# Nitric oxide gas inhalation for healthcare providers to prevent COVID-2019

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ClinicalTrials.gov Protocol Number: NCT04312243
Detailed Protocol Version 7

This document has been written following the PHRC instructions about Detailed Protocol writing.

Version 7.0, May 22, 2020

## Synopsis and Study Schema

**Title:** Nitric oxide gas inhalation for healthcare providers to prevent COVID-2019 disease

**Principal Investigator:** Lorenzo Berra, MD

**Study Objective(s):** To assess if intermittent inhaled NO might prevent the risk of COVID-19 among healthcare workers.

**Study Design:** Randomized clinical trial

**Inclusion Criteria.**

1. Age ≥18 years
2. Scheduled to work with SARS-CoV-2 RT-PCR patients for at least 3 days in a week.
3. Healthcare providers or other professional (doctors, nurses, respiratory therapists, technician, administrative staff, others) working at a participating hospital

**Exclusion Criteria.**

1. Proven previous SARS-CoV2 infection and subsequent negative rt-PCR
2. Pregnancy
3. Known hemoglobinopathies
4. Known anemia

**Primary endpoint**.
Incidence of subjects with COVID-19 disease at 14 days

**Secondary endpoints**.

1. Incidence of subjects with positive SARS-CoV-2 rt-PCR test at 14 days
2. Proportion of healthcare providers requiring quarantine
3. Total number of quarantine days

**Total expected number of subjects: 470**

**Coordinating center:** Massachusetts General Hospital, Boston, MA, USA

**Study Drug or Intervention:** The treatment group will inhale 160-parts per million (ppm) of nitric oxide for 15 minutes before and after each work shift.

**Evaluation Criteria:** Laboratory confirmation of real time reverse transcriptase–polymerase chain reaction (RT-PCR) positive for SARS-CoV-2.

**Safety Assessment:** Continuous Monitoring of MetHb with a non-invasive co-oximetry monitor in order to prevent methemoglobinemia (defined as MetHb > 5%). Continuous monitoring of Nitrogen Dioxide ($NO_2$) in inhaled breath (threshold level < 5 ppm) in order to prevent reaching dangerously high levels.

**Statistical Considerations:**
Reported rates of intra-hospital SARS-CoV-2 infection rate are extremely variable and depend on time elapsed since the epidemic outbreak. We hypothesized an infection rate of 15% in control subjects, provided that adequate protective measures are followed, and 5% in those treated with iNO. Assuming an alpha of 0.05 and a beta (power) of 0.9, we calculated with a two-sided test a need for N1=207 and N2=207 subjects that we increased to a total of 470 by considering a dropout of 12.5%. Estimated sample sizes where calculated are based on a test of equality of two proportions performed on a specific software.

**Duration of Study Period:** Study participants will be followed-up for 14 days year after-randomization

**Data Safety Monitoring Board**

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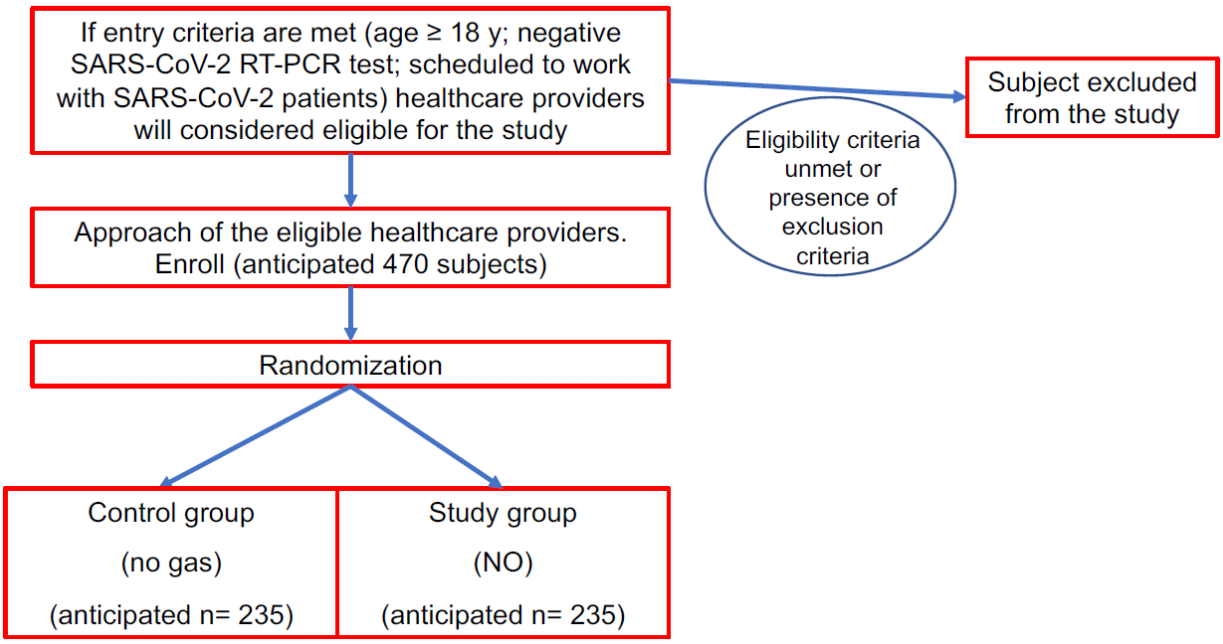


