American Journal of Medicine and Public Health

Research Article
Published: 25 Jan, 2024

Persistent Reduction of Lung Capillary Blood Volume as the Major "Deep Lung" Disorder Underlying Respiratory Long-COVID Syndrome

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Abstract

Background: Long-COVID syndrome is the persistency of symptoms of the original SARS-CoV-2 infection. Dyspnea frequently lasts for several weeks/months (the Respiratory Long-COVID Syndrome). Impairment of lung alveolar and capillary structures are related to dyspnea duration.

Aim: To compare subjects recovered from COVID Pneumonia (HOSP), subjects who only suffered Pauci-Symptomatic SARS-CoV-2 infection (PS), and Healthy Controls (HC) by lung function and dyspnea scores.

Methods: mMRC scores, spirometric parameters, usual DL_{CO}, and single-breath simultaneous NO (sDL_{NO}) and CO (sDL_{CO}) diffusion were assessed in: 40 HOSP subjects still complaining dyspnea 16 weeks after discharge (19 mMRC<1, 21 mMRC>1); 35 PS (five no-vax) complaining long-lasting dyspnea, and 28 HC. Comparison among groups was conducted by ANOVA test.

Results: The sensitivity of spirometric parameters and usual DLCO was low in discriminating the groups (all p=ns). Only sDL_{NO} and sDL_{CO}, their ratio (sDL_{NO}/sDL_{CO}), and the volume of lung of capillary blood (Vc) discriminated the groups with high significancy ($p<0.05$ in almost all comparisons). PS and HOSP patients with low mMRC seem to be similar spirometric profiles ($p>0.20$). The five no-vax PS subjects showed higher microvascular impairment.

Conclusion: The impairment of capillary lung volume proves the major pathogenetic sequela of SARS-CoV-2 inflammatory aggression underlying respiratory Long-COVID syndrome. Long-lasting dyspnea acts as the predicting clinical sign in these cases. Only simultaneous measures of sDL_{NO}/sDL_{CO} ratio and Vc allow to detect and grading with high sensitivity/specificity the microvascular impairment otherwise undetectable in respiratory Long-COVID syndrome. The protecting role of vaccination should be focused also from this point of view.

Keywords: Long-COVID syndrome; Lung function; Lung capillary blood volume; Dyspnea; Simultaneous single-breath DLCO and DLNO measurement; Lung diffusion

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Received Date: *29 Dec 2023*
Accepted Date: *19 Jan 2024*
Published Date: *25 Jan 2024*

Citation:
Negro RD, Turco P, Povero M. Persistent Reduction of Lung Capillary Blood Volume as the Major "Deep Lung" Disorder Underlying Respiratory Long-COVID Syndrome. Am J Med Public Health. 2024; 5(1): 1059.

Introduction

A great proportion (ranging 30-50% in Western Countries and over 60-70% is Asiatic regions) of subjects who experienced a Severe Acute Respiratory Syndrome – Coronavirus-2 (SARS-CoV-2) lung infections (in particular COVID-19 pneumonia) complain one or more symptoms of their original acute disease and limitation in their quality of life of variable duration [1-6].

Strict criteria for the exact definition of Long-COVID syndrome are still debated and the true prevalence is consequently difficult to assess [7,8]. Meanwhile, the Long-COVID syndrome is generally regarded as a condition characterized by the persistency of some clinical signs that likely reflect the still active involvement of different organs, regardless the severity of the original SARS-CoV-2 infection [9-10].

Long-lasting dyspnea of variable degree is the respiratory discomfort most frequently complained for several weeks or months (the respiratory Long-COVID syndrome) by 40% to 60% of patients recovered from COVID-19 pneumonia regardless their normalized Computed Tomography (CT) scan and lung volumes [1,3-6,10], but also by around 30% of non-hospitalized subjects who suffered milder respiratory SARS-CoV-2 infections [11,12].

The injury to the alveolar epithelial cells, the formation of hyaline membrane and fibrin deposition, the hyperplasia of type II pneumocytes, together with the pulmonary congestion and the microvascular thrombosis/occlusion are the major pathogenetic findings most frequently described during SARS-CoV-2 infection (during COVID-19 pneumonia in particular) [13-16]. The subsequent long-lasting dyspnea had then been generically presumed as related to the persistency of undefined virus-induced tissular damage originally occurred within the lung [17].

