Inhaled nitric oxide to treat intermediate risk pulmonary embolism: A multicenter randomized controlled trial

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ABSTRACT

Objective: To test the hypothesis that adjunctive inhaled NO would improve RV function and viability in acute PE.

Methods: This was a randomized, placebo-controlled, double blind trial conducted at four academic hospitals. Eligible patients had acute PE without systemic arterial hypotension but had RV dysfunction and a treatment plan of standard anticoagulation. Subjects received either oxygen plus 50 parts per million nitrogen (placebo) or oxygen plus 50 ppm NO for 24 h. The primary composite endpoint required a normal RV on echocardiography and a plasma troponin T concentration < 14 pg/mL. The secondary endpoint required a blood brain natriuretic peptide concentration < 90 pg/mL and a Borg dyspnea score ≤ 2. The sample size of N = 76 tested if 30% more patients treated with NO would achieve the primary endpoint with 80% power and alpha = 5%.

Results: We randomized 78 patients and after two withdrawals, 38 were treated per protocol in each group. Patients were well matched for baseline conditions. At 24 h, 5/38 (13%) of patients treated with placebo and 9/38 (24%) of patients treated with NO reached the primary endpoint (P = 0.375). The secondary endpoint was reached in 34% with placebo and 13% of the NO (P = 0.11). In a pre-planned post-hoc analysis, we examined how many patients with RV hypokinesis or dilation at enrollment resolved these abnormalities; 29% more patients treated with NO resolved both abnormalities at 24 h (P = 0.010, Cochrane's Q test).

Conclusions: In patients with severe submassive PE, inhaled nitric oxide failed to increase the proportion of patients with a normal troponin and echocardiogram but increased the probability of eliminating RV hypokinesis and dilation on echocardiography.

Clinical trial registration: NCT01939301.

1. Introduction

Acute pulmonary embolism (PE) causes direct thrombotic occlusion of pulmonary arteries, which increases pulmonary vascular resistance and right ventricular (RV) afterload, ultimately causing RV contractile dysfunction and inflammatory damage [1,2]. As a result, PE impairs RV contraction on transthoracic echocardiography, and produces elevated circulating cardiac troponin proteins and brain natriuretic peptides (BNP) [3]. Severe RV afterload and dysfunction clearly increases the risk of death and contributes to ventilation-perfusion abnormalities that cause dyspnea and hypoxemia, which may be long-lasting [4–7].

The rationale for an inhaled vasodilator as a treatment for PE stems

from evidence that non-mechanical processes contribute to increased pulmonary vascular resistance. The percentage of radiographically-evident pulmonary thrombotic vascular occlusion often fails to predict severity of RV dysfunction, biomarker abnormalities and clinical outcome [6,8,9]. Acute PE triggers pulmonary vasoconstriction, platelet hyperactivation and microvascular obstruction, caused in part by reduced nitric oxide availability to the pulmonary vasculature [10–12]. Drugs that increase cyclic guanosine monophosphate, such as inhaled NO, acutely decrease pulmonary arterial pressures in experimental models of hemolysis- and PE-induced pulmonary hypertension as well as in patients with chronic pulmonary hypertension [13–16]. Several case reports and a small, non-randomized clinical trial of PE showed temporal improvement in dyspnea from PE with NO treatment [15,17].

We therefore hypothesized that inhaled NO would reduce both RV dysfunction and injury from acute PE seen on echocardiography and high precision plasma troponin concentrations, respectively. Our secondary aim tested if inhaled NO would reduce RV strain, evidenced by a reduction in BNP and patient perception of dyspnea.

2. Study design

The inhaled Nitric Oxide for Pulmonary Embolism (iNOPE) trial was a multicenter randomized, double-blind, controlled trial of inhaled NO + oxygen versus nitrogen + oxygen in subjects with acute PE with normotension but with RV dysfunction (intermediate risk or submassive PE). The iNOPE protocol and methods have been described in detail and the trial was registered on clinicaltrials.gov (**NCT01939301**) [18]. The protocol was approved by the Institutional Review Boards at participating centers.

3. Results

As shown in Fig. 1, from March 2014 to October 2016, we screened 1550 patients with diagnosed PE and obtained informed consent from 86 patients. After 8 screen failures and two voluntary withdrawals after starting drug (n = 1) or placebo (n = 1), we treated 76 patients treated per protocol (n = 38 per group) for 24 h. Regarding timing of enrollment relative to the qualifying CT scan performance, we enrolled 32 patients within 12 h of CT scanning, 39 patients within 12–20 h, 8 between 20 and 24 h and one 48 h later.

Table 1 shows that patients were well matched for baseline vital signs, prior medical conditions and indexes of PE severity. The mean (SD) RV/LV ratio was 1.32 (0.39) [median 1.11, 1st-3rd quartiles 0.89–1.69] and all 78 patients had one or more filling defects in a lobar or more proximal pulmonary artery. After one patient in each group withdrew after enrollment, 38 patients completed the entire protocol in each group (N = 76 total).

