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Original Article

Dose-related effects of norepinephrine on early-stage endotoxemic shock in a swine model

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ABSTRACT

Background: The benefits of early use of norepinephrine in endotoxemic shock remain unknown. We aimed to elucidate the effects of different doses of norepinephrine in early-stage endotoxemic shock using a clinically relevant large animal model.

Methods: Vasodilatory shock was induced by endotoxin bolus in 30 Bama suckling pigs. Treatment included fluid resuscitation and administration of different doses of norepinephrine, to induce return to baseline mean arterial pressure (MAP). Fluid management, hemodynamic, microcirculation, inflammation, and organ function variables were monitored. All animals were supported for 6 h after endotoxemic shock.

Results: Infused fluid volume decreased with increasing norepinephrine dose. Return to baseline MAP was achieved more frequently with doses of 0.8 μg/kg/min and 1.6 μg/kg/min (P <0.01). At the end of the shock resuscitation period, cardiac index was higher in pigs treated with 0.8 μg/kg/min norepinephrine (P <0.01), while systemic vascular resistance was higher in those receiving 0.4 μg/kg/min (P <0.01). Extravascular lung water level and degree of organ edema were higher in animals administered no or 0.2 μg/kg/min norepinephrine (P <0.01), while the percentage of perfused small vessel density (PSVD) was higher in those receiving 0.8 μg/kg/min (P <0.05) and serum lactate was higher in the groups administered no and 1.6 μg/kg/min norepinephrine (P <0.01).

Conclusions: The impact of norepinephrine on the macro- and micro-circulation in early-stage endotoxemic shock is dose-dependent, with very low and very high doses resulting in detrimental effects. Only an appropriate norepinephrine dose was associated with improved tissue perfusion and organ function.

Introduction

Endotoxemic shock is characterized by peripheral vasodilatation induced by systemic inflammatory responses and dysregulated host reactions.[1] The vasodilation induced in this syndrome leads to relative insufficiency of effective circulating volume, resulting in microcirculatory disruption and organ hypoperfusion.[2] Adequate and effective early fluid resuscitation has become the first-line management strategy for endotoxemic shock[3]; however, excessive infusion inevitably induces fluid overload and tissue edema.[4,5]

Inconsistency between the macro- and micro-circulation during sepsis has been observed by several investigators.[2,6] Most studies reported that improvements in macrohemodynamics were associated with the enhancement of microcirculation during early sepsis; however, when microvascular and endothelial inflammation is predominant, studies have suggested a lack of hemodynamic coherence between macro- and micro-circulatory parameters. Norepinephrine (NE) is considered the first-choice vasopressor for the treatment of endotoxemic shock.[3,7] NE is a potent arterio-constrictive drug that counters vascular dilatation and augments tissue perfusion pressure.[8] Moreover, the

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veno-constrictive effects of NE increase venous return and cardiac output (CO).[9–11] NE may also aggravate microcirculatory disorders and, with increasing dosage, can impair tissue perfusion and lead to organ dysfunction.[12] The impact of using varying doses of NE on microcirculation, tissue perfusion, and organ dysfunction in the early phase of endotoxemic shock is not entirely clear. Therefore, we aimed to observe the effects of different doses of NE on fluid management, hemodynamic state, microcirculation, tissue perfusion, and organ function in an animal model of early endotoxemic shock.

Methods

Animal preparation, anesthesia, and instrumentation

After approval by the Wuhan University Animal Care Committee for Animal Research (approval No. 02516023B), experiments were performed in adherence with the National Institutes of Health Guidelines on the Use of Laboratory Animals (GB/T 35823-2018).

Thirty healthy Bama suckling pigs of both sexes (15 male, 15 female), weighing 25–35 kg and approximately 6 months old, were allowed to acclimatize for a minimum of 1 week before the experiment. All animals were housed in polyacrylic cages and maintained under standard conditions (room temperature [22 °C] with a 12-h light–dark cycle). Food and water were available ad libitum. Twelve hours before the experimental phase, solid food was withdrawn while access to water was still allowed.

Anesthesia was induced with an intramuscular injection of 0.8 mg/kg midazolam (Nhwa Pharma. Corporation, Jiangsu, China) and 30 mg/kg pentobarbital sodium (Sigma-Aldrich, MO, USA). After anesthesia induction, animals were intubated (endotracheal tube size 8F; Smiths Medical International Hythe, Kent, UK) and commenced on mechanical ventilation (VELA, CareFusion, IL, USA) in volume-controlled (8.0 mL/kg tidal volume [TV]) mode, with a respiratory rate of 12 breaths/min, to maintain partial pressure of carbon dioxide ($PaCO_2$) of 35–45 mmHg. The initial fraction of inspired oxygen (FiO_2) was 30%, and positive end-expiratory pressure was 5 cm H_2O, with adjustment to maintain oxygen saturation ≥94%. Right lateral inguinal dissection and the Seldinger technique were used to insert a dual-lumen central venous catheter (Arrow International, PA, USA). Anesthesia was maintained by continuous infusion of 8 mg/kg/h pentobarbital sodium and 15 μg/kg/h fentanyl (Humanwell Pharmaceuticals, Hubei, China). Anesthetic and analgesic medications were titrated to ensure animals were comfortable throughout the entire experimental process.

The right internal jugular vein was dissected and cannulated using a two-lumen central venous catheter (Arrow International), to facilitate continuous central venous pressure (CVP) monitoring and administration of thermodilution cold (0–4 °C) normal saline boluses. A pulse indicator continuous CO (PICCO) catheter (PULSION Medical Systems, Munich, Germany) was inserted into the right femoral artery using the Seldinger technique from the right lateral inguinal dissection area, to facilitate continuous blood pressure and CO monitoring. All hemodynamic data were automatically recorded with a data monitoring system ($PICCO_2$ hemodynamic monitoring system, PULSION Medical Systems).

Mouth pliers were used to keep the pig mouths open with fully exposed sublingual mucosa. Tongue forceps were used to pull and fix the pig tongues. A side stream dark-field (SDF; Xuzhou LiHua Electronic Technology, Jiangsu, China) device, equipped with a sterile probe cap, was applied to observe and record the sublingual mucosa microcirculation. After manually optimizing image focus, the SDF device was fixed on a custom bracket (Supplementary Figure S1). The linear telescopic knob on the bracket was slightly rotated every hour to decompress the observation site for at least 5 min and then tightened. Isotonic saline (10 mL) was sprayed around the observation site every hour, to keep mucosa moist.

All animals received 250 mL/h lactated Ringer's solution (LRS, Double-Crane, Beijing, China) during surgical preparation, to offset insensible fluid losses. Warming blankets (P&C-A, Hengbang, Beijing, China) were used to maintain normothermia.

