Randomized Controlled Trial of Inhaled Nitric Oxide for the Treatment of Microcirculatory Dysfunction in Patients With Sepsis*

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Objectives: Sepsis treatment guidelines recommend macrocirculatory hemodynamic optimization; however, microcirculatory dysfunction is integral to sepsis pathogenesis. We aimed to test the hypothesis that following macrocirculatory optimization, inhaled nitric oxide would improve microcirculation in patients with sepsis and that improved microcirculation would improve lactate clearance and multiple organ dysfunction.

Design: Randomized, sham-controlled clinical trial.

Setting: Single urban academic medical center.

Patients: Adult patients with severe sepsis and systolic blood pressure less than 90 mm Hg despite intravascular volume expansion and/or serum lactate greater than or equal to 4.0 mmol/L.

Interventions: After achievement of macrocirculatory resuscitation goals, we randomized patients to 6 hours of inhaled nitric oxide (40 ppm) or sham inhaled nitric oxide administration. We administered study drug via a specialized delivery device that concealed treatment allocation so that investigators and clinical staff remained blinded.

Measurements and Main Results: We performed sidestream dark-field videomicroscopy of the sublingual microcirculation prior to and 2 hours after study drug initiation. The primary outcome measure was the change in microcirculatory flow index. Secondary outcomes were lactate clearance and change in Sequential Organ Failure Assessment score. We enrolled 50 patients (28 of 50 [56%] requiring vasopressor agents; 15 of 50 [30%] died). Although inhaled nitric oxide significantly raised plasma nitrite levels, it did not improve microcirculatory flow, lactate clearance, or organ dysfunction. In contrast to previous studies conducted during the earliest phase of resuscitation, we found no association between changes in microcirculatory flow and lactate clearance or organ dysfunction.

Conclusions: Following macrocirculatory optimization, inhaled nitric oxide at 40 ppm did not augment microcirculatory perfusion in patients with sepsis. Further, we found no association between microcirculatory perfusion and multiple organ dysfunction after initial resuscitation. (*Crit Care Med* 2014; 42:2482–2492)

Key Words: clinical trial; inhaled nitric oxide; microcirculation; nitric oxide; resuscitation; sepsis; septic shock; severe sepsis

Sepsis is a common and devastating disease that is responsible for more than 200,000 deaths annually in the United States alone and is the leading cause of death in critically ill patients (1, 2). Circulatory failure is one of the hallmarks of the septic state. Although the most clinically apparent manifestation of circulatory failure in sepsis is systemic arterial hypotension, it is now recognized that intrinsic microcirculatory dysfunction is also a key element of sepsis pathogenesis (3).

The microcirculatory dysfunction of sepsis results from discrete pathogenic events that occur in microvessels and are not solely downstream effects of macrocirculatory hemodynamic perturbations. These pathogenic events include endothelial activation and dysfunction, disruption of microvascular tone and integrity, and leukocyte adhesion, among others (3). In studies using in vivo videomicroscopy in patients with sepsis, worsening microcirculatory perfusion indices have been associated with worse outcome (4–6). Specifically, early impairment of microcirculatory perfusion has been associated with subsequent development of multiple organ dysfunction, which is the major driver of mortality in patients with sepsis (7, 8). Thus, microcirculation-directed therapies could potentially attenuate multiple organ dysfunction in sepsis and lead to improved outcome (9).

One postulated treatment for sepsis-induced microcirculatory dysfunction is exogenous nitric oxide (NO) (or NO-donor agent) administration (10, 11). Although NO production is up-regulated in sepsis, and this up-regulation can be responsible in large part for arterial hypotension in patients with sepsis, NO is also critical for maintenance of microvascular patency, especially when the microcirculation sustains an insult (as in sepsis). Thus, sepsis-induced NO up-regulation may be an adaptive response for maintaining tissue perfusion. By modulating microvascular tone as well as antiadhesive effects on the microvascular endothelium, exogenous NO administration could in theory be beneficial by helping to keep the microcirculation "open" in patients with sepsis (9–11). IV nitrates (e.g., nitroglycerin and nitroprusside) are known to markedly reduce systemic blood pressure, which could be problematic in hemodynamically stressed patients with sepsis. One potential therapy to increase systemic NO bioavailability without inducing or exacerbating arterial hypotension is inhaled nitric oxide (INO), which can increase circulating nitrite and/or *S*-nitrosothiols with resultant effects on systemic microcirculatory blood flow (12–18). We hypothesized that following macrocirculatory optimization, INO administration would improve microcirculation in patients with sepsis and that improved microcirculation would improve lactate clearance from the blood and reduce multiple organ dysfunction. To test this hypothesis, we conducted a randomized controlled trial of INO for the treatment of microcirculatory dysfunction in patients with sepsis.

METHODS

Study Design

This study was a prospective, randomized, sham-controlled, parallel group clinical trial over 37 months (2009–2013) in a single urban academic medical center (Cooper University Hospital, Camden, NJ). Patients were enrolled in the Emergency Department or on arrival to the ICU. Written informed consent was obtained from the patient or next of kin. The research protocol was approved by the local institutional review board and performed in accordance with Good Clinical Practice guidelines. The trial was registered (NCT00608322) on ClinicalTrials.gov prior to the first patient enrollment.

