Sole causal therapy worsens outcome as compared to no therapy and combined causal and goal-directed supportive therapy in ovine septic shock

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Background: There is clear evidence that early causal therapy improves outcome in sepsis and septic shock, whereas recent studies on supportive hemodynamic therapy have produced very conflictive results. The objective of the present study was to determine whether a supportive hemodynamic therapy guided by clinically relevant invasive monitoring improves survival and organ function in a high-lethality model of septic shock in sheep as compared to sole causal therapy including surgical and antimicrobial treatment.

Methods: Twenty healthy ewes were anaesthetized and instrumented for hemodynamic surveillance. After laparotomy and fecal withdrawal from the caecum, animals were randomly assigned to one of four groups: sham, control, causal and combined therapy. In all groups but the sham group, feces were injected into the peritoneal cavity. Septic shock was defined as mean arterial pressure (MAP) ≤60 mmHg and arterial lactate concentration ≥1.8 mmol·L^{-1}. Animals of the control group received no therapy, while the causal group received broad-spectrum antibiotic therapy and peritoneal lavage. The combined therapy group received causal therapy plus supportive hemodynamic therapy.

Results: The sham animals showed no signs of systemic infection, while all other animals developed septic shock with arterial hypotension and lactic acidosis within 4.0 (4.0–6.8) hours. Induction of causal therapy did not impact on haemodynamics as compared to the control group. Notably, 50% of the control animals and none of the causal therapy animals survived the study. Combined therapy stabilized haemodynamics and improved organ function and survival as compared to control and causal therapy groups.

Conclusions: The present data suggest that sole causal sepsis therapy without hemodynamic support worsens outcome even more than natural evolution of sepsis and combined causal and supportive therapy. This underlines the importance of early hemodynamic stabilization in parallel with antibiotic and surgical treatment of the sepsis focus.

Keywords: Causal therapy; septic shock; sheep; supportive therapy; survival

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Introduction

Sepsis and septic shock are among the most common causes of death in intensive care units (1,2). To investigate and establish new therapeutic agents and strategies, preclinical sepsis models are necessary that implement current treatment guidelines thereby allowing the most reliable translational research (3).

Though many components of sepsis treatment have changed over the years, causal therapy and supportive hemodynamic treatment remain the basis of sepsis treatment (4,5). Prospective randomized investigations concerning omission or delay of established components of sepsis treatment are rare for obvious ethical reasons. However, due to analyses of Kumar *et al.* (6) and other investigators (7,8) we know that a delay in appropriate antibiotic treatment may result in increased mortality and this knowledge is accepted as "state of the art". A retrospective study of Seymour *et al.* analyzed the median time of initiation of broad-spectrum antibiotics and completion of intravenous fluid bolus regarding mortality (9). Interestingly, the authors recognized impaired survival in patients who received delayed antibiotic therapy while delayed completion of intravenous fluid bolus had no influence on survival. The current evidence for fluid resuscitation in sepsis remains highly conflicting (10) and findings like the results from the trials of Seymour and Andrews indicate that even established concepts in critical care medicine should be re-evaluated. Therefore, the present study aimed to evaluate the effects of causal sepsis therapy with absence of hemodynamic support versus combined causal and supportive therapy as well as the natural evolution of sepsis on hemodynamics, organ function variables as well as survival in septic shock. For this purpose, a clinically relevant large animal model of ovine septic shock including causal and supportive hemodynamic therapy as suggested by the current sepsis guidelines was established (4,5).

We hypothesized that combined causal and supportive hemodynamic therapy improve survival and organ function as compared to natural evolution of sepsis or causal treatment only in a large animal model of septic shock.

Methods

Study approval

The present study was approved by the Animal Care Committee of the State Government of North-Rhine Westphalia (LANUV NRW, Recklinghausen, Germany) with the approval no. 84-02.04.2011.A300. In addition, the responsible veterinarians of the facility were consulted before the beginning of the study about the adequate dosage of all used medications. All methods were performed in accordance with the National Institutes of Health Guide and as well as the American Physiologic Society's "Guide for the Care and Use of Laboratory Animals" using established protocols.

Anesthesia

After withdrawal of food for 12 hours, twenty healthy, female ewes aged 6–9 months [41.0 kg (35.0–43.0)] were anesthetized by intramuscular injection of S-ketamine (Ketanest® S, 10 $mg \cdot kg^{-1}$, Parke-Davis, Berlin, Freiburg, Germany) and midazolam (Dormicum®, 0.3 $mg \cdot kg^{-1}$, Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany). Endotracheal intubation was performed with a 9.0 tracheal tube (Rüsch, Rüschelit®, Teleflex Medical GmbH, Kernen, Germany) to enable mechanical ventilation (pressure controlled ventilation, tidal volume 10 $mL \cdot kg^{-1}$ adapted to expiratory carbon dioxide partial pressure of 35±5 mmHg). Anesthesia was maintained by inhalational isoflurane (targeted expiratory concentration 1.2%; Forene®; AbbVie Deutschland GmbH & Co. KG, Wiesbaden, Germany) and continued throughout the complete experiment. After induction of anesthesia, animals were kept anesthetized until the end of the study.

Cardiovascular instrumentation

All of the following catheterizations and surgical procedures were performed under sterile conditions and after ascertaining an appropriate level of general anesthesia. After placing a quad-lumen central venous catheter into the right jugular vein (6 Fr. Quadlumen Catheter Set, PVB Medizintechnik GmbH, Kirchseeon, Germany) in Seldinger's technique, anesthesia was supplemented with ketamine (1 $mg \cdot kg^{-1} \cdot h^{-1}$), midazolam (0.3 $mg \cdot kg^{-1} \cdot h^{-1}$) and lidocaine (1.5 $mg \cdot kg^{-1} \cdot h^{-1}$) during the further instrumentation (11). A pulse contour cardiac output catheter was placed in the right femoral artery (5 Fr. PiCCO catheter, Pulsion Medical Systems, Munich, Germany) and a Foley catheter (12 Fr. urinary catheter, Porgès S.A., Le Plessis Robinson-Cedex, France) was inserted to measure urinary output. After the instrumentation, the intravascular catheters were connected to a transpulmonary thermodilution and pulse contour cardiac output computer ($PiCCO_2$, Pulsion Medical Systems, Munich, Germany) to

Table 1 Randomisation and treatments of the study groups

Group	N	Sepsis induction	Antibiotic therapy	Surgical therapy	Supportive therapy
Sham	4	No	No	No	No
Control	4	Yes	No	No	No
Causal therapy	4	Yes	Yes	Yes	No
Combined therapy	8	Yes	Yes	Yes	Yes

provide continuous hemodynamic surveillance.