Experimental protocol

A 60-minute stabilization period was provided after anesthesia and surgical instrumentation placement. At the end of the stabilization period (time at baseline [Tb]), baseline measurements were conducted. Endotoxemic shock was induced using lipopolysaccharide (*Escherichia coli* serotype O55:B5, Sigma), diluted to 100 μg/mL with normal saline, and total doses of 20 μg/kg administered using an intravenous infusion syringe pump (Perfusor Space, B. Braun Medical Inc., Melsungen, Germany) over 60 min. A decline of 40% in mean arterial pressure (MAP) from baseline was considered to confirm endotoxemic shock induction. Resuscitation started at shock time (Ts), defined as confirmed endotoxemic shock based on the drop in MAP. All animals received LRS infusion (LI) at 10 mL/min and continuous intravenous infusion of NE at a fixed rate. The 30 pigs were randomly assigned to five groups of six pigs each, including the control group (N0), with no NE infusion, and groups N0.2, N0.4, N0.8, and N1.6 that received NE at rates of 0.2 μg/kg/min, 0.4 μg/kg/min, 0.8 μg/kg/min, and 1.6 μg/kg/min, respectively. Once MAP returned to baseline, LI was stopped and NE continued. If MAP again dropped to <20% of the baseline value during this period, LI was restarted at 10 mL/min, until MAP returned to baseline. Endotoxemic shock resuscitation (T6) was marked at the 6th hour following Ts. At the end of the experiment, animals were euthanized by an anesthetic agent overdose (bolus injection of 100 mg/kg pentobarbital sodium). The timeline of the experimental intervention is shown in Figure 1.

Monitoring and measurements

Heart rate (HR), MAP, and CVP were continuously monitored throughout the experiment. PICCO thermodilution variables (cardiac index [CI], global end-diastolic volume index [GEDVI], systemic vascular resistance index [SVRI], and extravascular lung water index [EVLWI]) were collected hourly at Tb and from Ts to T6. Blood samples were collected and analyzed at Tb, Ts, and T6 for blood gas (ABL800 Flex, Radiometer, Copenhagen, Denmark), blood urea nitrogen (BUN), serum creatinine (CREA) (Beckman Coulter Australia Pty Ltd., NSW, Australia), and serum cytokines (interleukin [IL]-1β, IL-6, IL-10,

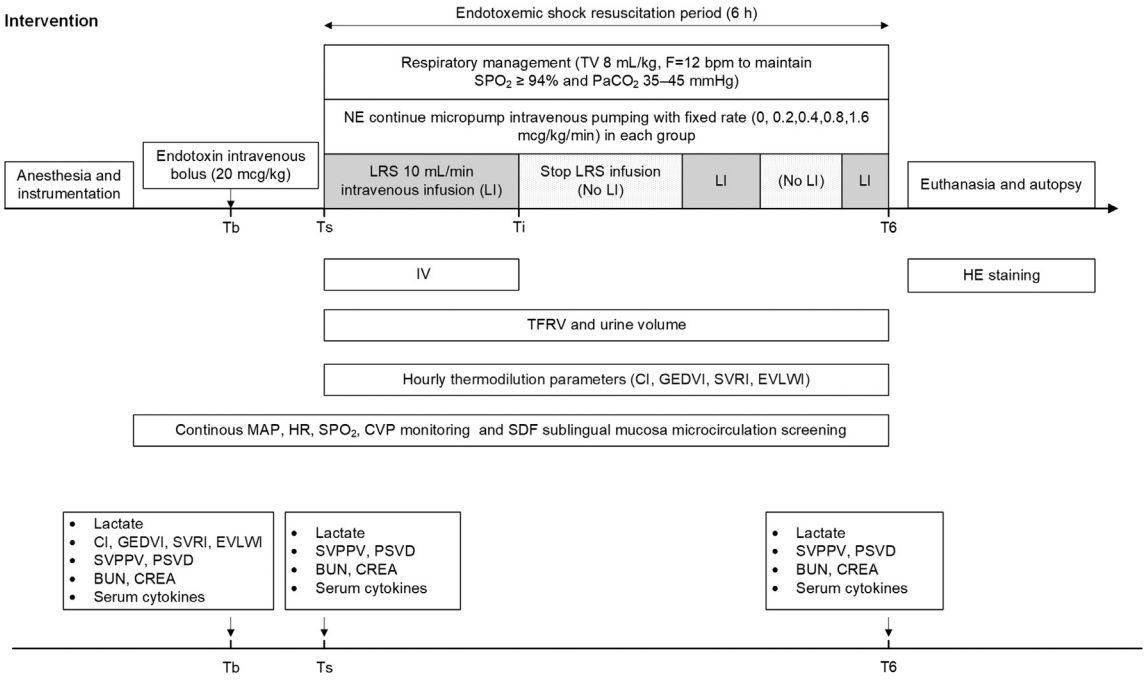

Figure 1. Timeline representation of the experiment protocol. After anesthesia and surgical instrumentation, a 1-h stabilization period was provided; the endpoint of the stabilization period was Tb. Endotoxemic shock was induced using an intravenous bolus of 20 μg/kg endotoxin infusion and the time point at which MAP decreased to 40% from baseline was Ts. Resuscitation started at Ts, with animals receiving LI (10 mL/min; gray filled areas, "LI") and undergoing continuous NE intravenous pumping using micropumps at fixed rates (0, 0.2, 0.4, 0.8, and 1.6 μg/kg/min) in the different groups. Once MAP returned to baseline (Ti), LI was stopped and only NE continued (dotted shadow areas, "No LI"); if MAP dropped to <20% of baseline when LI was stopped, LRS administration was restarted at 10 mL/min until the MAP baseline was again reached. The endotoxemic shock resuscitation period was 6 h (Ts to T6), with respiratory management.
BUN: Blood urea nitrogen; CI: Cardiac index; CREA: Serum creatinine; CVP: Central venous pressure; EVLWI: Extravascular lung water index; F: Ventilator frequency; GEDVI: Global end-diastolic volume index; HE: Hematoxylin–eosin; HR: Heart rate; IL: Interleukin; IV: Initial intravascular expand volume; LI: LRS infusion; LRS: Lactated Ringer's solution; MAP: Mean arterial pressure; NE: Norepinephrine; No LI: No LRS infusion; $PaCO_2$: Partial pressure of carbon dioxide; PSVD: Perfused small vessel density; SDF: Side stream dark-field; SPO_2: Saturation of peripheral oxygen; SVPPV: Small vessel proportions of perfused vessels; SVRI: Systemic vascular resistance index; T6: Time at the end of the shock resuscitation period, equal to Ts + 6 h; Tb: Time at baseline; TFRV: Total fluid resuscitation volume during the shock resuscitation period; Ti: Time at MAP first back to baseline level after resuscitation; TNF: Tumor necrosis factor; Ts: Time identified with endotoxemic shock; TV: Tidal volume.

tumor necrosis factor [TNF]-α) using ELISA kits (Elabscience, Hubei, China). The required initial intravascular expand volume (IV) from Ts to return MAP to baseline was documented. The first time that MAP returned to baseline was defined as Ti. Total fluid resuscitation volume (TFRV), comprising the volume of injected cold saline for thermodilution and total intravascular volume during the shock resuscitation period, was recorded. After euthanasia, an autopsy was performed to collect organ specimens (lung, liver, heart, and kidney) for hematoxylin–eosin (HE) staining and further analysis.

