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Nebivolol: an effective option against long-lasting dyspnoea following COVID-19 pneumonia - a pivotal double-blind, cross-over controlled study

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ABSTRACT

Background: Pulmonary microvascular occlusions can aggravate SARS-CoV-2 pneumonia and result in a variable decrease in capillary blood volume (Vc). Dyspnoea may persist for several weeks after hospital discharge in many patients who have "radiologically recovered" from COVID-19 pneumonia. Dyspnoea is frequently "unexplained" in these cases because abnormalities in lung vasculature are understudied. Furthermore, even when they are identified, therapeutic options are still lacking in clinical practice, with nitric oxide (NO) supplementation being used only for severe respiratory failure in the hospital setting. Nebivolol is the only selective β_1 adrenoceptor antagonist capable of inducing nitric oxide-mediated vasodilation by stimulating endothelial NO synthase *via* β_3 agonism. The purpose of this study was to compare the effect of nebivolol *versus* placebo in patients who had low Vc and complained of dyspnoea for several weeks after COVID-19 pneumonia.

Methods: Patients of both genders, aged ≥18 years, non-smokers, who had a CT scan that revealed no COVID-related parenchymal lesions but still complaining of dyspnoea 12-16 weeks after hospital discharge, were recruited. Spirometrical volumes, blood haemoglobin, SpO_2, simultaneous diffusing capacity for carbon monoxide (CO) and NO (DL_{CO} and DL_{NO}, respectively), DL_{NO}/DL_{CO} ratio, Vc and exhaled NO (eNO) were measured together with their dyspnoea score (DS), heart frequency (HF), and blood arterial pressure (BAP). Data were collected before and one week after both placebo (P) and nebivolol (N) (2.5 mg od) double-blind cross-over administered at a two-week interval. Data were statistically compared, and $p<0.05$ assumed as statistically significant.

Results: Eight patients (3 males) were investigated. In baseline, their mean DS was 2.5±0.6 SD, despite the normality of lung volumes. DL_{CO} and DL_{NO} mean values were lower than predicted, while mean DL_{NO}/DL_{CO} ratio was higher. Mean Vc proved substantially reduced. Placebo did not modify any variable (all p=ns) while N improved DLco and Vc significantly (+8.5%, $p<0.04$ and +17.7%, $p<0.003$, respectively). eNO also was significantly increased (+17.6%, $p<0.002$). Only N lowered the dyspnoea score (-76%, $p<0.001$). Systolic and diastolic BAP were slightly lowered (-7.5%, $p<0.02$ and -5.1%, $p<0.04$, respectively), together with HF (-16.8%, $p<0.03$).

Conclusions: The simultaneous assessment of DL_{NO}, DL_{CO}, DL_{NO}/DL_{CO} ratio, and Vc confirmed that long-lasting dyspnoea is related to hidden abnormalities in the lung capillary vasculature. These abnormalities can persist even after the complete resolution of parenchymal lesions regardless of the normality of lung volumes. Nebivolol, but not placebo, improves DS and Vc significantly. The mechanism suggested is the NO-mediated vasodilation *via* the β3 adrenoceptor stimulation of endothelial NO synthase. This hypothesis is supported by the substantial increase of eNO only assessed after nebivolol. As the nebivolol tolerability in these post-COVID normotensive patients was very good, the therapeutic use of nebivolol against residual and symptomatic signs of long-COVID can be suggested in out-patients.

Key words: Nebivolol; COVID-19; vascular effects; lung perfusion; capillary blood volume (Vc); simultaneous DL_{CO} and DL_{NO} assessment.

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

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Introduction

The clinical impact of SARS-CoV-2 infection widely ranges from mild involvement of upper airways to severe interstitial pneumonia and hypoxemic respiratory failure, not infrequently fatal [1-7]. The crucial pathogenetic events occurring in the lung are high local concentration of cytokines, chemokines and IgM-mediated immunocomplexes that induce a tremendous recruitment of inflammatory cells [2,8-9]; diffuse damage at alveolar level [10]; microvascular thrombosis and capillary occlusion at variable extent [11-13]; activation of platelets and tissue factors further causing coagulation and micro-thrombosis [2].

