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Nitric oxide for inhalation in the acute treatment of sickle cell pain crisis: A randomized clinical trial

Mark T. Gladwin, MD[1,2], **Gregory J. Kato, MD**[3], **Debra Weiner, MD, PhD**[4], **Onyinye C. Onyekwere, MD, MS**[5], **Carlton Dampier, MD**[6], **Lewis Hsu, MD**[7], **R. Ward Hagar, MD**[8], **Thomas Howard, MD**[9], **Rachelle Nuss, MD**[10], **Maureen M. Okam, MD, MPH**[11], **Carole K. Tremonti, RN**[11], **Brian Berman, MD**[12], **Anthony Villella, MD**[12], **Lakshmanan Krishnamurti, MD**[2], **Sophie Lanzkron, MD**[13], **Oswaldo Castro, MD**[5], **Victor R. Gordeuk, MD**[5], **Wynona A.**

[*]Address correspondence to: Dr. Mark T. Gladwin, Pulmonary, Allergy and Critical Care Medicine, Vascular Medicine Institute, University of Pittsburgh, Tel. 412-692-2210; Fax: 412-692-2260; gladwinmt@upmc.edu.

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Study concept and design (Steering Committee): Gladwin, Casella, Baldassarre, Weiner.
Acquisition of data: Mark Gladwin, MD, Gregory Kato, MD, Onyinye Onyekwere, MD, Lewis Hsu, MD, R. Ward Hagar, MD, Thomas Howard, MD, Brigitta G. Mueller, MD, Rachelle Nuss, MD, Maureen Okam, MD, Lakshmanan Krishnamurti, MD; Brian Berman, M.D., Victor R. Gordeuk, MD,; Wynona Coles, RT, Mariana Hildesheim, MS, William C. Blackwelder, PhD, James Baldassarre, MD, Debra Weiner, MD, PhD, James Casella, MD
Analysis and interpretation of data: Gladwin, Kato, Casella, Baldassarre, Weiner, Hildesheim, Blackwelder.
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Coles, RRT[3], **Marlene Peters-Lawrence, RN, RRT**[3], **James Nichols, RN**[3], **Mary K. Hall, CIP**[14], **Mariana Hildesheim, MS**[1,3], **William C. Blackwelder, PhD**[14], **James Baldassarre, MD**[15], and **James F. Casella, MD**[16] **for the DeNOVO Investigators**

[1]Division of Pulmonary, Allergy and Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA

[2]Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, PA

[3]Pulmonary andVascular Medicine Branch, National Heart Lung and Blood Institute/NIH, Bethesda, MD (personal views that do not represent the Government)

[4]Division of Emergency Medicine, Children's Hospital Boston, Harvard Medical School, Boston, MA

[5]Howard University, Washington, DC; NHLBI, Bethesda, MD

[6]Emory University School of Medicine, and Children's Healthcare of Atlanta, Atlanta, GA, USA

[7]Children's Hospital of Oakland, Oakland, CA

[8]Children's National Medical Center, Washington DC

[9]University of Alabama at Birmingham, Birmingham, AL

[10]Pediatric Branch, Colorado Sickle Cell Treatment and Research Center, Univ. of Colorado Health Science Center, Denver, CO

[11]Brigham and Women's Hospital, Harvard Medical School, Boston, MA

[12]Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, OH

[13]Division of Hematology and Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

[14]Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD

[15]Dept of Clinical Research, Ikaria, Clinton NJ

[16]Division of Pediatric Hematology, Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD

Abstract

Context—Inhaled nitric oxide (NO) has shown evidence of efficacy in mouse models of sickle cell disease (SCD), case series of patients with acute chest syndrome, and 2 small placebo-controlled trials for treatment of vaso-occlusive pain crisis (VOC).

Objective—To determine whether inhaled NO gas reduces the duration of painful crisis in patients with SCD who present to the emergency room or hospital for care.

Design, Setting and Participants—Prospective, multicenter, double-blind, randomized, placebo-controlled clinical trial for up to 72 hours of inhaled NO gas versus inhaled nitrogen placebo in 150 participants presenting with VOC of SCD at 11 centers between October 5, 2004 and December 22, 2008. The primary endpoint was the time to resolution of painful crisis, defined by: 1) freedom from parenteral opioid use for 5 hours; 2) pain relief as assessed by visual analog pain scale scores ≤ 6 cm; 3) ability to walk; and 4) patient and family's decision, with physician consensus, that the remaining pain could be managed at home.

Intervention—Inhaled NO gas versus inhaled nitrogen placebo.

Results—There was no significant change in the primary endpoint between the NO and the placebo groups, with a median time to resolution of crisis of 73.0 hours (95% CI: 46.0–91.0) and

65.5 hours (95% CI: 48.1–84.0), respectively (P=.87). There were no significant differences in secondary outcome measures, including length of hospitalization, VAS scores, cumulative opioid usage and the rate of acute chest syndrome. Inhaled NO was well tolerated with no increase in serious adverse events. Increases in venous methemoglobin concentration confirmed compliance and randomization, but did not exceed 5% in any study participant. Significant increases in plasma nitrate occurred in the treatment group, but there were no observed increases in plasma or whole blood nitrite.

Conclusions—Among patients with SCD hospitalized with VOC, the use of inhaled NO compared with placebo did not improve time to crisis resolution.

Keywords

Nitric oxide; sickle cell disease; vaso-occlusive pain crisis; acute chest syndrome

Introduction

Sickle cell disease (SCD) is an autosomal recessive disorder of the β globin gene. Mutant hemoglobin S polymerizes in erythrocytes causing occlusion of the small blood vessels, manifesting clinically as episodes of severe pain (vaso-occlusive crisis, or VOCs), damage to vital organs, and early death.[1–7] VOC is common among patients with SCD, occurring at a rate of approximately 2 episodes per person year in the absence of treatment,[7] with a mean length of hospitalization during VOC of 4.5 days for children 10–14 yrs of age.[8, 9] As many as 20% of patients hospitalized for VOC develop the acute chest syndrome (ACS), a life threatening acute lung injury that prolongs the length of stay to a mean of 14 days.[10, 11] The national expenditure for inpatient sickle cell medical care in 2004 is estimated at $571,000,000 in 2010 dollars.[8] Given the suffering, high rate of morbidity and cost of care for VOC in SCD, and the absence of a current treatment option, there is an imperative to identify and evaluate new treatments.

