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Original Research

Dyspnea and fatigue in Long-COVID: definition of risk factors and of DLCO-based phenotypes in a multicenter study of 765 patients from Italy

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ABSTRACT

Background: Dyspnea and fatigue represent common Long-COVID symptoms, but their presence is not always accompanied by lung function abnormalities. Aim of the study was to evaluate dyspnea and fatigue in relation to pulmonary function and exercise capacity.

Methods: Multicenter cohort study. Multivariable analyses were used to characterize, for both symptoms, functional phenotypes with and without pulmonary impairment according to the diffusing lung capacity for carbon monoxide (DLCO). Exercise capacity was assessed through the distance walked in 6 min (6MWD).

Results: Among 765 patients evaluated at a mean interval of six months from COVID-19, rates of dyspnea and fatigue were 41.3% and 41.6%, respectively. Roughly half of the patients with these two symptoms (51.6% and 54.7%, respectively) had normal pulmonary function at DLCO testing (≥80% of predicted). Low-DLCO (<80%) dyspnea was significantly associated with female sex, anxiety, duration of hospitalisation, use of corticosteroids and of monoclonal neutralizing antibodies, and its risk decreased at the increasing in time from acute infection. Normal-DLCO dyspnea was associated with younger age and obesity. Low-DLCO fatigue was associated with female sex, heart failure, anxiety and use of corticosteroids. Normal-DLCO fatigue was not associated with demographics, comorbidities, or COVID-19 severity. For both symptoms, the low-DLCO phenotypes had a significantly lower 6MWD.

Conclusions: The clinical phenotypes of dyspnea and fatigue with normal pulmonary function should be further explored, possibly with additional tests that assess cardiorespiratory and cardiovascular function. DLCO testing should be included in the evaluation of patients who report dyspnea and/or fatigue as possible Long-COVID symptoms.

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1. Introduction

Pulmonary symptoms represent a major element in the clinical spectrum of Long-COVID [1]. Dyspnea and fatigue are usually recognized as the most common Long-COVID symptoms, but there is still limited information about the underlying mechanisms, which might involve multiple cofactors and distinct pathways, ultimately leading to different clinical and functional phenotypes [2,3]. Several studies have investigated instrumentally the correlates of dyspnea and fatigue and the risk factors for persistent pulmonary functional abnormalities, but their findings were often controversial [1,4–20]. Most of the studies [1,2,6,10,11,15], but not all [8] identified severity of acute disease as a risk factor; advanced age [1,6,8], smoking [19] and comorbidities [1,15] were risk factors only in some studies, and with respect to sex, some studies reported no association [8], others higher risk for males [2,15], and others higher risk for females [12,19]. Such inconsistencies might be explained by differences involving the populations studied, the functional tests used, the time of observation, the level of adjustment for covariates, and the amplitude of the sample evaluated.

With respect to the outcome measures, there is a general agreement that, in the evaluation of pulmonary function, the diffusing capacity of the lungs for carbon monoxide (DLCO) represents a better and more sensitive index compared to other spirometric tests such as Forced Expiratory Volume in 1 s (FEV-1), Forced Vital Capacity (FVC) and Total Lung Capacity (TLC), that may provide results within normal limits, even in the presence of dyspnea or fatigue [1,4–6,10,11,13–15,18,19]. Another simple and standardized measure commonly used to assess exercise capacity in patients with cardiopulmonary conditions is the distance (6-min walking distance or 6MWD) walked during the 6-min walking test (6MWT). Due to its simplicity and reproducibility, this test has also been frequently used in the functional evaluation of patients with Long-COVID, often in association with other tests [21].

With the aim to contribute further information on the complex interplay of pulmonary function, exercise capacity and persistent symptoms in patients with Long-COVID, we evaluated the rate and determinants of reduced pulmonary function (as measured by DLCO) and the relation of dyspnea and fatigue with exercise capacity (as measured by 6MWD), using data from a national cohort of patients followed in Long-COVID centers [22]. In order to define possible clinical and functional phenotypes, we evaluated a large number of clinical and demographic cofactors in patients symptomatic for dyspnea and fatigue with and without DLCO functional impairment.

2. Methods

2.1. Study overview

The present clinical study, structured as an observational cohort, is part of the national project "Analysis and strategies for responding to the long-term effects of the COVID-19 infection (Long-COVID)", funded by the Italian Ministry of Health in 2021 (Grant I85F21003410005) and approved by the Ethics Committee of the Italian National Institute of Health (ref. PRE BIO CE 01.00 0015066, 2022). The project aimed at monitoring the long-term effects of SARS-CoV-2 infection (establishing the clinical cohort here analysed), increasing knowledge about the Long-COVID condition, and providing recommendations to standardize the approach nationwide [23]. Participation to the cohort study was offered to all the centers for assistance to Long-Covid previously identified in a national survey [24].