3.1. Primary and secondary outcomes

Table 2 presents the primary outcome and shows that 9/38 (24%) of patients treated with NO and 5/38 (13%) of patients treated with placebo had both normal RV function on echocardiography and a normal TnT after 24 h of treatment (P = 0.375, Fisher's exact test). Analysis of the components of the primary outcome failed to show any salient difference between treatments; Table *2* shows relatively equal distribution of normal results for each the four echocardiographic variables used to define RV function. Fig. 2, which plots the pre- and post-treatment TnT values for all patients, shows similar changes with each treatment from enrollment to 24 h. Likewise, Table 3 shows no difference in the secondary outcome of a BNP < 90 pg/mL and the Borg dyspnea score between patients, and no difference in the fraction of patients who scored favorably on the psychometric tests for health related quality of life. Fig. 3 plots the pre- and post-treatment BNP values for each patient at enrollment. Similarly, no difference was found in the fraction of patients who scored favorably on the psychometric tests for

health related quality of life after 90 days (Supplemental Fig. 1). Heart failure, kidney disease and coronary artery disease may increase steady-state concentrations of troponins and natriuretic peptides. To assess this possible confounding effect, we removed patients with prior heart failure, kidney disease (creatinine > 1.7 mg/dL), and coronary artery disease, which excluded eight patients from NO treatment and five from placebo (Supplemental Tables 1 and 2). At enrollment, mean troponin and BNP concentrations were elevated in patients with these comorbidities. However, as Supplemental Tables 3 and 4 show, their removal had no effect on the probability of normalization on either troponin T (Troponin T < 14 pg/mL at wean: 12/30 [40%] after NO treatment versus 10/33 [30%] after placebo, P = 0.46, Fisher's exact), or BNP (BNP < 90 pg/mL at wean: 7/23 after NO treatment versus 10/23 after placebo, P = 0.55).

3.2. Safety measurements (Table 4)

No patient was discontinued from NO for any safety reason and no serious adverse event was attributed to study drug. No patient had sustained methemoglobinemia (see Supplemental Fig. 2). After receiving intravenous promethazine and morphine, two NO-treated patients developed hypotension that required saline infusion. Care providers opened the blinding envelope during treatment in one placebo treated patient who developed worsened dyspnea.

3.3. Additional clinical outcomes

Mortality at 28 days for patients treated with NO and Placebo respectively was 0/38 and 3/38 (P = 0.24, Fisher's exact) and 2/38 and 4/38 at 90 days (P = 0.67). Causes of death included one death from complications associated with rescue embolectomy on day 3, another from respiratory failure on day 69, in two, the patient died at home and the cause of death was unknown but were not ascribed to recurrent PE, and the other two died from progression of cancer. No patient had an autopsy at death. One patient, treated with placebo, had a recurrent DVT, and was the only case of VTE recurrence within 90 days. Supplemental Figs. 3–6 show the, Borg score, heart rate, SpO2%, and systolic blood pressure values at enrollment, 1, 8 and 24 h of treatment. The salient observations include no apparent difference in any of these measurements, except for a consistently higher Borg score for NO treated patients.

3.4. Effectiveness of study procedures

Regarding the integrity of blinding, the clinical care physicians thought 32 patients received NO, and were correct in 14/32 (43%) instances; they thought 31 patients received Placebo, and were correct in 17 (54%) instances; physicians had no opinion of treatment assignment in 13 patients. We confirmed evidence of NO delivery with doubling of the plasma nitrate concentration in patients treated with NO (Fig. 4). The median nitrate concentrations (μg/mL, 1st-3rd quartiles) in the Placebo group were 15 (9–19) and 15 (11–20) (P = 0.113, sign-rank test) at enrollment and 24 h, respectively and the median nitrate concentrations in the NO group were 12 (8–16) and 27 (22–38) (P < 0.001, sign rank-test). Nitrite concentrations were not significantly changed from enrollment to 24 h in either group.