0In recent years there has been much interest in understanding the possible pathophysiological and therapeutic roles of nitric oxide (NO) in SCD.[12–23] Nitric oxide is the critical effector of endothelial-dependent vasodilation and exerts pleiotropic effects on vascular and circulating blood cells, including the inhibition of platelet aggregation, down-regulation of cellular adhesion molecules, and modulation of ischemia-reperfusion injury, all pathways adversely affected during VOC.[24–30] Inhaled NO is a relatively safe agent, already approved by the Food and Drug Administration for hypoxic respiratory failure in newborn infants. Pre-clinical studies in transgenic mouse models have consistently demonstrated effects on inhibition of Gardos channels, reduction in red cell density, improved perfusion, or reductions in lung injury, microvascular vaso-occlusion and mortality.[31–36] Early clinical trials suggested inhaled NO could improve hemoglobin oxygen affinity,[14] although this result was not reproduced by other investigators.[37] Case reports have suggested beneficial effects of NO inhalation in patients with ACS.[38–40] A single institution placebo-controlled study of inhaled NO in children with SCD in VOC suggested decreased pain severity and reduced opioid analgesic usage, with trends toward reductions in length of hospitalization.[9] Recently, an 18 patient multicenter placebo-controlled study of inhaled NO in adults demonstrated a significantly greater reduction in pain and a trend toward lower narcotic use.[41]

To further evaluate the efficacy of inhaled NO, we undertook a phase II, randomized, double-blind, placebo-controlled, multi-center study of NO inhalation for up to 72 hours in 150 participants with SCD presenting with VOC.

Methods

This was a prospective, multicenter, double-blind, placebo-controlled, randomized, phase II study of participants with SCD presenting with VOC. The study was approved by the National Heart, Lung and Blood Institutes (NHLBI) Institutional Review Board and by each participating center's Institutional Review Boards. An NHLBI Data Safety and Monitoring Board (DSMB) monitored the study conduct and safety and an unblinded independent biostatistician (W. Blackwelder) reported interim methemoglobin safety results for children and adults to the DSMB. This was requested by the DSMB to ensure that children on inhaled NO in this trial were not subjected to increased risk of methemoglobinemia. Eleven sites (National Institutes of Health, Johns Hopkins University, Boston Children's Hospital, University of Alabama at Birmingham, Howard University Hospital, St. Christopher's Hospital for Children, Children's Hospital Oakland/Alta Bates Medical Center, University of Colorado Health Science Center, Brigham and Women's Hospital, Cleveland Case Western University, Children's Hospital of Pittsburgh) participated between October 5, 2004 and December 22, 2008.

Participants with known SCD age 10 years and older were identified during presentation with VOC to the Emergency Department/Emergency Clinic (ED/EC) or other appropriate unit. Written informed consent was obtained, with patients under age 18 providing assent along with parental consent. Participants were also recruited in the outpatient setting while not in pain, signing a final consent form at the time of VOC presentation. Exclusion criteria included: HbSC disease; exposure to therapeutic NO within the previous 12 hours; use of phosphodiesterase 5 inhibitors, L-arginine, nitroprusside or nitroglycerine within the previous 12 hours; previous ED/EC or other appropriate unit treatment for a VOC within 48 hours, or hospitalization within14 days of presentation with VOC; ED/EC visits or hospitalizations for VOC >10 times in the preceding year; clinically diagnosed bacterial infection at presentation; current enrollment in any other investigational drug study, except for hydroxyurea studies; current pregnancy or nursing; chronic transfusion or exchange transfusion in the preceding 30 days; suspected splenic sequestration; new pulmonary infiltrate at presentation; or previous participation in the study.

Participants were randomized using block randomization by site and age at entry (age 10–15 and >15 years), in blocks of 4, in a 1:1 ratio of placebo to inhaled NO. Randomization was defined at the time a set of study placebo or NO gas cylinders was assigned and a cylinder was opened.

NO for inhalation (Ikaria - formerly INO Therapeutics, Port Allen, LA) was supplied at a concentration of 800 ppm balanced with nitrogen (99.92% grade 5 nitrogen, 0.08% pharmaceutical grade NO). Placebo study gas was 100% Grade 5 nitrogen gas. Either NO or placebo was delivered with air and mixed with oxygen to achieve a constant FiO_2 of 24%. Participants were treated via facemask using a continuous flow delivery system. Those randomized to inhaled NO received 80 ppm for 4 hours, followed by 4 hours of 40 ppm. For participants remaining in the hospital after the initial 8 hour dose, study gas was administered through a pulsed flow delivery system with 1L continuous oxygen via nasal cannula at a dose of 6 mL/pulse/breath of 800 ppm NO for body weight ≥27 kg, or 3 mL/pulse/breath if <27 kg, up to a maximum of 72 hours total study gas administration. This is the first clinical trial to use a pulse delivery system, which delivers a lower dose of NO gas to the circulation, equivalent to approximately 5 ppm depending on minute ventilation. The use of pulse nasal canula delivery was considered necessary to facilitate the practical use of prolonged gas therapy for inpatients. The oxygen flow was increased as required to maintain $SaO_2 \geq 85\%$, with a maximum of 4L permissible to continue in the study. If study gas was

interrupted for more than 1 hour, it was not restarted. If the gas was stopped or a patient withdrew from treatment, the time to resolution of the VOC was still collected.

Coded labels were applied to the study cylinders at the manufacturing site. A "blinded" version of the face mask NO delivery systems blanked out and covered the NO and NO_2 monitor displays. The placebo gas was administered in the same way and over the same time to ensure that participants and investigators were blinded.

Pain associated with the VOC was measured on a scale of 0–10 using a visual analogue scale (VAS), which consisted of a 10 cm horizontal line with the ends representing the extreme limits of "no pain" (0) and "worst pain" (10). The participant was asked to make a mark along the line to indicate the intensity of pain at baseline and at hours 2, 4, 6 and 8 after the start of the study drug and then at 4 hour intervals. Each participant's score was the measurement in cm from 0 of his mark, to the nearest 0.1 cm. Starting with hour 12, sleeping participants were not woken to complete the VAS and a missing value was assigned. Participants were not shown their previous response. Demographic, clinical and laboratory variables were collected by the site coordinators from source documents and recorded on study case report forms.

Primary Efficacy Variable

The primary efficacy variable was the time to VOC resolution, modified from the poloxomer-188trial,[42] defined by all of the following criteria being met: freedom from parenteral opioid use for at least 5 hours; pain relief [VAS scores ≤6 cm maintained during 2 consecutive readings obtained at least 2 hours apart, each at least 3 hours after the last dose of parenteral opioids]; ability to walk (except for chronically non-ambulatory participants); and agreement of the physician, and patient and parent or guardian that residual pain was low enough to be manageable at home. Time to VOC resolution for participants who were discharged with missing endpoint data or with incomplete criteria for VOC resolution was censored at the actual time of discharge from the hospital. Death before discharge without meeting the definition for VOC resolution was censored at a time later than the latest time of censoring or VOC resolution in participants who did not die before discharge. For participants in the hospital longer than 30 days without crisis resolution, the duration of crisis was determined by the time of the discharge order.

