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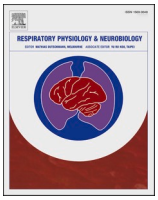

Ventilatory efficiency in long-term dyspnoeic patients following COVID-19 pneumonia

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ABSTRACT

Background: Long COVID is defined as persistency of symptoms, such as exertional dyspnea, twelve weeks after recovery from SARS-CoV-2 infection.
Objectives: To investigate ventilatory efficiency by the use of cardiopulmonary exercise testing (CPET) in patients with exertional dyspnea despite normal basal spirometry after 18 (T_{18}) and 36 months (T_{36}) from COVID-19 pneumonia.
Methods: One hundred patients with moderate-critical COVID-19 were prospectively enrolled in our Long COVID program. Medical history, physical examination and lung high-resolution computed tomography (HRCT) were obtained at hospitalization (T_0), 3 (T_3) and 15 months (T_{15}). All HRCTs were revised using a semi-quantitative CT severity score (CSS). Pulmonary function tests were obtained at T_3 and T_{15}. CPET was performed in a subset of patients with residual dyspnea (mMRC $\geq$ 1), at T_{18} and at T_{36}.
Results: Remarkably, at CPET, ventilatory efficiency was reduced both at T_{18} ($V'_E/V'CO_2$ slope = 31.4±3.9 SD) and T_{36} ($V'_E/V'CO_2$ slope = 31.28±3.70 SD). Furthermore, we identified positive correlations between $V'_E/V'CO_2$ slope at T_{18} and T_{36} and both percentage of involvement and CSS at HRCT at T_0, T_3 and T_{15}. Also, negative linear correlations were found between $V'_E/V'CO_2$ slope at T_{18} and T_{36} and DL_{CO} at T_3 and T_{15}.
Conclusions: At eighteen months from COVID-19 pneumonia, 20 % of subjects still complains of exertional dyspnea. At CPET this may be explained by persistently reduced ventilatory efficiency, possibly related to the degree of lung parenchymal involvement in the acute phase of infection, likely reflecting a damage in the pulmonary circulation.

1. Introduction

The SARS-CoV-2 pandemic has seen more than 700 million cumulative cases worldwide, causing more than 6 million deaths ("WHO covid19.who.int). The natural history, the physiopathology and the long-term sequelae of SARS-CoV-2 infection still need to be fully understood; many patients manifest persistency or development of a wide range of symptoms after the acute infection, affecting functional performance and quality of life (Crook et al., 2021). "Long COVID" has been defined from the World Health Organization (WHO) as a variety of long-term symptoms that persist or develop three months after COVID-19 and lasting for at least 2 months, representing nowadays a challenge for physicians, as well as a relevant social and economic burden (WHO https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease). The European Respiratory Society Statement on Long COVID indicates that age and initial severity of disease appear to be correlated with long-term consequences, but not necessarily with the persistence of symptoms (Antoniou et al., 2022). Although several pathophysiological mechanisms have been proposed as an explanation for Long COVID, there are still many uncertainties and

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further mechanistic studies seem to be necessary (Crook et al., 2021).

Dyspnea is one of the most frequent symptoms that clinicians face in Long COVID patients. A large longitudinal cohort study found that in Long COVID patients reporting dyspnea (defined as a modified British Medical Research Council (mMRC) score ≥ 1), gradually decreased from 288 (26 %) out of 1104 patients at 6 months to 168 (14 %) out of 1191 patients at 2 years. Also, lung function tests were performed, finding a higher prevalence of lung diffusion impairment, reduced residual volume, and reduced total lung capacity (Huang et al., 2022). In patients with Long COVID, dyspnea is usually multifactorial, and even though long-term pulmonary complications are unlikely (Daher et al., 2020), the development of Post-COVID Interstitial Lung Diseases (PC-ILDs) and pulmonary embolism (PE) are possible (Tanni et al., 2021; Cui et al., 2020). In a previous study we demonstrated that at 15 months follow-up, 8 % of patients previously hospitalized for moderate to critical COVID-19 showed residual radiological and functional signs consistent with PC-ILDs (Sanna et al., 2023).

Dyspnea and exercise intolerance in Long COVID patients represent a challenge for clinicians, particularly for those with normal lung function testing. In this regard, cardiopulmonary exercise testing (CPET) has been proven to be the gold-standard for determining the level of exercise intolerance and its possible causes (Laveneziana et al., 2022; Onathan et al., 2002; Gulati et al., 2005; Durstenfeld et al., 2022). To the best of our knowledge, data on persistency of dyspnea and ventilatory efficiency in a very long-term follow-up of Long COVID patients with normal spirometry is lacking, especially when considering studies aimed to understand underlying pathophysiological mechanisms limiting exercise tolerance. For this reason, patients from our Long COVID outpatient clinic, who still complained of exertional dyspnea at 18 months from a moderate to critical COVID-19, were investigated using CPET. We hypothesized that, because of endothelial lung injury, dyspnea on exertion could be explained by ventilatory inefficiency. Also, at 36 months from hospitalization, CPET was repeated in the same subjects, to investigate possible long-term changes in the main cardiovascular and ventilatory variables.

2. Materials and methods

One hundred patients consecutively admitted to our hospital, from March 2020 to August 2020, with a diagnosis of moderate to critical

