Effects of nitroglycerin on sublingual microcirculatory blood flow in patients with severe sepsis/septic shock after a strict resuscitation protocol: A double-blind randomized placebo controlled trial

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Objectives: **Microcirculatory alterations have been associated with morbidity and mortality in human sepsis. Such alterations occur despite pressure-guided resuscitation. Earlier data suggested that impaired microcirculatory blood flow could be corrected with intravenous nitroglycerin in these patients. We tested this concept after fulfillment of preset systemic hemodynamic resuscitation end points in the early phase of sepsis.**

Design: **Prospective, single center, randomized, placebo-controlled, double-blind clinical trial.**

Setting: **Closed-format 22-bed mixed intensive care unit in a tertiary teaching hospital.**

Patients: **Patients ≥18 yrs with sepsis, according to international criteria, and at least one early sign of organ dysfunction, as the principal reason for intensive care unit admission, were eligible for enrollment.**

Interventions: **Patients were randomly assigned to receive nitroglycerin (n = 35) or placebo (n = 35) after fulfillment of protocol-driven resuscitation end points. This trial is registered with ClinicalTrials.gov as NCT00493415.**

Measurements and Main Results: **Primary outcome was sublingual microcirculatory blood flow of small vessels, as assessed by side-stream dark field imaging. After protocolized resuscitation, we observed recruitment of sublingual microcirculation in both groups, as indicated by a significant improvement in the microcirculatory flow index after 24 hrs, in comparison to baseline. However, no difference in the sublingual microvascular flow index was observed between groups. The median microvascular flow index in sublingual small-sized vessels was 2.71 (1.85–3) in the nitroglycerin group and 2.71 (1.27–3), $p = .80$, in the placebo group. In medium-sized vessels, the respective values were 3 (2.75–3) vs. 2.86 (2.19–3), $p = .21$, and in large-sized vessels, 3 (3–3) vs. 3 (2.89–3), $p = .06$. In-hospital mortality, as a secondary outcome, was 34.3% in the nitroglycerin group and 14.2% in the placebo group, $p = .09$.**

Conclusions: **In the context of a strict resuscitation protocol, based upon fulfillment of systemic hemodynamic end points in patients with early-phase severe sepsis or septic shock, we conclude that intravenous nitroglycerin does not promote sublingual microcirculatory blood flow. (Crit Care Med 2010; 38:93–100)**

Key Words: microcirculation; sidestream dark field imaging; nitroglycerin; sepsis; early goal-directed therapy; fluid responsiveness

For many decades, sepsis has been classified as a distributive form of shock (1). As opposed to all other forms of shock, it is not characterized by a reduction in cardiac output, but by a distributive inability of blood to effectively reach the main exchange site, namely the microcirculation. This view of distributive shock was confirmed by direct microcirculatory observations in septic patients after the introduction of sublingual orthogonal polarization spectral imaging (2) and its technical successor sidestream dark field (SDF) imaging (3). It has become clear that the discordance between systemic hemodynamic parameters and microcirculatory alterations is most prominent during sepsis, in contrast to other forms of shock (4). Under septic conditions, these alterations have also been identified as markers for morbidity and mortality (5, 6), regardless of the correction of systemic hemodynamic parameters and oxygen-derived variables (5–7). The view that resuscitated sepsis is persistently unresponsive to improvement led to the direction of therapeutic modalities toward improvement of microcirculatory function. It was within this context that Spronk et al (8) gave credence to a new concept: the instant improvement of microcirculatory flow by a bolus of the vasodilatory nitric oxide (NO) donor nitroglycerin (NTG). NTG was indicated following persistence of impaired micro-

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Dr. Ince is chief scientific officer of MicroVision-Medical, a university-based company that develops optical spectroscopic tools, such as the SDF imaging methodology used in the present study. He holds patents and shares of relevance to this role. The remaining authors have not disclosed any potential conflicts of interest.

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circulatory flow despite fulfillment of the traditional resuscitation end points. Although a generally accepted theory considers an overwhelming NO production as an important reason for a decrease in vascular constriction during sepsis, a trial blocking the NO system increased the patient's blood pressure, but there was an increasing in mortality, illustrating the ambiguous effects of the NO system in sepsis (9). Vincent et al (10) highlighted this delicate balance between the potential negative and positive effects of NO in sepsis. Reported reduction of leukocyte adherence, microvascular permeability, and platelet adhesion by NO (11, 12) all have the theoretical potential to improve microcirculatory blood flow, as suggested by the first direct observations of the sublingual microcirculation in a small patient population after administration of NTG in sepsis (8). Due its open-label design however, this study failed to adequately assess the influence of other factors, such as the evolvement of microcirculatory alterations over time. Furthermore, a central venous oxygen saturation ($S(c)VO_2$) driven approach to sepsis resuscitation was outside the scope of their effort (13). We therefore designed a randomized placebo controlled clinical trial in patients with early-phase severe sepsis or septic shock, and incorporated a strict SVO_2 driven resuscitation protocol and careful exclusion of fluid-responsiveness. Our goals were to compare: 1.) sublingual SDF-derived microcirculatory effects of NTG with those of placebo over time, and 2.) the effects of NTG on systemic hemodynamic parameters with those of placebo over time.