Secondary Efficacy Variables

The following secondary efficacy variables were evaluated: length of hospitalization from admission to discharge (time of discharge order); VAS score over time; total dose of opioids in the first 8 hours after enrollment into the study and during the entire hospitalization; rate of ACS or pneumonia requiring blood transfusion; proportion discharged in the first 24 hours; proportion returned to ED or hospital within 30 days;; change in nitrate/nitrite levels and methemoglobin levels as measures of NO metabolism and reactions in the blood (Table 2). Secondary evaluation of possible pain relapse was determined by the proportion of participants treated again for pain in the ED/EC, hospital or other appropriate unit within 24 hours and within 30 days after hospital discharge.

Safety Monitoring

Since the primary known toxicity of inhaled NO is methemoglobinemia, venous methemoglobin was monitored at baseline and every 2 hours for the first 8 hours, then every 24 hours for the rest of the study. Bedside personnel and site investigators were not allowed access to methemoglobin levels, which were reported by designated laboratory personnel through an interactive voice response system at each site to a central safety monitor, who notified site investigators if dose change was indicated. Methemoglobin values were only

accessible to the DSMB and an unblinded monitoring statistician reporting to the DSMB. If any value was ≥ 5%, the treatment dose was to be decreased by 50%. If any value was >7.5%, the investigational therapy was to be discontinued. Other stopping rules included: assessment of the investigator, treating physician or participant that discontinuation of the inhalation therapy was in the patient's best interest; any serious adverse event thought to be related to the investigational therapy; clinically significant hypotension; sepsis or septic shock; or sustained pulse oxygen saturation below 85% for >15 minutes while on supplemental oxygen up to 4L by nasal cannula or 35% oxygen by mask. Participants who were discontinued from therapy remained in the study and continued with all data collection, unless consent to do so was withdrawn.

Serious adverse events were recorded during study gas inhalation and defined as any event that at any dose required hospitalization or resulted in disability or death (Table 3). As is standard in clinical trials in the SCD field, ACS was considered a serious adverse event during active treatment with NO because it is one of the major complications that occurs during hospitalizations for pain crisis. ACS requiring blood transfusion was also captured as a secondary outcome measure both on and off NO or placebo treatment (Table 2).

Statistical and Analytic Plans

At the end of the study, the dataset was analyzed by the steering committee, independently of the sponsor, using pre-specified analyses, endpoints and subgroups. Interim analysis of efficacy was not planned or performed.

Sample size was estimated based on data from a previous study of NO therapy in children[9] and AHRQ,[8] predicting a mean length of stay approximating 106 hrs. For the purposes of the calculation, time to crisis resolution, which was selected to minimize effects of factors unrelated to medical condition that impact discharge time, was assumed to approximate length of stay. The study was designed to have 80% power to find a significant decrease in duration of crisis if the true decrease was 24 hours. Sample size was based on a difference in $\log_{10}$ (duration), since the logarithm of duration was more normally distributed than untransformed values. Assuming a 2-sided Wilcoxon rank-sum test at the 5% significance level, normal distributions for $\log_{10}$ (duration) with difference 0.11 ($\log_{10}$ (106) − $\log_{10}$ (82)) and common standard deviation 0.22, we obtained a sample size of 68 per group, or a total of 136. Allowing for 9% of participants withdrawing or censored, the sample size became 75 participants per group, or a total of 150. Sample size and power calculations were done using PASS 2005 software (Number Cruncher Statistical Systems, Kaysville, Utah).

All analyses were intention to treat and 2-sided P-values less than 0.05 were considered significant. Clinical and laboratory characteristics were compared between the inhaled NO treatment group and the placebo group by the nonparametric Wilcoxon rank sum test for continuous variables and Pearson's chi-square statistic for categorical variables. Treatment efficacy was evaluated by calculating Kaplan-Meier survival curves for duration of crisis over time, using the log-rank test to determine significant differences in time to resolution between the 2 groups. The effect of inhaled NO vs. placebo was also examined in pre-defined subgroups by age (10–15 years and >15 years) and hydroxyurea use, as suggested by the poloxomer-188 trial in which young patients and those on hydroxyrea therapy appeared to respond better to treatment.[42] The effect of treatment on length of hospital stay and opioid use, both total and cumulative, were examined using the Wilcoxon 2-sample test. Treatment related changes in pain score and NO metabolites were evaluated using an unpaired 2-tailed t-test to examine differences at specific points in time and repeated measures ANOVA to examine changes over time. The frequency of dichotomous secondary outcomes and serious adverse events was examined using either the Pearson's chi-square or the Fisher's exact test.

Cox Proportional Hazards regression models were used to examine the association of treatment with duration of crisis while adjusting for significant covariates. The likelihood ratio test was used to determine the significance of individual regression coefficients. As a post-hoc analysis, associations of potential confounders, including study site, gender, baseline VAS score, age and hydroxyurea use, with time to crisis resolution were examined using Kaplan-Meier analysis. The effect of study site on length of hospitalization, total opioids and baseline VAS score was performed using either the Kruskal-Wallis test or ANOVA.

In a post-hoc analysis, comparisons of time to resolution with other measures of disease severity were examined using the Spearman rank order correlation coefficient and the Wilcoxon 2-sample test. All analyses were performed using SAS version 9.1.3 (SAS Institute Inc, Cary, NC) and Stata version 9.0 (StatCorp LP, College Station, TX).

Results

Of 1,078 patients with SCD who were assessed for eligibility or pre-enrolled in steady state, 150 presenting during VOC were enrolled and randomized. Four participants withdrew in each group but were included in the intention to treat analysis (Figure 1). There were no deaths. Table 1 describes the characteristics of the NO-treated and-placebo treated groups, which were balanced in terms of age, gender, genotype, hydroxyurea use, vital signs, pain scores, and laboratory values.

