

mRNA vaccines protect from the lung microvasculature injury and the capillary blood volume loss occurring in SARS-CoV-2 paucisymptomatic infections

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ABSTRACT

Introduction: The reduction of lung capillary blood volume (V_c) had been identified as the microvascular injury mostly underlying the respiratory Long-COVID syndrome following post-COVID-19 pneumonia. The same kind of injury has been recently also found in several individuals after milder paucisymptomatic SARS-CoV-2 infections. Though current guidelines strongly recommend vaccination, studies aimed to investigate the *in vivo* protection of anti-SARS-CoV-2 vaccines on lung microvascular targets still are missing to our best knowledge.

Aim: to assess the protection of mRNA vaccines from the reduction of lung capillary blood volume (V_c) caused by paucisymptomatic SARS-CoV-2 infections in vaccinated compared to unvaccinated individuals.

Methods: Non-smoking individuals with recent paucisymptomatic SARS-CoV-2 infection were divided into vaccinated and unvaccinated groups. Lung function parameters, including single-breath diffusing capacity and microvascular blood volume, were compared between groups.

Results: Fifty vaccinated and twenty-five unvaccinated well-matched individuals were studied. Differently than usual lung function parameters, only the single-breath simultaneous assessment of sDL_{CO} , sDL_{NO}/sDL_{CO} ratio and V_c allowed to identify the occurrence of the lung microvascular injury with high sensitivity and specificity ($p < 0.001$).

Conclusion: mRNA vaccines proved to exert a high protection from the loss of lung capillary blood volume (V_c) induced by SARS-CoV-2 paucisymptomatic infections ($p < 0.001$). The availability of this non-invasive investigational model should be regarded as a very helpful tool for assessing and comparing *in vivo* the protective effect of mRNA vaccines on the human microvascular structures of the deep lung.

Key words: Long COVID; mRNA vaccines; lung function; DL_{NO} and DL_{CO} single-breath simultaneous measure; lung microvascular injury; lung capillary blood volume

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Introduction

The SARS-CoV-2 outbreak of late 2019 caused a tremendous public health impact worldwide during the following couple of years, with over half a billion confirmed cases and around seven million deaths as reported by the World Health Organization [1].

SARS-CoV-2 infections showed variable severity, ranging from the serious COVID-19 disease with acute respiratory failure (frequently fatal) to a mild clinical picture mostly involving only upper airways [2-5]. In general, the onset of infection is characterized by fever, dysgeusia, fatigue, anorexia and respiratory symptoms, such as: cough, expectoration, and dyspnea, of variable severity, duration and evolution [6-10].

It was shown that around 40% of patients are still complaining variable dyspnea associated to limitation in their quality of life for several weeks/months after their recovery from COVID-19 pneumonia (the Respiratory Long-COVID Syndrome) [11-14]. Our group demonstrated a substantial reduction of lung capillary blood volume that persists for several weeks in these cases [15].

Though previously underestimated and mostly regarded as merely due to psychological factors [6,16-18], long-lasting dyspnea associated to the same kind of pathophysiological lung disorders was also assessed by our group in a not negligible proportion of subjects following paucisymptomatic COVID-19 syndromes [19,20], thus supporting the occurrence of a substantial reduction of lung capillary blood volume also in these milder conditions.

Current guidelines recommend vaccination against SARS-CoV-2 of all eligible individuals [21-27]. However, despite the huge number of investigations focusing the biological and immunological effects of anti-COVID vaccines, pathogenetic studies aimed to investigate and assess their protection on specific lung structural targets *in vivo* still are missing to our best knowledge. mRNA vaccines are of great interest from this point of view because they were found to be highly effective in preventing the SARS-CoV-2 inflammatory aggression and safe [28-30].

Aim of the study was to compare the protection of mRNA vaccines against the reduction of lung

capillary blood volume caused by paucisymptomatic SARS-CoV-2 infection in vaccinated and unvaccinated individuals.

