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Phenotyping lung function disorders in respiratory Long-COVID

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ABSTRACT: *Dyspnea is frequently complained for several weeks in Long-COVID, still being its underlying pathophysiology poorly investigated in the clinical setting.* ***Aim:*** *to investigate the lung functional pattern of Long-COVID patients and healthy controls (HC). Non-smokers aged ≥18years, discharged after COVID pneumonia were investigated, were classified as low dyspnea scorers (LDS) or high dyspnea scorers (HDS) if they were not complaining or otherwise still complaining dyspnea 12-16 weeks after discharge. A group of non-smoking HC was compared. Spirometric parameters; usual DL_{CO}; simultaneous single-breath DL_{NO} (sDL_{NO}) and DL_{CO} (sDL_{CO}); lung capillary blood volume (Vc), and expiratory nitric oxide (eNO) were assessed. Their linear association was explored by correlation analysis. Area under the curve (AUC) were used to determine the best predictors of being HC, LDS or HDS, and to establish optimal cut-off values. 40 Long-COVID patients (19 LDS; 21 HDS) and 28 HC were investigated. FEV1, VC and FEV_1/VC were equal in LSD and HSD. A decreasing trend ($p<0.0001$) for DL_{CO}, sDL_{CO}, sDL_{NO} and Vc, and a corresponding increasing trend ($p<0.0001$) for sDL_{NO}/sDL_{CO} and eNO were detected from HC to HDS patients. sDL_{NO}/sDL_{CO} and Vc resulted the best predictors of belonging to the HDS group. Optimal cut-off values were 113.5 for sDL_{NO}/sDL_{CO} ratio and 58.5 for Vc, respectively. Data suggest the reduction of lung capillary blood volume as the main disorder in these cases and contribute to phenotyping respiratory troubles in Long-COVID. These disorders would be otherwise neglected by usual lung function parameters, due their low specificity.*

KEY WORDS: Long-COVID-19 phenotyping; single-breath simultaneous DL_{CO} and DL_{NO}; blood gas transport; capillary blood volume; healthy controls

INTRODUCTION

A large proportion of patients (around 50%) who experienced COVID-19 pneumonia are complaining one or more symptoms of their original acute disease for long time, with variable limitation in their quality of life [**1-6**]. Though still not exactly defined [**7,8**], this long-term condition is currently named "Long-COVID" or "Post-COVID syndrome" and is characterized by the persistency of some clinical signs that are mirroring the persistent involvement of different organs, regardless the severity of the original infection [**1,8-16**].

Dyspnea at variable extent is the symptom most frequently complained for several weeks (or months) by patients previously admitted for COVID-19 pneumonia [**11**,**12,16-20**]. The cause of long-lasting

dyspnea after COVID pneumonia had been suggested as due to the persistency of virus-induced tissular damage originally occurred in the respiratory units of the lung [**9,10-12,15,21-27**].

Nevertheless, the identification of underlying respiratory disorders still is infrequently pursued in the clinical setting [**8**]. Lung function changes currently reported in these patients only consist of a generic spirometric restrictive pattern associated to a mild reduction of diffusing capacity for carbon monoxide (DL_{CO}) in around 25-30% of patients only [**28,29**]. Unfortunately, while spirometric indices are characterized by low specificity in these cases, the current measure of DL_{CO} proved of limited value in discriminating disorders of alveolar diffusing conductance (DM) from those involving the microvascular structures of respiratory units [**29-31**]. The single-breath simultaneous assessment of diffusion for carbon dioxide (sDL_{CO}) and nitric oxide (sDL_{NO}) has been recommended for this purpose [**32,33**]. Moreover, the pattern of lung function of Long-COVID patients was never compared to that one of healthy individuals.

Aim of the present study was to assess sDL_{CO} and sDL_{NO} (and derived parameters) in Long-COVID patients and in healthy controls, and to investigate the relationship existing between sDL_{CO}, sDL_{NO}, spirometric volumes and usual DL_{CO}.