MATERIALS AND METHODS

Patients

The study was performed between January 2007 and June 2008 in a closed-format 22-bed mixed intensive care unit (ICU) in a tertiary teaching hospital. It was designed as a prospective, single-center, randomized, placebo-controlled, double-blind clinical trial and is registered with ClinicalTrials.gov as NCT00493415 and with EudraCT as 2006–004298-88. All patients of ≥18 yrs of age with suspected sepsis as the principal reason for ICU admission at the study site were eligible for assessment. Inclusion criteria were the presence of sepsis, according to international criteria (14), and at least one sign of early-phase organ dysfunction, as summarized in Table 1. Reasons for exclusion were pregnancy, use of nitrate derivatives within 24 hrs before admission, strict indication for intravenous nitroglycerin (instable coronary syndrome), organ dysfunction >48 hrs, or therapeutic restrictions (do not resuscitate orders excluded). A local ethical and scientific committee approved the study protocol, and written informed consent was obtained from the patients or their surrogate decision makers, consistent with applicable laws.

Table 1. Inclusion criteria: Fulfillment of at least one item in each list is needed for enrollment in the study

Suspected source of infection:
- New infiltrate on radiograph plus positive Gram stain or culture
- Positive blood culture
- Confirmed bowel perforation
- Positive urine Gram stain or culture
- Positive spinal fluid Gram stain or culture
- Necrotizing fasciitis plus positive Gram stain or culture
- Arthritis plus positive Gram stain or culture
- Mediastinitis plus positive Gram stain or culture
- Pancreatitis plus positive fine needle aspiration

Signs of organ failure:
- Oliguria: urine output <0.5 mg/kg per hr for at least 2 consecutive hrs after adequate volume resuscitation, or serum creatinine >177 mmol/L in the absence of chronic renal failure (creatinine >177 mmol/L or hemodialysis)
- Metabolic acidosis: pH <7.35 and lactate >2.5 mmol/L
- Respiratory failure: Pao_2/Fio_2 <26.6 kPa
- Coagulation disorders: platelet count $<100 \times 10^9$/L or prothrombin time international normalized ratio >1.5 in the absence of coumarine derivatives
- Cardiovascular failure: persistent hypotension (systolic blood pressure <90 mm Hg) despite adequate volume supply, resulting in the use of vasopressors (dopamine >5 μg/kg per min, norepinephrine any dose)

Protocol

As soon as a patient with suspected sepsis was admitted to the ICU, patients were aimed for enrollment within 4 hrs after admittance. Before randomization, all patients followed a strict protocol to optimize systemic hemodynamic parameters in accordance with the basic principles of early goal-directed therapy (13). Systemic hemodynamic assessment was achieved through continuous invasive monitoring of arterial blood pressure and right heart catheterization with continuous cardiac output and central venous oxygen saturation (Vigilance, Edwards Lifesciences, Saint-Prex, Switzerland). Until a pulmonary artery catheter was in place, the use of fluids and vasoactive agents was at the discretion of the attending physician, whose goal was to maintain a minimal mean arterial pressure of 60 mm Hg. After calibration, treatment of circulatory failure was performed using the following strict hierarchical order: 1) establishment of fluid responsiveness by repeated infusions of at least 250 mL crystalloids, colloids, or blood products, until the increase in left ventricular stroke volume was <10%, or until the pulmonary artery wedge pressure exceeded 18 mm Hg; 2) treatment of inadequate systemic oxygen supply, defined as a cardiac index <2.5 L/m^2 per minute or central venous oxygen saturation <70%, with dopamine administered at up to 10 μg/kg per minute and additional enoximone in the event of an inadequate response to dopamine; and 3) reversal of hypotension with norepinephrine in case of mean arterial pressure <60 mm Hg despite the aforementioned steps.

Immediately after fulfillment of these therapeutic end points, patients entered a 24-hr period of study medication. During this period, therapeutic goals remained unchanged. The use of hydrocortisone up to a maximum of 100 mg intravenously 3 q.d. was permitted for shock reversal in case of vasopressor dependency; the general red blood cell transfusion trigger was hematocrit <25%. After fulfillment of all systemic hemodynamic end points, and additional repeat exclusion of fluid responsiveness, we recorded the baseline systemic and microcirculatory parameters. Treatment with NTG (1 mg/mL) or placebo (isotonic saline) was randomly assigned for each block of six. The study medication was prepared at the pharmacy department according to a randomization list and delivered to the ICU department in identical syringes. Blinding to investigators was maintained until hospital discharge of all patients. During the first 30 mins of administration, a front load of 2 mL was given continuously (4 mL/hr); during the next 23.5 hrs, the infusion rate was kept constant at 2 mL/hr. In cases of patient body weight <50 kg, infusion rates were reduced by 50%. For safety reasons, a sustained mean arterial pressure <60 mm Hg during infusion despite protocolized treatment constituted a reason to immediately and permanently stop the infusion therapy.