**Figure 1. Study schema**

## I. BACKGROUND AND SIGNIFICANCE

Historical background

The current COVID-19 pandemia sparked in December 2019 from Wuhan (China). It is believed that the virus was transmitted at first from a bat (zoonosis) followed by human-to-human transmission [3]. The disease spread throughout the Asian continent, to Europe and more recently to the United States [1].The first case in the US has been reported on January 19, 2020, in a patient who had travelled from Wuhan. By March 2020, the disease is threatening cities from East to the West Coast [2].

The infection, known as COVID-19, is caused by a novel coronavirus called SARS-CoV-2 due to several genetic and clinical similarities with the coronavirus SARS-CoV which caused SARS epidemic in 2002-2003 [2]. The microbiological diagnosis is performed with Real Time Reverse Transcription - Polymerase Chain Reaction (RT-PCR) from oro/naso pharyngeal swabs or stool samples, or broncho-alveolar lavage in intubated patients.

COVID-19 is predominantly a respiratory infection that spans from a mild involvement of the upper respiratory tract to a severe pneumonia leading to respiratory distress, shock and death [4]. In the early case series, fever, cough and dyspnea/tachypnea, together with myalgia and fatigue, have been identified as the most common presentation symptoms [6]. The incubation period ranges from 1 to 14 days with an estimated median of 5 to 6 days [3]. Most of the patients are affected by an upper respiratory tract disease for a relatively long period of time (a median of 8-10 days), after that as much as 25% of affected patients may develop severe symptoms that require ICU admission [4]. Incidence of overall mortality is about 3 up to 4% [4]. Among the severe cases, 61% of patients might die [5].

The disease is commonly transmitted in a hospital settings and healthcare providers are at highest risk. The hospital transmission of the infection is not unusual during an outbreak, as shown during SARS outbreak in 2003. In February 2020, about one thousand and seven hundreds Chinese healthcare workers were confirmed to be infected by SARS-CoV-2 with at least 6 reported casualties including Dr Li Wenliang, the first physician to identify this new disease [6]. More recently in the United States, two ER physicians are in critical conditions in the Intensive Care Unit, after being in contact with COVID-19 patients. Unfortunately, at present time, there is no vaccine or pharmacological prophylaxis to decrease the risk in healthcare providers to contract the infection. Remdesivir may speed up a patient's recovery and discharge from the hospital, however it has not been shown to prevent transmission of the virus.

Thus, a safe and prophylactic therapy to reduce COVID-19 disease in healthcare workers would be of a great benefit the individuals and the society.

Previous clinical and pre-clinical studies leading up and sustaining the proposed research

Nitric oxide (NO) is an anti-infective agent at high concentrations. *In-vitro* studies have identified a significant activity against pathogens broadly resistant to antibiotic therapy, such as bacteria responsible for hospital acquired pneumonia [7]. In a small size clinical trial, nitric oxide gas showed to be safe and effective against bacterial colonization/superinfections in the context of chronic respiratory diseases [8].

NO gas showed also anti-viral activities against SARS-CoV [9] [10]. In an *in-vitro* study, NO-donor compound S-nitroso-N-acteylpenicillamine added to a cell culture preparation increased

the survival rate of mammalian cells infected with SARS-CoV [11]. In patients affected by SARS, NO inhalation decreased the need of oxygen therapy, ventilatory support, and a faster resolution of chest x-ray abnormalities [12].
Due to the genetic similarities, both Coronaviridae SARS-CoV and SARS-CoV-2 might behave similarly to NO gas exposure. We hypothesized that high dose of exogenous inhaled NO is viricidal agent in COVID-19 disease. A dose of 160 ppm of nitric oxide has been found to be bactericidal, fungicidal, viricidal [13, 14]and safe for the patient when adequately managed. In fact, high dose of NO administered continuously for long time can generate an excess of methemoglobinemia. Miller et al found that intermittent administration, 160 ppm for 4 cycles of 30 min, have the same antibacterial effect and can be safely administered [15].
The administration of high doses of nitric oxide, with potent viricidal effect, could prevent development of COVID-19 disease in healthcare providers exposed to SARS-CoV-2 positive patients.

Rationale and hypothesis
Preliminary data support the anti-viral effects of high concentration, intermittent inhaled NO gas. We hypothesized that high concentration inhaled NO can decrease the risk for COVID-19 infection in healthcare providers.
Sparing healthcare workers is highly desirable, especially during a pandemic infection.
To test this hypothesis, we propose a randomized clinical trial.