Though variably mixed and mostly occurring in the acute phase of pulmonary infection, these tissular changes can partially persist over of the following healing and recovering phase, and can contribute to substantial alterations in alveolar-blood gas exchange, being dyspnoea the mayor clinical sign reported [14]. Dyspnoea is in fact complained by a considerable proportion of patients for several weeks after their ‘’apparent recovery” from COVID-19 pneumonia. Unfortunately, though dyspnoea can affect daily activities persistently in these subjects, its underlying causes still are poorly investigated in clinical practice [15]. Usually, in clinics, after the exclusion of any cardiac or psychologic cause (the two causes most frequently suggested), respiratory investigations are mostly limited to the assessment of spirometrical lung volumes and of diffusing capacity for carbon monoxide (DL_{CO}) in a smaller proportion of cases [16-19].

However, lung volumes are of limited value in the majority of cases, while the current assessment of DL_{CO} is unable to discriminate abnormalities occurring at the alveolar level (i.e., the membrane diffusing conductance - DM) from those attaining the vascular side of the blood gas exchange (i.e., such as the pulmonary capillary blood volume - Vc) [20-23]. In fact, due to the much faster binding of NO with intracapillary haemoglobin (Hb), also the assessment of diffusing capacity for carbon monoxide (DL_{NO}) was recommended in these cases [24-26] even in patients with minimal or no abnormalities in their chest computed tomography (CT) [23]. Recently, the simultaneous assessment of DL_{CO} and DL_{NO} proved suitable and reliable for investigating the underlying causes of long-lasting dyspnoea in out-patients defined “recovered” from COVID-19 pneumonia, being the reduction of capillary blood flow assessed in these cases strictly related to their dyspnoea score (DS), regardless of their normal lung volumes [27].

Unfortunately, once these abnormalities documented, specific therapeutic options are still missing to date in these cases. Currently, inhaled NO supplementation is only used in hospital setting in the aim to induce a strong vasodilation at pulmonary level in most severe cases of respiratory failure [28-29]. Nebivolol is the only selective β_1 adrenoceptor antagonist capable of inducing nitric oxide-mediated vasodilation by stimulating endothelial nitric oxide synthase *via* β_3 agonism [30-33].

The aim of the present study was to investigate *vs* placebo the effect of nebivolol *vs* placebo in affecting the long-lasting pulmonary blood volume reduction assessed in patients “radiologically recovered” from COVID-19 pneumonia, but still complaining dyspnoea for several weeks.

Methods

Out-patients aged ≥18 years previously defined “recovered” for COVID-19 pneumonia, but still complaining dyspnoea for 12-16 weeks after discharge were investigated between 1st September 2021 and 15th March 2022, after their informed consent. All patients suffered from COVID pneumonia originally affecting ≥50% of their lung volume (CT documented) and during their hospitalization they received high flow oxygen. At recruitment, patients had to provide a CT scan performed in the previous two weeks and showing the absence of any residual COVID-related parenchymal lesions.

Exclusion criteria were the refusal of the informed consent; subjects aged <18 years; current and former-smokers; subjects with main comorbidities affecting lung diffusion (i.e., blood Hb <12 g/L; heart failure; lung fibrosis; vasculitis; COPD; diabetes; renal and liver failure); persisting COVID-related parenchymal lesions; physical and/or cognitive limitations enabling lung function measurements and other procedures of the study.

Clinical and lung function variables collected in each patient before and after both treatments were:

- age (in years);
- gender;
- BMI;
- Hb (blood haemoglobin, in g/L);
- SpO_2 (O_2 saturation, in %);
- VC (vital capacity) and FEV_1 (forced expiratory volume in 1 sec); both reported as % predicted);
- DL_{CO} (diffusing capacity for carbon oxide; in % predicted);
- DL_{NO} (diffusion capacity for nitric oxide; in % predicted);
- DL_{NO}/DL_{CO} ratio (in % predicted);
- Vc (capillary blood volume; in % predicted);
- eNO (exhaled NO, in ppm);
- DS (dyspnoea score);
- dyspnoea duration after discharge (in weeks)
- systolic blood pressure (S-BP, in mmHg)
- diastolic blood pressure (D-BP, in mmHg)
- heart frequency (HF, in beats/min)

A Platinum DX Elite Plethysmography (MedGraphics, Saint Paul, MN, USA) was used for assessing spirometrical volumes. DL_{CO}, DL_{NO}, DL_{NO}/DL_{CO}, Vc, and eNO were obtained by means of the “Stand-Alone” Hypair Compact System (MGC Diagnostics International, Sorinnes, Belgium). This equipment consents the simultaneous assessment of DL_{CO} and DL_{NO} during the usual single breath manoeuvres [26,34]. According to standard procedures, measure of DL_{CO} and DL_{NO} required breath-hold times of 10 and 5 sec, respectively [20-23,27].