Participants

Patients with severe sepsis or septic shock were assessed for inclusion, which required that patients be 18 years or older with confirmed or suspected acute infection and have hypoperfusion evidenced by either a systolic blood pressure lower than 90 mm Hg after a minimum of 20 mL/kg rapid IV volume challenge or a blood lactate concentration of at least 4 mmol/L (19, 20). The criteria for exclusion from the study were pregnancy, shock etiology other than sepsis, status post cardiac arrest treated with cardiopulmonary resuscitation, support limitations (e.g., "do not resuscitate") prior to enrollment, need for immediate transport to the operating room for surgery, inability to put a videomicroscopy probe under the tongue for any reason (e.g., inability to open the mouth), and elapsed time from first meeting inclusion criteria more than 24 hours.

Randomization and Masking

Patients were randomly assigned to one of two groups, INO (INOmax, Ikaria, Clinton, NJ) at 40 ppm or sham INO administration. The group assignment sequence was generated by an independent statistician using a parallel design, balanced (1:1 ratio) block randomization schedule in blocks of four. Standard measures were used to ensure appropriate concealment of group assignment. The randomization assignments were kept in sequentially numbered opaque, sealed envelopes.

Interventions

The drug delivery system was a conventional INO delivery device (INOvent, Datex-Ohmeda, Madison, WI) specially configured with an opaque, locked shield over the face of the device that concealed the display screen for content of respiratory gases. With the locked shield in place, investigators and clinicians were blinded as to whether or not INO is being administered to the patient. After informed consent was obtained, a trained unblinded respiratory therapist who was designated prior to the start of the clinical trial opened the randomization envelope and according to the group assignment either 1) initiated INO at 40 ppm or 2) performed sham INO administration by mimicking all of the standard respiratory therapist calibration procedures for initiating INO therapy through the INOvent and continuing the flow of oxygen without turning on the flow of INO. The unblinded respiratory therapist closed and locked the opaque shield immediately after performing 1) or 2) above, and only the unblinded respiratory therapist was able to unlock the blinded INOvent to reveal treatment allocation. By design, the investigators, treating physicians, and

clinical staff were blinded to the treatment allocation, and the unblinded respiratory therapist had no access to primary outcome information (i.e., microcirculatory blood flow indices, described below). This approach using a blinded INOvent and unblinded respiratory therapist has previously been used successfully in multiple clinical trials of INO (21–23).

Although patients were randomized when written informed consent for participation was obtained, we required achievement of macrocirculatory resuscitation goals (central venous pressure ≥ 8 mm Hg; mean arterial pressure ≥ 65 mm Hg; central venous oxygen saturation ≥ 70% [20] or lactate clearance from the blood ≥ 10% [19]) prior to initiation of study drug. These macrocirculatory resuscitation goals are recommended by international treatment guidelines for sepsis and prospective randomized controlled trials (19, 20, 24). This type of quantitative resuscitation approach has been used at our institution since 2005 (8, 25), and all interventions for hemodynamic optimization (e.g., IV fluids and vasopressor agents) were selected and administered by the treating clinicians. We required that clinicians achieve macrocirculatory goals prior to study drug administration on the grounds that homogeneity in macrocirculatory variables is needed to rigorously test the microcirculatory-specific effects of an intervention.

For patients on mechanical ventilation at the time of enrollment, the study drug was administered through an endotracheal tube. For spontaneously breathing patients, the study drug was administered via one of three routes, depending on the patient's supplemental oxygen needs: 1) aerosol face mask with 6-inch corrugated tubing in the vent holes; 2) a noninvasive high-flow gas delivery apparatus (Vapotherm 2000i, Vapotherm, Exeter, NH); or 3) conventional nasal cannula. These noninvasive routes have previously been demonstrated as effective for INO delivery (26–28). The INO or sham INO administration continued for a total of 6 hours after initiation.

Measurements

At 0 hour, prior to study drug administration, we recorded vital signs and hemodynamic indices, measured serum lactate, performed laboratory tests needed for calculation of organ dysfunction scores, obtained and immediately centrifuged and froze plasma samples at –80°C for measurement of nitrite levels, and performed the baseline microcirculation assessment.

We visualized the sublingual microcirculation with side-stream dark-field (SDF) videomicroscopy, a minimally invasive method of imaging the microcirculation beneath mucosal surfaces. The SDF instrument (Microscan, Microvision Medical, Amsterdam, The Netherlands) has a 5× objective giving 326× magnification. The technique consists of a handheld videomicroscope containing a ring of stroboscopic light-emitting diodes (29). The light is absorbed by hemoglobin so that RBCs appear dark, yielding high-contrast video of blood flow in submucosal microvessels. The technique (including its predecessor, Orthogonal Polarization Spectral videomicroscopy) has been well validated (29–32). We selected the sublingual space for imaging because both direct and indirect assessments of tissue perfusion at the sublingual site have previously been

demonstrated to predict mortality in critically ill patients, including those with sepsis (4–6, 33–35).