## II. **SPECIFIC AIMS**

The proposed randomized clinical trial will test the efficacy of inhaled NO at 160-180 ppm for 2 daily cycles of 15 minutes each in:

Aim 1
To assess whether intermittent delivery of inhaled NO gas in air at a high dose may protect healthcare workers (physician, nurse, respiratory therapist, administrative staff, technician or other healthcare professional) from COVID-19.

Methods to achieve Aim 1
Primary outcome of the study is the proportion of healthcare providers who present COVID-19 at 14 days. COVID is defined as SARS-CoV-2 infection (positive real-time polymerase chain reaction (RT-PCR) plus one of the following:

- Fever: defined as temperature:
  - > 36.6 °C, axillary site
  - > 37.2°C, oral site
  - >37.7°C, tympanic or rectal site
- Cough
- Shortness of breath

Aim 2
Secondary outcome is the proportion of healthcare providers who present positivity to real time RT-PCR at 14 days.

Methods to achieve Aim 2
The secondary outcome (RT-PCR for SARS-CoV-2 positivity at 14 days) will be evaluated as the proportion of subjects who have a positive swab for SARS-CoV-2 at 14 days.

Aim 3
Proportion of healthcare providers requiring quarantine

Methods to achieve Aim 3
The aim number 3 will be evaluate as the proportion of the subjects who require quarantine during the study periods.

Aim 4
Total number of quarantine days in the two groups

Methods to achieve Aim 4
The aim number 3 will be evaluate as the difference in the mean of the quarantine days between the 2 groups

## III. SUBJECT SELECTION

Inclusion /Exclusion criteria
Inclusion criteria

1. Age ≥18 years
2. Scheduled to work with SARS-CoV-2 infected patients for at least 3 times/week (at least 6 shifts in 14 days).
3. Healthcare providers or other professional (doctors, nurses, respiratory therapists, technician, administrative staff, others) working at a participating hospital

Exclusion criteria

1. Proven previous SARS-CoV2 infection and subsequent negative rt-PCR
2. Pregnancy. All eligible women in childbearing age must undergo urinary pregnancy test (will be excluded from testing women over 50 years of age or with more than 12 months from the last menstrual cycle. Will be excluded, as well, women with well documented surgical sterilization).
3. Known hemoglobinopathies
4. Known anemia

Source of subject and recruitment methods
This study is targeted for a population of healthcare provider (physician, nurse, respiratory therapist, administrative staff, technician or other healthcare professional) working in a hospital with COVID-19 patients. At the admission of the first SARS-CoV-2 positive patient in a hospital

unit a recruitment email will be sent to the department chief to inform the staff about the research. Individual hospital staff members may be contacted via email and asked to participate using the recruitment email. Staff distribution lists may also be utilized to identify potential participants. Participants who are interested in learning more about the study will be able to contact the study team to learn more.

All the healthcare professionals that met all the inclusion and not the exclusion criteria become eligible for the study. The clinical staff of each unit will be adequately informed about the protocol details. No clinical care procedure will be interrupted or delayed due to the study procedure.

## IV. SUBJECT ENROLLMENT

Methods of enrollment, including procedures for patient registration

The study was reviewed and approved by the MGH Anesthesia-in-Chief (Oluwaseun Johnson-Akeju, investigator on this study), by the leadership of the Respiratory Care Services (Dr. Robert Kacmarek, investigator on this study) and by the Medical Director of Respiratory Care (Dr. Lorenzo Berra, PI of this study).

An email containing the details of the study, the link to ClinicalTrial.gov and the study staff contacts will be sent to all the medical directors of the hospital responsible for the COVID-19 patients in order to inform all their staff about the possibility to join this study. The study staff will approach the subject to evaluate the inclusion/exclusion criteria.

In case eligibility criteria are met, investigator(s) will proceed with the enrollment procedures. The member of the research staff will explain to the subject all the general details of the research protocol. Then, if the subject is willing to, an MD investigator will go through further details and answer potential questions regarding the research study. Written informed consent will be obtained from the subject. To avoid any form of coercion the department chiefs involved in the study (Dr.Robert Kacmarek and Dr. Oluwaseun Johnson-Akeju) are not allowed to know the names of the study participants. Furthermore, they cannot participate to study gas administration. Since all the subjects will be healthy with intact decision-making capacity, no surrogate consent will be accepted. A de-identified code will be assigned to the subject and registered on a dedicated enrollment log.

Procedures for obtaining informed consent

If the subject will autonomously decide to take part of the study, contacting the study staff, will be approached by an investigator who will go through the details of the study. Signing of an Informed Consent Form will be requested for participation in the study. Consent can be withdrawn at any time. The investigators will access Personal Medical Information (PMI) for study purposes.
We expect all the subjects involved in this study to be able to personally consent to participation.

