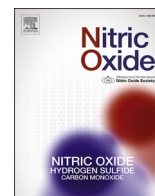




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## Regular article

## Inhaled high dose nitric oxide is a safe and effective respiratory treatment in spontaneous breathing hospitalized patients with COVID-19 pneumonia



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## ABSTRACT

**Background:** Inhaled nitric oxide (NO) is a selective pulmonary vasodilator. *In-vitro* studies report that NO donors can inhibit replication of SARS-CoV-2. This multicenter study evaluated the feasibility and effects of high-dose inhaled NO in non-intubated spontaneously breathing patients with Coronavirus disease-2019 (COVID-19).

**Methods:** This is an interventional study to determine whether NO at 160 parts-per-million (ppm) inhaled for 30 min twice daily might be beneficial and safe in non-intubated COVID-19 patients.

**Results:** Twenty-nine COVID-19 patients received a total of 217 intermittent inhaled NO treatments for 30 min at 160 ppm between March and June 2020. Breathing NO acutely decreased the respiratory rate of tachypneic patients and improved oxygenation in hypoxemic patients. The maximum level of nitrogen dioxide delivered was 1.5 ppm. The maximum level of methemoglobin (MetHb) during the treatments was 4.7%. MetHb decreased in all patients 5 min after discontinuing NO administration. No adverse events during treatment, such as hypoxemia, hypotension, or acute kidney injury during hospitalization occurred.

In our NO treated patients, one patient of 29 underwent intubation and mechanical ventilation, and none died. The median hospital length of stay was 6 days [interquartile range 4–8]. No discharged patients required hospital readmission nor developed COVID-19 related long-term sequelae within 28 days of follow-up.

**Conclusions:** In spontaneous breathing patients with COVID-19, the administration of inhaled NO at 160 ppm for 30 min twice daily promptly improved the respiratory rate of tachypneic patients and systemic oxygenation of hypoxemic patients. No adverse events were observed. None of the subjects was readmitted or had long-term COVID-19 sequelae.

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Abbreviation			
COVID-19	coronavirus disease 2019	PH	pulmonary hypertension
NO	nitric oxide	AKI	acute kidney injury
Ppm	parts per million	FiO <sub>2</sub>	Fraction of inspired oxygen
ARDS	acute respiratory distress syndrome	SpO <sub>2</sub>	Peripheral oxygen saturation
LOS	length of stay	MAP	mean arterial pressure
SARS-CoV	Severe acute respiratory syndrome coronavirus	RVSP	right ventricular systolic pressure
SNAP	S-nitroso-N-acetylpenicillamine	IQR	interquartile range
NO <sub>2</sub>	nitrogen dioxide	V/Q	ventilation/perfusion
MetHb	methemoglobin	IL-6	interleukin-6
rt-PCR	reverse transcription polymerase chain reaction	TTE	transthoracic echocardiography
CXR	chest radiograph	RR	respiratory rate
		BMI	body mass index
		MV	mechanical ventilation

## 1. Introduction

There are few respiratory therapeutic options for the acute symptomatic treatment of spontaneously breathing patients with pneumonia due to coronavirus disease 2019 (COVID-19). Supplemental inhaled oxygen, the use of high-flow nasal cannula (HFNC), awake prone positioning, continuous positive airway pressure, and non-invasive ventilation have been used in non-intubated COVID-19 patients. However, to date, none of these treatments has been shown to improve the clinical outcome of patients with COVID-19 pneumonia [1,2].

Inhaled nitric oxide (NO) is a selective pulmonary vasodilator, approved by the Food and Drug Administration in 1999 at doses up to 80 parts per million (ppm) for the treatment of newborns with hypoxic respiratory failure due to persistent pulmonary hypertension [3]. Inhaled NO is used as a rescue therapy to improve oxygenation of mechanically ventilated patients with severe acute respiratory distress syndrome (ARDS) [4]. Others suggested that administration of NO gas might induce bronchodilation, suppress inflammation and thrombosis, and exert antimicrobial effects [5–7].

