High-Dose Inhaled Nitric Oxide in Acute Hypoxemic Respiratory Failure due to COVID-19: A Multicenter Phase 2 Trial

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STUDY METHODS

Study Design

This was an investigator-initiated multicenter, single-blinded, randomized (1:1), controlled, parallel-arm clinical trial conducted at five sites in the United States (Massachusetts General Hospital, University of Alabama at Birmingham, Louisiana State University, and Beth Israel Deaconess Medical Center) and Sweden (Danderyd Hospital). All participants or their medically authorized representatives provided written informed consent, and the study was approved by the Institutional Review Boards at each of the respective study sites. The detailed study protocol and the statistical analysis plan are included in this Online Data Supplement document. This study was registered on ClinicalTrials.gov as NCT04306393 (Registered on March 12th, 2020).

Participants

The study enrolled adult patients with SARS CoV-2 infection (confirmed diagnosis using RT-PCR) admitted to the intensive care units and were intubated and mechanically ventilated. Individuals were excluded if they had been intubated for >72 hours or the physician of record opposed the enrollment due to safety concerns. In July 2020, exclusion criteria were expanded to additionally exclude those who: 1) were enrolled in another intervention study, 2) had a prior medical history of lung malignancy, pneumonectomy, or lung transplant, 3) were receiving tidal volume support <3 ml/kg of ideal body weight at the screening to assure NO delivery, 4) had severe burns (>40% of total body surface area), 5) had experienced cardiac arrest with cardiopulmonary resuscitation for longer than 30 minutes, 6) had a presumed severe deficit in cerebral function with fixed dilated pupil, 7) were receiving renal replacement therapy at the time of screening, 8) had a history of malignancy or other irreversible disease/conditions with an estimated six- month mortality > 50%, 9) had received inhaled nitric oxide gas before screening, or 10) were admitted to the hospital for reasons unrelated to COVID-19. Expansion of the exclusion criteria occurred during the first months of the trial to ensure a uniform patient population among centers

during the evolving pandemic. Figure 1 describes patient enrolment and follow-up as per CONSORT recommendations (Appendix, p 42).

Randomization and Masking

After informed consent was obtained, eligible participants were randomized on the same day using a centralized, secure computer platform stratified by site, age (≤ or >60 years-old), and sex. Because of the uncertainty of site enrolment due to the variable nature of the pandemic, a small, fixed block size of two was used for the first 20 allocations at each site. Future assignments were conducted using randomized permuted blocks of size 2 and 4 to respective study arms. All assignments were generated using a 1:1 ratio using a randomization scheme prepared by the study statistician. Allocation concealment was accomplished using a centralized computer system. The study interventions included either the institutional usual care alone (control arm) or the institutional usual care with the addition of high-dose inhaled nitric oxide (treatment arm). Usual care was delivered according to each institution's protocols (including ventilation strategies and use and dosage of antivirals and antimicrobials, anti-inflammatory agents including steroids, inhaled nitric oxide as a rescue therapy at 5-20 ppm when PaO_2/FiO_2<100 mmHg, inotropic-vasopressor agents and initiation of extracorporeal membrane oxygenator). Enrolment of participants occurred at the onset of the pandemic and before vaccines were available. In order to minimize aerosolization procedures and associated possible contamination and infection of healthcare professionals from the break of the ventilator circuit per recommendations of the World Health Organization, no placebo was used.16 Masking was only possible for participants and/or their legally authorized representative, as patients were unconscious and no one other than medical personnel was allowed in the participant's room. Healthcare professionals and study team members assessing outcomes were not blinded to the treatment.