A detailed description of our standard operating procedure for sublingual microcirculatory image acquisition and analysis has been described elsewhere (6, 8) and appears in the **electronic supplementary material** (Supplemental Digital Content 1, http://links.lww.com/CCM/B22). Briefly, we stored videos by random number code without source patient identifiers so that the data could be analyzed off-line blinded to all clinical data. We determined the microcirculatory flow index (MFI) using a semiquantitative image analysis methodology originally described by Spronk et al (10) (0 = absent; 1 = intermittent; 2 = sluggish; 3 = normal). Our group has previously demonstrated good interrater agreement for determining MFI among multiple independent raters (κ = 0.77–0.87) (6, 8). We calculated the MFI for all four quadrants of the image and averaged the values to yield a single MFI for five different sublingual sites. We averaged the five sites to give a single MFI value for each time point that a patient was imaged. To quantify the heterogeneity in microcirculatory flow at each time point, we calculated the flow heterogeneity index as the highest sublingual site MFI minus the lowest sublingual site MFI divided by the mean of the MFIs across all sublingual sites (6).

At 2 hours after study drug initiation, we repeated the SDF videomicroscopy and obtained serum lactate and plasma nitrite samples. At 2 hours, the unblinded respiratory therapist performed a bedside measurement of methemoglobin as a safety check, while maintaining the blind (details appear in the electronic supplementary material, Supplemental Digital Content 1, http://links.lww.com/CCM/B22). At 6 and 24 hours after study drug initiation, we repeated all measurements (including laboratory testing) needed for calculation of organ dysfunction scores.

For plasma nitrite measurements, we used a liquid tri-iodide reductive ozone-based chemiluminescent method (Sievers Nitric Oxide Analyzer, GE, Boulder, CO) as described previously (36) and in the electronic supplementary material (Supplemental Digital Content 1, http://links.lww.com/CCM/B22).

Outcome Measures

The primary outcome measure was mechanistic in nature—change in microcirculation (ΔMFI) over 0–2 hours of study drug. Because the degree of early multiple organ system failure (i.e., either early progression or resolution) is closely linked to eventual outcome and can be a result of tissue hypoperfusion (37–40), the main patient-oriented outcome measure was the change in the Sequential Organ Failure Assessment (SOFA) (41) score from 0 to 24 hours. The SOFA score components appear in the electronic supplementary material (Supplemental Digital Content 1, http://links.lww.com/CCM/B22). We prospectively defined an organ dysfunction "responder" as a decrease in two or more SOFA points from 0 to 24 hours (8). Secondary outcome measures included the change in SOFA score from 0 to 6 hours and lactate clearance from the blood over 0–2 hours of study drug, calculated as: $[(\text{lactate}_{0\text{-hour}} - \text{lactate}_{2\text{-hour}})/\text{lactate}_{0\text{-hour}}] \times 100\%$ (19).
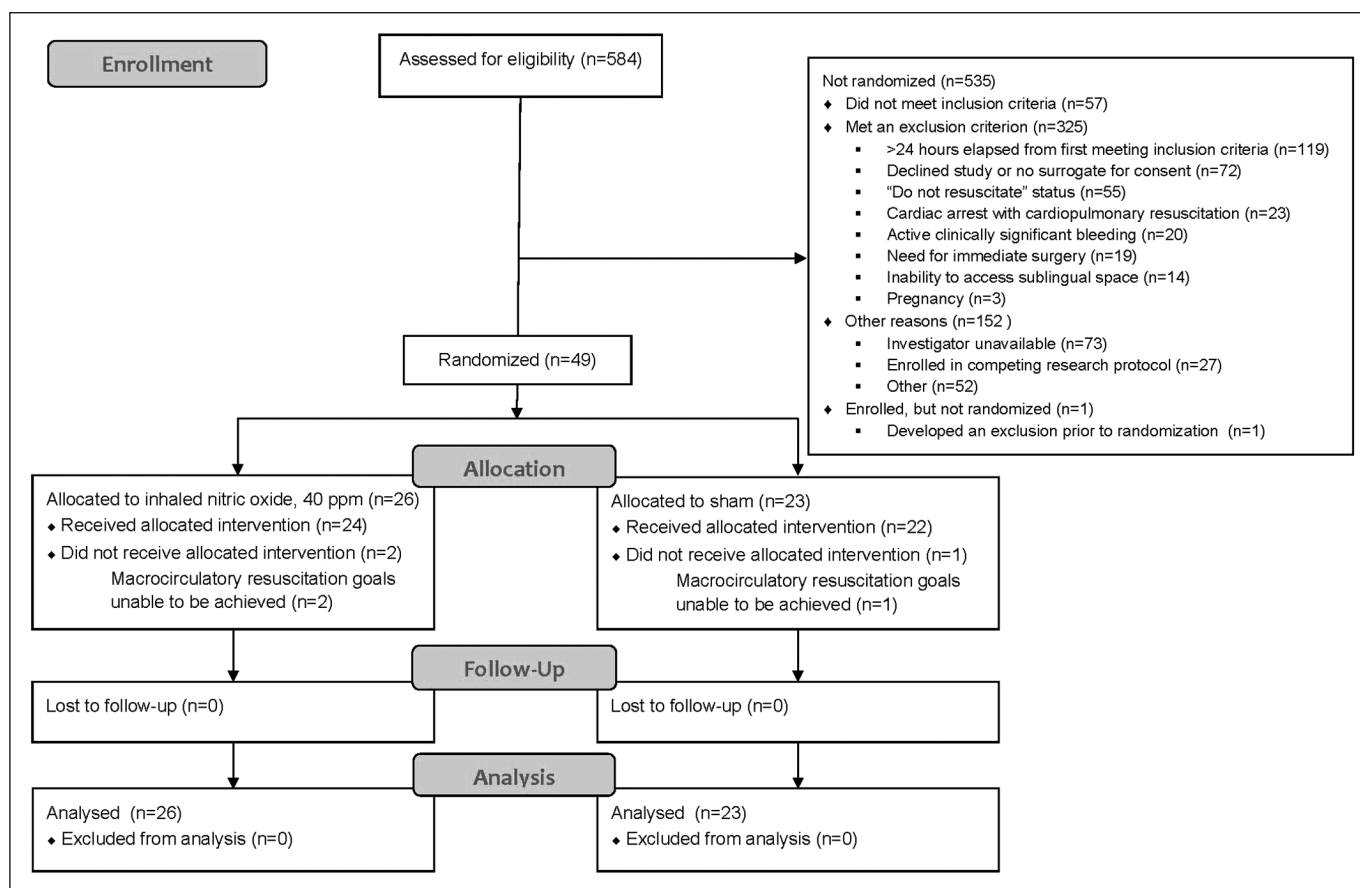
Figure 1. Consolidated Standards of Reporting Trials diagram of patient enrollment.