Methods

Study design

Non-smoker patients of both genders, aged ≥ 18 years, referring to our Specialist Medical Centre (CEMS) between September 1, 2021 and June 30, 2023 after a paucisymptomatic SARS-CoV-2 infection (without any pneumonia) managed at home for a few days over the last six months before the date of recruitment were enrolled 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] <12g/L); heart failure, COPD; lung fibrosis; vasculitis; liver and renal failure; diabetes; any previous hospital admission for COVID pneumonia; any inflammatory parenchymal lesion radiologically (CT scan) documented over the last three months before recruitment; physical and/or cognitive impairment enabling procedures for lung function tests; refusal of consent.

The sample was divided in two groups: 1) vaccinated subjects at least two months before their paucisymptomatic SARS-CoV-2 infection, and: 2) unvaccinated individuals because no-vax, in proportion of 2:1.

The protocol is shortly described further below. All subjects were investigated by means of usual spirometric parameters and current diffusing capacity for carbon monoxide (DL_{CO}), associated with the non-invasive simultaneous single-breath measurements of DL_{CO} (sDL_{CO}) and nitric oxide (sDL_{NO}), the sDL_{NO}/sDL_{CO} ratio, and the lung capillary blood volume (V_c). The simultaneous single-breath method for assessing sDL_{CO} and sDL_{NO} (5 seconds breath hold time) was added to current DL_{CO} measures (10 seconds breath hold time) because current DL_{CO} is intrinsically unable to discriminate abnormalities occurring at the alveolar level (such as, the membrane diffusing conductance - DM) from those attaining the vascular side

of the blood gas exchange (such as, the total volume of blood in the lung capillaries exposed to alveolar air - V_c). In fact, as the binding of NO with intracapillary haemoglobin (Hb) is extremely faster than that of CO, sDL_{NO} mainly informs on the condition of the epithelial surface of the alveolar membrane, while sDL_{CO} mainly informs on the vascular phase of diffusion through the membrane. Moreover, only when sDL_{NO} and sDL_{CO} are simultaneously measured, the sDL_{NO}/sDL_{CO} ratio can be calculated. Obviously, higher the ratio, lower the value of sDL_{CO} , and then of the lung capillary blood volume (V_c). These are the reasons why the single-breath method for assessing sDL_{CO} and sDL_{NO} simultaneously is recommended for investigating the different factors affecting the determinants of diffusing capacity [31].

Current dyspnea was graded at recruitment by means of the Modified British Medical Research Council dyspnea score (mMRC-DS) in all subjects according to the British Thoracic Society (BTS) recommendations [31]. The duration of dyspnea was also calculated in days from the resolution of acute symptoms.

Data collected

Age, sex, body mass index (BMI), blood Hb (in g/L), % O_2 saturation (SpO_2 %), the mMRC-DS and comorbidities were recorded together with the therapeutic approach to the paucisymptomatic COVID infection at home. Information on anti-COVID vaccinations received before the SARS-CoV-2 infection were also collected.

Lung function parameters collected were: Vital Capacity (VC), Forced Expiratory Volume in 1 sec (FEV_1), current DL_{CO} , sDL_{CO} and sDL_{NO} , sDL_{NO}/sDL_{CO} ratio, and V_c . 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). 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 noninvasively the simultaneous assessment of diffusing membrane conductance (DM) and V_c (such as the vascular side of alveolar/capillary membrane)

as a function of the standard single-breath method. This method is based on the principle by Roughton & Forster [32,33]: $1/DL=1/DM+1/\emptyset V_c$, where $1/DL$ is the total resistance to the NO absorption and the CO uptake; $1/DM$ is the resistance opposed by the alveolar membrane, and $1/\emptyset V_c$ is the diffusion resistance to the red cell membrane and Hb combination [32,33]. As NO and CO are characterized by different solubilities for plasma, the method is based on the principle of two distinct reactions of Theta fractions, one for NO and the other for CO, during the same single breath.