Procedures

Participants in the treatment arm received inhaled nitric oxide at 80 ppm for the first 48 hours after enrolment. The gas was started immediately after randomization within the first 72 hours of mechanical ventilation. After the first 48 hours of treatment, the gas was reduced to 40 ppm and maintained at this concentration until severe hypoxemia resolved (PaO_2/FiO_2>300 mmHg). The gas administration was synchronized with the ventilator and delivered through an injector module at constant concentration throughout the respiratory cycle into the inspiratory limb of the patient's breathing circuit. Weaning from inhaled nitric oxide was initiated when PaO_2/FiO_2>300 mmHg was recorded for a duration of greater than 24 hours. Gradual weaning by 50% every four hours was employed with cautious evaluation for rebound hypoxemia and acute hypotension. The previous dose was used when rebound hypoxemia or acute hypotension occurred. The detailed inhaled nitric oxide weaning protocol is described in the Appendix (pp 13-14).

Participants from all centers were evaluated by the research staff up to 90 days after study completion. In participating U.S. centers, alive participants were contacted on day 90 for a follow-up phone call. In addition, data were retrieved from physicians' notes to determine outcomes after hospital discharge (e.g., hospital readmission, admission to a rehabilitative facility, death) and to investigate functional status. The phone interviews were conducted by research staff through a structured questionnaire provided in the Appendix (pp 22-31). During the follow-up phone calls, participants were asked if they were experiencing any sensory symptoms or motor deficits. No phone calls were performed in Sweden, and data were retrieved only from the medical records (following the same format as the U.S. questionnaire). Electromyography and imaging (magnetic resonance imaging or computed tomography) reports were collected from medical records for participants reporting sensory or motor symptoms. For the final analysis, participants from any center were considered positive for neurological findings if sensory symptoms and/or motor deficits were confirmed and reported by a physician caring for the patient, such as a neurologist or physiatrist, in the medical charts.

Outcomes

The primary outcome of this study was the change in arterial oxygenation (PaO_2/FiO_2) at 48 hours. The secondary outcomes were all-cause mortality at 28 and 90 days, time to reach normoxaemia (defined by a PaO_2/FiO_2 >300 mmHg for at least 24 hours among survivors), and the proportion of normoxaemic participants in the two groups at 28 days. The safety outcomes for this clinical trial included methaemoglobinaemia defined as methaemoglobin (MetHb) exceeding 5%, inhaled nitrogen dioxide >3 ppm, haemodynamic instability (rebound hypotension) during weaning, the occurrence of acute kidney injury by 28-days, or the initiation of renal replacement therapy by 90 days. Exploratory study outcomes included change in viral load (Log10 copies of SARS-CoV-2 per mL) in plasma and sputum, duration of mechanical ventilation, use of venous-venous extracorporeal membrane oxygenator (VV-ECMO), and neurological signs and symptoms (motor and sensory) at 90 days.

Plasma and Sputum Preparation

Starting June 24th, 2020, at Massachusetts General Hospital, plasma and sputum (endotracheal aspirate in intubated participants, spontaneously expectorated in extubated participants) were collected prospectively. Biological samples of those participants who consented were stored at -80 oC for quantification of the SARS-CoV-2 viral load. There was no post-hoc selection of participants for this analysis. Quantification of viral load in blood and sputum was obtained as previously described.17 Briefly, samples were centrifuged at 21,000 g for 2 hours at 4 oC. The supernatant was removed, and Trizol-LS was added to the samples and vortexed briefly. The samples were incubated at 4 oC for 15 minutes and subsequently treated with chloroform. Samples were vortexed briefly and then centrifuged at 21,000 g for 15 minutes at 4 oC. The resulting supernatant containing RNA was then concentrated using isopropanol precipitation, and SARS- CoV-2 RNA was measured by RT-qPCR.