METHODS

Study design

Non-smoker patients of both genders, aged ≥18 years, previously admitted for COVID pneumonia (involving ≥50% of their lung volume) and discharged over the last six months as "clinically recovered" were investigated between September 1, 2021 and March 15, 2022, after their informed consent. All patients had received high-flow oxygen supplementation during their hospitalization. According to the British Thoracic Society (BTS) recommendations [**34**], all patients had to be provided with a control CT scan performed 12 weeks after discharge regardless of symptom status. The complete resolution of any residual COVID-related parenchymal lesion was CT confirmed in all patients. A comparable group of non-smoking healthy controls (HC) was also recruited, such as: subjects who had not experienced COVID-19 (negative IgG and IgM serology), and without any significant comorbidity.

Exclusion criteria were: current and former-smoke habit, age < 18 years, comorbidities able to affect the diffusion capacity, namely: anemia (blood hemoglobin [Hb] <12g/L), heart failure, COPD, lung fibrosis, vasculitis, liver and renal failure, diabetes; persistency of COVID-related parenchymal lesions, physical and/or cognitive impairment enabling procedures for lung function tests; refusal of consent.

Data collected

Further to age and sex, body mass index (BMI) and blood Hb (in g/L) were measured. Lung function parameters to collect included: $\%O_2$ saturation at rest (SpO_2 %), Vital Capacity (VC), Forced Expiratory Volume in 1 sec (FEV_1), % FEV1/VC ratio, usual DL_{CO}, single-breath simultaneous sDL_{CO} and sDL_{NO}, sDL_{NO}/sDL_{CO} ratio, and lung capillary blood volume (Vc). All parameters have been reported as % predicted. The alveolar expiratory nitric oxide concentration (eNO) was also measured in ppm. A Plethysmography Platinum DX Elite (MedGraphics, USA) was used for

assessing spirometric volumes and usual DLco (10 seconds breath hold time). Single-breath sDL_{CO} and sDL_{NO} (5 seconds breath hold time) were obtained simultaneously by means of the "Stand-Alone" Hypair Compact System (MGC Diagnostics International, Sorinnes, Belgium) that allows, further to alveolar eNO, the simultaneous assessment of DM and Vc as a function of the standard single-breath method. This method is based on the principle by Roughton & Forster [**35**] according to reference values fixed in the ERS/ATS Task-Force 2017 [**36**].

Current dyspnea was graded according to the Modified British Medical Research Council (mMRC) dyspnea score in all patients [**37**]. The duration of dyspnea after Hospital discharge was also calculated (in weeks). Patients were classified as low dyspnea scorers (LDS) or high dyspnea scorers (HDS) if they were not complaining or otherwise still complaining significant dyspnea 12-16 weeks after discharge.

Statistical analysis

Continuous data were presented as means and standard deviation (SD), while sex as absolute and relative frequencies. Differences in baseline characteristics between HC, LDS and HDS groups were tested by ANOVA test. Differences in lung function parameters were estimated by a generalized linear model (gamma family) adjusting for age, sex, BMI and Hb levels at enrollment. Results were reported as mean difference and confidence intervals (CI) adjusted for multiple comparisons using Šidák correction [**38**].

Receiver Operating Characteristic (ROC) curves and area under the curve (AUC) were used to identify the parameters able to classify HC, LDS and HDS with the highest predicting power. Youden's criterion was used to establish optimal cut-off values with sensitivity, specificity, and diagnostic odds ratios (DOR) also reported.

Finally, correlation analysis was performed to explore the linear association between diffusive (DL_{CO}, sDL_{CO}, sDL_{NO}, sDL_{NO}/sDL_{CO} ratio, Vc, and eNO) and spirometric (FEV_1, VC, FEV_1/VC) indices in LDS and HDS groups. All results were adjusted by age, sex, BMI and Hb levels at enrollment.
A p value < 0.05 was considered statistically significant. All statistical calculations were carried out by means of STATA (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Ethics: at recruitment, all subjects gave their informed consent also to the anonymous use of their own data for research purposes. The study was approved by the Ethical and Scientific Commission of the National Centre for Respiratory Pharmacoeconomics and Pharmacoepidemiology during the session of May 2nd, 2021.

RESULTS

Patient characteristics

A total of 40 Long-COVID patients (19 classified as LDS, and 21 as HDS), and 28 HC were investigated (Table 1). The three groups were comparable by age (p=0.9202), BMI (p=0.4752), and Hb (p=0.3092) distribution. The proportion of male was not different in LDS and HDS patients (47.3 vs. 38.1%, p =0.5653), but lower than in HC (p=0.0093) (Table 1).