SDF videos were recorded at different time points (Tb, Ts, and T6) and video images at each time point were intercepted for analysis. Small vessel proportions of perfused vessels (SVPPV) and perfused small vessel density (PSVD) were derived and calculated using microcirculation analysis software (Medsoft Microcirculation Information Management System, Guangdong, China).

Data analysis

Data were assessed for normal distribution using the Kolmogorov–Smirnov test. Where normality was ascertained, continuous data are summarized as mean ± standard deviation. A one-way ANOVA was conducted for multiple comparisons and the Student's *t*-test was applied for other comparisons. Statistical analysis was performed using SPSS software (version 19.0; IBM Corp., Armonk, NY, USA) and GraphPad Prism 5(Alliance Development Group, China). *P*-values <0.05 were considered significant.

Results

Baseline hemodynamic variables at Tb did not differ significantly among the experimental groups (Supplementary Table S1).

Fluid management

Required initial IV varied according to NE infusion dose, as follows: N0, 3600±0 mL; N0.2, 2732±453 mL; N0.4,778±254 mL; N0.8, 443±123 mL; and N1.6, 322±205 mL; the differences among groups were significant, with particularly marked differences between values in the N0.8 and N1.6 groups and those in the N0 (both P <0.01), N0.2 (both P <0.01), and N0.4 (P=0.032 and P=0.005, respectively) groups. TFRV was significantly lower in the N0.4 (1372±363 mL), N0.8 (1168±313 mL), and N1.6 (1073±419 mL) groups than in the N0 (3900±0 mL; all P <0.01) and N0.2 (3273±380 mL,

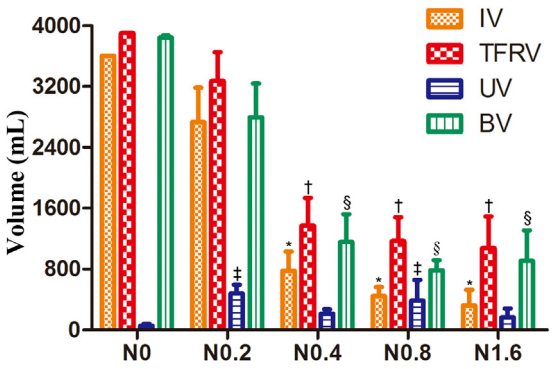

Figure 2. Fluid management of the different experimental groups.
*Compared to N0 and N0.2, IV was significantly lower (P <0.05).
†Compared to N0 and N0.2, TFRV was significantly lower (P <0.05).
‡Compared to N0, N0.4, and N1.6, UV was significantly higher (P <0.05).
§Compared to N0 and N0.2, BV was significantly lower (P <0.05).
BV: Fluid balance volume during the shock resuscitation period; IV: Initial intravascular expand volume; TFRV: Total fluid resuscitation volume during the shock resuscitation period; UV: Urine volume during the shock resuscitation period.

all P <0.01) groups. Total urine volume from Ts to T6 was significantly higher in groups N0.2 (479±115 mL) and N0.8 (383±275 mL) than in N0 (54±25 mL, P <0.001 and P=0.001, respectively) and N1.6 (165±116 mL, P=0.001 and P=0.016, respectively). Total fluid balance from Ts to T6 was positive in all groups and values in the N0.4 (1156±366 mL), N0.8 (785±134 mL), and N1.6 (908±400 mL) groups were significantly lower than those in N0 (3846±25 mL) and N0.2 (2795±442 mL) (all P <0.01) (Figure 2). Overall, some benefits in rapidly achieving and maintaining target blood pressure were observed in groups N0.4 and N0.8, as indicated by lower IV, TFRV, and total fluid balance. Fluid-related data are summarized in Supplementary Table 2.

Thermodilution variables

CI, GEDVI, SVRI, and EVLWI did not differ significantly among groups at Tb. Further, at Ts, all groups exhibited similar reductions in CI, GEDVI, and SVRI, and a similar minimal elevation in EVLWI. Based on the hourly resuscitation data (from Ts to T6), no improvements in CI or SVRI were detected in the N0 group, while in the other groups (i.e., those concomitantly administered fluids and NE) CI, GEDVI, and SVRI gradually recovered to baseline levels, and EVLWI increased to higher than Tb and Ts levels; these changes varied among the groups. At T6, CI (L/min/m^2) in N0.8 (4.20 0.49) was significantly higher than that in N0.2 (3.36±0.53, P=0.003), N0.4 (2.77±0.44, P <0.001), and N1.6 (3.34 0.55, P=0.003); GEDVI (mL/m^2) in N0.8 (515.33±50.00) was higher than that in N0.4 (416.17±38.00, P <0.001); SVRI value (dyn·s/cm^5·m^2) in N0.4 (3170.67±362.93) was higher than those in N0.2 (2057.50±207.76, P <0.001), N0.8 (2577.50±579.71, P=0.006), and N1.6 (2145.83±204.52, P <0.001); and EVLWI values (mL/kg) in N0 (24.33±3.27) and N0.2 (22.50±2.43) were higher than that in N0.4 (18.17±1.17, P <0.001 and P=0.003, respectively), while EVLWI in the N0 group was also higher than that in N0.8 (19.67±1.75, P=0.001) (Figure 3). These data are summarized in Supplementary Tables 3–6.

Microcirculation

Images of the sublingual mucosa microcirculation at Tb, Ts, and T6 are presented in Figure 4A. We detected a significant reduction in small vessels from Tb to Ts in each group, indicating similar microcirculation injury in all animals exposed to endotoxin; however, the small vessel changes from Ts to T6 differed among groups (Figure 4A; b to c worsened; e to f, partially recovered; and h to I, no noticeable improvement). Similar results were obtained on analysis of SVPPV and PSVD; that is, there were significant reductions from Tb to Ts in all groups, with variation among groups undergoing different shock resuscitation strategies. In N0, SDF parameters consistently declined, SVPPV decreased to (14.64%±2.34%), and PSVD (mm/mm^2) declined to (1.93±0.16) at T6, with both values significantly lower than those in the other groups. In each group receiving NE, the downward trend from Ts to T6 slowed, or even partially reversed, but to varying degrees. At T6, SVPPV (%) in N0.8 (77.54±5.46) was significantly higher than that in N0.4 (68.28±8.24, P=0.009) and N1.6 (65.33±4.76, P=0.001); PSVD (mm/mm^2) in N0.8 (10.19±0.99) was significantly higher than that in N0.2 (9.28±0.68, P=0.044), N0.4 (8.87±0.67, P=0.005), and N1.6 (6.02±0.93, P <0.001); and PSVD values in groups N0.2 and N0.4 were also higher than that in N1.6 (both P <0.001) (Figure 4B). The N0.8 group performed better in terms of microcirculation, with significant improvements in SDF parameters, including SVPPV and PSVD, at T6. This information is summarized in Supplementary Tables S7 and S8.

