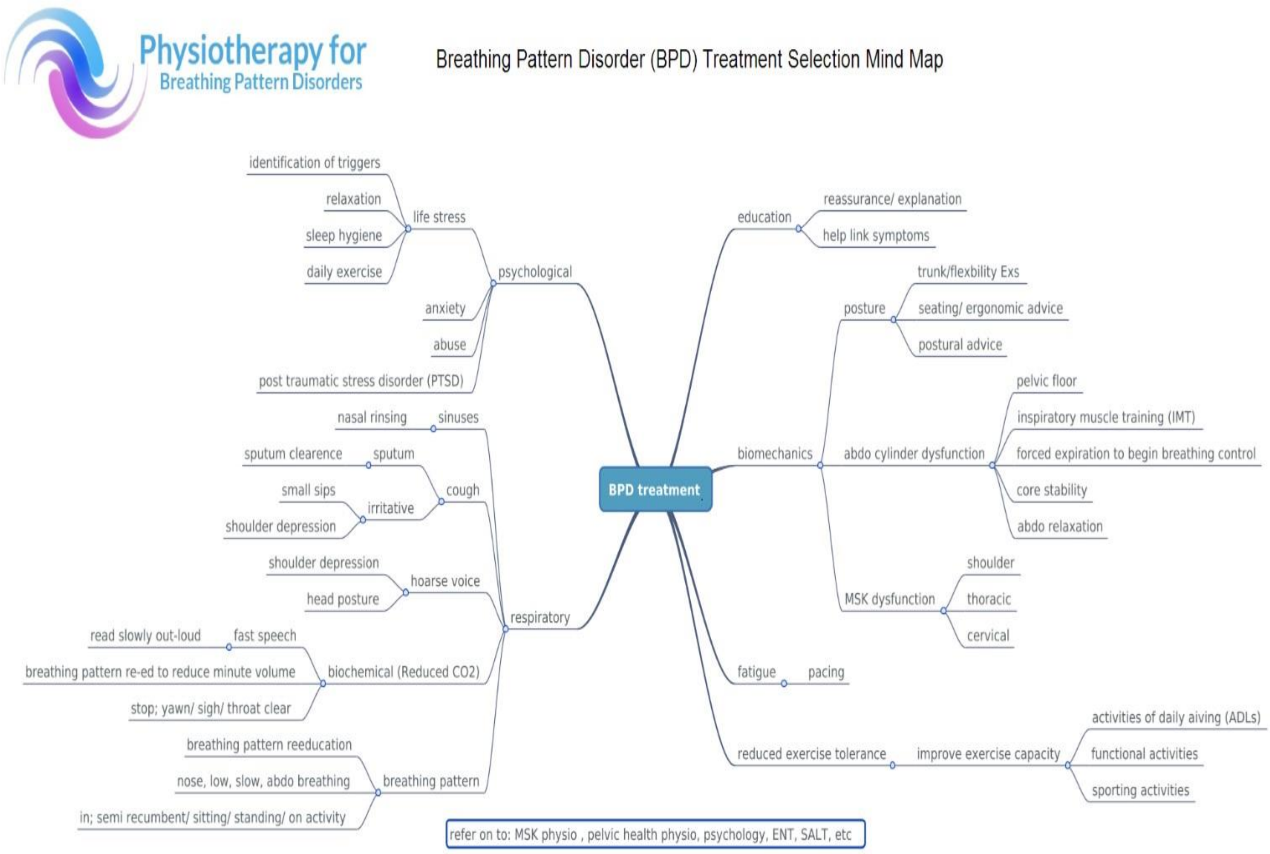


Figure 16. Breathing Pattern Disorder (BPD). Treatment Selection Mind Map, (https://www.physiotherapyforbpd.org.uk)—a [152].

For physical activity, the threshold of 6000 steps per day represents the recommended distance for normal physical activity [153,154].

Distance travelled was tracked with the use of digital tools, such as pedometer applications for smartphones.

To assess the patient compliance with the rehabilitation protocol, it was recommended that a daily diary be kept to record the performance or non-performance of both breathing exercise and walking.

3.3.2 Questionnaires

At the two study protocol assessments (V_1 and V_2) anthropometric and functional parameters were collected including age, gender, BMI and smoking habit.

A physical examination was performed on each participant to record vital signs, such as heart rate, respiratory rate, oxygen saturation and blood pressure.

Several questionnaires were administered to the participants:

• Asthenia Assessment Questionnaire using Visual Analogue Scale - VAS [155];

• Dyspnoea assessment questionnaire by modified Medical Research Council Dyspnoea – mMRC [156];

• Questionnaire to assess the impact of cough on daily life: Leicester Cough Questionnaire - LCQ [157,158];

• Questionnaire to assess health: EuroQoL [159].

The VAS scale, the mMRC Dyspnoea and the LCQ were administered to the patients in both V_1 and V_2 while EuroQoL was only considered only in V_1.

The VAS scale [155] was used to assess fatigue/asthenia. The scale is characterised by a horizontal segment on which a progressive numerical sequence from zero to ten is shown. Zero corresponds to "no fatigue" and 10 corresponds to "extreme fatigue". The patient is asked to mark an X on the number corresponding to his or her perceived state of fatigue (Fig.17).

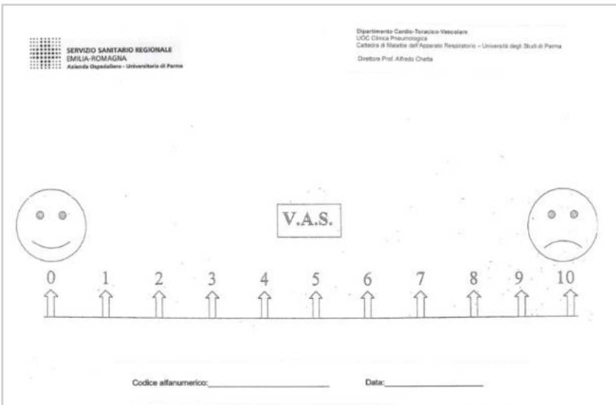


Figure 17. VAS scale for fatigue assessment.

Modified Medical Research Council Dyspnoea – mMRC [156] is a questionnaire used to evaluate the degree of dyspnoea could be:

- Grade 0: breathlessness only on strenuous exercise;
- Grade 1: breathless when hurrying on the level or walking up a slight hill;

- Grade 2: walking slower than the other people of same age on the level ground due to shortness of breath or need to stop for breath when walking at own pace;
- Grade 3: short of breath after walking for a few minutes on the level ground or about 90 meters;
- Grade 4: too breathless to leave the house, or breathless when dressing or undressing.

The patient is invited to mark an X on the grade corresponding to his/her state of perceived dyspnoea (Fig. 18).

Questionario Modificato del *Medical Research Council* per la valutazione del grado di dispnea

PER FAVORE BARRARE LA CASELLA CHE VI RIGUARDA (UNA SOLA)

mMRC Grado 0. Ho dispnea solo per sforzi intensi ☐

mMRC Grado 1. Mi manca il fiato se cammino veloce (o corsa) in piano o in lieve salita ☐

mMRC Grado 2. Su percorsi piani cammino più lentamente dei coetanei, oppure ho necessità di fermarmi per respirare quando cammino a passo normale ☐

mMRC Grado 3. Ho necessità di fermarmi per respirare dopo aver camminato in piano per circa 100 metri o per pochi minuti ☐

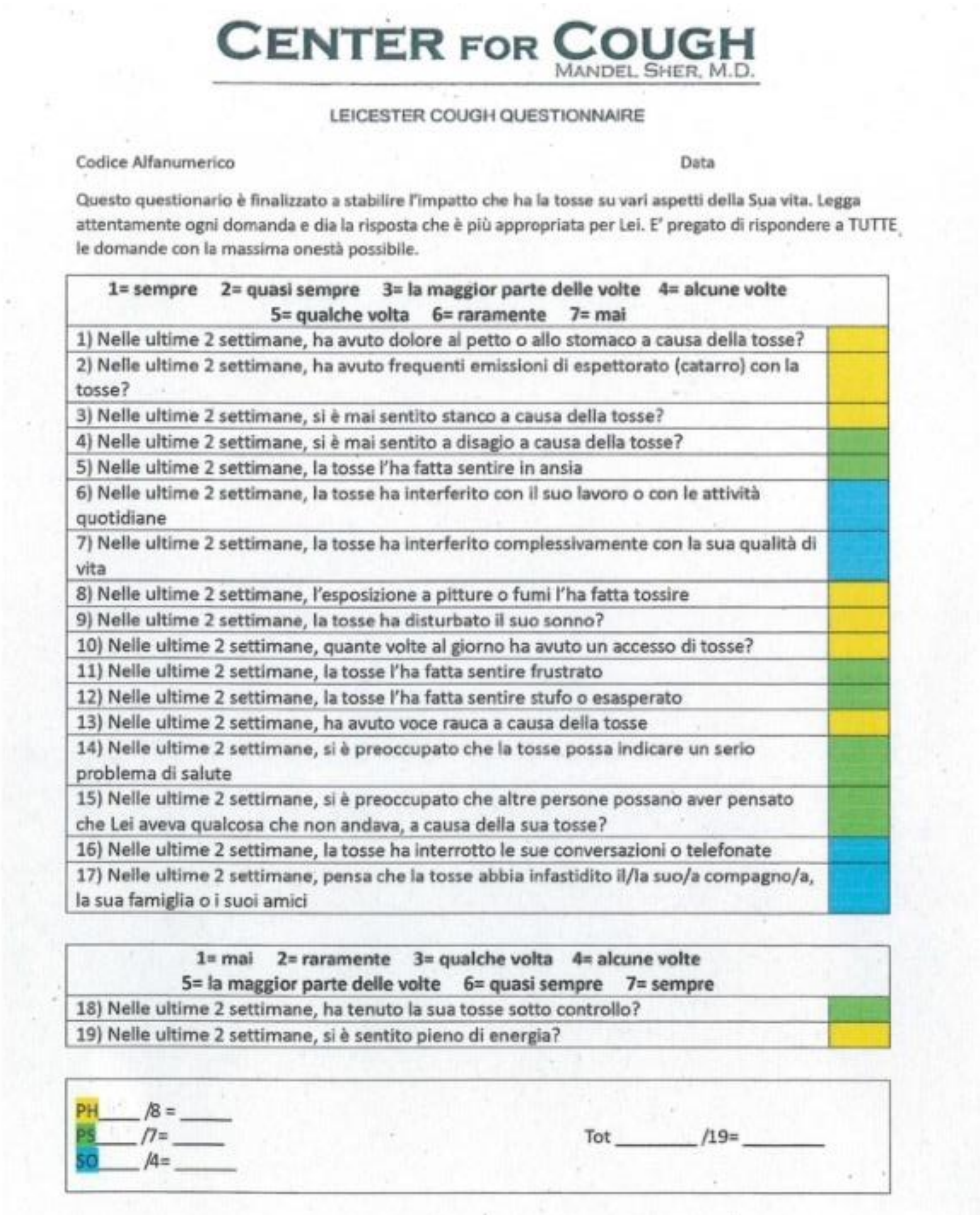
mMRC Grado 4. Mi manca il fiato a riposo per uscire di casa o per vestirmi/spogliarmi ☐

Figure 18. modified Medical Research Council Dyspnoea – mMRC (reported in Italian).