Imaging and Analysis

SDF imaging is a stroboscopic light emitting diode ring-based imaging modality that is incorporated in a handheld device. The device illuminates an area of interest for clinical observation of the microcirculation. It has been successfully validated against its technical predecessor, namely orthogonal polarization spectral imaging (3). If a wavelength within the hemoglobin absorption spectrum (e.g., 530 nm) is chosen, red blood cells will appear dark and white blood cells may be visible as refringent bodies. The vessel walls are not visualized directly and imaging therefore depends on the presence of red blood cells. Semiquantitative analysis was performed as described in detail elsewhere (15). In short, a minimum of three steady images of at least 20 secs in duration were obtained from the sublingual region by a research investigator other than the attending physician. A specially trained group of four individuals was available during the study period at all times, with the exception of a total of 7 days. After gentle removal of saliva by an isotonic saline–drenched gauze and avoiding pressure artifacts, images were acquired and stored on a digital videotape (Video Walkman GV-D 1000E, Sony, Tokyo, Japan). Subsequently, the images were captured in 5 to 10 sec representative AVI format video clips (SonyDVgate, Sony, Tokyo, Japan). Video clips were blindly analyzed offline by an investigator who had no involvement in the data collection. The images were presented in random order so as to prevent interimage coupling. SDF images were obtained from three different locations within the sublingual region, and each image was divided into four equal quadrants. Quantification of flow (no flow: 0, intermittent flow: 1, sluggish flow: 2, and continuous flow: 3) was scored per quadrant for each vessel diameter cohort (small: 10–25 μm, medium 26–50 μm, and large 51–100 μm). The microvascular flow index (MFI) was calculated as the sum of each quadrant score divided by the number of quadrants in which the vessel type was visible. The final MFI was averaged over a maximum of 12 quadrants (three regions, four quadrants per region) derived from the overall flow impressions of all vessels with a particular range of diameter in a given quadrant. The heterogeneity index was calculated, following the method of Trzeciak and colleagues (6), as the difference between the highest and lowest MFI, divided by the mean MFI of all sublingual sites at a single time point. Calculation of total (small) vessel density was performed with the AVA 3.0 software package (MicroVision Medical, Amsterdam, The Netherlands), as described and validated recently (16) using a cutoff diameter for small vessels of <20 μm. After stabilization of the images using the AVA 3.0 software, we defined the perfused (small) vessel density and the proportion of perfused (small) vessels (PPVs) in terms of the number and percentage of crossings with perfused (small) vessels per total length of three equidistant horizontal and three equidistant vertical lines. This method has been described elsewhere by de Backer et al (17) and is in accordance with reports of a round table conference.

Data Collection

The following data were recorded at baseline: general characteristics; severity of illness and predicted mortality consistent with Acute Physiology and Chronic Health Evaluation (APACHE) IV (18), Sequential Organ Failure Assessment (SOFA) (19) (calculated over the first 24 hrs following ICU admission), and Risk, Injury, Failure, Loss, and End-stage kidney disease (RIFLE) (20) scores; systemic hemodynamic parameters; sublingual SDF images; and results of standard laboratory tests, including blood gases and arterial lactate concentrations, blood cultures, and cultures of specimens sampled from each presumed site of infection. Systemic hemodynamics, SDF images, arterial lactate concentrations, and blood gases were recorded at 30 mins, and 2, 12, and 24 hrs after the start of the study medication and the SOFA plus RIFLE score was calculated daily during each patient's ICU stay. Survival status was confirmed for each subject at the end of their hospitalization. The primary end point was MFI within the 24-hr study medication period. Secondary end points were the SOFA score, systemic hemodynamics, dose of dopamine/norepinephrine and survival distribution from randomization to hospital discharge.

Statistical Analysis

We anticipated a mean MFI at baseline of 1.6 with a standard deviation (SD) of 0.83, based on earlier observations (21). We calculated a sample size of 70 patients to detect an absolute difference in MFI of 0.6 in a two-sided test with a 0.05 type I error and an 80% probability. The Statistical Package for Social Sciences (SPSS 15.1 for Windows, Chicago IL) was used for statistical analyses. For continuous variables, all data are presented as mean ± SD, or as medians and interquartile ranges (IQRs) in case of nonnormal distributions. The effects of treatment on systemic and microcirculatory parameters were compared between groups using an independent sample Student's *t* test. The effects on nonnormally distributed parameters (MFI, total vessel density, PPV, perfused vessel density, LOS, cumulative SOFAs, and maximum RIFLE score) were compared using the nonparametric Mann–Whitney test. Comparison of mortality rates across different treatment strategies was performed using the chi-square test. Cumulative event curves (censored end point at day 60) were estimated with the Kaplan–Meier procedure and the effect of treatment on survival probability was compared between groups with a log-rank test. Comparison against baseline of systemic and microcirculatory hemodynamic parameters after 24 hrs was performed with a paired Student's *t* test, or with a Wilcoxon signed rank test in case of variables that were not normally distributed. A two-sided *p* value of $<.05$ was considered statistically significant.

RESULTS

Out of 133 patients who were screened for eligibility, 70 patients were randomly assigned to receive treatment (Fig. 1). Protocolized resuscitation and start of study medication was achieved in all patients within 4 hrs after ICU admittance. Pulmonary artery catheterization was performed successfully in all patients.

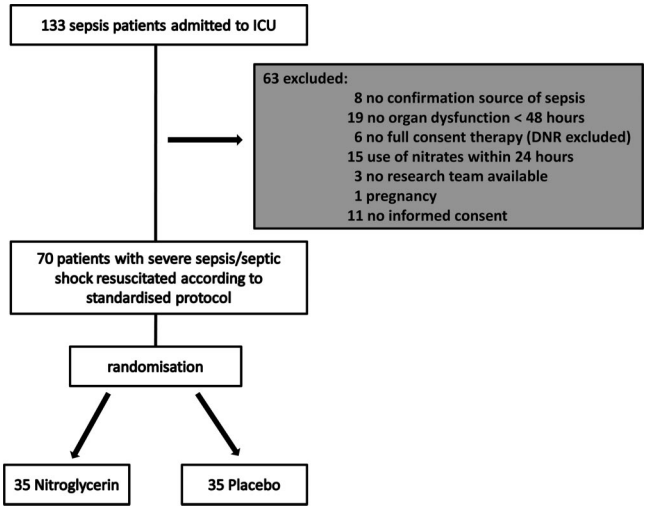

Figure 1. Trial profile. *ICU*, intensive care unit; *DNR*, do not resuscitate.