Statistical Analysis

The sample size calculation and primary analytical plan centered on the hypothesis that INO administration would be associated with an increase in MFI and an improvement in organ dysfunction and lactate clearance. All by-group statistical analyses were based on the intent-to-treat principle. An independent Data Safety Monitoring Board that had the authority to terminate the study for safety concerns performed an interim safety analysis after 40 patients were enrolled.

Where appropriate, categorical data were compared using a chi-square test or Fisher exact test, and continuous variables were compared with a Mann-Whitney U test or unpaired t test, two sided with p value less than 0.05 considered significant (StatPlus, AnalystSoft, Alexandria, VA). Preplanned secondary analyses included testing the correlation between the change in MFI and physiological data including change in serum lactate and SOFA score among all patients, independent of treatment assignment, using Pearson correlation coefficient.

A detailed description of the sample size calculation and the stopping rule appears in the electronic supplementary material (Supplemental Digital Content 1, http://links.lww.com/CCM/B22). Briefly, we calculated that 46 patients were needed to test the effects of INO on ΔMFI, and 138 patients were needed to test the effects of INO on ΔSOFA. The a priori–defined analysis plan called for an interim analysis after 46 evaluable patients in order to determine the efficacy of INO on the outcome measure of ΔMFI, and then, based on these results, either continuing the trial to 138 patients to test the patient-oriented outcome (ΔSOFA) or stopping the trial for futility. Based on our previous work, we expected an 8% dropout rate for inability to complete study procedures due to early death; thus, in order to accrue 46 evaluable subjects, we planned the interim analysis after 50 patients were enrolled.

RESULTS

At interim analysis ($n = 50$), the trial met the a priori–defined criteria for stopping for futility, due to a lack of increase in microcirculation in the INO-treated patients compared with sham, as described below. The Consolidated Standards of Reporting Trials diagram appears in **Figure 1**. One patient was enrolled but developed an exclusion criterion just prior to randomization. Thus, 49 patients underwent randomization. In three patients (two in INO group and one in sham group), macrocirculatory optimization could not be achieved, and thus, these patients did not receive study drug but are included in all analyses (intent-to-treat). All three of these patients experienced early death due to unrecoverable circulatory failure.

Baseline data for all patients and those in the INO and sham groups appear in **Table 1**. Baseline hemodynamic data (i.e., central venous pressure, mean arterial pressure, and central venous oxygen saturation) confirm that this population underwent macrocirculatory optimization prior to randomization.