Efficacy of inhaled NO gas versus placebo on VOC

Time to VOC resolution did not differ significantly according to treatment (p=.87; Figure 2), with an estimated median time to resolution of crisis of 73.0 hours (95% CI: 46.0–91.0) for the inhaled NO group and 65.5 hours (95% CI: 48.1–84.0) in the placebo group. Additionally, the planned secondary analyses did not differ significantly according to experimental treatment (Table 2), including median length of hospitalization [4.1 (IQR 2.0–6.0) vs. 3.1 (IQR 1.7–6.4) days, inhaled NO vs. placebo; p=.30], mean VAS scores [6.1 (95%CI: 5.3–6.8) vs 6.0 (95% CI: 5.4–6.6) cm at 24 hours, p=.90; eFigure 1A], and median total opioid use [2.8 (IQR: 1.4–6.1) vs. 2.9 (IQR: 1.1–9.9) mg of morphine equivalents/kg, p=.73]. Decreases in mean VAS scores over 2 hour intervals in the first 8 hours of treatment were also not different by treatment group, with reductions in pain score in the inhaled NO group ranging from 0.4 (95% CI 0.1–0.8) to 1.3 (95% CI 0.8–1.8) compared with reductions from 0.7 (95% CI 0.3–1.1) to 1.2 (95% CI 0.7–1.8) in the placebo group (p=.90). Cumulative opioid use up to 72 hours after presentation also yielded no effect of inhaled NO vs. placebo [0.44 (IQR: 0.3–0.7) vs. 0.47 (IQR: 0.3–0.6) mg/kg over 4 hours, p=.47; 0.71 (IQR: 0.6–0.9) vs. 0.70 (IQR: 0.3–0.9) mg/kg over 8 hours, p=.19; and 0.95 (IQR: 0.8–1.3) vs. 0.92 (IQR: 0.7–1.2) mg/kg over 12 hours, p=.35; eFigure 1B]. There were no differences between the groups in the percentage of participants that developed ACS requiring a transfusion over the entire study period (Table 2) or in those with ACS as a reported serious adverse event during study gas inhalation (Table 3). The rare event of being rehospitalized within 30 days was almost twice as frequent in the placebo group, but the difference was not statistically significant (Table 2).

Effect of inhaled NO gas on methemoglobin, nitrate and nitrite

We measured the reaction products of NO in blood and plasma, methemoglobin, nitrate and nitrite. Participants on NO gas inhalation had significantly higher levels of methemoglobin in the venous blood (p<.001; Figure 3) consistent with NO gas exposure, but no participant's methemoglobin values exceeded 5%, considered a toxic level. Participants randomized to inhaled NO showed an increase in mean venous methemoglobin at 4 hours [2.3 (95% CI

2.1–2.5) vs. 0.8 (95% CI 0.7–1.0), p<.001] and 8 hours [1.7 (95% CI 1.5–1.9 vs. 0.9 (95% CI 0.7–1.0), p<.001]. Participants receiving NO had higher nitrate levels in plasma (p=.03) but did not demonstrate significant increases in nitrite in plasma or whole blood (p=.77 and . 31) (eFigures 2A, B and C).

Potential confounding factors

Study site, gender, pain score, alkaline phosphatase and total bilirubin were independently associated with time to VOC resolution (eTable 1). The estimated median time to VOC resolution (Figure 4A–C) was significantly shorter at selected study sites [41.5 (95% CI 28.0–59.3) hours at NIH and JHU vs. 91.0 (95% CI 73.7–123.0) hours at other sites; p<. 001], with shorter time to VOC resolution in males [59.5 (95% CI 43.8–75.0) vs. 90.0 (95% CI 59.3–112.7) hours, males vs. females; p=.04], and longer time to VOC resolution in those presenting with higher pain scores [91.0 (95% CI 73.7–123.0) vs. 47.3 (95% CI 31.4–64.0) hours, VAS ≥7.7 vs. <7.7 cm; p<.001]. Patients on hydroxyurea therapy did not differ significantly from those who were not on therapy [73.7 (95% CI 59.3–93.0) vs. 56.0 (95% CI 42.7–75.3) hours, HU vs. no HU, p=.4; Figure 4D]. Median time to VOC resolution also did not differ significantly by age [76.3 (95% CI 46.1–132.0) vs. 72.0 (95% CI 52.8–78.0) hours, ≤15 vs. >15 years; p=.3]. The study site effect was significant and consistent across many variables. The NIH and JHU sites enrolled participants who were reporting less pain, had shorter hospitalization times, and received significantly less cumulative opioid (eTable 2). Adjustment for these potentially confounding effects did not affect the responses to NO gas inhalation vs. placebo.

Validation of time to VOC resolution and endpoint analysis

The time to VOC resolution as defined in this study appeared to correlate well with other markers of disease severity. Spearman rank order correlation coefficients of time to resolution of VOC with various other secondary endpoints were 0.92 (p<.001) for duration of hospitalization, 0.84 (p<.001) for total opioid use, and 0.40 (p<.001) for pain score at baseline. Time to resolution of VOC was also greater in participants with ACS compared to participants without ACS [142.4 (IQR 91.0–219.8) vs. 56.0 (IQR 24.8–99.6) hours; Wilcoxon p<.001] and in participants reporting one or more SAEs, compared with participants reporting no serious adverse events [95.7 (IQR 78.0–172.0) vs. 59.5 (IQR 26.5–104.4) hours; Wilcoxon p=.01].

Discussion

Inhaled NO gas had no effect on the time to VOC resolution or on any of the planned secondary analyses, including length of hospitalization, change in VAS scores, and total opioid use. Sustained inhaled NO delivered for 8 hours by face mask and for up to 72 hours by a nasal cannula pulse system was well tolerated. No value of methemoglobin exceeded 5% and no increase in serious adverse events were observed.

The design of this study overcame many of the challenges that have affected previous therapeutic trials in SCD VOC. The placebo and treatment groups were well balanced. While many of the endpoints were necessarily subjective, we found that these correlated well with objective measures of disease severity. We designed our trial to minimize missing and censored data at discharge. The time to VOC resolution was chosen as an endpoint, rather than the actual duration of hospitalization, to capture more precisely the time at which the crisis functionally ended, independent of non-medical factors that impact discharge time, such as the availability of family members or transportation and the timing of physician and nursing shift cycles. Our adoption of a discharge VAS pain score ≤ 6 cm, rather than ≤ 4 cm,

appears to have resulted in fewer censored participants than the prior study using this endpoint. [42]

It is notable that the median duration of crisis and length of hospitalization were much shorter than predicted based on data obtained from AHRQ and prior studies, perhaps partly due to our entry criteria, which required ED presentation but not necessarily a decision to hospitalize.[8, 9] The time to resolution in patients with ACS of 142.4 hours was much shorter than reported in prior studies and the 10% incidence of ACS during hospitalization lower than reported in prior studies. A requirement for mechanical ventilation was a rare event in this trial and none of the patients died. The lower duration of hospitalization for ACS is potentially related to the rapid evaluation and treatment of pre-enrolled patients, a lower threshold for hospitalization in the context of this trial, as well as aggressive transfusion therapy for complications, a practice that is now routine in the participating centers.

The lack of any observable effect of inhaled NO in this trial may be the result of a lack of systemic generation of nitrite, which has been shown to exhibit therapeutic effects in models of ischemia and reperfusion injury.[43, 44] In pre-clinical studies of inhaled NO for ischemia and reperfusion injury, the levels of nitrite in the circulation and target organs increased significantly.[45–47] The absence of increase in nitrite could be due to our mode of NO administration. The pulse delivery of NO provides a pulse of pure NO in nitrogen at the “front” of the tidal volume. This reduces mixing of NO with oxygen in the airways, which can form nitrogen dioxide, dinitrogen trioxide and nitrite. Failure to see an effect of NO could also be due to the fact that tissue injury in VOC is not reversible by the time it is recognized clinically.