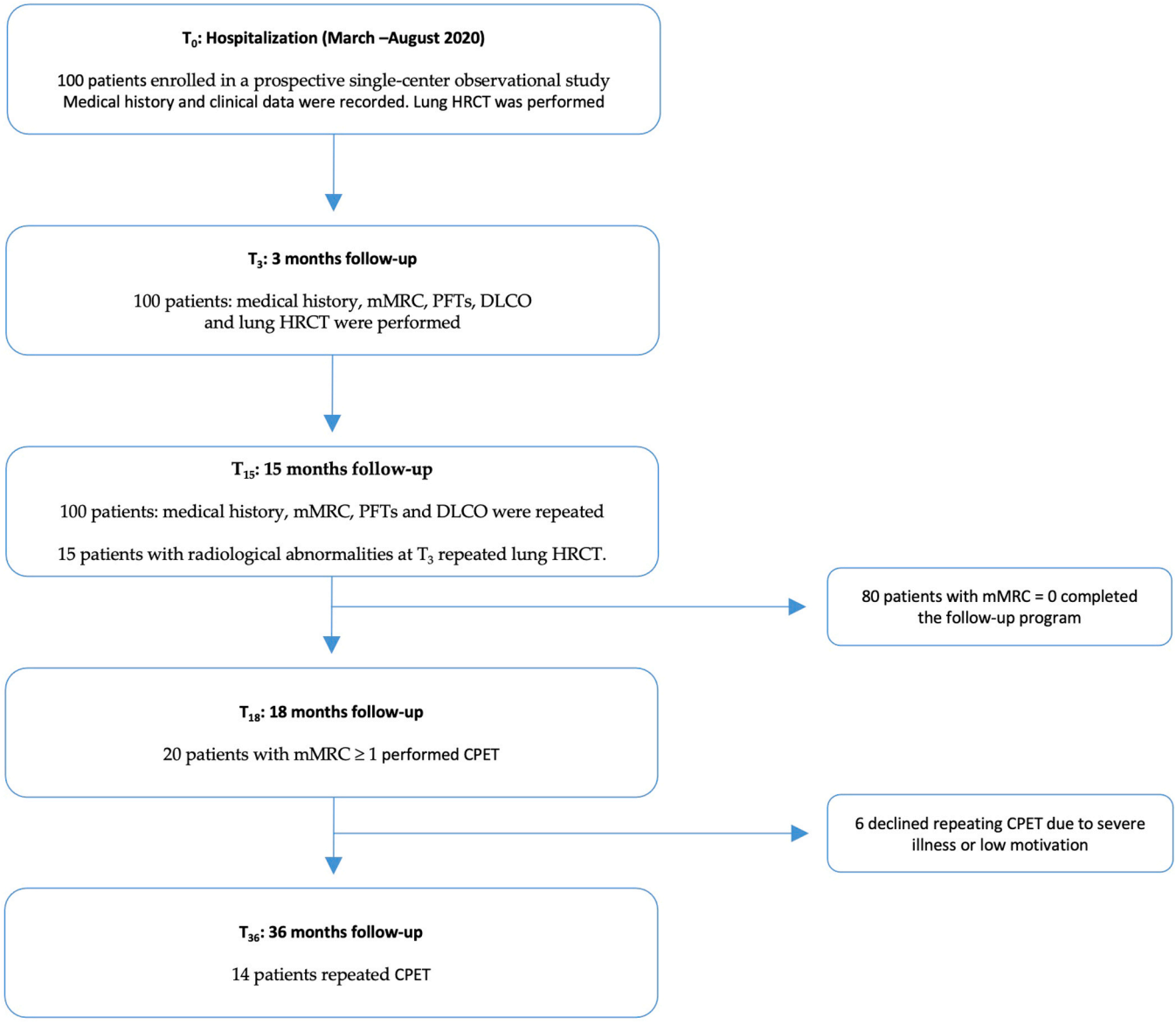

Fig. 1. Study design. CPET, cardiopulmonary exercise test; DLCO, diffusion capacity of carbon monoxide; HRCT, high resolution computed tomography; mMRC, modified british medical research council questionnaire; PFTs, pulmonary function tests.

SARS-CoV-2 infection (as defined by WHO) (Health Organization W., 2021), were enrolled in a prospective, single-center observational study. COVID-19 pneumonia was confirmed by positive SARS-CoV2 testing and lung parenchymal involvement at High Resolution Computed Tomography (HRCT) on admission (T_0). Demographic data, such as age, sex, BMI, smoking habit, date of onset of symptoms and hospital admission, comorbidities, pharmacological treatments and respiratory support were recorded. Lung cancer was an exclusion criteria for the enrollment in the study and drop-out criteria consisted in death during the follow-up period or the patient's refusal to undergo follow-up.

2.1. Study aim and design

The study aimed to investigate the prevalence of residual dyspnea at 18 months (T_{18}) follow-up in a cohort of 100 patients who had been hospitalized for COVID-19 pneumonia, and to investigate cardiopulmonary exercise testing (CPET) variables in dyspnoeic patients at 18 and 36 months follow up. Also, possible correlations were studied with pulmonary function tests (PFTs), and radiological variables that were collected at 3 (T_3) and at 15 (T_{15}) months. Study design is shown in Fig. 1; briefly, it consisted of two follow-up visits, at T_3 and T_{15}, where clinical history, physical examination, mMRC and PFTs were collected. Lung HRCT was performed at T_3 in all subjects. At T_{15}, lung HRCT was repeated in patients with evidence of radiological abnormalities at HRCT or with alteration in PFTs at T_3. At T_{18}, patients with residual exertional dyspnea (mMRC $\geq$ 1) were selected to undergo CPET. At 36 months follow-up (T_{36}) all 20 patients who had performed CPET at T_{18} were invited to repeat CPET: 14 patients performed CPET, 3 patients refused because of low motivation, 3 patients were excluded because of evidence of absolute contraindications (recent cardiovascular disease and recent major surgery). Exclusion criteria for performing CPET followed the absolute and relative contraindications described in the latest international statements (Radtke et al., 2022). Also, we excluded from performing CPET patients with the following characteristics: age $<$ 18 years, moderate or severe obstructive or restrictive pattern at PFTs, patients with chronic heart failure with NYHA $\geq$ Class III, peripheral arterial disease, definitive or possible diagnosis of pulmonary hypertension.

2.2. Pulmonary Function Testing (PFTs)

PFTs were performed by qualified personnel using a spirometer (Quark PFT, Cosmed, Pavona, Italy), according to the recommendations of the American Thoracic Society and the European Respiratory Society (Wanger et al., 2005; Graham et al., 2017). The following tests were performed: spirometry, with evaluation of forced vital capacity (FVC), forced expiratory volume at 1 s (FEV_1); body plethysmography to measure total lung capacity (TLC) and residual volume (RV); alveolar-capillary diffusion capacity for carbon monoxide (DL_{CO}) by using the single breath method (Wanger et al., 2005; Graham et al., 2017).

2.3. CT protocol and images analysis

All examinations were performed using two multidetector CT scanners (Somatom Sensation 16 and Somatom Sensation 64; Siemens Healthineers) with the patient in a supine position. Scan parameters corresponded to the manufacturer's recommended standard pre-setting for a chest routine. Image reconstruction was made with a slice thickness of 1 mm, applying the standard filtered back projection method with a soft tissue kernel of B20 and a lung kernel of B60. Coronal and sagittal multiplanar reconstructions were available for all examinations. In agreement with previous publications, COVID-19 pneumonia on the baseline CTs and its outcomes on follow-up images, were evaluated through the following CT findings: ground glass opacities (GGO), crazy-paving, consolidation and interlobular septal thickening (Francone et al., 2020; Salehi et al., 2020; Pecoraro et al., 2021), as defined by the Fleischner Society's glossary for thoracic imaging (Hansell et al., 2008). According to these findings, a visual percentage of lung parenchymal involvement was assessed. Also, the semiquantitative CT severity score proposed by Pan et al. (2020) was used to assess the total extent of disease on both baseline and follow-up CTs. HRCT was considered normal in the absence of lesions or in the presence of alterations involving less than 5 % of the lung parenchyma. Baseline and follow up images were anonymized, randomized, and analyzed separately by two different radiologists (the first with more than 15 years of experience and the second with more than 5 years of experience in chest imaging), who were blinded from clinical information. Differences in opinion were resolved by consensus.

2.4. Cardiopulmonary exercise testing (CPET)

Incremental symptom-limited exercise testing was performed on an electronically braked cycle ergometer through automated testing system (OMNIA, Cosmed, Pavona, Italy), in accordance with international recommendations (Weisman et al., 2003; Palange et al., 2007) and by using predicted values of normality proposed by Wasserman and Hansen (Wasserman et al., 2011; Hansen et al., 2015). Work rate increment (from 5 to 25 W/min) was individually established according to reported exercise tolerance, and predicted maximal O_2 uptake ($V'O_2$), to reach peak exercise within 8–12 min (Weisman et al., 2003). $V'O_2$, Carbon dioxide production ($V'CO_2$), minute ventilation (V'_E), tidal volume (V_T) and respiratory frequency (RF) were analyzed breath-by-breath during the test. Hemoglobin saturation was continuously monitored with pulse oximetry (SpO_2). All measured and derived parameters [e.g., ventilatory equivalents for O_2 and CO_2 ($V'_E/V'O_2$ and $V'_E/V'CO_2$, respectively), end-tidal O_2 and CO_2 partial pressures ($P_{ET}O_2$ and $P_{ET}CO_2$, respectively)] were recorded and averaged every 20 seconds. The anaerobic threshold (AT) was non-invasively determined using the dual-methods approach (V-slope and ventilatory equivalents methods) (Wasserman et al., 2011). Perceived breathlessness and leg fatigue during the test were rated according to the modified 10-point Borg (mBORG) scale (Borg GAV, 1982). $V'O_2$ at peak exercise ($V'O_2$ peak) was normalized for body weight and expressed as a percentage of predicted value. Peak V'_E response (V'_E peak) was expressed as a raw value and relative to the estimated maximal voluntary ventilation (eMVV), which was defined as forced expiratory volume (FEV_1) X 40 (Palange et al., 2007). The end of the isocapnic buffering period was identified when $V'_E/V'CO_2$ increased and $P_{ET}CO_2$ decreased. The linear phase of the $V'_E/V'CO_2$ relationship was detected on the V'_E (y-axis) versus $V'CO_2$ (x-axis) plot, between the beginning of loaded exercise and the end of the isocapnic buffering period. Linear regression was then applied, and $V'_E/V'CO_2$ slope and its intercept on the y-axis were calculated. Dysfunctional breathing was defined as the presence of a pattern of hyperventilation, erratic ventilation and/or periodic sighing (Ionescu et al., 2021). All CPET results were analyzed separately by 2 expert physicians, differences in opinion were resolved by consensus.