The dyspnoea duration after discharge was measured in weeks. Current dyspnoea was graded in each patient by means of the modified British Medical Research Council (mMRC) dyspnoea score [35].

Study design

This was a double-blind cross-over study. All lung function parameters, together with the DS, HF, S-BP, D-BP were collected before and after nebivolol 2.5 mg od, and before and after undistinguishable placebo, both randomly administered for one week, with a two-week interval in between.

Statistics

Continuous data were presented as means and standard deviation (SD), while gender as absolute frequency. Differences in all variables were tested by *t*-test for continuous data and $p<0.05$ was accepted for statistical significance.

All statistical calculations were carried out by means of STATA (StataCorp. 2017. Stata Statistical Software: Release 15. StataCorp LLC., College Station, TX, USA), $p<0.05$ was assumed as the limit of statistical significance.

Ethics statement

At recruitment, all subjects gave their informed consent; their consent for the anonymous use of their own data for research pur-

poses was also included. The study was approved by the Ethical and Scientific Commission of the National Centre for Respiratory Pharmacoeconomics and Pharmacoepidemiology during the session held on May 2nd, 2021.

Results

A total of eight out-patients were investigated. No significant comorbidities were recorded. Patients' general characteristics assessed in baseline are reported in Table 1 together with mean values for their blood Hb, lung volumes, S-BP, D-BP and HF. Mean dyspnoea duration and mean current DS score are also reported in the same table. In baseline, all parameters were in their normal range, except HF that was high when compared to usual resting conditions. All patients were showing a higher DS that despite the absolute normality of their lung volumes.

DL_{CO} and DL_{NO} mean values were lower than predicted in baseline, while mean DL_{NO}/DL_{CO} ratio was slightly higher. Mean Vc proved highly lowered than predicted, while the mean eNO value was at the lower limit of the normal range.

Table 2 reports mean values ± SD for each variable of lung diffusion measured before and after placebo (P), and before and after nebivolol (N), together with the corresponding statistical comparisons and significancy. N, but not P, improved significantly DL_{CO} by 8.5 ($p<0.04$) and and Vc by 17.7% ($p<0.003$), respectively, while the DL_{NO}/DL_{CO} ratio was lowered even if the variation did not reach the statistical significancy. To emphasize that eNO was significantly increased by 17.6% ($p<0.002$), thus confirming the ability of N to increase the NO expression at pulmonary vascular level in these cases.

Changes observed in DS, S-BP, D-BP, and HF with both treatments are reported in Table 3. Only N minimized DS by 76% from the corresponding mean basal value ($p<0.001$), while P was completely ineffective from this point of view (p=ns).

Finally, S-BP and D-BP were significantly lowered only after N by 7.5% ($p<0.02$) and 5.1% ($p<0.04$), respectively, while mean HF was lowered by 16.8% ($p<0.03$). Mean changes obtained in S-BP and D-BPs were mild, while those in HF were more pronounced. However, these changes, likely also related to the nebivolol β_1 adrenoceptor antagonism, had been perfectly tolerated by all patients.

Discussion

Further to alveolar damage, pulmonary microvascular thrombosis and occlusions (such as: lesions to the capillary endothelium; angiogenesis within the inter-alveolar septa; capillary microthrombi) represent the main pathogenetic events complicating the SARS-CoV-2 infection at variable extent and duration.

These events can lead to reduction of Vc in the lung [2,10-13] and frequently contribute to the occurrence of persisting alterations

Table 1. General characteristics of the sample at recruitment. Data are reported as means ± SD while comorbidities as relative frequency.

n	8
Males/females	3/5
Age (y)	50.5 ± 17.2
BMI	24.4 ± 2.8
Hb (g/L)	13.9±0.4
SpO_2 (%)	96.8±1.1
VC (% pred.)	95.7±11.1
FEV_1 (% pred.	93.2±10.6
Systolic BAP (mmHg)	132.3±4.6
Diastolic BAP (mmHg)	77.3±3.4
HF (b/min)	96.4±8.1
Dyspnoea duration after hospital discharge (weeks)	13.2±2.7
Dyspnoea score	2.5±0.6
DL_{CO} (% pred.)	72.1±14.7
DL_{NO} (% pred.)	73.1±15.5
DL_{NO}/DL_{CO} (% pred.)	121.6±3.6
Vc (% pred.)	45.0±7.9
eNO (ppm)	5.2±0.6

Table 2. Mean values ± SD for each variable of lung diffusion measured before and after placebo (P), and before and after nebivolol (N), with corresponding significance of statistical comparisons.