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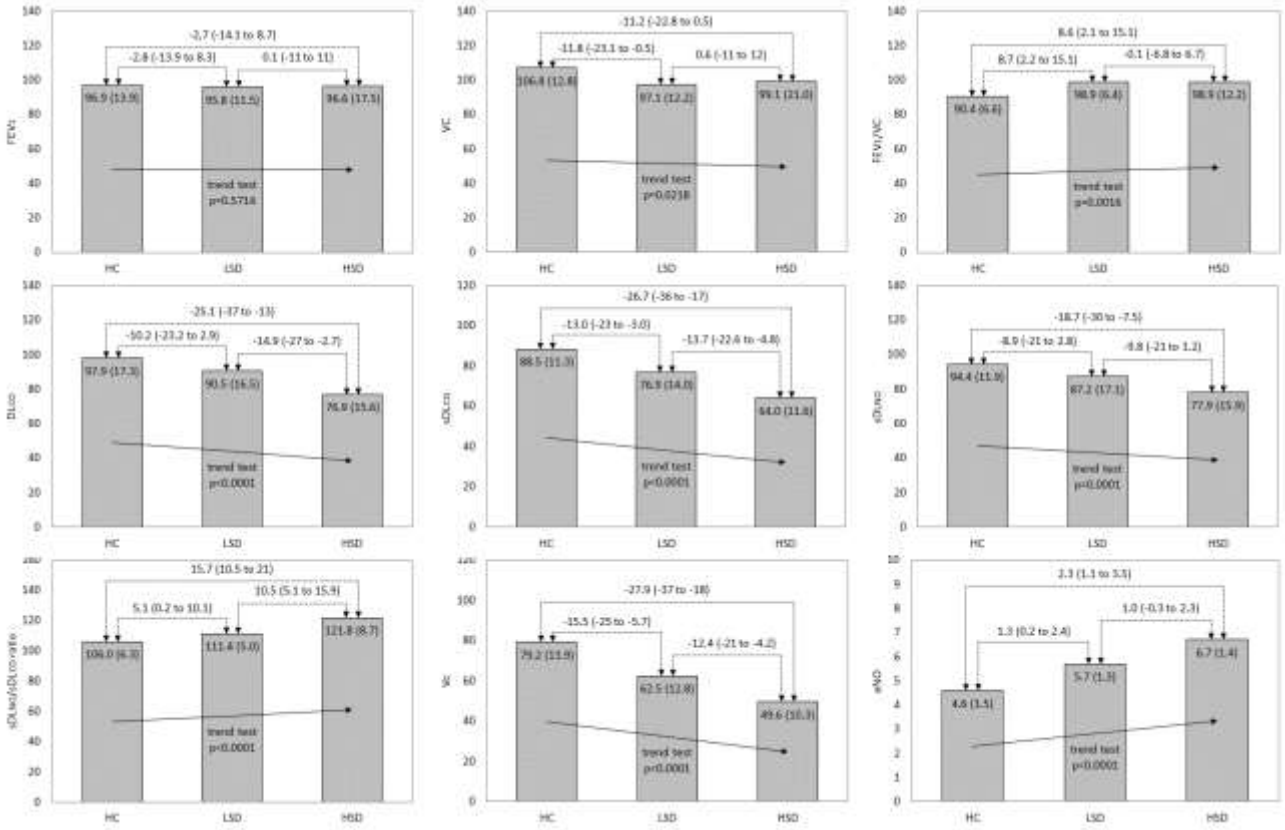
Table 1. Baseline characteristics of the groups considered in the analysis.

	HC	LDS	HDS	p-value
N	28	19	21	
Male (%)	22 (78.6%)	9 (47.3%)	8 (38.1%)	0.0093
Mean age (SD)	50.4 years (15.3)	48.4 years (16.7)	48.9 years (20.6)	0.9202
Mean BMI (SD)	25.8 m^2 (5.6)	24.2 m^2 (4.1)	24.3 m^2 (4.9)	0.4752
Mean Hb (SD)	13.9 g/dl (0.4)	14.1 g/dl (0.4)	14.1 g/dl (0.5)	0.3092
Mean SpO_2 (SD)	98.2% (1.2)	97.8% (1.1)	96.7% (1.6)	0.7701

BMI: body mass index, Hb: hemoglobin; HC: healthy controls, HDS: high dyspnea scorers, LDS: low dyspnea scorers, SD: standard deviation

The multiple comparisons of lung parameters among HC, LDS and HDS groups is reported in Figure 1. The distribution of FEV_1 values was similar in the 3 groups, no difference was found between LSD and HSD patients. As concerning the diffusive parameters, a decreasing trend ($p<0.0001$) for DL_{CO}, sDL_{CO}, sDL_{NO} and Vc, and an increasing trend ($p<0.0001$) for sDL_{NO}/sDL_{CO} ratio and eNO were detected from HC subjects to HDS patients (Figure 1).