Treatment assignment and randomization

Randomization will occur through random allocation sequence generated by a computerized random generation program (REDCap). Parallel allocation to treatment or control group will

occur with a 1:1 ratio. Randomization will not be stratified for pre-specified demographic conditions (e.g., sex, age). Results will be adjusted for such conditions in analysis phase as specified in the statistical analysis section.
Subjects will be randomly allocated to the treatment group with NO administration or to the control group in which no nitric oxide gas will be delivered.

Blinding
No masking is going to be used in this study.

## V. STUDY PROCEDURES

Study subjects will be the healthcare providers (physician, nurse, respiratory therapist, administrative staff, technician or other healthcare professional) part of the units assigned to the COVID-19 outbreak management. Fixed time of treatment administration (14 days) and evaluation (14 days) has been established. All the outcomes will be evaluated at the end of this period. Subjects will stop receiving NO before the 14 days period under the conditions specified in the section "Study time frame". In any case the subject will be monitored for 14 days. The workflow of the study has been summarized in Appendix 7.
Since pregnancy is an exclusion criterion every woman in childbearing age will undergo a urinary pregnancy test before study gas administration. Menopausal women, defined as 12 months without menstrual period AND over 50 years of age, as well as women with well documented surgical sterilization do not need to undergo a pregnancy test. During the study period the women in childbearing age must declare that they are not seeking pregnancy and that they are using acceptable contraception or are not sexually active with men.

Drug to be used: NO administration
NO will be delivered in 2 daily sessions (before and after the work shift) during 14 consecutive days. To consider the study gas administration effective the subject must receive at least 12 sessions in 14 days (weekend days included).
Each session will last 15 minutes, for a total of 30 minutes/day for each subject. The gas will be delivered as previously described in IRB #2016P001629 and 2020P000786. We have enrolled to date 220 patients in trial IRB #2016P001629. The system is easy to use and safe. No adverse events have been reported. Methemoglobin remained always below 5% and NO2 never higher than 2 ppm. The non-invasive ventilation is set at a range between 2 and 5 cmH2O of Positive End Expiratory Pressure (PEEP) (as illustrated in Appendix 5). A tank will be connected to the inspiratory limb of the circuit, and the output will be adjusted to deliver a target concentration between 140 and 180 ppm. Commercially available tanks or a nitric oxide generator (Appendix A) will be used to deliver the gas. The choice to use a tank or the nitric oxide generator will depend on the number of available nitric oxide tanks that day, and whether they are already in use. The nitric oxide generator will only be used if the tanks are otherwise unavailable. The NO generator is a box that creates nitric oxide from air by electrical spark. The air (nitrogen and oxygen) is flown in the box and the production of nitric oxide is proportional to the voltage difference and the rate of sparks per minute. This nonsignificant risk device and its use will comply with the abbreviated IDE requirements found in 21 CFR 812.2(b). Thus, this device will appropriately be labeled as an investigational device, and requirements for device accountability,

informed consent, monitoring, records, reports, and prohibition against promotion and IRB approval will be followed. Further the PHRC will be notified immediately if any problems associated with the use of the device are encountered.

Connection to the non-invasive ventilation circuit will be through a blender mounted on the ventilatory machine. Desired mixture of air, $O_2$ and NO in the blender will be titrated with the blender knob, so to reach a concentration between 140 and 180 ppm at the inspiratory limb. An HEPA filter will be positioned on the expiratory line.
Alternatively, a non-rebreathing mask will be used to deliver the gas (as illustrated in Appendix 6). The choice of the system to delivery NO will depend on the availability of respiratory equipment and on study subject preference.
The gas delivery process will follow these sequences: an investigator or a respiratory therapist will describe to the subject the equipment and the delivery of gas, like we generally do to patients that require home-CPAP. Once learned the CPAP settings, the subject will wear the mask. The respiratory therapist and the investigator will step out of the room and the CPAP system in stand-by until then will start delivery nitric oxide. The investigator with the respiratory therapist will monitor the subject's oxygenation and EKG during the gas therapy and will keep the contact with the subject on an audio/video monitoring. The subject at the end of the time will remove the mask and the ventilator will go in a stand-by mode. The subject will remain in the room for the next 10 minutes of observation.
Inspiratory concentration of NO and $NO_2$ (never higher than 3 ppm) will be continuously monitored. Methemoglobin also will be continuously monitored non-invasively with a dedicated pulse oximeter system and should remain always below 5%. NO deliver will be titrated in order to keep methemoglobin under 5%. Values will be recorded in a dedicated paper data sheet, specifically: (I) before starting NO delivery, (II) at the end of the delivery and (III) at 5 to 10 minutes after completion of the NO delivery to demonstrate a decrease of methemoglobin after cessation of gas delivery. If MetHb doesn't decrease, we will continue monitor until there is a decrement. Subjects assigned to the control group will not receive any gas therapy.