Surgical preparation

All animals underwent a median laparotomy. The cecum was detected and incised in order to withdraw 1.5 $g \cdot kg^{-1}$ feces, while omitting a contamination of the peritoneal cavity. Afterwards this incision was carefully sutured, and the surface of the cecum cleaned and decontaminated. Two 16 Fr. drains were placed between the mesentery of the small intestine and the abdominal wall was closed layer by layer with continuous sutures.

Experimental protocol

The postoperative, healthy baseline measurement (BL) was performed when the following conditions were fulfilled and maintained for one hour (see "Measurements" for details):

- Heart rate (HR) <100 beats per minute (bpm);
- Mean arterial pressure (MAP) ≥70 mmHg;
- Cardiac index (CI): ≥2.5 $L \cdot min^{-1} \cdot m^{-2}$;
- Arterial lactate ≤1.2 $mmol \cdot L^{-1}$;
- Blood temperature 38.0–39.8 °C.

Randomization

After BL measurement, sheep were randomized to the four study groups using a computer-based algorithm in a 1:1:1:2 ratio (sham, control, causal therapy, combined therapy; see *Table 1*). Following randomization, the feces was injected into the peritoneal cavity via the indwelling drain in order to induce peritoneal sepsis in all sheep except for the sham group. Basal fluid requirements were substituted in all groups by intravenous infusion of 2 $mL \cdot kg^{-1} \cdot h^{-1}$ of balanced crystalloid solution (Sterofundin® ISO, B. Braun Melsungen AG, Melsungen, Germany).

The shock time point was defined based on the Surviving Sepsis Campaign Guidelines 2008 by meeting the following conditions:

- Time from injection of feces ≥4 hours;
- Arterial lactate ≥1.8 $mmol \cdot L^{-1}$ [i.e., 1.5 times the upper normal limit of sheep (12)];
- MAP ≤60 mmHg.

Surgical and antimicrobial therapy

After shock time measurement (see "Measurements" for details), the animals of the causal and combined therapy groups received peritoneal lavage and antimicrobial chemotherapy. Lavage was performed once after shock time by fractional instillation of four liters of warm saline (38° Celsius) through the abdominal drains until no macroscopic fecal contamination was detectable in the effluent secretion.

Concurrently, intravenous antimicrobial chemotherapy was initiated by bolus injection of 20 $mg \cdot kg^{-1}$ meropenem (Meronem®, AstraZeneca GmbH, Wedel, Germany), followed by continuous intravenous infusion with 2.5 $mg \cdot kg^{-1} \cdot h^{-1}$. The sham and control groups received neither of the above-mentioned treatments (see *Table 1*).

Supportive hemodynamic therapy

Supportive hemodynamic treatment including fluid resuscitation was only performed in the combined therapy group (see *Table 1*). A balanced crystalloid solution (Sterofundin® ISO, B. Braun Melsungen AG, Melsungen, Germany) and 6% hydroxyethyl starch (HES) 130/0.4 (Volulyte®, Fresenius Kabi Deutschland, Bad Homburg, Germany) were used for fluid resuscitation. Crystalloids and colloids were infused alternately with boluses of 250 mL HES or 500 mL crystalloid. HES was given up to a cumulative maximum dose of 50 $mL \cdot kg^{-1}$ over the whole interventional period. Afterwards, fluid resuscitation was continued with crystalloids only. Indications for fluid resuscitation were each of the following:

- Global end-diastolic volume index (GEDI) <620 $mL \cdot m^{-2}$ or below BL value;
- Stroke volume variation (SVV) >13%;
- Hemoglobin (Hb) below BL value.

Fluid boluses were administered until all three conditions were met. Hemoglobin and GEDI were measured every 30 minutes, while SVV was measured continuously. Re-calibration of the PiCCO system was performed every 30 minutes. Norepinephrine infusion was initiated after shock time and titrated continuously to maintain a

MAP ≥65 mmHg up to a maximum dose of 5 $\mu g \cdot kg^{-1} \cdot h^{-1}$. Furthermore, dobutamine was used up to a maximum dose of 10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ if cardiac function index (CFI) was $<4.5 \cdot min^{-1}$. The maximum dosages were chosen from clinical experience, since no further vasoconstrictive or inotropic action could be expected with higher dosages due to tachyphylactic effects.

Measurements

All hemodynamic measurements were obtained in anesthetized animals. Hemodynamic parameters, urinary output as well as arterial and central-venous blood gas analyses were documented at BL, shock time and hourly thereafter. The study animals were monitored until death in deep anesthesia.

Laboratory measurements

Blood and urine samples were taken at BL, shock time and every 4 hours thereafter. The samples were immediately centrifuged and stored at −70 °C for later analysis.

The following variables were determined from the blood and urine samples, respectively:

- Blood gas analyses (electrolytes, oxygen- and carbon dioxide partial pressure, pH, BE, hemoglobin, oxygen saturation, lactate, glucose);
- Parameters of organ (dys-) function (bilirubin, creatinine, creatinine clearance).

End of protocol

Animals surviving the interventional period (8 hours after shock time) were killed with a bolus injection of 100 mL of 1-molar potassium chloride solution after anesthesia was deepened with propofol (4 $mg \cdot kg^{-1}$).

Outcome variables

Primary outcome measure was survival of the study animals over the interventional period. Secondary outcome measures included hemodynamic variables as well as diuresis and laboratory markers of organ function.

Statistical analysis

Statistical analysis was performed with IBM SPSS statistics software version 24 (IBM, Armonk, New York, United States). Due to the lack of pre-published data on the effects of supportive hemodynamic therapy versus no hemodynamic therapy (e.g., on survival), a rational *a priori* sample size analysis was not suitable. Thus, all analyses were explorative. All data are presented as median and interquartile range (IQR). Comparisons between groups were made using Mann-Whitney U test or Kruskal-Wallis test dependent on the number of groups to compare. If necessary, post-hoc comparisons were conducted using Dunn's test. Comparisons between time points were made using Wilcoxon signed-rank test. Asymptotic two-sided P smaller than 0.05 were assumed as statistically relevant differences.

Results

Effects of the instrumentation and laparotomy

The animals of the sham group showed no signs of systemic inflammation (i.e., increase in heart rate, temperature, or lactate) between BL and shock time and during the following 8 hours (see *Table S1*). All other measured variables were within physiological ranges according to reference values for anesthetized sheep (12).

Effects of sepsis induction

In median sheep developed septic shock after 4.0 hours (IQR 4.0 to 6.8). The effects of sepsis induction are described for animals of the control group between BL and shock time. All septic animals of the other study groups showed similar effects from BL to shock time (see *Table 2*).

