Original papers

Fluid resuscitation, but not inhaled nitric oxide, improves microcirculation in septic pigs

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Abstract

Background. Prolonged deterioration of microvascular flow during sepsis leads to organ dysfunction. Capillary flow restoration may prevent this complication.

Objectives. The main aim of this study was to investigate the microcirculatory effects of inhaled nitric oxide (iNO) combined with intravenous hydrocortisone in a porcine model of sepsis. The 2nd aim was to evaluate the influence of hemodynamic resuscitation with noradrenaline and crystalloids on capillary flow.

Materials and methods. Eleven piglets of Polish breed underwent surgical colon perforation to develop sepsis. They were randomly allocated to one of 3 treatment groups. Group 1 received iNO and hydrocortisone, whereas group 2 did not. Both groups were resuscitated with crystalloids and noradrenaline if hypotensive. Group 3 received no treatment at all. During a 30-hour observation, we assessed the microcirculation using sidestream dark field imaging (SDF).

Results. We found no effect of iNO with hydrocortisone on the microcirculation. Fluid and vasopressor treatment led to a higher microcirculatory flow index after 20 h of observation (3 and 2.75 in groups 1 and 2 compared to 1.9 in group 3), a greater proportion of perfused vessels (94% and 87% compared to 63% in groups 1, 2 and 3, respectively) and a greater perfused vessel density (15.2 mm/mm^2, 15.09 mm/mm^2 and 10.1 mm/mm^2 in groups 1, 2 and 3, respectively).

Conclusions. Crystalloid and vasopressor treatment postponed microvascular flow derangements, whereas iNO combined with intravenous hydrocortisone did not improve microvascular perfusion.

Key words: sepsis, multiorgan failure, microcirculation, inhaled nitric oxide, sidestream dark field

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Background

Each year, 31 million people are hospitalized due to sepsis, and over 5 million die with this diagnosis.[1] Sepsis and its complications, including multiorgan failure, result from complex hemodynamic and cellular mechanisms, with capillary perfusion being altered. Specific changes in capillary flow may precede the more severe symptoms of sepsis and septic shock.[2,3] Persistent microcirculatory derangement is strongly related to poor outcomes and can be observed in patients with multiorgan dysfunction even after the resolution of shock.[4,5] Therefore, capillary flow monitoring may be helpful in the early diagnosis of sepsis, following its course and determining its prognosis.

There are multiple studies concerning septic patients and septic animal models that provide reproducible information about the microcirculation changes occurring in sepsis.[6–8] Activation of the inflammatory response leads to the cessation of flow in some capillaries and augmentation of flow in others, resulting in heterogeneity within microvascular flow.[9] An increased distance between perfused capillaries impedes oxygen diffusion and its delivery to cells, creating a microcirculatory shunt.[10] It leads to oxygen deficits in tissues, increased lactate production, shock, and finally organ dysfunction.[2,11,12] Therefore, microvascular resuscitation and the restoration of homogeneity of flow in capillary beds are crucial for managing sepsis and preventing its complications.

In recent years, much attention has been paid to nitric oxide (NO) and its role in microvascular flow restoration in sepsis. Generally, the release of NO is increased in septic shock. However, in some areas, NO production may be impaired, which is one of the factors leading to heterogeneity and hypoperfusion of the microvascular bed.[13,14] There are multiple trials targeting both NO production inhibition and external NO delivery.[15–17] The delivery of exogenous NO, a very potent vasodilator, can potentially restore microcirculation and tissue perfusion, and reduce mortality.[17,18] Inhaled nitric oxide (iNO) is one of the donors tested not only because of its pulmonary activity but also because of its recognized peripheral effects.[19]

It has been previously reported that moderate doses of hydrocortisone administered in the early stages of septic shock improved capillary perfusion and increased NO delivery to tissues.[20] The use of hydrocortisone in septic shock seems beneficial not only due to its anti-inflammatory properties since glucocorticoids inhibit pro-inflammatory gene expression and activate anti-inflammatory protein production.[21] It also increases vessel responsiveness to catecholamines.[22] Low doses of corticosteroids can restore circulation and reduce mortality in septic shock.[23,24] In a preliminary study on piglets exposed to prolonged endotoxin/lipopolysaccharide (LPS) infusions, it has been demonstrated that combined iNO and intravenous steroids attenuate LPS-induced kidney injury, but neither of them does it on its own.[25] This effect may be partially explained by the fact that iNO upregulates glucocorticoid receptors and blunts the inflammatory response.[26]