During the session continuous $SpO_2$ and heart rate will be monitored by study staff, non-invasive arterial pressure will be cycled every 5 minutes. Values will be recorded in a dedicated paper data sheet, specifically: (I) before starting NO delivery, (II) at the end of the delivery and (III) at 5 to 10 minutes after completion of the NO delivery to demonstrate a decrease of methemoglobin after cessation of gas delivery.
The study gas will be administered in the respiratory care room in Ellison 4 (next to the surgical ICU).
Subjects assigned to the control group will not receive any gas therapy.

Throat Swab
We will be collecting a nasal/oropharyngeal swab at baseline and at 14 days. Sample collection for participants will depend on the availability of the specimen collection kits. Usual precautions will be utilized for collection of these samples. Following collection samples will be labelled and immediately frozen in a clean double bag in a -20°C or colder freezer for storage. These samples will be stored in the BSL2 lab under the direction of Dr. Hohmann. These samples will be analyzed to determine if the amount of detectable virus is different between groups. These

samples will be analyzed in batches, therefore we will not return individual results to participants. These samples will only be collected at MGH.

Study time frame

Study enrollment will last for 14 days. The NO gas administration will be performed every day twice a day for 14 days (before and after the work shift). Nitric oxide administration will be stopped if one of the following events happens:

1) Development of COVID-19 disease (see "Methods to assess Aim 1").
2) Positive RT-PCR for SARS-CoV-2 (see "Methods to assess Aim 2")
3) Subject quarantined (see "Methods to assess Aim 3 and 4")
4) Change of the assigned ward

For subjects who will exit the study before the completion of the defined time period the outcomes will be evaluated the same way as who will not exit at 14 days.

Data To be collected and when the data is to be collected

Collection from subject past medical history:

- Subject demographics
- Past and current medical history of heart, lung and neoplastic disease
- Cigarette smoke

Prospective collection of subject data will include:

- Hour of exposition to SARS-CoV-2 positive patients in 14 days
- COVID-19 disease development.
- Time to COVID-19 development (in days).
- Results of SARS-CoV-2 RT-PCR tests carried out by the study subject (according to Hospital policy)

## VI. BIOSTATISTICAL ANALYSIS

Study Endpoint

Primary endpoint: incidence of subjects with COVID-19 disease at 14 days

Secondary endpoints:

1. Incidence of subjects with positive SARS-CoV-2 rt-PCR test at 14 days
2. Proportion of healthcare providers requiring quarantine
3. Total number of quarantine days

Statistical methods

Data will be analyzed following the intention to treat analysis principle. Demographic and clinical data will be presented as proportions for categorical outcomes and mean plus standard deviation or median plus interquartile range for continuous outcomes. Comparison between groups for the primary outcome will be made with the $X^2$ test or the Fisher exact test for categorical variables. Secondary outcomes will be analyzed with the $X^2$ test or the Fisher exact

test for categorical variables and with the t-test or Wilcoxon rank-sum test for continuous variables, after assessing the total number of quarantine days for normal distribution.
A subgroup analysis will be performed with multiple linear regression, as well as logistic and cox regression models to adjust for age, pulmonary comorbidities, history of neoplastic disease and hour of exposition to SARS-CoV-2 positive patients in 14 days.

Power analysis
Based on available data from Italy and China, we predict an incidence of 15% of SARS-CoV-2 infection in healthcare providers, and we assume that the incidence will be reduced to 5% with iNO cycles. Consider an alpha level of 0.05 and a power of 0.9, we determined a sample size of n1=207, n2=207, by a two-sided test run on a dedicated software [16]. In order to account for possible dropouts, we increased the sample size of about 10%, thus, from 414 to a total of 470 subjects (235 each group).

## VII. RISKS AND DISCOMFORTS

Procedure and drug-related risks for this study: Non-Invasive Ventilation and nitric oxide administration.

Non-invasive ventilation risk:

- Due to the tightness of the facial mask the risk of pressure sores is described between 2 and 50%. This kind of complication usually follow longer time period, increasing the incidence with the time of the duration of the session. Such a small period should not be associated with skin lesions. The research team together with respiratory therapist will follow all the indications presented in literature in order to minimize the risk of this kind of complication. To avoid pressure sores, a non-rebreathing mask may be available.
- Due to noise and the tightness of the facial mask the patient could experience a sensation of discomfort. The limited period should reduce the risk of patient's discomfort. For better tolerance, a non-rebreathing mask is available.

The patients will be informed for possible risks as indicated in the informed consent.