Patients entered the study between January 2023 and March 2024, with data extracted on April 2, 2024. The study population included patients attending for a first visit after the study start and, more frequently, patients already followed, who returned for a programmed follow up visit after acute infection or for occurrence/persistence of potential Long-Covid symptoms. All patients provided written informed consent. Symptom collection was retrospective, based on clinical records and patient interview, for patients who had previous visits before the start of the study (January 2023), and prospective for patients newly accessing the centers. Data on demographics, acute infection and comorbidities were taken from clinical records. Study data were entered by medical staff at the participating centers using an online dedicated platform. Inclusion criteria for the present analysis were age at least 18, a recorded date of acute SARS-CoV-2 infection (defined by date of first positive swab), a clinical assessment performed at least 4 weeks after acute SARS-CoV-2 infection, and available information for DLCO.

2.2. Data collection

For data collection, a shortened version of the Post COVID-19 Case Report Form (CRF) from the WHO Global Clinical Platform for COVID-19 (modules 1-3) was used [25], that included patient demographics, comorbidities, severity and timing of acute COVID, plus 30 different symptoms (fatigue, dyspnea, sleep disturbances, memory loss, joint pain or swelling, muscle pain, difficult concentration, cough, anxiety, taste reduction, smell reduction, palpitations/tachycardia, depressed mood, skin disorders/alopecia, thoracic pain, paresthesia, brain fog, headache, disorders of equilibrium or gait, visual disturbances, weight loss, diarrhea, hearing disturbances, pharygodynia, loss of appetite, nausea or vomiting, fever, menstrual disorders, chilblains, delirium or hallucinations) [25]. Data were entered in the electronic platform by physicians, based on patients' report. After data collection, the different answers to the symptom questions in Module 2 of the WHO form were categorized as a binary variable, considering as patients with persisting symptoms those with the answer "Yes, still present" and as patients without persisting symptoms those with the answers "No ", "Yes but not present anymore", "Yes intermittent".

2.3. Definitions

Severity of acute SARS-CoV-2 disease was defined as mild (grade 1), moderate (grade 2), severe (grade 3) or critical (grade 4) according to the WHO grading [25]. Respiratory assistance was categorized as none, low or high flow oxygen, continuous positive airway pressure (CPAP), mechanical ventilation (MV) and extracorporeal membrane oxygenation (ECMO). Phase of the pandemic was categorized as Pre-Omicron or Omicron according to occurrence of date of acute infection before or after December 23, 2021 [26]. The comorbidities considered were neoplastic disease, ischemic heart disease, heart failure, renal failure, stroke, anxiety, depressive disorders, chronic liver disease, respiratory failure, chronic pulmonary disease, diabetes, hypertension, obesity, autoimmune diseases, asthma, obstructive sleep apnea syndrome (OSAS), plus a general category of other major conditions, reviewed by two of the authors. SARS-CoV-2 vaccination status was categorized according to timing of first dose administration (before SARS-CoV-2 infection, after SARS-CoV-2 infection, not vaccinated). According to the WHO form, dyspnea and fatigue were evaluated as binary measures (present or absent) based on subjective patients' reports.

2.4. Procedures

Diffusion capacity of the lungs for carbon monoxide (DLCO) was measured using the single-breath test, with values below 80% considered as abnormal [6,10,13,15,16,18,19]. For the 6-min walking test each patient walked on flat ground as fast as possible without oxygen inhalation and performed the test independently. The distance completed was expressed in meters [14].

2.5. Data analysis

Data were summarized as proportions for categorical variables and as means with standard deviations (SD) for quantitative variables. For both dyspnea and fatigue, we defined, according to DLCO levels, two

groups of symptomatic patients, who had respectively normal or impaired DLCO status, and explored possible differences in demographic and clinical characteristics between these two groups. Mean values were compared by Student's T test and proportions by the chi-square test in contingency tables. Multivariable logistic regression models were used to assess the associations between clinical and demographic covariates and presence of dyspnea and fatigue with and without a reduced DLCO (<80% of predicted). The models included all covariates with a level of association <0.15 (p value) in univariate analyses plus age and sex. Associations were expressed as adjusted odds ratios (AOR) with 95% confidence intervals (CI). The goodness of fit of the models was tested with the Hosmer-Lemeshow test and multicollinearity with correlation matrices. No input was used to substitute missing data. All analyses were performed using the SPSS software, version 29.0 (IBM Corp, 2022, Armonk, NY, US).

3. Results

3.1. Population characteristics

The study population included 765 patients followed as outpatients in clinical units of Pneumology, Infectious Diseases, Cardiology, Geriatrics and Internal Medicine, mostly (96%) already followed at study sites before the start of the study. Their general characteristics are reported in Table 1. Overall, the study population was characterised by a slight prevalence of male sex (54.0%), a relatively advanced age (mean: 59.1 years), common presence of comorbidities (64.4%), relatively high body mass index (mean: 27.1 kg/m^2), and frequent occurrence of hospitalisation during acute COVID-19 (71.0%); roughly half of the patients had presented severe or critical acute SARS-CoV-2 infection, and more than one quarter had received CPAP or mechanical ventilation as respiratory support. Treatments administered during acute phase included systemic steroids in 59.4% of the cases, antivirals (mostly remdesivir) in 26.5%, and less frequent use of IL-6 inhibitors (12.4%) or neutralizing antibodies (5.2%). At a clinical assessment performed after a mean interval of 179 days from acute COVID-19, 564 patients (73.7%) presented at least one persisting symptom, with dyspnea and fatigue present in 41.3% and 41.6% of the individuals, respectively. A reduced DLCO (<80%) was observed in 39.6% of the patients (Table 1).