Nitric oxide inhalation therapy was previously reported to be beneficial for patients with viral respiratory infections. In a multicenter trial involving 43 infants with viral bronchiolitis, treatment with inhaled high-dose NO (160 ppm) for 30 min, five times per day, improved oxygenation and reduced the hospital length of stay (LOS) [8].

During the severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) epidemic of 2002–2003, Chen et al. reported that breathing NO gas improved oxygenation of spontaneously breathing patients with acute lung injury. These beneficial effects persisted beyond the treatment period, suggesting that inhaled NO may have an antiviral effect [9]. In addition, *in vitro* studies report that S-nitroso-N-acetylpenicillamine (SNAP), an NO donor, inhibited the replication of both SARS-CoV-1 and SARS-Coronavirus-2 (SARS-CoV-2) in a dose-dependent fashion [10,11].

Three recent clinical studies investigated the effect of continuous inhalation of low dose NO (20–30 ppm) on oxygenation of intubated patients with COVID-19 ARDS [12–14]. Our study differs from those prior reports, as we tested high dose of inhaled NO gas (160 ppm) for brief periods (30 min twice per day) on spontaneously breathing non-intubated patients. To allow high dose breathing of NO to non-intubated patients, we built a face mask apparatus allowing safe delivery of high-dose inhaled NO (up to 250 ppm) [15]. In two recent case series, we used this apparatus to administer 160–200 ppm NO gas to five COVID-19 patients with acute respiratory failure [16] and to six pregnant women with severe and critical COVID-19 pneumonia. Inhaled NO improved oxygenation, reduced respiratory rate, and decreased plasma levels of inflammatory markers [17].

In the present study, we evaluated the safety and efficacy of breathing NO at 160 ppm, twice daily for 30 min in 29 spontaneously breathing, non-intubated, hospitalized patients with mild-to-moderate COVID-19-induced pneumonia.

## 2. Materials and methods

The patients received inhaled NO as part of a randomized clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT04305457; Massachusetts General Hospital [MGH] IRB Protocol 2020P000786). The steering committee ended the trial on July 7th, 2020, due to a marked reduction of COVID-19-related hospital admissions in Massachusetts. Since the pre-specified randomized sample size (240) was not reached, we herein present the treatment group's data including the inhaled concentration of NO and nitrogen dioxide (NO<sub>2</sub>), transcutaneous methemoglobin (MetHb) levels and vital signs before, during, and 5 min after ceasing NO treatment. Four indicators were used as safety endpoints: the incidence of rebound pulmonary hypertension (PH) following cessation of NO inhalation, the occurrence of acute hypotension or acute desaturation during the use of NO gas, and the proportion of patients who developed acute kidney injury (AKI). Serum creatinine was measured to detect AKI. AKI evaluation was included because previous studies suggested that renal injury may be associated with inhaling NO in patients with ARDS [4].

The study also evaluated 6 outcome variables: 1) the incidence of tracheal intubation and mechanical ventilation within 28 days after study enrollment; 2) the 28-day all-cause mortality and number of days to clinical recovery, defined as the absence of fever, decreased respiratory rate to less than 24 breaths/min, absence of cough for three consecutive days, or hospital discharge; 3) hospital LOS, 4) hospital readmission rate, 5) a negative reverse transcriptase-polymerase chain reaction (rt-PCR) for SARS-CoV-2 at 28 days after initiation of NO treatment, 6) vital signs and selected plasma inflammatory markers. Vital signs and laboratory results were evaluated before the initiation of NO treatment and daily thereafter as the closest value to 8 a.m. To visually assess pulmonary involvement, chest radiographs (CXRs) before NO treatment were evaluated by a subspecialty-trained thoracic radiologist with 23 years of experience.

### 2.1. Population

Adult patients admitted to the medical ward with 1) SARS-CoV-2 infection confirmed by rt-PCR assay, and 2) presence of COVID-19 symptoms such as cough or tachypnea (respiratory rate  $\geq$  24 breaths/minute) were included in the study. Patients on long-term oxygen therapy and those requiring HFNC therapy at the time of screening were excluded. Pregnant patients and patients who do not resuscitate, do not intubate, or comfort measures only orders were also excluded.

