Temporal changes in tissue cardiorespiratory function during faecal peritonitis

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Abstract

**Purpose:** Sepsis affects both macro- and microcirculatory transport of oxygen to tissues causing regional hypoxia. However, this relationship is poorly characterized with respect to inter-organ variability, disease severity, and the evolution to organ dysfunction. We hypothesized that an early circulatory insult precedes the development of organ dysfunction, and is more severe in predicted non-survivors. Consequently, we assessed temporal changes in myocardial function and regional tissue oxygenation in peripheral and deep organs in a rat model of faecal peritonitis. We also examined the utility of a dynamic oxygen challenge test to assess the microcirculatory.

**Methods:** Awake, tethered, fluid-resuscitated male Wistar rats were randomized to receive intraperitoneal injection of faecal slurry, or to act as controls. At either 6 or 24h post-insult, rats were anaesthetized and underwent echocardiography, arterial cannulation and placement of tissue oxygen probes in peripheral (muscle, bladder) and deep (liver and renal cortex) organ beds. Measurements were repeated during fluid loading and an oxygen challenge test (administration of high oxygen concentrations).

**Results:** Early sepsis (6h) was characterized by a fall in global oxygen delivery with concurrent decreases in muscle, renal cortical and, especially, liver tissue PO$_2$. By contrast, during established sepsis (24h), myocardial and circulatory function had largely recovered despite increasing clinical unwellness, hyperlactataemia and biochemical evidence of organ failure. O$_2$ challenge revealed an early depression of response that, by 24h, had improved in all organ beds bar the kidney.

**Conclusions:** This long-term septic model exhibited an early decline in tissue oxygenation, the degree of which related to predicted mortality. Clinical and biochemical deterioration however progressed despite cardiovascular recovery. Early circulatory dysfunction may thus be an important trigger for downstream processes that result in multi-organ failure. Furthermore, the utility of tissue PO$_2$ monitoring to highlight the local oxygen supply-demand balance, and dynamic O$_2$ challenge testing to assess microcirculatory function merit further investigation.

**Key words:** sepsis, tissue oxygen tension, microcirculation, oxygen challenge test, echocardiography.
Introduction

Sepsis affects oxygen delivery to tissues through cardiac, macro- and microcirculatory alterations [1, 2], and consumption through effects on mitochondrial respiration [3]. The prognostic importance of early restoration of an adequate circulation was highlighted by Rivers et al [4], in contradistinction to achieving haemodynamic optimization in patients in established organ failure [5, 6]. Consequently, an early circulatory insult appears to precede the development of overt organ dysfunction. Using an established, fluid-resuscitated, faecal peritonitis model we recently found that low stroke volumes at 6h were highly predictive of poor outcome (Rudiger et al, submitted) [7]. This model therefore allows comparison of temporal changes in circulatory and tissue oxygenation variables in relation to sepsis severity, and to assess inter-organ differences. This is important when considering the use of peripheral/accessibe organs as surrogates for changes occurring in deep, vital organs.

We previously reported that monitoring of tissue (interstitial) oxygen tension assesses the local oxygen supply-demand balance in different organ beds [8]. While falls in tissue PO$_2$ are recorded in low flow states (e.g. haemorrhage) [9], normal/elevated values are reported in fluid-resuscitated sepsis; patients [10-12]/animal models [13,14]. As most oxygen consumed is utilized by mitochondria for ATP production, a raised tissue PO$_2$ implies a reduction in local tissue oxygen consumption relative to supply, potentially due to dysfunction of the electron transport chain and/or reduced metabolic demand for energy [15]. By contrast, during early sepsis, macrocirculatory (hypovolaemia secondary to capillary leak and vascular inflammation) and microcirculatory abnormalities likely lead to regional hypoxia and an oxygen supply-demand imbalance. The importance in relation to subsequent organ failure remains to be established. Consequently, we hypothesized that an early circulatory insult precedes the development of organ dysfunction, and is more severe in predicted non-survivors.