Biochemistry

Serum lactate increased from Tb to Ts in all groups at differing rates. Lactate level further increased in N0 and N1.6 at Ts, but decreased in N0.2, N0.4, and N0.8 following Ts (Supplementary Figure S2). At T6, serum lactate levels (mmol/L) in N0 (4.55±0.41) and N1.6 (3.97±0.42) were significantly higher than in N0.2 (2.95±0.48, both P <0.001), N0.4 (2.78±0.66, both P <0.001), and N0.8 (2.35±0.40, both P <0.001) (Supplementary Table S9).

Serum BUN and CREA increased from Tb to Ts and further increased from Ts to T6 in all groups; however, the degree of elevation differed among groups. At T6, BUN (mmol/L) in N0 (6.86±0.53) and N1.6 (6.82±0.62) was significantly higher than that in N0.4 (5.66±0.61, both P <0.001) and N0.8 (5.83±0.48, P=0.003 and P=0.004, respectively) (Supplementary Figure S2). CREA levels (μmol/L) in N0 (73.13±4.94), N0.2 (71.19±5.85), and N1.6 (71.57±4.52) were significantly higher than those in N0.8 (63.71±3.97, P=0.003, P=0.014, and P=0.010, respectively), while CREA levels in N0 were also significantly higher than those in N0.4 (65.89±5.04, P=0.017) (Supplementary Figure S2). These data are outlined in Supplementary Tables S10 and S11.

Inflammatory cytokines

All serum cytokines increased from Tb to Ts. At T6, IL-1β, IL-6, and IL-10 levels were higher than those at Ts, while TNF-α decreased to near baseline levels. There were no significant differences among groups in IL-1β, IL-6, IL-10, or TNF-α levels

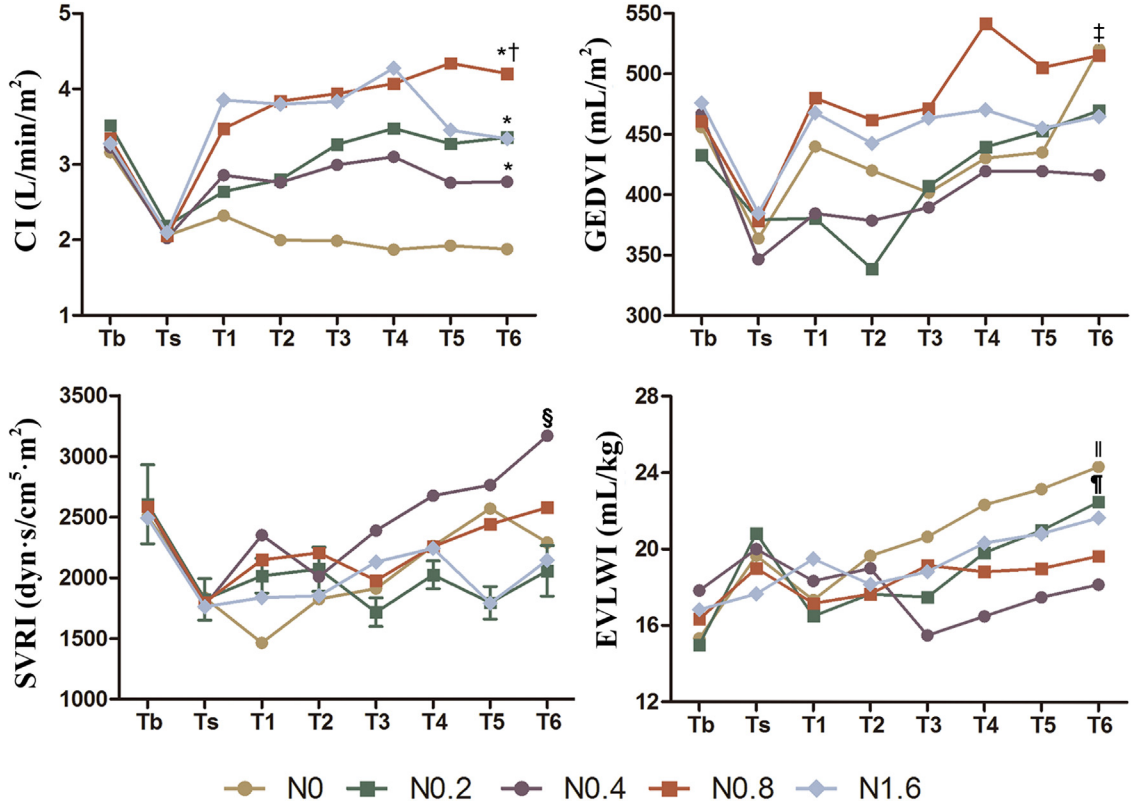

Figure 3. Hourly changes in thermodilution variables in different experimental groups.
*N0.2, N0.4, N0.8, and N1.6 were significantly higher than N0 (P <0.05).
†N0.8 was significantly higher than N0, N0.2, N0.4, and N1.6 (P <0.05).
‡N0 and N0.8 were significantly higher than N0.4 (P <0.05).
§N0.4 was significantly higher than N0, N0.2, N0.8, and N1.6 (P <0.05).
‖N0 was significantly higher than N0.4 and N0.8 (P <0.05).
¶N0.2 was significantly higher than N0.4 (P <0.05).
CI: Cardiac index; EVLWI: Extravascular lung water index; GEDVI: Global end-diastolic volume index; SVRI: Systemic vascular resistance index; T6: Time at the end of the shock resuscitation period, equal to Ts+6 h; Tb: Time at baseline; Ts: Time identified with endotoxemic shock.

at any time point (Supplementary Figure S2). These findings are summarized in Supplementary Tables S12–S15.

Histopathology

Lung samples from groups N0 and N0.2 showed inflammatory cell infiltration, along with edematous thickening of the bronchial submucosa, diffuse expansion of the alveolar interstitium, and fluid in some alveolar cavities (black arrow; Figure 5). With increasing NE infusion dose, inflammatory cell infiltration and pulmonary interstitial edema were progressively alleviated in groups N0.4 and N0.8. In the N1.6 group, impairment of the pulmonary alveolar epithelium and alveolar cavity fluid were both aggravated relative to groups N0.4 and N0.8.

Discussion

To our knowledge, this is the first investigation to demonstrate that the use of different doses of NE together with fluid resuscitation induces dose-dependent changes in a porcine endotoxemic shock model. At 0.8 μg/kg/min NE, we noted a decrease in fluid requirement and improved microcirculation; however, a higher dose (1.6 μg/kg/min) was associated with microcirculatory disorganization and tissue hypoperfusion.

Several authors have suggested examining SDF using at least three sites (ideally five sites) before calculating the average value.[13–15] By fixing the microscope with a bracket, we were able to capture the required images and their changes at the same site throughout the experiment, avoiding sampling errors.

To evaluate the impact of NE on preload at different doses, we attempted to minimize the effect of fluid infusion on the preload. Therefore, a dynamic fluid resuscitation process, based on hemodynamic indicators, was adopted, which was different from previous reports that used continuous infusion methods throughout the process[16], or stopped the infusion entirely after a specific period.[17] Our fluid resuscitation strategy was based on fixed administration of NE. Fluid infusion was only used when MAP dropped, and was stopped once MAP returned to baseline, and then MAP was maintained using a fixed dose of NE alone. This approach could maximize the effect of NE on reducing preload dependence and facilitate observation of its impact. Our strategy was more consistent with dynamic, individualized fluid management in clinical treatment.