Leicester Cough Questionnaire [157,158] is a questionnaire used to assess the impact of cough on different aspects of daily life.

It consists of 19 questions on the impact of coughing, to which the patient answers with a number from one to seven.

Specifically, for the first 17 questions, answer 1 corresponds to “always” while answer 7 corresponds to “never”. For the last 2 questions, answer 1 means “never” while answer 7 means “always” (Fig. 19).



CENTER FOR COUGH
MANDEL SHER, M.D.

LEICESTER COUGH QUESTIONNAIRE

Codice Alfanumerico Data

Questo questionario è finalizzato a stabilire l'impatto che ha la tosse su vari aspetti della Sua vita. Legga attentamente ogni domanda e dia la risposta che è più appropriata per Lei. E' pregato di rispondere a TUTTE le domande con la massima onestà possibile.

1= sempre 2= quasi sempre 3= la maggior parte delle volte 4= alcune volte 5= qualche volta 6= raramente 7= mai	
1) Nelle ultime 2 settimane, ha avuto dolore al petto o allo stomaco a causa della tosse?	
2) Nelle ultime 2 settimane, ha avuto frequenti emissioni di espettorato (catarro) con la tosse?	
3) Nelle ultime 2 settimane, si è mai sentito stanco a causa della tosse?	
4) Nelle ultime 2 settimane, si è mai sentito a disagio a causa della tosse?	
5) Nelle ultime 2 settimane, la tosse l'ha fatta sentire in ansia	
6) Nelle ultime 2 settimane, la tosse ha interferito con il suo lavoro o con le attività quotidiane	
7) Nelle ultime 2 settimane, la tosse ha interferito complessivamente con la sua qualità di vita	
8) Nelle ultime 2 settimane, l'esposizione a pitture o fumi l'ha fatta tossire	
9) Nelle ultime 2 settimane, la tosse ha disturbato il suo sonno?	
10) Nelle ultime 2 settimane, quante volte al giorno ha avuto un accesso di tosse?	
11) Nelle ultime 2 settimane, la tosse l'ha fatta sentire frustrato	
12) Nelle ultime 2 settimane, la tosse l'ha fatta sentire stufo o esasperato	
13) Nelle ultime 2 settimane, ha avuto voce rauca a causa della tosse	
14) Nelle ultime 2 settimane, si è preoccupato che la tosse possa indicare un serio problema di salute	
15) Nelle ultime 2 settimane, si è preoccupato che altre persone possano aver pensato che Lei aveva qualcosa che non andava, a causa della sua tosse?	
16) Nelle ultime 2 settimane, la tosse ha interrotto le sue conversazioni o telefonate	
17) Nelle ultime 2 settimane, pensa che la tosse abbia infastidito il/la suo/a compagno/a, la sua famiglia o i suoi amici	

1= mai 2= raramente 3= qualche volta 4= alcune volte 5= la maggior parte delle volte 6= quasi sempre 7= sempre	
18) Nelle ultime 2 settimane, ha tenuto la sua tosse sotto controllo?	
19) Nelle ultime 2 settimane, si è sentito pieno di energia?	

PH____ /8 = _____
PS____ /7= _____
SO____ /4= _____

Tot ________ /19= ________

Figure 19. Leicester Cough Questionnaire, questionnaire assessing the impact of cough on daily living activities (reported in Italian).

EuroQoL is a useful questionnaire to assess health status/condition [159]. It consists of two parts. In the first part five multiple choice questions are present: the first three questions concerning daily activities (mobility, personal care and usual activities) and the participant is invited to mark an X on the answer corresponding to his or her level of difficulty in carrying them out. The fourth question is related to assessment of pain or discomfort; the last fifth question is about anxiety or depression. The second part of the questionnaire consists of a vertical numerical scale from zero to 100 where zero corresponds to “the worst health” and 100 corresponds to “the best health”. The patient is invited to mark an X on the appropriate number (Fig. 20 and Fig. 21).

Sotto ciascun argomento, faccia una crocetta sulla casella (UNA SOLA) che descrive meglio la sua salute OGGI.

CAPACITÀ DI MOVIMENTO

Non ho difficoltà nel camminare ☐
Ho lievi difficoltà nel camminare ☐
Ho moderate difficoltà nel camminare ☐
Ho gravi difficoltà nel camminare ☐
Non sono in grado di camminare ☐

CURA DELLA PERSONA

Non ho difficoltà nel lavarmi o vestirmi ☐
Ho lievi difficoltà nel lavarmi o vestirmi ☐
Ho moderate difficoltà nel lavarmi o vestirmi ☐
Ho gravi difficoltà nel lavarmi o vestirmi ☐
Non sono in grado di lavarmi o vestirmi ☐

ATTIVITÀ ABITUALI (*per es. lavoro, studio, lavori domestici,attività familiari o di svago)*

Non ho difficoltà nello svolgimento delle attività abituali ☐
Ho lievi difficoltà nello svolgimento delle attività abituali ☐
Ho moderate difficoltà nello svolgimento delle attività abituali ☐
Ho gravi difficoltà nello svolgimento delle attività abituali ☐
Non sono in grado di svolgere le mie attività abituali ☐

DOLORE O FASTIDIO

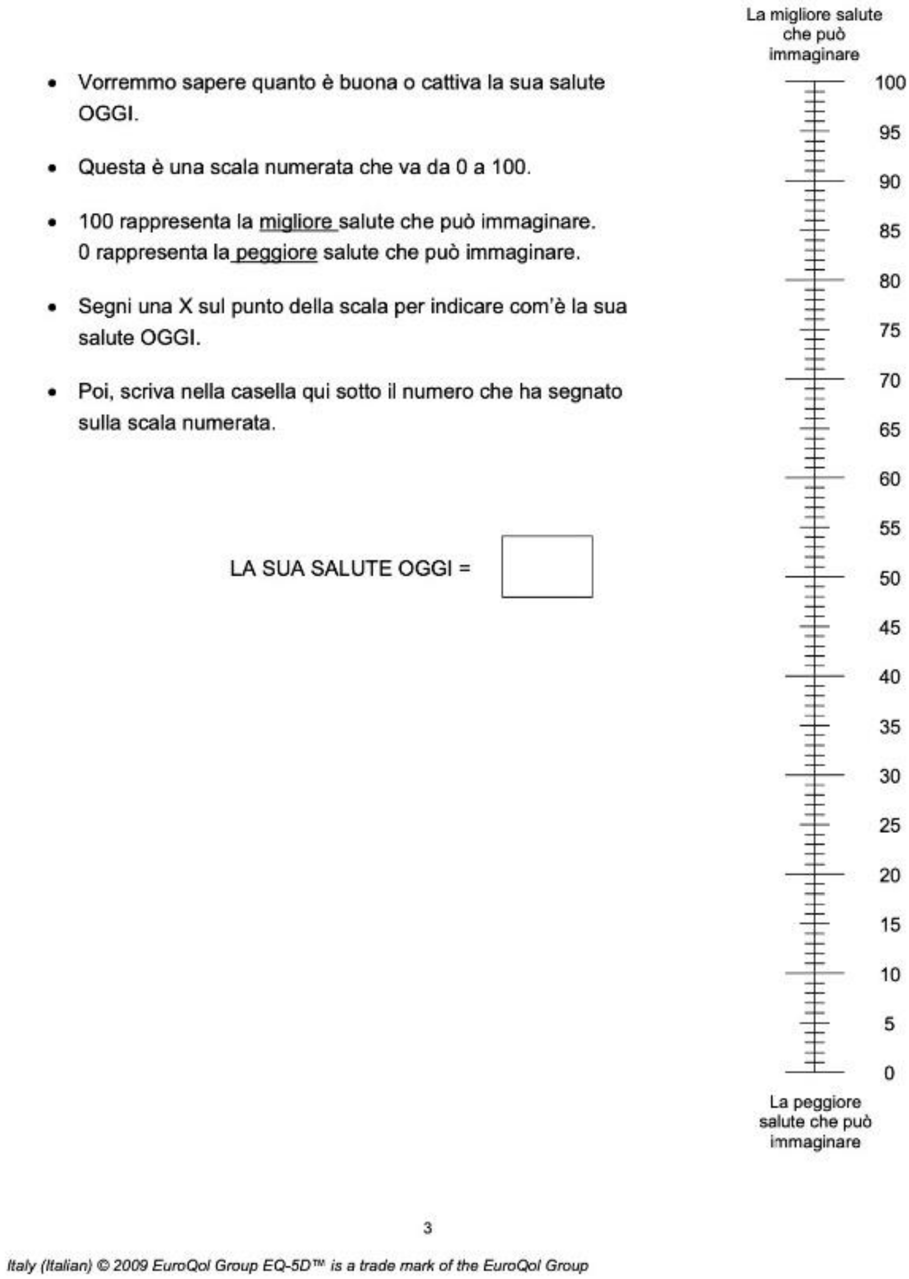
Non provo alcun dolore o fastidio ☐
Provo lieve dolore o fastidio ☐
Provo moderato dolore o fastidio ☐
Provo grave dolore o fastidio ☐
Provo estremo dolore o fastidio ☐

ANSIA O DEPRESSIONE

Non sono ansioso/a o depresso/a ☐
Sono lievemente ansioso/a o depresso/a ☐
Sono moderatamente ansioso/a o depresso/a ☐
Sono gravemente ansioso/a o depresso/a ☐
Sono estremamente ansioso/a o depresso/a ☐

2

Figure 20. First part (reported in Italian) of EuroQoL questionnaire [159].



- Vorremmo sapere quanto è buona o cattiva la sua salute OGGI.
- Questa è una scala numerata che va da 0 a 100.
- 100 rappresenta la migliore salute che può immaginare.
  0 rappresenta la peggiore salute che può immaginare.
- Segni una X sul punto della scala per indicare com'è la sua salute OGGI.
- Poi, scriva nella casella qui sotto il numero che ha segnato sulla scala numerata.

LA SUA SALUTE OGGI =

3

Italy (Italian) © 2009 EuroQol Group EQ-5D™ is a trade mark of the EuroQol Group

Figure 21. Second part (reported in Italian) of EuroQoL questionnaire [159].