After feces instillation, the animals developed signs of systemic inflammation and capillary leakage. Core body temperature and hemoglobin concentration increased significantly between BL and shock time (see *Table 2*).

Between BL and shock time, the animals developed a hypodynamic and hypotensive macrocirculation, as measured by a significant decrease in MAP and CI (see *Table 2*). Further hemodynamic parameters, such as an increase in heart rate and a decrease of GEDI and SVI confirmed the development of hypovolemia (see *Table 2*).

Urinary output of all septic animals decreased between BL and shock time, while creatinine concentration increased in the same period, according to a decrease in creatinine clearance (see *Table 2* and *Figure 1*).

Effects of sepsis treatment

After initiation of the individual group-specific therapy, the

Table 2 Haemodynamics, variables of oxygen transport, metabolic and organ function at baseline (BL) and shock time in sheep with faecal peritonitis (septic animals)

Variable	Septic animals at BL (n=16)	Septic animals at shock time (n=16)	P (BL *vs.* shock time)
MAP (mmHg)	86 (75 to 94)	42 (36 to 55)	<0.001*
HR ($1 \cdot min^{-1}$)	80 (76 to 86)	86 (75 to 95)	0.244
CI ($L \cdot min^{-1} \cdot m^{-2}$)	3.6 (3.2 to 4.3)	2.5 (2.2 to 2.6)	<0.001*
GEDI ($mL \cdot m^{-2}$)	720 (645 to 800)	574 (513 to 646)	0.003*
CVP (mmHg)	3 (2 to 5)	0 (0 to 3)	0.068
SVI ($mL \cdot m^{-2}$)	42 (34 to 51)	29 (26 to 34)	0.004*
SVV (%)	12 (6 to 13)	10 (6 to 12)	0.637
EVLWI ($mL \cdot kg^{-1}$)	12 (12 to 13)	14 (12 to 18)	0.134
Hb ($g \cdot dL^{-1}$)	7.6 (7.0 to 8.9)	10.7 (9.3 to 12.0)	<0.001*
Lactate ($mmol \cdot L^{-1}$)	0.8 (0.7 to 1.2)	1.9 (1.8 to 2.0)	0.007*
pH_a [$-log_{10}c(H^+)$]	7.43 (7.40 to 7.47)	7.40 (7.38 to 7.45)	0.023*
BE ($mmol \cdot L^{-1}$)	8.1 (4.5 to 9.7)	6.3 (1.1 to 7.4)	0.002*
DO_2I ($mL \cdot min^{-1} \cdot m^{-2}$)	374 (329 to 450)	318 (303 to 397)	0.496
O_2-ER (%)	21 (13 to 25)	37 (28 to 41)	0.001*
VO_2I ($mL \cdot min^{-1} \cdot m^{-2}$)	65 (54 to 89)	110 (104 to 130)	0.009*
$S_{cv}VO_2$ (%)	80 (77 to 87)	65 (60 to 70)	0.001*
Creatinine ($mg \cdot dL^{-1}$)	0.9 (0.8 to 1.0)	1.5 (1.1 to 1.8)	0.001*
Diuresis ($mL \cdot kg^{-1} \cdot h^{-1}$)	1.4 (0.7 to 3.7)	0.2 (0.0 to 0.3)	0.001*
Crea-Clearance ($mL \cdot min^{-1} \cdot m^{-2}$)	72 (48 to 127)	13 (2 to 27)	0.003*
Bilirubin ($mg \cdot dL^{-1}$)	0.05 (0.05 to 0.10)	0.05 (0.05 to 0.05)	0.058
Temperature (℃)	38.9 (38.5 to 39.5)	40.5 (39.4 to 41.2)	<0.001*

Data are presented as median (interquartile range). Wilcoxon signed-rank test was used to compare variables between BL and Shock time. *, indicates a statistical significant difference (P<0.05). BE, base excess; Bilirubin, serum bilirubin concentration; BL, healthy baseline measurement; CI, cardiac index; Crea-Clearance, creatinine clearance; Creatinine, serum creatinine concentration; DO_2I, oxygen delivery index; EVLWI, extravascular lung water index; HR, heart rate; CVP, central venous pressure; GEDI, global end-diastolic index; Hb, haemoglobin; MAP, mean arterial pressure; O_2-ER, oxygen extraction rate; Ph_a, arterial potentia hydrogenii; $S_{cv}O_2$, central venous oxygen saturation; SVI, stroke volume index; SVV, stroke volume variation; VO_2I, oxygen consumption index.

animals of the combined therapy group showed signs of improved organ function as compared to the other groups (see details below).

MAP, SVI, CI and GEDI were increased in the combined therapy group as compared to causal and control group (each P<0.05, *Table 3*). Hemoglobin levels and lactate concentrations were lower in the combined therapy group (each P<0.05, see *Figure 1* and *Table 4*).

Urinary output was higher in the combined therapy group as compared to the other septic groups, in which anuria developed after shock time (see *Table 4*). Serum creatinine concentration was significantly lower and creatinine clearance significantly increased in the combined therapy group as compared to the causal and control group (each P<0.05, see *Figure 2* and *Table 4*).

Base excess of the combined therapy animals remained in near physiologic ranges, while the animals of the control and causal therapy group showed a significant decrease in BE during the same time (up to -11.8 $mmol \cdot L^{-1}$, P<0.05). Accordingly, pH was significantly decreased in these groups (see *Table 4*).

The variables of oxygen transport (i.e., oxygen delivery

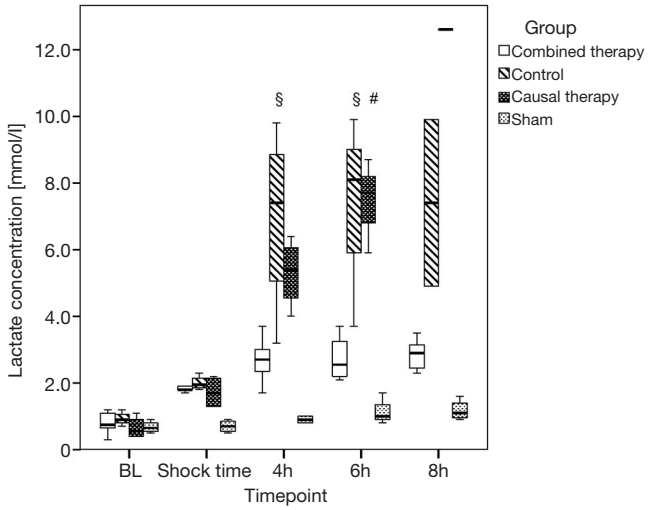

Figure 1 Serum creatinine concentration of the sham, control, causal and combined therapy group during the 8-hour interventional period. All animals of the causal therapy group were dead at time point 8 h after shock time. Values are presented as median (interquartile range). Kruskal-Wallis test was used for comparison between groups. Post-hoc comparisons were conducted using Dunn's test. BL, baseline. #, combined therapy versus causal therapy P<0.05; §, combined therapy versus control group P<0.05.