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

A pre-specified sample size calculation was performed based on the mean difference of spirometric and diffusive parameters according to the formula for unmatched samples $n=(1+1/c)(z_{1-\alpha}+z_{1-\beta})^2/\Delta^2$, where $\alpha=5\%$ and $\beta=20\%$ are the type I and II errors, respectively. Δ is the standardized mean difference defined as the mean difference d of each parameter between the two groups divided by its standard deviation, and c is the ratio between vaccinated and unvaccinated patients. As vaccinated patients were expected to be more frequent than unvaccinated patients, a ratio of 2:1 (i.e., $c=2$) was chosen for the sample size calculation. Conservatively it was assumed that the mean value for each parameter considered in the group of unvaccinated patients would be 10% lower than the corresponding mean value in the group of vaccinated patients (for RV and sDL_{NO}/sDL_{CO} ratio, it was assumed an increase of 10%). The standard deviation of their mean difference was calculated assuming that the standard deviation for each parameter was equal to 40% of the mean value in both groups, and a plausible linear correlation $\rho=0.5$ for each parameter between the two groups. According to these assumptions, a total

of at least 60 patients (40 vaccinated and 20 unvaccinated) should be enrolled in the study.

Continuous data were presented as means and standard deviation (SD), while gender and prevalence of comorbidities as absolute and relative frequencies. Differences assessed in baseline between the two subsets of patients were tested by non-parametric Wilcoxon test (for continuous variables) and Fisher exact test (for gender and comorbidities). Differences in lung function parameters were estimated by a generalized linear model (gamma family) adjusting for all the characteristics available at enrollment. Results were reported as adjusted mean difference (AMD) and confidence intervals (CI). Moreover, the association between lung parameters and dyspnea was also investigated by ANOVA test using the variable mMRC-DS as categorical independent variable. Pairwise comparison (i.e. mMRC-DS=0 vs. mMRC-DS=1, mMRC-DS=0 vs. mMRC-DS=2, etc.) were expressed in terms of p-value adjusted for multiple comparison using the Sidak correction.

A $p < 0.05$ 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

A total of 75 subjects were recruited: fifty vaccinated and twenty-five unvaccinated individuals. Patients of both groups had been managed at home by

their GPs and were prescribed with anti-inflammatory drugs (100%), antimicrobics (64% macrolides; 24% - lactams; 10% quinolones), and systemic steroids (92%) for a few days. The distribution of prescriptions was not significantly different in the two groups ($p=ns$). Nirmatrelvir/ritonavir had never been used in the patients recruited.

All vaccinated subjects received three doses of mRNA vaccines (two doses and one buster) over an average of 163 days ± 39 sd before the onset of their paucisymptomatic COVID syndrome. Vaccines used were Pfizer (55%) and Moderna (44%), respectively.

Baseline characteristics of subjects are reported in Table 1. At recruitment, the two groups were well matched for age, sex, BMI, Hb and prevalence of comorbidities. Moreover, the distribution of comorbidities was comparable in the two groups, such as: bronchial asthma (6); overweight (5), and blood hypertension (4) in the group of vaccinated subjects, while bronchial asthma (3); overweight (2), and blood hypertension (2) in the unvaccinated group, respectively. The mean dyspnea score was 0.3 (SD=0.5) in vaccinated subjects, while 1.1 (SD=0.8) in unvaccinated individuals ($p < 0.0001$). Moreover, mean SpO₂ was higher in vaccinated than in unvaccinated individuals: 97.6% (SD=0.8) and 96.8% (SD=1.4), respectively ($p < 0.0018$).

Mean values of spirometric parameters (namely, VC and FEV₁) were in the normal range in both groups and no statistical difference was found between groups (Table 2). Similarly, no difference was observed

Table 1. Baseline characteristics of patients.