In order to evaluate the primary objective ("to assess if ACBT and moderate physical activity can improve Long-COVID related symptoms") questionnaires were administered to patients (VAS scale, mMRC and LCQ).

3.3.3 Functional Assessment: Impulse Oscillometry System (IOS), Spirometry and Multiple-Breath Nitrogen Washout (MBNW)

Patients underwent also functional respiratory assessment of central airways by spirometry (FEV_1, FVC, and FEV_1/FVC) and small airways by IOS and MBNW regarding the secondary objective ("to assess if respiratory impairments are related to small airways dysfunction and if it could improve after rehabilitation protocol").

Impulse Oscillometry System (IOS)

The Impulse Oscillometry System (IOS) measures the lung mechanical properties during quiet tidal breathing by the application of minimal pressures at the mouth.
Airway resistance detected at 5 Hz (R5) and 20 Hz (R20) (expressed in KPa s l^{-1}) represent indices of total and proximal airway resistance, respectively, whereas the fall in resistance from 5 Hz to 20 Hz (R5 – R20) is considered measure of the small airway resistances [160].
A measure of this index greater than 0.070 KPa s l^{-1} was chosen to identify the presence of small airway disease (SAD) [161-163].
The device may provide parameters that are complementary to more traditional measure of lung function, such as spirometry, lung volume, or diffusing capacity [160]. In fact, IOS has revealed to detect SAD even in the setting of normal spirometry. Surprisingly, in smoker symptomatic population with normal spirometry, IOS revealed peripheral airway dysfunction related to wheeze [164].
Participants underwent IOS according to current recommendations [160] by means of the Jaeger MasterScreen–IOS (CareFusion Technologies, San Diego, CA, USA). Participants were seated and asked to wear a nose clip while breathing gently. Mean values of at least three 30-second trails were assessed.
The reactance detected at 5 Hz (X5, expressed as KPa s l^{-1}) and the integrated area of low-frequency reactance (AX, expressed as kPa·l^{-1}) from 5 Hz to resonant frequency (F_{Res}, expressed as Hz) were also considered as markers of peripheral airway abnormalities.

Spirometry

Spirometry was performed after IOS to avoid compromising peripheral airway tone during forced expiration manoeuvre. A flow-sensing spirometer connected to a computer for data analysis (V_{max}22 and 6,200; SensorMedics, Yorba Linda, CA, USA) according to the guidelines [82].

FEV_1, FVC, FEF_{25-75}, FEF_{50} and FEF_{75} were recorded and expressed as absolute values (L) and as a percentage of predicted value (% pred). The presence of at least two of three forced expiratory flows, i.e., FEF_{25-75}, FEF_{50} and FEF_{75} less than 65% of predicted values was detected SAD [165].
The FEV_1/ FVC and FEV_3/ FEV_6 values was calculated according the equation of Hansen et al. [166]. A FEV_3/ FEV_6 value less than the lower limit of normal was considered index of SAD [167].

Multiple-Breath Nitrogen Washout testing

Multiple-breath nitrogen washout (MBNW) was performed according to a standard procedure [168].

Participants sat with a nose clip and lips sealed around a mouthpiece connected to a gas analyser (EXHALYZER® D, ECO MEDICS AG, Dürnten, Switzerland). They breathed 100% oxygen from the end-expiratory lung volume until the nitrogen concentration in the exhaled volume reached $1/40^{th}$ or 2.5% of the initial concentration of lung resident nitrogen for three consecutive breaths, while breathing with a fixed tidal volume and respiratory rate. The duration of each test was approximately two to ten minutes and was carried out a minimum of two times to ensure reproducibility.

Lung clearance index (LCI), as well as indices of conductive (S_{cond}) and acinar (S_{acin}) ventilation heterogeneity and ventilated Functional Residual Capacity o FRC (FRCMBNW), is considered to be an index of global ventilation inhomogeneity. LCI was defined as the ratio of the cumulative expired volume of the inert gas to the FRCMBNW. S_{cond} and S_{acin} (in L^{-1}) were derived from the phase III slopes of the nitrogen spirogram. The values rise with increasing ventilation heterogeneity [169]. Reference values for the results of MBNW in adults are limited, despite the growing availability of MBNW [170].

3.4 Statistical Analysis of the third study

Due to the explorative nature of the study no formal sample size calculation was performed.
Continuous variables characterized by normal distribution were reported as means ± SD, whereas continuous variables characterized by non-normal distribution were reported as medians and IQR. The number of participants and percentage were reported as categorical variables.
Comparisons between variables were determined by unpaired *t-test* or by Chi-square test, when appropriate.
Concerning the correlation analysis, the Pearson (r) correlation coefficient will be used for linear or normally distributed variables; the Spearman (ρ) correlation coefficient will be used for not linear or not normally distributed variables.

A $p< 0.05$ was taken as significant. Statistical analysis and diagrams were obtained by Prism 9.5 (©2018 GraphPad Software, La Jolla, CA, USA).

3.5 Results of the third study

In our research study a total of 33 LC patients were enrolled and completed the protocol.
The first participant was recruited on 5 April 2022 and the last patient was enrolled on 26 July 2023. As the study is ongoing, the results are about patients who completed their rehabilitation in September 2023.

In our study population of 33 patients (13 female), mean age 56.76 ± 10.74 years, aged between 30 and 80 years, mean BMI (pre-rehabilitation) 27.75 ± 4.88 Kg/m^2 with a BMI between 18.4 and 38.97 kg/m^2, no one reports history of active smoking. It should be noted that 22 out of 33 participants needed hospitalisation during the acute phase of the disease, while the period of time since infection is mean 21.27 ± 7.94 months. Anthropometric data of 33 patients are shown in Table 3.

Table 3. Characteristics of the 33 Long-COVID patients.

Gender (M/F)	20/13
Age (years)	56.76 ± 10.74
BMI (Kg/m^2) (baseline)	27.75 ± 4.88
BMI (Kg/m^2) (follow-up)	27.63 ± 4.81
Smoking habit (no/yes/former/second-hand)	19/0/13/1
Hospitalisation (yes/no)	22/11
Period of time since infection (months)	21.27 ± 7.94

Clinical and functional parameters were assessed after and before the rehabilitation protocol.

It should be noted that the most statistically findings in symptom control after the rehabilitation concern dyspnoea by mMRC, health status by VAS scale (0-100) and LCQ (p= 0.0032, p= 0.0416, p=0.0001, respectively) (Table 4) (Fig. 22, Fig. 23 and Fig. 24).
Perceived fatigue, as measured by the VAS scale (0-10) did not change significantly (p= 0.1195), although a trend of improvement can be described.

In terms of functional parameters, lung volumes could be considered in a normal range; only the average FEF_{75} value, seems below the normal limit (65% pred) at baseline.

Regarding peripheral airway function, it is noteworthy that R5-R20 (0.074±0.097 vs 0.053±0.071 KPa s l^{-1}), AX index (0.62 ± 0.88 vs. 0.53 ± 0.59 KPa s l^{-1}), and LCI (8.51 ± 2 vs. 8.39±1,77), all improved after the rehabilitation protocol, although not statistically (p= 0.1737, p=0.4243, p=0.9056, respectively) (Fig. 25, Fig. 26 and Fig. 27). At baseline, about one third of study population is characterised by small airway dysfunction (R5-R20 ≥ 0.07 KPa s l^{-1}) and improved after rehabilitation. Contrasting data are obtained in terms of FEF_{25-75} e FEF_{75}, showing a functional worsening, while expiratory dynamic lung volumes (FVC and FEV_3) improved at follow-up.

Table 4. Clinical and functional values of the 33 Long-COVID patients (table continued on the next page).

	Pre-Rehabilitation	Post-Rehabilitation	*p*
mMRC (0-4)	1 (0.5 - 1)	0 (0 - 1)	***0.0032***
VAS fatigue scale (0- 10)	4 (3 - 6)	3 (1.5 – 6.5)	*0.1195*
VAS health status scale (0-100)	70 (52.5 - 80)	75 (62.5 – 87.5)	***0.0416***
LCQ (Tot/19)	6.79 (6.58 – 6.95)	6.80 (6.68 – 6.95)	***0.0001***
FVC (% pred)	96.56 ± 11,46	98.61 ± 11.88	*0.0770*
FEV_1 (% pred)	95.57 ± 11.96	95.53 ± 12.25	*0.9748*
FEV_1/FVC (%)	78.79 ± 7.03	77.04 ± 6.61	***0.0021***
FEV_3 (% pred)	95.54 ± 11.03	96.20 ± 11.36	*0.4971*
FEV_3/FEV_6 (%)	94.23 ± 2.40	94.17 ± 2.11	*0.6683*

FEF_{50} (%)	90.26 ± 34.32	85.31 ± 32.77	*0.0951*
FEF_{25-75} (%)	92.08 ± 36.10	85.10 ± 37.06	***0.0095***
FEF_{75} (%)	68.30 ± 34.27	58.97 ± 32.29	***0.0091***
R5-R20 (KPa s l^{-1})	0.074±0.097	0.053±0.071	*0.1737*
X5 (KPa s l^{-1})	-0.11 ± 0.05	-0.12 ± 0.06	*0.3060*
AX (KPa l^{-1})	0.62 ± 0.88	0.53 ± 0.59	*0.4243*
LCI 2.5%	8.51 ± 2	8.39 ± 1.77	*0.9056*

The values of mMRC scale, VAS fatigue scale, VAS health status scale and LCQ are expressed as median and quartiles (25% - 75% percentile). Values for spirometry (FVC, FEV1, FEV1/FVC, FEV_3, FEV_3/FEV_6, FEF_{50}, FEF_{25-75}, FEF_{75}), Impulse Oscillometry (R5-R20, X5, AX) and Nitrogen Washout (LCI) are expressed as mean ± standard deviation. In bold significant comparisons.

Among different functional parameters (questionnaires, spirometry, IOS or MBNW) mMRC only correlates with R5-R20 (r= 0.4084, p= 0.0183) and AX (r = 0.3953, p = 0.0228) (Table 5).

Table 5. Correlations between mMRC and functional parameters in 33 Long-COVID patients. In bold significant correlations (table continued on the next page).

	mMRC
R5-R20	*r = 0.4084* ***p = 0.0183***
AX	*r = 0.3953* ***p = 0.0228***
X5	*r = -0.3141* *p = 0.0750*
FEV_1	*r = -0.1907* *p = 0.2878*

LCI	*r = 0.2584* *p = 0.1604*
FEV_1/FVC	*r = 0.08195* *p = 0.6503*
FEF_{50}	*r = -0.08863* *p = 0.6238*
FEV_3	*r = -0.2604* *p = 0.1432*

.