We chose an endotoxemic model with rapid CO reduction induced by using an endotoxin bolus before infusion.[17,18] Bryne et al.[17] used an incremental increase in the rate of continuous endotoxin infusion to maintain near-normal cardiac function, while SVRI declined, and reported that this model reflected the

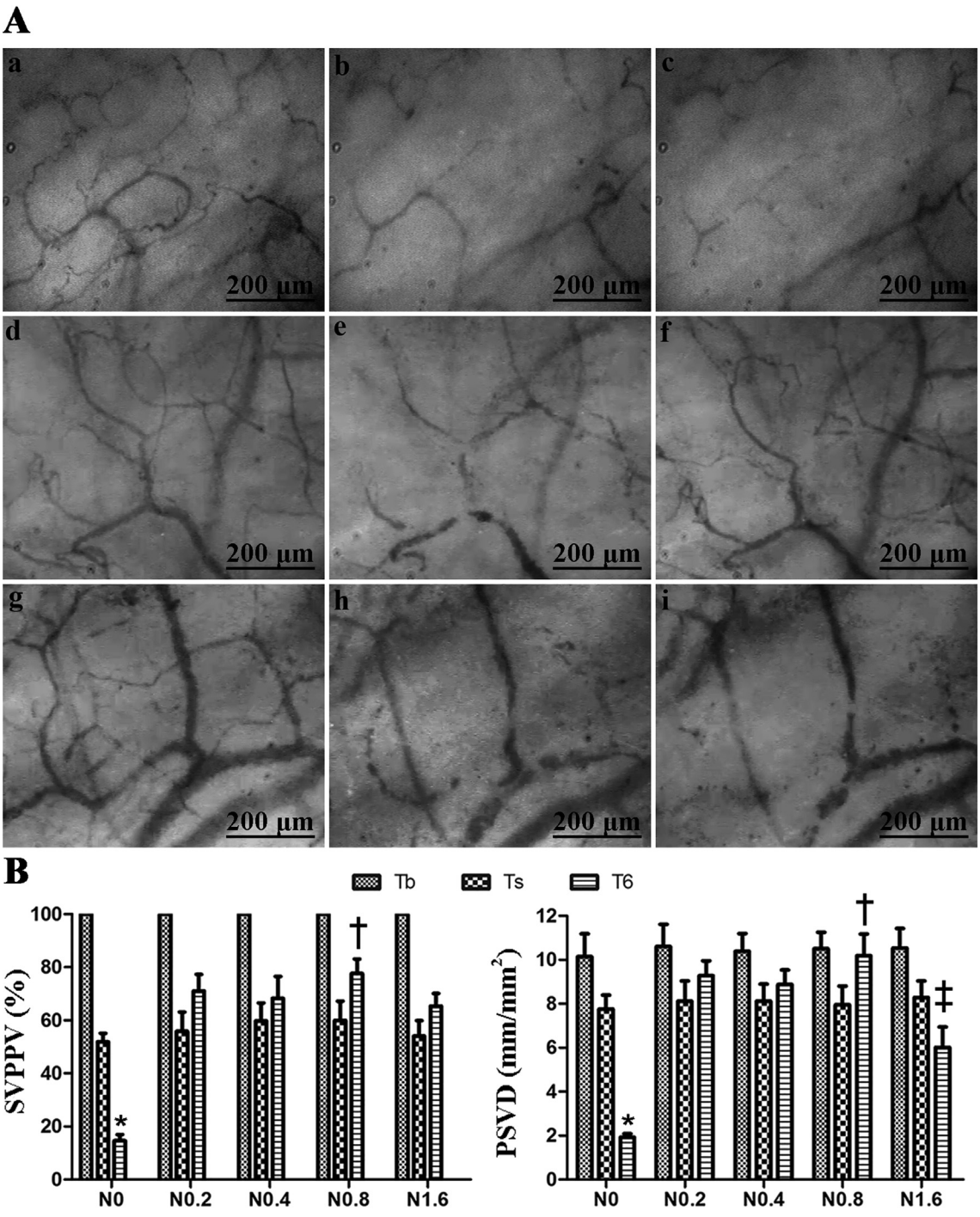

Figure 4. Microcirculation changes in SDF images and variables. A: SDF images from the same sublingual mucosa microcirculation site at different time points. a, b, and c show the changes in group N0; d, e, and f show the change in group N0.8; g, h, and i show the change in group N1.6, from Tb to Ts to T6. A, d, and g, basal state; b, e, and h, stasis and discontinuity of blood flow with decreased perfusion vessel density; c, further aggravation of blood stasis, discontinuity, and significant decrease in perfusion vessel density; f, reperfusion in some vessels and a significant increase in perfusion vessel density; i, no significant improvement in stasis or discontinuity, but perfusion vessel density was reduced to even slightly lower than that observed at Ts (h). B: Changes in SDF variables in groups administered different NE pumping regimens.

*Significantly lower than the other groups at T6 (P <0.05).

†Significantly higher than N0, N0.4, and N1.6 at T6 (P <0.05).

‡Significantly lower than N0.2, N0.4, and N0.8 at T6 (P <0.05).

NE: Norepinephrine; PSVD: Perfused small vessel density; SDF: Side stream dark-field; SVPPV: Small vessel proportions of perfused vessels; T6: Time at the end of the shock resuscitation period, equal to Ts + 6 h; Tb: Time at baseline; Ts: Time identified with endotoxemic shock.