	Pre-P	Post-P	p	Pre-N	Post-N	p
DL_{CO} (% pred.)	72.1±14.7	72.5±17.0	ns	70.9±13.7	76.0±14.5	0.04
DL_{NO} (% pred.)	73.1±15.5	73.4±13.5	ns	74.2±15.5	73.2±14.8	0.75
DL_{NO}/DL_{CO} (% pred.)	121.6±3.6	123.2±4.5	ns	120.6±7.8	117.5±6.3	0.31
Vc (% pred.)	45.0±7.9	44.4±8.7	ns	44.1±8.6	51.9±9.0	0.003
eNO (ppm)	5.2±0.6	5.0±0.5	ns	5.1±0.6	6.0±0.9	0.002

Table 3. Mean values ± SD for dyspnoea score, systolic BAP, diastolic BAP and HF measured before and after placebo (P), and before and after nebivolol (N), with corresponding significance of statistical comparisons.

	Pre-P	Post-P	p	Pre-N	Post-N	p
Dyspnoea score	2.5±0.6	2.6±0.4	ns	2.5±0.8	0.6±0.3	0.001
Systolic BAP (mmHg)	132.3±4.6	134.3±5.4	ns	134.6±5.2	124.2±6.7	0.02
Diastolic BAP (mmHg)	77.3±3.4	78.4±4.1	ns	78.6±3.9	74.7±3.0	0.04
HF (b/min)	96.4±8.1	97.1±9.2	ns	95.7±7.7	78.6±8.9	0.03

in alveolar-blood gas exchange [14] and dyspnoea, both long-lasting even beyond the healing and recovering phase of COVID-19 pneumonia.

From a general point of view, two are the critical issues in these cases: first, the assessment of these disorders, and second, their therapeutic approach. Their assessment is difficult indeed in clinical practice due to technological and methodological limits. Spirometrical measurements (such as: lung volumes) are unable to identify these disorders while the sole measure of DL_{CO} is insufficient to recognize these abnormalities specifically [16-23]. As mentioned above, also the DL_{NO} assessment is recommended in these cases [23-26]. Unfortunately, specific dedicated technologies are not currently available in clinical practice and require high specialist skills, longer time and higher costs.

The recent technological opportunity that allows the simultaneous assessment of DL_{CO} and DL_{NO} represents a novel, easier, and reliable methodological approach for measuring and discriminating the damage occurred at the alveolar level from disorders occurred at the vascular side of the alveolar membrane, included the quantification of the pulmonary capillary blood volume [24-26]. This method proved particularly suitable for investigating also in clinical practice the hidden alveolar and capillary damage due to COVID-19 pneumonia together with the so-called "unexplained" causes of long-lasting dyspnoea, such as the mayor symptom complained by these patients [15,27]. This method was adopted in a recent study aimed to investigate from this point of view a selected sample of patients defined "radiologically recovered" from COVID-19 pneumonia and with no residual CT pulmonary abnormalities even if still complaining significant dyspnoea and tachycardia for more than 12 weeks from their hospital discharge. Regardless of their normal lung volumes, a substantial reduction of pulmonary capillary blood volume was documented in these cases, this limitation resulting strictly related to the current patients' DS [27].

As mentioned above, once identified the hidden damage, a further crucial point is to be faced: such as, the problem of the therapeutic approach aimed to improving the pulmonary capillary blood volume and the persistent dyspnoea in these cases. At present, specific pharmacologic opportunities are practically missing for outpatients as NO supplementation via inhalation is in fact only limited to most severe cases of respiratory failure to be managed in hospital setting and aimed to stimulate a strong vasodilation at pulmonary level both in adults and in children [28,29].