3.2. Functional and clinical correlates of dyspnea and fatigue

The characteristics of patients with and without persisting dyspnea at the time of assessment are reported in Table 2. Dyspnea was significantly associated with female sex, younger age, smoking, presence of any comorbidity, anxiety, asthma, obesity, Omicron pandemic phase, admission to intensive care unit, mechanical ventilation, and administration of systemic corticosteroids during the acute phase of the disease. The functional tests showed that individuals with dyspnea, compared to individuals not presenting this symptom, had lower mean values of DLCO, a more frequent occurrence of DLCO levels below 80%, and a significant shorter distance walked at the 6MWT (Table 2).

The clinical and functional correlates of fatigue are reported in Table 3. Fatigue was significantly associated with female sex, younger age, smoking, anxiety, depression, autoimmune diseases and SARS-CoV-2 vaccination before infection. Patients with fatigue had less frequently severe or critical COVID-19 disease, lower rates of hospitalisation, and received less frequently antivirals and IL-6 inhibitors during acute infection. As for dyspnea, the comparison of individuals with and without fatigue showed that the first group had significantly worst functional status, as shown by lower mean values of DLCO, more frequent occurrence of DLCO levels below 80%, and a significant shorter distance walked at the 6MWT (Table 3).

Table 1
Population characteristics.

Female sex: (n, %)		352 (46.0)
Age: years (mean, SD)		59.1 (13.4)
≥65 years (n, %)		260 (34.0)
Body mass index (kg/m^2, mean, SD) (n: 699)		27.1 (4.8)
Current smoking (n: 725) (n, %)		54 (7.4)
Comorbidities: (mean number, SD)		1.2 (1.3)
Any (n, %)		493 (64.4)
Neoplastic disease		47 (6.1)
Ischemic heart disease		50 (6.5)
Heart failure		23 (3.0)
Renal insufficiency		15 (2.0)
Stroke		21 (2.7)
Anxiety		29 (3.8)
Depression		34 (4.4)
Chronic liver disease		12 (1.6)
Respiratory failure or COPD		54 (7.1)
Diabetes		72 (9.4)
Hypertension		308 (40.3)
Obesity		97 (12.7)
Autoimmune diseases		61 (8.0)
Asthma		48 (6.3)
Atrial fibrillation		25 (3.3)
OSAS		16 (2.1)
Other major conditions		36 (4.7)
Acute infection pandemic phase:	Pre-omicron	645 (84.3)
	Omicron	120 (15.7)
SARS-CoV-2 vaccination (any dose or type) (n: 577):	Not vaccinated	418 (72.4)
	Vaccinated before infection	86 (14.9)
	Vaccinated after infection	73 (12.7)
Hospitalised during acute phase:		538 (71.0)
Admitted to intensive care unit (n: 756):		62 (8.2)
WHO COVID severity grade (n: 760):	Mild	216 (2.2)
	Moderate	128 (16.7)
	Severe	299 (39.1)
	Critical	117 (15.3)
Respiratory assistance (n: 740):	None	276 (37.3)
	Low-flow O2	212 (28.6)
	High-flow O2	64 (8.6)
	CPAP	143 (19.3)
Mechanical ventilation		45 (6.1)
Treatments during acute phase (n: 734):	Antivirals	194 (26.5)
	Oral or IV steroids	436 (59.4)
	Neutralizing monoclonal antibodies	38 (5.2)
	IL-6 inhibitors	91 (12.4)
Days from acute COVID-19 at assessment (mean, SD)		179 (107)
Persisting Long-COVID symptoms:	Any	564 (73.7)
	Dyspnea	316 (41.3)
	Fatigue	318 (41.6)
Meters walked in 6 min (mean, SD) (n: 400)		491 (107)
DLCO (%, mean, SD)		82.0 (15.7)
DLCO<80% (%)		303 (39.6)

SD: standard deviation; COPD: Chronic Obstructive Pulmonary Disease; OSAS: Obstructive Sleep Apnea Syndrome; O2: oxygen; CPAP: Continuous Positive Airway Pressure; IV: intravenous; IL-6: Interleukin 6; DLCO: Diffusing capacity of the lungs for carbon monoxide.

3.3. Definition of functional phenotypes

Despite an overall worst DLCO and 6MWD functional status, most individuals with dyspnea or fatigue did not show a reduced DLCO capacity: among the 316 individuals reporting dyspnea, 163 (51.6%) had DLCO values of at least 80% and among the 318 individuals with fatigue, 174 (54.7%) had DLCO values at or above this threshold. In multivariable logistic regression models, dyspnea with low DLCO was significantly associated with female sex, anxiety, duration of hospitalisation, use of corticosteroids and of monoclonal neutralizing antibodies during

Table 2
Clinical and functional correlates of dyspnea.