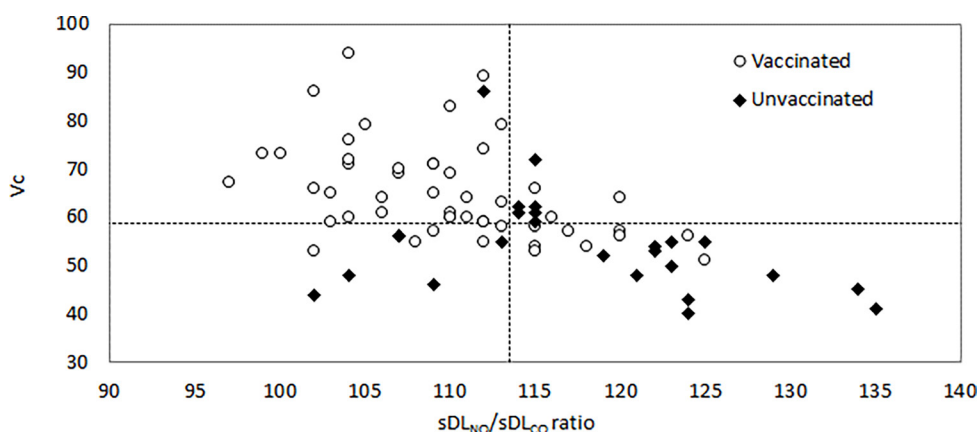
Parameters	Vaccinated	Unvaccinated	p
N	50	25	
Mean age (SD)	57.0 (14.5)	51.5 (11.5)	0.0580
Male (%)	28 (56.0%)	15 (60.0%)	0.4690
Mean BMI (SD)	25.0 (4.5)	26.3 (3.7)	0.2163
At least 1 comorbidity (%)	21 (42.0%)	6 (24.0%)	0.1000
Mean Hb (SD)	13.9 (0.3)	14.1 (0.3)	0.0571
Mean SpO ₂ (SD)	97.6 (0.8)	96.8 (1.4)	0.0018
Mean mMRC-DS (SD)	0.3 (0.5)	1.1 (0.8)	<0.0001

BMI: body mass index; Hb: blood hemoglobin; mMRC-DS: Modified British Medical Research Council dyspnea score; SD: standard deviation; SpO₂: saturation.

Table 2. Comparison of diffusive and spirometric parameters between vaccinated and non-vaccinated patients and statistical significance.

Parameters	Vaccinated	Unvaccinated	MD (95% CI)	AMD (95% CI)
FEV ₁	95.4 (14.4)	91.4 (14.0)	4.1 (-2.7 to 10.8), p=0.239	6.2 (-1.0 to 13.4), p=0.093
VC	102.6 (13.5)	98.4 (13.4)	4.2 (-2.2 to 10.7), p=0.195	6.5 (-0.2 to 13.3), p=0.058
DL _{CO}	93.7 (13.7)	90.9 (20.8)	2.8 (-5.0 to 10.6), p=0.480	2.8 (-6.0 to 11.6), p=0.536
sDL _{CO}	82.8 (11.9)	73.6 (10.5)	9.2 (4.0 to 14.5), p=0.001	10.5 (4.4 to 16.7), p=0.001
sDL _{NO}	90.7 (11.6)	82.6 (13.8)	8.1 (2.3 to 14.0), p=0.006	8.8 (2.2 to 15.4), p=0.009
sDL _{NO} /sDL _{CO} ratio	109.9 (6.3)	117.7 (8.6)	-7.8 (-11.3 to -4.3), p<0.001	-8.7 (-12.6 to -4.8), p<0.001
V _c	65.1 (9.9)	54.1 (10.1)	11.0 (6.5 to 15.6), p<0.001	12.0 (7.0 to 16.9), p<0.001

AMD: adjusted mean difference; DL_{CO}: current diffusing capacity for carbon monoxide; FEV₁: Forced Expiratory Volume in 1 sec; MD: mean difference; sDL_{CO}: single-breath diffusing capacity for carbon monoxide; sDL_{NO}: single-breath diffusing capacity for nitric oxide; VC: Vital Capacity; V_c: lung capillary blood volume.

**Figure 1.** Distributions of vaccinated and unvaccinated subjects according to optimal cut-off values for sDL_{NO}/sDL_{CO} ratio (113.5) and V_c (58.5) [see reference 20].

sDL_{CO}: single-breath diffusing capacity for carbon monoxide; sDL_{NO}: single-breath diffusing capacity for nitric oxide; V_c: lung capillary blood volume.

in the current DL_{CO} between vaccinated and unvaccinated (p < 0.536). Conversely, sDL_{CO}, sDL_{NO}, sDL_{NO}/sDL_{CO} and V_c were highly discriminant, with significant differences in favor of vaccinated subjects (ranging between p < 0.009 and p < 0.001) (Table 2). It should be also underlined that the distribution of sDL_{NO}/sDL_{CO} and V_c values below their optimal cut-off values for normality [19,20] appeared quite different between groups and dramatically in favor of vaccinated subjects (Figure 1 and Table 3). Specifically, about two third of vaccinated patients lie in the quadrant sDL_{NO}/sDL_{CO} ratio < 113.5 and V_c > 58.5, while almost half of the unvaccinated patients lie in the opposite quadrant (i.e., sDL_{NO}/sDL_{CO} ratio > 113.5 and V_c < 58.5).