### 2.2. Nitric oxide administration

Building on recent reports on safety and anti-viral effects of breathing 160 NO for short periods of time, we decided to deliver [8,16,17]

inhaled NO at 160 ppm for 30 min twice per day up to 14 days (28 treatments) or until hospital discharge, or a negative rt-PCR for SARS-CoV-2 (nasopharyngeal swab), or lack of respiratory symptoms for three consecutive days. Inhaled NO was delivered via the face mask apparatus previously reported (Fig. 1) [15], which had provided high-dose inhaled NO gas (up to 250 ppm) to spontaneously breathing patients. Due to our COVID-19 hospital policy limiting the transfer of equipment between patients' rooms, it was not feasible to deploy more sensitive analytical methods (e.g. Sievers 280i Nitric Oxide Analyzer, GE Analytical Instruments, Boulder; CO and Cavity Attenuated Phase Shift [CAPS] NO<sub>2</sub> monitor (Aerodyne Research Inc, Billerica, MA)) to

continuously measure the delivered NO and NO<sub>2</sub> concentrations during treatment. Before each administration, we performed a bench test to confirm the flows of air, oxygen, and NO gas (as described by Gianni et al. and reported in Online Supplementary Material Table S1) necessary to obtain 160 ppm NO at a given fraction of inspired oxygen (FiO<sub>2</sub>) and to assess the generated NO<sub>2</sub> [15]. The set flow of each gas was not changed during treatment.

To assess NO therapy's hemodynamic and respiratory effects, vital signs (respiratory rate, heart rate, and non-invasive blood pressure) were measured before treatment, 15 min after starting to breathe NO, and 5 min after each session. Peripheral oxygen saturation (SpO<sub>2</sub>) was measured continuously from before treatment until 5 min after each session. The reaction between oxyhemoglobin and NO generates MetHb by oxidizing ferrous iron of the heme group to the ferric state. Since MetHb is unable to bind oxygen, a non-invasive transcutaneous MetHb-oximeter (Masimo rainbow SET, Irvine, CA 92618) was used to monitor MetHb levels during NO treatment continuously. Based on the results of previous studies, we considered 5% MetHb as the highest permitted level [4].

Rebound PH is a possible side effect upon discontinuation of inhaled NO [18,19]. Patients were monitored for clinical and echocardiographic signs of rebound PH, including tachypnea, peripheral oxygen saturation (SpO<sub>2</sub>) <80%, a decrease in mean arterial pressure (MAP) by ≥ 50 mmHg and an increased estimated right ventricular systolic pressure (RVSP) by ≥ 10% [16,17].

### 2.3. Outcomes, data collection and statistical analysis

Data are presented as the median and interquartile range (IQR) for continuous variables and the number and percentage of instances for categorical variables. To evaluate the trend of a continuous variable over time (before, during, and after the treatment or during the hospitalization), a mixed effect model (R package [lme4] counting each patient as a random effect, R package [emmeans] for post hoc analysis) was used. To evaluate the trend of statistical significance was considered at a two-tailed  $P < 0.05$ . All the analyses were conducted using R (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results

### 3.1. Study population

Between March and June 2020, 29 hospitalized patients were treated for COVID-19 with 160 ppm of inhaled NO gas twice daily. Study population characteristics are summarized in Table 1.