When it comes to septic shock, intravenous fluids with vasopressors have been proven to resuscitate the microcirculation. However, whether hemodynamic stabilization equals microcirculatory improvement is still in question. For years, early goal-directed therapy (EGDT) has been recognized as an effective treatment in managing septic shock and reducing mortality and the probability of multiorgan failure.[27] Further studies have not confirmed the influence of protocol-based fluid resuscitation on mortality and morbidity reduction.[28–30] However, in the above trials, patients received fluids before randomization. For this reason, fluid resuscitation was retained in the latest Surviving Sepsis Campaign guidelines, but the strength of this recommendation was reduced to weak.[31] Ospina-Tascon et al. proved that early administration of fluids in sepsis may improve capillary perfusion.[32] Interestingly, microcirculatory changes were independent of macrocirculatory variables. Early fluid loading without a cardiac response could increase microvascular perfusion, whereas late administration of fluids, even if associated with increased cardiac index and mean arterial pressure, did not lead to microvascular resuscitation. For this reason, hemodynamic monitoring seems insufficient in sepsis, and observation of the microcirculation seems necessary as it provides the full picture of the disease. For this purpose, in our study, we applied sidestream dark field imaging (SDF) as described elsewhere.[33]

Objectives

Based on our previous experience in an experimental model of piglet endotoxemia undergoing combined therapy with iNO and intravenous hydrocortisone, we attempted to verify whether this type of therapeutic management, along with standard fluid and hemodynamic resuscitation, can improve or restore homogeneous capillary flow in septic pigs.

Our study was based on a porcine fecal peritonitis model. This model has been widely used in the study of the pathophysiology of sepsis and the impact of new therapeutic modalities on its course.[34,35]

Materials and methods

Ethical issues

This study was approved by the Animal Research Ethics Committee of the Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wrocław, Poland (permission No. 7/05) and reported in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. All methods were carried out in accordance with applicable Polish law and followed the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH) and Minimum Quality Threshold in Pre-Clinical Sepsis Studies (MQTiPSS) recommendations.[36,37]

Throughout our research, care was taken to guarantee the maximum comfort of the animals. The depth of analgesia was adjusted to the level of pain stimuli and the depth of anesthesia was adjusted according to the level of consciousness. The animals that survived the study period were euthanized with pentobarbital.

Animal preparation

A detailed description of the instrumentation was previously presented elsewhere.[33] This study is part of a larger project covering a sequence of studies on iNO delivery to septic piglets.[25,38–40]

The experiment was conducted in the Department of Surgery at the Wrocław University of Environmental and Life Sciences, Poland. Originally, the study group consisted of 12 female piglets of Polish breed. One of them was excluded as it developed severe hypotonia unresponsive to treatment during instrumentation and died. All animals were of the same age (2 months). Their median body weight was 18.5 kg (range: 17–22 kg).

All piglets fasted for 6 h before the experiment; however, they were allowed to drink water at will. To induce anesthesia, we administered tiletamine-zolazepam doses of 4 mg × kg^{-1} dissolved in medetomidine 0.08 mg × kg^{-1} intramuscularly. All animals were successfully intubated and mechanical ventilation was started using Servo 900C ventilators (Siemens-Elema AB, Solna, Sweden). A ventilation protocol was based on the pressure-controlled mode with an initial inspired fraction of oxygen (FIO_2) of 0.3 and a positive end-expiratory pressure (PEEP) of 5 cm H_2O. During the experiment, ventilator settings were adjusted in accordance with blood gas analysis.

Following induction, general anesthesia was maintained with the intravenous infusion of ketamine (1.5–2.4 mg × kg^{-1} × h^{-1}), medetomidine (5.3–8.2 µg × kg^{-1} × h^{-1}), fentanyl (0.8–1.3 µg × kg^{-1} × h^{-1}), and midazolam (0.08–0.13 mg × kg^{-1} × h^{-1}). All animals received a solution of 2.5% dextrose in 0.9% saline (Glu/NaCl 1:1) at a constant rate of 100 mL × h^{-1} to prevent hypoglycemia. In addition, all animals received 500 mg of cefuroxime (GlaxoSmithKline, Solna, Sweden) intravenously (i.v.) every 8 h to counter accidental bacterial contamination during instrumentation and as the treatment modality.