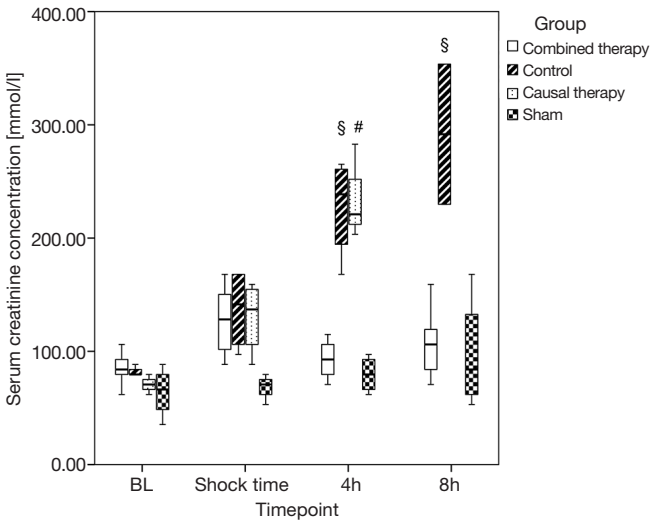

Figure 2 Arterial lactate concentration of the sham, control, causal and combined therapy group during the 8-hour interventional period. All animals of the causal therapy group were dead at time point 8 h after shock time. Values are presented as median (interquartile range). Kruskal-Wallis test was used for comparison between groups. Post-hoc comparisons were conducted using Dunn's test. BL, baseline. #, combined therapy versus causal therapy P<0.05; §, combined therapy versus control group P<0.05.

index DO_2I, oxygen extraction rate O_2-ER, oxygen consumption index VO_2I) showed no differences between the groups except for a higher central venous oxygen saturation ($S_{cv}O_2$) in the combined therapy group as compared to the control group (see *Table 4*).

There were no differences in hemodynamic or laboratory variables between the animals of the control group and those of the causal therapy group at any time during the study.

Relative organ weights of the animals were comparable between groups except for the weight of the lungs. The right lung was heaviest in the control group [7.0 g·kg^{-1} (IQR 6.8 to 7.3)] with significant difference to the sham group [4.8 (IQR 4.1 to 5.6), P<0.05]. Animals of the causal group had the heaviest left lungs [5.7 (IQR 4.8 to 6.3)] with significant difference to the sham group [3.8 (3.7, 4.2), P<0.05, see *Table S2*].

Fluid balance

The animals of the combined therapy group received 5,500 mL (IQR 4,125 to 7,750) study fluids in the 8-hour interventional period. Cumulative basal fluid administration (2 mL kg^{-1} h^{-1}) was as follows: combined therapy group 616 mL (IQR 552 to 688), causal group 547 mL (IQR 474 to 574), control group 605 mL (IQR 476 to 640) and sham group 688 mL (IQR 646 to 704). Total fluid balance after 8 hours for the respective groups were as follows: combined therapy group 5,737 mL (IQR 4,414 to 7,945), causal group 532 mL (IQR 465 to 555), control group 580 mL IQR (460 to 622) and sham group 428 mL (IQR 358 to 489) (see *Table S3*).

Survival

All animals of the combined therapy group and all sham animals survived the 8-hour interventional period. Among the animals of the control group, 50% survived during the same time. None of the causal therapy animals survived the 8-hour interventional period (P<0.001 *vs.* combined therapy group, P<0.05 *vs.* control group, P<0.05 *vs.* sham group, see *Figure 3*).

Discussion

The present study investigated the effects of causal and

Table 3 Haemodynamics of the control, causal and combined therapy group during the 8-hour interventional period

Variable	Group	Shock time	4 h	6 h	8 h
MAP (mmHg)	Control	43 (36 to 50)	31 (18 to 44)	36 (26 to 40)	34 (29 to 38)
	Causal therapy	50 (37 to 57)	30 (25 to 35)	40 (34 to 41)	NA
	Combined therapy	40 (36 to 54)	60 (56 to 61)	61 (59 to 63)	60 (58 to 61)
	P	0.797	0.003[§,$]	0.008[§,$]	0.036[$]
HR ($1 \cdot min^{-1}$)	Control	75 (60 to 100)	105 (70 to 151)	131 (99 to 140)	129 (113 to 144)
	Causal therapy	93 (88 to 99)	136 (127 to 158)	146 (121 to 153)	NA
	Combined therapy	86 (73 to 90)	114 (99 to 124)	115 (106 to 127)	115 (105 to 126)
	P	0.347	0.089	0.658	0.433
CI ($L \cdot min^{-1} \cdot m^{-2}$)	Control	2.6 (2.3 to 3.6)	2.1 (0.6 to 3.5)	2.6 (1.9 to 3.8)	3.4 (3.4 to 3.4)
	Causal therapy	2.5 (2.2 to 3.4)	1.4 (0.7 to 2.4)	1.9 (1.7 to 4.5)	NA
	Combined therapy	2.4 (2.1 to 2.5)	5.8 (5 to 7.2)	6.5 (4.7 to 8.6)	6.6 (4.9 to 8.4)
	P	0.227	0.003[§,$]	0.064	0.047[†]
GEDI ($mL \cdot m^{-2}$)	Control	558 (533 to 716)	501 (187 to 690)	609 (534 to 661)	756 (621 to 892)
	Causal therapy	556 (510 to 674)	337 (167 to 477)	430 (407 to 576)	NA
	Combined therapy	606 (494 to 644)	759 (728 to 847)	752 (687 to 807)	741(642 to 781)
	P	0.928	0.009[§]	0.083	0.256
CVP (mmHg)	Control	1 (0 to 4)	1 (0 to 8)	0 (0 to 2)	NA
	Causal therapy	0 (0 to 1)	NA	0 (0 to 2)	NA
	Combined therapy	1 (0 to 4)	7 (4 to 8)	9(2 to 10)	5 (2 to 13)
	P	0.604	0.016[§]	0.078	NA
SVI ($mL \cdot m^{-2}$)	Control	32 (28 to 37)	18 (6 to 23)	19 (18 to 24)	26 (23 to 29)
	Causal therapy	30 (27 to 33)	15 (10 to 68)	19 (15 to 30)	NA
	Combined therapy	28 (24 to 69)	57 (49 to 69)	71 (52 to 74)	57 (44 to 74)
	P	0.690	0.035[§]	0.008[§,$]	0.046[†]
SVV (%)	Control	10 (7 to 14)	5 (0 to 15)	14 (7 to 19)	11 (10 to 12)
	Causal therapy	10 (8 to 11)	17 (13 to 27)	28 (18 to 31)	NA
	Combined Therapy	10 (5 to 13)	12 (7 to 15)	8 (6 to 14)	11 (9 to 16)
	P	0.888	0.184	0.276	0.282
EVLWI ($mL \cdot kg^{-1}$)	Control	15 (13 to 21)	18 (7 to 28)	20 (18 to 32)	46 (22 to 70)
	Causal therapy	13 (11 to 18)	14 (6 to 21)	21 (16 to 23)	NA
	Combined therapy	13 (12 to 17)	14 (11 to 15)	14 (11 to 15)	13 (10 to 15)
	P	0.682	0.765	0.083	0.047[†]