We observed major effects of study site. The NIH and JHU sites admitted more participants to the study with less pain, who used less opioids and exhibited shorter durations of crisis. The reasons for these site variations are not clear, but could represent an increased capability to identify and enroll patients early in their crises at these sites, which was an expressed goal of this trial, before a decision about admission to the hospital was made. While the NIH site pre-enrolled patients and directly admitted patients with VOC, the JHU site enrolled patients out of the emergency room, suggesting that care delivery structure did not determine this effect.

The overall median crisis duration of 65.5 hours in the placebo arm is of potential concern, as the study was designed to have adequate statistical power to observe a reduction of approximately 1 day from an expected 4 day duration of crisis in placebo controls. However, given that there was no evident effect of NO, even when adjusted for sites with longer durations of VOC, it seems unlikely that this accounts for the lack of apparent efficacy. A lack of effect on secondary outcomes such as pain scores and narcotic use also supports a primary lack of efficacy. An unexpected observation was that female participants had longer durations of crisis and hospitalization, which has been reported in retrospective studies, but this finding deserves further study.[48] Increasing VAS at admission was associated with a longer length of stay, consistent with prior studies.[48] The characteristics of VOC and its management should inform the design of future VOC trials.

Limitations of this study include the relatively small sample size (150 patients) and the broad, but overlapping confidence intervals for the medians of the primary outcome variable, leaving open the possibility that a small treatment effect may have been missed. If such a treatment effect exists, it is likely to be less that our predetermined minimal clinical significance of a 25 % reduction in duration of crisis. Finally, this study used a subjective endpoint, as there are no true objective indicators of cessation of crisis.

In summary, the results of this study indicate that inhaled NO in the doses and methods of administration used in this study does not reduce VOC severity in SCD. These results underscore the need for new agents and a sustained clinical trials apparatus for studying VOC, with sufficient numbers of patients to provide adequate power to rapidly test promising therapeutics in patients with SCD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Role of Ikaria in the “design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript": Study drug, placebo and delivery devices were provided by Ikaria®. Ikaria, formerly INO Therapeutics, provided the Data Coordinating Center so were involved in the conduct of the study, collection of data, and management of the site data collection. Dr. Baldassarre is an employee of Ikaria and contributed to the analysis of data as an author. Ikaria and Dr. Baldassarre reviewed the manuscript but did not have rights to approve the manuscript. The study design and statistical analysis was performed independently by study Investigators and was performed under the auspices of a National Institutes of Health Government Collaborative Research and Development Agreement. The blinded statistical reviewer was employed by the NIH and not Ikaria.

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Appendix

DeNOVO clinical site support teams (sub-investigators, study coordinators, and respiratory therapists): National Institutes of Health: Catarina Minniti, MD, Kevin Coles, RRT; Howard University Hospital: Abiodun Akintilo, MS, Alice Ukaegbu, MSN, FNP, George Tandongfuet, RRT; John's Hopkins Univ. of School of Medicine: Phillip Seaman, PA; Gary Oldenburg, RRT; St. Christopher's Hospital for Children: Maureen Meier (Rockstein), RN, CCRC, Marylou MacDermott, MSN, CRNP, Steven Johnson CRTT, MEd, Annette Horton, RRT; Alta Bates Medical Center: Vickie Nolan, Terri Nielsen, RN, BSN, Lou Schroel; Children's Hospital Boston: Eric Fleegler, MD, Matthew Heeney, MD, Pam Boardman, MPH, Nancy Craig, RTT; Univ. of Colorado Health Science Center: Julie McAfee, BA, Laura Cole, Donna Parker, RRT; Brigham and Women's Hospital: Ronald McCaffrey, MD, Ainlsey Ross, BA; Case Western University: Philip Toltzis, MD; University of Alabama at Birmingham: Lindley Webb, CPNP, Roy McDonald, Lindley Webb, CPNP, Mary Jones, Craig Wooten, RT; Children's Hospital of Pittsburgh: Maria Schmucker, Al Saville, RRT, Mike Simpson, RRT