2.5. Statistical analysis

Descriptive statistics were obtained using proportions for dichotomous and categorical variables, mean and standard deviation for normally distributed continuous variables whereas median and interquartile range for non-normally distributed continuous variables. Correlations were explored between $V'_E/V'CO_2$ slope at T_{18} and at T_{36} and the following variables: HRCT percentage of parenchymal involvement at T_0, T_3 and T_{15}; CT semi-quantitative severity score at T_0, T_3 and T_{15}, DLCO at T_3 and T_{15}. Correlations were explored and visually represented by using Pearson's and Spearman's rank tests, for normally and non-normally distributed data, respectively. Any difference in CPET responses at 18 and 36 months were then compared using the Wilcoxon signed rank test or the McNemar test. Analyses were performed using

Stata (StataCorp LLC, 4905 Lakeway Drive, College Station, TX 322, USA), version 17.0. Correlations were explored and visually represented using Microsoft Excel (version 16.76). A two-sided p-value < 0.05 was considered statistically significant. For the correlations explored, given the limited sample size, p-value was not calculated.

3. Results

3.1. Anthropometric characteristics, clinical data and pulmonary function tests

Twenty patients (ten males and ten females) with residual respiratory dyspnea at T_{18} were selected to undergo CPET. The main anthropometric characteristics and clinical data are shown in Table 1. Most of our patients (12/20) had suffered of a critical COVID-19, as defined by WHO (Health Organization W., 2021), and the rest of them (8/20) of a moderate disease. Average values of spirometry and DL_{CO}, performed at T_{15}, are shown in Table 2, and resulted normal. The only respiratory comorbidity that was present in part of our population was COPD. Of notice, at T_3 mean DLCO value was 72.4 % ± 15.5 %, with a total of 14 subjects showing an abnormal value <80 % of predicted. At T_{15}, although the mean value of DLCO resulted normal, a total of 10 patients still showed DLCO values lower than normal.

3.2. Radiological findings

Results of lung HRCTs performed at T_0, T_3 and T_{15} are shown in Table 1, considering both percentage of parenchymal involvement and the semiquantitative CT severity score. Percentage of parenchymal involvement was of 30.5 % ± 18.1 % at T_0 and showed a reduction in time. At T_{15} only 11 patients performed chest HRCT, based on their radiological and functional findings at T_3.

Table 1
Anthropometric characteristics and clinical data of participants at 18 months.

	Total (n. 20)
Anthropometric characteristics	
Age, years	62 ± 11.3
Sex M/F	10/10
BMI kg/m^2	26.6 ± 3.75
Dyspnea	
mMRC = 1	16/20 (80 %)
mMRC = 2	3/20 (15 %)
mMRC = 3	1/20 (5 %)
mMRC = 4	0/20
Comorbidities	
Arterial hypertension	8/20 (40 %)
Type 2 diabetes mellitus	3/20 (15 %)
COPD	6/20 (30 %)
Overweight/Obese	9/20 (45 %)
Current smoker	1/20 (5 %)
Former smoker	7/20 (35 %)
Severity	
WHO moderate	8/20 (40 %)
WHO critical	12/20 (60 %)
ICU stay	6/20 (30 %)
IMV	5/20 (25 %)
CPAP/NIV	12/20 (60 %)
Radiological findings	
CT % involvement T_0	30.5 ± 18.1
CT % involvement T_3	10 (0.6 – 13.7)
CT % involvement T15 (11 patients)	5 (5 – 10)
CT score T_0	8.9 ± 5.1
CT score T_3	2 (0.2 – 4.5)
CT score T_{15} (11 patients)	1 (1 – 3)

Data are shown as means ± SD or median interquartile range (IQR), according to Gaussian/non-Gaussian distribution, respectively. BMI, body mass index; COPD, chronic obstructive pulmonary disease; CPAP, continuous positive airway pressure; CT, computed tomography; F, female; ICU, intensive care unit; IMV, invasive mechanical ventilation; M, male; mMRC, modified british medical research council questionnaire; NIV, non invasive ventilation; WHO, world health organization.

Table 2
PFTs and CPET responses of participants at 15 and 18 months, respectively.

Functional tests	
PFTs	
FVC, L	3.85 ± 1.21
FVC, % pred.	109 ± 18.9
FEV_1, L	3.00 ± 0.92
FEV_1, % pred.	106.4 ± 18.6
FEV_1/FVC, %	78.2 ± 5.6
DL_{CO}, % pred.	81.5 ± 14.5
K_{CO}, % pred.	88.1 ± 13.6
CPET	
$V'O_2$, mL/min	1675.1 ± 480.5
$V'O_2$/Kg, mL/min/Kg	21.7 ± 5.8
$V'O_2$, % pred.	88.5 (79.5 – 113.2)
$V'O_2$ at LT, mL/min	1034 (824.75 – 1158.25)
VO_2 at LT % VO_2 max pred.	58.7 ± 9.7
V'_E, L/min	72.7 ± 25.7
BR, % eMVV	38.8 ± 12.3
$V'_E/V'CO_2$ at LT	30.8 ± 3.8
$V'_E/V'CO_2$ slope	31.4 ± 3.9
$V'_E/V'CO_2$ intercept	0.04 ± 2.2
O_2 pulse, mL/beat	12.1 ± 3.0
O_2 pulse, % pred.	110.4 ± 21.6
Work rate, Watt	116.5 ± 39.3
$V'O_2$/work, mL/min/Watt	10.64 ± 1.7
$P_{ET}CO_2$ at peak, mmHg	32.4 ± 4.2
Dyspnea, mBORG	5.1 ± 2.6
Leg fatigue, mBORG	5.9 ± 2.3
Dysfunctional breathing, n. of patients (total n. 20)	6/20
Time from hospitalization, days	537 ± 48

Data refer to peak exercise, unless stated otherwise. Data are shown as means ± SD or median interquartile range (IQR), according to Gaussian/non-Gaussian distribution, respectively. BR, breathing reserve; DLCO, diffusion capacity of carbon monoxide; eMVV, estimated maximal voluntary ventilation; FEV1, forced expiratory volume in the 1st second; FVC, forced vital capacity; KCO, transfer coefficient of the lung; LT, lactic threshold; mBORG, modified BORG scale; PETCO2, end tidal partial pressure of carbon dioxide; PFTs, pulmonary function tests; pred., predicted; V'CO2, production of carbon dioxide; V'E, minute ventilation; V'O2, oxygen consumption.

3.3. Cardiopulmonary exercise testing (CPET)

Main physiological responses to exercise at T_{18} of the 20 selected patients are shown in Table 2. A total of 5 patients expressed a $V'O_2$% pred. < 80 % but > 75 %, determining a mild exercise intolerance. Of notice, $V'_E/V'CO_2$ slope was > 30, and considered abnormal, in 13 patients, and $V'_E/V'CO_2$ intercept was negative in 9 participants. An example of $V'_E/V'CO_2$ response during CPET for a representative patient is illustrated in Fig. 2. In our population, a dysfunctional breathing pattern, more precisely an erratic breathing pattern was clearly found in 6 patients; a representative patient is shown in Fig. 3.

Main physiological responses to exercise of the 14 patients who repeated CPET at T_{36}, and differences with the baseline values are shown in Table 3.