We report temporal changes in systemic and haemodynamics and regional tissue oxygenation in this long-term model of fluid-resuscitated sepsis, and the relationship to physiological and biochemical organ dysfunction. We also examined inter-organ differences and the impact of increasing concentrations of inspired oxygen on tissue PO$_2$. This dynamic challenge may have utility as a simple bedside tool to monitor microcirculatory function.
Methods

Male Wistar rats were used throughout. Pre-study, housed in cages of six on a 12–12h light–dark cycle. All experiments followed local ethics committee approval and government licences under the 1986 Scientific Procedures Act.

Spontaneously breathing animals (37°C), anaesthetized by 5% isoflurane (reduced to 2%), received 0.96mm diameter PVC tubing inserted into the right internal jugular vein, tunnelled subcutaneously to the nape of the neck and attached to a tether system; the animal recovers from anaesthesia and freely moves around its cage with access to food and water. 24h later, sepsis was induced by intraperitoneal administration of faecal slurry [16]. Sham-operated animals were instrumented as above but received no intraperitoneal injection to prevent accidental bowel perforation.

The experimental design is depicted in supplementary Fig-1. At 2h post-induction of sepsis (and in sham-operated controls), fluid resuscitation (10ml/kg/h infusion of 6% hydroxyethylstarch (Fresenius Kabi, Warrington, UK) and 5% glucose (Baxter Healthcare, Thetford, UK) at a 1:1 ratio) was started and continued throughout.

At 6h post-sepsis, observational clinical scoring was performed [16] (Table-1). Under 1.2% isoflurane transthoracic echocardiography was performed (14MHz probe/0-2cm depth; Vivid 7 Dimension, GE Healthcare, Bedford, UK). Echocardiography is validated as a simple, non-invasive measure of myocardial function in rats [17,18] and mice [19]. Intra- and inter-operator variability between AD and AR was <10% (supplementary data Tables-1 and -2). End-diastolic volume (EDV) was calculated using the formula for a prolate spheroid: \( v = \frac{4}{3} \pi a^2 b \), where \( v \) is volume, and \( a \) and \( b \) represent half the internal dimensions of the ventricle. Aortic blood flow velocities were determined in the aortic arch using pulsed-wave Doppler. Stroke volume was determined as the product of velocity time integral (VTI) and vessel cross-sectional area \( (\pi x [0.5 \times \text{diameter}]^2) \). Rats of this age have an aortic diameter of 0.26 cm [17]; a cross sectional area of \( (0.13)^2 \times \pi \) was assumed for all animals studied. Heart rate was determined by measuring the time between six consecutive cardiac cycles. Cardiac output was calculated as the product of stroke volume and heart rate.
**Study 1**

After echocardiography at 6h, a midline laparotomy was performed and the bladder cannulated using 1.57mm-ED polythene tubing (Portex Ltd, Hythe, UK), inserted through a small apical incision. Large area surface (LAS)™ oxygen-sensing optodes (0.7mm diameter; 8mm² in contact with tissue; Oxford Optronix, Oxford, UK) were placed into thigh muscle, bladder, liver and renal cortex for continuous monitoring of tissue PO$_2$ (tPO$_2$) [8, 9]. The sensor comprises a ‘tonometer sleeve’ that averages spatial micro-environmental tPO$_2$ fluctuations, making it less sensitive to movement, placement and heterogeneity of nutrient microvessels. Probes are pre-calibrated within the range 0-26.7kPa and temperature-correct automatically. Animals were allowed to stabilize for at least 30 minutes before baseline physiological variables were collected. Arterial blood gas analysis (ABL-625 Analyzer, Radiometer, Copenhagen, Denmark) measured arterial base deficit and pH, lactate, PO$_2$ and PCO$_2$, and allowed calculation of global oxygen delivery.