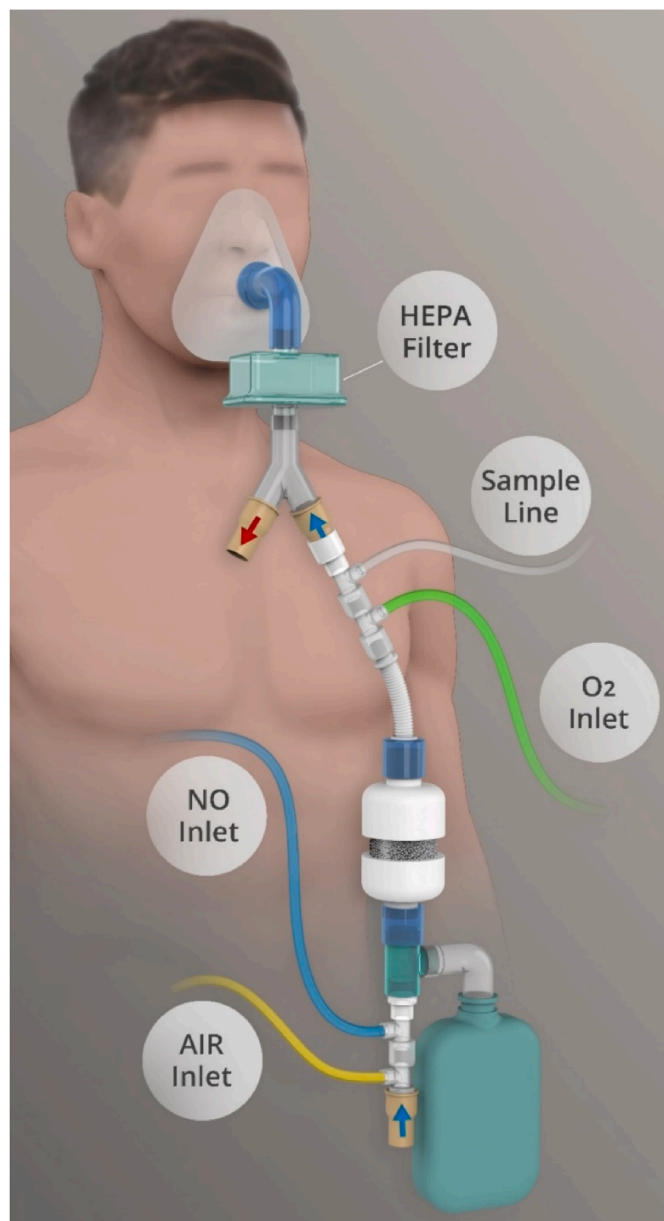
The median time between the onset of COVID-19 symptoms and confirmed diagnosis by positive rt-PCR test was 6 [IQR 3–10] days. A total of 217 treatments were given to the 29 patients. The median number of treatments per patient was 6 [range 1–27] (Table 2 and Online Supplementary Material Figure S1).

### 3.2. Vital signs, chest imaging, and inflammatory markers

At the time of enrollment, 13 patients (45%) required supplemental oxygen (median 2 [IQR 1.5–3.5] L/min), and 24 patients (82%) had abnormal findings on CXR. Radiographic evaluation at the time of hospital admission showed in 16 patients (55%) bilateral, mid-to-lower-zone predominant and peripherally distributed lung opacities, which were considered classic for COVID-19 (Online Supplementary Material Figure S2 and Table S2) [20].

During the administration of NO, tachypneic patients had a decreased respiratory rate (Fig. 2A), demonstrating a role of NO gas in relieving respiratory distress. Several patients fell asleep and felt less distress.

The ratio of SpO<sub>2</sub>/FiO<sub>2</sub>, a marker of systemic oxygenation [21], in



**Fig. 1.** Delivery system description. In summary, a non-rebreathing circuit composed of standard respiratory circuit connectors, a reservoir bag, a scavenging chamber containing calcium hydroxide, and a snug-fitting mask was used to reach 160 ppm NO gas. Nitric oxide balanced in nitrogen (Praxair, Inc. Danbury, Connecticut, United States) was blended with medical air and oxygen to obtain the desired mixture, based on *in vitro* measurements. The delivery system allows stability of administered concentration of NO, with NO<sub>2</sub> levels always below the Occupational Safety and Health Administration (OSHA) threshold.

**Table 1**

Baseline Demographics and clinical characteristics. Time to confirmed diagnosis = Time between onset of symptoms and a confirmed diagnosis (positive SARS-CoV-2 test). BMI = Body Mass Index; SpO<sub>2</sub> = Peripheral saturation of oxygen; O<sub>2</sub> = Oxygen.

