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RESEARCH ARTICLE

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Long-term alveolar-capillary diffusion impairments after severe SARS-CoV-2 pneumonia

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ABSTRACT

Background: Persistent respiratory symptoms and impaired gas exchange are common in patients recovering from COVID-19 pneumonia. The Lung Diffusing Capacity for Carbon Monoxide (DLCO) and Carbon Monoxide Transfer Coefficient (KCO) do not adequately distinguish alveolar membrane dysfunction from vascular abnormalities. This study aimed to characterize persistent diffusion impairment in post-ICU patients with prior SARS-CoV-2 pneumonia and reduced DLCO.

Methods: After hospital discharge, patients underwent spirometry, DLCO measurement, and a 6-minute walking test every six months. If DLCO remained impaired at 18–24 months, a combined Lung Diffusing Capacity for Nitric Oxide (DLNO) and DLCO assessment was performed to differentiate alveolar-capillary membrane (DmCO) and pulmonary capillary blood volume (Vc) alterations.

Results: Among 20 patients with persistent DLCO reduction, 3 had an obstructive ventilatory pattern, 6 had restriction, and 12 had low KCO. In restrictive cases, KCO was reduced but remained within normal limits without compensation. The DLNO/DLCO ratio exceeded 113.5% predicted in all patients. DmCO was impaired in 7 patients, while Vc was reduced in 16.

Conclusion: Both DLCO determinants were affected, with vascular impairment predominating. Vc reduction was present in most patients, with mean values below the lower limit of normality, whereas DmCO was less affected and often normal. The elevated DLNO/DLCO ratio suggests that persistent DLCO reduction is primarily driven by prolonged pulmonary capillary circulation dysfunction rather than alveolar membrane alterations, highlighting the vascular component as the primary site of long-term impairment.

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Introduction

SARS-CoV-2 is a virus first reported in Wuhan, China, in December 2019 and rapidly spreading worldwide. From the earliest reports to date, around 770 million cases have been confirmed, with almost 7 million deaths worldwide [1].

COronaVIrus Disease 19 (COVID-19) is associated with different respiratory involvement, ranging from mild upper respiratory tract symptoms (developing a flu-like illness with fever, malaise, dry cough, or shortness of breath) to severe acute respiratory distress syndrome (ARDS) requiring ventilatory support [2–8].

In addition, because of an imbalance between pro-inflammatory and anti-inflammatory responses caused by the so-called "cytokine storm", some patients may develop a systemic inflammatory response

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syndrome, sepsis with shock, and/or multi-organ dysfunction syndrome, leading to the need for admission to an intensive care unit (ICU) [9].

More than 100 million people worldwide have recovered from COVID-19 to date [1], but concern remains that some organs, including the lungs, may have long-term impairment. Rehabilitation appears to be effective in reducing the impact of long-term consequences of infection, even in mild-to-moderate ones [10].

Many studies have observed persistent functional respiratory impairment after severe SARS-CoV-2 infection, resulting from diffuse alveolar and capillary damage, hyaline membrane formation, fibrous proliferation of the alveolar septum, and lung consolidation [11,12]. These abnormalities mainly result in reduced alveolar-capillary diffusion of respiratory gases [13–16].

On a pathophysiologic level, diffuse damage at the alveolar level, which includes injury to the alveolar epithelial cells, hyaline membrane formation, fibrin deposition, hyperplasia of type II pneumocytes [17], and pulmonary congestion with microvascular thrombosis and occlusion, was found [18]. Nonetheless, the recruitment of inflammatory cells and the production of IgM-mediated immune complexes may further lead to coagulation and micro-thrombosis [3]. Therefore, a significant disruption in alveolar-blood gas exchange may be observable [19].

Long-term dyspnea has been reported by a not trivial proportion of patients who were incorrectly defined as "clinically recovered" from COVID-19 pneumonia [20].

Relatively to that, together with a restrictive pattern at pulmonary function tests, a variable decrease in lung diffusing capacity for carbon monoxide (DLCO) was the prominent respiratory function abnormality reported as occurring in approximately 30% of patients after several weeks following hospital discharge [21–24].