3.5. Frequency of resolution of RV hypokinesis and dilation

This study used several echocardiographic measurements of RV function that are not commonly used in routine medical practice. However, we believe that RV hypokinesis and dilation are more commonly reported in routine echocardiographic interpretations. Accordingly, in a post-hoc analysis using only the 71 study echocardiograms with adequate imaging to assess both RV hypokinesis and dilation, 19/35 (54%) of NO-treated and 10/36 (28%) of placebo-
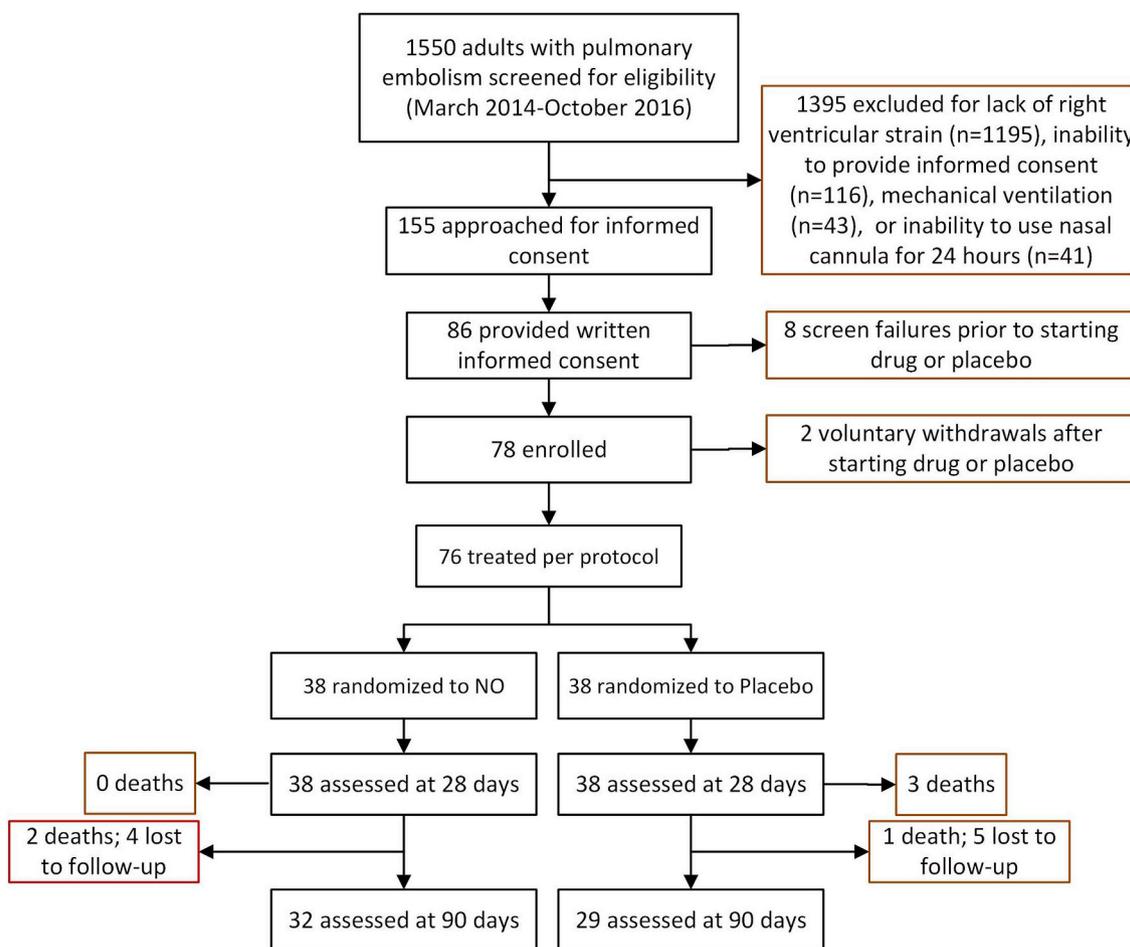
Fig. 1. Flow diagram showing the numbers of patients screened, consented, enrolled and treated per protocol.

treated patients were free of either abnormality (P = 0.03, Fisher's exact). Furthermore, as part of usual care, 22/38 (58%) and 24/38 (63%) of NO and Placebo patients, respectively had a transthoracic echocardiogram performed within 12 h prior to enrollment with in-adequate imaging to assess both RV hypokinesis and dilation. At en-rollment, 4/22 (18%) and 4/24 (17%) of NO and Placebo patients had neither abnormality; at 24 h, 11/22 (50%) and 5/24 (21%) of patients treated with NO or Placebo, respectively, were free of both abnormal-ities (P = 0.010, Cochrane's Q test).

4. Discussion

In this randomized, double-blind, placebo-controlled trial, the pro-portion of patients randomized to 24 h of inhaled NO who had normal RV function on echocardiography, and no evidence of myocardial in-jury (hs TnT) was not significantly higher than patients treated with placebo. Likewise, NO treatment did not lower BNP concentrations, patient perceived dyspnea (Borg score), and did not improve patient reported health related quality of life on psychometric testing at 90 days (PEmb QoL and SF 36). The serial measurements of methe-moglobin, dyspnea perception, heart rate, and oxygenation were not different between groups over the 24 h treatment period. Study proce-dures appeared to reduce bias reasonably well. Randomization pro-duced two groups that were similar in the distributions of prior cardi-opulmonary disease. However, the troponin T concentrations tended to be higher in the placebo treated group, possibly introducing bias in favor of NO. The Borg dyspnea score appeared to be higher at baseline in the NO treated group, possibly introducing bias in favor of placebo. We confirmed effective delivery of NO, based upon increase in plasma

nitrate concentrations, similar to those measured in a previous trial of inhaled NO for acute chest syndrome in sickle cell disease [22]. The mechanism of blinding appeared effective based upon the balanced percentage of the time that clinicians correctly guessed which treatment the patient received.