Despite the huge number of papers dedicated to the description of the prevalence and duration of symptoms that characterize the respiratory Long-COVID syndrome [1-10], much less attention had been paid to detect the underlying pathophysiological mechanisms sustaining the respiratory Long-COVID syndrome in these cases. On the other hand, this is a difficult issue indeed given that current spirometric indices prove of low specificity and show a non-specific volumetric restrictive pattern of variable degree in only 25% to 30% of cases [18,19]. Unfortunately, also usual measurements of DLCO proved of limited value because unable in discriminating disorders of alveolar-capillary Diffusing Membrane Conductance (DM) from those involving the microvascular structures of respiratory units, and in grading their severity [20-22]. The persistent reduction of lung capillary blood Volume (Vc) has been recently identified as the peculiar feature of lung function that is able to characterize and grade the respiratory Long-COVID syndrome in subjects still complaining long-lasting dyspnea of different severity after sixteen weeks after their complete recovery from COVID pneumonia [23,24]. In these studies, the severity of dyspnea proved strictly related to the diffusive parameters that are only assessed by the single-breath simultaneous measure of Diffusion for Carbon Dioxide (sDL_{CO}) and nitric oxide (sDL_{NO}) [25,26].

At present, nothing is known to our best knowledge concerning the possible microvascular lung involvement due to pauci-symptomatic SARS-CoV-2 infections, such as those milder conditions not complicated by any pneumonia. To date, the possible occurrence of this peculiar lung injury also in these cases has never been investigated although a similar underlying disorder cannot be ruled out to occur also in pauci-symptomatic subjects.

Aim of the study was to compare patients who suffered pauci-symptomatic SARS-CoV-2 infection to patients recovered from COVID pneumonia and to healthy controls who never experienced SARS-CoV-2 infection in terms of their lung function and dyspnea scores.

Materials and Methods

Study design

All patients of both genders and aged ≥ 18 years referring to our Centre between September 1st, 2021 and June 30th, 2023 were recruited after their informed consent. Exclusion criteria were: Current and former-smoke habit; age <18 years; comorbidities able to affect the diffusion capacity, namely: Anemia (Blood Hemoglobin [Hb] <12 g/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.

Patients were initially divided in three groups

1. subjects previously hospitalized for COVID pneumonia and discharged as "clinically recovered", but still complaining daily dyspnea of variable degrees for 16 weeks from their discharge (Group HOSP).

2. subjects who suffered a Pauci-Symptomatic (PS) SARS-CoV-2 infection over the last six months, managed at home and without any documented parenchymal lesion, but still complaining dyspnea of variable duration (Group PS).

3. Healthy Controls (HC) who had never experienced COVID-19 infection i.e., negative IgG and IgM serology (Group HC).

All subjects were investigated by means of usual spirometric parameters and DL_{CO}, associated with the simultaneous single-breath measurements of sDL_{CO} and sDL_{NO} and related parameters (namely, sDL_{NO}/sDL_{CO} ratio, and Vc). At recruitment, a CT proved the complete resolution of any parenchymal lesion in all subjects of Group HOSP and PS.

Current dyspnea was graded by means of the Modified British Medical Research Council (mMRC) dyspnea score in all subjects according to the British Thoracic Society (BTS) recommendations [27]. The duration of dyspnea was also calculated in weeks for HOSP and PS subjects (from the hospital discharge in subjects of Group HOSP, and from the resolution of acute symptoms in those of Group PS). Finally, patients in the group HOSP were further divided into patients with Low Dyspnea Scorers (HOSP-LDS) and patients with High Dyspnea Scorers (HOSP-HDS) if they were still complaining low (<1) or high (>1) dyspnea scores for 16 weeks after discharge, respectively.

Data collected

Age, sex, Body Mass Index (BMI) and blood Hb (in g/L) were recorded. Lung function parameters to collect included: Vital Capacity (VC), Forced Expiratory Volume in 1 sec (FEV1), % 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. A Plethysmography Platinum DX Elite (MedGraphics, USA) was used for assessing spirometric parameters and usual DL_{CO} (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 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 [28], according to reference values fixed in the ERS/ATS Task-Force 2017 [29].

Information on COVID vaccinations received before SARS-CoV-2 infection were also collected for PS subjects, but not for HOSP-HDS and HOSP-LDS subjects as they suffered COVID pneumonia during the first pandemic phase when vaccinations did not yet exist.

Ethics

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. At recruitment, all subjects gave their informed consent also to the anonymous use of their own data for research purposes.