	Dyspnea			
	Yes	No	Odds ratio (95%CI)	P value
Female sex: (n, %)	160 (50.6)	192 (42.8)	1.37 (1.03-1.83)	**0.031**
Age ≥65 years (n, %)	90 (28.5)	170 (37.9)	0.65 (0.48-0.89)	**0.007**
Current smoking (n: 725) (n, %)	31 (10.0)	23 (5.5)	1.89 (1.08-3-32)	**0.026**
Comorbidities (n, %):				
Any	219 (69.3)	274 (61.0)	1.44 (1.06-1.96)	**0.019**
Neoplastic disease	25 (7.9)	22 (4.9)	1.67 (0.92-3.01)	0.091
Ischemic heart disease	21 (6.6)	29 (6.5)	1.03 (0.58-1.84)	0.918
Heart failure	9 (2.8)	14 (3.1)	0.91 (0.39-2.13)	0.830
Renal insufficiency	5 (1.6)	10 (2.2)	0.71 (0.24-2.08)	0.528
Stroke	9 (2.8)	12 (2.7)	1.07 (0.44-2.56)	0.884
Anxiety	21 (6.6)	8 (1.8)	3.92 (1.71-8.98)	**0.001**
Depression	19 (6.0)	15 (3.3)	1.85 (0.93-3.70)	0.082
Chronic liver disease	6 (1.9)	6 (1.3)	1.43 (0.46-4.47)	0.540
Respiratory failure or COPD	23 (7.3)	31 (6.9)	1.06 (0.60-1.85)	0.842
Diabetes	31 (9.8)	41 (9.1)	1.08 (0.66-1.77)	0.752
Hypertension	128 (40.5)	180 (40.1)	1.02 (0.76-1.36)	0.908
Obesity	60 (19.0)	37 (8.2)	2.61 (1.68-4.05)	**<0.001**
Autoimmune diseases	28 (8.9)	33 (7.3)	1.23 (0.72-2.07)	0.448
Asthma	29 (9.2)	19 (4.2)	2.29 (1.26-4.16)	**0.007**
Atrial fibrillation	7 (2.2)	18 (4.0)	0.54 (0.22-1.31)	0.176
OSAS	10 (3.2)	6 (1.3)	2.41 (0.87-6.70)	0.091
Other major conditions	14 (4.4)	22 (4.9)	0.90 (0.45-1.79)	0.763
Omicron pandemic phase (n, %)	62 (19.6)	58 (12.9)	1.65 (1.11-2.43)	**0.013**
Vaccinated for SARS-CoV-2 before infection (n: 577) (n, %)	42 (17.7)	44 (12.9)	1.45 (0.91-2.29)	0.114
Hospitalised during acute phase (n: 758) (n, %)	211 (67.6)	327 (73.3)	0.76 (0.55-1.04)	0.090
Admitted to intensive care unit (n: 756) (n, %)	3.9 (12.5)	23 (5.2)	2.61 (1.53-4.47)	**<0.001**
WHO COVID grade severe or critical (n: 760) (n, %)	169 (53.8)	247 (55.4)	0.94 (0.70-1.25)	0.671
Respiratory assistance with mechanical ventilation (n: 740) (n, %)	26 (8.6)	19 (4.3)	2.06 (1.12-5.80)	**0.020**
Treatments during acute phase (n, %):				
Antivirals (n: 733)	77 (25.8)	117 (27.0)	0.94 (0.67-1.31)	0.716
Oral or IV steroids (n: 734)	195 (65.0)	241 (55.5)	1.49 (1.10-2.01)	**0.010**
Neutralizing monoclonal antibodies (n: 736)	18 (6.0)	20 (4.6)	1.32 (0.69-2.54)	0.406
IL-6 inhibitors (n: 734)	36 (12.0)	55 (12.7)	0.94 (0.60-1.47)	0.786
Reduced DLCO (<80%) (n, %)	153 (48.4)	150 (33.4)	1.87 (1.39-2.51)	**<0.001**

	Dyspnea			
	Yes	*No*	*Difference (95%CI)*	*P value*
Days from COVID-19 (mean, SD)	178 (115)	179 (100)	−1.6 (−17, 14)	0.843
Meters walked in 6 min (n: 400) (mean, SD)	473 (116)	509 (95)	−35 (−56, −14)	**<0.001**
DLCO (mean, SD)	78.9 (16.9)	84.2 (14.5)	−5 (−7, −3)	**<0.001**

SD: standard deviation; COPD: Chronic Obstructive Pulmonary Disease; OSAS: Obstructive Sleep Apnea Syndrome; IV: intravenous; IL-6: Interleukin 6; DLCO: Diffusing capacity of the lungs for carbon monoxide. Significant p values are expressed in bold.

acute infection, and its risk decreased at the increasing in time from acute infection. Dyspnea with preserved DLCO was associated with younger age and obesity (Table 4). Among the individuals with dyspnea who had 6MWT results available (n: 197), those with reduced DLCO had a significantly lower distance walked in 6 min compared to individuals with dyspnea and normal DLCO (450 m, SD 117, vs. 493, SD 111, $p = 0.005$).