Values are presented as median (interquartile range). Kruskal-Wallis test was used for comparison between groups. Post-hoc comparisons were conducted using Dunn's test: [§], causal therapy versus combined therapy $P<0.05$; [$], combined therapy versus control group $P<0.05$; [†], post-hoc comparisons not significant. MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; CVP, central venous pressure; EVLWI, extravascular lung water index; GEDI, global end-diastolic index; NA, value not available, because all animals of the respective group were dead at that time point or measurement was not possible; SVI, stroke volume index; SVV, stroke volume variation.

Table 4 Metabolics, kidney function and temperature of the control, causal and combined therapy group during the 8-hour interventional period

Variable	Group	Shock time	4 h	6 h	8 h
pH [$-\log_{10}c(H^+)$]	Control	7.40 (7.31 to 7.40)	7.13 (7.11 to 7.25)	7.08 (7.06 to 7.22)	7.14 (6.99 to 7.30)
	Causal therapy	7.39 (7.38 to 7.42)	7.24 (7.20 to 7.29)	7.14 (7.04 to 7.17)	NA
	Combined therapy	7.44 (7.38 to 7.47)	7.45 (7.43 to 7.48)	7.40 (7.36 to 7.46)	7.42 (7.40 to 7.45)
	P	0.391	0.004[$]	0.036[†]	0.079
BE ($mmol \cdot L^{-1}$)	Control	2.7 (−0.8 to 5.2)	−10.4 (−13.5 to −5.7)	−11.8 (−12.8 to −7.7)	−9.7 (−12.6 to −6.7)
	Causal therapy	6.3 (1.6 to 7.1)	−7.3 (−9.9 to −4.7)	−11.3 (−14.1 to −10.1)	NA
	Combined therapy	7.0 (3,5 to 8.5)	2.9 (2.2 to 4.5)	2.3 (1 to 3.4)	2.2 (−0.3 to 3.7)
	P	0.284	0.003[§,$]	0.008[§,$]	0.047[†]
Hb ($g \cdot dL^{-1}$)	Control	12 (10.5 to 12.6)	11.8 (11.1 to 12.4)	11.8 (11.1 to 12.2)	11.2 (10.8 to 11.6)
	Causal therapy	11.9 (11 to 13.5)	11.5 (10.9 to 14.7)	11.3 (10.8 to 13.2)	NA
	Combined therapy	9.5 (8.7 to 10.3)	6.7 (6 to 7.6)	6.2 (6 to 7.7)	7.3 (5.7 to 8.1)
	P	0.044[†]	0.003[§,$]	0.007[$]	0.047[†]
DO_2I ($mL \cdot min^{-1} \cdot m^{-2}$)	Control	363 (316 to 523)	464 (326 to 523)	427 (304 to 595)	483 (449 to 517)
	Causal therapy	386 (342 to 500)	341 (263 to 435)	541 (459 to 630)	NA
	Combined therapy	313 (251 to 353)	608 (484 to 727)	570 (366 to 823)	600 (456 to 675)
	P	0.374	0.077	0.752	0.296
VO_2I ($mL \cdot min^{-1} \cdot m^{-2}$)	Control	136 (106 to 178)	111 (73 to 175)	69 (49 to 116)	126 (25 to 226)
	Causal therapy	110 (102 to 118)	79 (48 to 104)	74 (68 to 110)	NA
	Combined therapy	118 (98 to 130)	79 (73 to 175)	86 (63 to 121)	80 (65 to 107)
	P	0.626	0.834	0.871	1.0
$S_{cv}O_2$ (%)	Control	69 (52 to 72)	67 (52 to 78)	84 (73 to 87)	72 (56 to 89)
	Causal therapy	72 (66 to 77)	74 (69 to 83)	83 (76 to 85)	NA
	Combined therapy	61 (59 to 65)	85 (82 to 87)	83 (79 to 86)	84 (80 to 87)
	P	0.086	0.047[$]	0.921	0.360
O_2-ER (%)	Control	30 (25 to 48)	30 (19 to 46)	16 (13 to 27)	25 (6 to 44)
	Causal therapy	28 (22 to 34)	24 (16 to 29)	16 (13 to 22)	NA
	Combined therapy	40 (38 to 44)	14 (12 to 18)	15 (12 to 19)	14 (11 to 22)
	P	0.055	0.124	0.928	0.441
Diuresis ($mL \cdot kg^{-1} \cdot h^{-1}$)	Control	0.2 (0.1 to 0.3)	0.1 (0 to 0.3)	0 (0 to 0)	NA
	Causal therapy	0.2 (0.1 to 0.3)	NA	NA	NA
	Combined therapy	0.1 (0 to 0.4)	0.9 (0.5 to 1.8)	1 (0.4 to 1.7)	0.5 (0.1 to 1)
	P	0.983	0.003[§]	0.006[§]	NA
Creatinine-Clearance ($mL \cdot min^{-1} \cdot m^{-2}$)	Control	12 (4 to 20)	3 (0 to 8)	NA	0 (0 to 0)
	Causal therapy	24 (12 to 55)	0 (0 to 0)	NA	NA
	Combined therapy	11 (4 to 39)	55 (34 to 73)	NA	65 (43 to 69)
	P	0.665	0.003[§,$]	NA	0.036[$]
Temperature (°C)	Control	40.7 (40 to 41.1)	41.0 (40.2 to 41.7)	41.0 (40.3 to 41.8)	42.2 (41.4 to 42.9)
	Causal therapy	41.5 (40.6 to 42.3)	42.5 (41.6 to 43.5)	41.9 (41.8 to 42.7)	NA
	Combined therapy	39.4 (39.2 to 40.5)	39.3 (38.7 to 39.9)	39.4 (38.9 to 39.9)	39.4 (39.1 to 40.0)
	P	0.052	0.005[§]	0.024[$]	0.118

Values are presented as median (interquartile range). Kruskal-Wallis test was used for comparison between groups. Post-hoc comparisons were conducted using Dunn's test: [§], causal therapy versus combined therapy $P<0.05$; [$], combined therapy versus control group $P<0.05$; [†], post-hoc comparisons not significant. BE, base excess; DO_2I, oxygen delivery index; Hb, haemoglobin; NA, value not available, because all animals of the respective group were dead at that time point or measurement was not possible; O_2-ER, oxygen extraction rate; pH, arterial potential hydrogen; $S_{cv}O_2$, central venous blood oxygen saturation; VO_2I, oxygen consumption index.