Figure 1. Comparison of lung parameters among HC, LDS and HDS: data reported as mean differences and 98.11% CI (according to Šidák correction).



HC: healthy controls, HDS: high dyspnea scorers, LDS: low dyspnea scorers

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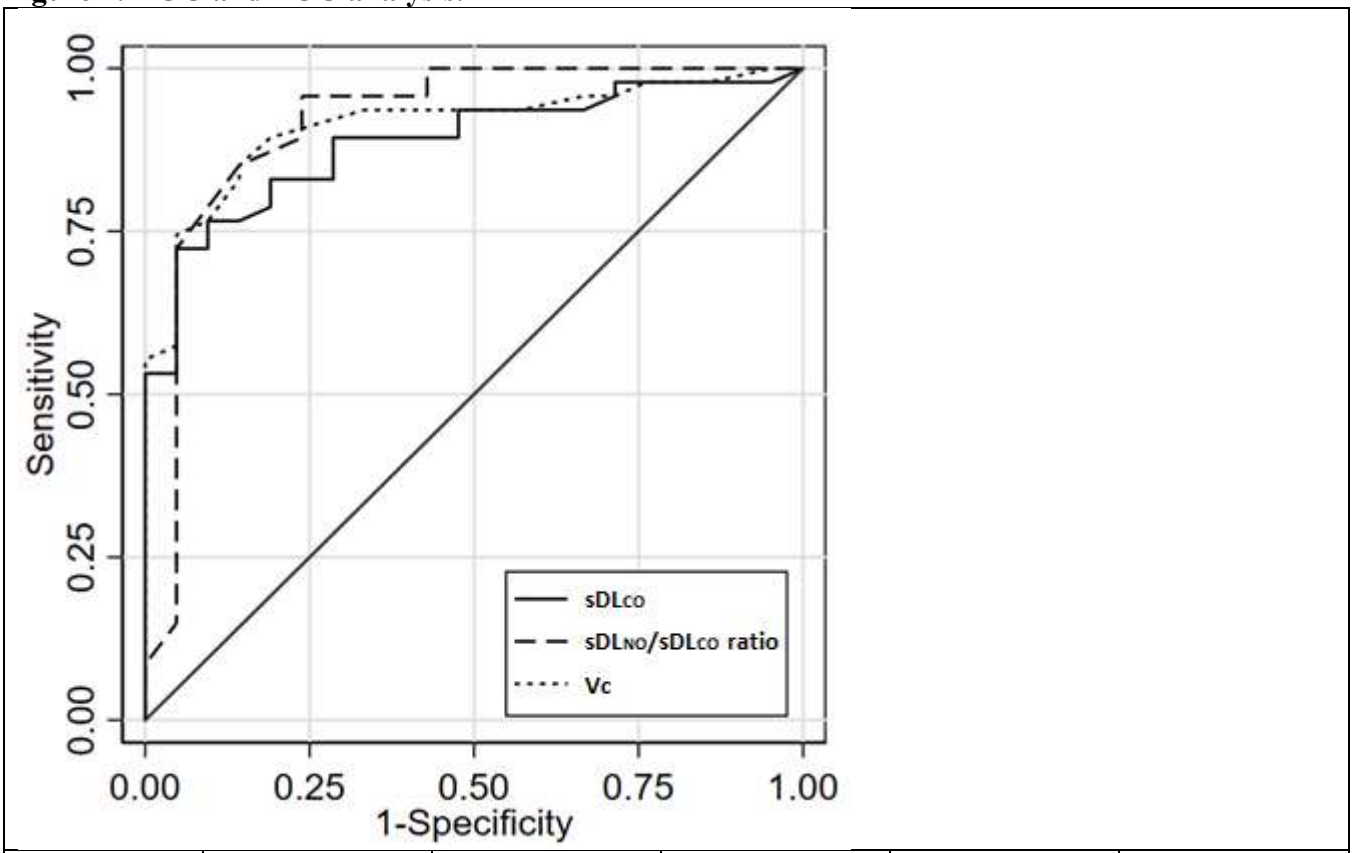
HDS prediction

AUC analysis proved sDL_{NO}/sDLco ratio and Vc as the best predictors of belonging to the HDS rather than to the LDS group (Figure 2).