However, the increasing awareness of the essential role of NO in different physiological processes stimulated multiple pharmacological strategies for different diseases [36-39]. Further to old NO donors (such as sodium nitroprusside, nitroglycerin and isosorbide dinitrate), new molecules were developed in the last decades, in particular newer vasodilating β-blockers that increase NO bioavailability substantially [40,41]. Moreover, the interest on NO pulmonary effects was further expanded due to the NO ability to attenuate the effects of the platelet activating factor, that is a further pathogenetic determinant of capillary obstruction [29], particularly in COVID patients [2].

The original hypothesis of the present study was the assumption that nebivolol, due to its peculiar mechanism of action, would provide an interesting opportunity for intervention against the COVID-induced alterations of lung capillary bed and related persisting dyspnoea, otherwise therapeutically "orphan". On the other hand, nebivolol is the only selective β_1 adrenoceptor antagonist that is capable to induce nitric oxide-mediated vasodilation by stimulating endothelial nitric oxide synthase *via* β_3 agonism [30-33] and endothelium-dependent vasodilation mediated *via* the L-arginine/NO pathway [41,42]. The vasodilatory mechanism of action strongly differentiates nebivolol from all other vasodilatory β-blockers (such as labetalol and carvedilol) that act via α1-receptor antagonism [32,33].

It was also documented that nebivolol is capable to provide anti-thrombotic, anti-platelet and anti-aggregation activity associated to its enhanced NO bioactivity [43]. In particular, while endothelial-derived NO acts as a major vasodilator, cyclic guanosine monophosphate (cGMP) and protein kinase (PKG), that are its downstream effectors, are also provided with peculiar vasodilatative, anti-proliferative, anti-coagulant, and anti-inflammatory effects on pulmonary vasculature [44].

Data from the present study showed for the first time at our best knowledge that nebivolol, but not placebo, affects the hidden abnormalities in pulmonary capillary vasculature induced by COVID-19 pneumonia substantially and significantly. In other words, nebivolol proved effective in increasing the patients' pulmonary capillary blood volume persistently reduced by COVID-19 infection. This therapeutic effect, assessed experimentally after a low dose (2.5 mg od) administered for a short period (one week) empirically decided, is supported by the basic pharmacology of nebivolol and confirms the original hypothesis of the study. In particular, the significant increase of the exhaled NO release at pulmonary level (eNO) assessed after nebivolol further confirms the therapeutic mechanism of action of this molecule at pulmonary level. On the other hand, it was showed that β_3-adrenergic receptor agonists produce a significant reduction in pulmonary vascular resistance in experimental studies on pulmonary hypertension (PH), thus emerging as an innovative potential approach for managing PH patients [45].

Moreover, it should be emphasized that the efficacy of nebivolol on pulmonary capillary blood volume proved strictly correlated to the drop in patients' dyspnoea score. This outcome further confirms the relationship existing between the documented pulmonary capillary alterations and the persistence of dyspnoea, even in the absence of lung volume limitations in this kind of patients.

The present study has some limitations: i) the small sample of patients investigated; ii) the dose of nebivolol and the duration of treatment, both empirically decided; iii) the lack of a documented dose-dependent effect of nebivolol.

Point of strengths are: i) the strict selection of patients investigated aimed to avoid any clinical confounding factor; ii) the adoption of the simultaneous assessment of DL_{CO} and DL_{NO} that presently represents the most appropriate method for assessing and discriminating the pathologic damage occurring at the alveolar and at the vascular pulmonary level; iii) the very first application of this investigational method in clinical pharmacology; iv) the excellent correspondence between the pharmacology of nebivolol and results obtained.

Conclusions

Patients "radiologically recovered" from COVID-19-induced pneumonia can be frequently characterized by persistent reduction of pulmonary capillary blood volume and long-lasting dyspnoea as the major clinical sign. To note that these abnormalities would be unexplained and neglected in clinical practice unless investigated by means of a proper methodological approach, such as the simultaneous assessment of both the DL_{CO} and DL_{NO}.

Due to its peculiar mechanism of action, nebivolol 2.5 mg od proved effective in increasing pulmonary capillary blood volume and the corresponding dyspnoea substantially, both persisting for several weeks after hospital discharge in patients previously

defined "recovered" from COVID-19 pneumonia. Nebivolol seems to provide a novel and effective option against these pulmonary abnormalities in clinical practice, even if still off-label. Finally, nebivolol was well tolerated in all patients investigated though normo-tensive.

The multifaceted pharmacological action of nebivolol (such as, the vasodilatative, anti-coagulant, anti-inflammatory and antioxidant activities) are regarded as likely contributing to minimize the abnormalities in lung capillary volume that can frequently persist in out-patients after COVID-19 pneumonia.