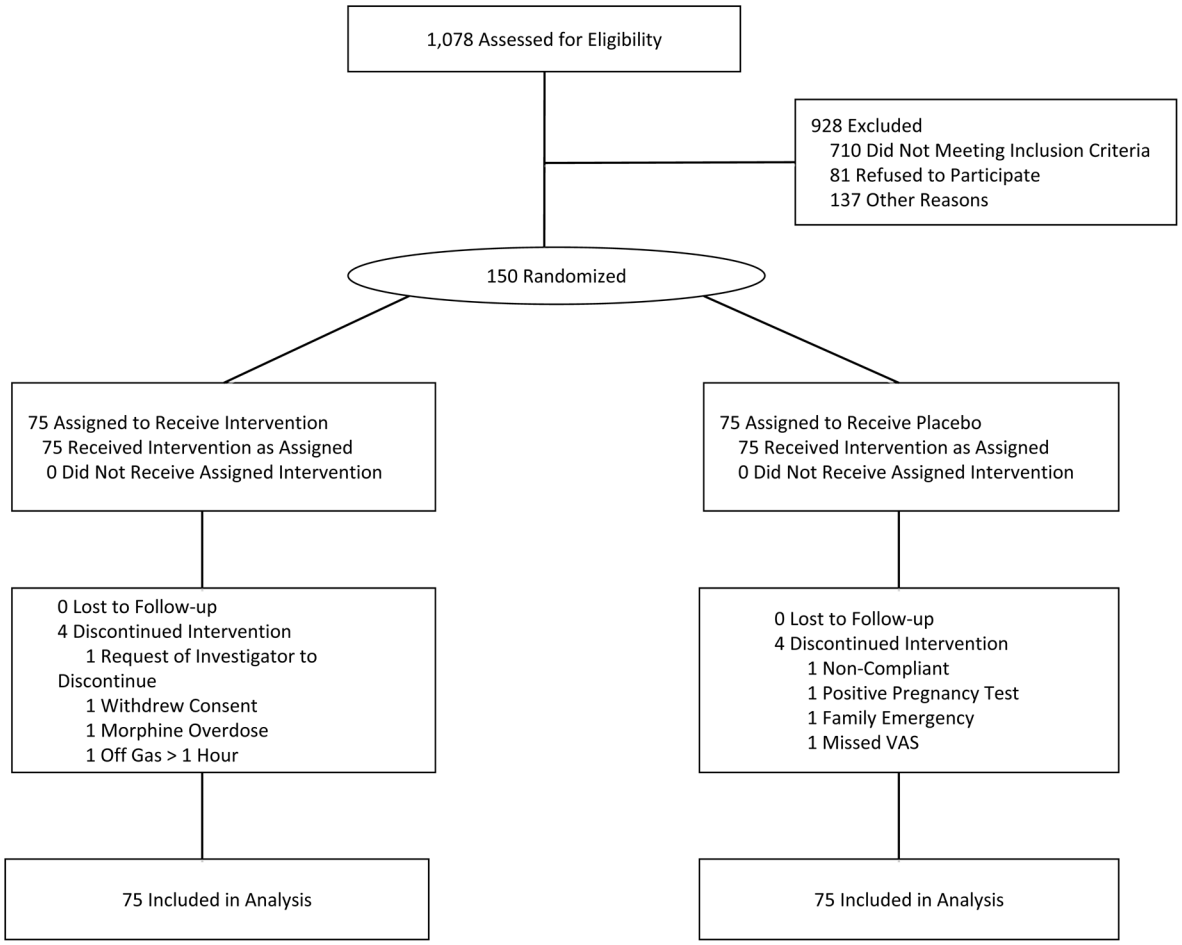


Figure 1. Patient Enrollment and Randomization

The patient population consisted of 150 patients presenting with VOC who were enrolled and randomized in a prospective, placebo-controlled, double-blind clinical trial of inhaled NO versus placebo at sites in the United States. Four patients withdrew in each group, and the numbers resolving pain crisis were 65 and 64 for the iNO and placebo groups, respectively. All 150 participants were evaluated by intention to treat. The large number of patients assessed for eligibility (1,078) reflects the pre-enrollment of out-patients at many of the sites.

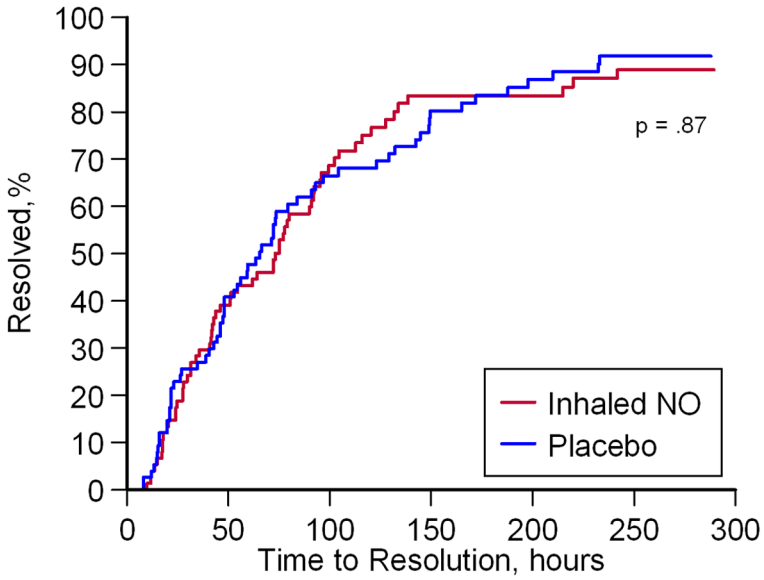


Figure 2. Efficacy of Inhaled NO Gas Versus Placebo on VOC

There was no significant effect of inhaled NO on time to VOC resolution by Kaplan-Meier analysis (A), p=.87, log-rank test.

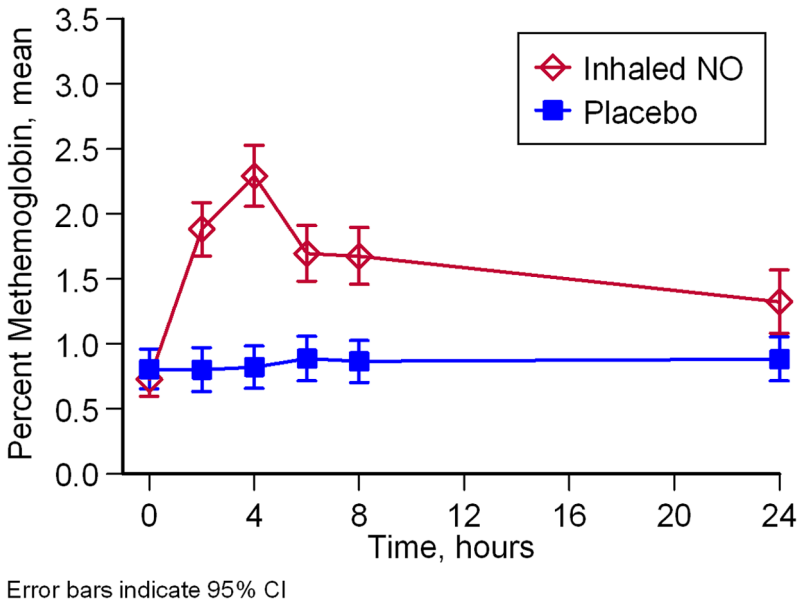
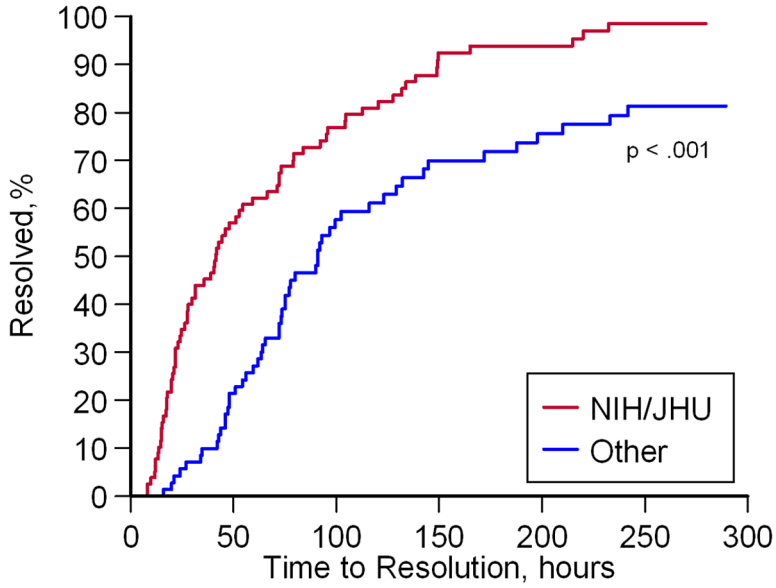
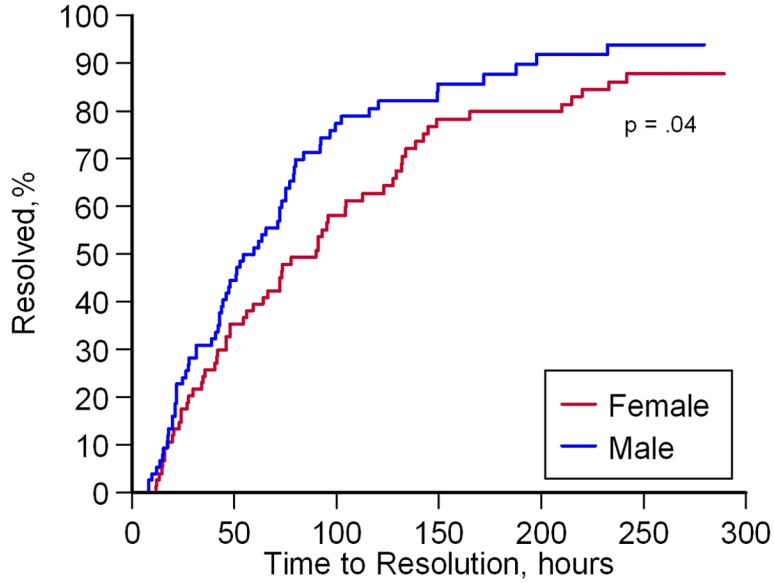