At last, positive moderate linear correlations were identified between $V'_E/V'CO_2$ slope at T_{18} and HRCT percentage of parenchymal involvement at T_0 (r=0.46 – p=0.02) (Fig. 4), at T_3 (r=0.55 – p=0.02) and at T_{15} (r=0.54 – p=0.08). Similar correlations were found between $V'_E/V'CO_2$ slope at T_{18} and CT severity score at T_0 (r=0.40 – p=0.04), T_3 (r=0.45 – p=0.04) and T_{15} (0.42 – p=0.19). On the other hand, negative moderate correlations were identified between $V'_E/V'CO_2$ slope at T_{18} and DLCO % pred. at T_3 (r= −0.46 – p=0.02) (Fig. 5) and T_{15} (r= −0.37 – p=0.05). Moreover, similar correlations were found between $V'_E/V'CO_2$ slope at T_{36} and both HRCT percentage of parenchymal involvement at T_0 (r=0.55 – p=0.02), T_3 (r=0.55 – p=0.06) and T_{15}

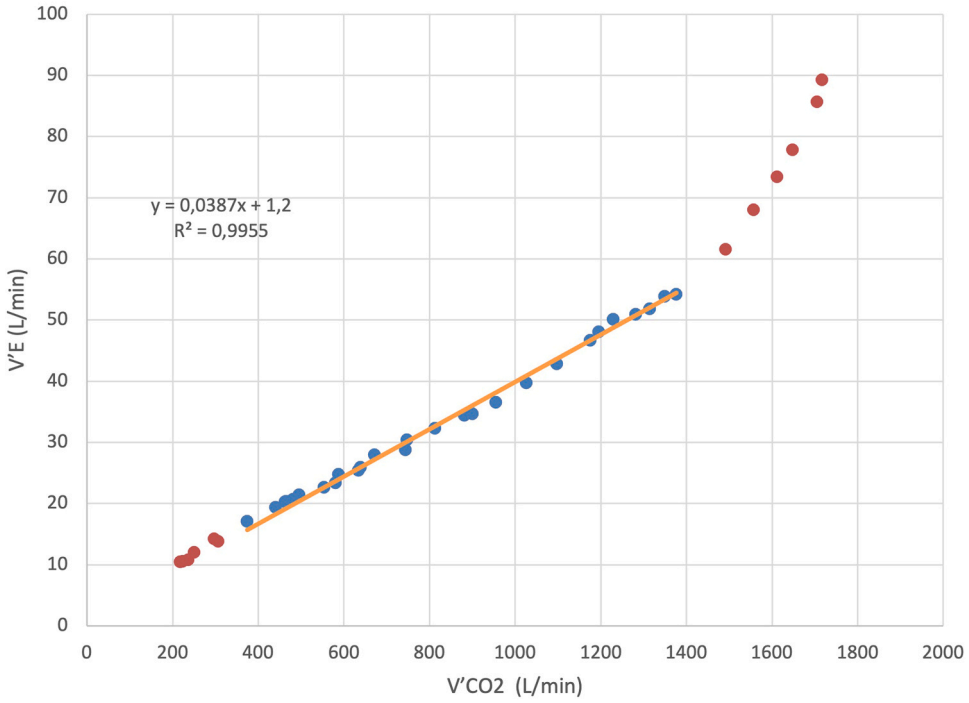

Fig. 2. Representative case of ventilatory inefficiency profile ($V'_E/V'CO_2$ slope) in a Long COVID patient. Each data point is the arithmetic mean of breath-by-breath values recorded over a 20 second period. V'_E, minute ventilation; $V'CO_2$, carbon dioxide output.

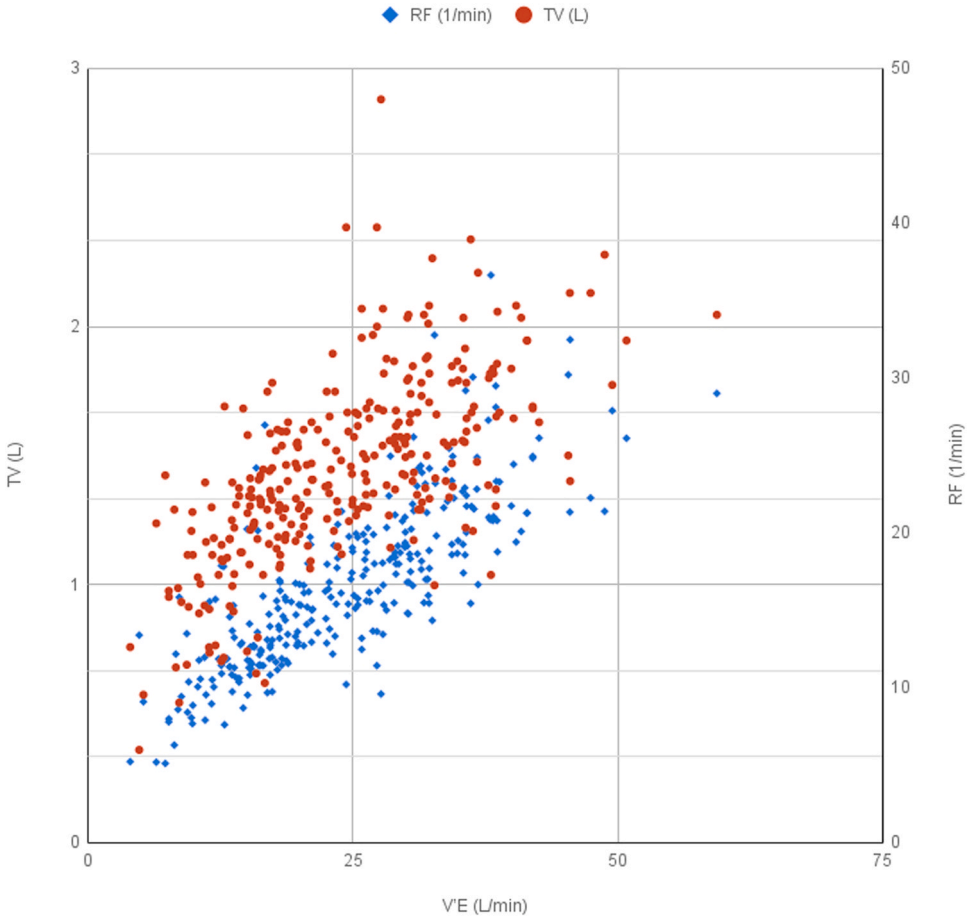

Fig. 3. Representative case of an erratic dysfunctional breathing pattern in a Long COVID patient, mostly evident on the TV and RF against V'_E plot. Data is representative of breath-by-breath analysis and was not filtered. RF, respiratory frequency; TV, tidal volume; V'_E minute ventilation.

Table 3
Differences in CPET responses of 14 participants at 18 and 36 months.

Variable	18 months (N=14) Mean ± SD	36 months (N=14) Mean ± SD	P-value
CPET			
$V'O_2$, mL/min	1723.21 ±	1646.64 ±	0.67
$V'O_2$/Kg, mL/min/Kg (VO2/Kg peak)	491.82 21.81 ± 5.32	435.82 19.3 ± 7.05	0.35 0.87
$V'O_2$, % pred.	95.36 ± 18.95	93.36 ± 18.05	**0.04**
$V'O_2$ at LT, mL/min	1068.71 ±	967.93 ±	0.17
VO_2 at LT % VO_2 max pred.	278.47	177.13	0.31
V'_E, L/min	58.57 ± 9.92	54.00 ± 8.44	0.33
BR, % eMVV	76.09 ± 24.68	82.76 ± 25.56	0.67
$V'_E/V'CO_2$ at LT	39.42 ± 9.39	35.20 ± 15.49	0.84
$V'_E/V'CO_2$ slope	31.52 ± 3.85	31.16 ± 3.57	0.94
$V'_E/V'CO_2$ intercept	31.69 ± 4.13	31.28 ± 3.70	0.08
O_2 pulse, mL/beat	1.74 ± 1.28	1.62 ± 1.39	0.09
O_2 pulse, % pred.	12.74 ± 3.29	11.51 ± 2.75	0.30
Work rate, Watt	112.57 ± 21.01	103.43 ± 20.29	**0.03**
$V'O_2$/work, mL/min/Watt	122.86 ± 37.45	120.43 ± 35.66	0.76
$P_{ET}CO_2$ at peak, mmHg	10.60 ± 1.28	12.74 ± 3.28	0.45
Dyspnea, mBORG	31.15 ± 3.63	30.92 ± 3.82	0.75
Leg fatigue, mBORG	5.00 ± 2.90	4.04 ± 2.55	0.99
Dysfunctional breathing, n. (%)	6.07 ± 2.34 4 (28.57 %)	5.71 ± 2.13 4 (28.57 %)	

Data refer to peak exercise, unless stated otherwise. BR, breathing reserve; eMVV, estimated maximal voluntary ventilation; LT, lactic threshold; mBORG, modified BORG scale; PETCO2, end tidal partial pressure of carbon dioxide; pred., predicted; V'CO2, production of carbon dioxide; V'E, minute ventilation; V'O2, oxygen consumption.