The rationale for the components of each outcome were well justi-fied [18]. The four echocardiographic parameters assessed the global function of the RV pumping against an acutely increased afterload: 1. The RV diameter to measure volume overload, 2. The TAPSE to mea-sure longitudinal shortening, 3. The FAC to reflect circumferential contraction, and 4. The RIMP to indicate speed of stroke volume ejec-tion [23]. The high precision troponin T was intended to measure de-gree of myocyte necrosis from shear effect and inflammatory injury, both of which increase with higher RV afterload [24]. Regarding tro-ponin assays, we used troponin I as an eligibility criterion, whereas we used troponin T in the primary outcome. The reason for this difference was that during the time of enrollment, we used the FDA cleared tro-ponin assays that were used in usual clinical practice at the enrolling hospitals, which all measured troponin I. The preplanned primary outcome required high precision troponin, and the device we used for this measures troponin T. In the time since our study was completed, this device has been cleared to market in the US. Data in Table 1 show that 14/38 (39%) of patients treated with NO normalized their troponin values, compared with 11/38 (29%), which if analyzed independently would have yielded P = 0.63 (Fisher's exact test). We do acknowledge the possibility that had we measured troponin concentrations later, for example at 48 h, we may have seen wider separation in the frequency of normalization of troponin measurements between groups.

In the secondary outcome, the plasma BNP and dyspnea severity

Table 1
Clinical characteristics of enrolled patients.

Age and vital signs	NO treated (N = 39)[a]		Placebo treated (N = 39)[a]	
	Mean	SD	Mean	SD
Age (years)	55	15	59	16
Body mass index (kg/m^2)	36	12	33	11
Heart rate (beats/min)	86	12	93	13
Systolic blood pressure (mm Hg)	125	20	127	20
Pulse oximetry (%)	97	3	96	6
Patient features and past medical history	N	%	N	%
Female gender	21	54%	20	51%
Caucasian race	28	72%	21	54%
Creatinine > 1.7 mg/dL	2	6%	1	3%
Hemoglobin < 10.0 g/dL	9	26%	5	14%
Surgery in past 4 weeks	7	18%	4	10%
Trauma in past 4 weeks	4	10%	5	13%
Coronary artery disease	3	8%	5	13%
Heart failure	6	15%	1	3%
Chronic obstructive pulmonary disease	7	18%	4	10%
Prior venous thromboembolism	10	26%	12	31%
Active cancer	3	8%	5	13%
Connective tissue disease	6	15%	7	18%
Post partum (within 4 weeks)	1	3%	1	3%
Never smoked	25	64%	20	51%
Markers of RV strain	Median or proportion	1st-3rd quartile or percentage	Median or proportion	1st-3rd quartile or percentage
Troponin concentration (pg/mL)	23	12–71	51	18–101
Brain natriuretic peptide (pg/mL)	206	83–386	234	84–556
Right ventricular systolic pressure (mm Hg[b])	54.5	50–62	50	47–52.5
Right ventricular/Left ventricular ratio	1.12	0.89–1.69	1.10	0.85–1.68
Right ventricular dilation (> 43 mm) on echocardiography	14/24	58%	13/26	50%
Right ventricular hypokinesis on echocardiography	15/24	63%	20/26	77%

[a] These data are for enrolled patients; one patient in each group withdrew after enrollment.
[b] Estimated from equation $V^2 \times 4 + 10$ mm Hg where V = tricuspid regurgitant jet velocity (meters/second).

were intended to measure RV muscle strain and a patient-oriented measurement of ventilation-perfusion mismatching from increased alveolar deadspace; moreover, these two measurements [25]. While BNP values could have been altered by coincident heart failure, kidney disease or coronary artery disease, their removal had no effect on the proportion of patients who normalized BNP (Supplemental Tables 1–4). The two psychometric tests are self-evident as measures of patient perceived health wellness. We used four physiologically-oriented echocardiographic indexes that are not always used in usual care and can be difficult to obtain because of limitations in acoustic windows. For this reason, we conducted a post-hoc analysis of RV hypokinesis and dilation. Our rational for choosing these two variables between NO-treated and Placebo-treated in the post-hoc analysis is because both variables were assessable in 93% of study echocardiograms, and RV hypokinesis and dilation are often reported on clinical echocardiogram

interpretations. These post-hoc analyses suggest that NO may have improved the probability of correcting RV hypokinesis and dilation more than chance alone would predict.

Taken together, these data provide some evidence of clinical value of inhaled NO for PE. On one hand, inhaled NO did not significantly increase the proportion of patients with good primary or secondary outcomes, and did not improve symptoms, heart rate, oxygenation or perception of wellness. However, this study does not rule out the possibility of modest improvements in RV function with inhaled NO. As a phase II study, the sample size was predicated on a relatively large 30% difference in primary outcome to justify progression to a large, expensive phase III trial. After study completion, we found an only an 11% difference in the primary outcome. By comparison, the only two randomized controlled trials of fibrinolysis versus placebo that repeated echocardiograms at 24 h after treatment both found only a 10%

Table 2
The primary outcome.