The distribution of mean values for each lung function parameter collected are reported by mMRC-DS categories in Figure 2 and comparison among groups is detailed in Table 4.

The distribution of sDL_{CO}, sDL_{NO}/sDL_{CO} ratio and V_c seem to be strongly associated with mMRC-DS categories (ANOVA test p < 0.005). After adjusting for multiple comparison, distribution of V_c in patients with MRC-DS=2 is significantly different from the distribution in patients with both MRC-DS=0 and MRC-DS=1, and distribution of sDL_{CO} in patients with MRC-DS=2 is significantly different from the distribution in patients with MRC-DS=0 (Table 4).

Table 3. Distribution of subjects with sDL_{NO}/sDL_{CO} ratio and V_c values under and over optimal cut-off values for normality in both groups (see reference 20).

Vaccinated	ratio<113.5	ratio>113.5	Unvaccinated	V_c <58.5	V_c >58.5
V_c >58.5	66%	6%	ratio<113.5	4%	24%
V_c <58.5	10%	18%	ratio>113.5	24%	48%

sDL_{CO} : single-breath diffusing capacity for carbon monoxide; sDL_{NO} : single-breath diffusing capacity for nitric oxide; V_c : lung capillary blood volume.

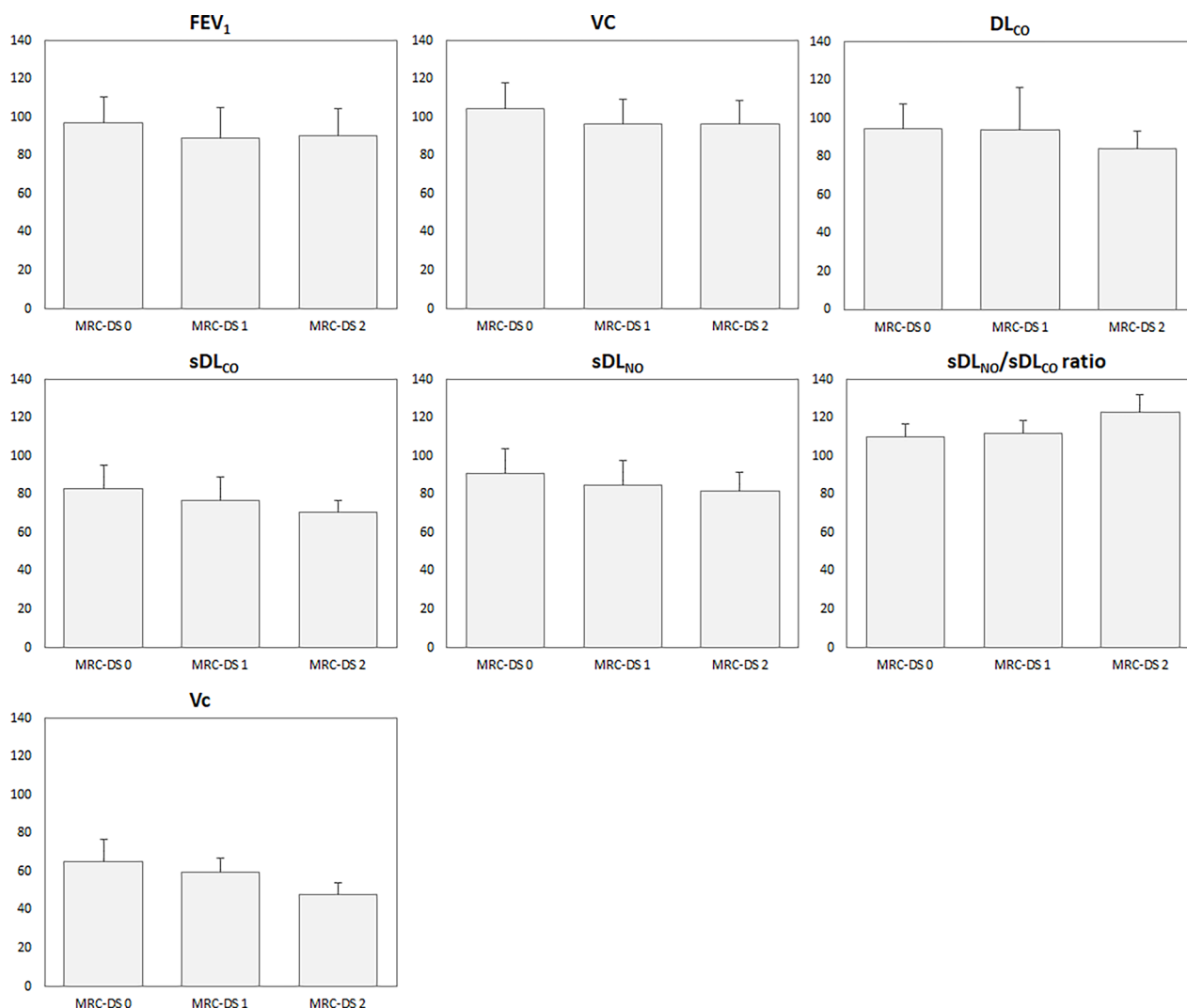


Figure 2. Mean value of diffusive and spirometric parameters according to MRC-DS (bars represent standard deviations).

DL_{CO}: current diffusing capacity for carbon monoxide; FEV₁: Forced Expiratory Volume in 1 sec; sDL_{CO}: single-breath diffusing capacity for carbon monoxide; sDL_{NO}: single-breath diffusing capacity for nitric oxide; VC: Vital Capacity; V_c: lung capillary blood volume.