Nitric Oxide:
Breathing NO at 160 ppm for 30 minutes has been found to be safe in adults and children as shown in two scientific reports [14-15]. At MGH, we have been successfully delivered high dose of NO at 160 ppm to a minor over the past year (more than 70 sessions of NO gas treatments). The subject never had MetHb higher than 5% and $NO_2$ was always below 5ppm.
The theoretical risks of NO breathing include the following: pulmonary hypertension, pulmonary edema, methemoglobinemia, hypoxia, and hypotension. In the study in neonates breathing NO for up to 4 days, study subjects had hematuria (6%), hyperglycemia (7%), sepsis (2%), infection (3%), stridor (3%) and hypotension (10%). They all occurred in the placebo group as well and none of these findings was significantly higher in the NO group. In a small study of healthy volunteers, Frostell et al [16] report that there were no adverse clinical events in the group inhaling NO. Of note, the average baseline methemoglobin level prior to NO administration was 0.61% and this increased to 0.77% after 10 minutes of inhalation of NO at 80 ppm. This was a statistically significant, although clinically irrelevant increase in methemoglobin levels. In our

recent study [17], there were no side effects or drug toxicity associated to the use of NO at 80 ppm for up to 24 hours.

The inhalation of NO will be administered and monitored by a trained Respiratory Therapist (RRT). There will be dedicated therapists who also administer NO as part of their clinical responsibilities. They will be familiarized with the technical aspects of this protocol and supervised by some of the investigators (Dr. Lorenzo Berra or Dr. Robert Kacmarek or Dr. Ryan Carroll or Dr. Edward Bittner or Dr. Fumito Ichinose), who have significant experience with NO administration both clinically and as part of investigational protocols. The study staff will continuously monitor the NO, nitrogen dioxide ($NO_2$) and oxygen concentration of the inhaled gas. The respiratory therapist will continuously monitor the oxygen saturation, via non-invasive oximetry during the procedure. Methemoglobin levels will be assessed using a non-invasive continuous co-oximetry monitor. If methemoglobin level rises more than 5%, NO will be halved and closely monitored until a reduction occurs. If methemoglobin persists at > 5%, NO will be halved until methemoglobin is < 5%.
Since we will treat many subjects in the same room, we will monitor room nitric oxide concentration to keep it below 1 ppm.
Any study subject who experiences a side effect which is felt to be related to the study drug will not be allowed to continue in the protocol.

There are no known risks associated with the use of the electric NO generator device. The machine is not currently approved by the FDA for any medical purpose. It has been used safely in other small research studies at MGH however (Appendix A). If any problems associated with the use of the device occur, the IRB will be notified immediately.

There are no additional anticipated physical risks associated with the throat swab.

Psychosocial risks
We do not anticipate psychosocial risks to the study subjects from participation in this protocol. Each subject will personally sign the consent form, we will not ask for a surrogate's permission to enter a patient into the study. The consent form will be obtained prior to the initiation of any study procedures, including those done for screening. Strict confidentiality will be maintained by the research team at all times, including keeping all data in a secure, locked cabinet with limited access.

Radiation risks
None. We do not request use of x-rays for research purposes.

## VIII. POTENTIAL BENEFITS

Benefits to participating individuals:
Subjects breathing NO might benefit from a decreased risk of COVID-19 disease development after professional exposure to SARS-CoV-2 positive patients.

Benefits to society:
There is no definitive drug available for the treatment of the disease or for the prevention of the development of COVID-19 disease. New information suggests that remdesivir may speed up a patient's recovery and discharge from the hospital and can be used in the hospital for severe COVID-19 infection, however it has not been used in healthcare providers.

One of the major benefits of NO might be the reduction of healthcare disease development and subsequent reduced need for quarantine. If administration of NO in this trial showed clinical benefits, the reduced number of quarantined healthcare providers will give a great support to the whole healthcare system in this world pandemic.

## IX. MONITORING AND QUALITY ASSURANCE

Independent monitoring of source data
Informed consent forms, case report forms, and data will be reviewed by the principal investigator following enrollment of every 50 subjects.

Safety monitoring (e.g. Data Safety Monitoring Board)
An interim safety report will be sent to IRB after the enrollment of the 30th subject.
The Data Safety Management Board (DSMB) will perform an interim analysis for superiority after the 25$^{th}$ patient. In case of a significant decreased infection rate in the treated subjects, the trial will be stopped.
Safety data include levels of Methemoglobin, $NO_2$ levels, minor deviation from the protocol and minor adverse reactions to non-invasive ventilation. Other data to be reviewed includes maintenance of patient confidentiality throughout the study.
Members of the Data Safety management Board (DSMB) consist of an intensivist, a virologist and an NO expert. Names and contacts of the DSMB members are:

Richard Pino MD, PhD
Associate Professor of Anesthesia at Harvard Medical School
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John Thomas MD, PhD
Professor Emeritus in Microbiology at West Virginia University
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Carl Nathan, MD
R.A. Rees Pritchett Professor of Microbiology
Weill Cornell Medicine
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Outcomes monitoring

All the subjects will undergo an oro/nasopharyngeal swab with RT-PCR for SARS-CoV-2 at COVID-19 symptoms development. Follow up will continue for 14 days after recruitment as described in "V Study procedure".