| Measurement	Definitions	NO		Placebo		P value
		n/38*	%	n/38*	%	
Primary outcome	Normal right ventricular function on echocardiography and normal troponin T**	9	24%	5	13%	0.375
Components of primary outcome	Right ventricular diastolic diameter < 43 mm	31	82%	27	71%	
	Tricuspid annular plane systolic excursion > 16 mm	29	76%	28	74%	
	Right ventricular index of myocardial performance normal	22	58%	22	58%	
	Right ventricular fractional area of contraction > 33%	23	61%	21	55%	
	Plasma troponin T concentration < 14 pg/mL	14	37%	11	29%	
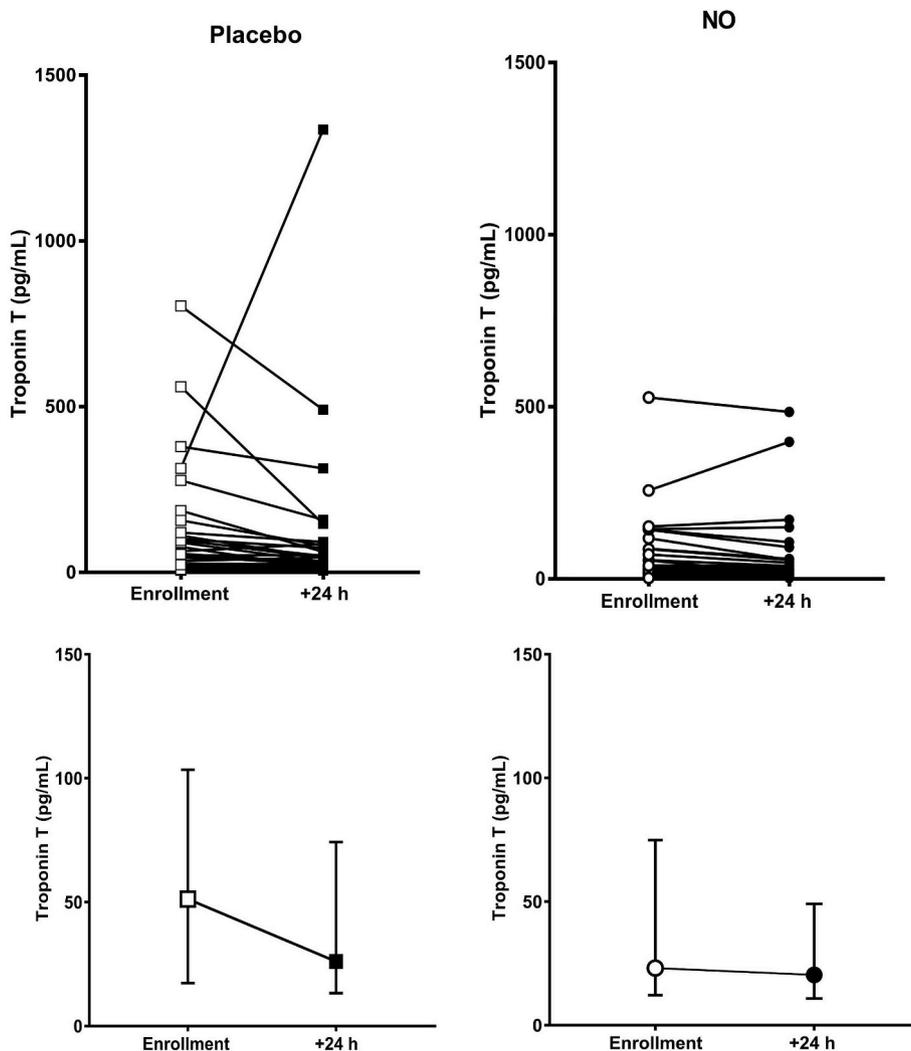
Fig. 2. Dot plot showing plasma concentration of troponin T at enrollment, and then again after 24 h of treatment for patients each treated with either placebo (n = 38, squares), or 50 parts per million of inhaled nitric oxide (n = 38, circles). The bottom panels show the median and interquartile ranges. The cutoff for abnormal was 14 pg/mL.

increase in rate of RV resolution compared with placebo [26,27]. Moreover, no NO-treated patient required escalation of therapy while on protocol, compared with four Placebo-treated patients, including two who underwent both catheter directed fibrinolysis and then surgical embolectomy (Table 4). Third, the post-hoc analysis suggests NO was more likely than Placebo at correcting RV hypokinesis and dilation

at 24 h. Finally, NO was not associated with any serious adverse events. These findings may leave open the possibility of future work with higher doses of NO or the use of drugs that directly stimulate or activate guanylate cyclase [28]. It could be speculated that NO may benefit patients with more severe PE, such as those with hypotension or requiring mechanical ventilation, but we believe the rarity of patients

Table 3
Secondary outcome and mortality.

Measurement	Definitions	NO		Placebo		P value
		n/38	%	n/38	%	
Secondary outcome	Brain natriuretic peptide < 90 pg/mL and Borg dyspnea score ≤ 2	6	16%	13	34%	0.111
Components of secondary outcome	Brain natriuretic peptide < 90 pg/mL	13	34%	15	39%	
	Borg dyspnea score ≤ 2	27	71%	32	84%	
Health related quality of life (90 days)	Favorable PEmb QoL score[a]	11	29%	14	37%	0.450
	Favorable SF36[b]	15	39%	15	39%	1.000
Survival	Mortality at 28 days	0	0%	3	8%	0.210
	Mortality at 90 days	2	5%	4	11%	0.670

[a] Score < 40%.
[b] Normalized score > 50%; N = 33, NO and N = 32, Placebo; see Fig. 1 for additional detail.