The similar analyses performed for fatigue showed that fatigue with low DLCO was associated with female sex, heart failure, anxiety and use of corticosteroids during acute SARS-CoV-2 infection. Fatigue with preserved DLCO was not associated with demographics or indexes of COVID-19 or with major individual comorbidities. Its risk was lower for patients who received antivirals during acute infection and for those with a history of other major conditions before infection (Table 4). Among the individuals with fatigue who had 6MWT results available (n: 167), those with reduced DLCO had a significantly lower distance walked in 6 min compared to individuals with fatigue and normal DLCO (439 m, SD 131, vs. 499, SD 119, $p = 0.001$).

4. Discussion

Our study explored and characterized the clinical and functional correlates of dyspnea and fatigue in a population of patients followed at a mean interval of six months from acute SARS-CoV-2 infection. The findings confirmed that, with a prevalence of roughly forty per cent for both symptoms, dyspnea and fatigue are common persisting symptoms in patients accessing care for Long-COVID, indicating a significant burden of morbidity [1,2,5,7,13,27]. The analysis of the clinical correlates showed some risk factors common to the two symptoms (female sex, younger age, smoking, anxiety) and some associations specific for the two individual symptoms: dyspnea was significantly associated with a more severe acute SARS-CoV-2 disease, represented by admission to intensive care unit and administration of corticosteroids during acute disease, while fatigue was inversely associated with hospitalisation and antiviral therapeutic interventions. These findings, together with the specific association of fatigue with autoimmune diseases and SARS-CoV-2 vaccination, might be consistent with the hypothesis that Long-COVID is a heterogeneous disease, with distinct pathogenetic pathways involved in the expression of specific symptoms [9,28]. More specifically, immune mechanisms might be more commonly involved in the persistence of fatigue, while persisting dyspnea might be more commonly the consequence of severe acute infection that caused directly or indirectly structural changes [11,13,14,29]. In this perspective, the strains responsible for acute COVID-19 disease might also play a role [30,31].

The analysis of the functional correlates of dyspnea and fatigue showed that the presence of each of these two symptoms overall conferred a significantly worst functional status at both DLCO

Table 3
Clinical and functional correlates of fatigue.

	Fatigue			
	Yes	No	Odds ratio (95%CI)	P value
Female sex: (n, %)	168 (52.8)	184 (41.2)	1.60 (1.20-2.14)	**0.001**
Age ≥65 years (n, %)	93 (29.2)	167 (37.4)	0.69 (0.51-0.94)	**0.020**
Current smoking (n: 725) (n, %)	34 (11.0)	20 (4.8)	2.46 (1.39-4.37)	**0.002**
Comorbidities (n, %):				
Any	216 (67.9)	277 (62.0)	1.30 (0.96-1.76)	0.090
Neoplastic disease	16 (5.0)	31 (6.9)	0.71 (0.38-1.32)	0.282
Ischemic heart disease	19 (6.0)	31 (6.9)	0.85 (0.47-1.54)	0.597
Heart failure	8 (2.5)	15 (3.4)	0.74 (0.31-1.77)	0.504
Renal insufficiency	7 (2.2)	8 (1.8)	1.23 (0.44-3.44)	0.686
Stroke	10 (3.1)	11 (2.5)	1.29 (0.54-3.07)	0.569
Anxiety	18 (5.7)	11 (2.5)	2.38 (1.11-5.11)	**0.026**
Depression	22 (6.9)	12 (2.7)	2.69 (1.13-5.53)	**0.007**
Chronic liver disease	8 (2.5)	4 (0.9)	2.86 (0.85-9.57)	0.089
Respiratory failure or COPD	26 (8.2)	28 (6.3)	1.33 (0.76-2.32)	0.310
Diabetes	29 (9.1)	43 (9.6)	0.94 (0.57-1.55)	0.815
Hypertension	133 (41.8)	175 (39.1)	1.12 (0.83-1.50)	0.457
Obesity	46 (14.5)	51 (11.4)	1.31 (0.86-2.01)	0.212
Autoimmune diseases	35 (11.0)	26 (5.8)	2.00 (1.18-3.40)	**0.010**
Asthma	25 (7.9)	23 (5.1)	1.57 (0.88-2.82)	0.129
Atrial fibrillation	7 (2.2)	18 (4.0)	0.54 (0.22-1.30)	0.168
OSAS	7 (2.2)	9 (2.0)	1.09 (0.40-2.97)	0.858
Other major conditions	11 (3.5)	25 (5.6)	0.60 (0.29-1.25)	0.174
Omicron pandemic phase (n, %)	52 (16.4)	68 (15.2)	1.09 (0.73-1.61)	0.669
Vaccinated for SARS-CoV-2 before infection (n: 577) (n, %)	44 (18.6)	42 (12.4)	1.62 (1.02-2.56)	**0.040**
Hospitalised during acute phase (n: 758) (n, %)	201 (63.8)	337 (76.1)	0.55 (0.40-0.76)	**<0.001**
Admitted to intensive care unit (n: 756) (n, %)	27 (8.6)	35 (7.9)	1.09 (0.64-1.84)	0.754
WHO COVID grade severe or critical (n: 760) (n, %)	157 (49.8)	259 (58.2)	0.71 (0.53-0.95)	**0.023**
Respiratory assistance with mechanical ventilation (n: 740) (n, %)	20 (6.6)	25 (5.7)	1.15 (0.63-2.11)	0.650
Treatments during acute phase (n, %):				
Antivirals (n: 733)	62 (20.9)	132 (30.3)	0.61 (0.43-0.86)	**0.005**
Oral or IV steroids (n: 734)	179 (60.3)	257 (58.8)	1.06 (0.79-1.43)	0.693
Neutralizing monoclonal antibodies (n: 736)	18 (6.0)	20 (4.6)	1.34 (0.69-2.57)	0.386
IL-6 inhibitors (n: 734)	27 (9.1)	64 (14.7)	0.58 (0.36-0.93)	**0.025**
Reduced DLCO (<80%) (n, %)	144 (45.3)	159 (35.6)	1.50 (1.12-2.01)	**0.007**