Table 2. Baseline characteristics

Variables	Nitroglycerin (n = 35)	Placebo (n = 35)	p
Men	21 (60)	22 (62.9)	.81
Age	64 ± 15.8	59 (15.4)	.2
APACHE IV	88 ± 24.0	94 ± 62.0	.59
Predicted mortality APACHE IV, %	43 ± 21.0	39 ± 16.0	.36
SOFA	9 ± 3.0	10 ± 3.0	.66
Source of infection			
Lung	12 (34)	12 (34)	
Abdominal	19 (54)	12 (34)	
Urinary tract	2 (6)	2 (6)	
Other	2 (6)	9 (26)	
Mean arterial pressure, mm Hg	72 ± 11.5	71 ± 12.0	.97
Heart rate, beats/min	109 ± 16.7	112 ± 17.8	.52
Central venous pressure, mm Hg	12 ± 4.0	13 ± 5.5	.26
Cardiac index, L/min per m^2	4.1 ± 1.3	4.3 ± 1.3	.63
Pulmonary artery wedge pressure, mm Hg	15 ± 6.0	16 ± 5.9	.51
Mixed venous oxygen saturation, %	71 ± 8.4	71 ± 6.3	.87
Oxygen consumption, mL/min per m^2	153 ± 46.0	165 ± 75.0	.41
Oxygen delivery, mL/min per m^2	581 ± 168.0	608 ± 214.0	.55
Oxygen extraction, %	28 ± 8.8	27 ± 7.1	.78
Dopamine dose, n (μg/kg per min)	30 (5.4 ± 3.8)	30 (6.0 ± 3.5)	.53
Norepinephrine dose, n (μg/kg per min)	11 (0.37 ± 0.52)	12 (0.27 ± 0.43)	.37
Central-to-toe temperature gradient, °C	5.5 ± 2.6	5.4 ± 2.5	.87
Ventilation-perfusion ratio, %	30 ± 10.0	32 ± 10.0	.31
Ventilator, use of	34 (97.1)	35 (100)	.32
PEEP, cm H_2O	12 ± 3.0	13 ± 3.0	.28
Lactate, baseline, mmol/L	2.8 ± 2.3	2.4 ± 2.1	.50
Lactate, highest before baseline, mmol/L	3.5 ± 2.4	3.2 ± 2.9	.63
pH	7.31 ± 0.9	7.31 ± 1.0	.96
Base excess, mmol/L	−5.8 ± 5.3	−6.2 ± 6.0	.26
Hematocrit, %	31 ± 4.0	32 ± 6.0	.80
ARF RIFLE score on admission	0.51 ± 0.98	0.89 ± 1.08	.14

APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment; PEEP, positive end-expiratory pressure; ARF, acute renal failure; RIFLE, Risk, Injury, Failure, Loss, and Endstage.

All data are presented as mean ± SD or as numbers (%).

Baseline characteristics were well balanced between the groups (Table 2). Exceptions were an insignificantly higher age and RIFLE score in the placebo group and more prominent abdominal sepsis in the NTG group. Causative pathogens were identified in 60 (86%) patients and blood cultures were positive for 16 (22.8%) patients. Nine patients in the NTG group and seven patients in the placebo group received enoximone during the study period; two patients in the placebo group received activated protein C. Three patients in the NTG group and one patient in the placebo group died within 24 hrs of admission due to progressive cardiac failure or cardiac arrest. Withdrawal from the study because of sustained hypotension was not reported.

In both groups, MFI in small vessels significantly increased over the 24-hr period of study medication, in comparison to baseline. In the NTG group, the change was from 1.67 (0.67–2.42) to 2.71 (1.85–3), $p < .0001$, and in the placebo group from 1.42 (0.83–2.63) to 2.71 (1.27–3), $p = .006$ (Table 3, Fig. 2). MFI in medium and large-sized vessels increased in a small but significant way in the NTG group, and not in the placebo group (Table 3).

Despite this overall increment in small vessel MFI over time, there was no significant difference in primary outcome between the NTG and placebo groups at 24 hrs after the administration of study medication (Table 3). Median MFI in sublingual small vessels was 2.71 (1.85–3) vs. 2.71 (1.27–3), $p = .80$, in medium-sized vessels 3 (2.75–3) vs. 2.86 (2.19–3), $p = .21$, and in large-sized vessels 3 (3–3) vs. 3 (2.89–3), $p = .06$ (Table 3, Fig. 2). The number of responders, defined as any MFI increment for small vessels between baseline and 24 hrs, was similar in both the NTG and placebo groups: 24 (68.5%) vs. 20 (57.1%), $p = .46$. The heterogeneity index and parameters of (perfused) vessel density (total vessel density, PPV, and perfused vessel density) did not differ between groups, except for a significant but small difference in PPV after 30 mins and after 24 hrs (Table 3).

The heterogeneity index decreased significantly over time in both groups, but parameters of (perfused) vessel density remained unaltered.

At the time of inclusion, 56 out of 70 patients fulfilled the criteria for septic shock, 29 in the placebo group and 27 in the NTG group. For patients in shock, median MFI of small vessels was 1.5 (0.7–2.7) at the start of study medication and 2.8 (1.8–3) after 24 hrs. For nonshock, this was 1.7 (1.1–2.3) and 2.5 (1.6–2.8), respectively.