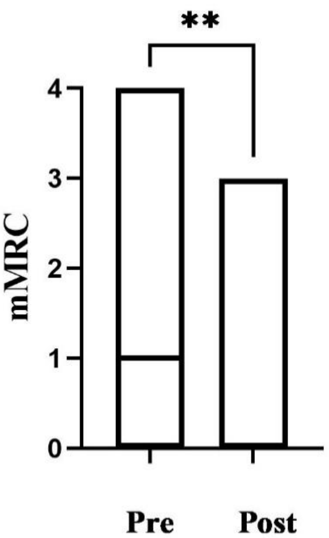


Figure 22. Median and range of mMRC values before and after the rehabilitation programme in 33 Long-COVID patients. Median and minimum value of mMRC coincide in patients assessed post-rehabilitation.

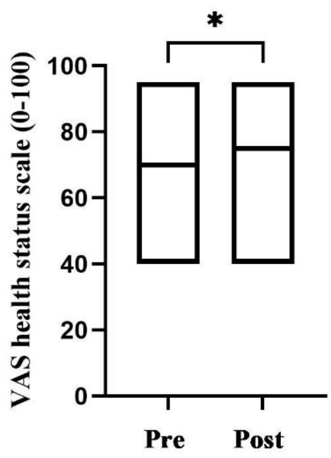


Figure 23. Median and range of VAS health status (0-100) values before and after the rehabilitation programme in 33 Long-COVID patients.

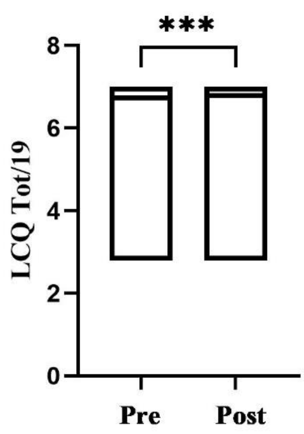


Figure 24. Median and range of LCQ Tot/19 values before and after the rehabilitation programme in 33 Long-COVID patients.

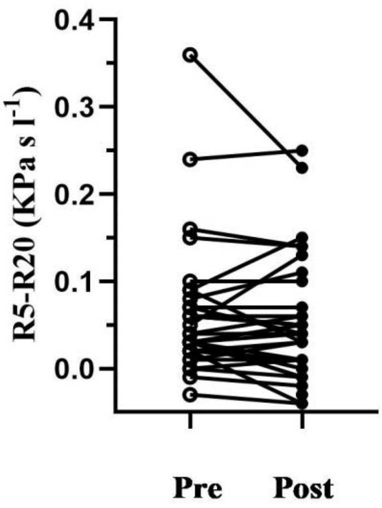


Figure 25. Individual R5-R20 values measured before and after the rehabilitation programme.

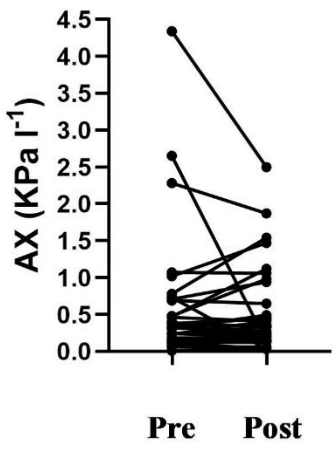


Figure 26. Individual AX values measured before and after the rehabilitation programme.

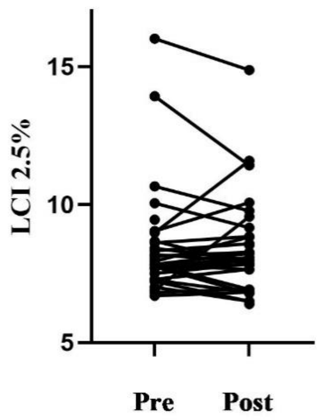


Figure 27. Individual LCI 2.5% values measured before and after the rehabilitation programme.

3.6 Discussion of the third study

For many individuals, surviving the acute phase of COVID-19 does not mean full recovery, but may represent the beginning of clinical and radiological sequelae for several weeks. So-called Long-COVID is a condition that can consists of a wide range of symptoms of varying intensity and can be highly disabling, with a negative impact on the quality of life of those affected. The most common symptoms are dyspnoea, cough and fatigue. According to data from a meta-analysis by Chen et al. [171], the overall prevalence of Post COVID-19 was 43% and was higher in hospitalised patients during the acute phase than in non-hospitalised patients (54% vs. 34%).

The wide variability of symptoms, age range and epidemiological importance suggest a multidisciplinary approach.
In our study, we selected adult patients with predominantly respiratory symptoms such as cough, dyspnoea and fatigue. The rehabilitation programme we proposed is simple and easily to do at home, as it was self-managed.

Our preliminary data showed that the perception of dyspnoea, healthy status and cough were significantly reduced at the end of the rehabilitation approach.

Patients presented with spirometric functional parameters in the normal range overall, but with increased resistance of the small airways at baseline measured by oscillometric technique and ventilation heterogeneity by means of multiple-breath washout.

At baseline, about one third of study population is characterised by small airway dysfunction (R5-R20 ≥ 0.07 KPa s l^{-1}) and improved after rehabilitation.

We could consider that the functional parameters, lung volumes could be in a normal range at baseline in a cohort of LC patients complained of respiratory symptoms.
The contrasting results with partial worsening lung function after rehabilitation are of statistical but not clinical significance, given the significant improvement in the perception of dyspnoea, fatigue and cough; however, average baseline score of R5-R20 is above normal limits, indicating improvement in line with the perceptual spectrum of symptoms.

It should also be emphasized that mMRC correlates positively and significantly with R5-R20 and AX, the parameters that express distal airway function. The higher the degree of perception of dyspnoea, therefore, the greater the dysfunction of the small airways.

Given these preliminary results, the positive effects of rehabilitation on lung function in LC patients have yet to be fully defined.

Several studies in the literature assessed respiratory rehabilitation [121,141]. The conclusions confirmed that a rehabilitation programme is considered useful and safe for improving physical performance and quality of life, with little effect on lung function.

In addition, a retrospective study [114] suggests that specific physiotherapy practices may also benefit COVID-19 patients in the acute phase by facilitating return to ambulation and discharge.

Our study lacks a control group of patients treated with placebo. However, it is not easy to implement a placebo treatment in rehabilitation trials. On the other hand, as this is a pragmatic, real-world study, a placebo arm was not planned.
It is also important to consider the ability of participants to adhere to the rehabilitation programme throughout the proposed period, as well as the practicality of implementing the protocol in hospital settings that have previously been affected by the pandemic.

As mentioned above, Tamburlani et al. conducted a systematic review in 2023, in which they assessed the efficacy and benefits of PR protocols in the post-acute phase of COVID-19 to improve respiratory function and physical performance, symptoms and QoL in COVID-19 and also to improve the severity of lung complications [140]. They found that many studies about rehabilitation had low methodological quality and significant differences due to the lack of control groups, which is necessary to better understand the long-term effects. For the assessment of pulmonary function, another outcome analysed by most of the studies considered, the same instruments were used; however, heterogeneous results were obtained.

The findings indicate that a comprehensive rehabilitation programme, comprising respiratory physiotherapy, aerobic training as well as strength and resistance training, provides benefits in alleviating the common repercussions in COVID-19 patients. However, due to the limited number of high-quality studies in the literature, there is insufficient evidence to establish a preferred or elective protocol.

Our study has important clinical implications and we can certainly conclude that a rehabilitation programme including breathing exercises and physical activity can be supportive for Long-COVID patients. In addition to improving health status, it may encourage the resumption of outdoor physical activities, which are limited by the variable period of isolation. This approach could also be particularly useful for older patients who have lost the ability to perform daily activities.

Therefore, it is advisable to increase the implementation of comprehensive and personalized rehabilitation protocols for COVID-19 patients, with a significant portion dedicated to respiratory rehabilitation to regain overall functionality and improve quality of life.

3.7 Conclusion of the third study

Our study is effective in demonstrating that rehabilitation protocol including breathing exercises and physical activity could improve the overall symptoms of dyspnoea, health status and cough.

We also emphasized the role of the small airways, that were found to be impaired in about one third of LC population. Peripheral airways shouldn’t be considered just simple air passages, but also an important determinant of lung mechanics [172].

The consequences of SARS-CoV-2 infection are far-reaching, and the impact on the respiratory system has been a major focus in Long-COVID condition.

Until now, non-drug interventions have focused on breath control, breathing pattern training, inspiratory muscle training, exercise-based rehabilitation and various forms of exercise rehabilitation, modified from those used in other respiratory disorders.

While the efficacy of nonpharmacologic options is still being investigated, it is likely that Long-COVID individuals will be best served by an integrative multidisciplinary approach with pulmonologists and rehabilitation specialists in the centre of this team using therapeutic and rehabilitative strategies on individual patient's condition and needs, and ensuring cultural appropriateness and equity of access to different communities.

References

1. World Health Organization. Coronavirus disease (COVID-19) outbreak. www.who.int
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020 Feb 20; 382 (8):727-33
3. The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. The Epidemiological Characteristics of an Outbreak of 2019 Novel Coronavirus Diseases (COVID-19) — China, 2020[J]. China CDC Weekly, 2020, 2(8): 113-122
4. Critical Care Committee of Chinese Association of Chest Physician, Respiratory and Critical Care Group of Chinese Thoracic Society, Respiratory Care Group of Chinese Thoracic Society. Zhonghua Jie He He Hu Xi Za Zhi. Conventional respiratory support therapy for Severe Acute Respiratory Infections (SARI): Clinical indications and nosocomial infection prevention and control. 2020 Feb 16;43(0):E015
5. Gattinoni L, Coppola S, Cressoni M, et al. Covid-19 Does Not Lead to a "Typical" Acute Respiratory Distress Syndrome (published online ahead of print, 2020 Mar 30). Am J Respir Crit Care Med. 2020;10.1164/rccm.202003- 0817LE.
6. Simpson S, Kay FU, Abbara S, Bhalla S, Chung JH, Chung M, Henry TS, Kanne JP, Kligerman S, Ko JP, Litt H. Radiological Society of North America Expert Consensus Statement on Reporting Chest CT Findings Related to COVID-19. Endorsed by the Society of Thoracic Radiology, the American College of Radiology, and RSNA - Secondary Publication. J Thorac Imaging. 2020 Jul;35(4):219-227. doi: 10.1097/RTI.0000000000000524. PMID: 32324653; PMCID: PMC7255403
7. Zhou, P., Yang, X., Wang, X. et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273 (2020)
8. Harmer D, Gilbert M, Borman R, Clark KL, Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett 2002; 532: 107–10.
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China [published correction appears in Lancet. 2020 Jan 30;:]. Lancet. 2020;395(10223):497-506
10. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China [published correction appears in Intensive Care Med. 2020 Apr 6]. Intensive Care Med. 2020;46(5):846-848
11. Wu C, Chen X, Cai Y, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China [published online ahead of print, 2020 Mar 13]. JAMA Intern Med. 2020;180(7):1-11
12. Jose RJ, Manuel A. COVID-19 cytokine storm: the interplay between inflammation and coagulation. Lancet Respir Med. 2020;8(6):e46-e47
13. Delgado-Roche L, Mesta F. Oxidative Stress as Key Player in Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Infection. Arch Med Res. 2020;51(5):384-387
14. Yang Y, Shen C, Li J, et al. Plasma IP-10 and MCP-3 levels are highly associated with disease severity and predict the progression of COVID-19. J Allergy Clin Immunol. 2020;146(1):119-127.e4
15. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020 Feb 15; 395 (10223): 507 -13