TABLE 1. Baseline Data for All Patients at the Time of Enrollment

	All Patients	Inhaled Nitric Oxide	Sham
	n = 49	*n* = 26	*n* = 23
Age, yr, mean (SD)	59 (17)	59 (15)	58 (20)
Female gender, *n* (%)	25 (51)	15 (58)	10 (43)
Comorbidities, *n* (%)			
Diabetes	14 (29)	7 (27)	7 (30)
Coronary artery disease	8 (16)	3 (12)	5 (22)
Myocardial infarction	5 (10)	3 (12)	2 (9)
Hypertension	24 (49)	14 (54)	10 (43)
Malignancy	4 (8)	0 (0)	4 (17)
Renal insufficiency	2 (4)	1 (4)	1 (4)
Chronic pulmonary disease	11 (22)	6 (23)	5 (22)
Cerebral vascular disease	6 (12)	3 (12)	3 (13)
Congestive heart failure	5 (10)	3 (12)	2 (9)
Liver cirrhosis	2 (4)	2 (8)	0 (0)
HIV	3 (6)	3 (12)	0 (0)
Source of infection, *n* (%)			
Lung	21 (43)	9 (34)	12 (52)
Urine	8 (16)	6 (23)	2 (9)
Skin	5 (10)	3 (12)	2 (9)
Abdomen	8 (16)	3 (12)	5 (22)
Endocarditis	1 (2)	0 (0)	1 (4)
Central nervous system	2 (4)	2 (7)	0 (0)
Unknown	4 (8)	3 (12)	1 (4)
Hemodynamics, median (IQR)			
Mean arterial pressure, mm Hg	76 (68–86)	72 (66–80)	81 (74–95)
Central venous pressure, mm Hg	12 (10–15)	13 (11–16)	11 (8–14)
$Scvo_2$, %	77 (66–86)	72 (64–82)	78 (70–88)
Serum lactate, mmol/L, median (IQR)	3.0 (1.6–4.7)	2.7 (1.5–4.4)	3.3 (2.1–4.9)
Vasopressor agent support, *n* (%)	28 (57)	17 (65)	11 (48)
Inclusion criteria met, *n* (%)			
Hypotension despite IV fluids	41 (84)	24 (92)	17 (74)
Lactate ≥ 4 mmol/L	17 (35)	8 (31)	9 (39)
Mechanical ventilation, *n* (%)	34 (69)	20 (77)	14 (61)
0-hr Sequential Organ Failure Assessment score, median (IQR)	6 (3–8)	7 (4–8)	6 (3–7)
0-hr microcirculatory flow index, median (IQR)[a]	1.9 (1.7–2.1)	2.0 (1.7–2.2)	1.9 (1.7–2.0)

IQR = interquartile range.

[a]p value for difference in 0-hr microcirculatory flow index between inhaled nitric oxide and sham by Mann-Whitney U test is $p = 0.12$.

TABLE 2. Plasma Nitrite Levels (μM) and Change in Plasma Nitrite Levels for the Inhaled Nitric Oxide and Sham Groups

	All Patients	Inhaled Nitric Oxide	Sham
0 hr	0.20 (0.07–0.25)	0.21 (0.08–0.26)	0.14 (0.07–0.25)
2 hr	0.27 (0.15–0.52)	0.41 (0.24–0.63)	0.19 (0.12–0.31)
Δ 0–2 hr	0.09 (0.04–0.23)	0.25 (0.07–0.32)[a]	0.07 (0.03–0.10)

[a]$p = 0.01$ compared to sham group by Mann-Whitney U test.

All values are expressed as median and interquartile range. We found that the change in plasma nitrite was significantly increased in the inhaled nitric oxide–treated group compared with sham, $p = 0.01$.

TABLE 3. Outcome Measures

	All Patients ($n = 49$)	Inhaled Nitric Oxide ($n = 26$)	Sham ($n = 23$)	p
Δ Microcirculatory flow index (0–2 hr)	−0.06 (−0.17 to 0.07)	−0.06 (−0.20 to 0.03)	−0.03 (−0.14 to 0.15)	0.37
Lactate clearance (%) (0–2 hr)	−9 (−15 to 0)	−9 (−16 to 12)	−9 (−15 to 0)	0.59
Δ SOFA score (0–6 hr)	0 (−1 to 0)	0 (−1 to 0)	0 (−1 to 0)	0.96
Δ SOFA score (0–24 hr)	−1 (−2 to 0)	−1 (−2 to 0)	−1 (−1 to 0)	0.30
Organ dysfunction responder,[a] n (%)	13 (27)	8 (31)	5 (22)	0.48
In-hospital mortality, n (%)	15 (31)	9 (35)	6 (26)	0.52

SOFA = Sequential Organ Failure Assessment.

[a]Defined as a reduction in SOFA score of two or more points over 0–24 hours.

Data are expressed as median and interquartile range except where indicated otherwise.

TABLE 4. Resource Utilization

	All Patients ($n = 49$)	Inhaled Nitric Oxide ($n = 26$)	Sham ($n = 23$)
Mechanical ventilation days	4 (0–10)	4 (0–14)	4 (1–8)
ICU days	5 (3–14)	8 (3–15)	5 (3–10)
Hospital days	11 (5–22)	14 (5–23)	9 (6–21)

Data are presented as median and interquartile range.

Slightly more than half of study patients were treated with vasopressor agents at baseline. The median 0-hour SOFA score of 6 confirms the presence of multiple organ dysfunction in this septic population. There was no significant difference between groups in the median baseline (0-hr, pretreatment) MFI (INO 2.0 [interquartile range, IQR, 1.7–2.2] vs sham 1.9 [IQR, 1.7–2.0]; $p = 0.12$).

Table 2 displays plasma nitrite levels. The median change in plasma nitrite was significantly higher in the INO group compared with the sham group (INO +0.25 [IQR, 0.07–0.32] μM vs sham +0.07 [IQR, 0.03–0.10] μM; $p = 0.01$). These data suggest that this bioactive metabolite of INO was delivered systemically and therefore could exert circulatory effects in the INO-treated population.