In terms of secondary outcomes, there was no difference in systemic hemodynamic variables between treatment groups, with the exception of a lower central-to-toe temperature gradient in the NTG group in comparison to placebo (4.4 ± 1.9 and 6.6 ± 3.8 respectively, $p = .004$) after 12 hrs (Table 4). Mean blood pressure, oxygen consumption, and arterial lactate levels did not differ at any time. The percentage of patients who had a significant drop in mean blood pressure (>10 mm Hg or any increment of norepinephrine dose to maintain mean arterial blood pressure >60 mm Hg) within the first 30 mins (after a considerable dose of 2 mg NTG) was 25.7 (9 of 35) in the NTG group and 17.1 (6 of 35) in the placebo group. Both groups were administered equivalent doses of dopamine/norepinephrine and no significant difference was recorded in terms of fluid balance after 24 hrs (Table 4).

Both for ICU and hospital LOS, as well as for cumulative SOFA scores over the first 5 days of ICU admission, there was a significant reduction in favor of the NTG group (Table 5). It is important to note that the NTG group presented a substantially higher ICU mortality rate, although the difference between the groups remained insignificant (31.4% in the NTG group and 11.4% in the placebo group, $p = .08$). In addition, the NTG group displayed a higher in-hospital mortality rate, although the difference was insignificant (34.3% in the NTG group and 14.2% in the placebo group, $p = .09$) (Table 5, Fig. 3).

DISCUSSION

In the present study, we found that protocolized resuscitation resulted in recruitment of sublingual microcirculation, as indicated by a significant improvement in the MFI in comparison to baseline. However, we did not find any additional MFI improvement as a result

Table 3. Microvascular variables over time

Variables	Baseline NTG (n = 35)	Placebo (n = 35)	30 mins NTG (n = 35)	Placebo (n = 35)	2 hrs NTG (n = 35)	Placebo (n = 35)	12 hrs NTG (n = 34)	Placebo (n = 35)	24 hrs NTG (n = 32)	Placebo (n = 34)
MFI small vessels	1.67 (0.67–2.42)	1.42 (0.83–2.63)	1.83 (1.08–2.75)	1.83 (0.83–2.83)	2.25 (1.42–2.75)	2.25 (1.25–2.92)	2.34 (0.83–3)	2.08 (1.5–2.83)	2.71 (1.85–3)[b]	2.71 (1.27–3)[a]
MFI medium vessels	2.33 (1.83–2.83)	2.33 (2–2.83)	2.67 (2.25–2.83)	2.42 (2.17–2.92)	2.83 (2.42–3)	2.75 (2.33–3)	2.79 (2.08–3)	2.67 (2.5–2.92)	3 (2.75–3)[b]	2.86 (2.19–3)
MFI large vessels	2.92 (2.75–3)	2.92 (2.75–3)	3 (2.83–3)	3 (2.75–3)	3 (3–3)	3 (3–3)	3 (2.81–3)	3 (2.92–3)	3 (3–3)[a]	2.89 (3–3)
TVD, mm/mm^2	14 (12.8–15.6)	15 (12.3–16.1)	13.9 (12.2–15)	14.1 (12.8–15.9)	14.3 (13.2–15.1)	14 (12.9–16)	14 (13.1–15.7)	13.9 (13.2–15.5)	13.9 (12.5–15.7)	14.7 (13.1–16.1)
PPV, %	98 (93–100)	97 (89–100)	100 (96–100)	97 (90–99)[c]	99 (96–100)	98 (93–100)	99 (93–100)	99 (93–100)	100 (98–100)	98 (86–100)[c]
PVD, 1/mm	9.1 (8.3–10.5)	9.8 (8.4–10.8)	9.7 (8.7–10.5)	9.7 (8–10.5)	9.7 (8.4–10.7)	9.5 (8.7–11.3)	10 (8.3–10.8)	9.1 (8.2–10.5)	10.2 (8.7–11.2)	10.1 (8.5–10.7)
Heterogeneity index	1.76 (0.88–2.84)	1.96 (0.66–3)	1.71 (0.36–2.17)	1.53 (0.36–2.75)	0.82 (0.26–2.11)	1.24 (0.34–2.4)	1.22 (0–2.89)	1.44 (0.35–2)	0.74 (0–1.62)[b]	0.54 (0–1.76)[a]

NTG, nitroglycerin; MFI, microcirculatory flow index; TVD, total vessel density of (small) vessels; PPV, proportion of perfused(small) vessels; PVD, perfused (small) vessel density.

[a]$p < .05$; [b]$p < .0001$ after 24 hrs in comparison to baseline, nonparametric test for dependent samples; [c]$p < .05$ between groups, nonparametric test for independent samples. Cutoff value for small vessels <20 μm. All data are presented as medians (interquartile range).