Table 4
Safety endpoints during treatment.

Event	NO		Placebo		P value
	n/39[a]	%	n/39[a]	%	
Unblinding	0	0%	1	3%	1.000
Escalated to thrombolysis	0	0%	3	8%	0.240
Escalated to thrombectomy	0	0%	1	3%	1.000
Bleeding[b]	0	0%	1	3%	1.000
Hypotension	2	5%	1	3%	1.000
Respiratory distress requiring intervention	1	3%	1	3%	1.000
METH018 – Supportive Rx: continued O2 support					
METH027 – Supportive Rx: Cpap continuation at night.					
Hypoxemia requiring intervention	3	8%	1	3%	0.615
ESKE002 – Supportive Rx: O2 evaluation for home oxygen. Increase in NC flow from 4L (rest) - 6L (exertion) of O2.					
ESKE008 - Supportive Rx: O2 evaluation for home oxygen. Increase in NC flow from 1L (rest) - 2L (exertion) of O2.					
METH019 - Supportive Rx: O2 evaluation for home oxygen & discharged with home O2.					
METH028 – Supportive Rx: Bipap use at night for untreated sleep apnea					
Arrhythmia requiring intervention	2	5%	1	3%	1.000
ESKE003 – Supportive Rx: Patient restarted on home medication, Diltiazem 30 mg PO QID, due to reported A-fib with RVR with HR = 124.					
METH014 – Supportive Rx: metoprolol started					
METH049 – Supportive Rx: Amniodarone increased from 200 mg to 600 mg PO					
Death	0	0%	0	0%	1.000
Other adverse event requiring medication or supportive care[c]	12	31%	10	26%	0.802

[a] N = 39 used in each group for denominator despite voluntary withdrawal of one subject in each group prior to completion of the 24 h protocol because 39 in each group receive NO for some time period.

[b] Vaginal bleeding without need for transfusion or procedural intervention.

[c] Zero instances of vasopressor use, cardiopulmonary resuscitation, death bronchospasm, neurological deterioration, gastrointestinal distress or methemoglobinemia > 10% for 1 h during the 24 h treatment period.

with this severe form of PE, combined with real world difficulty of obtaining their written informed consent constitute an insurmountable barrier to conducting such a trial.

5. Conclusions

Compared with placebo, 24 h of nasally inhaled NO did not increase the proportion of patients with intermediate-high risk PE who normalized their RV function, circulating troponin T or BNP concentrations after 24 h of treatment. A secondary analysis suggests that inhaled NO may increase likelihood of resolving echocardiographically-observed RV hypokinesis and dilation. Future studies should focus on this endpoint.

5.1. Patient selection

All patients were > 17 years old, normotensive, had image-proven proximal PE and were intermediate-high risk using the European Society of Cardiology definition (sPESI ≥ 1 and RV dysfunction) [19]. The definition of image proven was a filling defect suggestive of acute PE in a contrast-enhanced pulmonary artery. RV dysfunction was defined by an elevated BNP (> 90 pg/mL) or troponin I (> 0.1 ng/mL), and evidence of RV dilation (RV > 42 mm in diastole, or RV:LV ratio > 1.0 on CT scan measured as part of usual care by a board certified radiologist who was not affiliated with the study) or RV hypokinesis on echocardiography [6,19]. Exclusions included pregnancy, vasopressor use, inability to tolerate a nasal cannula, altered mental status, written plan to not administer anticoagulation, written plan for fibrinolysis, and the use of drugs known to increase cyclic guanosine monophosphate.

5.2. Treatment protocol

All patients received treatment doses of either unfractionated or low molecular weight heparin. Antiplatelet drugs were discontinued. Study drug or placebo was administered with a commercially available, FDA-cleared device (Inovent,® Mallinckrodt Pharmaceuticals, Clinton, NJ).

Blinded canisters of NO or placebo were supplied by Mallinckrodt. The method of randomization and blinding, blood collection and echocardiography has been described in detail [18]. NO or nitrogen placebo was titrated at 2 ppm/min over 25 min to a dose of 50 ppm *via* nasal cannula, which was maintained for 24 h. The rationale for the 2 ppm per minute titration and weaning rate was based upon considerations of patient safety versus the potential feasibility and generalizability of the protocol. As previous described, we believed the 2 ppm per minute rate of change allowed research personnel enough time to observe the patient for changes (symptoms and vital signs), but would not excessively prolong the protocol [18]. After 24 h (+/− 3 h) of treatment, the NO or nitrogen was weaned at 2 ppm per minute to 0 ppm NO over 25 min. To investigate integrity of blinding, the research coordinator asked the primary physician in charge of the patient's care during hospitalization for his or her opinion whether the patient received NO or Placebo.