Main outcome measures are displayed in **Table 3**. We found no significant difference between INO and sham groups in the change in MFI (INO −0.06 [IQR, −0.20 to +0.03] vs sham −0.03 [IQR, −0.14 to +0.15]; $p = 0.37$). There was also no significant difference in the flow heterogeneity index between treatment groups (INO +0.06 [IQR, −0.18 to +0.16] vs sham −0.01 [IQR, −0.14 to +0.16]; $p = 0.67$). In addition, we found no significant difference between INO and sham groups in the secondary outcome measures of lactate clearance, change in SOFA score, or the proportion of patients that were organ dysfunction responders (i.e., decrease by two or more SOFA points from 0 to 24 hr). Resource utilization between groups is displayed in **Table 4**.

The serious adverse events experienced by all patients during study drug administration, and those in the INO and sham groups, are displayed in **Table 5**. The most common serious adverse event was worsening cardiovascular instability, but there was no significant imbalance in the occurrence between groups. No patients experienced significant methemoglobinemia.

For the secondary analyses of the relationships between microcirculation and physiological variables (e.g., organ dysfunction) in the entire population, there was considerable change in SOFA scores over 0–24 hours; SOFA went down in 26 of 49 (53%), went up in 9 of 49 (18%), and remained the same in 14 of 49 (29%). **Table 6** displays the correlations between the change in MFI and the change in serum lactate and SOFA score for all patients in the study. In this population of patients who already had optimization of the macrocirculation, we found no significant correlation between change in MFI and lactate clearance or change in SOFA score. The relationships between percent change in MFI and the 0- to 6-hour and 0- to 24-hour change in SOFA score are displayed graphically in **Figure 2, A** and **B**, respectively.

TABLE 5. Serious Adverse Events

	All Patients (n = 49)	Inhaled Nitric Oxide (n = 26)	Sham (n = 23)
Worsening cardiovascular instability[a]	8 (16)	5 (19)	3 (13)
Need for surgery	2 (4)	0 (0)	2 (9)
Hospital-acquired infection	5 (10)	3 (12)	2 (9)
Initiation of hemodialysis	3 (6)	2 (8)	1 (4)
Initiation of mechanical ventilation	1 (2)	0 (0)	1 (4)
Stroke	1 (2)	1 (4)	0 (0)
Clinician opt out	1 (2)	0 (0)	1 (4)
Supraventricular tachycardia	1 (2)	0 (0)	1 (4)
Methemoglobinemia (> 5%)	0 (0)	0 (0)	0 (0)
Methemoglobin, %, median (interquartile range)	0.2 (0–0.8)	0.5 (0.2–1.1)	0 (0–0.1)

[a]Defined as any one of the following over 0–6 hours of study drug administration: (a) sustained (> 30 min) mean arterial pressure < 65 mm Hg; (b) addition of a vasopressor agent; or (c) increase in dose of any vasopressor agent > 50% over 0-hour dose.
Data are expressed as n (%) unless otherwise specified.

TABLE 6. Secondary Analyses Among all Randomized Patients (n = 49) of Correlations (Pearson Correlation Coefficient) Between the Change in Microcirculatory Flow Index and Change in Serum Lactate and Organ Dysfunction

	Lactate Clearance (%) (0–2 Hr)	Δ SOFA Score (0–6 Hr)	Δ SOFA Score (0–24 Hr)
Δ Microcirculatory flow index (0–2 hr)	R = 0.10	R = −0.11	R = 0.03
	p = 0.52	p = 0.48	p = 0.86

SOFA = Sequential Organ Failure Assessment.

DISCUSSION

The hypothesis for this randomized trial originated from a sentinel report in 2002 in which the authors found that administration of a NO-donor agent could augment microcirculatory perfusion in patients with septic shock (10). It was the first clinical study that aimed to investigate whether NO is beneficial, rather than harmful, in patients with sepsis. Given that intrinsic microcirculatory dysfunction is known to be a pivotal event in the pathogenesis of sepsis, as demonstrated in numerous laboratory investigations and clinical studies (4–6, 8, 33, 35, 42–45), the postulated mechanism of benefit of exogenous NO administration is that it might reverse sepsis-induced microcirculatory shutdown and keep microvessels open, that is, "rescue" the microcirculation (46).

This hypothesis could be considered somewhat contrarian to the conventional thinking of the role of NO in the pathogenesis of this disease. Historically, the study of NO modulation in sepsis was focused on the hypothesis that endogenous NO up-regulation was deleterious, largely because of its role in producing hypotension through arteriolar vasodilation. However, NO is also vitally important for maintaining microvessel patency in shock states, and it is possible that endogenous NO up-regulation is an adaptive response to preserve effective microcirculatory perfusion (9). Although inhibition of nitric oxide synthase (NOS) in sepsis clearly raises arterial pressure (47–49), it can simultaneously worsen microcirculatory perfusion and oxygen transport to tissues (50–55), and the only published phase III trial of nonspecific NOS inhibition in human subjects with sepsis was stopped early for a signal of harm in the NOS inhibition group (56). Although the sepsis proinflammatory response triggers a sharp increase in systemic NO production, the up-regulation of inducible NOS can be heterogeneously expressed between and within organ systems, and NO can be consumed by reactive oxygen species, giving the possibility of relative NO deficiency in microvascular beds despite total body NO "excess" (46, 57, 58). Thus, application of exogenous NO could in theory relieve some of the regional heterogeneity in tissue perfusion that typically characterizes the septic state (43, 44), and could also help to relieve pathophysiologic microcirculatory shunting in sepsis, that is, the diversion of blood flow away from distressed microvascular units via opening of arteriovenous shunts within capillary beds (59).