For further hemodynamic monitoring, arterial lines, central venous catheters and pulmonary artery catheters were introduced. The following hemodynamic parameters were registered: heart rate (HR), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), systolic pulmonary artery pressure (SPAP), central venous pressure (CVP), diastolic pulmonary artery pressure (DPAP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO), cardiac index (CI), systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), and stroke volume index (SVI). Access to the urinary bladder was obtained transabdominally. According to core temperature measurements, heating blankets and external cooling were used to keep temperatures within the normal range. After instrumentation, the first blood sample was taken to evaluate arterial blood gas, complete blood count, aspartate transaminase (AST), alanine transaminase (ALT), creatinine, and urea. Hemodynamic measurements and microvascular imaging were performed.

Study protocol

The timeline of interventions is presented in Fig. 1. After a 1-hour rest, all piglets underwent a surgical midline laparotomy. The descending colon was visualized, a 3-centimeter incision was performed and 1.5 g/kg of fecal content was removed, mixed with blood, and then deposited close to the diaphragm to cause a septic-like condition. Then, the laparotomy was closed and all measurements were repeated every 10 h from that moment (Fig. 1).

Next, the piglets were randomly allocated to one of 3 groups:

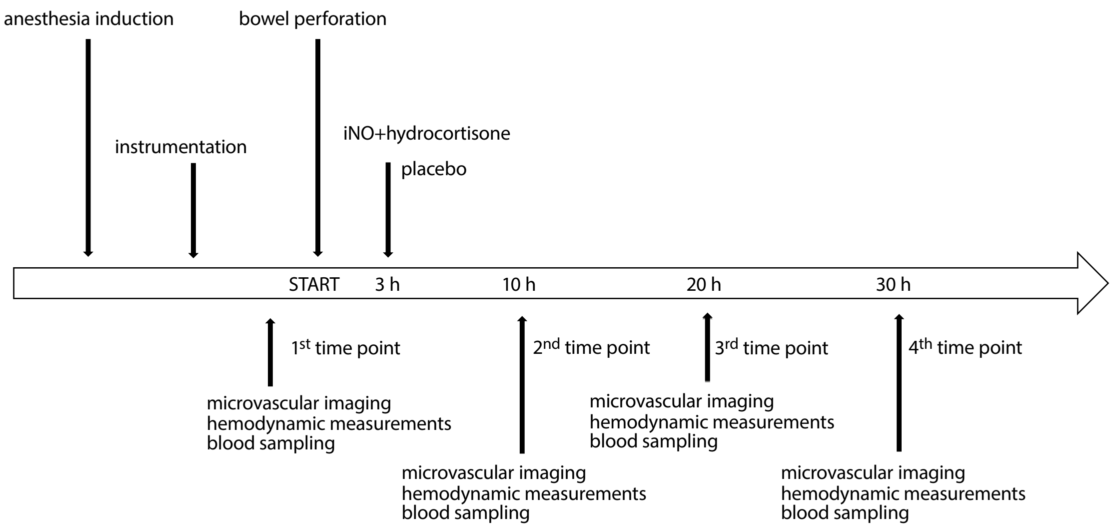

Fig. 1. Timeline of interventions and data acquisition events

Group 1 – piglets receiving iNO (800 ppm iNO in 9000 nitrogen; Pulmonox-Messer Griesheim, Bad Soden am Taunus, Germany) at a concentration of 30 ppm and hydrocortisone (75 mg i.v. every 7 h). Hypotensive piglets (MAP < 65 mm Hg for more than 3 min) were treated with a bolus of Ringer's lactate (300 mL) and norepinephrine infusion (if unresponsive to fluids) to maintain MAP ≥ 65 mm Hg.

Group 2 – piglets receiving neither iNO nor hydrocortisone but treated for hypotension with fluids and noradrenaline infusion similar to group 1.

Group 3 – piglets not receiving iNO and hydrocortisone, and not resuscitated with fluids or noradrenaline infusions.

In most cases, fluid boluses were sufficient to raise the MAP above 65 mm Hg in the resuscitated groups. Only 1 pig from group 1 received noradrenaline.

Microcirculation assessment

Our study evaluated microcirculation with the MicroScan™ device (MicroVisionMedical, Amsterdam, the Netherlands) based on SDF. We assessed the microvascular bed of the sublingual region of the oral cavity using 4 different sequences at every time point. Every record was stored using a random number code and the analyzing researcher was blinded to all clinical data. To optimize image quality and avoid artifacts, we ensured maximum camera stability, removed saliva from the sublingual area using sterile gauze, and minimized pressure on the mucosa.