16. Guo T, Fan Y, Chen M, et al. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19) Jama Cardiol. March 27, 2020.
17. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med. 2017; 377:1119–1131.
18. Zhang L, Yan X, Fan Q, Liu H, Liu X, Liu Z, Zhang Z. D-dimer levels on admission to predict in-hospital mortality in patients with Covid-19. J Thromb Haemost. 2020 Jun;18(6):1324-1329. doi: 10.1111/jth.14859. PMID: 32306492; PMCID: PMC7264730.
19. Diao B, Wang C, Wang R, Feng Z, Zhang J, Yang H, Tan Y, Wang H, Wang C, Liu L, Liu Y, Liu Y, Wang G, Yuan Z, Hou X, Ren L, Wu Y, Chen Y. Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 infection. Nat Commun. 2021 May 4;12(1):2506. doi: 10.1038/s41467-021-22781-1. PMID: 33947851; PMCID: PMC8096808.
20. He F, Wu C, Li P, Li N, Zhang D, Zhu Q, Ren W, Peng Y. Functions and Signaling Pathways of Amino Acids in Intestinal Inflammation. Biomed Res Int. 2018 Feb 26;2018:9171905. doi: 10.1155/2018/9171905. PMID: 29682569; PMCID: PMC5846438.
21. Chain X, Hu L, Zhang Y, et al. Specific ACE2 Expression in Cholangiocytes May Cause Liver Damage After 2019-nCoV Infection bioRxiv 2020.02.03.931766; doi: https://doi.org/10.1101/2020.02.03.931766
22. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q, Chang J, Hong C, Zhou Y, Wang D, Miao X, Li Y, Hu B. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol. 2020 Jun 1;77(6):683-690. doi: 10.1001/jamaneurol.2020.1127. PMID: 32275288; PMCID: PMC7149362.
23. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004 Jun;203(2):631-7. doi: 10.1002/path.1570. PMID: 15141377; PMCID: PMC7167720
24. Recalcati S. Cutaneous manifestations in COVID-19: a first perspective. J Eur Acad Dermatol Venereol. 2020 May;34(5):e212-e213. doi: 10.1111/jdv.16387. PMID: 32215952.
25. Wang Y, Dong C, Hu Y, et al. Temporal Changes of CT Findings in 90 Patients with COVID-19 Pneumonia: A Longitudinal Study. Radiology. 2020;296(2):E55-E64
26. Zhao YM, Shang YM, Song WB, Li QQ, XieH, Xu QF, et al. Follow-up study of the pulmonary function and related physiological characteristics of COVID-19 survivors three months after recovery. EClinicalMedicine. 2020; 25: 100463.
27. Huang Y, Tan C, Wu J, Chen M, Wang Z, Luo L, et al. Impact of coronavirus disease 2019 on pulmonary function in early convalescence phase. Respir Res. 2020; 21(1): 163.
28. Huntley CC, Patel K, Bil Bushra S-E-S, et al. Pulmonary function test and computed tomography features during follow-up after SARS, MERS and COVID-19: a systematic review and meta-analysis. ERJ Open Res 2022; 8: 00056–2022.
29. Aiello M, Marchi L, Calzetta L, Speroni S, Frizzelli A, Ghirardini M, Celiberti V, Sverzellati N, Majori M, Mori PA, Ranzieri S, Pisi R, Pelà G, Corradi M, Chetta A. Coronavirus Disease 2019: COSeSco - A Risk Assessment Score to Predict the Risk of Pulmonary Sequelae in COVID-19 Patients. Respiration. 2022;101(3):272-280. doi: 10.1159/000519385. Epub 2021 Oct 21. PMID: 34673642; PMCID: PMC9059039.
30. Tuty Kuswardhani RA, Henrina J, Pranata R, Anthonius Lim M, Lawrensia S, Suastika K. Charlson comorbidity index and a composite of poor outcomes in COVID-19 patients: A systematic review and meta-analysis. Diabetes

Metab Syndr. 2020 Nov-Dec;14(6):2103-2109. doi: 10.1016/j.dsx.2020.10.022. Epub 2020 Oct 28. PMID: 33161221; PMCID: PMC7598371.

31. Rogliani P, Calzetta L, Coppola A, Puxeddu E, Sergiacomi G, D'Amato D, Orlacchio A. Are there pulmonary sequelae in patients recovering from COVID-19? Respir Res. 2020 Oct 30;21(1):286. doi: 10.1186/s12931-020-01550-6. PMID: 33126869; PMCID: PMC7598236.
32. Carvalho-Schneider C, Laurent E, Lemaignen A, Beaufils E, Bourbao-Tournois C, Laribi S, Flament T, Ferreira-Maldent N, Bruyère F, Stefic K, Gaudy-Graffin C, Grammatico-Guillon L, Bernard L. Follow-up of adults with noncritical COVID-19 two months after symptom onset. Clin Microbiol Infect. 2021 Feb;27(2):258-263. doi: 10.1016/j.cmi.2020.09.052. Epub 2020 Oct 5. PMID: 33031948; PMCID: PMC7534895.
33. Cazzola M, Calzetta L, Matera MG, Muscoli S, Rogliani P, Romeo F. Chronic obstructive pulmonary disease and coronary disease: COPDCoRi, a simple and effective algorithm for predicting the risk of coronary artery disease in COPD patients. Respir Med. 2015 Aug;109(8):1019-25. doi: 10.1016/j.rmed.2015.05.021. Epub 2015 Jun 23. PMID: 26111914.
34. Hui DS, Joynt GM, Wong KT, Gomersall CD, Li TS, Antonio G, Ko FW, Chan MC, Chan DP, Tong MW, Rainer TH, Ahuja AT, Cockram CS, Sung JJ. Impact of severe acute respiratory syndrome (SARS) on pulmonary function, functional capacity and quality of life in a cohort of survivors. Thorax. 2005 May;60(5):401-9. doi: 10.1136/thx.2004.030205. PMID: 15860716; PMCID: PMC1758905.
35. Das KM, Lee EY, Singh R, Enani MA, Al Dossari K, Van Gorkom K, Larsson SG, Langer RD. Follow-up chest radiographic findings in patients with MERS-CoV after recovery. Indian J Radiol Imaging. 2017 Jul-Sep;27(3):342-349. doi: 10.4103/ijri.IJRI_469_16. PMID: 29089687; PMCID: PMC5644332.
36. Antonio GE, Wong KT, Hui DS, Wu A, Lee N, Yuen EH, Leung CB, Rainer TH, Cameron P, Chung SS, Sung JJ, Ahuja AT. Thin-section CT in patients with severe acute respiratory syndrome following hospital discharge: preliminary experience. Radiology. 2003 Sep;228(3):810-5. doi: 10.1148/radiol.2283030726. Epub 2003 Jun 12. PMID: 12805557.
37. Mo X, Jian W, Su Z, Chen M, Peng H, Peng P, et al. Abnormal pulmonary function in COVID-19 patients at time of hospital discharge. Eur Respir J. 2020;55:2001217.
38. Frija-Masson J, Debray MP, Gilbert M, Lescure FX, Travert F, Borie R, et al. Functional characteristics of patients with SARSCoV-2 pneumonia at 30 days post infection. Eur Respir J. 2020:2001754.
39. British Thoracic Society. British Thoracic Society Guidance on Respiratory Follow Up of Patients with a ClinicoRadiological Diagnosis of COVID-19 Pneumonia [Internet]. 2020. Available from: https://www.brit-thoracic.org.uk/ document-library/quality-improvement/covid-19/resp-followup-guidance-post-covid-pneumonia/.
40. Torres-Castro R, Vasconcello-Castillo L, Alsina-Restoy X, Solis-Navarro L, Burgos F, Puppo H, Vilaró J. Respiratory function in patients post-infection by COVID-19: a systematic review and meta-analysis. Pulmonology. 2021 Jul-Aug;27(4):328-337. doi: 10.1016/j.pulmoe.2020.10.013. Epub 2020 Nov 25. PMID: 33262076; PMCID: PMC7687368.
41. Qin W, Chen S, Zhang Y, Dong F, Zhang Z, Hu B, Zhu Z, Li F, Wang X, Wang Y, Zhen K, Wang J, Wan Y, Li H, Elalamy I, Li C, Zhai Z, Wang C. Diffusion capacity abnormalities for carbon monoxide in patients with COVID-19 at 3-month follow-up. Eur Respir J. 2021 Jul 22;58(1):2003677. doi: 10.1183/13993003.03677-2020. PMID: 33574077; PMCID: PMC7877322.

42. COVID-19 Rapid Guideline: Managing the Long-Term Effects of COVID-19; NICE Guideline, No. 188; National Institute for Health and Care Excellence (NICE): London, UK, 18 December 2020; ISBN-13: 978-1-4731-3943-5

43. https://www.iss.it/long-covid-cosa-sappiamo

44. Chippa V, Aleem A, Anjum F. Post Acute Coronavirus (COVID-19) Syndrome. 2022 Jun 19. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 34033370

45. A clinical case definition of post COVID-19 condition by a Delphi consensus, 6 October 2021. World Health Organization. https://www.who.int/publications/i/item/WHO-2019-nCoV-Post_COVID-19_condition-Clinical_case_definition-2021.1

46. O'Mahoney LL, Routen A, Gillies C, Ekezie W, Welford A, Zhang A, Karamchandani U, Simms-Williams N, Cassambai S, Ardavani A, Wilkinson TJ, Hawthorne G, Curtis F, Kingsnorth AP, Almaqhawi A, Ward T, Ayoubkhani D, Banerjee A, Calvert M, Shafran R, Stephenson T, Sterne J, Ward H, Evans RA, Zaccardi F, Wright S, Khunti K. The prevalence and long-term health effects of Long Covid among hospitalised and non-hospitalised populations: A systematic review and meta-analysis. EClinicalMedicine. 2022 Dec 1;55:101762. doi: 10.1016/j.eclinm.2022.101762. Erratum in: EClinicalMedicine. 2023 May;59:101959. PMID: 36474804; PMCID: PMC9714474

47. Garner P, 2020. For 7 Weeks I Have Been through a Roller Coaster of Ill Health, Extreme Emotions, and Utter Exhaustion. The BMJ. Available from:. Accessed 31 July 2020. https://blogs.bmj.com/bmj/2020/05/05/paul-garner-people-who-have-a-more-prot racted-illness-need-help-to-understand-and-cope-with-the-constantly-shifting-bizarre-symptoms/.