Adverse event reporting guidelines

In accordance with PHRC policy on Adverse Event Reporting and Unanticipated Problems Involving Risks to Subjects, the Principal Investigator (Lorenzo Berra, MD) will report adverse events or other unanticipated problems to the DSMB and to the PHRC within 5 working days/7 calendar days of the date the investigator first becomes aware of the problems. Mild or moderate adverse events will be presented in progress reports at continuing reviews.

**Stopping rules.** The review and decision regarding altering or stopping the protocol will be performed by the principal investigator together with the Data Safety Management Board (DSMB). Mild or moderate adverse events will be presented in progress reports at continuing reviews. Protocol exit criteria will be:

a. Acute worsening hypotension: decrease in mean blood pressure of > 20 mmHg not attributable to other causes, such as: progression of the disease, hypovolemia, hemorrhage, sepsis, acute heart failure.
b. Sudden hypoxemia defined as a 5% reduction of peripheral oxygen saturation ($SpO_2$) of from the basal value.

Or any life-threatening symptom potentially attributed to NO administration by the physician investigator.

## References


1. *https://www.healthmap.org/covid-19/.*
2. *https://www.arcgis.com/apps/opsdashboard/index.html#/bda7594740fd40299423467b48e9ecf6.*
3. WHO, *Coronavirus disease 2019 (COVID-19) Situation Report - 37.* 2020.
4. Wang, D., et al., *Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China.* JAMA, 2020.
5. Yang, X., et al., *Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study.* The Lancet Respiratory Medicine, 2020.
6. Zhou, P., et al., *Protecting Chinese Healthcare Workers While Combating the 2019 Novel Coronavirus.* Infection Control & Hospital Epidemiology, 2020: p. 1-4.
7. Privett, B.J., et al., *Examination of bacterial resistance to exogenous nitric oxide.* Nitric oxide : biology and chemistry, 2012. **26**(3): p. 169-173.
8. McMullin, B.B., et al., *The Antimicrobial Effect of Nitric Oxide on the Bacteria That Cause Nosocomial Pneumonia in Mechanically Ventilated Patients in the Intensive Care Unit.* Respiratory Care, 2005. **50**(11): p. 1451-1456.
9. Akerstrom, S., et al., *Nitric oxide inhibits the replication cycle of severe acute respiratory syndrome coronavirus.* J Virol, 2005. **79**(3): p. 1966-9.
10. Akerstrom, S., et al., *Dual effect of nitric oxide on SARS-CoV replication: viral RNA production and palmitoylation of the S protein are affected.* Virology, 2009. **395**(1): p. 1-9.
11. Keyaerts, E., et al., *Inhibition of SARS-coronavirus infection in vitro by S-nitroso-N-acetylpenicillamine, a nitric oxide donor compound.* Int J Infect Dis, 2004. **8**(4): p. 223-6.
12. Chen, L., et al., *Inhalation of nitric oxide in the treatment of severe acute respiratory syndrome: a rescue trial in Beijing.* Clin Infect Dis, 2004. **39**(10): p. 1531-5.
13. Deppisch, C., et al., *Gaseous nitric oxide to treat antibiotic resistant bacterial and fungal lung infections in patients with cystic fibrosis: a phase I clinical study.* Infection, 2016. **44**(4): p. 513-20.
14. Regev-Shoshani, G., et al., *Gaseous nitric oxide reduces influenza infectivity in vitro.* Nitric Oxide, 2013. **31**: p. 48-53.
15. Miller, C., et al., *Gaseous nitric oxide bactericidal activity retained during intermittent high-dose short duration exposure.* Nitric Oxide, 2009. **20**(1): p. 16-23.
16. Lenth, R.V., *Java Applets for Power and Sample Size [Computer software]. Retrieved 02/24/2020 from http://www.stat.uiowa.edu/~rlenth/Power.* 2006-9.

## APPENDIX A. NITRIC OXIDE GENERATOR

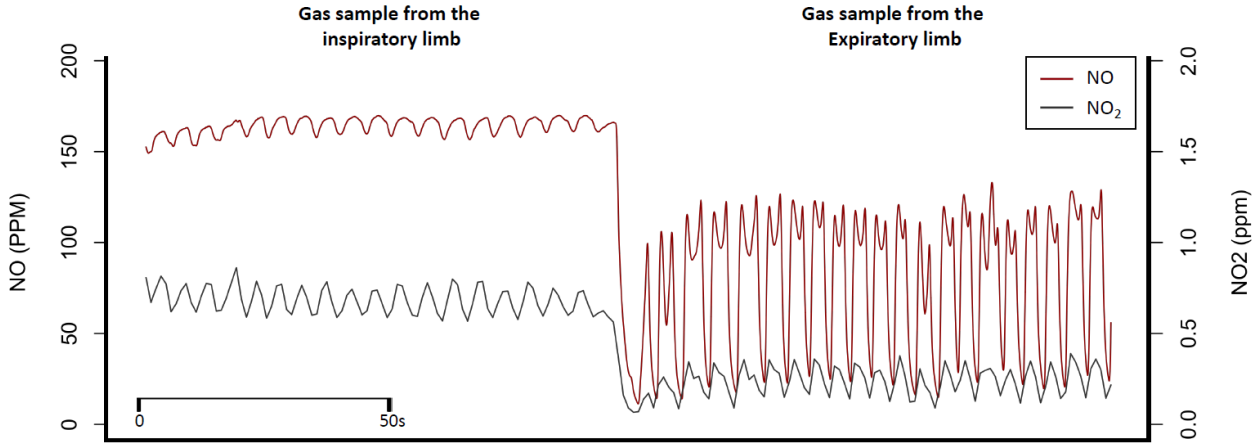
As of April 27, 2020, we have enrolled 16 HCWs, of which 8 receive NO treatments, for a total 64 administrations. We did not experience any adverse events and methemoglobin levels remained within safe range. Please see Table 2 for more details.