	Baseline demographics and clinical characteristics (n = 29)
Age (years-old)	50 [41–60]
Gender, No. (%) Female	13 (44.8)
BMI (kg/m <sup>2</sup> )	31 [25–34]
<b>Race, No. (%)</b>	
White	27 (93.1)
Black or African American	2 (6.9)
<b>Ethnicity, No. (%)</b>	
Hispanic or Latino	22 (75.9)
Not Hispanic or Latino	7 (24.1)
<b>Comorbidities, No. (%)</b>	
Hypertension	12 (41.4)
Diabetes	10 (34.5)
Chronic Pulmonary Disease	3 (10.3)
Time to confirmed diagnosis, Days	6 [3–10]
Respiratory Rate, Breaths/min	20 [20–24]
SpO <sub>2</sub> , %	96 [94–97]
O <sub>2</sub> requirement, No. (%)	13 (44.8)
O <sub>2</sub> requirement, L/min	2 [1.5–2.5]
Altered Chest Radiographs, No. (%)	24 (82.8)

**Table 2**

Safety and feasibility. NO = nitric oxide. NO<sub>2</sub> = nitrogen dioxide. FiO<sub>2</sub> = Fraction of inspired oxygen. RVSP = Right Ventricular Systolic Pressure. Ppm = parts per million. Min = minutes. Max = maximum value.

Total number of treatments, No.	217		
NO <sub>2</sub> inhaled, ppm	1.1 [0.98–1.14], Max = 1.47		
FiO <sub>2</sub> , %	23 [21–30]		
<b>Concerns</b>			
Mask discomfort	3		
Tingling of hands bilaterally	1		
Nausea	1		
	<b>Before Treatment</b>	<b>15 min after NO initiation</b>	<b>5 min after treatment ends</b>
sMethemoglobin, %	0.5 [0.2–0.8] Max 2.3	1.8 [1.4–2.2] Max 4.7	1.6 [1.2–2.0] Max 3.5
RVSP (mmHg)	19.4 [15.4–23.5]	17.9 [15.2–20.1]	18.8 [16.6–21.9]

the subgroup of patients who experienced pre-treatment hypoxemia (defined as an SpO<sub>2</sub>/FiO<sub>2</sub> <315) [21], improved during NO gas administration (Fig. 2B). The improved systemic oxygenation is likely due to improved ventilation to perfusion (V/Q) matching, due to selective pulmonary vasodilation by NO of ventilated lung regions [22].

During the first four days after randomization, the respiratory rate, starting from a median of 20 breaths/minute, decreased over time by 1 breath/minute each day of treatment. The largest difference was observed 48–72 h after the initiation of NO therapy (Fig. 2C). The reduction in respiratory rate may indicate a resolution of respiratory symptoms possibly related to a lasting effect from multiple NO gas treatments. Mean arterial pressure and heart rate did not change during hospitalization (Online Supplementary Material Figure S3).

At the time of enrollment, patients had evidence of systemic inflammation with elevated plasma levels of Interleukin-6 (IL-6), C-reactive protein, ferritin, and D-dimers, while the median white blood cell counts were within the normal range (Online Supplementary

Material Table S3). During hospitalization, we did not measure reduced white blood cell count, C-reactive protein, and ferritin (Online Supplementary Material Figure S4). An increase over time in platelet count within the normal range (Online Supplementary Material Figure S4D) was observed. However, no definitive conclusions can be drawn related to the inhibition of intra pulmonary platelet aggregation by inhaled NO.

### 3.3. The safety of inhaled NO treatment at 160 ppm

The maximum level of MetHb measured during NO treatment at 160 ppm was 4.7%; none of the treatments were terminated because of increased MetHb levels. MetHb levels rapidly decreased over 5 min in all patients after discontinuation of NO inhalation, suggesting normal red cell MetHb reductase activity in all patients (Table 2, Fig. 3) [23].

Transthoracic echocardiography (TTE) was performed in 14 patients receiving inhaled NO therapy. The estimated RVSP was normal in all patients before NO treatment (Table 2). None of the patients developed clinical symptoms or echocardiographic signs of rebound PH after ending NO therapy.