Statistical analysis

Continuous data were presented as means and Standard Deviation (SD), while sex as absolute and relative frequencies. Differences in

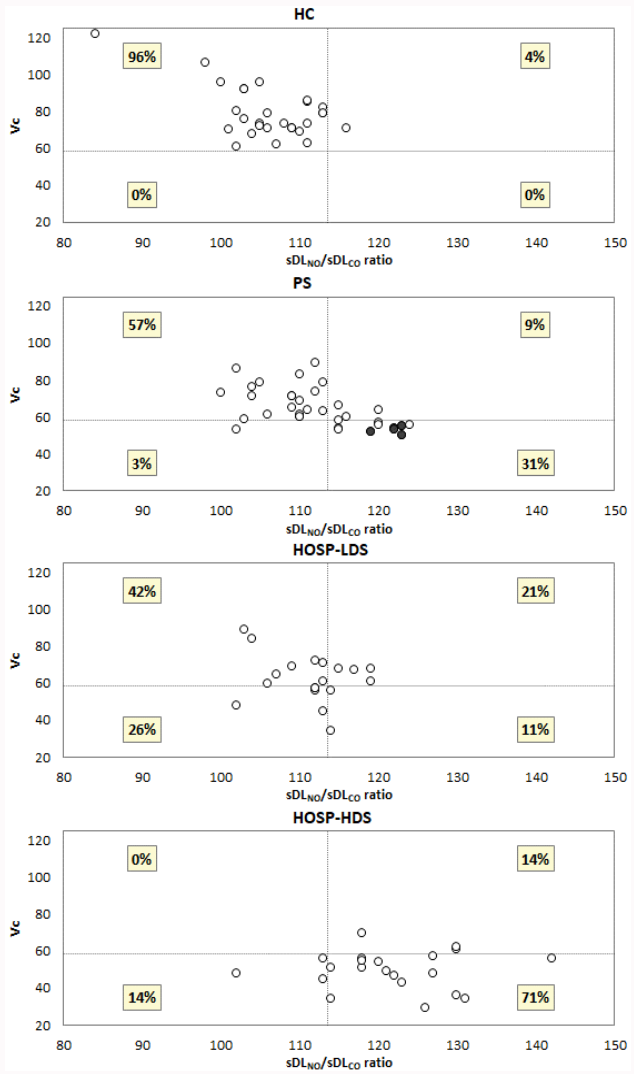


Figure 1: Comparison among distributions of HC, PS, HOSP-LDS and HOSP-HDS optimal cut-off for sDL_{NO}/sDL_{CO} ratio and Vc (in boxes, the proportion of observations in each quadrant). Black circles in PS quadrant refer to no-vax subjects.

baseline characteristics among the four groups (HC, PS, HOSP-LDS, and HOSP-HDS) were tested by ANOVA test. Differences in lung function parameters were estimated by ANOVA test adjusted for multiple comparisons using Šidák correction.

According to our previous analysis [24], the sDL_{NO}/sDL_{CO} ratio and the Vc value proved able to discriminate HOSP-HDS patients from HOSP-LDS patients and HC (sensitivity, specificity, and AUC greater than 0.85). Estimated optimal cut-off values were 113.5 (95% CI 110-117) for the sDL_{NO}/sDL_{CO} ratio, and 58.5 (95% CI 54-63) for Vc, respectively. Such threshold was used to categorized PD patients enrolled in this analysis and proportion of patients with sDL_{NO}/sDL_{CO} ratio below and with Vc above the cut-off, values, respectively, was compared to the distribution of the other 3 groups by ANOVA test adjusted for multiple comparisons using Šidák correction.

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).

Results

Patient characteristics

General characteristics of the four clusters of subjects investigated are reported in Table 1. Subjects resulted well matched in terms of mean age, BMI, Hb, SpO_2, even if males were prevailing in HC and PS groups. Comorbidities were found in 9 cases of HOSP-HDS and HOSP-LDS subjects: Mild arterial hypertension (n=6), atopy (n=2), and chronic thyroiditis (n=1). In PS group, comorbidities were found in 7 cases: Mild arterial hypertension (n=3), atopy (n=2), and obesity (n=2). Comorbidity prevalence was slightly higher in the HOSP-LDS group (26.3%) with respect to PS (20%) and HOSP-HDS (19.0%) (Table 1).

To note that, though not significantly different, subjects belonging to the HOSP-HDS group had a lower mean SpO_2 value at rest. Mean dyspnea scores were significantly different in the three clusters of subjects PS, HOSP-LDS, and HOSP-HDS ($p<0.001$), both in terms of its severity and duration.

HOSP-HDS and HOSP-LDS subjects had never received any COVID vaccination because belonging the first pandemic phases when vaccines were not yet available, while 30/35 of PS subjects had received full COVID vaccinations courses (90% 3 doses, 10% 2 doses). The remaining five refused vaccinations because no-vax.