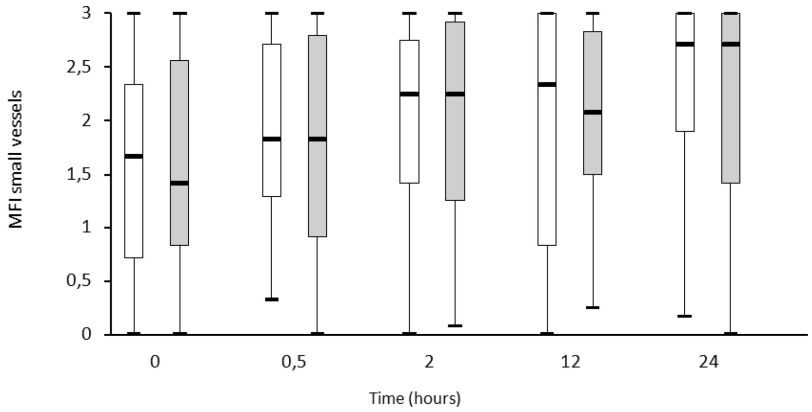

Figure 2. Box plots of sublingual microvascular flow index (*MFI*) in small vessels (<20 μm) during the 24-hr study period. *White boxes*, nitroglycerin, *gray boxes*, placebo.

of treatment with NTG in comparison to placebo in terms of sublingual microcirculatory alterations in patients with severe sepsis or septic shock, with the exception of a very small but significant increment in the proportion of perfused small vessels in the NTG group. In addition, we did not detect any significant differences in systemic hemodynamic parameters between the two treatment strategies, with the exception of a transient significantly lower central-to-toe temperature gradient evidenced in the NTG group. We observed no difference in MFI, after 24 hrs of study medication, between patients who fulfilled international criteria for shock and patients who did not.

Our findings are not consistent with a previous report in which eight patients responded promptly to an intravenous bolus of 0.5 mg NTG after pressure-guided resuscitation with a significant increase in sublingual MFI (8). However, despite use of an equivalent NTG dose, there are marked differences in respect to the study designs. To our knowledge, the present study is the first randomized placebo-controlled trial that has aimed to improve microcirculatory blood flow in patients with severe sepsis/septic shock, after a rigorous resuscitation protocol based upon the principles of early goal-directed therapy and repetitive dynamic testing of fluid responsiveness. Resuscitation end points (minimal mean blood pressure of >60 mm Hg, central venous pressure >12 mm Hg at the lowest dopamine dose) in the previous study did not rule out fluid responsiveness, nor persistence of heart failure. The fact that MFI of large vessels in the study by Spronk et al (8) was below 2.5 in the majority of patients and that it responded to NTG to the same extent as in small vessels is consistent with persistent fluid responsiveness. Specifically, flow in large vessels is maintained despite gross flow alterations in small vessels (17), as was the case in our study. In a recent report, septic patients who had been characterized as responders to fluid expansion in terms of classic systemic hemodynamic parameters demonstrated concomitant improvement of sublingual microcirculatory blood flow (22). Furthermore, treatment of (relative) heart failure by targeting a central venous oxygen saturation ≥70% has been proven to lead to improved outcomes (13). However, despite a reported relationship between improvement of the SOFA score and sublingual microcirculatory blood flow (23), a causative relation between optimization of venous oxygen saturation and microcirculatory blood flow remains to be established. Besides these differences in resuscitation end points, other factors, such as timing and concomitant medication, may have played a role. The previously reported immediate response to NTG after 5 mins was outside the scope of our observations and use of the S_2-serotonergic receptor blocker ketanserin was not part of our protocol. Indeed, under conditions of local hypoxia, red blood cell scavenging of nitric oxide has been demonstrated to evoke a situation-dependent vasoconstrictive effect in the context of serotonin administration (24).

An important assumption that underpinned our study design was the notion that NTG would act as an NO donor. NTG and other organic nitrates are believed to use the same signaling pathway as NO generated by NO synthases. In this manner, NO-related promotion of vascular smooth muscle relaxation, attenuation of leukocyte-endothelium and leukocyte-platelet interaction (25), and reduction of edema formation (26) were all considered potential mechanisms to promote microcirculatory blood flow. However, in contrast to isosorbide dinitrate, NTG showed a striking dissociation of its vascular activity and NO-donor properties (27). This dissociation together with a diminished suppression of activity by the NO scavenger carboxy-PTIO (28) challenges the widely accepted NTG/NO hypothesis.

Apart from the discussed mechanism of action, the net effect of NTG on the delicate balance between vasodilatation and vasoconstriction also depends on