Intravascular volume optimization was achieved by administering a 25ml/kg bolus of 6% hydroxyethyl starch. This volume was derived from pilot studies demonstrating that septic animals remained hypovolaemic at this timepoint despite the aggressive background (10 ml/kg/h) infusion regimen. Following circulatory filling and another stabilization period (15 mins), the FiO$_2$ was progressively increased (0.3, 0.6, 1.0) at 15-minute intervals. This ‘oxygen challenge test’ examines the ability of a filled circulatory system to transport high partial pressures of oxygen to tissues [20, 21]. The increment in tissue PO$_2$ was measured in each organ following each successive rise in FiO$_2$.

Echocardiography and arterial blood gas analysis were performed following each intervention. Prior to sacrifice, whole arterial blood (1.5ml) was removed and centrifuged to separate the serum fraction; this was frozen in liquid N$_2$ and subsequently assessed for biochemical markers of organ dysfunction.

**Study 2**

In separate experiments, animals underwent instrumentation, induction of sepsis (or not, for controls) and, at 6h, echocardiography to enable separation into good and poor prognosis groups, followed by fluid loading (25ml/kg). Animals were returned to their cages, with background fluid infusion continued. At 24h post-sepsis they were re-anaesthetized, instrumented as described above, and received a further bolus of 10ml/kg
hydroxyethylstarch solution to ensure intravascular optimization. As per Study 1, animals were then exposed to
increases in inspired oxygen, echocardiography and blood gas analyses, and terminal blood sampling

Markers of organ function were analysed using non-parametric Kruskal-Wallis testing followed by Dunn’s test
for multiple comparisons, and expressed as median ± range. All other raw and calculated data are presented as
mean ± standard error (n=6 for sham, n=8 for sepsis). We used eight septic animals per group to account for the
expected increase in variability compared with sham-operated animals. As some septic animals died before 24h,
additional animals were studied until the group size of eight was achieved. Statistics on parametric data 2-way
ANOVA followed by Tukey’s test for post-hoc comparisons. All data were analysed using GraphPad Prism 5.0
(GraphPad Software, San Diego, CA, USA). Probability values <0.05 were considered statistically significant.

Results

All sham-operated controls (body weight; 318±8g) survived until the end of each 6h or 24h experiment. Of the
38 animals subjected to faecal peritonitis (body weight; 322±3g), 17 were classified as good prognosis (45%)
and 21 poor prognosis (55%), based on a stroke volume cut-off of 0.14ml. All septic animals used for 6h
experiments (early sepsis) survived until that timepoint. Because of premature deaths, 9 good-prognosis and 13
poor-prognosis sepsis animals were instrumented for 24h studies (established sepsis) to achieve eight/group.
Thus, 24h mortality was 11% (1/9) and 38% (5/13) in the good and prognosis groups, respectively.

All instrumented animals recovered fully prior to injection of faecal slurry. Sham-operated animals remained
normal/active throughout. Faecal peritonitis produced a hunched appearance, piloerection, reduced
movement/alertness. At the 6h timepoint clinical scoring couldn’t distinguish between animals stratified
echocardiographically into high- and low-mortality prognostic groups. Clinical severity was pronounced at 24h
in poor prognosis animals (Fig-1; clinical scoring).

Sepsis caused a severity-dependent decrease in blood pressure at 6h and 24h, albeit not significantly, while core
temperature initially increased in severely affected animals (p<0.05 vs sham, 6h) but fell at 24h (Fig-1). Despite
the fluid resuscitation regimen commencing at 2h, septic animals showed haemoconcentration at both
timepoints while percent oxyhaemoglobin levels, arterial pH and PCO₂ were similar in all groups
Arterial base deficit and lactate levels were deranged in poor-prognosis sepsis animals at 6h and in all septic animals at 24h (p<0.05).

Myocardial function and global oxygen delivery

Despite the high volume infusion rate commenced at 2h, baseline (B; pre-fluid bolus) stroke volume, end-diastolic volume and cardiac output showed severity-dependent decreases at 6h (p<0.05 vs sham). At 24h post-sepsis, myocardial function was still perturbed in poor-prognosis animals (Fig-2, p<0.05 vs sham). Fluid loading (F; Fig-2) produced significant increases in all values in both sham and septic animals, with restoration of haemodynamics in the septic animals.