For the microcirculation evaluation, we used AVA v. 3.0 (MicroVision Medical BV, Amsterdam, The Netherlands), which automatically identifies vessels. It calculates total vessel density by calculating the number of vessels crossing a grid of 6 lines (dividing the screen image into 16 equal areas). Further images were assessed using 2 semi-quantitative methods described by Boerma and De Backer.[3,6] The analysis provided information about the proportion of perfused vessels (PPV), the perfused vessel density (PVD = PPV × total vessel density (TVD)) and the microvascular flow index (MFI). All of the above variables were calculated separately for small vessels whose diameter was less than 20 μm (small vessel MFI – sMFI, small vessel density – sVD, proportion of perfused small vessels – sPPV, and perfused small vessel density – sPVD).

Statistical analyses

Statistical analysis was performed using Statistica v. 13.1 PL (StatSoft Polska, Kraków, Poland). The distribution of the microcirculatory variables was tested with the Shapiro–Wilk normality test. Since they showed a non-normal distribution, data were presented as medians with interquartile range (IQR) and analyzed with non-parametric tests. Differences between groups were tested with a Kruskal–Wallis analysis of variance (ANOVA) test, which was followed by a post hoc analysis. Correlation analysis was assessed using Spearman's rho correlation coefficient. A p-value <0.05 was considered statistically significant. All values were reported as medians unless otherwise stated.

Results

All eleven piglets included in the study developed a systemic inflammatory response syndrome (SIRS) diagnosed by meeting at least 2 of its criteria – heart rate >90 bpm and

Table 1. Group characteristics demonstrating the number of animals at particular time points, number of animals that developed systemic inflammatory response syndrome (SIRS), and temperature, heart rate (HR) and white blood cell count (WBC)

Variable	Group	START	10 h	20 h	30 h
Number of animals	1	4	4	4	3
	2	4	4	3	3
	3	3	3	3	0
Number of animals with SIRS	1	0	4	4	3
	2	0	3	3	3
	3	0	3	3	–
Temperature [°C]	1	36.0 ±0.6	38.4 ±1.5	41.3 ±0.5	41.5 ±0.6
	2	36.7 ±0.6	39.0 ±1.1	41.0 ±0.6	41.9 ±0.7
	3	37.5 ±0.8	39.3 ±1.6	41.5 ±0.7	–
HR [bpm]	1	76 ±8	155 ±43	142 ±28	152 ±3
	2	77 ±13	124 ±15	123 ±12	151 ±33
	3	95 ±12	158 ±47	161 ±45	–
WBC [m/mm^3]	1	15.0 ±7.4	13.2 ±6.7	11.1 ±4.3	9.5 ±4.5
	2	18.8 ±2.7	13.7 ±5.1	18.2 ±6.6	9.9 ± 5.9
	3	18.6 ±4.6	21.6 ±12.6	22.4 ±3.3	–

Temperature, HR and WBC are expressed as mean ± standard deviation (M ±SD). Group 1 – iNO + hydrocortisone; group 2 – placebo; group 3 – no resuscitation.

Table 2. Baseline microcirculatory variables. Initially, there were no differences in TVD, sTVD, PVD, sPVD, PPV, sPPV, MFI, sMFI, and the De Backer score between the groups ($p > 0.05$)

Variable	Group 1	Group 2	Group 3	p-value
TVD [mm/mm^2]	17.4 (16.8–19.0)	18.6 (17.4–21.3)	18.0 (16.0–20.3)	0.35
sVD [mm/mm^2]	16.0 (14.7–18.5)	17.4 (16.0–20.0)	16.7 (15.3–19.5)	0.30
PVD [mm/mm^2]	15.5 (13.7–16.9)	17.6 (15.4–20.1)	16.4 (14.1–18.8)	0.19
sPVD [mm/mm^2]	14.0 (12.8–15.8)	16.5 (14.1–19.0)	14.7 (12.6–18.6)	0.18
PPV [%]	91 (78–98)	94 (88–97)	91 (84–94)	0.48
sPPV [%]	92 (77–98)	93 (88–97)	91 (84–94)	0.52
MFI	2.9 (2.4–3.0)	3.0 (2.75–3.0)	2.9 (2.7–3.0)	0.86
sMFI	2.8 (2.3–3.0)	3.0 (2.6–3.0)	3.0 (2.6–3.0)	0.70
De Backer score	12.0 (10.6–13.1)	12.4 (11.1–13.6)	11.8 (10.9–13.4)	0.66