Statistical Analysis

Participants randomized to inhaled nitric oxide were hypothesized to have at least 20% greater improvement in PaO_2/FiO_2 at 48 hours after gas initiation compared with the usual care alone. Assuming a two-tailed alpha of 0.05, the enrolment of 182 participants would provide 90% power to detect an effect size of 38 mmHg PaO_2/FiO_2 change based on the effect estimates in a previous investigation in hypoxemic intubated and mechanically ventilated patients.18 Presuming a 10% dropout, the target sample size was 100 in each group (n=200 total). The original sample size estimates were derived using a frequentist approach to the analysis. However, due to reporting requirements posed by the FDA and IRB and the need for rapid learning in the context of the COVID-19 crisis, the analysis plan was revised (April 2020) to employ Bayesian estimation, which allowed for sequential group analyses. Prior probability distributions were specified for the intercept, the individual predictors, and the error term(s) (i.e., sigma for linear regression). Traditionally, weakly informative priors specified as ~ usual (0, 2.5 x SD of the outcome) were used. For the appropriateness of the choice of priors, a prior predictive check was conducted by simulating the distributions. Generally, the predicted values from the priors appeared reasonable, as the predicted values resided in a diverse range of plausible values for the outcome.

The baseline characteristics were summarized as the median and interquartile range for continuous data and counts and percentages for categorical data. Standardized mean difference (SMD) is reported to quantify the differences between the two study arms, with values greater than 0.20 suggesting a potential imbalance between groups. All study outcomes were analyzed using Bayesian estimation of generalized linear models. Bayesian estimation utilized in this trial allowed interpretation of the posterior probability distributions for respective effects. The primary and secondary outcomes analysis was conducted using a Bayesian framework that estimates the treatment effect conditional on several pre-specified additional variables included in the model (defined a priori: age, age^2, sex, BMI, and APACHE II score and variables with SMD>0.20). The PaO_2/FiO_2PaO2/FiO2 ratio was modeled for the primary outcome using a normal distribution and identity link function. The PaO2/FiO2 ratio at 48 hours was regressed on baseline (i.e., enrollment) PaO2/FiO2 ratio, randomized group assignment, and additional covariates, as specified

above. Secondary endpoints were conducted using outcome distributions and link functions appropriate to the outcome (e.g., normal, gamma, negative binomial) using the same covariates (i.e., age, age^2, sex, BMI, and APACHE II score and variables with SMD>0.20) and baseline PaO2/FiO2 ratio. The primary and secondary study outcomes are reported as adjusted effect estimates and 95% credible interval (CrI).

To assess the change in viral load in 149 plasma samples from 33 patients and 86 sputum samples from 22 patients, a Bayesian linear mixed-effect model was used to determine the effect of high dose inhaled nitric oxide on viral load (log10 ΔCT [threshold cycle]) over time. This model included fixed effects of the treatment arm, time from randomization, and an interaction term (arm × time), using a repeated measures covariance structure (first-order autoregressive structure). A random intercept at the level of the participant was used in these models.

All study outcomes were analyzed in the modified intention-to-treat population, excluding participants for whom respiratory treatment was unavailable at the study site or who did not meet the secondary review of the inclusion/exclusion criteria within 24 hours of enrolment. A total of seven patients were excluded after randomization for the modified intention-to-treat analysis (mITT, Figure 1). Due to the ongoing pandemic, relatives and family members were not allowed to come to the hospital. This limited the information on the medical history of critically ill patients entering the intensive care unit, rendering a mandatory secondary review of inclusion/exclusion criteria after randomization. Additionally, the pandemic impacted the supply of nitric oxide gas tanks at the respective study sites, which was sometimes intermittent.

All statistical analyses were performed using R 4.0.2 (R Core Team) with Bayesian estimation conducted in RStan. Bayesian posterior distributions are summarized using posterior means and two-sided 0.95 CrI. No formal adjustments were made to the inferences that would be appropriate for statistical significance thresholds using frequentist adaptive designs. Full details of the statistical analysis plan – including prior probability definitions, full model details, and stopping rules – are outlined in Appendix (p 32-41). The

trial was overseen by an independent data safety monitoring board (DSMB) which evaluated unmasked interim data for futility, efficacy, and safety. The DSMB met to review interim data analysis after every 25 patients were enrolled. The study continued until the target recruitment was reached. The only stopping rule for the trial was defined as a significant increase of mortality with nitric oxide gas.