Figure 2. ROC and AUC analysis.



Variables	AUC	Cut-off	Sensitivity	Specificity	DOR
FEV_1	0.57 (0.45 to 0.70)	91.5 (82.5 to 101)	0.77 (0.50 to 1)	0.38 (0.15 to 0.61)	1.00 (0.96 to 1.04)
VC	0.69 (0.56 to 0.81)	94.5 (91 to 98)	0.85 (0.72 to 0.98)	0.52 (0.61 to 0.74)	0.99 (0.95 to 1.02)
FEV_1/VC	0.70 (0.58 to 0.82)	99 (96 to 102)	0.62 (0.42 to 0.82)	0.79 (0.65 to 0.93)	1.06 (1 to 1.12)
DL_{CO}	0.78 (0.68 to 0.88)	82 (75.7 to 88.3)	0.85 (0.71 to 1)	0.71 (0.56 to 0.87)	0.94 (0.91 to 0.98)
sDL_{CO}	0.84 (0.75 to 0.92)	76.7 (71.6 to 81.8)	0.77 (0.64 to 0.90)	0.90 (0.77 to 1)	0.89 (0.83 to 0.94)
sDL_{NO}	0.72 (0.63 to 0.82)	81 (70 to 92)	0.83 (0.58 to 1)	0.62 (0.37 to 0.87)	0.94 (0.91 to 0.98)
sDL_{NO}/sDL_{CO} ratio	0.85 (0.77 to 0.94)	113.5 (110 to 117)	0.86 (0.72 to 0.99)	0.85 (0.71 to 0.99)	1.39 (1.17 to 1.65)
Vc	0.85 (0.78 to 0.93)	58.5 (54 to 63)	0.85 (0.74 to 0.96)	0.86 (0.73 to 0.98)	0.86 (0.80 to 0.93)
eNO	0.73 (0.63 to 0.83)	6.2 (5.2 to 7.2)	0.67 (0.48 to 0.86)	0.79 (0.62 to 0.96)	2.01 (1.35 to 3.01)

AUC: area under the curve, DOR: diagnostic odds ratio

According to the ROC analysis, both parameters were characterized by the highest AUC sensitivity and specificity (Figure 2). Optimal cut-off values were 113.5 (95% CI 110 to 117) for the sDL_{NO}/sDL_{CO} ratio, and 58.5 (95% CI 54 to 63) for Vc, respectively. Both parameters proved able to discriminate HDS patients from the other groups (Figure 3).