Mean values for all lung function parameters are reported in Table 2 together with their statistical comparisons and corresponding p-values. The four clusters proved practically equal in terms of spirometric parameters. Only mean FEV1/FVC values were statistically higher in HOSP-HDS and HOSP-LDS subjects with respect to HC, though this difference was very mild and clinically not significant.

Usual DLCO mean values were progressively decreasing from HOSP-HDS to HC subjects, but only discriminated HOSP-HDS subjects from the other groups. Mean values for sDL_{CO} also showed the same decreasing trend, but allowed to discriminate HOSP-HDS, HOSP-LDS and PS from HC subjects, and HOSP-HDS from HOSP-LDH and PS subjects with high level of significance, while HOSP-LDS and PS subjects were not different (p=0.980). Moreover, also mean values for sDL_{NO} showed a decreasing trend and discriminated HOSP-HDS patients from those belonging to the other three clusters, but were unable to discriminate HOSP-LDS and PS from HC subjects, and HOSP-LDS from PS subjects. These data tend to confirm the different sensitivity and specificity achievable with current DLCO, sDL_{CO} and sDL_{NO}. measurements from this point of view.

Mean values for sDL_{NO}/sDL_{CO} ratio highly discriminated HOSP-HDS subjects from those of the other three clusters: Furthermore, this parameter proved also able to discriminate the other groups from each other significantly, except HOSP-LDS from PS subjects. Finally, Vc mean values proved as much discriminant: they were able not only to distinguish HOSP-HDS subjects from those belonging to the other three clusters, but also to differentiate each group (except HOSP-LDS from PS subjects) in terms of involvement of capillary lung vasculature.

Comparisons among the distributions of HC, PS, HOSP-LDS and HOSP-HDS optimal cut-off values for sDL_{NO}/sDL_{CO} ratio and Vc were reported in Figure 1 together with the frequency of observations for each quadrant (in the small squares). While values recorded in HCs were all concentrated in the first top-left quadrant (with

Table 1: Baseline characteristics of the groups investigated and statistical comparisons.

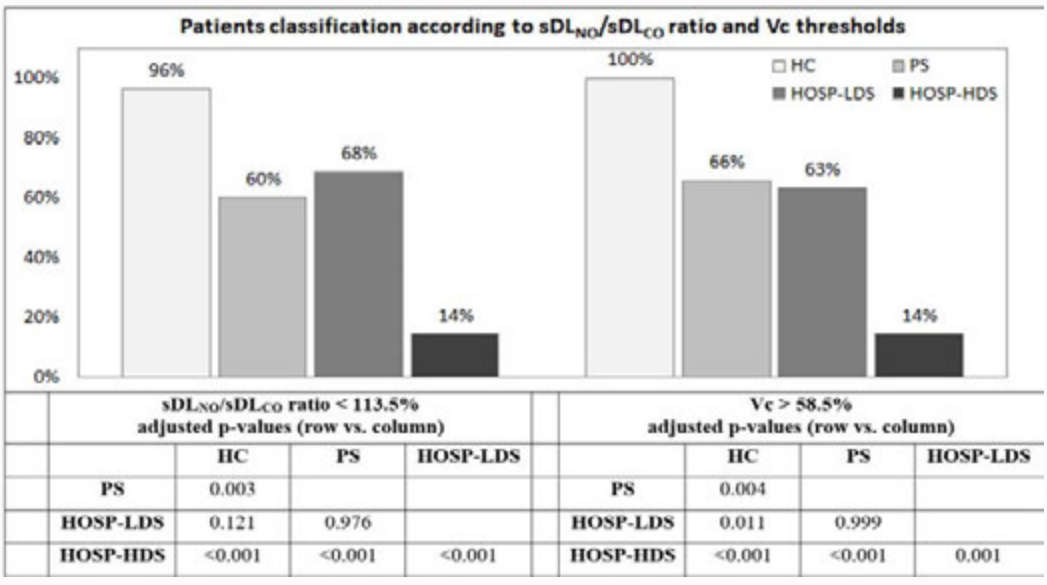
	HC	PS	HOSP-LDS	HOSP-HDS	p-value
N	28	35	19	21	
Male (%)	22 (78.6%)	19 (54.3%)	9 (47.3%)	8 (38.1%)	0.026
Mean age (SD)	50.4 years (15.3)	57.1 years (13.4)	48.4 years (16.7)	48.9 years (20.6)	0.151
Mean BMI (SD)	25.8 m^2 (5.6)	24.7 m^2 (4.0)	24.2 m^2 (4.1)	24.3 m^2 (4.9)	0.624
Mean Hb (SD)	13.9 g/dl (0.4)	14.0 g/dl (0.3)	14.1 g/dl (0.4)	14.1 g/dl (0.5)	0.291
Mean SpO_2 (SD)	98.2% (1.2)	97.5% (0.8)	97.8% (1.1)	96.7% (1.6)	0.87
Comorbidities (%)	0 (0.0%)	7 (20.0%)	5 (26.3%)	4 (19.0%)	0.055
Mean dyspnea score (SD)	NA	0.37 (0.60)	0.11 (0.32)	1.71 (0.46)	<0.001