Compared with controls, global oxygen delivery was reduced (p<0.05) in severely septic animals at 6h (Fig-3; baseline) but was restored by fluid loading. At 24h, baseline delivery was similar in all animals. Despite an increase in stroke volume after fluid loading in all study groups, no further improvement was seen in global oxygen delivery (Fig-3) due to a dilutional effect on haemoglobin.

Tissue oxygen tension

At 6h, sepsis caused severity-dependent decreases in muscle, renal cortical and particularly liver tPO$_2$ (p<0.05 for sham vs poor prognosis sepsis) in line with the reduction in global oxygen delivery, however bladder tPO$_2$ values were maintained (Fig-3). Despite the significant increase in global oxygen delivery with the fluid bolus, only liver tPO$_2$ rose significantly. By 24h, baseline oxygen delivery and tissue oxygen tensions in both sets of septic animals did not significantly differ from control (Fig-3), despite the major increase in illness severity. Tissue oxygen tensions were unaffected by fluid loading at 24h in all animals (Fig-3).

Oxygen challenge test

Following fluid loading, the FiO$_2$ was increased to test the ability of the filled circulatory system to transport higher partial pressures of oxygen to the tissues. At 6h, despite maintenance of global oxygen delivery, the increment in tPO$_2$ in the septic animals was blunted compared to sham-operated controls in a severity-dependent manner (Fig-4). Lower values were seen in the poor-prognosis group compared to sham-operated controls in muscle (p<0.05), liver (p<0.05), renal cortex (p<0.01) and bladder (p=0.07). At 24h the disparity in tPO$_2$
response to an oxygen challenge was less marked, except in the renal cortex that remained significantly lower in poor prognosis animals (supplementary data Fig-3).

**Biochemical markers of organ function**

At 24h, poor prognosis animals exhibited greater evidence of organ dysfunction with significant increases in urea and aspartate aminotransferase (AST). Decreased albumin levels in the poor prognosis group indicate increased capillary leak and/or decreased synthesis due to hepatic dysfunction (Table-2).

**Discussion**

Oxygen transport comprises a complex system of physiologic processes that are perturbed during sepsis, ranging from myocardial dysfunction [1] to macro- and microcirculatory abnormalities [2,22]. The resultant oxygen supply-demand imbalance could be an important trigger of downstream mechanisms that cause organ failure. Here, we demonstrate altered tissue oxygenation in various organ beds as the septic process evolves, from an early circulatory insult (as reflected by decreased tPO\(_2\) and responsiveness to oxygen challenge) to haemodynamic recovery at 24h despite increasing, severity-related, biochemical and clinical features of unwellness/organ dysfunction. Notably, at this later timepoint, baseline tPO\(_2\) measurements and oxygen challenge sensitivity had normalized in most beds studied.

Despite an aggressive background fluid regimen (from 2h) we found marked decreases in end-diastolic volume, stroke volume, cardiac output and global oxygen delivery at 6h. Though the macrocirculation could be restored with additional fluid loading (implying a significant role for hypovolaemia secondary to capillary leak), we still found significantly depressed left ventricular function in this model [7]. This is consistent with other studies documenting myocardial depression in sepsis in both animal models [23] and humans [24, 25]. By 24h, when sepsis had become established, haemodynamic function had improved, despite persisting hyperlactataemia and a marked increase in clinical severity.