48. Harding L, 2020b. Weird as Hell': the Covid-19 Patients Who Have Symptoms for Months. The Guardian. 15 May. Available from:. Accessed 17 August 2020. https ://www.theguardian.com/world/2020/may/15/weird-hell-professor-advent-ca lendar-covid-19-symptoms-paul-garner.

49. Harding L, 2020a. Available from: https://twitter.com/lukeharding1968/status/1292 731730433052672?s=20 https://twitter.com/lukeharding1968/status/129273173 0433052672?s=20.

50. Colbert S, et al., 10 August 2020. n.d.) the Covid long haul: why are some patients not getting better?. Available from:. Accessed 19 August 2020. https://www.theguardia n.com/news/audio/2020/aug/10/the-covid-long-haul-why-are-some-patients-not -getting-better.

51. Chopra V, Flanders SA, O'Malley M, Malani AN, Prescott HC. Sixty-day outcomes among patients hospitalized with COVID-19. Ann Intern Med 2021;174:576-8. doi:10.7326/M20-5661

52. Carfì A, Bernabei R, Landi F, Gemelli Against COVID-19 Post-Acute Care Study Group. Gemelli against C-P-ACSG. Persistent symptoms in patients after acute COVID-19. JAMA 2020;324:603-5. doi:10.1001/ jama.2020.12603

53. Davis HE, Assaf GS, McCorkell L, et al. Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. MedRxiv 2020;12.24.20248802.

54. Pelà G, Goldoni M, Solinas E, Cavalli C, Tagliaferri S, Ranzieri S, Frizzelli A, Marchi L, Mori PA, Majori M, Aiello M, Corradi M, Chetta A. Sex-Related Differences in Long-COVID-19 Syndrome. J Womens Health (Larchmt). 2022 May;31(5):620-630. doi: 10.1089/jwh.2021.0411. Epub 2022 Mar 25. PMID: 35333613.

55. Biadsee A, Biadsee A, Kassem F, Dagan O, Masarwa S, Ormianer Z. Olfactory and oral manifestations of COVID- 19: Sex related symptoms-a potential pathway to early diagnosis. Otolaryngol Head Neck Surg 2020;163:722– 728.
56. Lechien JR, Chiesa-Estomba CM, Place S, et al. Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. J Intern Med 2020;288:335–344.
57. Gebhard C, Regitz-Zagrosek V, Neuhauser HK, Morgan R, Klein SL. Impact of sex and gender on COVID-19 outcomes in Europe. Biol Sex Differ 2020;11:29.
58. Maleki Dana P, Sadoughi F, Hallajzadeh J, et al. An insight into the sex differences in COVID-19 patients: What are the possible causes? Prehosp Disaster Med 2020;35:438–441.
59. Medzikovic L, Cunningham CM, Li M, et al. Sex differences underlying preexisting cardiovascular disease and cardiovascular injury in COVID-19. J Mol Cell Cardiol 2020;148:25–33.
60. Gemmati D, Bramanti B, Serino ML, Secchiero P, Zauli G, Tisato V. COVID-19 and individual genetic susceptibility/receptivity: Role of ACE1/ACE2 genes, immunity, inflammation and coagulation. Might the double X-chromosome in females be protective against SARSCoV- 2 compared to the single X-chromosome in males? Int J Mol Sc 2020;21:3474
61. Pradhan A, Olsson P-E. Sex differences in severity and mortality from COVID-19: Are males more vulnerable? Biology of sex differences 2020;11:53.
62. Jacobsen H, Klein SL. Sex differences in immunity to viral infections. Front Immunol 2021;12:720952
63. Wostyn P. COVID-19 and chronic fatigue syndrome: Is the worst yet to come? Med Hypotheses 2021;146:110469
64. Sorge RE, Totsch SK. Sex differences in pain. J Neurosci Res 2017;95:1271–1281.
65. Barsky AJ, Peekna HM, Borus JF. Somatic symptom in women and men. J Gen Intern Med 2001;16:266–275.
66. Lopez-Leon S, Wegman-Ostrosky T, Perelman C, Sepulveda R, Rebolledo PA, Cuapio A, Villapol S. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. Sci Rep. 2021 Aug 9;11(1):16144. doi: 10.1038/s41598-021-95565-8. PMID: 34373540; PMCID: PMC8352980
67. Phetsouphanh C, Darley DR, Wilson DB, Howe A, Munier CML, Patel SK, Juno JA, Burrell LM, Kent SJ, Dore GJ, Kelleher AD, Matthews GV. (2022). Immunological dysfunction persists for 8 months following initial mild-to-moderate SARS-CoV-2 infection. Nature Immunology, 23(2), 210–216
68. Proal A D, VanElzakker M. B. (2021). Long COVID or post-acute sequelae of COVID-19 (PASC): An overview of biological factors that may contribute to persistent symptoms. Frontiers in Microbiology, 12, 698169
69. Taquet M, Dercon Q, Harrison PJ. (2022). Six-month sequelae of post-vaccination SARS-CoV-2 infection: A retrospective cohort study of 10,024 breakthrough infections. Brain, Behavior, and Immunity, 103, 154–162
70. Steinbeis F, Knape P, Mittermaier M, Helbig ET, Tober-Lau P, Thibeault C, Lippert LJ, Xiang W, Müller-Plathe M, Steinbrecher S, Meyer HJ, Ring RM, Ruwwe-Glösenkamp C, Alius F, Li Y, Müller-Redetzky H, Uhrig A, Lingscheid T, Grund D, Temmesfeld-Wollbrück B, Suttorp N, Sander LE, Kurth F, Witzenrath M, Zoller T. Functional limitations 12 months after SARS-CoV-2 infection correlate with initial disease severity: An observational study of cardiopulmonary exercise capacity testing in COVID-19 convalescents. Respir Med. 2022 Oct;202:106968. doi: 10.1016/j.rmed.2022.106968. Epub 2022 Aug 28. PMID: 36081267; PMCID: PMC9420203

71. Rinaldo RR, Mondoni M, Parazzini EM, Pitari F, Brambilla E, Luraschi S, Balbi M, Papa GFS, Sotgiu G, Guazzi M et al. Deconditioning as main mechanism of impaired exercise response in COVID-19 survivors. Eur. Respir. J. 2021, 58, 2100870
72. Skjørten I, Ankerstjerne OAW, Trebinjac D, Brønstad E, Rasch-Halvorsen Ø, Einvik G, Lerum TV, Stavem K, Edvardsen A, Ingul CB. Cardiopulmonary exercise capacity and limitations 3 months after COVID-19 hospitalisation. Eur. Respir. J. 2021, 58, 2100996
73. Motiejunaite J, Balagny P, Arnoult F, Mangin L, Bancal C, Vidal-Petiot E, Flamant M, Jondeau G, Cohen-Solal A, d'Ortho MP et al. Hyperventilation as one of the mechanisms of persistent dyspnoea in SARS-CoV-2 survivors. Eur. Respir. J. 2021, 58, 2101578.
74. Frésard I, Genecand L, Altarelli M, Gex G, Vremaroiu P, Vremaroiu-Coman A, Lawi D, Bridevaux PO. Dysfunctional breathing diagnosed by cardiopulmonary exercise testing in 'long COVID' patients with persistent dyspnoea. BMJ Open Respir.Res. 2022, 9, e001126
75. Mancini DM, Brunjes DL, Lala A, Trivieri MG, Contreras J.P, Natelson BH. Use of Cardiopulmonary Stress Testing for Patients with Unexplained Dyspnea Post-Coronavirus Disease. JACC Heart Fail. 2021, 9, 927–937.J. Clin. Med. 2022, 11, 7388 9 of 9
76. von Gruenewaldt A, Nylander E, Hedman K. Classification and occurrence of an abnormal breathing pattern during cardiopulmonary exercise testing in subjects with persistent symptoms following COVID-19 disease. Physiol. Rep. 2022, 10, e15197.
77. Boulding R, Stacey R, Niven R, Fowler SJ. Dysfunctional breathing: A review of the literature and proposal for classification. Eur. Respir. Rev. 2016, 25, 287–294.
78. Tobin, M.J. Breathing pattern analysis. Intensive Care Med. 1992, 18, 193–201
79. Bellemare F, Grassino A. Evaluation of human diaphragm fatigue. J. Appl. Physiol. Respir. Environ. Exerc. Physiol. 1982, 53,1196–1206.
80. Lind F, Hesser C.M. Breathing pattern and lung volumes during exercise. Acta Physiol. Scand. 1984, 120, 123–129.
81. Frizzelli A, Di Spigno F, Moderato L, Halasz G, Aiello M, Tzani P, Manari G, Calzetta L, Pisi R, Pelà G, Piepoli M, Chetta A. An Impairment in Resting and Exertional Breathing Pattern May Occur in Long-COVID Patients with Normal Spirometry and Unexplained Dyspnoea. J Clin Med. 2022 Dec 13;11(24):7388. doi: 10.3390/jcm11247388. PMID: 36556003; PMCID: PMC9784722.
82. Graham B.L, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, Hallstrand TS, Kaminsky DA, McCarthy K, McCormack MC, et al. Standardization of spirometry 2019 update an official American Thoracic Society and European Respiratory Society technical statement. Am. J. Respir. Crit. Care Med. 2019, 200, E70–E88
83. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests, European community for steel and oral official statement of the European respiratory society. Eur. Respir. J. 1993, 6, 5–40
84. Ross RM. ATS/ACCP Statement on cardiopulmonary exercise testing. Am. J. Respir. Crit. Care Med. 2003, 167, 211–277
85. Wasserman K, Hansen JE, Sue DY, Casaburi R, Whipp BJ. (Eds.) Normal values. In Principles of Exercise Testing & Interpretation; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 1994; pp. 143–162.