(r=0.58 – p=0.2) and CT severity score at T_0 (r=0.51 – p=0.03), T_3 (r=0.61 – 0.02) and T_{15} (r=0.34 – p=0.49), and between $V'_E/V'CO_2$ slope at T_{36} and DL_{CO} % pred. at T_3 (r= −0.30 – p=0.15) and T_{15} (r=-0.25 – p=0.19).

4. Discussion

The main finding of our study was the reduction in ventilatory efficiency during CPET observed at 18 and 36 months follow-up in patients with exertional dyspnea despite normal spirometry. Moreover, the reduction in ventilatory efficiency correlated with the severity of COVID-19 pneumonia at lung HRCT, at hospital admission, 3 and 15 months follow-up, and with the reduction of DL_{CO} at 3 and at 15 months follow-up. During exercise, pulmonary ventilation rises to meet the increased production of CO_2 and H^+ (Whipp et al., 1984; Forster et al., 2012). $V'_E/V'CO_2$ slope at CPET reflects the steepness with which ventilation increases following the augmented CO_2 production, therefore representing a measure of ventilatory efficiency. $V'_E/V'CO_2$ slope is determined by two main factors: the increase of arterial CO_2 partial pressure ($PaCO_2$) during exercise and the fraction of V_T that doesn't contribute to gas exchange, expressed as the dead space ventilation (V_D) fraction of tidal volume (V_D/V_T). A high V_D/V_T is responsible of a high $V'_E/V'CO_2$ slope at CPET, suggestive of ventilatory inefficiency (Whipp and Ward, 1982; Weatherald et al., 2018). The interest for $V'_E/V'CO_2$ slope has grown in the past decades, finding $V'_E/V'CO_2$ slope to be an independent prognostic predictor in different chronic cardiopulmonary diseases (Puente-Maestu et al., 2016). Also, $V'_E/V'CO_2$ has been shown to be a useful tool in identifying ventilatory inefficiency in early stages of disease, such as chronic obstructive pulmonary disease (COPD) (Neder

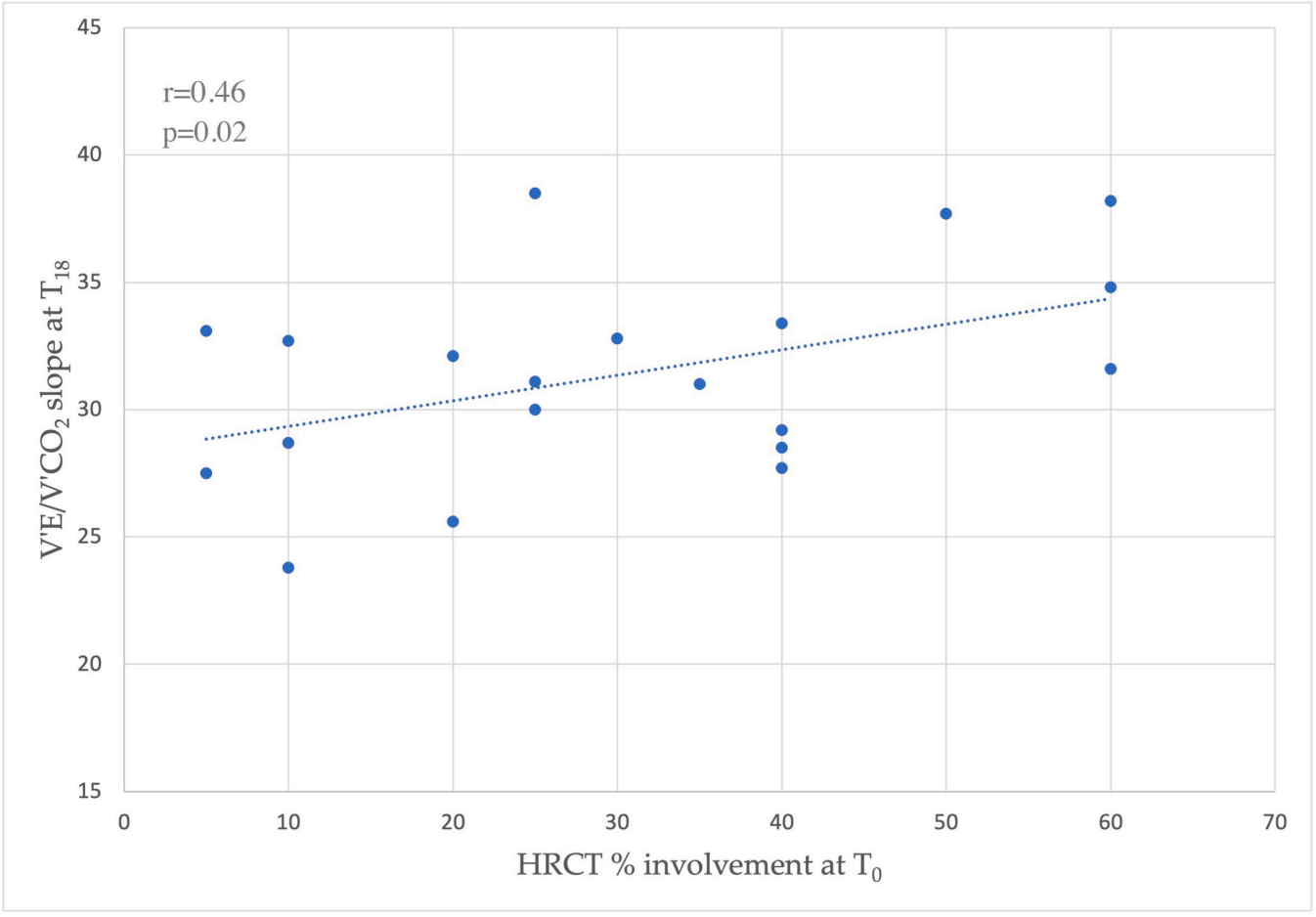

Fig. 4. $V'_E/V'CO_2$ slope – HRCT% involvement at T_0 correlation.