Tissue oxygen tension measures oxygen partial pressures in the interstitial (extravascular) space. The proximity of this measurement (between the microcirculation and cells) supports the notion that it represents the local oxygen supply-demand balance (Table-3). Muscle tPO\(_2\) is elevated in fluid-resuscitated septic patients [10]
implying an availability of oxygen in excess of tissue requirements. Variable findings in tissue \( \text{PO}_2 \) are reported in septic animal models; increases in bladder and gut [13, 14, 26], and decreases in muscle, liver and kidney [27-31]. However, studies reporting low values generally utilised short-term endotoxin models and often without adequate fluid loading. It is noteworthy that tissue \( \text{PO}_2 \) can increase in both high and low cardiac output endotoxaemia [13]. In low cardiac output states, this implies that the fall in oxygen consumption exceeds the drop in delivery. Temporal changes in multiple organs in long-term models have not been previously assessed. During early sepsis we found organ-specific changes that varied both at baseline and in response to fluid challenge. For example, at 6h, baseline muscle and renal cortical \( \text{tPO}_2 \) fell in proportion to the 32% fall in global oxygen delivery, whereas bladder \( \text{tPO}_2 \) remained unchanged while liver \( \text{tPO}_2 \) showed a striking 84% reduction. We reported similar findings during acute endotoxaemia [8]. Potential explanations for this disparity in liver \( \text{tPO}_2 \) include intrahepatic flow redistribution [32], elevated hepatic metabolic activity (acute phase response) [33], and/or reductions in portal venous oxygen saturation [34]. Hepatocytes are particularly tolerant to the effects of hypoxia [35]; this may explain why liver \( \text{tPO}_2 \) may be severely compromised yet gross hepatic failure and necrosis are rarely seen in sepsis. The responses to fluid loading between organs were also variable at 6h. The renal cortex was least responsive, remaining unchanged despite restoration of global oxygen delivery. Of note, tissue oxygen tensions had normalised after 24h of sepsis despite the worsening clinical severity. In addition, no response to fluid was seen at this timepoint. Taken together, these results demonstrate a clear difference in the relationship between oxygen supply and demand that varies between organs, and changes over time. These data support the concept that systemic and regional haemodynamics are uncoupled; restoration of ‘normal’ macrocirculatory indices do not necessarily result in improvement at the end-organ level [36].

The microcirculation plays a crucial role in local distribution of oxygen and other nutrients to meet cellular metabolic demand [37]. Microcirculatory dysfunction in early sepsis is considered to play a prominent pathophysiologic role [38, 39]. In pilot studies we tried to image the microcirculation using sidestream dark field imaging however the marked inter-organ variation in microvascular architecture has so far precluded accurate quantitation of changes in microcirculatory flow. We postulated that the ‘oxygen challenge’ test (measuring downstream increases in \( \text{tPO}_2 \) following an increase in \( \text{PaO}_2 \)) may offer a further, dynamic approach to assess the microcirculation. Here, the rise in \( \text{tPO}_2 \) could be quantified in all organs, irrespective of differences in microcirculatory architecture. Rather than increasing mass oxygen transport (except for cases of hypoxaemia), the oxygen challenge test assesses the ability of the circulation to transport high partial pressures
of oxygen to tissues. Under healthy conditions, peripheral tPO$_2$ changes directly with FiO$_2$ and PaO$_2$ [40] but during shock states the tPO$_2$ response to an increased PaO$_2$ is reduced [40,41]. In low flow states (haemorrhage, heart failure) the macrocirculatory inability to transport oxygen will be a major contributor to a reduced tPO$_2$ response. However, in normovolaemic sepsis where global oxygen delivery is maintained, a reduced tPO$_2$ response is highly indicative of microcirculatory dysfunction. Oxygen challenge tests have been performed in septic patients in peripheral organ beds [20,21]; those with a higher incremental tPO$_2$ had less organ failures and lower mortality rates [20]. In the present study, a severity-dependent reduction in the tPO$_2$ increment was observed in all organ beds during early sepsis, despite restoration of global oxygen delivery. This suggests, at least during early sepsis, that microcirculatory dysfunction is a ubiquitous phenomenon and supports the concept that changes in peripheral organs can act as a surrogate for those that occur in deeper, vital organs. Notably, by 24h this deficit had resolved in all organ beds with the exception of the renal cortex. This suggests ongoing microcirculatory dysfunction or, possibly, increased arterio-venous shunting as a protective response to decrease glomerular blood flow in this particular organ [42].