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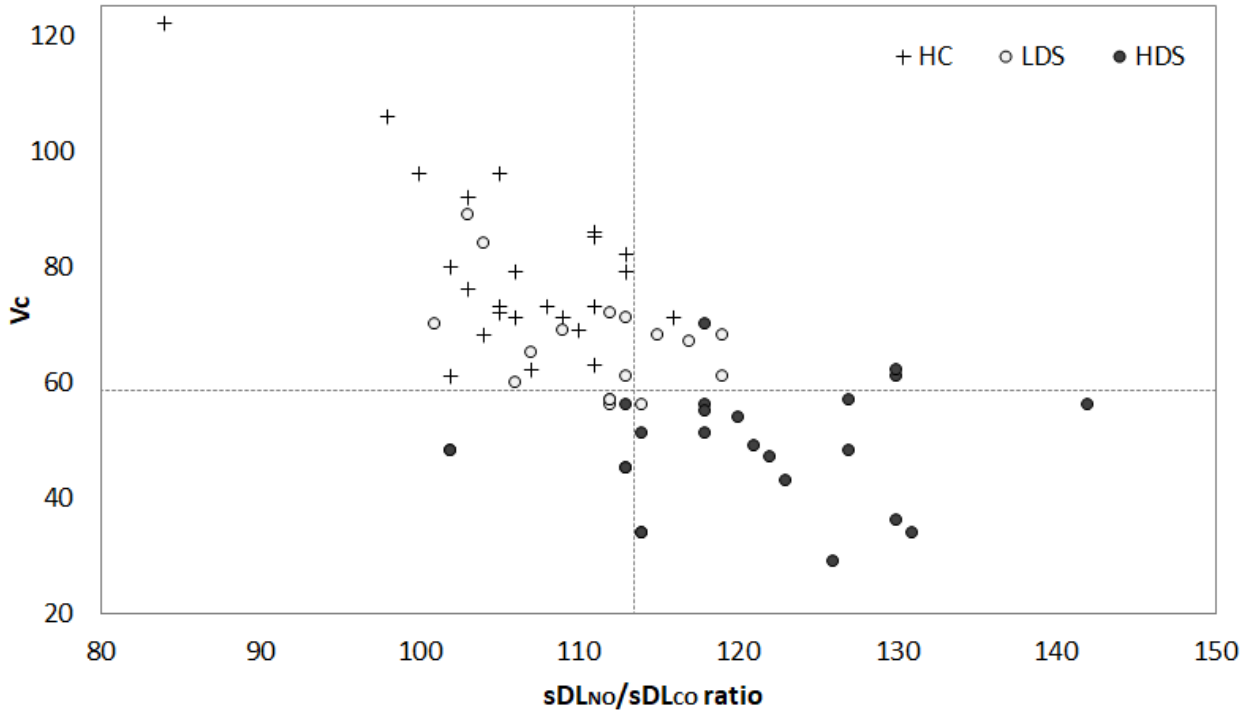
Figure 3. Comparison among distributions of HC, LDS and HDS, and optimal cut-off for sDL_{NO}/sDL_{CO} ratio and Vc.



HC: healthy controls, HDS: high dyspnea scorers, LDS: low dyspnea scorers

Linear correlation

In both LDS and HDS, FEV1 and VC proved positively correlated with DLCO, sDLCO, sDLNO and Vc (Supplementary material Tables S1-S3 and S5), but not correlated with the sDL_{NO}/sDL_{CO} ratio and eNO (Supplementary material Tables S4 and S6). Moreover, FEV1/VC proved not correlated with any diffusive parameter (Supplementary material Tables S1-S6). For each pair of parameters that resulted correlated, the interaction term slope × HDS is significantly negative (Figure 4), thus emphasizing that the slope of linear regression lines in HDS patients are always lower than the slope calculated in LDS patients. In other words, the same increase in FEV_1 or VC is associated with a higher increase of DL_{CO}, sDL_{CO}, sDL_{NO} or Vc in LDS than in HDS.

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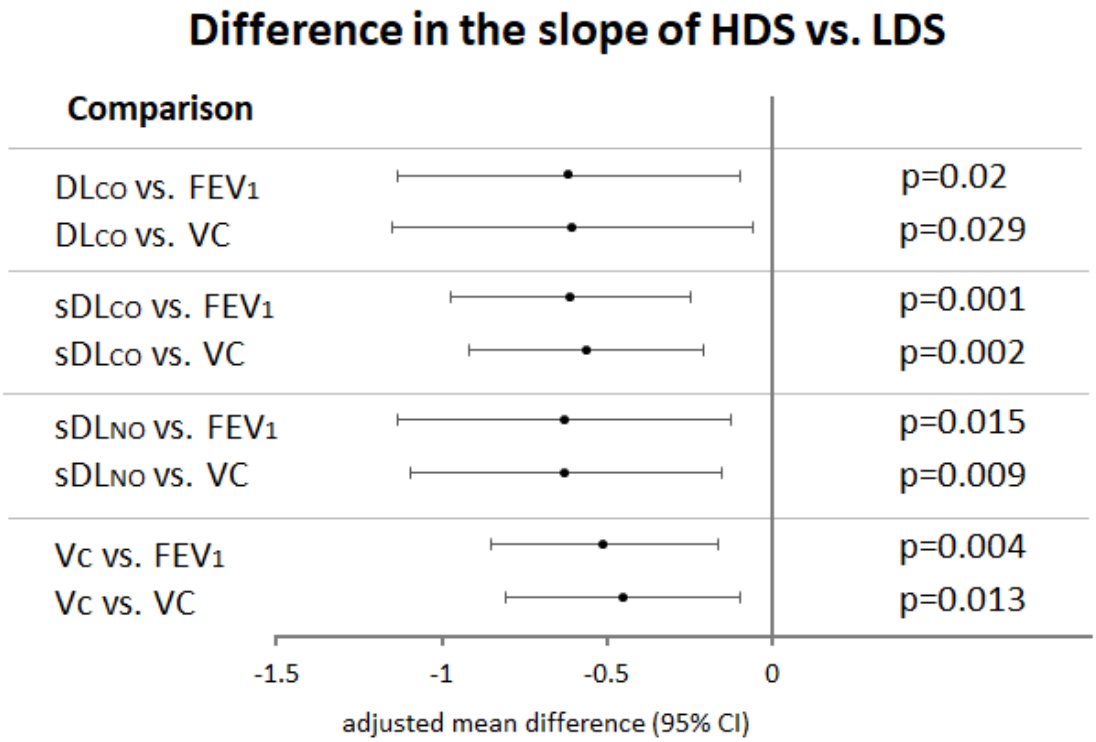
Figure 4. Interaction between lung parameters and the dyspnea score.