	Fatigue			
	Yes	*No*	*Difference (95%CI)*	*P value*
Days from COVID-19 (mean, SD)	175 (106)	182 (107)	−7 (−22, 8)	0.367
Meters walked in 6 min (n: 400) (mean, SD)	474 (127)	505 (89)	−31 (−52, −10)	**0.007**
DLCO (mean, SD)	80 (16)	83 (15)	−3 (−5, −1)	**0.005**

SD: standard deviation; COPD: Chronic Obstructive Pulmonary Disease; OSAS: Obstructive Sleep Apnea Syndrome; IV: intravenous; IL-6: Interleukin 6; DLCO: Diffusing capacity of the lungs for carbon monoxide. Significant p values are expressed in bold.

(pulmonary function) and 6MWD (exercise capacity) testing. The associations of dyspnea and fatigue with impaired DLCO and 6MWD and the correlation between these two measures were already described in some studies [1,5,7–9,12,18,19], that however, possibly because of sample size limitations, did not assess in more detail the possible functional phenotypes associated with these symptoms. In our study, more than half of individuals with dyspnea and fatigue had normal DLCO levels. We defined for both dyspnea and fatigue two distinct functional phenotypes based on DLCO findings, characterized by normal and abnormal pulmonary function, respectively. The multivariable analyses showed that for both symptoms the two phenotypes had different determinants, providing further insights in the assessment of these two major Long-COVID symptoms.

Female sex, comorbidities (anxiety, heart failure) and severity of acute disease (possibly also expressed by use of systemic steroids and monoclonal antibodies) were associated with the low-DLCO dyspnea and the low-DLCO fatigue phenotypes, suggesting a role of disease severity and preexisting conditions in determining functional damage, while the normal-DLCO dyspnea phenotype was associated with younger age and obesity, suggesting independent mechanisms. It is important to underline that the latter association indicates for patients with dyspnea and normal pulmonary function a modifiable risk factor. As for the additional mechanisms potentially involved, several causes (airflow obstruction, anemia, heart failure, arrhythmias, neuromuscular and psychological diseases) may produce dyspnea without impairing gas diffusion. Although we found no significant association of comorbidities and of anxiety or depression with the normal-DLCO dyspnea phenotype, this evaluation was based on patient history and not on more specific tests concurrently conducted, that could have better clarified the potential mechanisms involved.

The normal-DLCO fatigue phenotype appeared to be less characterized: use of antivirals during acute infection and lower comorbidity burden were associated with lower odds, but the clinical implications of these findings are uncertain. In the interpretation of data, it should be also considered that fatigue, compared to dyspnea, is a more generic symptom, for which non-pulmonary mechanisms or defects non captured by DLCO testing may be more commonly involved [1]. Some authors suggested that dyspnea and fatigue may be caused by a combination of peripheral, psychological and neurocognitive factors that may require a multidisciplinary diagnostic approach [5,10,11].

Our study has some limitations. Compared to the general population infected by SARS-CoV-2, our study sample was characterized by frequent occurrence of advanced age and severe acute COVID-19, and was followed at centers caring for Long-COVID patients. This selection may have determined a higher prevalence of functional abnormalities and of Long-COVID symptoms compared to the general population. This methodological limitation, that introduces a selection bias, is however common in clinical studies of COVID-19. A representative recruiting of

Table 4
Variables associated with persistence of dyspnea and fatigue in multivariable logistic regression models.

	Dyspnea with reduced DLCO		Dyspnea without reduced DLCO		Fatigue with reduced DLCO	Fatigue without reduced DLCO	Fatigue with reduced DLCO	Fatigue without reduced DLCO
	AOR, 95% CI	p	AOR, 95% CI	p	AOR, 95% CI	p	AOR, 95% CI	p
Female sex	1.60 (1.04-2.47)	0.034	*		1.85 (1.09-3.16)	0.024	*	
Age >65 years	*		0.63 (0.40-0.99)	0.044	*		*	
Current smoking					*		*	
SARS-CoV-2 vaccine pre-infection					*			
Infected during Omicron	*		*					
Severe or critical COVID-19	*		*		*		*	
Hospitalised for COVID-19	*		*				*	
Lenght of hospitalisation (days)	1.04 (1.02-1.05)	<0.001	*		*		*	
Admitted to ICU	*				*			
Mechanical ventilation for COVID-19	*				*			
Months from acute COVID-19#	0.92 (0.86-0.99)	0.020	*		*			
Treatments during acute infection								
Antivirals							0.53 (0.31-0.92)	0.025
Systemic steroids	3.23 (1.88-5.54)	<0.001			2.23 (1.19-4.20)	0.013	*	
Monoclonal antibodies	2.44 (1.09-5.48)	0.030						
IL-6 inhibitors					*			
Comorbidities (any)	*				*			
Number of comorbidities#	*				*		*	
Neoplasms	*						*	
Ischemic heart disease	*						*	
Heart failure					3.63 (1.02-12.86)	0.046	*	
Renal insufficiency								
Stroke	*				*			
Anxiety	5.24 (2.00-13.72)	<0.001			4.24 (1.40-12.85)	0.011		
Depression					*			
Chronic liver disease	*						*	
Chronic pulmonary disease	*		*		*		*	
Diabetes								
Hypertension								
Obesity	*		2.25 (1.32-3.82)	0.003				
Autoimmune diseases					*			
Asthma			*				*	
Atrial fibrillation							*	
OSAS	*							
Other major conditions							0.10 (0.01-0.74)	0.025