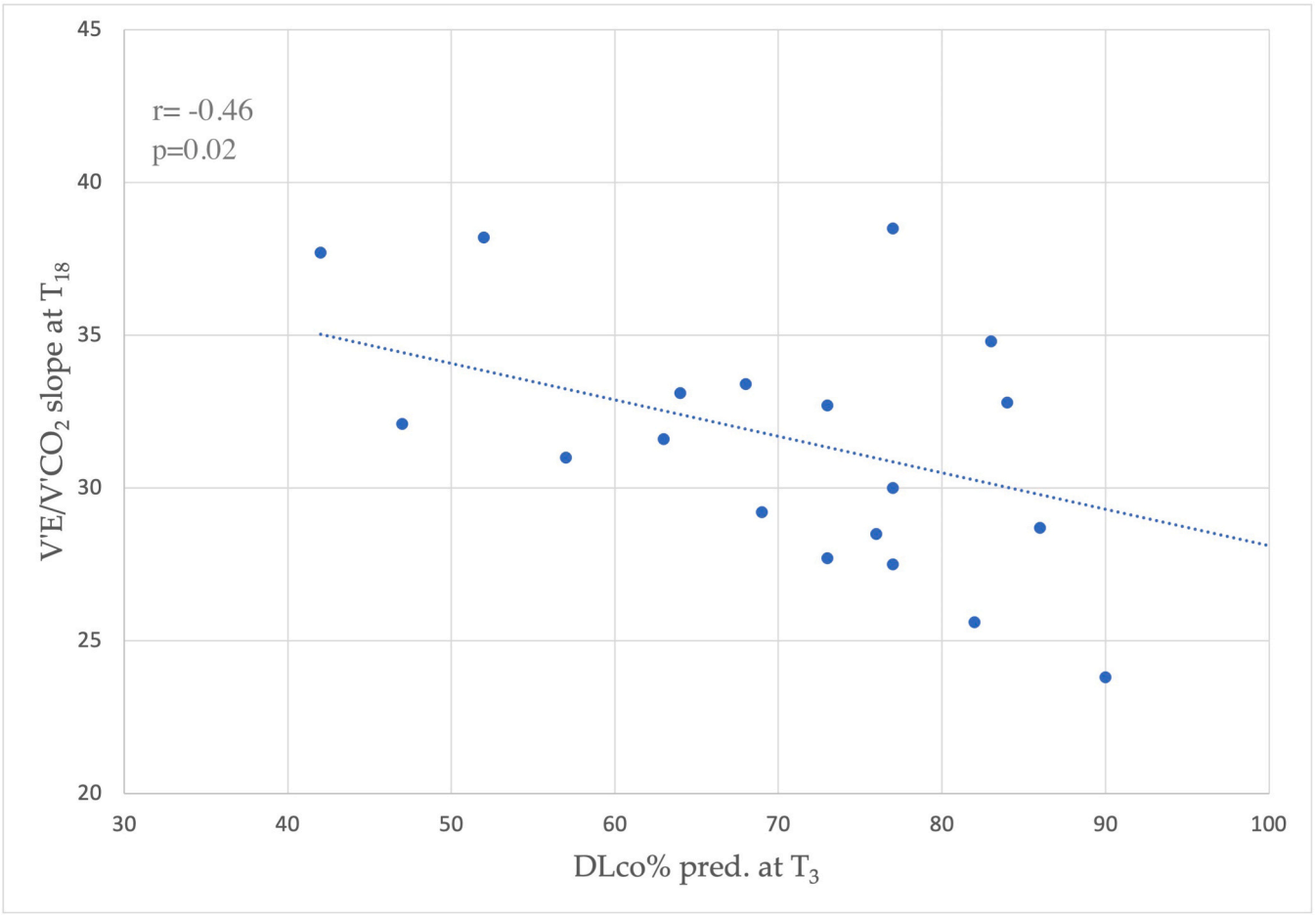

Fig. 5. $V'_E/V'CO_2$ slope – DLCO % pred. at T_3 correlation.

et al., 2017) and pulmonary vascular disease (Weatherald et al., 2020). High $V'_E/V'CO_2$ slope is not specific for pulmonary vascular disease, but it can be a useful marker of early disease (Sun et al., 2001) and it represents a significant prognostic factor (Weatherald et al., 2021; Deboeck et al., 2012; Groepenhoff et al., 2013, 2008; Ferreira et al., 2014; Schwaiblmair et al., 2012).

Data on CPET variables in Long COVID is limited. A recent study investigated medium-term effects of COVID-19 on 58 patients, showing a lower $V'O_2$ peak, a lower oxygen uptake efficiency slope and a higher $V'_E/V'CO_2$ slope when compared to controls (Raman et al., 2021). Dyspnea (mMRC $\geq$ 1) was reported in 47 % of patients at 3 months follow-up from COVID-19 hospitalization finding reduced ventilatory efficiency in 15 % of participants (Skjørten et al., 2021). Another study suggests that the presence of a dysfunctional breathing pattern during exercise in almost 30 % of patients could explain the persistency of dyspnea after COVID-19 (Frésard et al., 2022). *Motiejunaite et al.* observed elevated values of $V'_E/V'CO_2$ and respiratory alkalosis in dyspnoeic Long COVID patients, hypothesizing that symptoms were induced by a hyperventilation syndrome (Motiejunaite et al., 2021). A recent meta-analysis concluded that mean VO_2 at peak of exercise was significantly lower in patients with Long COVID symptoms, underlying that the main mechanisms involved are deconditioning, abnormal peripheral oxygen extraction, dysfunctional breathing and chronotropic incompetence (Durstenfeld et al., 2022).

Soon after the beginning of the SARS-CoV-2 pandemic, acute COVID-19 pneumonia was better characterized by the discovery of distinctive vascular features such as endothelial cell damage, thrombosis, microangiopathy and angiogenesis (Ackermann et al., 2020), leading to the hypothesis that pulmonary vascular damage could be the first insult of COVID-19 pneumonia (Price et al., 2021), explaining the severe respiratory failure despite preserved lung compliance (Gattinoni et al., 2020). Also, endothelial barrier dysfunction has been suggested to be one of the main mechanisms to impair exercise performance at CPET and may explain the persistency of lung diffusion impairment at long-term follow-up in patients who were hospitalized for COVID-19 (Huang et al., 2022). A recent investigation has excluded that dyspnea in Long COVID patients is linked to variations in DL_{CO}, even though most of the patients examined had not been hospitalized for COVID19. In our study, even if our population did not have significant limitations for undergoing incremental symptom-limited exercise testing, we identified abnormally high $V'_E/V'CO_2$ values, which correlated with the severity and extension of pneumonia on CT images and with the reduction of DL_{CO}, without changing significantly in time. Given the sheer heterogeneity of the mechanisms that have been described to cause dyspnea and exercise intolerance in Long COVID patients, it is conceivable that poor ventilatory efficiency reflects multiple abnormalities in ventilatory control, in addition to pulmonary gas exchange inefficiency. One possible hypothesis could be that the degree of severity of COVID-19 pneumonia on lung HRCT could be directly correlated to the degree of vascular damage, finding in $V'_E/V'CO_2$ a long-term marker that could partially explain breathlessness or exertional dyspnea in patients suffering from Long COVID.

The limitations of our study are the absence of a control group of age and sex matched individuals who have survived COVID19 without residual dyspnea, and the small sample size of patients who underwent CPET. Also, a larger sample size could give the opportunity to outline more precisely the specific mechanisms which can be reflected by poor ventilatory efficiency. Further studies with a larger sample size and a

longer follow-up may be necessary to confirm and to further investigate pathophysiological mechanisms of breathlessness in Long COVID patients, also to exclude confounding factors on the genesis of dyspnea, such as the presence of dysfunctional breathing, which could be misleading in the interpretation of V'E/V'CO2, as these patients are either hyperventilating or ventilating abnormally and erratically during CPET.

5. Conclusions

Exertional dyspnea is a common symptom in patients suffering from Long COVID. Data on its pathophysiology is limited, and recent literature suggests the presence of different mechanisms that could explain this invalidating symptom. CPET is a valid tool to better understand the pathophysiology of otherwise unexplained dyspnea in Long COVID patients with normal spirometry. In our study, the finding of a reduced $V'O_2$% pred. at peak of exercise in 25 % of our population and a high $V'_E/V'CO_2$ slope at 18 and 36 months follow-up, which correlated with the severity of pneumonia at lung HRCT and with the reduction of DLCO, could identify a possible contributing factor for the persistency of exertional dyspnea in these patients, leading to the hypothesis of a link between the initial extension of pneumonia, pulmonary vascular damage and persistency of ventilatory inefficiency at long-term follow-up.

CRediT authorship contribution statement

Daniel Piamonti: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Luigi Panza:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Roberto Flore:** Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Valentina Baccolini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Daniela Pellegrino:** Writing – original draft, Investigation, Data curation, Conceptualization. **Arianna Sanna:** Validation, Methodology, Data curation, Conceptualization. **Altea Lecci:** Validation, Investigation, Data curation, Conceptualization. **Giulia Lo Muzio:** Validation, Investigation, Data curation, Conceptualization. **Dario Angelone:** Validation, Investigation, Data curation, Conceptualization. **Flavio Marco Mirabelli:** Validation, Methodology, Data curation, Conceptualization. **Matteo Morviducci:** Validation, Methodology, Data curation, Conceptualization. **Paolo Palange:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emanuele Messina:** Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Valeria Panebianco:** Validation, Methodology, Formal analysis, Data curation. **Carlo Catalano:** Writing – original draft, Methodology, Formal analysis, Data curation. **Matteo Bonini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paolo Onorati:** Writing – original draft, Validation, Methodology, Data curation.