We also measured inspiratory and expiratory concentrations of NO and $NO_2$ gas in a health-care worker enrolled in our present study breathing 160 ppm of NO from a NO/$N_2$ tank mixed with air (please see Figure 1). Please note that the levels of $NO_2$ remain below 1 ppm during the entire respiratory cycle both in inspiratory gas and in exhalation gas.

| | |
|---|---|
| Number of subjects treated | 8 |
| Number of nitric oxide gas administrations | 64 |
| Nitric oxide dose administered (ppm) | 160 |
| NO2 (ppm) | 0.98 |
| SpO2 baseline (%), mean ± SD | 97.1 ± 1.38 |
| SpO2 end of administration (%), mean ± SD | 96.1 ± 1.53 |
| Methemoglobin baseline (%), mean ± SD | 0.83 ± 0.43 |
| Methemoglobin end of administration (%), mean ± SD | 1.68 ± 0.37 |
| Methemoglobin 5 min after administration (%), mean ± SD | 1.56 ± 0.38 |

*Table 1.*

*Figure 1: Inspiratory and expiratory concentration of NO and $NO_2$.*




Since our study opened enrollment, we have been challenged by the large numbers of healthcare workers willing to participate to our trial. Using only tanks, we have no means to deliver NO gas to more than two volunteers at each session, as each volunteer requires a NO tank setup, which is cumbersome, large, and needs additional equipment (oxygen flowmeter and a source of oxygen), and additional personnel.

The use of an electric NO-from-air generator would allow us to avoid (I) handling and transporting heavy tanks, (II) tank storage, (III) additional respiratory tubing and an oxygen source (tank NO is mixed with nitrogen so to maintain the FiO2 at 21% (air) we require an external oxygen line).

In the Department of Anesthesia, Critical care research unit at MGH, we recently conceived, built and tested two electric NO generators that produces NO gas equivalent to NO commercial tanks. However, since it produces NO from ambient air it does not require an additional source of oxygen and allows us to treat at least 12 additional HCW per day.

In awake sheep, we successfully tested the device to determine the vasodilator response of the pulmonary circulation at clinical concentrations of inhaled NO, from 20 to 80 parts per million (ppm) (please see manuscript, Yu et al. "Intratracheal injection of nitric oxide, generated from air by pulsed electrical discharge, for the treatment of pulmonary hypertension in awake ambulatory lambs" Nitric Oxide. 2020 Apr 1;97:11-15.)

In three sheep, we also delivered higher concentrations of NO gas (up to 240 ppm of gas) and measured the levels of nitric dioxide ($NO_2$), which remained low, below 1 ppm (Table 2).

**Table 2.** Nitric oxide (NO) was delivered intratracheally to awake sheep. NO and $NO_2$ were sampled and measured inside the lamb's trachea.

| Awake sheep | Nitric oxide (NO, ppm) | Nitrogen dioxide ($NO_2$, ppm) |
|---|---|---|
| Sheep #1 | 150 | 0.61 |
| | 200 | 0.80 |
| | 220 | 0.91 |
| Sheep #2 | 160 | 0.62 |
| | 210 | 0.87 |
| | 240 | 0.90 |
| Sheep #3 | 130 | 0.52 |
| | 180 | 0.65 |
| | 200 | 0.76 |

The gas produced by our electric NO generator is pure and no particles were found in mice lungs after 28 days of breathing continuously NO at 50 ppm (Yu et al. Nitric Oxide. 2016. Detection and removal of impurities in nitric oxide generated from air by pulsed electrical discharge.)

In a previous study in the MGH catheterization laboratory, (Partners IRB #2014P00253636), we studied an early prototype of the same device. In six patients with chronic pulmonary hypertension we found that the device is able to produce an equivalent vasodilator response of NO obtained from a $NO/NO_2$ tank (Berra L, et al. Electric Plasma-generated Nitric Oxide: Hemodynamic Effects in Patients with Pulmonary Hypertension. Am J Respir Crit Care Med. 2016 Nov 1;194(9):1168-1170.).

This device has been inspected by the MGH Respiratory Care Dept N: 54923 (inspected in 12/2019).