The symbol * indicates all the variables included in each model according to prespecified criteria (sex and age plus any variable associated with the outcome at a significance level <0.15 in univariate analyses) and no more significantly associated with the outcomes in the multivariable analyses. AOR: adjusted odds ratio; CI: confidence interval; DLCO: Diffusing capacity of the lungs for carbon monoxide; #: AOR per each additional unit; ICU: intensive care unit; IL-6: Interleukin 6. OSAS: Obstructive Sleep Apnea Syndrome.

the general population with COVID-19 in clinical cohorts is practically unfeasible in the absence of population registries able to catch asymptomatic or mild acute disease. Additionally, the participation of healthy and asymptomatic subjects would be subjected to patients' willingness, introducing another type of bias. The skewed characteristics of the study population, however, is unlikely to have affected the analysis of the determinants of symptoms and phenotypes, because risk factors were variably distributed in the population studied. Although most of the patients evaluated had normal DLCO levels, we based our study on availability of the results of the test, that might have been preferentially performed in patients with known or suspected clinical abnormalities. We also analysed dyspnea and fatigue only as binary measures (present or absent). Specific instruments that include severity grading would have provided more precise information, permitting a better analysis of the correlation of these two symptoms with the functional measures evaluated (6MWT, DLCO). Some associations found also do not automatically imply causality. In particular, the associations between systemic corticosteroids or monoclonal antibodies and the low-DLCO phenotype are probably influenced by indication, because both these treatments indicate more severe acute disease. Our study was based on functional measures (DLCO, 6MWT), and did not include an evaluation of pulmonary volumes, that would have provided additional

information. Finally, DLCO information prior to COVID-19 was not available, but this test is not routinely performed in the absence of significant clinical conditions. We had no information on a possible previous functional impairment in patients with preexisting respiratory or systemic conditions such as COPD, asthma, smoking history, obesity, or other respiratory comorbidities, and although we adjusted the analyses controlling for most of these preexisting conditions, we cannot exclude preexisting DLCO deficits.

The strengths of the present study are represented by evaluation of a large sample, concomitant availability of information on 6MWD, use of a wide array of covariates for adjusting risk estimates, use of clinical data with symptom information directly collected from patients, use of a COVID-19-specific WHO questionnaire, and multicentre data collection from centers with multidisciplinary characteristics. Compared to other studies, the concomitant collection of clinical and functional information on a large sample provided power to the multivariable analyses and allowed the identification of distinct phenotypes that may have clinical relevance in common practice [1,2,15].

In conclusion, we characterized, in patients followed at Long-COVID centers, determinants of dyspnea and fatigue, and showed that roughly half of the patients with these two symptoms had no impaired pulmonary function at DLCO testing. Functional impairment was associated with comorbidities and with more severe acute disease, but also showed an inverse association with time from infection, suggesting that even structural changes detected by DLCO testing or other procedures may reduce with time due to lung repair mechanisms [6,8,11,13,32]. Dyspnea and fatigue with normal pulmonary function may indicate non-pulmonary or systemic causes. DLCO alone does not capture pulmonary vascular disease, ventilatory inefficiency, deconditioning, dysautonomia, or extra-pulmonary mechanisms. The normal-DLCO dyspnea and fatigue phenotypes should therefore be further explored, possibly with additional tests that assess cardiorespiratory and cardiovascular function [7–10,16,17]. Obesity should be considered as a possible cause of dyspnea when pulmonary function is normal, and appropriate interventions considered. DLCO testing may provide useful information and should be included in the evaluation and classification of patients who report dyspnea and/or fatigue as possible Long-COVID symptoms.

Availability of data and materials

The study data can be made available upon reasonable request. Ethics Committee consultation may be necessary in order to obtain permission to share. Requests to access the datasets should be directed to marco.floridia@iss.it.

Ethics approval and consent to participate

The Italian National Ethics Committee approved the project (AOO-ISS—19/04/2022–0015066 Class: PRE BIO CE 01.00). Written informed consent was required for patient inclusion, using a patient information and consent form also approved by the Italian National Ethics Committee.