Further studies are needed for confirming the present pivotal results.

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References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
2. Matricardi PM, Dal Negro RW, Nisini R. The first, holistic immunological model of COVID-19: implications for prevention, diagnosis, and public health measures. Pediatr Allergy Immunol 2020;31:454-70.
3. Gorbalenya AE, Baker SC, Baric RS, de Groot RJ, Drosten C, Gulyaeva AA, et al. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 2020;5:536-44.
4. Woelfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Mueller MA, et al. Clinical presentation and virological assessment of hospitalized cases of coronavirus disease 2019. medRxiv 2020.03.05.20030502.
5. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
6. Guan W, Ni Z, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382:1708-20.
7. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
8. Okba NMA, Müller MA, Li W, Wang C, Geurtsvan Kessel CH, Corman VM, et al. Severe acute respiratory syndrome coronavirus 2−specific antibody responses in coronavirus disease patients. Emerg Infect Dis 2020;26:1478-88.
9. El Karoui K, Hill GS, Karras A, Jacquot C, Moulonguet L, Kourilsky O, et al. A clinicopathologic study of thrombotic microangiopathy in IgA nephropathy. J Am Soc Nephrol 2012;23:137-48.
10. Tian S, Xiong Y, Liu H, Niu L, Guo J, Liao M, et al. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. Med Pathol 2020;33:1007-14.
11. Orwoll BE, Spicer AC, Zinter MS, Alkhouli MF, Khemani RG, Flori HR, et al. Elevated soluble thrombomodulin is associated with organ failure and mortality in children with acute respiratory distress syndrome (ARDS): a prospective observational cohort study. Crit Care 2015;19:435.
12. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 2020;18:844-7.
13. Wang J, Hajizadeh N, Moore EE, McIntyre RC, Moore PK, Veress LA, et al. Tissue plasminogen activator (tPA) treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): A case series. J Thromb Haemost 2020;18:1752-5.
14. Hughes JMB, Pride NB. Examination of the carbon monoxide diffusing capacity (DLCO) in relation to its KCO and VA components. Am J Respr. Crit Care Med 2012;186:132–9.
15. Wu X, Lin X, Zhou Y, Yu H, Li R, Zhan Q, et al. 3-month, 6-month. 9-month. And 12-month respiratory outcomes in patients following COVID-19 related hospitalization: a prospective study. Lancet Respir Med 2021;9:747-54.
16. Frija-Masson J, Debray M-P, Gilbert M, Lescure FX, Travert F, Borie R, et al. Functional characteristics of patients with SARS-CoV- 2 pneumonia at 30 days post-infection. Eur Resp J 2020;56:2001754.
17. Mo X, Jian W, Su Z, Chen MU, Peng H, Peng P, et al. Abnormal pulmonary function in COVID-19 patients at time of hospital discharge. Eur Respir J 2020;55:2001217.
18. Huang Y, Tan C, Wu J, Chen M, Wang Z, Luo L, et al. Impact of coronavirus disease 2019 on pulmonary function in early convalescence phase. Respir Res 2020;1:163.
19. van den Borst B, Peters JB, Brink M, Schoon Y, Bleeker-Rovers CP, Schers H, et al. Comprehensive health assessment three months after recovery from acute COVID-19. Clin Infect Dis 2021;73:e1089-98.
20. Guenard H, Varene N, Vaida P. Determination of lung capillary blood volume and membrane diffusing capacity by measurement of NO and CO transfer. Resp Physiol 1987;70,113–20.
21. Borland CDR, Hughes JMB. Lung diffusing capacities (DL) for nitric oxide (NO) and carbon monoxide (CO): The evolving story. Compr Physiol 2019;10:73–97.
22. Gibson QH, Roughton FJW. The kinetics and equilibria of the reactions of nitric oxide with sheep haemoglobin. J Physiol 1957;136:507-24.
23. Barisone G, Brusasco V. Lung diffusing capacity for nitric oxide and carbon monoxide following mild-to-severe COVID-19. Physiol Rep2021;9:e14748.
24. Borland CD, Dunningham H, Bottrill F, Vuylsteke A, Yilmaz C, Dane DM, et al. Significant blood resistance to nitric oxide transfer in the lung. J Appl Physiol 1985;108:1052-60.
25. Zavorsky GS, Hsia CCW, Hughes JMB, Borland CDR, Guénard H, van der Lee I, et al. Standardisation and application of the single-breath determination of nitric oxide uptake in the lung. Eur Respir J 2017;49:1600962.
26. Roughton FJ, Forster RE. Relative importance of diffusion and chemical reaction in determining rate of exchange of gases in the human lung. J Appl Physiol 1957;11:290-302.
27. Dal Negro RW, Turco P, Povero M. Long-lasting dyspnoea in patients otherwise clinically and radiologically recovered from COVID pneumonia: a probe for checking persisting disorders in capillary lung volume as a cause. Multidiscip Respir Med 2022;17:875.
28. Rossaint R, Gerlach H, Schmidt-Ruhnke H, Pappert D, Lewandowski K, Steudel W, et al. – Efficacy of inhaled nitric oxide in patients with severe ARDS. Chest 1995;107:1107-15.
29. Ichinose F, Roberts JD, Zapol WM. Inhaled nitric oxide: a selective pulmonary vasodilator: currents uses and therapeutic potential. Circulation 2004;109:3106-11.
30. Bristow M, Nelson P, Minobe W, Johnson C. Characterization of β1-adrenergic receptor selectivity of nebivolol and various