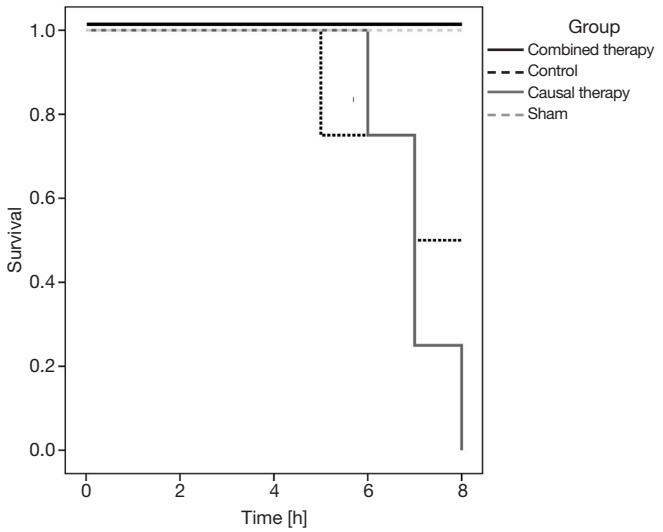

Figure 3 Kaplan-Meier survival curves of the sham group and the intervention groups.

supportive hemodynamic treatment strategies in an innovative and clinically relevant model of abdominal septic shock in sheep. All animals except for the sham group developed lactic acidosis and organ dysfunction, indicating that the induction of peritoneal sepsis results in severe systemic inflammation. Treatment of the animals following a study protocol with causal and supportive hemodynamic therapy improved macrohemodynamics, organ function and survival of the affected animals significantly. Notably, causal therapy without supportive hemodynamic treatment did not result in the abovementioned beneficial effects and even worsened survival. This suggests that causal therapy without hemodynamic support worsens outcome more than the natural evolution of sepsis at least in the present model.

The animals in the present investigation met criteria for sepsis-associated organ dysfunction by arterial hypotension, lactic acidosis and an increase in creatinine (13). This means in consequence, that the present model is able to induce sepsis-related organ dysfunction comparable to a clinical setting.

Since the animals of the sham group did not develop signs of systemic inflammation, one can assume that the instrumentation and surgery of the ewes did not induce a pathologic state *per se*. After peritoneal feces injection, systemic hemodynamics developed to septic shock as in human beings. Hemoconcentration, tachycardia, arterial hypotension and low cardiac output suggest vasodilation and capillary leakage as a central feature. After induction of supportive hemodynamic therapy including fluid resuscitation and vasopressors, a typical hyperdynamic vasodilatory shock developed. Notably, relatively high doses of norepinephrine were necessary to counteract arterial hypotension, which may reflect the severity of shock and the catecholamine hyposensitivity often observed in the clinical setting (14). The significant increase of hemodynamic parameters like CI, GEDI and SVI indicated adequate filling. Furthermore, the mentioned parameters even exceeded BL values (see *Table 3*), which confirms the development of a hyperdynamic circulatory state which is common in early septic shock (15).

Hemodynamics in the causal therapy group did not improve over time and showed no differences to the animals of the control group (see *Table 3*). The potential beneficial effects of the causal treatment (i.e., reduced number of bacteria and toxins) may need some minimum time to occur. It seems to be logical, that this time must be bridged by adequate supportive hemodynamic therapy to allow the organism to survive until antimicrobial drugs show their effects. Interestingly, none of the causal therapy animals survived the interventional period, while 50% of the control group animals survived the same time. One probable explanation for this observation may be that peritoneal lavage inevitably involves manipulation of the septic focus, and might thereby worsen systemic inflammation by additional release of toxins into the bloodstream (16,17). This effect may even have been enhanced by the release of toxins due to the bactericidal effects of meropenem with consecutive lysis of bacterial cell membranes (18) and histamine liberation. Especially in Gram-negative sepsis, a 3- to 20-fold increase of endotoxin concentration due to bacteriolysis following application of antibiotic medication could be measured (19). Fekade *et al.* proved, that the application of anti tumor necrosis factor alpha—antibodies decreases the release of interleukin-6 and -8 (20) and therefore lessens the effect of the "Jarisch-Herxheimer-reaction" including fever and hypotension. Another important reason for the worst outcome of the causal-group animals might be the effect of the warm water for peritoneal lavage, which may cause hypotension and worsening of vasodilatory shock. Mechanisms will most likely be liberation of damage- and pathogen-associated molecular patterns (DAMPs and PAMPs) as well as local vasodilation. In the full therapy group, these effects were offset by supportive hemodynamic therapy. These findings indicate that initiation of causal therapy without adequate hemodynamic support may result in decreased survival in

septic patients, a situation which may occur especially in regions with resource-limited settings. Amir and colleagues investigated the effects of the World Health Organization's Integrated Management of Adolescent and Adult Illness (IMAI) in patients with severe infection. Interestingly, the investigators found no difference in survival and organ function (despite lactate clearance) between the patients who received fluid resuscitation according to the IMAI guidelines (n=28) and those who did not (n=94). The administered doses of fluids after 6 hours were 3 L in the IMAI group *vs.* 1.5 L in the no-IMAI group (21). It must be noted, that the rate of HIV-positive patients in the treated groups were quite high (62%) and no details regarding causal therapy was presented by the authors. Though the findings from the present must be transferred with caution, it should be noted that causal sepsis therapy without fluid resuscitation might be harmful and that potential sources of sepsis, ethnological differences and potential comorbidities must be taken into consideration.