In addition, persistent respiratory symptoms and alterations in gas transport can be observed even in subjects who suffered from mild COVID-19 pneumonia with no or minimal CT abnormalities [16].

Unfortunately, the assessment of DLCO and CO uptake (Krogh's index: KCO) is not enough to differentiate abnormalities in the alveolar membrane diffusing conductance (DM) from those involving the vascular side of diffusion, causing a reduced capillary blood volume in the lung (Vc). Therefore, the simultaneous determination of diffusing capacity for nitric oxide (DLNO) and carbon monoxide (DLCO) evaluation is recommended [25–27].

Recently, a study employing such a single-breath technology, potentially allowing differentiation between DM and Vc disorders [28,29], has been conducted in patients recovered from COVID-19 pneumonia, mainly showing a reduction in lung capillary blood volume [30] 12–16 weeks after hospital discharge. However, no studies have investigated the gas exchange abnormalities and their origin at a longer follow-up.

Our study aimed to characterize the persistent diffusion alteration in patients with previous SARS-CoV-2 pneumonia admitted to the ICU and showing a decreased DLCO to understand which alveolar-capillary membrane structure is most impaired in these patients: the capillary microcirculation or the alveolar membrane.

Materials and methods

This is a single cohort prospective monocentric study, enrolling patients aged 18 years or older who were previously admitted to the ICU of ASST Spedali Civili located in Brescia, Italy, for the development of acute respiratory distress syndrome (ARDS) during SARS-CoV-2 pneumonia from November 2020 to May 2022. Eligible patients were required to meet the following criteria: being older than 18 years, having a laboratory-confirmed SARS-CoV-2 infection *via* real-time reverse transcription-polymerase chain reaction (RT-PCR), demonstrating pulmonary involvement as determined by clinical assessment supported by chest X-ray or HRCT imaging, and exhibiting a critical condition necessitating noninvasive ventilation (NIV) and/or orotracheal intubation (IOT) for mechanical ventilation. Patients unable to perform spirometry or those with a prior history of obstructive, restrictive, or mixed ventilatory defects attributable to pre-existing respiratory diseases were excluded from the study. All surviving patients were offered to participate in our hospital post-COVID-19 follow-up program. Each patient who agreed to participate in this study signed a written informed consent form and a permission form to use anonymized personal data. This work was carried out in accordance with the Declaration of Helsinki and is part of the project "Long-term Follow up in Survivors of Critical Illness" registered on ClinicalTrials.gov – ID: NCT04608994 and approved by the Brescia Ethics Committee (NP 2595). The study has been conducted following the STROBE checklist. The LOTO Investigators Working Group members contributed to the patients' enrollment and clinical evaluation.

Patients' anthropometric data (including age at the time of evaluation, gender, height, and weight) were

collected to generate reference values. Patients able to perform spirometry tests were regularly assessed at 6, 12, and 24 months after ICU discharge with respiratory function tests that included global spirometry recording Forced Expiratory Volume in the first second (FEV_1), Forced Vital Capacity (FVC), FEV1/FVC ratio, Total Lung Capacity (TLC), and usual DLCO measurement. Global Lung Function Initiative (GLI) values have been used as a reference for the spirometry evaluations. All patients who still had an impaired DLCO (below the lower limit of normality) after 24 months underwent the combined measurement of DLNO and DLCO by single breath test method.

Afterward, a 6-minute walking test (6MWT) was conducted following ERS/ATS recommendations [31,32], considering as significant an oxygen desaturation equal to or higher than 4% [33].

Spirometry was performed with a bell-shaped spirometer (Biomedin SRL, Padua, Italy). Lung volumes were measured by pressure-constant body plethysmography (Biomedin SRL, Padua, Italy). Usual DLCO (10 s breath hold time) was also obtained (Biomedin SRL, Padua, Italy).