BMI: Body Mass Index; Hb: Hemoglobin; HC: Healthy Controls; HOSP-LDS: Hospitalized Patients with Low Dyspnea Scorers
HOSP-HDS: Hospitalized Patients with High Dyspnea Scorers; PS: Pauci-Symptomatic Patients; SD: Standard Deviation

Table 2: Mean (SD) lung function parameters as % predicted in the four groups and statistical comparisons.

Lung function parameters	HC	PS	HOSP-LDS	HOSP-HDS	p-value (ANOVA)	Pairwise comparison (adjusted p-values)					
						PS *vs.* HC	HOSP-LDS *vs.* HC	HOSP-HDS *vs.* HC	HOSP-LDS *vs.* PS	HOSP-HDS *vs.* PS	HOSP-HDS *vs.* HOSP-LDS
FEV_1	96.9 (13.9)	96.9 (16.4)	95.8 (11.5)	96.6 (17.5)	0.9942	0.999	0.999	0.999	0.999	0.999	0.999
VC	106.8 (12.8)	102.9 (15.5)	97.1 (12.2)	99.1 (21.0)	0.1511	0.909	0.205	0.436	0.713	0.943	0.999
FEV_1/FVC	90.7 (6.6)	94.0 (7.7)	98.9 (6.4)	98.9 (13.2)	0.002	0.61	0.012	0.009	0.249	0.227	0.999
DL_{CO}	97.9 (17.3)	92.9 (19.1)	90.5 (16.5)	76.9 (15.6)	<0.001	0.837	0.638	<0.001	0.997	0.008	0.091
sDL_{CO}	88.5 (11.3)	79.4 (12.1)	76.9 (14.0)	64.0 (11.6)	<0.001	0.023	0.011	<0.001	0.98	<0.001	0.007
sDL_{NO}	94.4 (11.9)	89.7 (13.2)	87.2 (17.1)	77.9 (15.9)	0.001	0.745	0.448	0.001	0.99	0.019	0.225
sDL_{NO}/sDL_{CO} ratio	106.0 (6.3)	112.4 (6.8)	111.4 (5.0)	121.8 (8.7)	<0.001	0.002	0.056	<0.001	0.996	<0.001	<0.001
Vc	79.2 (13.9)	64.4 (10.4)	62.5 (12.8)	49.6 (10.3)	<0.001	<0.001	<0.001	<0.001	0.994	<0.001	0.005

HC: Healthy Controls; HOSP-LDS: Hospitalized Patients with Low Dyspnea Scorers; HOSP-HDS: Hospitalized Patients with High Dyspnea Scorers; PS: Pauci-Symptomatic Patients; SD: Standard Deviation



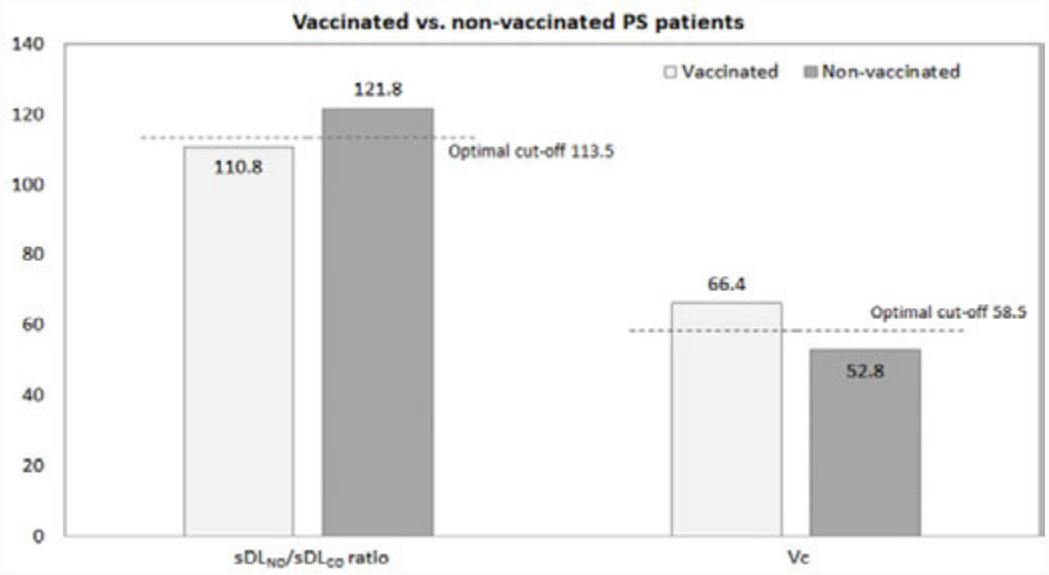
sDLNO/sDLCO ratio < 113.5% adjusted p-values (row vs. column)			
	HC	PS	HOSP-LDS
PS	0.003		
HOSP-LDS	0.121	0.976	
HOSP-HDS	<0.001	<0.001	<0.001