There were no hypotensive or hypoxemic episodes during NO inhalation. No device or system failures occurred during NO administration. However, three subjects reported discomfort due to the face mask, leading to discontinuation of their NO treatment.

None of the patients developed AKI during hospitalization, suggesting that breathing 160 ppm NO gas for 30 min twice a day did not impair kidney function (Online Supplementary Material Figure S5).

### 3.4. Outcomes

Only one patient out of 29 required intubation and mechanical ventilation due to progression of pulmonary disease. No deaths were observed among our population. Hospital LOS coincided with a clinical recovery time of a median of 6 days [IQR 4–8 days] (Table 3).

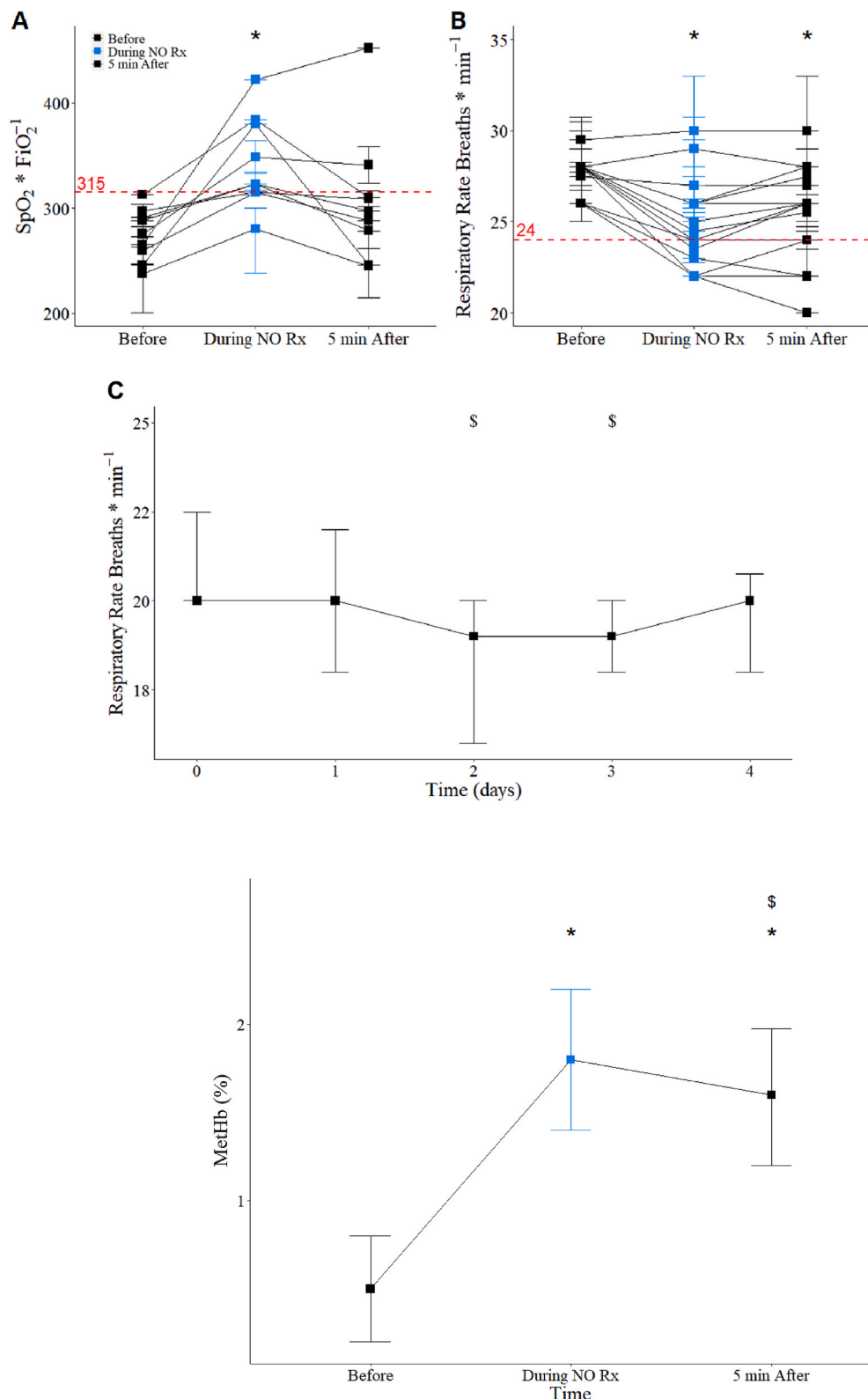
None of the patients subsequently required hospital readmission due to COVID-19 reactivation.