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CRediT authorship contribution statement

Marco Floridia: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **Liliana Elena Weimer:** Data curation, Writing – review & editing. **Aldo Lo Forte:** Investigation, Writing – review & editing. **Paolo Palange:** Investigation, Writing – review & editing. **Piergiuseppe Agostoni:** Investigation, Writing – review & editing. **Maria Rosa Ciardi:** Investigation, Writing – review & editing. **Matteo Tosato:** Investigation, Writing – review & editing. **Donato Lacedonia:** Investigation, Writing – review & editing. **Paola Gnerre:** Investigation, Writing – review & editing. **Emanuela Barisione:** Investigation, Writing – review & editing. **Giuseppe Pio Martino:** Investigation, Writing – review & editing. **Guido Vagheggini:** Investigation, Writing – review & editing. **Graziano Onder:** Conceptualization, Funding acquisition, Methodology, Writing – review & editing. **Marco Floridia:** Investigation, Data curation. **Marina Giuliano:** Investigation, Data curation. **Tiziana Grisetti:** Investigation, Data curation. **Flavia Pricci:** Investigation, Data curation. **Tiziana Grassi:** Investigation, Data curation. **Dorina Tiple:** Investigation, Data curation. **Marika Villa:** Investigation, Data curation. **Liliana Elena Weimer:** Investigation, Data curation. **Cosimo Polizzi:** Investigation, Data curation. **Fabio Galati:** Investigation, Data curation. **Maria Rosa Ciardi:** Investigation, Data curation. **Patrizia Pasculli:** Investigation, Data curation. **Piergiuseppe Agostoni:** Investigation, Data curation. **Francesca Colazzo:** Investigation, Data curation. **Irene Mattavelli:** Investigation, Data curation. **Elisabetta Salvioni:** Investigation, Data curation. **Paolo Palange:** Investigation, Data curation. **Daniela Pellegrino:** Investigation, Data curation. **Marco Bezzio:** Investigation, Data curation. **Federica Olmati:** Investigation, Data curation. **Arianna Sanna:** Investigation, Data curation. **Arianna Schifano:** Investigation, Data curation. **Dario Angelone:** Investigation, Data curation. **Antonio Fabozzi:** Investigation, Data curation. **Patrizia Rovere Querini:** Investigation, Data curation. **Maria Bernadette Cilona:** Investigation, Data curation. **Simona Santoro:** Investigation, Data curation. **Anna Fumagalli:** Investigation, Data curation. **Aurora Merolla:** Investigation, Data curation. **Valentina Canti:** Investigation, Data curation. **Maria Pia Ruggiero:** Investigation, Data curation. **Marco Messina:** Investigation, Data curation. **Marina Biganzoli:** Investigation, Data curation. **Danilo Buonsenso:** Investigation, Data curation. **Silvia Zucco:** Investigation, Data curation. **Alice Ianniello:** Investigation, Data curation. **Graziano Onder:** Investigation, Data curation. **Matteo Tosato:** Investigation, Data curation. **Vincenzo Galluzzo:** Investigation, Data curation. **Laura Macculi:** Investigation, Data curation. **Aldo Lo Forte:** Investigation, Data curation. **Valeria Maria Bottaro:** Investigation, Data curation. **Paolo Bonfanti:** Investigation, Data curation. **Luca Bonaffini:** Investigation, Data curation. **Anna Spolti:** Investigation, Data curation. **Nicola Squillace:** Investigation, Data curation. **Donato Lacedonia:** Investigation, Data curation. **Terence Campanino:** Investigation, Data curation. **Emanuela Barisione:** Investigation, Data curation. **Teresita Aloè:** Investigation, Data curation. **Elena Tagliabue:** Investigation, Data curation. **Stefano Figliozzi:** Investigation, Data curation. **Federica Testerini:** Investigation, Data curation. **Paola Andreozzi:** Investigation, Data curation. **Marzia Miglionico:** Investigation, Data curation. **Antonia Barbitta:** Investigation, Data curation. **Chiara Cenciarelli:** Investigation, Data curation. **Gianluca Pagnanelli:** Investigation, Data curation. **Giuseppe Piccinni:** Investigation, Data curation. **Paola Gnerre:** Investigation, Data curation. **Eugenia Monaco:** Investigation, Data curation. **Sandra Buscaglia:** Investigation, Data curation. **Antonella Visconti:** Investigation, Data curation. **Kwelusukila Loso:** Investigation, Data curation. **Giuseppe Pio Martino:** Investigation, Data curation. **Giuseppina Bitti:** Investigation, Data curation. **Laura Postacchini:** Investigation, Data curation. **Antonella Cognigni:** Investigation, Data curation. **Maria Antonietta di Rosolini:** Investigation, Data curation. **Sergio Mavilla:** Investigation, Data curation. **Domenico Maurizio Toraldo:** Investigation, Data curation. **Guido Vagheggini:** Investigation, Data curation. **Giulio Bardi:** Investigation, Data curation. **Giuseppa Levantino:** Investigation, Data curation. **Cristina Stefan:** Investigation, Data curation. **Andrea Martinuzzi:** Investigation, Data curation. **Gianfranco Parati:** Investigation, Data curation. **Elisa Perger:** Investigation, Data curation. **Davide Soranna:**

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Graziano Onder reports financial support was provided by Ministry of Health. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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