In this randomized controlled trial, we aimed to test if INO administration could augment microcirculatory blood flow in patients with sepsis. We selected INO rather than other NO-donor therapies for this clinical trial on the grounds that INO can increase systemic NO bioavailability (as evidenced by increasing circulating nitrite) (18), and in contrast to other agents such as IV nitroglycerin that could drop arterial pressure, INO would not be expected to induce or exacerbate systemic arterial hypotension (45, 60, 61). In experimental models, the administration of INO has been shown to attenuate microcirculatory dysfunction in shock states via modulation of microvascular tone (62–64), preservation of microvascular integrity
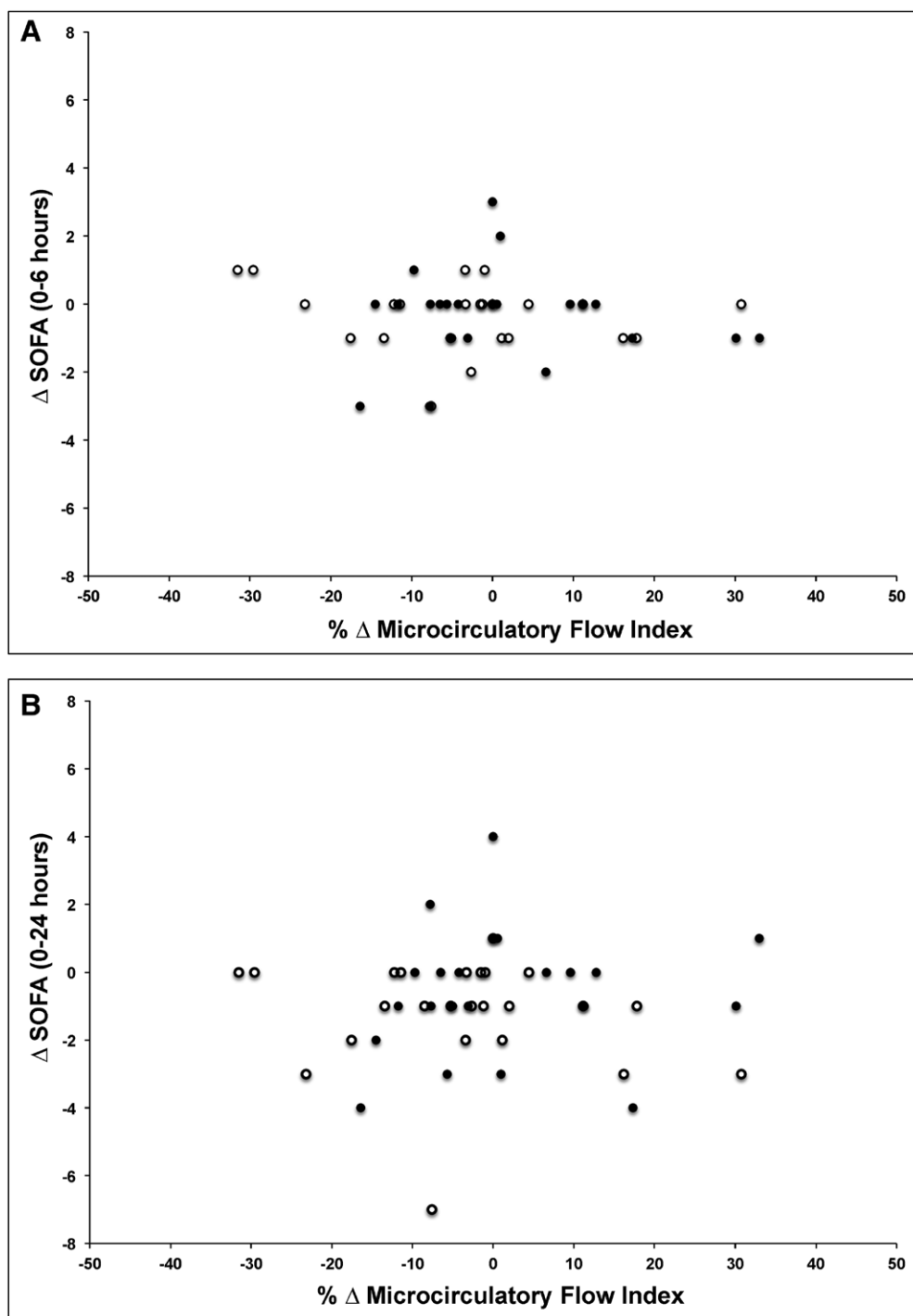
Figure 2. Relationship between percent change in microcirculatory flow index and change in Sequential Organ Failure Assessment (SOFA) score from 0 to 6hr (**A**) and 0–24hr (**B**) in all patients (n = 49). *Open circles* are inhaled nitric oxide−treated patients; *closed circles* are sham controls.

could represent clinical evidence of "downstream" effects of improved tissue perfusion (8, 9, 19). Although previous large-scale clinical trials of INO therapy in critically ill patients with acute respiratory distress syndrome (ARDS) did not show an outcome benefit, two of these trials excluded patients with sepsis (60, 61) and the third trial enrolled few patients with sepsis (45). Taken together, only 28 of 742 patients (4%) in the ARDS trials had sepsis; thus, the effects of INO in the treatment of patients with sepsis had not yet been tested.