Group 1 – iNO + hydrocortisone; group 2 – placebo; group 3 – no resuscitation. TVD – total vessel density; sVD – small vessel density; PVD – perfused vessel density; sPVD – perfused small vessel density; PPV – proportion of perfused vessels; sPPV – proportion of perfused small vessels; MFI – microvascular flow index; sMFI – small vessel MFI; iNO – inhaled nitric oxide.

temperature >38°C (Table 1).[41] There were no differences between the 3 studied groups in the initial microcirculatory parameters (Table 2).

iNO and hydrocortisone

The 30-hour analysis of groups 1 and 2 showed no influence of iNO and hydrocortisone on microvascular bed perfusion. In both groups, the microcirculation generally deteriorated (Fig. 2). Microvascular flow impairment after 30 h was expressed by a statistically significant drop in the MFI (from 2.9 to 2.6 in group 1, and from 3.0 to 2.3 in group 2; $p < 0.05$), sMFI (from 2.8 to 2.5 in group 1, and from 3.0 to 2.3 in group 2; $p < 0.05$), PVD (from 15.5 mm/mm^2 to 12.8 mm/mm^2 in group 1, and from 17.6 mm/mm^2 to 15.3 mm/mm^2 in group 2; $p < 0.05$), sPVD (from 14.0 mm/mm^2 to 12.1 mm/mm^2 in group 1, and from 16.5 mm/mm^2 to 13.6 mm/mm^2 in group 2; $p < 0.05$), PPV (from 78% to 52% in group 1, and from 88% to 65% in group 2; $p < 0.05$), and sPPV (from 92% to 80% in group 1, and from 93% to 85% in group 2; $p < 0.05$). There were no statistically significant differences in capillary perfusion between groups 1 and 2 after 30 h (Fig. 2).

Hemodynamic resuscitation

All piglets from group 3 died prematurely (after 12 h, 23 h and 23.5 h). For this reason, the complete analysis of all groups after 30 h could not be conducted, and it was performed 20 h after the laparotomy.

In group 3, the perfusion deteriorated to a significantly greater extent than in the other groups after 20 h of observation (Fig. 2). The median MFI was equal to 3 and 2.8 in groups 1 and 2, respectively, compared to 1.9 in group 3 ($p < 0.05$), the median PPV reached 94%, 87% and 63% in groups 1, 2 and 3, respectively ($p < 0.05$), and the median PVD was 15.2 mm/mm^2, 15.09 mm/mm^2 and 10.1 mm/mm^2 in groups 1, 2 and 3, respectively ($p < 0.05$). Similar results were observed during small vessel analysis: the median sMFI reached 3.0 and 2.6 in groups 1 and 2, and 1.4 in group 3 ($p < 0.05$), the median sPPV was equal to 85%, 88% and 59% in groups 1, 2 and 3, respectively ($p < 0.05$), and the median sPVD at 20 h reached 13.9 mm/mm^2, 14.2 mm/mm^2 and 9.0 mm/mm^2 in groups 1, 2 and 3, respectively ($p < 0.05$). There were no significant intergroup differences in the TVD, sVD and the De Backer score at 20 h (Table 3).

Microvascular flow compared with systemic flow

Throughout the study, the only consistent correlations between microvascular flow and systemic flow were observed in group 3 (Table 4). Impairment of microvascular flow (expressed by changes in MFI and sMFI) correlated with MAP, SAP, DAP, and SVRI with $p < 0.05$ in all cases. None of these parameters depended on CO, CI, PCWP, MPAP, and SPAP. Nevertheless, they were inversely related to HR and DPAP.

Markers of organ function

In terms of the laboratory tests, only in group 3 was capillary perfusion deterioration related to a decrease in pH, base excess (BE) and urine output, and an increase in creatinine, urea and AST (with $p < 0.05$ for all the above variables; Table 5).

Discussion

Flow impairment and heterogeneity are the main pathologies accounting for microcirculatory derangements in sepsis. Endothelial dysregulation, arteriolar constriction, increased leukocyte and platelet adhesion, and the heterogeneous expression of inducible NO synthase cease the flow in some capillaries and reduces functional