Simultaneous DLCO and DLNO (5 s breath hold time) measurement was obtained using the Medisoft ExpAir system (MGC Diagnostic Corporation, MN, USA), which allows assessment of DM and Vc according to the standard dual single-breath method. This method is based on Roughton and Forster's principle [34], involving two reactions for theta fractions, CO and NO, according to the reference values set in the ERS/ATS Taskforce 2017 [35]. Because an electrochemical analyzer is employed for NO detection, the standard 10-second apnea period for DLCO measurements is shortened to approximately 5 s for DLNO. Two gas mixtures are required for these measurements: the first one included helium (He, 14%), carbon monoxide (CO, 0.28%), oxygen (O_2, 18–21%), and nitrogen (N_2), whereas the second mixture included nitric oxide in nitrogen (NO in N_2, 400 ppm). For each patient, at least two tests were performed 10 min apart.

Statistics

A dedicated database reporting demographics and relative percentages of the predicted respiratory and exercise tolerance values has been designed.

Patients' demographic data and clinical characteristics were analyzed *via* descriptive statistical analysis by calculating mean and standard deviation (SD) for continuous-type variables.

According to the previous analysis performed by Dal Negro and coworkers using the same device, optimal cutoff values in healthy controls for DLNO/DLCO ratio and Vc were 113.5 (95% CI 110-117) % pred. and 58.5 (95% CI 54-63) % pred., respectively [30].

A post-hoc power analysis was conducted to verify the adequacy of the sample size for detecting a significant difference in the DLNO/DLCO ratio compared to the normality threshold of 113.5%.

Continuous variables were compared using the Student's t-test. Data were expressed as mean ± standard deviation. Statistical significance was taken as $p < 0.05$.

Analyses were performed using Graph Pad Prism 6.0 (Graph Pad Software, La Jolla, CA).

Results

Among 222 patients with COVID-19 pneumonia discharged from the ICU, 20 patients (9%) with persistently reduced DLCO were assessed, with a male gender prevalence (65%). The mean age of the sample was 67 ± 7 years. Demographic and comorbidities data are reported in Table 1, while spirometry data at different time points are reported in the Supplemental Material (Table S1 and Figure S1).

Three patients (15%, 2 males) showed obstructive ventilatory pattern (FEV_1/FVC ratio < LLN), probably already present before infection since it cannot be related to the outcomes of lung parenchyma infection. Moreover, 6 patients (30%, all males) of the sample had a restrictive ventilatory pattern (normal FEV_1/FVC ratio and TLC < LLN).

Relatively to DLCO, besides a VA reduction in 6 patients, we documented 12 patients (60%) with a low

Table 1. Demographics.

	Total ($N = 20$)	Males ($N = 13$)	Females ($N = 7$)	*p*-value
Age (years)	67 ± 6.7	67.8 ± 7.6	65.4 ± 4.4	0.455
Height (cm)	167.6 ± 8.3	171.9 ± 6.4	159.6 ± 4.4	<0.001**
Weight (kg)	81.3 ± 11.6	83.5 ± 8.0	77.3 ± 16.3	0.261
Body Mass Index (kg/m^2)	29 ± 4.0	28.3 ± 2.5	30.3 ± 6.0	0.288
Cardiovascular Comorbidities (%)	13 (65.0%)	10 (76.9%)	3 (42.9%)	0.128
Renal Comorbidities (%)	7 (35.0%)	6 (46.1%)	1 (14.3%)	0.154
Endocrine Comorbidities (%)	2 (10.0%)	0 (0.0%)	2 (28.6%)	0.042*
Neurological Comorbidities (%)	7 (35.0%)	5 (38.5%)	2 (28.6%)	0.658
Hematologic Comorbidities (%)	5 (25.0%)	3 (23.1%)	2 (28.6%)	0.787
Oncologic Comorbidities (%)	2 (10.0%)	1 (7.7%)	1 (14.3%)	0.693

All data are reported as mean ± standard deviation, and frequency (%).

KCO. Concerning the patients with restrictive pattern, KCO was abnormally reduced, although within normal limits, because it was not compensatory, as expected.