The comparison of causal therapy only and causal plus supportive therapy in the present study showed clearly, that a causal therapy with antibiotics and peritoneal lavage without supportive hemodynamic treatment is not able to improve hemodynamics and organ function in ewes with abdominal sepsis. Causal and supportive therapies have been cornerstones in sepsis therapy for decades. There is good evidence that a delay in causal therapy worsens outcome in patients with septic shock (6). Recent investigations described a potential benefit for septic shock patients receiving restrictive fluid therapy (22) and the debate about restrictive versus liberal resuscitation strategies is still going on. According to the authors of the CLASSIC-trial, restrictive fluid resuscitation might be superior to standard resuscitation protocols in septic patients (22). This raises the question, that if "less therapy" is better than "more therapy", it might be best to do "no therapy". Considering the causal therapy group as an "ultra-restrictive" fluid resuscitation regimen (basal requirements with 2 $mL \cdot kg^{-1} \cdot h^{-1}$ of balanced crystalloid solution), the data from the present study suggest that supportive hemodynamic therapy must include at least some amount of fluids in combination with causal therapy in order to avoid adverse outcome. In the present study, the absence of supportive therapy worsened organ function, hemodynamics and survival in the animals with abdominal sepsis Though the importance of causal therapy is out of the question regarding success of sepsis therapy, the survival of the animals who received causal therapy alone was even worse than in the control group. This may lead to the assumption, that causal and supportive therapy should be initiated together in order to buffer hemodynamic impairment and provide time for the causal therapy to work. This is of utmost importance especially for regions with resource-limited settings and should be considered in recommendations for sepsis therapy within these regions.

Limitations

There are some limitations regarding the present study that should be mentioned. Since this is a model in sheep, the results of this and similar studies should be transferred to human medicine with caution. Though especially sheep models show similar hemodynamic development compared to human beings, results from animal models often differ from clinical trial data for a variety of reasons (3,23). Moreover, the impact of sole hemodynamic without causal therapy was not investigated and may be a focus of future studies. However, since the benefit of causal therapy is clear from clinical data, we did not see a high clinical relevance from a study group of only supportive hemodynamic therapy. Organ function and injury were not investigated in detail in the present study and should be focused in future experiments. The sample size of each group is a limitation, though the results are clearly discriminating and significant." Another limitation of the present investigation was the use of HES in septic shock, which was an accepted strategy at the time of initiation of the study. The recommended dose of 50 $mL \cdot kg^{-1}$ BW HES was used in this study. However, since the dose was not adjusted to the relative low bodyweight of the animals, the applied dose of HES was relatively high. We assume that the possible adverse effects of HES on organ function might be negligible after a period of 8 hours. Since the present investigation was a pilot trial, no biometric calculation of the sample size was performed. Due to the low sample size and the reduced number of animals in the control group at 8 hours after shock time, the measured differences should be interpreted with caution. The study investigates intra-abdominal infection and it should be mentioned that the results might be different for other sources of sepsis (e.g., pneumonia). The use of meropenem might miss some Gram-positive bacteria, so in future studies the additional use of antimicrobial drugs with gram positive activity should be considered.

Conclusions

In the present severe model of septic shock with lactic acidosis and organ failure, the initiation of causal therapy

including antibiotics and peritoneal lavage without hemodynamic support worsened outcome and organ failure as compared to combined causal and supportive hemodynamic therapy and even natural evolution of sepsis without therapy. In conclusion, the presented investigation underlines the assumption that causal therapy without hemodynamic support might be harmful, and early supportive fluid and vasopressor therapy in septic shock is an essential part of initial sepsis therapy.

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Footnote

Conflicts of Interest: TG Kampmeier received travel reimbursements and honoraria as a consultant from Fresenius Kabi Germany. M Westphal is currently Chief Medical Officer of Fresenius Kabi Germany. S Rehberg has received travel fees provided by Orion Pharma and Amomed Pharma and is Medical Advisor for Fresenius Kabi Germany and Amomed Pharma. Other authors have no conflicts of interest to declare.

Ethical Statement: The present study was approved by the Animal Care Committee of the State Government of North-Rhine Westphalia (LANUV NRW, Recklinghausen, Germany) with the approval no. 84-02.04.2011.A300.

References

1. Angus DC, van der Poll T. Severe sepsis and septic shock. N Engl J Med 2013;369:840-51.
2. Fleischmann C, Thomas-Rueddel DO, Hartmann M, et al. Hospital Incidence and Mortality Rates of Sepsis. Dtsch Arztebl Int 2016;113:159-66.
3. Kampmeier TG, Ertmer C, Rehberg S. Translational research in sepsis - an ultimate challenge? Exp Transl Stroke Med 2011;3:14.
4. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013;39:165-228.
5. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med 2017;43:304-77.
6. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589-96.
7. Weiss SL, Fitzgerald JC, Balamuth F, et al. Delayed antimicrobial therapy increases mortality and organ dysfunction duration in pediatric sepsis. Crit Care Med 2014;42:2409-17.
8. Puskarich MA, Trzeciak S, Shapiro NI, et al. Association between timing of antibiotic administration and mortality from septic shock in patients treated with a quantitative resuscitation protocol. Crit Care Med 2011;39:2066-71.
9. Seymour CW, Gesten F, Prescott HC, et al. Time to Treatment and Mortality during Mandated Emergency Care for Sepsis. N Engl J Med 2017;376:2235-44.
10. Byrne L, Van Haren F. Fluid resuscitation in human sepsis: Time to rewrite history? Ann Intensive Care 2017;7:4.
11. Weibel S, Jokinen J, Pace NL, et al. Efficacy and safety of intravenous lidocaine for postoperative analgesia and recovery after surgery: a systematic review with trial sequential analysis. Br J Anaesth 2016;116:770-83.
12. Kampmeier T, Arnemann P, Heßler M, et al. Provision of physiologic data and reference values in awake and anaesthetized female sheep aged 6-12 months. Vet Anaesth Analg 2017;44:518-28.
13. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315:801-10.
14. Andreis DT, Singer M. Catecholamines for inflammatory shock: a Jekyll-and-Hyde conundrum. Intensive Care Med 2016;42:1387-97.
15. Yang S, Cioffi WG, Bland KI, et al. Differential alterations in systemic and regional oxygen delivery and consumption during the early and late stages of sepsis. J Trauma 1999;47:706-12.
16. Prins JM, van Agtmael MA, Kuijper EJ, et al. Antibiotic-induced endotoxin release in patients with gram-negative urosepsis: a double-blind study comparing imipenem and ceftazidime. J Infect Dis 1995;172:886-91.
17. Lepper PM, Held TK, Schneider EM, et al. Clinical implications of antibiotic-induced endotoxin release in septic shock. Intensive Care Med 2002;28:824-33.
18. Crosby HA, Bion JF, Penn CW, et al. Antibiotic-induced

release of endotoxin from bacteria in vitro. J Med Microbiol 1994;40:23-30.
19. Hurley JC. Antibiotic-induced release of endotoxin: a reappraisal. Clin Infect Dis 1992;15:840-54.
20. Fekade D, Knox K, Hussein K, et al. Prevention of Jarisch-Herxheimer reactions by treatment with antibodies against tumor necrosis factor alpha. N Engl J Med 1996;335:311-5.
21. Amir A, Saulters KJ, Muhindo R, et al. Outcomes of patients with severe infection in Uganda according to adherence to the World Health Organization's Integrated Management of Adolescent and Adult Illness fluid resuscitation guidelines. J Crit Care 2017;41:24-8.
22. Hjortrup PB, Haase N, Bundgaard H, et al. Restricting volumes of resuscitation fluid in adults with septic shock after initial management: the CLASSIC randomised, parallel-group, multicentre feasibility trial. Intensive Care Med 2016;42:1695-705.
23. Poli-de-Figueiredo LF, Garrido AG, Nakagawa N, et al. Experimental models of sepsis and their clinical relevance. Shock 2008;30 Suppl 1:53-9.