In this sample of adult patients with severe sepsis and septic shock, we found that INO administration at 40 ppm over 6 hours raised plasma nitrite levels, indicative of systemic delivery. However, INO did not augment microcirculation as measured by SDF imaging. We also found no difference between INO and sham control groups in any of the clinically meaningful secondary outcome measures. There was no signal of harm detected in this critically ill hemodynamically unstable population, including no significant imbalance between groups in the occurrence of serious adverse events related to macrocirculatory hemodynamic deterioration.

To date, few clinical trials of novel therapies have targeted the microcirculation in patients with sepsis. Microcirculation-directed therapies that have been investigated previously include dobutamine, activated protein C, and levosimendan (72–75).

(65, 66), endothelial-dependent effects (13, 67, 68), reducing leukocyte adherence (69, 70), and direct anti-inflammatory effects (71). We also assessed two important patient-oriented outcome measures in this clinical trial—acute organ system dysfunction and lactate clearance from the blood—on the grounds that the development of organ dysfunction and elevation of serum lactate in sepsis are considered indicators of impaired tissue perfusion, and improvement in these indicators

To our knowledge, only one other randomized trial in patients with sepsis has tested NO-donor agent administration for the treatment of microcirculatory dysfunction. In that study of IV nitroglycerin administration, no improvement in microcirculation was found (76). Importantly, in both the nitroglycerin trial and the present clinical trial, study drug was not administered until after conventional macrocirculatory resuscitation goals were achieved. This leaves open the possibility

that if exogenous NO was administered as part of frontline resuscitation, for example, in the Emergency Department setting, that results might differ. A recent laboratory investigation using an ovine model of septic shock found that very early administration of tetrahydrobiopterin, a NOS cofactor, attenuated the development of microcirculatory dysfunction and markers of tissue dysoxia and improved survival (77). This generates the hypothesis that perhaps administration of exogenous NO early enough to prevent microcirculatory dysfunction (rather than reverse microcirculatory dysfunction that has already occurred) would be a more successful approach.

Although the primary results of this study represent what is commonly referred to as a "negative" clinical trial, we submit that our secondary analyses of the entire study population as a whole provide important mechanistic insights about the role of microcirculatory dysfunction in patients with sepsis. In contrast to previous clinical studies using SDF imaging in the very early resuscitation phase of sepsis therapy in which our group and others have reported that the degree of acute multiple organ failure was linked to the degree of microcirculatory blood flow impairment (7, 8), we did not find an association between changes in microcirculation and organ dysfunction in the present study. This may be related to the fact that all of the patients in this clinical trial underwent macrocirculatory hemodynamic optimization prior to randomization and microcirculatory assessment, and thus, the present data represent a somewhat later stage of sepsis therapy compared with our previous work. Taken together, these data add support to the hypothesis that acute organ system failure in sepsis may be a perfusion-mediated phenomenon in the earliest stage of disease, but later organ failure may not be perfusion related, and may relate to mitochondrial dysfunction and/or apoptosis (78).

This randomized trial has important limitations to consider. Although the sublingual site yields microcirculatory perfusion data that have previously been shown to predict mortality in critically ill patients (4–6, 33–35), it is not clear to what extent these microcirculatory data can be extrapolated to other regional beds. We also acknowledge that MFI is only semiquantitative (rather than quantitative), and there exists the possibility of subtle microcirculatory changes that are not reflected in the MFI value. We also did not assess mitochondrial function or cellular apoptosis in this study. Although there was a slight difference in baseline mean arterial pressure between groups, this is likely due to chance given the randomized design, and we believe not a major concern given that all patients were required to have a mean arterial pressure greater than or equal to 65 mm Hg prior to study drug initiation. Although we observed a rise in nitrite bioavailability among INO-treated patients, we acknowledge that nitrite is not the only mechanism by which INO can have extrapulmonary effects (e.g., S-nitrosylated albumin) and other potential mechanisms were not measured. We also acknowledge that we did not measure peroxynitrite formation in the blood, which could have been a factor in microcirculatory blood flow. In addition, the presence of diabetes can affect endothelial function, and we did

not have enough patients with diabetes in the sample to fully evaluate the role of diabetes in the microcirculatory response to therapy. Lastly, we did not test dose-response in this study, and it is possible that different doses could have produced different results.

CONCLUSIONS

In adult patients with severe sepsis or septic shock, we found that INO administration at 40 ppm did not augment microcirculatory perfusion, despite raising plasma nitrite levels. In contrast to prior clinical studies conducted during the initial resuscitation phase of therapy, we found no association between microcirculatory perfusion and multiple organ dysfunction in this population, in whom macrocirculatory hemodynamics had already been optimized.

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