5.3. Patient safety concerns

Vital signs and methemoglobin, carboxyhemoglobin with a Masimo Rainbow® Cooximeter device (Masimo Corporation, Irving, CA), were monitored hourly. An unblinding envelope and emergency treatment algorithm were posted at the bedside to inform the healthcare team how to handle acute deterioration.

5.4. Study measurements

The Borg dyspnea score (0–10) was measured hourly. A study-funded transthoracic Doppler-echocardiogram was performed after weaning NO, always within 3 h. All patients had blood specimensdrawn for troponin, BNP and nitrate measurements within 1 h of starting NO at baseline and then immediately prior (< 1 h in all cases) to weaning, 24 h later. Plasma concentrations of nitrite and nitrate were measured with high performance liquid chromatography (EiCOM ENO-30, San Diego, CA); troponin T (TnT) was measured with a high precision instrument (Roche COBAS, e411, Indianapolis, IN) and BNP was measured with iSTAT®, (Abbott, Princeton, NJ).
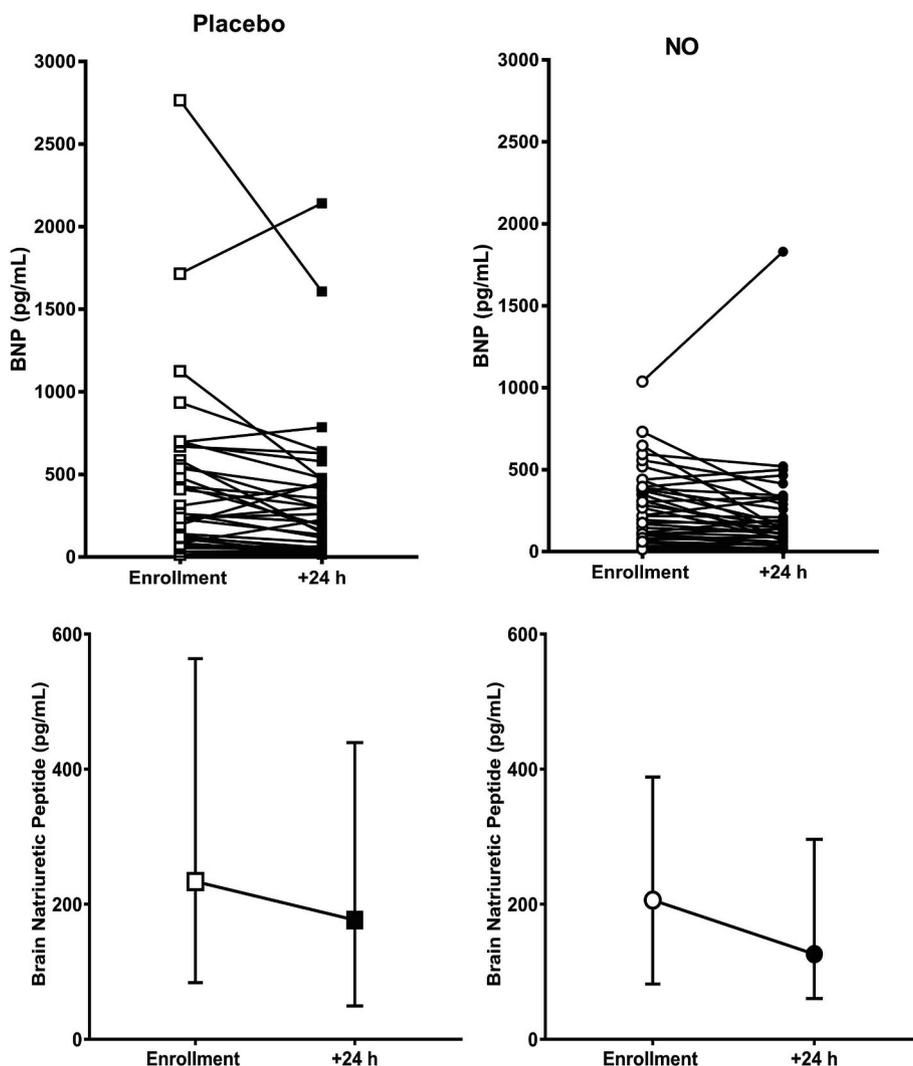
Fig. 3. Dot plot showing plasma concentration of brain natriuretic peptide at enrollment, and then again after 24 h of treatment for patients each treated with either placebo (n = 38, squares), or 50 parts per million of inhaled nitric oxide (n = 38, circles). The bottom panels show the median and interquartile ranges. The cutoff for abnormal was 90 pg/mL.