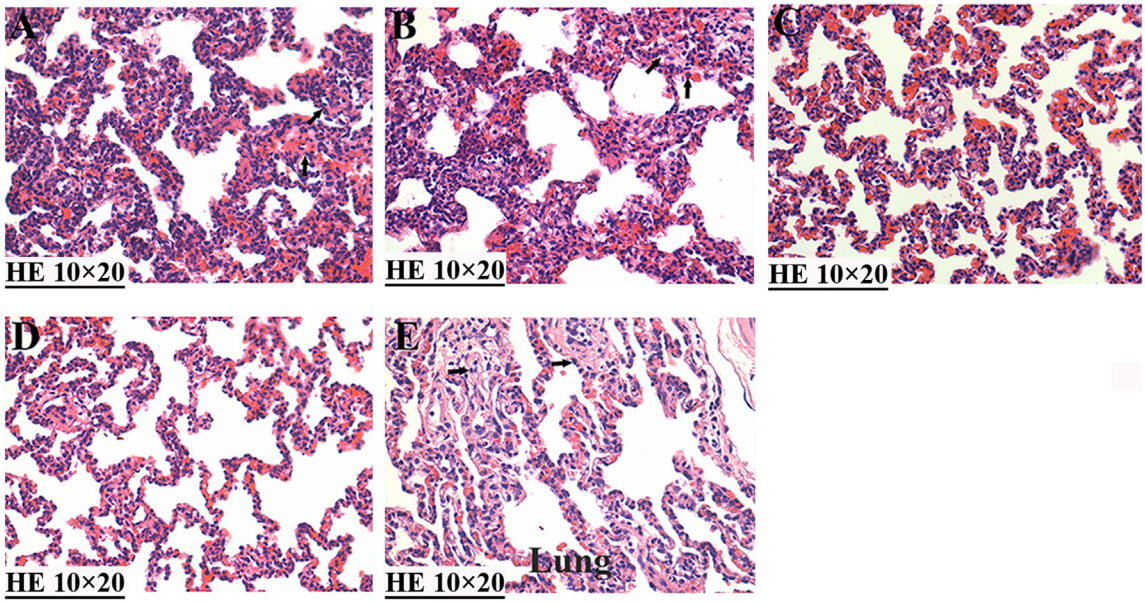

Figure 5. Histopathological changes in lung tissues shown by HE staining (200 × magnification). Lung tissue from groups: N0 (A), N0.2 (B), N0.4 (C), N0.8 (D), and N1.6 (E). Black arrow, impaired pulmonary alveolar epithelium and fluid edema, with pale red in the alveolar cavity. HE: Hematoxylin–eosin.

actual effect of fluid resuscitation in the clinical setting; however, their model may only be representative of mild endotoxemic shock status, which allows the involvement of compensatory mechanisms. Myocardial depression is among the characteristic pathophysiological changes during severe endotoxemic shock.[19,20] Our model showing low CO with a decline in SVRI may better represent severe endotoxemic shock requiring fluid resuscitation and NE infusion at an early stage.

Effects on macro-hemodynamics

NE has complex effects on macrocirculation, which are associated with changes in cardiac function, mean systemic filling pressure [10], and afterload.[9,21] NE increases stressed volume, thereby enhancing cardiac preload; however, by increasing cardiac afterload, NE can also impact CO.[22,23]

Our data demonstrate that early administration of NE with fluid resuscitation can increase CI and reduce the amount of fluid infusion required during the early stages of endotoxemic shock, relative to fluid resuscitation only. Nevertheless, this effect varies with NE dose; for example, contraction of resistant (arteries) and capacitance (veins) vessels were both insufficient in group N0.2, preventing the attainment of target blood pressure; therefore, more resuscitation IV fluids were administered. In comparison, resistant and capacitance vessels were both sufficiently contracted in groups N0.8 and N1.6, allowing rapid achievement of target blood pressure by increasing preload and mitigating the need for fluids. Meanwhile, we found no significant differences in IV and TFRV between groups N0.8 and N1.6. This finding was not consistent with observations of animals in groups N0 to N0.8, indicating that the decline in preload dependency and fluid infusion requirements are not linearly correlated with NE dose. In group N0.4, capacitance vessel contraction appeared to be insufficient to provide appropriate preload, despite achieving the target blood pressure. Indeed, we observed unsatisfactory CI, low GEDVI, and elevated SVRI in group N0.4 by PICCO monitoring, while MAP targets were achieved. This finding was consistent with the results of De Mey and Vanhoutte[24], who reported differences in the pharmacological properties of postjunctional alpha-adrenergic receptors among arteries and veins. Further, Hellegouarch et al.[25] found that different parts of the vena cava (i.e., rostral *vs.* caudal) showed varying affinity and contraction capacities in response to NE.

Microcirculation and tissue perfusion

Microcirculatory insufficiency is associated with unfavorable outcomes, and its correction can potentially improve survival.[26] Endotoxemic shock results in vasoparalysis and an inflammatory state, characterized by vasodilation and increased vascular permeability.[27,28] These changes in capillary permeability can, in turn, lead to microcirculatory dysfunction and low tissue perfusion.[29] Hence, improved microcirculation and tissue perfusion are primary targets in endotoxemic shock treatment.[30] Multiple studies have evaluated the impact of higher MAP induced by NE on the microcirculation. Xu et al.[31] reported improved microcirculation in response to increasing MAP using N in patients with a history of hypertension administered NE, while Dubin et al.[12] and Jhanji et al.[32] observed the opposite effect. In our research, animals in groups N0.2, N0.4, and N0.8 showed improvements in SDF parameters and lactate levels after 6 h of resuscitation. Unlike the other groups, animals in group N1.6 exhibited lower SDF parameters and elevated lactate level at T6. The primary reason for such discrepancies is the discordance between the macro- and micro-circulations in shock states.[28,33] Early use of NE can improve macrocirculation parameters, without correcting microcirculatory deficits.[28] The microvascular flow driving pressure is the gap between precapillary inflow pressure and microvenous outflow pressure.[34] NE can increase precapillary pressure and flow by contracting the microarteries[35]; however, NE can also decrease capillary flow by inducing excessive micro-

arterial vasoconstriction.[12,36] Therefore, the balance between driving pressure and capillary flow will determine what is observed in studies of microcirculatory parameters.

Fluid resuscitation may be associated with delayed vascular injury. This hypothesis was first proposed in the FEAST trial as, after early rapid fluid resuscitation, some patients developed circulatory insufficiency.[37] Subsequently, Bryne et al.[17] found an obvious decrease in SVRI and a rapid increase in vasopressor requirement in the fluid-resuscitated group, which could not be solely explained by systemic inflammation progression; they attributed this finding to an increase in glycocalyx shedding and exacerbated vascular dysfunction. In our study, early use of NE was associated with an apparent elevation in SVRI, possibly due to the protective influence of NE against the detrimental effects of fluids on endothelial cells.

Effects on organ function

Fluid overload following resuscitation leads to tissue edema.[38–40] NE can increase cardiac preload and decrease the need for fluids.[9] In some studies, early administration of NE to patients with sepsis reduced both the need for fluid infusion and mortality rates.[41–43] Macdonald et al.[44] found that a regimen involving restricted fluids and early vasopressor administration in patients with suspected sepsis with hypotension reduced total fluid volume administered in the first 24 h; however, Semler et al.[45] found that a conservative fluid management protocol did not decrease the mean daily fluid balance in patients with sepsis. In our study, the benefits of using NE were dose-dependent; hence, in the N0.2 group, the amount of fluid infusion was not lower, while animals in the N0.4 and N0.8 groups needed less fluid volume and, therefore, had lower tissue edema and higher tissue perfusion.

Microcirculatory and tissue perfusion dysfunction can also lead to kidney dysfunction.[46] Corrêa et al.[47] found that kidney function and urine output were improved when MAP rose in response to NE administration during septic shock; however, this effect was dose-dependent in our study, as the N1.6 group produced the least amount of urine, despite significant improvements in macro-circulatory parameters, which was not consistent with the findings of previous studies.[22,48]

Study limitations

Our study had some limitations. First, as we used an animal model, the translation of the changes in hemodynamic variables to humans requires further testing. Second, during SDF parameter measurement, some videos and images were not optimized. Third, although unlikely, the use of LRS as the resuscitation fluid may have influenced serum lactate levels. Fourth, we did not use organ weight or calculate dry-to-wet tissue ratio to assess the fluid volume accumulated in the thoracic and abdominal cavities. Finally, the experimentation time was relatively short, making it challenging to observe all the effects, particularly organ function changes and mortality. A longer experimentation time (e.g., 12–24 h) could potentially allow more information to be obtained about the longer-term impacts of our interventions.