Regarding the parameters resulting from the combined measurement of DLNO and DLCO, we found that DLNO/DLCO ratio was greater than 113.5% predicted in all patients evaluated (147.5 ± 17.7% pred.). Still, DmCO was altered (<80% pred.) in 7 patients (35%), whereas Vc was reduced in 16 (80%). No significant differences among spirometry parameters were observed between males and females (Table 2 and Figure 1). Considering the observed mean DLNO/DLCO ratio of 147% (SD ± 17.4%), a significance level (α) of 0.05, and statistical power (1-β) of 80-90%, the required sample size ranged between 4 and 5 patients. Given that our study included 20 patients, the sample size was adequate to ensure robust statistical power for detecting clinically relevant differences.

Finally, 6MWT was performed on only 15 patients (3 females and 12 males) based on patients' walking capabilities. Again, no significant differences were observed between males and females except for pre and post-exertion heart rates, which were higher in women (Table 3). Interestingly, significant exercise-induced oxygen desaturation (≥ 4%) was observed in 2 patients only.

Table 2. Spirometry data.

	Total ($N=20$)	Males ($N=13$)	Females ($N=7$)	p-value
FEV1 (% pred.)	100.2 ± 17.8	97.4 ± 17.2	105.6 ± 19.1	0.342
FVC (% pred.)	104.4 ± 17.5	99.8 ± 17.2	113.0 ± 15.8	0.111
FEV1/FVC (%)	76.8 ± 9.3	76.2 ± 10.2	78.0 ± 8.0	0.681
TLC (% pred.)	86.7 ± 14.7	84.7 ± 16.6	90.4 ± 10.6	0.421
DLCO (% pred.)	60.2 ± 9.4	60.5 ± 10.4	59.9 ± 8.1	0.896
KCO no restr. (% pred.)	72.9 ± 13.8	73.3 ± 13.5	72.6 ± 15.2	0.927
KCO restr. (% pred.)	87.5 ± 9.7	87.5 ± 9.7	–	–
DLNO/DLCO (% pred.)	147.0 ± 17.4	150.4 ± 20.1	140.6 ± 8.9	0.700
DmCO (% pred.)	82.8 ± 14.3	81.8 ± 15.9	84.5 ± 11.6	0.593
Vc (% pred.)	48.5 ± 12.4	47.9 ± 14.8	49.6 ± 6.6	0.691

All data are expressed as mean ± standard deviation.
FEV1: Forced Expiratory Volume in 1 s; FVC: Forced Vital Capacity; TLC: Total lung capacity; DLCO: Lung Diffusion Capacity for Carbon Monoxide; KCO: Carbon Monoxide Transfer Coefficient; DLNO/DLCO: Lung Diffusion Capacity for Nitric Oxide and Carbon Monoxide Ratio; DmCO: Alveolar-capillary membrane Conductance; Vc: Pulmonary capillary blood volume.

Discussion

After more than two years of the pandemic, it is now accepted that a significant percentage of patients hospitalized for SARS-CoV-2 pneumonia may experience negative long-term effects after discharge. These effects include clinical, physiological, and radiological impairments. Pulmonary rehabilitation has been proven effective in improving the quality of life in post-COVID patients, even in mild to moderate forms [10]. Clinically, several patients complained of chronic and exertional dyspnea associated with alterations in the oxygen lung diffusion across the alveoli-capillary membrane, which can be identified by respiratory function tests and explained by combined DLCO and DLNO measurements [30].