Figure 3. Effect of Inhaled NO Gas on methemoglobin

Methemoglobin concentrations were significantly higher for participants on inhaled NO gas than for participants in the placebo group (A; $p < .0001$). Blood samples for nitrate and nitrite testing were taken before crisis when possible (labeled baseline), on Day 1 before treatment started (labeled Pre-Tx), and on each subsequent day until discharge.





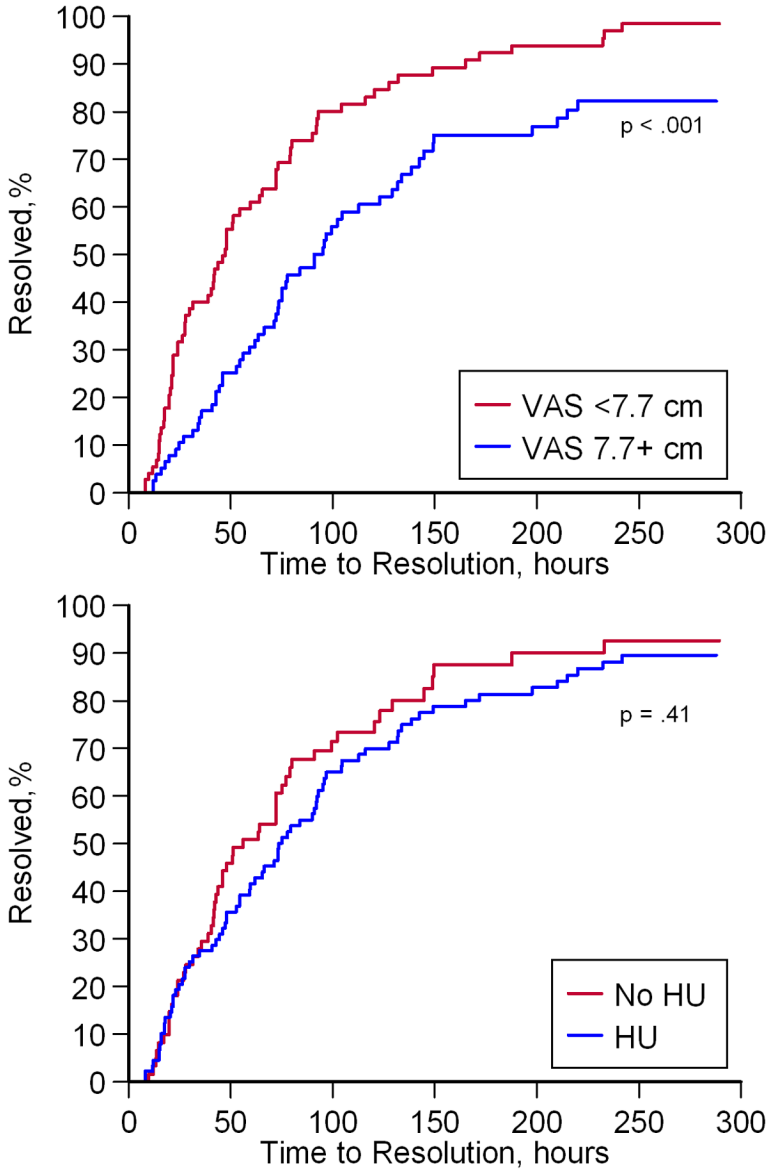


Figure 4. Kaplan-Meier Analysis of VOC Resolution by Study Site, Gender and Pain at Baseline

Shorter times to VOC resolution were observed for participants at NIH and JHU (A; p<.001, log-rank test), males (B; p=.04, log-rank test), and participants with baseline VAS scores less than 7.7 cm (C; p<.001, log-rank test). No significant differences in time to resolution were observed in participants on hydroxyurea therapy (D; p=.4, log-rank test).