The restoration of tissue oxygenation variables at 24h, despite worsening clinical severity and increasing biochemical dysfunction, implies activation of downstream processes that result in organ failure. We have previously shown that this occurs in the absence of significant cell death and histological damage [16]. These data, in combination with those presented here, are consistent with an important role for both microcirculatory and mitochondrial dysfunction (and/or a decrease in cellular metabolism) in the pathogenesis of organ failure [3,15,43]. Inc described a microcirculatory and mitochondrial distress syndrome whereby a reduction in oxygen supply during early sepsis, as witnessed in this study, plus direct effects on mitochondrial respiration could trigger a reduction in cellular oxygen demand [43]. The results obtained here support this concept.

**Limitations**

A limitation of our oxygen challenge relates to the type of oxygen probe used in this study. Fluorescence lifetimes used to calculate oxygen tension are inversely proportional to the local PO$_2$. This enables greatest accuracy at longer fluorescent lifetimes and is best suited to lower oxygen tensions that lie within the normal physiological range (0-8kPa). However, in preliminary experiments, we performed an *in vitro* calibration (0-26.7kPa) and found a bias of 1kPa (<5%) when compared with a gold-standard Clark-type electrode (not shown).
An important distinction needs to be made between a newly presenting, unresuscitated, untreated septic patient, and a patient receiving ongoing critical care (fluids, antibiotics, pressors, mechanical supports etc). We used fixed fluid resuscitation regimens rather than individual titration. However, despite large volumes being continually infused, a low stroke volume during early sepsis and the positive response to a fluid challenge does indicate hypovolaemia. Consequently, we were able to replicate a clinical scenario, at least in terms of insult and fluid resuscitation with some delay in between (4h in this case), but not the full spectrum of support offered to the intensive care patient.

Summary
In this long-term model of fluid-resuscitated faecal peritonitis there were marked temporal changes in tissue oxygenation that were severity-related and varied between organs. The diversity of change in tPO$_2$ in various organ beds suggests that some tissues are particularly sensitive to local changes in oxygen supply and demand, and/or adapt to specific insults. The early fall in tissue oxygenation was associated with both macro- and microcirculatory impairment, the latter persisting despite restoration of the macrocirculation, as indicated by a diminished tissue PO$_2$ response to an oxygen challenge. However, by 24h, this impairment had largely recovered yet the predicted poor prognosis animals were now manifesting clinical and biochemical signs of organ dysfunction, suggesting cellular abnormalities were coming to force at this later stage. Although ‘static’ tissue PO$_2$ values differ between organ beds (reflecting their individual oxygen supply-demand balance), the similar response between peripheral and deeper organs to a dynamic oxygen challenge test does imply some degree of commonality that merits further investigation as a potential clinical tool for assessing regional tissue oxygenation in sepsis.

Acknowledgements
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References


Legend for Figures

Figure 1. Appearance, vital signs and biochemical markers of organ hypoperfusion.
*p<0.05 vs sham, †p<0.05 between good and poor prognosis sepsis.

Figure 2. Temporal changes in myocardial function and the impact of fluid loading.
B; baseline measurements, F; following fluid challenge. *p<0.05 vs sham-operated controls, †p<0.05 between good and poor prognosis sepsis, #p<0.05 comparing each group before and after fluid loading.

Figure 3. The effects of fluid loading on global $O_2$ delivery and tissue oxygen tensions during early (6h) and established (24h) sepsis.
B; baseline measurements, F; following fluid challenge. *p<0.05 vs sham-operated controls, #p<0.05 comparing each group before and after fluid loading.

Figure 4. Relationship between $PaO_2$ and tissue $PO_2$ (tPO$_2$) with increases in inspired oxygen concentration (oxygen challenge test) during early (6h) sepsis.
*p<0.05 vs sham-operated controls.
Supplementary Figure 1. Experimental protocol.