Table 4. Systemic hemodynamic variables over time

Variables	Baseline NTG (n = 35)	Placebo (n = 35)	30 mins NTG (n = 35)	Placebo (n = 35)	2 hrs NTG (n = 35)	Placebo (n = 35)	12 hrs NTG (n = 34)	Placebo (n = 35)	24 hrs NTG (n = 32)	Placebo (n = 34)
Mean arterial pressure, mm Hg	72 ± 11.5	71 ± 12.0	66 ± 14.7	70 ± 11.8	66 ± 12.2	67 ± 8.9	64 ± 11.7	66 ± 10.8	69 ± 10.7	67 ± 10.7
Heart rate, beats/min	109 ± 16.7	112 ± 17.8	109 ± 18.1	111 ± 20.3	108 ± 19.7	106 ± 17.3	108 ± 17.4	105 ± 18.9	109 ± 20.0	108 ± 17.2
Central venous pressure, mm Hg	12 ± 4	13 ± 5.5	11 ± 4	12 ± 4.7	11 ± 4.3	12 ± 4.3	11 ± 4	12 ± 5.5	12 ± 4	12 ± 5.1
Cardiac index, L/min per m^2	4.1 ± 1.3	4.3 ± 1.3	4.1 ± 1.3	4.1 ± 1.2	4.1 ± 1.3	3.9 ± 1	4.0 ± 1.2	3.7 ± 0.8	4.2 ± 1.0	4.1 ± 0.87
Pulmonary artery wedge pressure, mm Hg	15 ± 6	16 ± 6	15 ± 6	16 ± 6	14 ± 5	16 ± 5	15 ± 5	16 ± 5	14 ± 6	15 ± 6
Mixed venous oxygen saturation, %	71 ± 8.4	71 ± 6.3	69 ± 8.6	71 ± 8.2	70 ± 8.6	69 ± 7.7	71 ± 10.4	72 ± 5.9	73 ± 7.1	75 ± 6.1
Oxygen consumption, mL/min per m^2	153 ± 46	165 ± 75	147 ± 49	160 ± 173	145 ± 50	151 ± 38	141 ± 37	142 ± 43	147 ± 43	135 ± 33
Oxygen delivery, mL/min per m^2	581 ± 168	608 ± 214	554 ± 160	588 ± 173	542 ± 155	563 ± 158	544 ± 161	540 ± 117	588 ± 159	583 ± 140
Oxygen extraction, %	28 ± 8.8	27 ± 7.1	28 ± 8.9	28 ± 7.3	29 ± 9.4	29 ± 7.5	28 ± 11.1	26 ± 6.2	26 ± 6.9	24 ± 7.0
Dopamine dose, n (μg/kg per min)	30 (5.4 ± 3.8)	30 (6.0 ± 3.5)	31 (5.6 ± 3.9)	30 (5.8 ± 3.3)	31 (6.1 ± 3.9)	32 (6.3 ± 3.2)	32 (6.7 ± 4.0)	32 (7.0 ± 3.3)	31 (6.0 ± 4.2)	30 (7.1 ± 3.6)
Norepinephrine dose, n (μg/kg per min)	11 (0.07 ± 0.1)	12 (0.05 ± 0.09)	17 (0.07 ± 0.1)	18 (0.07 ± 0.7)	18 (0.08 ± 0.1)	20 (0.08 ± 0.2)	25 (0.12 ± 0.09)	25 (0.09 ± 0.11)	17 (0.12 ± 0.12)	18 (0.12 ± 0.20)
Central-to-toe temperature gradient, °C	5.5 ± 2.6	5.4 ± 2.5	5.2 ± 2.3	5.2 ± 3	4.9 ± 2.9	6.5 ± 6.1	4.4 ± 1.9[a]	6.6 ± 3.8	4.3 ± 2.1	5.4 ± 2.5
Ventilation–perfusion ratio, %	30 ± 10	32 ± 10	33 ± 11	32 ± 10	29 ± 8	30 ± 8	28 ± 8	28 ± 10	27 ± 8	30 ± 10
Lactate, mmol/L	2.8 ± 2.3	2.4 ± 2.1	2.8 ± 2.5	2.3 ± 2	2.6 ± 2.2	2.1 ± 1.7	2.0 ± 1.9	1.8±.2.0	1.9 ± 1.6	2.69 ± 4.2
Hematocrit, %	31 ± 4.2	32 ± 6.0	30 ± 4.1	32 ± 5.7	30 ± 3.8	32 ± 5.3	30 ± 3.9	33 ± 4.8	30 ± 3.9	31 ± 3.8
Fluid balance, L	—	—	—	—	—	—	—	—	5.8 ± 2.7	6.5 ± 2.3

NTG, nitroglycerin.

[a]p value <.05, nonparametric test for independent samples between treatment groups. All data are presented as mean ± SD.

Table 5. Morbidity and mortality outcome variables

Variables	Nitroglycerin (n = 35)	Placebo (n = 35)	p
ICU mortality, n (%)	11 (31.4)	4 (11.4)	.08
Hospital mortality, n (%)	12 (34.3)	5 (14.2)	.09
LOS ICU, all patients, median (IQR)	8 (4–12)	12 (7–16)	.03
LOS ICU, survivors, median (IQR)	9 (5–12)	18 (9–35)	.11
LOS hospital, all patients, median (IQR)	21 (8–35)	29 (17–48)	.04
LOS hospital, survivors, median (IQR)	11 (7–14)	18 (12–40)	.67
Cumulative SOFA day 1–5, all patients	30 (22–41)	46 (32–53)	.003
Cumulative SOFA day 1–5, survivors	31 (28–40)	46 (34–53)	.07
Cumulative SOFA ICU, all patients	46 (28–80)	66 (49–121)	.02
Cumulative SOFA ICU, survivors	50 (30–68)	67 (54–93)	.03
ARF RIFLE score maximum, median (IQR)	3 (0–3)	1 (0–3)	.37
CVVH, use of, n (%)	12 (34.3)	11 (31.4)	.87

ICU, intensive care unit; LOS, length of stay; SOFA, Sequential Organ Failure Assessment; ARF, acute renal failure; RIFLE, Risk, Injury, Failure, Loss, and Endstage; CVVH, continuous veno-venous hemofiltration.

mechanisms referred to as tolerance and pseudotolerance. Pseudotolerance involves the dose-dependent, non-NTG specific neurohormonal counter-regulation due to the use of vasodilatory substances and has been reported to occur within 48 hrs of administration (26). It includes increases in plasma catecholamine, vasopressin, and aldosterone levels, as well as enhanced plasma renin activity (28). NTG-specific hypercontractile responses to angiotensin II and phenylephrine as a result of NTG-induced endothelial dysfunction following chronic NTG exposure is termed nitrate tolerance (29), and is believed to be elicited by oxidative stress (30). NTG treatment has previously been shown to stimulate vascular peroxynitrite formation as a reaction product of NO and superoxide (31, 32). Insufficient concentrations of the NOS III cofactor tetrahydrobiopterin may lead to further uncoupling of NOS III, with subsequent superoxide release (33). It is conceivable that, due to previous endothelial dysfunction and a relative lack of substrates in sepsis, situation-dependent formation of peroxynitrite and superoxide instead of NO may occur, even within the timeframe of the present study. The observed absence of differences in both microcirculatory and systemic parameters between NTG and placebo treatment does not disallow the potential absence of NO-mediated vascular relaxation and warrants further study.