other beta-blockers in human myocardium. Am J Hypertens 2005;18;51A-52A.
31. Pedersen ME, Cockroft J. The vasodilatory beta-blockers. Curr Hypertens Rep 2007;9:269-77.
32. Munzel T, Gori T. Nebivolol: the somewhat different beta-adrenergic receptor blocker. J Am Coll Cardiol 2009;54:1491-9.
33. Fongemie J, Felix-Getzik E. A review of nebivolol pharmacology and clinical evidence. Drugs 2015;75:1349-71.
34. Graham BL, Brusasco V, Burgos F, Cooper BG, Jensen R, Kendrick A, et al. ERS/ATS standards for single-breath carbon monoxide uptake in the lung. Eur Respir J 2017;49:1600016.
35. Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnoea. Chest 1988;93:580-6.
36. Ignarro IJ, Napoli C, Loscalzo J. Nitric oxide donors and cardiovascular agents modulating the bioactivity of nitric oxide: an overview. Circ Res 2002;90:21-8.
37. Keeble JE, Moore PK. Pharmacology and potential therapeutic applications of nitric oxide-releasing non-steroideal anti-inflammatory and related nitric oxide-donating drugs. Br J Pharmacol 2002;137:295-310.
38. Scatena R, Bottoni P, Martorana GE, Giardina B. Nitric oxide donor drugs: an update on pathophysiology and therapeutic potential. Expert Opin Investig Drugs 2005;14:835-46.
39. Mason RP, Cockroft JR. Targeting nitric oxide with drug therapy. J Clin Hypert 2006;8:40-52.
40. Kalinowski I, Dobrocki LW, Szczepanska-Konkel M, Konkel M, Jankowski M, Martyniec L, et al. Third-generation β-blockers stimulate nitric oxide release from endothelial cells through ATP efflux: a novel mechanism for antihypertensive action. Circulation 2003;107:2747-514.
41. Tzemos N, Lim PO, MacDonald TM. Nebivolol reverses endothelial dysfunction in essential hypertension: a randomized, double-blind, cross-over study. Circulation 2001;104:511-14.
42. Ritter JM. Nebivolol: endothelium-mediasted vasodilating effect. J Cardiovasc Pharmacol 2001;38:s13-6.
43. Mollnau H, Schulz E, Daiber A, Baldus S, Oelze M, August M, et al. Nebivolol prevents vascular NOS III uncoupling in experimental hyperlipidemia and inhibits NADPH oxidase activity in inflammatory cells. Arterioscler Thromb Vasc Biol 2003;23:615-21.
44. Tang H, Vanderpool RR, Wang J, Yuan JX. Targeting L-arginine-nitric oxide-cGMP pathway in pulmonary arterial hypertension. Pulm Circul 2017;7:569-71.
45. Garcia-Alvarez A, Pereda D, Garcia-Lunar I, Sanz-Rosa A, Fernandez-Jimenez R, Garcia-Prito J, et al. Beta-3 adrenergic agonists reduce pulmonary vascular resistance and improve right ventricular performance in a porcine model of chronic pulmonary hypertension. Basic Res Cardiol 2016;111:49.

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