Table 4. Comparison between diffusive and spirometric parameters among the MRC-DS groups: data expressed as mean (standard deviation). Comparisons among groups are expressed in terms of p adjusted for multiple comparison (Sidak correction).

Parameters	mMRC-DS 0	mMRC-DS 1	mMRC-DS 2	ANOVA test	mMRC-DS 1	mMRC-DS 2	mMRC-DS 2
					vs. 0	vs. 0	vs. 1
FEV ₁	97.3 (13.2)	89.4 (15.6)	90.7 (13.6)	p=0.0778	p=0.101	p=0.462	p=0.992
VC	104.6 (13.4)	96.7 (12.7)	96.4 (12.4)	p=0.0381	p=0.071	p=0.216	p=0.999
DL _{CO}	94.3 (13.4)	93.7 (22.3)	83.9 (9.2)	p=0.1819	p=0.998	p=0.195	p=0.309
sDL _{CO}	83.3 (11.8)	77.0 (12.4)	70.8 (6.3)	p=0.0048	p=0.113	p=0.008	p=0.409
sDL _{NO}	91.1 (12.7)	84.8 (12.9)	81.7 (10.2)	p=0.0427	p=0.166	p=0.105	p=0.890
sDL _{NO} /sDL _{CO} ratio	110.3 (6.4)	112.0 (6.9)	122.8 (9.4)	p<0.0001	p=0.749	p<0.001	p<0.001
V _c	65.5 (11.2)	59.8 (7.0)	47.7 (6.4)	p<0.0001	p=0.081	p<0.001	p=0.004

DL_{CO}: current diffusing capacity for carbon monoxide; FEV₁: Forced Expiratory Volume in 1 sec; mMRC-DS: Modified British Medical Research Council dyspnea score; sDL_{CO}: single-breath diffusing capacity for carbon monoxide; sDL_{NO}: single-breath diffusing capacity for nitric oxide; VC: Vital Capacity; V_c: lung capillary blood volume.

When compared to current DL_{CO}, the diffusive parameters obtained by the simultaneous single-breath method, such as the sDL_{CO}, sDL_{NO}, sDL_{NO}/sDL_{CO} ratio and V_c, confirmed their high discriminant power in identifying the occurrence of the lung microvascular involvement and the loss of the microvascular blood volume (Table 4).