Vc > 58.5% adjusted p-values (row vs. column)			
	HC	PS	HOSP-LDS
PS	0.004		
HOSP-LDS	0.011	0.999	
HOSP-HDS	<0.001	<0.001	0.001

Figure 2: Distribution of patients below and above the normality threshold for sDL_{NO}/sDL_{CO} ratio and Vc, respectively.
HC: Healthy Controls; HOSP-LDS: Hospitalized Patients with Low Dyspnea Scorers; HOSP-HDS: Hospitalized Patients with High Dyspnea Scorers; PS: Pauci-Symptomatic Patients

Vc and sDL_{NO}/sDL_{CO} values always ≥ 60% and ≤ 113% predicted, respectively), a progressive drop of VC values and a corresponding increasing trend of sDL_{NO}/sDL_{CO} values can be easily perceived from PS up or HOSP-HDS subjects, according to the different severity of their previous COVID infection. In particular, none of the HOSP-HDS subjects can be found in the first top-left quadrant as their majority are concentrated in the bottom-right quadrant. After excluding non-vaccinated patients in the group PS (black dots in Figure 1), the distribution of PS patients seems more centered in the top-left quadrant.

The distribution of subjects showing values for sDL_{NO}/sDL_{CO} and Vc below and above the corresponding thresholds for normality are



Fifure 3: Effect of vaccination on mean sDL_{NO}/sDL_{CO} ratio and Vc among PS patients.

reported in Figure 2. Vc and sDL_{NO}/sDL_{CO} ratio values of HC subjects proved in the normal range for 96% and in 100% of cases, respectively, while a decreasing proportion of subjects characterized by lower values for sDL_{NO}/sDL_{CO} ratio and by corresponding higher values in VC became evident, up to 14% of HOSP-HDS subjects. Once again, the trend was directly related to the severity of their original SARS-Cov-2 infection.

Based on ANOVA test (Tables in Figure 2), the proportion of patients below the threshold for sDL_{NO}/sDL_{CO} ratio or above the Vc cut-off value was significantly different among all groups with the exception of PS and HOSP-LDS patients (p=0.976 and p=0.999, respectively).

When compared to vaccinated subjects, the five no-vax subjects had higher mean dyspnea score (1.4 ± 0.5 SD *vs.* 0.3±0.5 SD, respectively) associated to a higher microvascular lung impairment, such as: Mean Vc values 52.8 (SD=1.9) *vs.* 66.4 (SD=10.0), and mean sDL_{NO}/sDL_{CO} values 121.8 (SD=1.6) *vs.* 110.8 (SD=6.0), respectively (Figure 3). No statistical comparison was performed due to the small sample size of the no-vax subgroup.

Discussion

Long-term pulmonary and extra-pulmonary symptoms following a SARS-CoV-2 infection had been reported by a huge number of studies, though also claiming a spontaneous and time-dependent decreasing severity in several cases [4,5].

The Long-COVID syndrome is one of the ways currently used for describing this clinical condition characterized by the persistency (for a few weeks up to 6 months, and longer) of at least one symptom (namely, brain fog, cough, fatigue, palpitations, but mainly dyspnea, exertional as well as at rest) after a SARS-CoV-2 acute infection [1-4,6] that can frequently limit patients' daily activities and Quality-of-Life (QoL) [30]. Several factors can lead to various clinical pictures of the Long-COVID syndrome, namely: The extension of the original multi-organ aggression a); the duration of patient's hospitalization; the therapeutic approach during the acute disease; the follow-up duration after patient's recovery (in particular the extension of anti-coagulants administration also at home), and the role of preexisting comorbidities [2].

The pathogenesis of the Long-COVID syndrome had been poorly investigated even if the persistency of inflammatory/immunological abnormalities and the vascular endothelial injury, with the consequent formation of microthrombi, were generally regarded as the principal causes of altered function in multiple organs [13-16,31].