Data availability

Data will be made available on request.

References

Antoniou, K.M., Vasarmidi, E., Russell, A.M., Andrejak, C., Crestani, B., Delcroix, M., et al., 2022. European Respiratory Society Statement on Long COVID-19 Follow-Up. Eur. Respir. J. 2102174. Aug 10.

Durstenfeld, M.S., Sun, K., Tahir, P., Peluso, M.J., Deeks, S.G., Aras, M.A., et al., 2022. Use of Cardiopulmonary Exercise Testing to Evaluate Long COVID-19 Symptoms in Adults: A Systematic Review and Meta-analysis. JAMA Netw. Open [Internet] 5 (10), E2236057. Oct 12 [cited 2023 Sep 3].

Francone, M., Iafrate, F., Masci, G.M., Coco, S., Cilia, F., Manganaro, L., et al., 2020. Chest CT score in COVID-19 patients: correlation with disease severity and short-term prognosis. Eur. Radio. [Internet] 30 (12), 6808–6817. ⟨https://pubmed.ncbi.nlm.nih.gov/32623505/⟩. Dec 1 [cited 2022 Oct 29].

Graham, B.L., Brusasco, V., Burgos, F., Cooper, B.G., Jensen, R., Kendrick, A., et al., 2017. 2017 ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur. Respir. J. [Internet] 49 (1). ⟨https://erj.ersjournals.com/content/49/1/1600016⟩. Jan 1 [cited 2022 Oct 29].

Gulati, M., Black, H.R., Shaw, L.J., Arnsdorf, M.F., Noel, C., Merz, B., et al., 2005. Progn. Value a Nomogram Exerc. Capacit. Women [Internet] Vol. 4. ⟨www.nejm.org⟩ (Available from:).

Health Organization W. Guideline Clinical management of COVID-19 patients: living guideline, 18 November 2021. 2021.

"WHO Coronavirus (COVID-19) Dashboard," ⟨https://covid19.who.int⟩.

Ackermann, M., Verleden, S.E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., et al., 2020. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N. Engl. J. Med. 383 (2), 120–128. Jul 9.

Borg GAV, 1982. Psychophysical bases of perceived exertion. Med Sci. Sports Exerc [Internet] 14 (5), 377–381. ⟨https://pubmed.ncbi.nlm.nih.gov/7154893/⟩ [cited 2022 Oct 29].

Crook, H., Raza, S., Nowell, J., Young, M., Edison, P., 2021. Long covid - Mechanisms, risk factors, and management. BMJ. BMJ Publ. Group Vol. 374.

Cui, S., Chen, S., Li, X., Liu, S., Wang, F., 2020. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. J. Thromb. Haemost. [Internet] 18 (6), 1421. Jun 1 [cited 2022 Sep 17].

Daher, A., Balfanz, P., Cornelissen, C., Müller, A., Bergs, I., Marx, N., et al., 2020. Follow up of patients with severe coronavirus disease 2019 (COVID-19): Pulmonary and extrapulmonary disease sequelae. Respir. Med [Internet]. Nov 1 [cited 2022 Sep 17]; 174. Available from: https://pubmed.ncbi.nlm.nih.gov/33120193/.

Deboeck, G., Scoditti, C., Huez, S., Vachiéry, J.L., Lamotte, M., Sharples, L., et al., 2012. Exercise testing to predict outcome in idiopathic versus associated pulmonary arterial hypertension. Eur. Respir. J. [Internet] 40 (6), 1410–1419. ⟨https://erj.ersjournals.com/content/40/6/1410⟩. Dec 1 [cited 2023 Jan 10].

Ferreira, E.V.M., Ota-Arakaki, J.S., Ramos, R.P., Barbosa, P.B., Almeida, M., Treptow, E. C., et al., 2014. Optimizing the evaluation of excess exercise ventilation for prognosis assessment in pulmonary arterial hypertension. Eur. J. Prev. Cardiol. [Internet] 21 (11), 1409–1419. ⟨https://academic.oup.com/eurjpc/article/21/11/1409/592604 3⟩. Nov 1 [cited 2023 Jan 10];.

Forster, H. v, Haouzi, P., Dempsey, J.A., 2012. Control of breathing during exercise. Compr. Physiol. 2 (1), 743–777 (Jan).

Frésard, I., Genecand, L., Altarelli, M., Gex, G., Vremaroiu, P., Vremaroiu-Coman, A., et al., 2022. Dysfunctional breathing diagnosed by cardiopulmonary exercise testing in 'long COVID' patients with persistent dyspnoea. BMJ Open Respir. Res 9 (1). Mar 1.

Gattinoni, L., Coppola, S., Cressoni, M., Busana, M., Rossi, S., Chiumello, D., 2020. COVID-19 does not lead to a "typical" acute respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine. American Thoracic Society, pp. 1299–1300. Vol. 201.

Groepenhoff, H., Vonk-Noordegraaf, A., Boonstra, A., Spreeuwenberg, M.D., Postmus, P. E., Bogaard, H.J., 2008. Exercise testing to estimate survival in pulmonary hypertension. Med Sci. Sports Exerc [Internet] 40 (10), 1725–1732. ⟨https://journals.lww.com/acsm-msse/Fulltext/2008/10000/Exercise_Testing_to_Estimate_Survival_in_Pulmonary.3.aspx⟩ [cited 2023 Jan 10].

Groepenhoff, H., Vonk-Noordegraaf, A., van de Veerdonk, M.C., Boonstra, A., Westerhof, N., Bogaard, H.J., 2013. Prognostic Relevance of Changes in Exercise Test Variables in Pulmonary Arterial Hypertension. PLoS One [Internet] 8 (9), e72013. ⟨https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0072013⟩. Sep 5 [cited 2023 Jan 10].

Hansell, D.M., Bankier, A.A., MacMahon, H., McLoud, T.C., Müller, N.L., Remy, J., 2008. Fleischner Society: glossary of terms for thoracic imaging. Radiol. [Internet]. 246 (3), 697–722. ⟨https://pubmed.ncbi.nlm.nih.gov/18195376/⟩. Mar [cited 2022 Oct 29].

Hansen, J.E., Sue, D.Y., Wasserman, K., 2015. Predict. Values Clin. Exerc. Testing1–3 129 (2 SUPPL) https://doi.org/101164/arrd19841292P2S49.

Huang, L., Li, X., Gu, X., Zhang, H., Ren, L., Guo, L., et al., 2022. Health outcomes in people 2 years after surviving hospitalisation with COVID-19: a longitudinal cohort study. Lancet Respir. Med (May).

Ionescu, M.F., Mani-Babu, S., Degani-Costa, L.H., Johnson, M., Paramasivan, C., Sylvester, K., et al., 2021. Cardiopulmonary exercise testing in the assessment of dysfunctional breathing. Frontiers in Physiology. Frontiers Media S.A. Vol. 11.

Laveneziana P., Paolo M. di, Palange P. The clinical value of cardiopulmonary exercise testing in the modern era Number 1 in the Series 'Ventilatory efficiency and its clinical prognostic value in cardiorespiratory disorders' Edited by Pierantonio Laveneziana and Paolo Palange. [cited 2022 Sep 25]; Available from: ⟨https://doi.org/10.1183/16000617.0187-2020⟩].

Motiejunaite, J., Balagny, P., Arnoult, F., Mangin, L., Bancal, C., d'Ortho, M.P., et al., 2021. Hyperventilation: A Possible Explanation for Long-Lasting Exercise Intolerance in Mild COVID-19 Survivors? Front Physiol. 11. Jan 18.

Neder, J.A., Berton, D.C., de Tarso Müller, P., Elbehairy, A.F., Rocha, A., Palange, P., et al., 2017. Ventilatory inefficiency and exertional dyspnea in early chronic obstructive pulmonary disease. : Ann. Am. Thorac. Soc. Am. Thorac. Soc. S22–S29.