## 4. Discussion

This study investigated the effectiveness and safety of inhaling 160 ppm NO gas twice daily for 30 min to reduce respiratory disease progression in hospitalized, non-intubated COVID-19 patients.

Three significant findings emerge from this study. First, NO inhalation was associated with an acute reduction of respiratory rate in tachypneic spontaneously breathing COVID-19 patients. The reduction in respiratory rate was evident in patients with tachypnea at the beginning of NO treatment. Furthermore, the reduction of respiratory rate was sustained throughout hospitalization and until the time of discharge, suggesting a long-lasting beneficial effect of NO gas beyond selective pulmonary vasodilation and improved V/Q matching during treatment. We also observed improved oxygenation in those patients who were hypoxemic before beginning each NO treatment. Second, the use of inhaled NO was associated with no hospital readmissions after discharge, this should be compared to a COVID-19-related readmission rate of 10–30% reported in the literature [24–26]. Third, no adverse events occurred during NO inhalation. MetHb remained below the 5% safety limit in all patients, which was set based on previous studies [4]. There were also no cases of AKI. These findings suggest that high-dose inhaled NO (160 ppm) can be safely administered to spontaneously breathing patients admitted with COVID-19.

The administration of NO to patients with COVID-19-related pneumonia was associated with an acute reduction of respiratory rate and



**Fig. 2.** Effect of nitric oxide (NO) inhalation on respiratory rate (RR) and oxygenation. Linear mixed model fit by maximum likelihood. (A) SpO<sub>2</sub>(%)/ FiO<sub>2</sub>(%) (n = 33 treatments) improved by 49 [95% CI: 29–70] during treatment, and by 22 [95% CI: 1–42] after treatment in all patients with hypoxemia (SpO<sub>2</sub>/FiO<sub>2</sub> < 315) before NO gas administration. (B) RR (n = 75 treatments) was reduced by 2 [95% CI: 2–3] breaths/min during NO treatment (Rx) and remained reduced by 2 [95% CI: 1–3] breaths/min after treatment in all patients with tachypnea (RR at baseline >24 breaths/min) at the commencement of the administration. (C) RR was reduced during hospitalization by 1 [95% CI: 0 to 1] breath/min for each day of treatment. At 48 h, the RR was lower by 3 [95%CI: 2–5] breaths/min. \*P < 0.05 vs Before. \$ P < 0.05 vs Day 0.

**Fig. 3.** Methemoglobin during nitric oxide (NO) inhalation therapy (Rx). Linear mixed model fit by maximum likelihood. \*P < 0.05 vs Before. \$ P < 0.05 vs During NO Rx.

**Table 3**

Outcomes. MV = mechanical ventilation. Viral shedding identifies viral clearance on rt-PCR from nasal or pharyngeal swab.