Conclusions

In our porcine endotoxemic shock model, we found that NE could reduce preload dependence and decrease the amount of infusion required, with an upper threshold. Thus, only using appropriate doses of NE improved microcirculation and organ function, while very low and very high doses resulted in detrimental effects. By targeting macro-circulatory correction alone, there is a chance that tissue perfusion deficiency is missed because of inadequate NE dose. An NE dose range of 0.4–0.8 μg/kg/min may be optimal, leading to comparable blood pressure and SVRI, or well-maintained SDF, due to lower fluid volume and tissue edema, as well as higher tissue perfusion. We do not suggest increased NE to treat compromised SDF, as SDF is only one microcirculation indicator. Tissue perfusion indicators, such as serum lactate and SDF indicators, as well as MAP, allow a comprehensive assessment of resuscitation during the treatment of endotoxemic shock, and these factors should be taken into consideration. More studies are needed to determine if our results can be translated into clinical practice.

Author Contributions

BH and JL designed this study and protocol development; BH, HX, CS, YZ, QL, SM were responsible for the conduct of experiments; HX, YZ, BH, ZP were responsible for sample analysis and data analysis; BH and HX conducted the manuscript writing; KK, ZP, and JL critically revising the manuscript; BH, HX, KK, ZP, JL final approved the version to be published; All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Ethics Statement

After approval by the Wuhan University Animal Care Committee for Animal Research (approval No. 02516023B), experiments were performed in adherence with the National Institutes of Health Guidelines on the Use of Laboratory Animals (GB/T 35823-2018).

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jointm.2023.06.007.

References

[1] Meyer J, Booke M, Waurick R, Prien T, Van Aken H. Nitric oxide synthase inhibition restores vasopressor effects of norepinephrine in ovine hyperdynamic sepsis. Anesth Analg 1996;83(5):1009–13. doi:10.1097/00000539-199611000-00019.

[2] De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, et al. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. Crit Care Med 2013;41(3):791–9. doi:10.1097/CCM.0b013e3182742e8b.

[3] Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Intensive Care Med 2017;43(3):304–77. doi:10.1007/s00134-017-4683-6.

[4] Maitland K, Kiguli S, Opoka RO, Engoru C, Olupot-Olupot P, Akech SO, et al. Mortality after fluid bolus in African children with severe infection. N Engl J Med 2011;364(26):2483–95. doi:10.1056/NEJMoa1101549.

[5] Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, et al., National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome (ARDS) Clinical Trials Network Comparison of two fluid-management strategies in acute lung injury. N Engl J Med 2006;354(24):2564–75. doi:10.1056/NEJMoa062200.

[6] Sturm T, Leiblein J, Schneider-Lindner V, Kirschning T, Thiel M. Association of microcirculation, macrocirculation, and severity of illness in septic shock: a prospective observational study to identify microcirculatory targets potentially suitable for guidance of hemodynamic therapy. J Intensive Care Med 2018;33(4):256–66. doi:10.1177/0885066616671689.

[7] Levy MM, Evans LE, Rhodes A. The surviving sepsis campaign bundle: 2018 update. Intensive Care Med 2018;44(6):925–8. doi:10.1007/s00134-018-5085-0.

[8] De Backer D, Biston P, Devriendt J, Madl C, Chochrad D, Aldecoa C, et al. Comparison of dopamine and norepinephrine in the treatment of shock. N Engl J Med 2010;362(9):779–89. doi:10.1056/NEJMoa0907118.

[9] Hamzaoui O, Georger JF, Monnet X, Ksouri H, Maizel J, Richard C, et al. Early administration of norepinephrine increases cardiac preload and cardiac output in septic patients with life-threatening hypotension. Crit Care 2010;14(4):R142. doi:10.1186/cc9207.

[10] Maas JJ, Pinsky MR, de Wilde RB, de Jonge E, Jansen JR. Cardiac output response to norepinephrine in postoperative cardiac surgery patients: interpretation with venous return and cardiac function curves. Crit Care Med 2013;41(1):143–50. doi:10.1097/CCM.0b013e318265ea64.

[11] Monnet X, Jabot J, Maizel J, Richard C, Teboul JL. Norepinephrine increases cardiac preload and reduces preload dependency assessed by passive leg raising in septic shock patients. Crit Care Med 2011;39(4):689–94. doi:10.1097/CCM.0b013e318206d2a3.

[12] Dubin A, Pozo MO, Casabella CA, Pálizas F Jr, Murias G, Moseinco MC, et al. Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study. Crit Care 2009;13(3):R92. doi:10.1186/cc7922.

[13] De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, et al. How to evaluate the microcirculation: report of a round table conference. Crit Care 2007;11(5):R101. doi:10.1186/cc6118.

[14] Pelland A, George RB, Lehmann C, Coolen J. Sidestream dark field imaging of the microcirculation to assess preeclampsia microvascular dysfunction. J Clin Med Res 2018;10(5):391–5. doi:10.14740/jocmr3368w.

[15] Milstein DM, te Boome LC, Cheung YW, Lindeboom JA, van den Akker HP, Biemond BJ, et al. Use of sidestream dark-field (SDF) imaging for assessing the effects of high-dose melphalan and autologous stem cell transplantation on oral mucosal microcirculation in myeloma patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109(1):91–7. doi:10.1016/j.tripleo.2009.08.041.

[16] Bressack MA, Morton NS, Hortop J. Group B streptococcal sepsis in the piglet: effects of fluid therapy on venous return, organ edema, and organ blood flow. Circ Res 1987;61(5):659–69. doi:10.1161/01.res.61.5.659.

[17] Byrne L, Obonyo NG, Diab SD, Dunster KR, Passmore MR, Boon AC, et al. Unintended consequences: fluid resuscitation worsens shock in an ovine model of endotoxemia. Am J Respir Crit Care Med 2018;198(8):1043–54. doi:10.1164/rccm.201801-0064OC.

[18] Somell A, Sollevi A, Suneson A, Riddez L, Hjelmqvist H. Beneficial effects of hypertonic saline/dextran on early survival in porcine endotoxin shock. Acta Anaesthesiol Scand 2005;49(8):1124–34. doi:10.1111/j.1399-6576.2005.00807.x.

[19] Pulido JN, Afessa B, Masaki M, Yuasa T, Gillespie S, Herasevich V, et al. Clinical spectrum, frequency, and significance of myocardial dysfunction in severe sepsis and septic shock. Mayo Clin Proc 2012;87(7):620–8. doi:10.1016/j.mayocp.2012.01.018.

[20] Sanfilippo F, Corredor C, Fletcher N, Landesberg G, Benedetto U, Foex P, et al. Diastolic dysfunction and mortality in septic patients: a systematic review and meta-analysis. Intensive Care Med 2015;41(6):1004–13. doi:10.1007/s00134-015-3748-7.