86. Baba R, Nagashima M, Goto M, Nagano Y, Yokota M, Tauchi N, Nishibata K. Oxygen uptake efficiency slope: A new index of cardiorespiratory functional reserve derived from the relation between oxygen uptake and minute ventilation during incremental exercise. J. Am. Coll. Cardiol. 1996, 28, 1567–1572
87. Cole CR, Blackstone EH, Pashkow FJ, Snader CE, Lauer MS. Heart-rate recovery immediately after exercise as a predictor of mortality. N. Engl. J. Med. 1999, 341, 1351–1357.
88. Lederer DH, Altose MD, Kelsen SG, Cherniack NS. Comparison of occlusion pressure and ventilatory responses. Thorax 1977, 32, 212–220
89. Gayan-Ramirez, G.; Decramer, M. Effects of mechanical ventilation on diaphragm function and biology. Eur. Respir. J. 2002, 20,1579–1586
90. Huang YZ, Kuan CC. Vaccination to reduce severe COVID-19 and mortality in COVID-19 patients: a systematic review and meta-analysis. Eur Rev Med Pharmacol Sci 2022; **26:** 1770–76.
91. National Institute of Health and Care Excellence. COVID-19 rapid guideline: managing COVID-19. NICE Guideline (NG191). March 23, 2021. https://www.nice.org.uk/guidance/NG191 (accessed May 5, 2023).
92. Docherty AB, Harrison EM, Green CA, et al. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. BMJ 2020; 369: m1985
93. Belli S, Balbi B, Prince I, Cattaneo D, Masocco F, Zaccaria S, et al. Low physical functioning and impaired performance of activities of daily life in COVID-19 patients who survived hospitalisation. Eur Respir J. 2020; 56(4): 2002096
94. American Thoracic Society. Report of an adhoc international task force on early and short-term rehabilitative interventions in COVID-19 survivors. 2020 Apr. Available from: https: //www.thoracic.org/members/ assemblies/assemblies/pr/journal-club/ report-of-an-ad-hoc-international-taskforce-to-develop-an-expert-based opinion. php?fbclid=IwAR1OKBl_clPGr5VdBQpmzkZ_JaTO5MPVSkNa9tYYFqooBRgNazimDPNW4zA.
95. Vitacca M, Carone M, Clini EM, Paneroni M, Lazzeri M, Lanza A, et al. Joint statement on the role of respiratory rehabilitation in the COVID-19 crisis: the Italian position paper. Respiration. 2020; 99(6): 493–9.
96. Thomas P, Baldwin C, Bissett B, Boden I, Gosselink R, Granger CL, et al. Physiotherapy management for COVID-19 in the acute hospital setting: clinical practice recommendations. J Physiother. 2020; 66(2): 73–82.
97. Polastri M, Nava S, Clini E, Vitacca M, Gosselink R. COVID-19 and pulmonary rehabilitation: preparing for phase three. Eur Respir J. 2020; 55(6): 2001822.
98. George PM, Barratt SL, Condliffe R, Desai SR, Devaraj A, Forrest I, et al. Respiratory followup of patients with COVID-19 pneumonia. Thorax. 2020 Aug 24. Available from: 10.1136/thoraxjnl-2020-215314. Epub ahead of print.
99. 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 Aug 13; 56(6): 2002197. Online ahead of print
100. www.paho.org
101. Wasilewski MB, Cimino SR, Kokorelias KM, Simpson R, Hitzig SL, Robinson L. (2021). Providing rehabilitation to patients recovering from COVID-19: A scoping review. PM&R, 14(2), 12669–13258. pmrj. https://doi.org/10.1002/pmrj.12669
102. NICE. Rehabilitation after critical illness in adults, 2009.

103. Cortés-Telles A, López-Romero S, Figueroa-Hurtado E, Pou-Aguilar YN, Wong AW, Milne KM, Ryerson CJ, Guenette JA. (2021). Pulmonary function and functional capacity in COVID-19 survivors with persistent dyspnoea. Respiratory Physiology & Neurobiology, 288(December 2020), 103644. https://doi.org/10.1016/j.resp.2021.103644
104. Torres-Castro R, Solis-Navarro L, Sitjà-Rabert M, Vilaró J (2021). Functional limitations post-COVID-19: A comprehensive assessment strategy. Archivos de Bronconeumología, 57(January), 7–8. https://doi.org/10.1016/j.arbres.2020.07.025
105. Barker-Davies RM, O'Sullivan O, Senaratne KPP, Baker P, Cranley M, Dharm-Datta S, Ellis H, Goodall D, Gough M, Lewis S, Norman J, Papadopoulou T, Roscoe D, Sherwood D, Turner P, Walker T, Mistlin A, Phillip R, Nicol AM, Bennett AN, Bahadur S. The Stanford Hall consensus statement for post-COVID-19 rehabilitation. Br J Sports Med. 2020 Aug;54(16):949-959. doi: 10.1136/bjsports-2020-102596. Epub 2020 May 31. PMID: 32475821; PMCID: PMC7418628
106. Wouters EFM, Wouters BBREF, Augustin IML, et al. Personalised pulmonary rehabilitation in COPD. Eur Respir Rev 2018;27:170125.
107. Hill NS. Pulmonary rehabilitation. Proc Am Thorac Soc 2006;3:66–74
108. Spruit MA, Singh SJ, Garvey C, et al. An official American thoracic Society/ European respiratory Society statement: key concepts and advances in pulmonary rehabilitation. Am J Respir Crit Care Med 2013;188:e13–64.
109. Dechman G, Hernandez P, Camp PG. Exercise prescription practices in pulmonary rehabilitation programs. Canadian Journal of Respiratory, Critical Care, and Sleep Medicine 2017;1:77–83.
110. Cheng HH, Chou W. Rehabilitation can reduce mortality rate in patients who were intubated due to pneumonia. Ann Phys Rehabil Med 2018;61:e280–1.
111. Rice H, Harrold M, Fowler R, et al. Exercise training for adults hospitalized with an acute respiratory condition: a systematic scoping review. Clin Rehabil 2020;34:45–55.
112. Lau HM- C, Ng GY- F, Jones AY- M, et al. A randomised controlled trial of the effectiveness of an exercise training program in patients recovering from severe acute respiratory syndrome. Aust J Physiother 2005;51:213–9.
113. Liu K, Zhang W, Yang Y, et al. Respiratory rehabilitation in elderly patients with COVID-19: a randomized controlled study. Complement Ther Clin Pract 2020;39:101166
114. Jiandani MP, Salagre SB, Kazi S, Iyer S, Patil P, Khot WY, Patil E, Sopariwala M. Preliminary Observations and Experiences of Physiotherapy Practice in Acute Care Setup of COVID 19: A Retrospective Observational Study. J Assoc Physicians India. 2020 Oct;68(10):18-24. PMID: 32978920
115. Zampogna E, Paneroni M, Belli S, Aliani M, Gandolfo A, Visca D, Bellanti MT, Ambrosino N, Vitacca M, Pulmonary rehabilitation in patients recovering from COVID-19, respir, Int. Rev. Thorac. Dis. 100 (2021) 416–422, https://doi.org/ 10.1159/000514387.
116. Lv D, Chen X, Wang X, Mao L, Sun J, Wu G, Lin Z, Lin R, Yu J, Wu X, Jiang Y. Pulmonary function of patients with 2019 novel coronavirus induced-pneumonia: a retrospective cohort study. Ann Palliat Med. 2020 Sep;9(5):3447-3452. doi: 10.21037/apm-20-1688. PMID: 33065795
117. Lopes AJ, Litrento PF, Provenzano BC, Carneiro AS, Monnerat LB, da Cal MS, Ghetti ATA, Mafort TT. Small airway dysfunction on impulse oscillometry and pathological signs on lung ultrasound are frequent in post-

COVID-19 patients with persistent respiratory symptoms. PLoS One. 2021 Nov 29;16(11):e0260679. doi: 10.1371/journal.pone.0260679. PMID: 34843598; PMCID: PMC8629296

118. Bouteleux B, Henrot P, Ernst R, Grassion L, Raherison-Semjen C, Beaufils F, Zysman M, Delorme M. Respiratory rehabilitation for Covid-19 related persistent dyspnoea: A one-year experience. Respir Med. 2021 Nov-Dec;189:106648. doi: 10.1016/j.rmed.2021.106648. Epub 2021 Oct 13. PMID: 34689061; PMCID: PMC8511554

119. Sonnweber T, Sahanic S, Pizzini A, Luger A, Schwabl C, Sonnweber B, et al. Cardiopulmonary recovery after COVID-19 - an observational prospective multi-center trial, Eur. Respir. J. (2020), https://doi.org/10.1183/13993003.03481- 2020

120. Antoniou KM, Vasarmidi E, Russell AM, Andrejak C, Crestani B, Delcroix M, Dinh-Xuan AT, Poletti V, Sverzellati N, Vitacca M, Witzenrath M, Tonia T, Spanevello A. European Respiratory Society statement on long COVID follow-up. Eur Respir J. 2022 Aug 4;60(2):2102174. doi: 10.1183/13993003.02174-2021. PMID: 35144991; PMCID: PMC9349784

121. Gloeckl R, Leitl D, Jarosch I, Schneeberger T, Nell C, Stenzel N, Vogelmeier CF, Kenn K, Koczulla AR. Benefits of pulmonary rehabilitation in COVID-19: a prospective observational cohort study. ERJ Open Res. 2021 May 31;7(2):00108-2021. doi: 10.1183/23120541.00108-2021. PMID: 34095290; PMCID: PMC7957293.

122. Holland AE, Spruit MA, Troosters T, et al. An official European Respiratory Society/American Thoracic Societytechnical standard: field walking tests in chronic respiratory disease. Eur Respir J 2014; 44: 1428–1446

123. McNarry MA, Berg RMG, Shelley J, et al. Inspiratory muscle training enhances recovery post-COVID-19: a randomised controlled trial. Eur Respir J 2022; 60: 2103101 [DOI: 10.1183/ 13993003.03101-2021].

124. Enright SJ, Unnithan VB, Heward C, et al. Effect of high-intensity inspiratory muscle training on lung volumes, diaphragm thickness, and exercise capacity in subjects who are healthy. Phys Ther 2006; 86: 345–354.

125. Nopp S, Moik F, Klok FA, Gattinger D, Petrovic M, Vonbank K, Koczulla AR, Ay C, Zwick RH. Outpatient Pulmonary Rehabilitation in Patients with Long COVID Improves Exercise Capacity, Functional Status, Dyspnea, Fatigue, and Quality of Life. Respiration. 2022;101(6):593-601. doi: 10.1159/000522118. Epub 2022 Feb 24. PMID: 35203084; PMCID: PMC9059007.

126. Vonbank K, Zwick RH, Strauss M, Lichtenschopf A, Puelacher C, Budnowski A, et al. Guidelines for outpatient pulmonary rehabilitation in Austria. Wien Klin Wochenschr. 2015; 127(13–14): 503–13

127. Klok FA, Boon GJAM, Barco S, Endres M, Geelhoed JJM, Knauss S, et al. The post-COVID- 19 functional status (PCFS) scale: a tool to measure functional status over time after COVID-19. Eur Respir J. 2020; 56(1): 2001494.