Although not designed to detect differences in survival between the NTG and placebo groups, this study revealed an insignificant but substantial difference in absolute numbers with regard to ICU and in-hospital mortality, in favor of the placebo group. However, the observed lower cumulative SOFA scores in the NTG group do not create an unequivocal picture, even after exclusion of nonsurvivors. Due to our relatively small sample size, an imbalance between the two groups cannot be ruled out. We observed no differences in circulatory parameters that could explain the difference in mortality. The overall in-hospital mortality in both groups was lower than that predicted by the APACHE IV scores: We observed an in-hospital mortality rate of 34.3% in the NTG group, as compared with a predicted 43 ± 21% rate. In the placebo group, the 14.2% predicted value was lower than the 39 ± 16% observed metric.

Nevertheless, many potential deleterious mechanisms as a result of the administration of NTG cannot be ruled out as a cause for a higher mortality rate. If NTG fuels the formation of peroxynitrite, su-

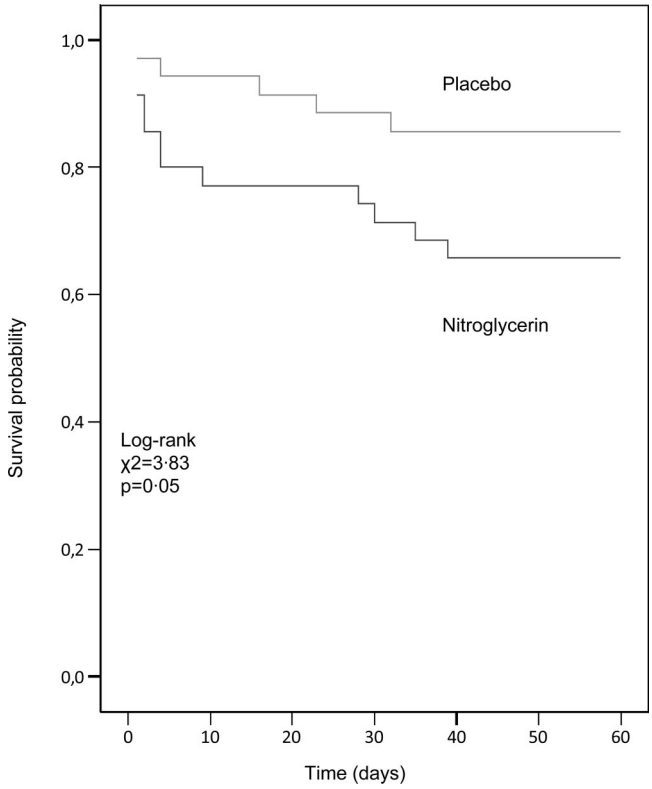

Figure 3. Kaplan–Meier plot of survival from randomization to day 60.

peroxide, and other radical oxygen species in septic patients (31, 32), this may lead to aggravation of the already existing endothelial dysfunction in sepsis. Also, NTG is reported to open mitochondrial permeability transition pores, and mitochondrial production of radical oxygen species (34). Mitochondrial failure due to NTG is in line with the observed higher mortality without differences in hemodynamic parameters. Apart from mechanistic reasons, that may explain a potential relationship between the administration of NTG and a higher mortality rate, direct observation of the sublingual microcirculation does not *per se* disclose microcirculatory alterations in other organs (21). In case such alterations were to be adaptive to (e.g.) mitochondrial failure, opening of other microcirculatory beds could theoretically interfere with the suggested adaptive mechanisms. Having stated this, however, an analysis of the causes of mortality did not implicate a common cause of death associated with such mechanisms.

Limitations of the present study are largely related to the trial design. Specifically, the complete blinding between the attending physician and the investigator involved the inclusion of all eligible patients, regardless of the extent of their baseline sublingual microcirculatory alterations. Although the MFI at baseline was consistent with our power calculations across both groups, we note our study's consequent loss of discriminative ability due to the inclusion of patients without a significant decrease in baseline MFI. Furthermore, the (predominantly) semiquantitative methodology used in our analysis of flow and capillary density may have resulted in the loss of detailed information that was beyond the recording range of our instruments. In this respect, we note that both MFI and heterogeneity index evolved over time, consistent with other reports in the literature (6, 17), whereas PPV remained unaltered over time and was considerably higher than that in previous reports (4, 5). This discrepancy may be due to differences in resuscitation end points. The use of software in this study to accurately measure vessel diameter, instead of discriminating small from large vessels by eye, may also lead to the inclusion of generally better perfused larger vessels in PPV. It should also be emphasized that the relationship between (potentially) promicrocirculatory resuscitation strategies and patient outcome remains to be established.

In conclusion, our presented data do not support the hypothesis that treatment of patients in the early phase of severe sepsis or septic shock with intravenous nitroglycerin promotes sublingual microcirculatory blood flow and perfusion. Our results are of note in the context of a rigorous resuscitation protocol that aims to fulfill systemic hemodynamic end points as initial procedure.

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