The ever-growing evidence of COVID-19-induced microangiopathy involving the pulmonary capillary bed strongly suggests this may be the most probable

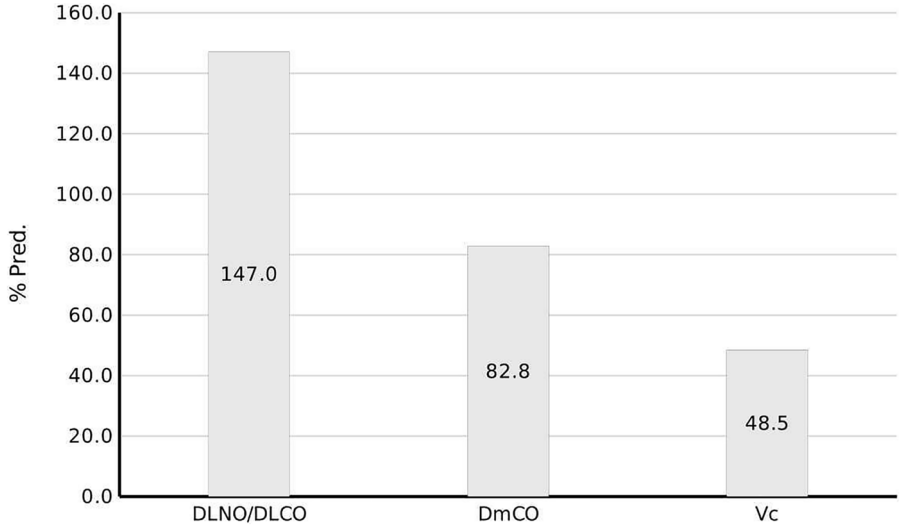

Figure 1. Mean DLNO/DLCO, DmCO, and Vc values of the enrolled population. Data are expressed as mean % pred. ± SD. DLNO/DLCO: Lung diffusion capacity for nitric oxide and carbon monoxide ratio; DmCO: Alveolar-capillary membrane conductance; Vc: Pulmonary capillary blood volume.

Table 3. 6-minute walking test data.

	Total ($N=15$)	Males ($N=12$)	Females ($N=3$)	p-value
6MWD (m)	466.3 ± 125.3	478.7 ± 134.5	416.7 ± 75.7	0.463
SpO_2 pre-test (%)	96.8 ± 0.94	96.7 ± 1.0	97.0 ± 1.0	0.696
SpO_2 post-test (%)	95.3 ± 1.5	95.2 ± 1.6	95.3 ± 0.6	0.935
HR pre-test (beats/min)	73.8 ± 10.6	70.7 ± 7.7	85.7 ± 13.7	0.032*
HR post-test (beats/min)	85 ± 12.4	80.5 ± 8.6	101.3 ± 10.6	0.004*

All data are expressed as mean ± standard deviation.
6MWD: 6-Min Walk Distance; SpO_2: Oxygen Saturation; HR: Heart Rate.

cause of the subtle gas exchange abnormalities, leading to maladaptation of alveolar perfusion [36–39]. Our study aimed to assess which component of the alveolus-capillary barrier was mainly responsible for the reduced lung gas diffusion in patients with previous severe SARS-CoV-2 pneumonia: the pulmonary capillary bed or the alveolar membrane.

Considering that CO diffusion is more sensitive to blood hemoglobin concentration, capillary oxygen pressure (PcO2), and pulmonary capillary blood volume (Vc), it follows that a DLCO and KCO alteration might more likely be ascribed to the alteration of the blood/vascular component, leading to decrease of the pulmonary capillary blood volume (Vc). Indeed, the mean value of Vc in the entire sample was reduced, suggesting that a decrease in lung capillary blood volume was a key determinant of the persistently reduced DLCO and abnormal KCO.

In contrast, NO diffusion is mostly dominated by the membrane conductance component, while it is insensitive to PcO2 and hemoglobin concentration and less sensitive to pulmonary capillary blood volume. A DLNO and KNO alteration more closely reflects an impairment of the alveolar membrane structure, affecting the DM more than the Vc component of CO diffusion.

Our data analysis found that both determinants of CO lung diffusion (DM and Vc) were altered. Still, there was an imbalance in the alteration of these two components, with the vascular component being more impaired than the membrane one. Vc reduction was present in most patients with mean values below the lower limit of normality, while DM showed a lesser reduction and was normal in 65% of them.