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Supplementary

Table S1 Hemodynamics, metabolic and organ function of the sham animals and the sheep with fecal peritonitis (septic animals) at shock time

Variable	Sham animals at shock time (n=4)	Septic animals at shock time (n=16)	P (sham *vs.* septic animals at shock time)
MAP (mmHg)	81 [66, 85]	42 [36, 55]	0.892
HR ($1 \cdot min^{-1}$)	75 [70, 78]	86 [75, 95]	0.122
CI ($L \cdot min^{-1} \cdot m^{-2}$)	3.8 (3.1, 4.8)	2.5 (2.2, 2.6)	0.007*
GEDI ($mL \cdot m^{-2}$)	736 [650, 970]	574 [513, 646]	0.029*
CVP (mmHg)	0.27 [1, 4]	0 [0, 3]	0.554
SVI ($mL \cdot m^{-2}$)	49 [42, 62]	29 [26, 34]	0.003*
SVV (%)	18 [7, 23]	10 [6, 12]	0.099
EVLWI ($mL \cdot kg^{-1}$)	11 [9, 15]	13.6 [12, 18]	0.148
Hb ($g \cdot L^{-1}$)	0.68 (0.56, 0.84)	1.07 (0.93, 1.20)	0.003*
Lactate ($mmol \cdot L^{-1}$)	0.7 (0.6, 0.9)	1.9 (1.8, 2.0)	<0.001*
pH_a [$-log_{10}c(H^+)$]	7.47 (7.46, 7.54)	7.40 (7.38, 7.45)	0.011*
BE ($mmol \cdot L^{-1}$)	9.2 (7.8, 13.2)	6.3 (1.1, 7.4)	0.011*
DO_2I ($mL \cdot min^{-1} \cdot m^{-2}$)	331 [276, 444]	318 [303, 397]	0.810
O_2-ER (%)	14 [10, 23]	37 [28, 41]	0.002*
VO_2I ($mL \cdot min^{-1} \cdot m^{-2}$)	59 [37, 68]	110 [104, 130]	0.001*
$S_{cv}O_2$ (%)	83 [79, 87]	65 [60, 70]	0.001*
Creatinine ($\mu mol \cdot L^{-1}$)	70.4 (61.6, 70.4)	132 (96.8, 158.4)	<0.001*
Diuresis ($mL \cdot kg^{-1} \cdot h^{-1}$)	0.5 (0.4, 1.0)	0.2 (0.0, 0.3)	0.014*
Crea-Clearance ($mL \cdot min^{-1} \cdot m^{-2}$)	69 [45, 87]	13 [2, 27]	0.011*
Bilirubin ($\mu mol \cdot L^{-1}$)	1.36 (0.85, 1.7)	0.85 (0.85, 0.85)	0.437
Temperature (°C)	39.0 (38.2, 40.3)	40.5 (39.4, 41.2)	0.052

Data are presented as median (interquartile range). *, indicates a statistically significant difference (P<0.05). BE, base excess; Bilirubin, serum bilirubin concentration; CI, cardiac index; Crea-Clearance, creatinine clearance; Creatinine, serum creatinine concentration; DO_2I, oxygen delivery index; EVLWI, extravascular lung water index; HR, heart rate; CVP, central venous pressure; GEDI, global end-diastolic index; Hb, hemoglobin; MAP, mean arterial pressure; O_2-ER, oxygen extraction rate; pH_a, arterial potential hydrogenii; $ScvO_2$, central venous oxygen saturation; SVI, stroke volume index; SVV, stroke volume variation; VO_2I, oxygen consumption index.

Table S2 Relative organ weights of the animals (related to baseline bodyweight)

Variable (unit)	Group	Heart	Right kidney	Left kidney	Right lung	Left lung	Ileum
Relative organ weight ($g \cdot kg^{-1}$)	Sham	4.6 (4.4, 5.0)	1.6 (1.5, 1.9)	1.5 (1.5, 1.6)	4.8# (4.1, 5.6)	3.8* (3.7, 4.2)	0.5 (0.5, 0.6)
	Control	4.8 (4.6, 5.3)	1.8 (1.5, 2.0)	1.9 (1.5, 2.0)	7.0 (6.8, 7.3)	5.1 (4.7, 5.4)	0.3 (0.1, 0.4)
	Causal	5.3 (4.6, 6.2)	1.9 (1.6, 2.1)	1.8 (1.6, 2.1)	6.8 (6.5, 7.6)	5.7 (4.8, 6.3)	0.6 (0.4, 0.8)
	Combined	5.4 (5.1, 6.4)	1.8 (1.5, 1.8)	1.8 (1.7, 2.0)	6.2 (6.0: 6.8)	4.9 (4.5, 5.3)	0.5 (0.5, 0.7)

Values are presented as median (interquartile range). Kruskal-Wallis test was used for comparison between groups. Post-hoc comparisons were conducted using Dunn's test: *, Sham versus Causal $P<0.05$, #, Sham versus Control $P<0.05$.

Table S3 Fluid balance of the study groups

Group	Fluid input (mL)	Urinary output (mL)	Fluid balance (mL)
Sham	688 (646 to 704)	260 (167 to 347)	428 (358 to 489)
Control	605 (476 to 640)	25 (16 to 35)	580 (460 to 622)
Causal therapy	547 (474 to 574)	15 (6 to 23)	532 (465 to 555)
Combined therapy	6,123 (4,677 to 8,438)	386 (200 to 486)	5,737 (4,414 to 7,945)

Fluid input contained basal fluid requirements (2 $mL \cdot kg^{-1} \cdot h^{-1}$ in all groups) and study fluid (only combined therapy group). Data are presented as median (interquartile range).