As the respiratory structures (and the deep lung in particular) are the first human targets of the corona virus and the site where major pathogenetic events due to the SARS-CoV-2 aggression usually occur (namely, alveolar damage and formation of microvascular thrombosis and occlusion) [13-15,32,33], it is easily presumable that long-term respiratory consequences of different severity may persist in these circumstances [1,34,35], thus favoring the onset of the respiratory Long-COVID syndrome.

Respiratory Long-COVID syndrome is the most frequent discomfort complained for several weeks or months by 40% to 60% of patients recovered after COVID-19 pneumonia, but also by a variable proportion of subjects who suffered milder SARS-CoV-2 infections [1,6,33-35].

However, while the assessment of the dyspnea prevalence and duration in respiratory Long-COVID syndrome concentrated the major interest of researchers [3-5,18], the underlying pathophysiology remained poorly investigated, particularly in the clinical setting [6,36]. On the other hand, the low specificity and sensitivity of usual diagnostic lung function tests currently available (namely, spirometry and DL_{CO} measures) from this point of view contributed to specific poor knowledge [19,34]. In particular, due to the slow binding of CO with intracapillary Hb, the current assessment of DL_{CO} results insufficient in discriminating disorders of DM from those of the vascular side of alveolar/capillary membrane, namely the pulmonary volume of capillary blood (Vc) [21,22,25,26]. Unfortunately, in the absence of any pathological CT finding, or of any specific pulmonary indicator, or of a clear cardiogenic origin (the only aspect investigated in >95% of cases), long-lasting dyspnea has been frequently presumed to be of psychological origin in these cases [16].

Studies specifically oriented to investigate new aspects of long-term changes in respiratory gas transport and the search for specific indicators of lung function have been solicited as an urgent need from this point of view [5,37]. These studies would in fact contribute to improve effectively our understanding on the long-lasting pathophysiological disorders induced by the SARS-CoV-2 infection within the deep lung. In 2021, significant disorders in gas transport were found to persist for several weeks after COVID pneumonia, and these disorders were mainly related to the induced long-lasting alveolar remodeling [38]. In 2022, a substantial volume reduction of pulmonary capillary blood (Vc) was assessed for the first time after the subjects' clinical and radiological recovery from COVID pneumonia by means of the simultaneous single-breath measurements of sDL_{CO} and sDL_{NO}, regardless normalized lung volumes [23]. Results of this pivotal study highlighted the major and prevailing role of persisting lung microvascular impairment in these cases, and the strict relationship between the extent of the lung capillary blood volume drop and the severity of persistent dyspnea. This evidence was further emphasized by comparing these results to those from healthy controls who never suffered any SARS-CoV-2 infection [24].

Unlike in Healthy Controls (HC), data of the present study concerning changes in in diffusive parameters of lung function occurring in subjects who only suffered pauci-symptomatic SARS-CoV-2 infections further clarify the events. Actually, the effect of some capillary impairment was also assessed in around 30% of these subjects, even in the absence of any previous lung parenchymal involvement. The lung function pattern of this cluster of patients, though significantly milder than that one of subjects with higher severe dyspnea scores after COVID pneumonia (such as, the HOSP-HDS subjects), proves very close to the condition of those complaining lower severe dyspnea scores after COVID pneumonia (such as, the HOSP-LDS subjects). In other words, also milder SARS-CoV-2 infections prove absolutely not free of risk for the deep lung as the acute viral aggression seem anyway able to cause some endothelial impairment of the lung capillary vasculature with corresponding respiratory disorders and discomfort (namely, dyspnea) of unpredictable duration.

Results of the present study are also supporting the hypothesis that the pattern of lung function disorders might be declined according to different pathophysiological phenotypes in respiratory Long-COVID. In fact, while a first phenotype can be represented by the less frequent condition characterized by the persistency of a prevailing alveolar remodeling [38], the significant remodeling occurring at the vascular side of the alveolar-capillary membrane represents the second and the most frequent pathophysiological phenotype found in respiratory Long-COVID syndrome, also following milder SARS-CoV-2 respiratory infections. In other words, we can speculate that the microangiopathy originally occurred in the lung capillary bed (and the consequent drop in pulmonary volume of capillary blood) corresponds to the major pathogenetic event sustaining the previously unexplained long-lasting abnormalities in gas transport (namely, dyspnea) in these cases, regardless the absence of any radiological (CT scan) finding and the normality of lung volumes.