Evaluation of both these measurements and the DLNO/DLCO ratio helped to clarify some relevant aspects of post-COVID lung function abnormalities and to distinguish membrane conductance disorders (DM) from capillary circulation disorders (Vc). Since the diffusion of the CO and NO reflects different components involved in the diffusion of gases across the membrane, a decrease in the DLNO/DLCO ratio below 95% of predicted directs toward a predominant problem of the alveolar-capillary one. In comparison, an increase in the DLNO/DLCO ratio above 113.5% of the predicted directs toward a predominant problem of capillary circulation [35].

In our study, all patients had an increased DLNO/DLCO ratio >113.5% of the predicted, suggesting a relevant problem involving the pulmonary capillary bed. These findings further prove that microvascular circulation is the most compromised structure in patients hospitalized for SARS-CoV-2 pneumonia.

Long-term lung function follow-up in COVID-19 survivors has emerged as an important strategy in post-acute care. Although high-resolution CT is highly effective in detecting structural abnormalities, such as lung fibrosis, several studies have shown that pulmonary function tests can reveal subtle functional impairments that are not apparent on imaging. This is particularly true for measurements such as diffusion capacity, which may remain reduced despite the absence of radiographic sequelae [40,41]. These deficits may be attributable to microvascular injury, subtle alveolar damage, or persistent inflammatory changes at a microscopic level. Early identification of such impairments is crucial to start early rehabilitative measures and tailor respiratory therapies before clinical symptoms become detectable, provide valuable information on the natural history of post-COVID lung recovery, and stratify patients with functional impairment, which may be at increased risk for long-term respiratory morbidity, for closer follow-up or inclusion in rehabilitation programs. The dissociation between normal chest imaging and abnormal pulmonary function underlines the need for comprehensive follow-up protocols in post-COVID care. Furthermore, the longitudinal evaluation of lung function offers an opportunity to better understand the mechanisms behind post-COVID respiratory impairment. This understanding is critical for designing targeted therapeutic strategies and refining long-term management guidelines for COVID-19 survivors.

The study's limitations are related to the low sample size and the absence of previously performed function tests, so it was impossible to compare our data with previously obtained ones concerning the development of SARS-CoV-2 pneumonia. Furthermore, no recent CT scans were available.

The strength of our study lies in its documentation of lung diffusion changes in patients with previous COVID-19 pneumonia two years after hospital discharge. This confirms a long-term pathological involvement of alveolar microcirculation as the main underlying disorder.

Conclusions

Approximately 2 years after discharge, some patients admitted for severe SARS-CoV-2 pneumonia may still have decreased lung diffusion. This finding, as supported by the increased DLNO/DLCO ratio >113.5% pred. in all patients with persistently reduced DLCO, is mainly attributable to the prolonged alteration of the pulmonary capillary circulation rather than to the alteration of the alveolar membrane. Further studies are needed to investigate this matter and potentially find useful therapies.

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Ethics approval

This work was carried out in accordance with the Declaration of Helsinki and is part of the project "Long-term Follow-up in Survivors of Critical Illness" registered on ClinicalTrials.gov - ID: NCT04608994 and approved by the Brescia Ethics Committee (NP 2595).

Patient consent for publication

Consent was obtained directly from the patients upon enrolling.

Author contributions

CRediT: **Laura Pini**: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing; **Jordan Giordani**: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Guido Levi**: Data curation, Formal analysis, Software, Validation, Visualization, Writing – original draft, Writing – review & editing; **Michele Guerini**: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – review & editing; **Simone Piva**: Data curation, Writing – review & editing; **Elena Peli**: Data curation, Writing – review & editing; **Manuela Violini**: Formal analysis, Investigation, Visualization, Writing – review & editing; **Stefano Piras**: Writing – review & editing; **Yehia El Masri**: Writing – review & editing; **Alessandro Pini**: Writing – review & editing; **Dina Visca**: Writing – review & editing; **Deodato Assanelli**: Funding acquisition, Resources, Writing – review & editing; **Maria Lorenza Muiesan**: Funding acquisition, Resources, Writing – review & editing; **Nicola Latronico**: Writing – review & editing; **Claudio Tantucci**: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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