HDS: high dyspnea scorers, LDS: low dyspnea scorers

DISCUSSION

A huge number of studies reported long-term pulmonary and extra-pulmonary symptoms after COVID-19 pneumonia in a high proportion of patients, though of decreasing severity with time in several cases [**16,17**]. As the systemic inflammatory aggression of SARS.CoV-2 to the lung structures recognizes the alveolar damage and the microvascular thrombosis/occlusion as the major pathogenetic events [**9**,**21-24,39-40**], long-term respiratory consequences may be presumed as highly probable over time [**1-6**].

Long-COVID is one of the terms currently used for describing a clinical condition characterized by the persistency at a variable severity and duration (from a few weeks up to 6 months, and longer) [**7,11-13**] of at least one symptom after the COVID-19 acute infection [**1,8-15**]. The criteria for Long-COVID definition are still debated as several factors can actually lead to different pictures of persistent sequelae, namely: the heterogeneity of multi-organ aggression, the original extension of parenchymal lung involvement, the duration of patient's hospitalization, the therapeutic approach during the acute disease, the length of follow-up after patient's recovery [**7,8,41,42**].

Long-lasting dyspnea is the most frequent discomfort complained by 40-60% of patients after their hospital discharge and their presumed full recovery after COVID-19 pneumonia [**1-6**,**13-20,43**]. However, while the prevalence and the duration of dyspnea in Long-COVID concentrated the interest of researchers [**13,16-20,44**], its underlying pathophysiology still remains poorly investigated in the

clinical setting [**8**,**12,45**]. Usual diagnostic procedures currently available (namely, spirometry and the current DL_{CO} measure) show low specificity and sensitivity from this point of view [**2-3,29**]. In particular, due to the slow binding of CO with intracapillary Hb, the current assessment of DL_{CO} proved insufficient to discriminate disorders of DM from those of the vascular side of alveolar/capillary membrane, namely Vc [**30-34,46,47**].

Unfortunately, in the absence of any pathological CT finding, or of any specific pulmonary indicator, or of a clear cardiogenic origin, the cause of long-lasting dyspnea is generally presumed to be of psychological origin in these cases [**48**]. Further studies specifically oriented to investigate new aspects of long-term changes in respiratory gas transport would be required [**19**]. Those studies would in fact contribute to a better understanding of long-lasting pathophysiological effects induced by the viral infection within the deep lung. In 2021, significant disorders in gas transport were found to persist for several weeks in Long-Covid patients, and these disorders were mainly related to long-lasting residual alveolar remodeling [**49**]. In 2022, a substantial reduction in Vc was shown for the first time by means of the single-breath simultaneous assessment of sDL_{CO} and sDL_{NO} in long-lasting dyspnea up to four months after the clinical and radiological recovery from COVID pneumonia (Long-COVID), regardless their normalized lung volumes [**50**]. This study highlighted the major role of residual lung capillary disorders in these cases.