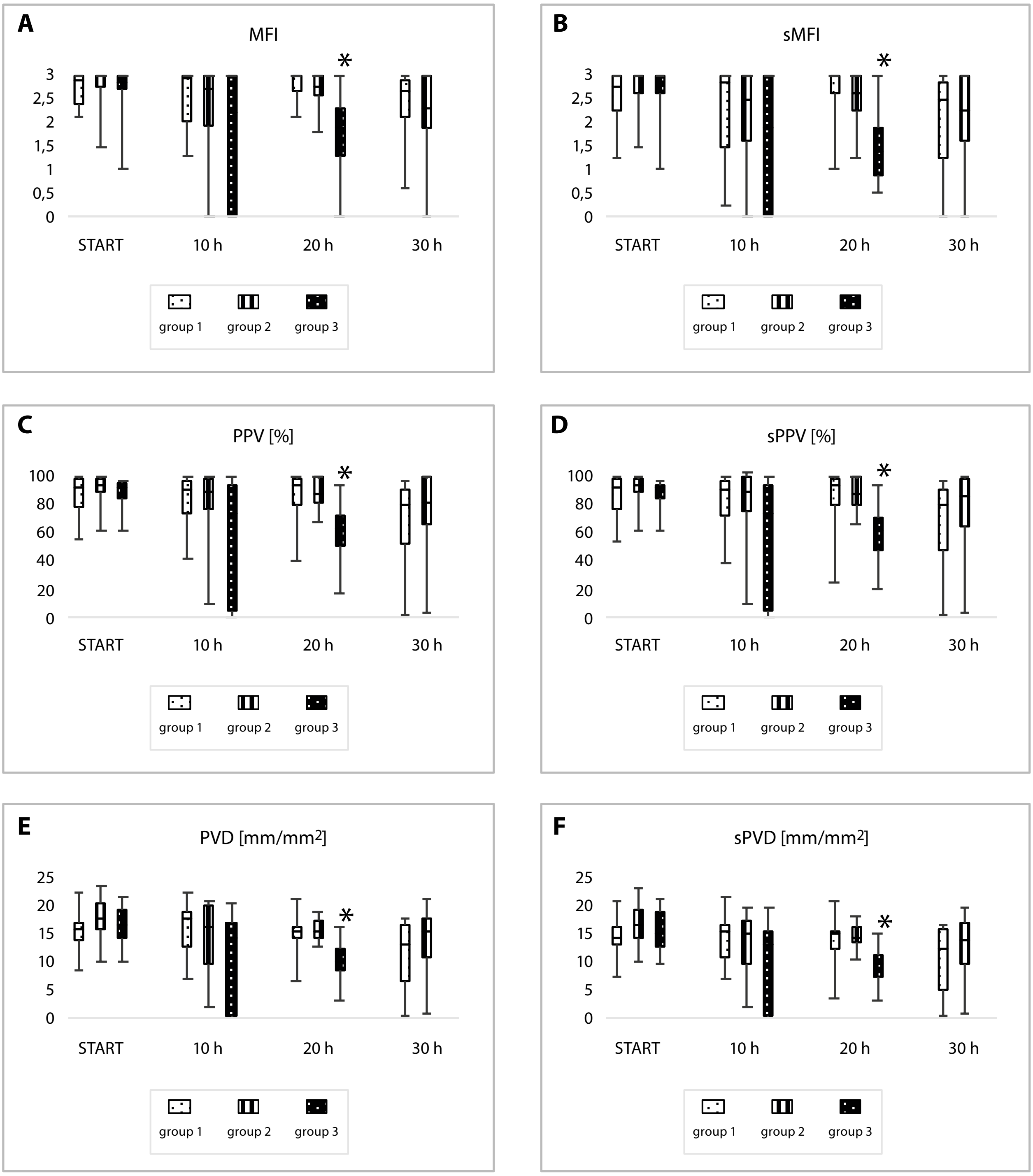

Fig. 2. Time course of microcirculatory variables in the studied groups. At 20 h of observation, significant difference (*) was seen between the groups, with a marked decrease in the microvascular flow index (MFI) (A), small vessel MFI (sMFI) (B), proportion of perfused vessels (PPV) (C), proportion of perfused small vessels (sPPV) (D), perfused vessel density (PVD) (E), and perfused small vessel density (sPVD) (F) in group 3 ($p < 0.05$). The piglets from this group died before the final endpoint (30 h). The analysis of the survivors from groups 1 and 2 showed no difference in MFI, sMFI, PPV, sPPV, PVD, and sPVD between these groups. Microcirculatory variables are presented as medians (25^{th}–75^{th} percentiles)

Group 1 – inhaled nitric oxide (iNO) + hydrocortisone; group 2 – placebo; group 3 – no resuscitation.