Respiratory structures (and those of the deep lung in particular) are the first human targets of the corona virus aggression. It is then easily presumable that long-term respiratory consequences of variable severity may occur in these circumstances [1, 34, 35], thus contributing to the onset of the respiratory Long-COVID syndrome.

Discussion

Long-term pulmonary symptoms (mostly dyspnea for several weeks) had been reported in several patients after SARS-CoV-2 infections, though paucisymptomatic [34,35]. As the aggression of SARS-CoV-2 to the lung structures recognizes alveolar damage, pulmonary congestion, and diffuse microvascular thrombosis in particular, as the major lung injuries occurring [4,36-40], long-term respiratory consequences in gas transfer may frequently occur and can be expected in a wide range of COVID severity, the paucisymptomatic syndromes included [19-20], due to ventilation/perfusion mis-match, being long-lasting dyspnoea the most frequent clinical sign.

Unfortunately, as mentioned above, these persisting troubles in blood gas transfer cannot be fully identified neither by spirometric procedures nor by current DL_{CO} measure due to their low specificity and sensitivity [13,41-43]. In particular, due to the slow binding of CO with intracapillary Hb, current measures of DL_{CO} proved insufficient to discriminate disorders of diffusing membrane conductance (DM) from those involving the vascular side of alveolar/capillary membrane and then for investigating and defining the underlying cause of ventilatory/perfusion mis-match occurring in these cases [31,33,44-47].

The persistent reduction of lung capillary blood volume (V_c), such as the total volume of blood in the lung capillaries exposed to alveolar air, has been recently identified as the peculiar pathophysiological disorder that is able to characterize and grade the respiratory Long-COVID syndrome in subjects still complaining long-lasting dyspnea also following paucisymptomatic SARS-CoV-2 infections. In other words, the microangiopathy originally occurred in the deep lung and the consequent drop in pulmonary volume of capillary blood correspond to the major pathogenetic events sustaining the previously unexplained long-lasting abnormalities in gas transport (namely, dyspnea) in these cases, regardless their normal lung volumes.

The single-breath simultaneous assessment of sDL_{CO}, sDL_{NO}/sDL_{CO} ratio and V_c allowed to identify *in vivo*, non-invasively, in short time, at

low cost and with high sensitivity and specificity the persisting underlying impairment of pulmonary microvasculature also due to paucisymptomatic SARS-CoV-2 infections, otherwise undetectable and then neglected [19,20,45,46]. In other words, also mild SARS-CoV-2 infections can alter the integrity of the lung microvasculature and consequently to lead to the inadequate response to tissue metabolic demands in some patients (as indicated by the persistency of their dyspnea) [19,20]. This aspect is crucial because the capillary blood volume proves reduced within the deep lung likely due to microvascular destruction. As a consequence, the vascular side of the diffusive function can result substantially affected also after milder SARS-CoV-2 infections. This peculiar pathophysiological disorder can be used as a marker of SARS-CoV-2 injury of the lung and for checking the effect of possible preventive and/or therapeutic interventions. Data of the present investigation are further supported by the results of an elegant capillaroscopic study that described in various tissue samples the occurrence of a long-lasting reduction in vascular density and the persistent capillary rarefaction as the two peculiar features that characterize both the acute SARS-CoV-2 infection and the Long-COVID syndrome [48].

In the present pivotal investigation *in vivo*, the difference between vaccinated and unvaccinated individuals proved dramatically in favor of the former groups of subjects. In other words, the mean extent of microvascular blood loss and the prevalence of cases characterized by diffusive values frankly lower the optimal cut-off limits for normality proved quite lower in vaccinated subjects. Present data are clearly suggesting that the microvascular derangement occurring in the lung can be largely prevented by anti-COVID vaccinations, in particular by mRNA vaccines. On the other hand, two-dose regimens of the Moderna and Pfizer-BioNTech mRNA vaccines (such as those vaccine that give your cells instructions for how to make the S protein found on the surface of the COVID-19 virus) had been documented to be able in providing a good protection (of around 90%) against severe COVID-19 in real-life, both in terms of mortality and morbidity [49]. Moreover, mRNA vaccines highly contributed to protect the economies

worldwide and to implement public health measures according to innovative protocols of intervention.