Recent evidence is further supporting this hypothesis aimed to explaining the long-lasting dyspnea in respiratory Long-COVID syndrome. Indeed, an *in vivo* study pointed out the long-lasting reduction in vascular density and the persistent capillary rarefication as the two peculiar features that characterize both the acute SARS-CoV-2 infection and the long-COVID syndrome [39]. The Authors suggested that this local microvascular limitation would presumably cause an inadequate response to the tissue metabolic demand. Moreover, they also speculate that the higher velocities in blood red cells found in Long-COVID patients might represent a compensatory mechanism to meet their metabolic demands. Finally, a third phenotype can be presumed when the alveolar and the vascular involvement are equally contributing to lung function disorders in gas transport. Though some specific studies are in advanced progress, the duration of these peculiar disorders in the lung microvascular bed after SARS-CoV-2 infections still is unknown, both in severe and in milder pauci-symptomatic cases.

The preventive role of vaccinations against SARS-CoV-2 infections is also worthy to be focused from this point of view. To highlight that the five no-vax pauci-symptomatic subjects of the present study showed a trend in higher impairment of their pulmonary microvasculature, peculiarly characterized by a higher reduction in pulmonary capillary blood Volume (Vc), associated to higher dyspnea scores.

This pivotal evidence contributes to support with specific lung function parameters the role of vaccinations in preserving biological structures of the deep lung. These preliminary results are in agreement with data from some recent clinical surveys oriented to investigate the role of vaccinations in preventing the occurrence of the Long-COVID syndrome and to assess the risk of developing SARS-CoV-2 infection in out-patients [10,40,41].

Some messages are emerging from the present study: a) 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 (and previously unknown) impairment of pulmonary microvascular circulation in the lung and of consequent disorders in blood-gas transport; b) these underlying abnormalities can be also found in pauci-symptomatic subjects, though to a lower prevalence and severity; c) at present, differently from in the past, these hidden abnormalities can be easily identified, graded and phenotype by means of appropriate lung function procedures; d); long-lasting dyspnea following SARS-CoV-2 infections has a pathophysiological explanation that should be investigated as soon as possible in these cases, and the passive waiting for the spontaneous resolution avoided; e) the protecting role of vaccinations against SARS-CoV-2 infections is worthy of further investigation also from this point of view.

The present study recognizes some limitations:

a) the clusters of patients derive from a monocentric investigation and their number is limited; b) the strategy of anti-IL6, heparin and systemic steroidal treatments during and after hospitalization in HOSP-HDS and HOSP-LDS subjects were impossible to assess precisely due to the first heavy pandemic phase; c) though longer than in the majority of previous studies, the maximum time interval from discharge was of 16 weeks in these patients, and not longer. Point of strength are: a) the present study represents the very first investigation designed for assessing and comparing the pattern of respiratory diffusive disorders in respiratory Long-COVID syndrome carried out in subjects following SARS-CoV-2 infections of different severity; b) it also represents an unprecedented investigation on pulmonary effects occurring in pauci-symptomatic infections *vs.* healthy controls; c) it is the first study aimed to compare in the clinical setting current lung function parameters to those obtained with the

non-invasive diagnostic procedure that is also able to investigate both the alveolar and the vascular side of lung diffusion; d) all subjects included had been carefully selected in clinical terms and proved well matched; e) at recruitment, a CT scan systematically confirmed the absence of any residual COVID–induced parenchymal lesion in all patients included; f) dyspnea was used as a clinical predictor; f) the statistical models adopted for comparing the different clusters of subjects investigated; g) to our best knowledge, the study is the first attempt of investigating the protecting role of vaccinations on the lung capillary vasculature.

Conclusion

Respiratory disorders are difficult to assess in respiratory Long-COVID syndrome by means of current lung function tests (such as, spirometry and usual DLCO) because unable to identify some critical pulmonary events that can escape in a great proportion of cases, thus limiting our understanding on underlying pathogenetic determinants. The simultaneous assessment of sDL_{NO}/sDL_{CO} ratio and Vc provides the opportunity to detect non-invasively, in short time, at low cost and with high sensitivity and specificity, those persisting respiratory disorders that would otherwise remain underestimated or unknown [22-26]. In other words, this functional approach contributes to a new pathophysiological vision on respiratory Long-COVID syndromes. The relative pathogenetic role of the alveolar and/or the pulmonary blood capillary impairment occurring during respiratory SARS-CoV-2 infections (and variably contributing to the gas transport) can be easily investigated, discriminated, quantified and phenotype by this diagnostic opportunity.

While current therapeutic strategies against respiratory Long-COVID still are empirical and of unpredictable results [42,43], promising opportunities might be disclosed by this recent diagnostic approach in our opinion, and innovative pulmonary therapeutic options based on novel mechanisms of action might be investigated effectively [44].

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