5.5. Outcome measurements

The *primary efficacy outcome* was normal RV function on echocardiography and a TnT concentration < 14 pg/mL [3] at 24 h. Normal RV function required normal RV size (< 42 mm in diastole) and tricuspid annular plane systolic excursion (TAPSE) > 16 mm and normal right ventricular index of myocardial performance (RIMP) (< 0.40 using spectral Doppler or < 0.55 using tissue Doppler) and normal fractional area of contraction (FAC) (> 35%); All echocardiograms, including those obtained at enrollment, were read by a cardiologist and echocardiography expert (author RAM) who was blinded to group assignment. Missing echocardiographic parameters were analyzed as if they were normal. The *secondary efficacy outcome* required a Borg dyspnea score ≤ 2 and a BNP level < 90 pg/mL. We also measured mortality rate and perceived perception of wellness with a standardized survey at 3 months after treatment (*via* SF36 and PEmb QoL) [20]. Lastly, we a preplanned secondary analysis, assessed the frequency of resolution of two commonly measured abnormalities on echocardiography, RV hypokinesis and dilation. We compared the proportion of patients between treatment groups who had neither abnormality at 24 h, and we compared the frequency with which each treatment was associated with resolution of both RV hypokinesis and dilation (i.e.,

conversion from having one or both abnormalities at diagnosis to having neither at 24 h).

5.6. Sample size and analytical methods

The minimum sample size of N = 72 treated per protocol was predicated on detection of a 30% difference in the proportions of patients in each group who reached the primary efficacy endpoint. Means, medians and frequency data were compared between groups using a two-tailed unpaired *t*-test, a Mann Whitney U, or Fisher's exact test as appropriate. In the post-hoc analysis, we use a Cochrane's Q test whether changes in paired frequencies differ by randomization. Because this was a phase II study with a physiological endpoint, and we did not anticipate an group imbalance in patients crossing over or withdrawing, we did not use an intent to treat design [21].

Author contributions

JAK: study design, protocol, funding, data collection, analysis, manuscript preparation: CLH, AEJ, RM, TL, EEG: protocol, data collection, analysis, manuscript preparation.
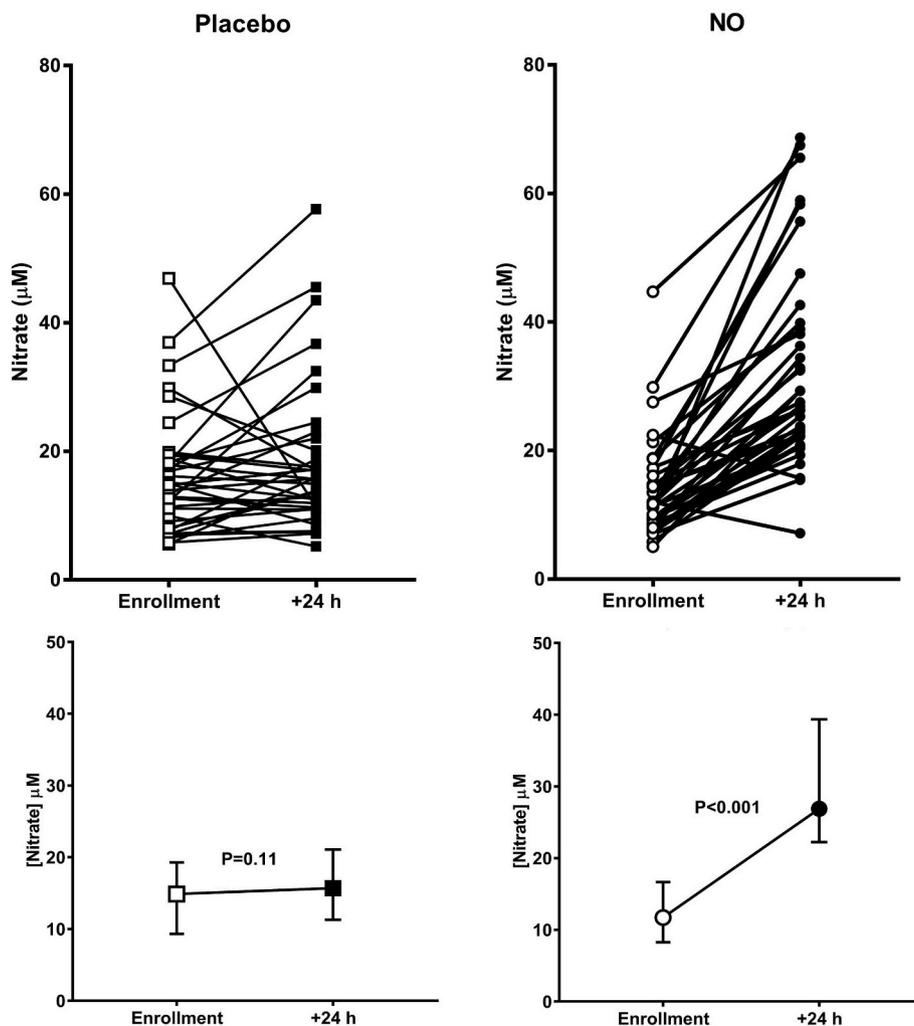
Fig. 4. Dot plot showing plasma concentration of nitrates at enrollment, and then again after 24 h of treatment for patients each treated with either placebo (n = 38, squares), or 50 parts per million of inhaled nitric oxide (n = 38, circles). The bottom panels show the median and interquartile ranges.

Conflicts of interest

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.niox.2019.01.006.

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