Though the main goal of vaccines is to limit the spread of a pathogen within a population [50-52], the identification of a lung tissular target where their specific tissular protection could be assessed is of equal importance in our opinion, particularly when the parameters to use are easy to obtain, not time consuming and highly specific. This aspect assumes further importance when we consider that several factors can contribute to bias the general evidence of real efficacy and effectiveness of vaccination (namely, patients' different immune conditions and response; technical aspects; social and political issues, etc.). On the other hand, an autopsy-based analysis documented the importance of vaccine-induced immunity in protecting from the effects of the inflammatory viral-induced aggression, thus supporting the importance and the efficacy of the vaccination against SARS-CoV-2 pulmonary and cardiac injury [53]. Unfortunately, poor evidence is still available for *in vivo* specific tissular responses to anti-COVID-19 vaccinations in humans.

The present investigation is providing the first *in vivo* evidence to our best knowledge concerning the efficacy of mRNA vaccines in preserving specific biological structures of the deep lung. This recent evidence can represent a quite relevant human model for investigating and quantifying in short time and non-invasively the *in vivo* efficacy of anti-COVID vaccines in preserving from those long-lasting microvascular injuries and hidden alveolar-perfusion abnormalities that underly SARS-CoV-2 infections, though of "apparent" mild clinical severity.

The present study recognizes some points of weakness: it consists of a monocentric investigation and the sample size is obviously limited. Moreover, only two mRNA vaccines were used because only those two were provided by our Public Health Institutions over the study period.

On the other hand, points of strength are: the strict selection of subject investigated; the pivotal method for the non-invasive lung function measurements; the identification of a specific lung tissular disorder (such as, of lung microvasculature) for testing the protection power of mRNA, never investigated before.

Conclusions

Paucisymptomatic SARS-CoV-2 infections can cause long-lasting troubles in gas transfer and consequent long-lasting dyspnea in more than 40% of unvaccinated individuals.

The pathogenesis of these respiratory disorders proved mainly related to the reduction of lung microvascular blood volume occurring in the deep lung. These features, previously unknown and undetected, can now be easily identified by means of the single-breath simultaneous assessment of sDL_{CO} , sDL_{NO}/sDL_{CO} ratio and V_c with high sensitivity and specificity, being the lung microvascular injury, the main feature underlying.

The main and unprecedented message emerging from the present study is that mRNA vaccines provide high protection of the deep lung from the long-lasting loss of lung microvascular blood volume induced by SARS-CoV-2 infections, though paucisymptomatic.

The study protocol was basically based on the Roughton & Forster equation that underlies the simultaneous single-breath assessment of DL_{NO} and DL_{CO} . Even if small discrepancies vs conventional DL_{CO} measurements are possible due to some methodological differences (mainly to different gas sampling and breath-holding time, virtually slightly affecting the values for alveolar volume), the simultaneous single-breath assessment of DL_{NO} and DL_{CO} has been recognized as able to provide different physiological information that provide a better understanding of lung involvement in respiratory diseases [54].

Even if further studies are needed, mRNA vaccines provide high protection from the long-lasting loss of lung microvascular blood volume induced by paucisymptomatic SARS-CoV-2 infections.

The availability of this human *in vivo* lung model for assessing and comparing non-invasively the protection of mRNA vaccines on deep lung structures assumes high relevance in our opinion because the morbidity of SARS-CoV-2 infections is still high in the general population and specific mRNA vaccinations can easily prevent their persisting lung tissue consequences and minimize their public health burden.

Abbreviations

BMI: body mass index
 Hb: hemoglobin
 SpO₂ %: % O₂ saturation
 mMRC-DS: modified British Medical Research Council dyspnea score
 VC: vital capacity
 FEV₁: forced expiratory volume in 1 second
 DL_{CO}: current measure of diffusion capacity for carbon oxide
 sDL_{CO}: non-invasive simultaneous single-breath measurements of diffusion for carbon oxide
 sDL_{NO}: the non-invasive simultaneous single-breath measurements of nitric oxide
 sDL_{NO}/sDL_{CO} ratio: the ratio between sDL_{NO}/sDL_{CO} values
 V_c: the lung capillary blood volume
 mRNA vaccines: messenger RNA vaccines

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