Results of the present study are supporting the hypothesis that the pathophysiological pattern of respiratory Long-COVID might be declined according to different phenotypes. Actually, further to the condition where the role of the alveolar remodeling is prevailing in causing persisting disorders in gas transport [**49**], also the condition characterized by the prevailing remodeling in the vascular side of alveolar-capillary membrane can be identified as a different pathophysiological phenotype of respiratory Long-COVID. In other words, the microangiopathy originally occurred in the lung capillary bed is suggested as the major pathogenetic event still supporting the hidden long-lasting alveolar-perfusion abnormalities (namely, dyspnea) in these cases, regardless the absence of any radiological (CT scan) findings and reduction in lung volumes. A third phenotype can be obviously presumed when the alveolar and the vascular involvement are equally contributing to lung function disorders.

As a consequence, the general message is that the long-lasting dyspnea should not be underestimated or neglected in Long-COVID patients, but instead regarded as a valuable "*clinical predictor*" of still active disorders in blood-gas exchange. The underlying abnormalities should be searched as soon as possible in these cases and the convincement of the spontaneous healing effect of time avoided.

The comparison to the functional pattern of the HC group further emphasizes this hypothesis. Present data allowed to describe the gap existing in the respiratory gas transport as proportional to the efficiency of alveolar-blood gas exchange [**50**]. Moreover, the addition of HC to the study also contributed to define a clear parametrical threshold between normality and Long-COVID conditions. Finally, quite interesting (and unprecedented to our best knowledge) was the trend of mean alveolar eNO concentrations observed in the three groups investigated. Alveolar eNO was increasing substantially from the normal range of healthy subjects [**51,52**] up to the highest mean values recorded in those patients still highly dyspneic (HDS). At first glance, this peculiar behaviour of alveolar eNO might be generically supposed as due to the long-term persistency of variable inflammation in the

deep lung of these Long-COVID patients. Anyhow, alveolar eNO values had been shown to only approximate the upper limits of normality in post-alveolitis patients [**52**]. On the contrary, as NO is known as a strong vasodilator agent of pulmonary vasculature [**53,54**], a compensatory endogenous vasodilator response might be suggested to occur spontaneously and progressively in the deep lung of LDS and HDS patients, respectively. This response might be presumed as aimed to mitigate the pathophysiological effects caused by the persistent reduction of the lung capillary bed occurring at variable extent in the lung units of these patients.

The present study has some limitations: a) the sample of patients is limited and monocentric; b) the characteristics and the duration of heparin and systemic steroidal treatments during and after hospitalization were impossible to assess in the majority of patients; c) even if longer than in the majority of studies, the maximum time interval from discharge was of 16 weeks. Point of strength are: a) patients were carefully selected in clinical terms; b) both groups of patients were comparable at recruitment; c) the CT scan proved the complete resolution of any residual COVID–induced parenchymal lesion in all patients; d) the unprecedented comparison with healthy controls; e) the simultaneous single-breath assessment of sDL_{CO}, sDL_{NO}, and Vc used for discriminating the alveolar and the vascular side of lung diffusion in the clinical setting; f) dyspnea was used as a *clinical predictor*; f) the statistical models adopted for comparing the two groups of patients vs healthy controls.

CONCLUSIONS

Respiratory Long-COVID is difficult to define by current lung function (namely, spirometry and usual DL_{CO}): critical changes can escape in a great proportion of cases, thus limiting our understanding on hidden underlying determinants. The simultaneous availability of sDLNO/sDLco ratio and Vc provides the opportunity to detect non-invasively, in short time, and at low cost, those persisting disorders in blood gas exchanges that would remain otherwise neglected [**31-33,49,50**]. In other words, the relative pathogenetic role of alveolar and/or capillary abnormalities contributing to the gas transport can be easily discriminated, quantified and phenotyped by this diagnostic approach. In our opinion, even if further studies are needed for improving the still insufficient knowledge on the recovery phase following COVID pneumonia, this functional approach contribute to a new pathophysiological vision of respiratory Long-COVID. Moreover, while current therapeutic strategies against respiratory Long-COVID still are empirical and of unpredictable results [**12,55**], promising opportunities might be disclosed by this recent diagnostic approach, and new therapeutic options based on novel mechanisms of action might be effectively investigated [**56**].

Stemming from these pivotal results, the pathophysiology underlying the long-lasting dyspnea associated to the respiratory Long-COVID seems no longer enigmatic as in the past.

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Contributes
RWD planned the study and wrote the manuscript. PT provided critical feedback, contributed to the data control and to the final version of the manuscript. MP carried out all statistical calculations and contributed to the manuscript. All Authors approved the final version of the manuscript.

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