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Table 1

Demographics and Baseline Characteristics of Study Participants by Treatment

	Inhaled NO		Placebo			Overall	
Characteristic	**N**	**Median (IQR)[1]**	**N**	**Median (IQR) [1]**	***P* Value[2]**	**N**	**Median (IQR) [1]**
Age, y	75	22.9 (17.8–33.9)	75	24.5 (17.1–31.8)	0.81	150	24.2 (17.4–33.7)
Men, N (%)	75	37 (49.3)	75	38 (50.7)	0.87	150	75 (50.0)
Sickle Cell Genotype (SS/Sβ$^+$), N (%)	75	70(93.3)/5(6.7)	75	66(88.0)/9(12.0)	0.26	150	136 (90.7)/14(9.3)
Hydroxyurea use, N (%)	75	43 (57.3)	75	46 (61.3)	0.62	150	89 (59.3)
Systolic blood pressure, mm Hg	75	118 (108–128)	75	117 (108–131)	0.90	150	118 (108–129)
Diastolic blood pressure, mm Hg	75	64 (59–71)	75	65 (57–74)	0.91	150	65 (58–72)
Visual analogue pain score (0–10), cm	75	7.7 (6.0–9.1)	75	7.6 (6.5–9.1)	0.83	150	7.7 (6.3–9.1)
Hemoglobin, g/dL	75	8.8 (7.7–9.8)	75	8.8 (7.9–10.2)	0.21	150	8.8 (7.9–10.1)
White blood cell count, × 10^{-3}/3L	73	12.9 (9.1–16.8)	75	12.4 (9.4–16.3)	0.68	148	12.8 (9.3–16.4)
Red blood cell count, × 10^{-6}/3L	75	2.7 (2.3–3.0)	75	2.8 (2.4–3.3)	0.12	150	2.7 (2.3–3.2)
Platelet count, × 10^{-3}/3L	73	383 (290–493)	75	348 (227–475)	0.07	148	367 (254–483)
Reticulocyte count, × 10^{-3}/3L	71	240 (91–306)	72	154 (17–293)	0.11	143	200 (23–300)
MCV, μm^3	75	95.3 (86.3–103)	75	91.0 (82.0–103.0)	0.21	150	93.7 (84.1–103.0)
MCH, μ μg	75	33.1 (29.9–36.4)	75	31.4 (27.9–35.9)	0.09	150	32.4 (28.7–36.2)
MCHC, gm/dL	75	34.7 (34.0–35.6)	75	34.5 (33.5–35.6)	0.35	150	34.7 (33.8–35.6)
Creatinine, mg/dL	75	0.6 (0.5–0.7)	75	0.6 (0.5–0.8)	0.98	150	0.6 (0.5–0.7)
Blood urea nitrogen, mg/dL	75	7 (5–9)	74	7 (5–10)	0.92	149	7 (5–9)
GGT, U/L	68	27.0 (17.5–56.0)	68	33.5 (19.5–66.5)	0.40	136	31.0 (19.0–64.5)
Lactate dehydrogenase, U/L	68	373 (295–509)	68	414 (297–566)	0.32	136	389 (297–553)
Alkaline phosphatase, U/L	73	95 (70–148)	75	103 (79–147)	0.37	148	99 (73–148)
Total bilirubin, mg/dL	72	2.8 (1.7–4.3)	75	2.3 (1.7–4.0)	0.37	147	2.6 (1.7–4.1)
Direct bilirubin, mg/dL	70	0.4 (0.3–0.5)	69	0.4 (0.4–0.6)	0.88	139	0.4 (0.3–0.6)
Indirect bilirubin, mg/dL	70	2.2 (1.4–4.1)	69	2.0 (1.4–3.3)	0.50	139	2.1 (1.4–3.6)
Alanine aminotransferase, U/L	73	24 (18–36)	75	26 (17–35)	0.60	148	25 (17–35)
Aspartate aminotransferase, U/L	69	36 (28–55)	72	41 (30–56)	0.48	141	39 (30–56)

[1] Except where otherwise noted

[2]From Wilcoxon two-sample test or chi-square test

Table 2

Effect of Inhaled NO Gas on Secondary Outcomes

Secondary Outcome		Inhaled NO (N=75)	Placebo (N=75)	*P* Value[a]
Length of Hospitalization, days	Median (IQR)	4.1 (2.0–6.0)	3.1 (1.7–6.4)	.30
VAS at 24 hours, cm	Mean (95% CI)	6.1 (5.3–6.8)	6.0 (5.4–6.6)	.90
VAS at 2, 4, 6, 8 hours (change from baseline, cm)	Mean (95% CI)	−0.4 [−0.8- (−0.1)]	−0.7 [−1.1- (−0.3)]	
		−0.6 [−0.8- (−0.1)]	−0.8 [−1.3- (−0.3)]	.90
		−1.2 [−1.7- (−0.7)]	−1.1 [−1.6- (−0.6)]	
		−1.3 [−1.8- (−0.8)]	−1.2 [−1.8- (−0.7)]	
Opioids in 1st 8 hours, mg/kg	Median (IQR)	0.28 (0.09–0.54)	0.23 (0.07–0.70)	.74
Total Opioids, mg/kg	Median (IQR)	2.8 (1.4–6.1)	2.9 (1.1–9.9)	.73
ACS Requiring Transfusion	N [% (95% CI)[b]]	8 [10.7 (4.7–19.9)]	7 [9.3 (3.8–18.3)]	.79
Discharged within 24 hours	N [% (95% CI)[b]]	5 [6.7 (2.2–14.9)]	7 [9.3 (3.8–18.3)]	.55
Returned to ED within 30 days	N [% (95% CI)[b]]	8 [10.7 (4.7–19.9)]	11 [15.1 (7.8–25.4)]	.44
Rehospitalized within 30 days	N [% (95% CI)[b]]	9 [12.2 (5.7–21.8)]	17 [23.0 (14.0–34.2)]	.08
Methemoglobin, %, at 0, 4, 24 hours	Mean (95% CI)	0.73 (0.59–0.86)	0.81 (0.65–0.96)	
		2.29 (2.05–2.52)	0.82 (0.66–0.98)	<.001
		1.32 (1.07–1.57)	0.88 (1.06–0.58)	
Plasma Nitrate[c] at 0, 2, 4 days μmol/L	Mean (95% CI)	24.5 (18.3–32.6)	24.3 (18.0–32.9)	
		60.9 (48.5–76.3)	22.2 (16.1–30.5)	.03
		36.2 (22.3–58.8)	20.9 (13.8–31.8)	
Plasma Nitrite[c] at 0, 2, 4 days μmol/L	Mean (95% CI)	0.22 (0.18–0.26)	0.21 (0.18–0.24)	
		0.30 (0.25–0.36)	0.24 (0.19–0.30)	.77
		0.23 (0.16–0.34)	0.27 (0.22–0.32)	
Whole Blood Nitrite[d] at 0, 4, 8 hours μmol/L	Mean (95% CI)	0.28 (0.14–0.56)	0.23 (0.14–0.37)	
		0.40 (0.24–0.67)	0.27 (0.17–0.41)	.31
		0.45 (0.23–0.85)	0.37 (0.22–0.62)	

[a] From Wilcoxon 2-sample test for comparison of medians; unpaired t-test for comparison of means at specific time points; Pearson's chi-square for comparison of proportions; Repeated Measures ANOVA for comparison of means over time

[b] Clopper-Pearson (Exact) 95% confidence limits

[c] From 83 samples from participants at sites with plasma storage

[d] From 34 samples from participants at sites with plasma storage

Table 3

Number and Percentage of Participants with Serious Adverse Events

	Inhaled NO (N=75)	Placebo (N=75)	
System Organ Class	**N [% (95% CI)[a]]**	**N [% (95% CI)[a]]**	***P* Value[b]**
Preferred Term			
Blood and Lymphatic System Disorders			
Acute Chest Syndrome	5 [6.7 (2.2–14.9)]	5 [6.7 (2.2–14.9)]	>0.99
Gastrointestinal Disorders			
Dysphagia	1 [1.3 (0.03–7.2)]	0 [0.0 (0.00–4.8)]	>0.99
General Disorders and Administration			
Site Conditions			
Pyrexia	1 [1.3 (0.03–7.2)]	1 [1.3 (0.03–7.2)]	>0.99
Sensation of Foreign Body	1 [1.3 (0.03–7.2)]	0 [0.0 (0.00–4.8)]	>0.99
Investigations			
Hemoglobin Decreased	1 [1.3 (0.03–7.2)]	0 [0.0 (0.00–4.8)]	>0.99

[a] Clopper-Pearson (Exact) 95% confidence limits

[b] From Fisher's exact test