capillary density.[9] The restoration of flow in closed capillaries by NO, a strong vasodilator, can improve tissue oxygenation and the elimination of anaerobic metabolism products. Trzeciak et al. found no effect of iNO on microcirculatory flow in adult patients with sepsis.[42] However, iNO application was delayed and was commenced after initial macrocirculatory resuscitation. He et al. created an ovine model of peritonitis and applied tetrahydrobiopterin (BH4, an intravenous NO donor) shortly after the injection of feces into the abdominal cavity, an event triggering sepsis.[43] In their study, BH4 improved microvascular flow, kidney perfusion and urine production, and

Table 3. Table demonstrating changes in TVD, sVD and De Backer scores over time. At all time points, there were no significant differences in the above variables between studied groups ($p > 0.05$). The TVD, sVD and De Backer scores did not change over time

Group	START	p-value	20 h	p-value	30 h	p-value
TVD						
iNO+hydrocortisone	17.4 (16.8–19.0)	0.35	16.3 (15.7–18.4)	0.33	17.0 (13.4–19.1)	0.08
Control	18.6 (17.4–21.3)		18.6 (15.4–19.7)		18.4 (16.6–21.0)	
No treatment	18.0 (16.0–20.3)		17.3 (14.3–19.0)		–	
sVD						
iNO+hydrocortisone	16.0 (14.7–18.5)	0.30	15.5 (14.6–16.4)	0.47	16.5 (11.9–17.4)	0.09
Control	17.4 (16.0–19.9)		15.8 (14.3–17.3)		16.9 (14.8–19.5)	
No treatment	16.7 (15.3–19.5)		15.3 (13.2–17.6)		–	
De Backer score						
iNO+hydrocortisone	12.0 (10.5–13.1)	0.66	11.0 (9.7–12.8)	0.74	11.3 (9.4–14.4)	0.78
Control	12.4 (10.8–13.6)		11.2 (9.1–13.5)		12.7 (11.3–13.7)	
No treatment	11.8 (10.8–13.4)		11.6 (10.5–13.4)		–	

Data are presented as medians (25^{th}–75^{th} percentiles). Group 1 – iNO + hydrocortisone; group 2 – placebo; group 3 – no resuscitation. iNO – inhaled nitric oxide; TVD – total vessel density; sVD – small vessel density.

Table 4. Spearman's rho correlation coefficients between MFI and sMFI and hemodynamic parameters (HR, MAP, SAP, DAP, and SVRI). All correlations were statistically significant with a p-value <0.05

Flow index	HR	MAP	SAP	DAP	SVRI
MFI	−0.54	0.57	0.49	0.61	0.46
sMFI	−0.62	0.63	0.55	0.68	0.54

MFI – microvascular flow index; sMFI – small vessel MFI (sMFI); HR – heart rate; MAP – mean arterial pressure; SAP – systolic arterial pressure; DAP – diastolic arterial pressure; SVRI – systemic vascular resistance index.

Table 5. Spearman's rho correlation coefficients between MFI and sMFI and pH, HCO_3^{2-}, BE, diuresis, creatinine, urea, AST, lymphocytes, monocytes, and granulocytes. All correlations were statistically significant with a p-value <0.05

Flow index	pH	HCO_3^{2-}	BE	Urine output	Creatinine	Urea	AST	Lymphocytes	Monocytes	Granulocytes
MFI	0.54	0.66	0.63	−0.46	−0.48	−0.48	−0.44	−0.49	0.56	0.49
sMFI	0.52	0.66	0.66	−0.51	−0.52	−0.52	−0.50	−0.53	0.52	0.53

MFI – microvascular flow index; sMFI – small vessel MFI (sMFI); BE – base excess; AST – aspartate transaminase.

postponed the death of the treated animals. In our study, we found no effect of iNO in conjunction with hydrocortisone on the PPV, perfused capillary density and the MFI, even though iNO was started shortly after laparotomy. We hypothesize that no influence of the administered iNO on capillary recruitment and perfusion can be explained by the restricted access to closed vessels.