128. Araújo BTS, Barros AEVR, Nunes DTX, Remígio de Aguiar MI, Mastroianni VW, de Souza JAF, Fernades J, Campos SL, Brandão DC, Dornelas de Andrade A. Effects of continuous aerobic training associated with resistance training on maximal and submaximal exercise tolerance, fatigue, and quality of life of patients post-COVID-19. Physiother Res Int. 2023 Jan;28(1):e1972. doi: 10.1002/pri.1972. Epub 2022 Sep 11. PMID: 36088642; PMCID: PMC9539049.

129. Carvalho, V. O., & Mezzani, A. (2011). Aerobic exercise training intensity in patients with chronic heart failure: Principles of assessment and prescription. European Journal of Preventive Cardiology, 18(1), 5–14. https://doi.org/10.1097/HJR.0b013e32833a9c63

130. Güneş M, Yana M, Güçlü MB. Physical activity levels respiratory and peripheral muscle strength and pulmonary function in young post-COVID-19 patients: A cross-sectional study. Wien Klin Wochenschr. 2023 May;135(9-10):251-259. doi: 10.1007/s00508-023-02204-5. Epub 2023 Apr 28. PMID: 37115337; PMCID: PMC10141881.
131. Del Corral T, Fabero-Garrido R, Plaza-Manzano G, Fernández-de-Las-Peñas C, Navarro-Santana M, López-de-Uralde-Villanueva I. Home-based respiratory muscle training on quality of life and exercise tolerance in long-term post-COVID-19: Randomized controlled trial. Ann Phys Rehabil Med. 2023 Feb;66(1):101709. doi: 10.1016/j.rehab.2022.101709. Epub 2022 Sep 30. PMID: 36191860; PMCID: PMC9708524
132. Romanet C, Wormser J, Fels A, Lucas P, Prudat C, Sacco E, Bruel C, Plantefève G, Pene F, Chatellier G, Philippart F. Effectiveness of exercise training on the dyspnoea of individuals with long COVID: A randomised controlled multicentre trial. Ann Phys Rehabil Med. 2023 Jun;66(5):101765. doi: 10.1016/j.rehab.2023.101765. Epub 2023 Jun 2. PMID: 37271020; PMCID: PMC10237688.
133. Longobardi I, Goessler K, de Oliveira Júnior GN, et al Effects of a 16-week home-based exercise training programme on health-related quality of life, functional capacity, and persistent symptoms in survivors of severe/critical COVID-19: a randomised controlled trial British Journal of Sports Medicine 2023;57:1295-1303.
134. Piercy KL, Troiano RP, Ballard RM, et al. The physical activity guidelines for Americans. JAMA 2018;320:2020–8.2021 Oct;55(19):1099-1105. doi: 10.1136/bjsports-2021-104080. Epub 2021 Apr 13. PMID: 33849909; PMCID: PMC8050880
135. Sallis R, Young DR, Tartof SY, Sallis JF, Sall J, Li Q, Smith GN, Cohen DA. Physical inactivity is associated with a higher risk for severe COVID-19 outcomes: a study in 48 440 adult patients. Br J Sports Med.
136. Pedersen BK, Saltin B. Exercise as medicine - evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand J Med Sci Sports 2015;25 Suppl 3:1–72.
137. Ross R, Blair SN, Arena R, et al. Importance of assessing cardiorespiratory fitness in clinical practice: a case for fitness as a clinical vital sign: a scientific statement from the American heart association. Circulation 2016;134:e653–99.
138. Zhao M, Veeranki SP, Magnussen CG, et al. Recommended physical activity and all cause and cause specific mortality in US adults: prospective cohort study. BMJ 2020;370:m2031.
139. Katzmarzyk PT, Friedenreich C, Shiroma EJ, et al. Physical inactivity and non- communicable disease burden in low- income, middle- income and high- income countries. Br J Sports Med 2021. doi:10.1136/bjsports-2020-103640. [Epub ahead of print: 29 Mar 2021].
140. Tamburlani M, Cuscito R, Servadio A, Galeoto G. Effectiveness of Respiratory Rehabilitation in COVID-19's Post-Acute Phase: A Systematic Review. Healthcare (Basel). 2023 Apr 8;11(8):1071. doi: 10.3390/healthcare11081071. PMID: 37107905; PMCID: PMC10137696
141. Greenhalgh T, Knight M, A'Court C, Buxton M, Husain L. Management of post-acute covid-19 in primary care. BMJ. 2020 Aug 11;370:m3026. doi: 10.1136/bmj.m3026. PMID: 32784198.
142. Eaton T, Young P, Zeng I, Kolbe J. A randomized evaluation of the acute efficacy, acceptability and tolerability of flutter and active cycle of breathing with and without postural drainage in non-cystic fibrosis bronchiectasis. Chron Respir Dis 2007;4: 23e30.
143. Phillips GE, Pike SE, Jaffe A, Bush A. Comparison of active cycle of breathing and high-frequency oscillation jacket in children with cystic fibrosis. Pediatr Pulmonol 2004;37:71e5.

144. van Hengstum M, Festen J, Beurskens C, Hankel M, Van Den Broek W, Corstens F. No effect of oral high frequency oscillation combined with forced expiration manoeuvres on tracheobronchial clearance in chronic bronchitis. Eur Respir J 1990;3:14e8.

145. Cecins NM, Jenkins SC, Pengelley J, Ryan G. The active cycle of breathing techniques-to tip or not to tip? Respir Med 1999;93: 660e5.

146. Elkins M, Jones A, van der Schans CP. Positive expiratory pressure physiotherapy for airway clearance in people with cystic fibrosis. Cochrane Database Syst Rev 2006. doi: 10.1002/14651858.CD003147. Issue 2: Art. No.: CD003147

147. Pryor JA, Webber BA. An evaluation of the forced expiration technique as an adjunct to postural drainage. Physiotherapy 1979;65:304e7.

148. Pryor J, Webber B, Bethune D. Physiotherapy techniques. In: Pryor J, Prasad S, editors. Physiotherapy for respiratory and cardiac problems. 3rd ed. Edinburgh: Churchill Livingstone; 2002.

149. Lewis LK, Williams MT, Olds TS. The active cycle of breathing technique: a systematic review and meta-analysis. Respir Med. 2012;106(2):155-172.

150. Phsysiotherapy Department at Liverpool Hospital. Updated February 2010

151. The Active Cycle of Breathing Techniques, available at https://www.acprc.org.uk/Data/Publication_Downloads/GL-05ACBT.pdf. Assoc Chart Physiother Respir Care. Published online 2011. Accessed January 13, 2022. www.brit-thoracic.org.uk/clinical-information/physiotherapy/physiotherapy-guideline.aspx

152. https://www.physiotherapyforbpd.org.uk)—a

153. Tudor-Locke C, Bassett DR. How Many Steps/Day Are Enough? Sport Med 2004 341. 2012;34(1):1-8.

154. Aoyagi Y, Shephard RJ. Habitual physical activity and health in the elderly: The Nakanojo Study. Geriatr Gerontol Int. 2010;10(SUPPL. 1):S236-S243

155. Huskisson EC. Measurement of pain. Lancet 1974;2:1127–31.

156. Bestall JC, Paul EA, Garrod R, Garnham R, Jones PW, Wedzicha JA. Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. Thorax. 1999 Jul;54(7):581-6.

157. Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MD, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). Thorax. 2003 Apr;58(4):339-43.

158. Ward N. The Leicester Cough Questionnaire. J Physiother. 2016 Jan;62(1):53.

159. EQ-5D. Accessed January 13, 2022. https://euroqol.org/.

160. Thamrin C, Dellacà RL, Hall GL, et al. Technical standards for respiratory oscillometry: test loads for calibration and verification. Eur Respir J. 2020;**56**(4):2003369. doi: 10.1183/13993003.03369-2020

161. Oppenheimer BW, Goldring RM, Herberg ME, et al. Distal airway function in symptomatic subjects with normal spirometry following world trade center dust exposure. Chest. 2007;**132**(4):1275–1282. doi: 10.1378/chest.07-0913

162. Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. Lancet Respir Med. 2014;2(6):497–506. doi: 10.1016/s2213-2600(14)70103-1

163. Crisafulli E, Pisi R, Aiello M, et al. Prevalence of small-airway dysfunction among COPD patients with different GOLD stages and its role in the impact of disease. Respiration. 2016;93(1):32–41. doi: 10.1159/000452479

164. Jetmalani K, Thamrin C, Farah CS, et al. Peripheral airway dysfunction and relationship with symptoms in smokers with preserved spirometry. Respirology. 2018;**23**(5):512–518. doi: 10.1111/resp.13215
165. Xiao D, Chen Z, Wu S, Huang K et al. Prevalence and risk factors of small airway dysfunction, and association with smoking, in China: findings from a national cross-sectional study. Lancet Respir Med. 2020 Nov;8(11):1081-1093. doi: 10.1016/S2213-2600(20)30155-7. Epub 2020 Jun 26. PMID: 32598906.
166. Hansen JE, Sun XG, Wasserman K. Discriminating measures and normal values for expiratory obstruction. Chest. 2006;129(2):369–77.
167. Dilektasli AG, Porszasz J, Casaburi R, Stringer WW, Bhatt SP, Pak Y, et al. A novel spirometric measure identifies mild COPD unidentified by standard criteria. Chest.2016; 150(5):1080–90
168. Robinson PD, Latzin P, Verbanck S, Hall GL, Horsley A, Gappa M, Thamrin C, Arets HG, Aurora P, Fuchs SI, King GG, Lum S, Macleod K, Paiva M, Pillow JJ, Ranganathan S, Ratjen F, Singer F, Sonnappa S, Stocks J, Subbarao P, Thompson BR, Gustafsson PM. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. Eur Respir J. 2013 Mar;41(3):507-22.
169. Verbanck S, Schuermans D, Van Muylem A, et al. Conductive and acinar lung-zone contributions to ventilation inhomogeneity in COPD. Am J Respir Crit Care Med 1998;157 (5 Pt 1):1573–1577.
170. Houltz B, Green K, Lindblad A, et al. Tidal N2 washout ventilation inhomogeneity indices in a reference population aged 7-70 years. Eur Respir J 2012; 40: Suppl. 56, P3797
171. 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. 2022 Nov 1;226(9):1593-1607. doi: 10.1093/infdis/jiac136. PMID: 35429399; PMCID: PMC9047189.
172. Frizzelli A, Nicolini G, Chetta A. Small Airways: Not Just Air Ducts - Pathophysiological Aspects and Clinical Implications. Respiration. 2022;101(10):953-958. doi: 10.1159/000525666. Epub 2022 Jul 12. PMID: 35820370.