[21] Lauzier F, Lévy B, Lamarre P, Lesur O. Vasopressin or norepinephrine in early hyperdynamic septic shock: a randomized clinical trial. Intensive Care Med 2006;32(11):1782–9. doi:10.1007/s00134-006-0378-0.

[22] Colling KP, Banton KL, Beilman GJ. Vasopressors in sepsis. Surg Infect 2018;19(2):202–7. doi:10.1089/sur.2017.255.

[23] Shi R, Hamzaoui O, De Vita N, Monnet X, Teboul JL. Vasopressors in septic shock: which, when, and how much? Ann Transl Med 2020;8(12):794. doi:10.21037/atm.2020.04.24.

[24] De Mey JG, Vanhoutte PM. Differences in pharmacological properties of postjunctional alpha-adrenergic receptors among arteries and veins. Arch Int Pharmacodyn Ther 1980;244(2):328–9.

[25] Hellegouarch A, DeFeudis FV, Clostre F. A regional difference in the in vitro response of the rabbit vena cava to norepinephrine. Gen Pharmacol 1982;13(1):41–3. doi:10.1016/0306-3623(82)90008-8.

[26] Spronk PE, Zandstra DF, Ince C. Bench-to-bedside review: sepsis is a disease of the microcirculation. Crit Care 2004;8(6):462–8. doi:10.1186/cc2894.

[27] Marechal X, Favory R, Joulin O, Montaigne D, Hassoun S, Decoster B, et al. Endothelial glycocalyx damage during endotoxemia coincides with microcirculatory dysfunction and vascular oxidative stress. Shock 2008;29(5):572–6. doi:10.1097/SHK.0b013e318157e926.

[28] Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care 2015;19(Suppl 3):S8. doi:10.1186/cc14726.

[29] Inagawa R, Okada H, Takemura G, Suzuki K, Takada C, Yano H, et al. Ultrastructural alteration of pulmonary capillary endothelial glycocalyx during endotoxemia. Chest 2018;154(2):317–25. doi:10.1016/j.chest.2018.03.003.

[30] Peake SL, Delaney A, Bailey M, Bellomo R, et al., ARISE Investigators, ANZICS Clinical Goal-directed resuscitation for patients with early septic shock. N Engl J Med 2014;371(16):1496–506. doi:10.1056/NEJMoa1404380.

[31] Xu JY, Ma SQ, Pan C, He HL, Cai SX, Hu SL, et al. A high mean arterial pressure target is associated with improved microcirculation in septic shock patients with previous hypertension: a prospective open label study. Crit Care 2015;19(1):130. doi:10.1186/s13054-015-0866-0.

[32] Jhanji S, Stirling S, Patel N, Hinds CJ, Pearse RM. The effect of increasing doses of norepinephrine on tissue oxygenation and microvascular flow in patients with septic shock. Crit Care Med 2009;37(6):1961–6. doi:10.1097/CCM.0b013e3181a00a1c.

[33] Kanoore Edul VS, Ince C, Dubin A. What is microcirculatory shock? Curr Opin Crit Care 2015;21(3):245–52. doi:10.1097/MCC.0000000000000196.

[34] Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle. Cardiovasc Res 2010;87(2):198–210. doi:10.1093/cvr/cvq062.

[35] Peters J, Mack GW, Lister G. The importance of the peripheral circulation in critical illnesses. Intensive Care Med 2001;27(9):1446–58. doi:10.1007/s001340101034.

[36] Kanoore Edul VS, Dubin A, Ince C. The microcirculation as a therapeutic target in the treatment of sepsis and shock. Semin Respir Crit Care Med 2011;32(5):558–68. doi:10.1055/s-0031-1287864.

[37] Maitland K, George EC, Evans JA, Kiguli S, Olupot-Olupot P, Akech SO, et al. Exploring mechanisms of excess mortality with early fluid resuscitation: insights from the FEAST trial. BMC Med 2013;11:68. doi:10.1186/1741-7015-11-68.

[38] Kelm DJ, Perrin JT, Cartin-Ceba R, Gajic O, Schenck L, Kennedy CC. Fluid overload in patients with severe sepsis and septic shock treated with early goal-directed therapy is associated with increased acute need for fluid-related medical interventions and hospital death. Shock 2015;43(1):68–73. doi:10.1097/SHK.0000000000000268.

[39] Chen H, Zhang H, Wang K, Yao Y, Xiao X. [Minimum quality threshold in preclinical sepsis studies (MQTiPSS): quality threshold for fluid resuscitation and antimicrobial therapy endpoints]. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2019;31(11):1307–16. doi:10.3760/cma.j.issn.2095-4352.2019.11.001.

[40] Chappell D, Bruegger D, Potzel J, Jacob M, Brettner F, Vogeser M, et al. Hypervolemia increases release of atrial natriuretic peptide and shedding of the endothelial glycocalyx. Crit Care 2014;18(5):538. doi:10.1186/s13054-014-0538-5.

[41] Bai X, Yu W, Ji W, Lin Z, Tan S, Duan K, et al. Early versus delayed administration of norepinephrine in patients with septic shock. Crit Care 2014;18(5):532. doi:10.1186/s13054-014-0532-y.

[42] Sennoun N, Montemont C, Gibot S, Lacolley P, Levy B. Comparative effects of early versus delayed use of norepinephrine in resuscitated endotoxic shock. Crit Care Med 2007;35(7):1736–40. doi:10.1097/01.CCM.0000269028.28521.08.

[43] Silversides JA, Fitzgerald E, Manickavasagam US, Lapinsky SE, Nisenbaum R, Hemmings N, et al. Deresuscitation of patients with iatrogenic fluid overload is associated with reduced mortality in critical illness. Crit Care Med 2018;46(10):1600–7. doi:10.1097/CCM.0000000000003276.

[44] Macdonald SPJ, Keijzers G, Taylor DM, Kinnear F, Arendts G, Fatovich DM, et al. Restricted fluid resuscitation in suspected sepsis associated hypotension (REFRESH):

a pilot randomised controlled trial. Intensive Care Med 2018;44(12):2070–8. doi:10.1007/s00134-018-5433-0.
[45] Semler MW, Janz DR, Casey JD, Self WH, Rice TW. Conservative fluid management after sepsis resuscitation: a pilot randomized trial. J Intensive Care Med 2020;35(12):1374–82. doi:10.1177/0885066618823183.
[46] Srivastava A. Fluid resuscitation: principles of therapy and "kidney safe" considerations. Adv Chronic Kidney Dis 2017;24(4):205–12. doi:10.1053/j.ackd.2017.05.002.
[47] Corrêa TD, Vuda M, Takala J, Djafarzadeh S, Silva E, Jakob SM. Increasing mean arterial blood pressure in sepsis: effects on fluid balance, vasopressor load and renal function. Crit Care 2013;17(1):R21. doi:10.1186/cc12495.
[48] Miyake K, Iwagami M, Ohtake T, Moriya H, Kume N, Murata T, et al. Association of pre-operative chronic kidney disease and acute kidney injury with in-hospital outcomes of emergency colorectal surgery: a cohort study. World J Emerg Surg 2020;15(1):22. doi:10.1186/s13017-020-00303-6.