We observed that microcirculatory variables changed more profoundly in piglets not resuscitated with fluids and noradrenaline, with a statistically greater decrease in the MFI, sMFI, PPV, sPPV, PVD, and sPVD than in other groups. Only in this group was capillary flow strongly related to changes in hemodynamic indices during septic shock – MAP, SAP, DAP, SVRI, and HR. In groups receiving fluids and noradrenaline, hemodynamic variables were stabilized but these changes did not lead to microcirculatory flow normalization. This observation supports the theory that resuscitation of systemic flow does not resuscitate microcirculation to the same degree as the systemic circulation. Capillary flow impairment remains but progresses at a slower pace. The discrepancies between hemodynamics and microcirculatory variables have been described by other authors.[3,5,44] According to De Backer et al., microcirculatory derangements may precede systemic hemodynamic compromise and the relation between hemodynamic and microcirculatory indices may be loose.[5] Still, changes in CO and MAP may influence capillary perfusion.[5] In a single-center prospective observational study, De Backer showed that when sepsis develops at the early stages of resuscitation, hemodynamic measurements are related to microvascular flow indices, and this correlation disappears in later stages.[3] In human research, observations are limited to septic and resuscitated groups and are usually compared to healthy control groups. For obvious reasons, a control non-resuscitated group cannot be permitted. It is possible that for this reason, no associations between systemic hemodynamic and capillary flow indices have been observed during the advanced stages of sepsis. This correlation only exists when sepsis proceeds undisturbed. Perhaps the search for a direct relationship between the indicators of micro- and macrocirculation is on the wrong track. At an early stage of the therapeutic process and treatment optimization, global hemodynamics improvement does not lead to immediate microcirculatory normalization. The fact that microcirculation and systemic circulation are not coherent in resuscitated patients does not mean that hemodynamic resuscitation is not effective in terms of capillary perfusion resuscitation. As shown above, microcirculation deteriorated in the resuscitated groups, but more slowly. This suggests that resuscitation may buy time necessary for the immune system and antibiotics to overcome the infection. This observation suggests that the management of sepsis should first concentrate on the monitoring and stabilization of the systemic circulation and – at later stages – on the evaluation and resuscitation of the microcirculation.[5] Unfortunately, we have not yet found an answer to how to resuscitate the microcirculation, and we still do not have therapeutic options to restore the microcirculation. Fluids and vasopressors are an exception and may have impact on the microcirculation. They increase the pressure differences between arterioles and venules, reduce viscosity, and potentially influence the impact of the endothelium on the morphotic elements of blood.[32] These effects seem to be limited to the early stages of sepsis, as at later stages, endothelial dysfunction and leakage evolve.

In the non-resuscitated group, microcirculatory parameters were significantly related to the progress of acidosis and renal failure, as well as increases in AST, lymphocytes, granulocytes, and monocytes. This correlation was not observed in the resuscitated groups, where acidosis and organ dysfunction did not proceed as fast as in the non-treated group. In group 3, the combination of capillary perfusion deterioration and the related metabolic acidosis supports the theory that severe microcirculatory derangement plays an important role in anaerobic metabolism during sepsis. Unfortunately, for technical reasons, lactate was not monitored during the study, and important information about anaerobic metabolism was lacking.

In our study, the TVD, sVD and De Backer score did not follow any certain pattern and their changes over time were not statistically significant. This observation does not support the results presented by Massey et al. in the ProCESS trial.[45] In the above study, the TVD, PVD and De Backer scores appeared to be strongly associated with outcomes in septic patients. However, their prognostic value appeared to be significant after 72 h of observation. The observed difference in TVD and De Backer score values might result from a shorter time of observation.

Limitations

This study has several limitations. Apart from the small sample size and lack of lactate levels, our observations of microcirculatory alterations were limited to the sublingual area. The sublingual mucosa and the digestive mucosa have the same embryologic origin and changes in their capnometry correlate quite well, showing similar alterations.[13,46,47] Capillary perfusion in the sublingual area seems to reflect the flow in the splanchnic mucosa and is easily accessible. Nevertheless, SDF does not allow the direct in vivo observation of the microcirculation in vital organs.

Moreover, in our study, we used healthy young pigs. Human sepsis is associated with elderly age and comorbidities. For this reason, the studied group did not reflect all conditions precisely. We chose a porcine model of sepsis as there are many similarities in the immune response between pigs and humans.[48,49] Nevertheless, in our pilot studies, we found out that small colon perforation rarely leads to sepsis and multiorgan failure. To increase the probability of provoking sepsis in the animals, we decided to use a model utilizing a larger incision and fecal deposit in the abdomen.

Conclusions

In our study, crystalloid iNO with intravenous hydrocortisone did not improve microvascular perfusion in septic pigs, whereas crystalloid and vasopressor treatment postponed microvascular flow impairment.

Supplementary data

Supplementary tables are available at https://doi.org/10.5281/zenodo.7334314. The package contains the following files:

Supplementary Table 1. Time course of hemodynamic variables in groups 1, 2 and 3.

Supplementary Table 2. Time course of organ/metabolic function markers in groups 1, 2 and 3.

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