	Outcomes
Tracheal intubation and MV, No. (%)	1/29 (3.4)
Mortality at 28 days, No. (%)	0/29 (0.0)
Time to clinical recovery, Days	6 [4–8]
Hospital length of stay, Days	6 [4–8]
Viral shedding at 28 days, No. (%)	7/10 (70)
Hospital readmission, No. (%)	0/29 (0.0)

improved oxygenation. When inhaled at concentrations greater than 80 ppm, NO has been shown to have mild bronchodilator effects in asthmatic patients [5]. Bronchodilation due to NO may also explain the acute decrease in the respiratory rate observed in our COVID-19 patients. In addition, patients receiving NO had a sustained decrease in respiratory rate throughout their hospital stay. Although we did not measure any NO-related systemic anti-inflammatory effects, it is possible that intra-pulmonary anti-inflammatory and anti-thrombotic effects of repeated NO treatments may explain the beneficial effects of NO on lung function, in addition to the known improvement of oxygenation secondary to amelioration of V/Q matching [27–29]. In another study of inhaled NO in Pregnant women with COVID-19 we did measure a reduced plasma level of C-reactive protein daily in all patients after the initiation of NO therapy [17].

In a recent *in vitro* study, Akaberi et al. showed that liquid phase NO donors (S-nitroso-N-acetylpenicillamine, 400  $\mu$ M) inhibit replication of SARS-CoV-2 and prevent viral-induced cytopathic effects upon infected epithelial cells. The authors noted that the treatment of cells with NO reduced viral mRNA production and inhibited over 90% of viral replication by covalent nitrosation of the SARS-CoV-2 protease [11]. The kinetics by which inhaled NO enters respiratory epithelial cells to produce antiviral effects are unknown, and the precise association between the NO concentration provided by liquid-phase donors and the corresponding concentration of inhaled NO gas has not been determined [30]. However, *in vitro*, preclinical, and clinical studies suggest that NO gas is helpful for treating viral respiratory diseases [8,10,30].

No readmissions were reported in our NO-treated patients. Our observation is encouraging when compared to the readmission rates reported in the United States and United Kingdom for patients admitted to hospitals with COVID-19, ranging from 10 to 30% [24–26]. Of note, in a population of 33 patients admitted for COVID-19 at our institution, matched for age, gender, and severity, we observed that 6 subjects (19.4%) were readmitted within 28 days due to COVID-19 (Data not shown). The decreased readmission rate in the NO-treated patients may have been due to the direct antiviral effect of inhaled NO. However, because of reduced assay availability early in the pandemic, only 10 patients underwent a follow-up test within 28 days from enrollment, and the results showed that 7 out of 10 treated and tested patients turned negative. We are, therefore, unable to provide definitive results regarding the effect of NO inhalation on SARS-CoV-2 clearance in patients with active COVID-19 pneumonia.

In the present study, twice-daily administration of 160 ppm NO for 30 min in patients with mild COVID-19-associated pneumonia was found to be safe. This regimen produced a measurable beneficial effect on the respiratory system, without adverse events during the 217 treatment sessions. The echocardiographic and clinical evaluation did not show evidence of rebound PH. Pulse oximetric monitoring showed that MetHb levels did not rise above the 5% safety limit during or immediately following NO inhalation. In our patients with modest increases of MetHb, the level rapidly decreased within 5 min after completing NO therapy. These findings confirm previous case series regarding the safety of NO administration to healthy volunteers and pregnant COVID-19 patients [17,31].

Nephrotoxicity has historically been a concern in patients with ARDS treated with NO. A meta-analysis of data from four trials involving

critically ill, mechanically-ventilated patients with septic ARDS (n = 945) suggested an increased risk of AKI (relative risk 1.59, 95% CI 1.17 to 2.16) [4]. However, the explanation for the association between NO administration and the development of AKI is unknown. In contrast, a nephroprotective effect of NO treatment was observed in a separate meta-analysis, including 579 cardiac surgical patients [32]. Notably, none of the patients receiving NO in this study developed AKI.

## 5. Conclusion

There are currently no pharmacologic interventions for COVID-19 that primarily target the respiratory system. We observed that NO gas therapy produced an acute improvement of systemic oxygenation in hypoxemic patients and reduced the respiratory rate. The rate reduction was both acute during NO therapy and sustained during the hospital stay. No re-hospitalization was observed among our population. The delivery of NO was found to be safe. These findings support the need for a randomized clinical trial to investigate the potential role of high-dose inhaled NO for the treatment of spontaneous breathing non-intubated COVID-19 patients.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.niox.2021.08.003>.

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