Onathan J., Yers M., Anish M., Rakash P., Ictor V., Roelicher F., et al. The New England Journal of Medicine EXERCISE CAPACITY AND MORTALITY AMONG MEN REFERRED FOR EXERCISE TESTING A BSTRACT [Internet]. Vol. 346, N Engl J Med. 2002. Available from: ⟨www.nejm.org⟩.

Palange, P., Ward, S.A., Carlsen, K.H., Casaburi, R., Gallagher, C.G., Gosselink, R., et al., 2007. Recommendations on the use of exercise testing in clinical practice. Eur. Respir. J. [Internet] 29 (1), 185–209. ⟨https://erj.ersjournals.com/content/29/1/185⟩. Jan 1 [cited 2022 Sep 25].

Pan, F., Ye, T., Sun, P., Gui, S., Liang, B., Li, L., et al., 2020. Time Course of Lung Changes at Chest CT during Recovery from Coronavirus Disease 2019 (COVID-19). Radiol. [Internet] 295 (3), 715–721. ⟨https://pubmed.ncbi.nlm.nih.gov/32053470/⟩. Jun 1 [cited 2022 Oct 29].

Pecoraro, M., Cipollari, S., Marchitelli, L., Messina, E., del Monte, M., Galea, N., et al., 2021. Cross-sectional analysis of follow-up chest MRI and chest CT scans in patients previously affected by COVID-19. Radio. Med [Internet]. 126 (10), 1273–1281. ⟨https://pubmed.ncbi.nlm.nih.gov/34251585/⟩. Oct 1 [cited 2022 Oct 29].

Price, L.C., Ridge, C., Wells, A.U., 2021. Pulmonary vascular involvement in COVID-19 pneumonitis: Is this the first and final insult?, Vol. 26. John Wiley and Sons Inc, Respirology, pp. 832–834.

Puente-Maestu, L., Palange, P., Casaburi, R., Laveneziana, P., Maltais, F., Neder, J.A., et al., 2016. Use of exercise testing in the evaluation of interventional efficacy: an official ERS statement. Eur. Respir. J. [Internet] 47 (2), 429–460. ⟨https://pubmed.ncbi.nlm.nih.gov/26797036/⟩. Feb 1 [cited 2023 Jan 9].

Radtke, T., Crook, S., Kaltsakas, G., Louvaris, Z., Berton, D., Urquhart, D.S., et al., 2022. ERS statement on standardisation of cardiopulmonary exercise testing in chronic lung diseases ([cited). Thierry Troosters [Internet] 16, 1901441. https://doi.org/10.1183/16000617.0101-2018.

Raman, B., Cassar, M.P., Tunnicliffe, E.M., Filippini, N., Griffanti, L., Alfaro-Almagro, F., et al., 2021. Medium-term effects of SARS-CoV-2 infection on multiple vital organs, exercise capacity, cognition, quality of life and mental health, post-hospital discharge. EClinicalMedicine 31. Jan 1.

Salehi, S., Abedi, A., Balakrishnan, S., Gholamrezanezhad, A., 2020. Coronavirus Disease 2019 (COVID-19): A Systematic Review of Imaging Findings in 919 Patients. AJR Am. J. Roentgenol. [Internet] 215 (1), 87–93. ⟨https://pubmed.ncbi.nlm.nih.gov/32174129/⟩. Jul 1 [cited 2022 Oct 29].

Sanna, A., Pellegrino, D., Messina, E., Siena, L.M., Baccolini, V., D'Antoni, L., et al., 2023. The Role of Pulmonary Function Testing and Lung Imaging in the Long-Term Follow-Up of Patients with COVID-19 Pneumonia Role of Pulmonary Function Tests and High-Resolution Computed Tomography in Post-COVID-19 Interstitial Lung Disease. Respiration 102 (4), 287–295.

Schwaiblmair, M., Faul, C., von Scheidt, W., Berghaus, T.M., 2012. Ventilatory efficiency testing as prognostic value in patients with pulmonary hypertension. BMC Pulm. Med [Internet] 12 (1), 1–7. ⟨https://bmcpulmmed.biomedcentral.com/articles/10.1186/1471-2466-12-23⟩. Jun 7 [cited 2023 Jan 10].

Skjørten, Ankerstjerne OAW, I., Trebinjac, D., Brønstad, E., Rasch-Halvorsen, Ø., Einvik, G., et al., 2021. Cardiopulmonary exercise capacity and limitations 3 months after COVID-19 hospitalisation. Eur. Respir. J. 58 (2). Aug 1.

Sun, X.G., Hansen, J.E., Oudiz, R.J., Wasserman, K., 2001. Exercise Pathophysiology in Patients With Primary Pulmonary Hypertension. Circ. [Internet] 104 (4), 429–435. ⟨https://www.ahajournals.org/doi/abs/10.1161/hc2901.093198⟩. Jul 24 [cited 2023 Jan 10];.

Tanni, S.E., Fabro, A.T., de Albuquerque, A., Ferreira, E.V.M., Verrastro, C.G.Y., Sawamura, M.V.Y., et al., 2021. Pulmonary fibrosis secondary to COVID-19: a narrative review. Expert Rev. Respir. Med 15 (6), 791–803.

Wanger, J., Clausen, J.L., Coates, A., Pedersen, O.F., Brusasco, V., Burgos, F., et al., 2005. Standardisation of the measurement of lung volumes. Eur. Respir. J. [Internet] 26 (3), 511–522. ⟨https://pubmed.ncbi.nlm.nih.gov/16135736/⟩. Sep [cited 2022 Oct 29].

Wasserman K., Hansen J., Sue D.Y., Stringer W., Sietsema K., Sun X.G., et al. Principles of exercise testing and interpretation: Including pathophysiology and clinical applications: Fifth edition. 2011. 1–592 p.

Weatherald, J., Sattler, C., Garcia, G., Laveneziana, P., 2018. Ventilatory response to exercise in cardiopulmonary disease: the role of chemosensitivity and dead space. Eur. Respir. J. [Internet] 51 (2). ⟨https://pubmed.ncbi.nlm.nih.gov/29437936/⟩ [cited 2023 Jan 9].

Weatherald, J., Boucly, A., Montani, D., Jaïs, X., Savale, L., Humbert, M., et al., 2020. Gas Exchange and Ventilatory Efficiency During Exercise in Pulmonary Vascular Diseases. Arch. Bronc-.-. 56 (9), 578–585. Sep 1.

Weatherald, J., Philipenko, B., Montani, D., Laveneziana, P., 2021. Ventilatory efficiency in pulmonary vascular diseases. Eur. Respir. Rev. 30 (161). Sep 1.

Weisman, I.M., Marciniuk, D., Martinez, F.J., Testing Measurements Frank Sciurba, E., Sue, D., Myers Bruce Johnson, J., et al., 2003. ATS/ACCP Statement on cardiopulmonary exercise testing. Am. J. Respir. Crit. Care Med [Internet] 167 (2), 211–277. ⟨www.atsjournals.org⟩ [cited 2022 Oct 29].

Whipp, B.J., Ward, S.A., 1982. Cardiopulmonary coupling during exercise. J. Exp. Biol. [Internet] 100 (1), 175–193. ⟨https://journals.biologists.com/jeb/article/100/1/175/3922/Cardiopulmonary-coupling-during-exercise⟩. Oct 1 [cited 2023 Jan 10].

Whipp, B.J., Ward, S.A., Wasserman, K., 1984. Ventilatory responses to exercise and their control in man. Am. Rev. Respir. Dis. [Internet] 129 (2 Pt 2). ⟨https://pubmed.ncbi.nlm.nih.gov/6421213/⟩ [cited 2023 Jan 9].

WHO "Coronavirus Disease (COVID-19): Post COVID-19 Condition," ⟨https://www.who.int/news-room/questions-and-answers/item/coronavirus-disease⟩-(covid-19)-post-covid-19-condition.