Fluid infusion: 10 ml/kg/h infusion of 1:1 ratio of 6% hydroxyethyl starch and 5% glucose in normal saline. FB:
Fluid bolus, 6h bolus; 25 ml/kg, 24h bolus; 10 ml/kg of hydroxyethyl starch. Thereafter, the fraction of inspired
oxygen (FiO\textsubscript{2}) was changed at 15 minute intervals. Instrumentation, stabilization, fluid loading and oxygen
challenge took approximately 2 hours.

Supplementary Figure 2: Arterial blood gas analysis.

The data shown are baseline measurements at each timepoint, i.e. before fluid loading.

PaO\textsubscript{2} and PaCO\textsubscript{2} represent the arterial partial pressures of O\textsubscript{2} and CO\textsubscript{2}; SaO\textsubscript{2}, percent haemoglobin saturation.

*p<0.05 vs sham-operated controls.

Supplementary Figure 3: Relationship between PaO\textsubscript{2} and tPO\textsubscript{2} (tPO\textsubscript{2}) with increases in inspired oxygen
concentration (oxygen challenge test) during established (24h) sepsis.

*p<0.05 vs sham-operated controls.
### Tables

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Scoring range</th>
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<td>Hunched</td>
<td>0-1</td>
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<tr>
<td>Bloated</td>
<td>0-1</td>
</tr>
<tr>
<td>Conjunctival injection</td>
<td>0-1</td>
</tr>
<tr>
<td>Piloerection</td>
<td>0-1</td>
</tr>
<tr>
<td>Lack of movement</td>
<td>0-2</td>
</tr>
<tr>
<td>Lack of alertness</td>
<td>0-2</td>
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Table 1. Clinical scoring characteristics.

Scoring denotes absence (0), presence (1), or where appropriate, marked presence (2).

<table>
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<tr>
<th>Sham-operated control</th>
<th>Good prognosis sepsis</th>
<th>Poor prognosis sepsis</th>
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<tbody>
<tr>
<td>Urea (mmol/l)</td>
<td>3.7 (2.9-7.3)</td>
<td>4.5 (2.7-7.8)</td>
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<tr>
<td>Creatinine (µmol/l)</td>
<td>31 (26-41)</td>
<td>33 (26-41)</td>
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<tr>
<td>AST (IU/l)</td>
<td>71 (4-100)</td>
<td>104 (51-195)</td>
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<tr>
<td>Albumin (g/l)</td>
<td>17 (13-24)</td>
<td>16 (13-24)</td>
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<tr>
<td>Arterial K⁺ (mmol/l)</td>
<td>3.7 (2.9-4.0)</td>
<td>3.8 (3.3-3.9)</td>
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Table 2. Markers of organ function during established sepsis (24h).

Data expressed as median and range. AST; aspartate aminotransferase. *p<0.05 vs sham-operated controls.
<table>
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<tr>
<th>Local Oxygen delivery</th>
<th>Local Oxygen Consumption</th>
<th>Tissue Oxygen Tension</th>
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<tr>
<td>↓</td>
<td>↔</td>
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Table 3. Relationship between local oxygen delivery and consumption, and tissue oxygen tension

↑↑, marked increase; ↓↓, marked decrease; ↔, no change.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intra-observer variability (%)</th>
<th>Inter-observer variability (%)</th>
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<tr>
<td>Heart rate (per min)</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.1</td>
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<tr>
<td>End diastolic volume (ml)</td>
<td>4.5 ± 1.2</td>
<td>7.7 ± 1.7</td>
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<tr>
<td>Stroke volume (ml)</td>
<td>2.1 ± 0.4</td>
<td>3.6 ± 0.7</td>
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<tr>
<td>Cardiac output (ml/min)</td>
<td>4.0 ± 1.1</td>
<td>8.1 ± 2.1</td>
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Supplementary Table 1. Intra- and inter-observer variability of echocardiography-derived measurements in naive animals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intra-observer variability (%)</th>
<th>Inter-observer variability (%)</th>
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<tbody>
<tr>
<td>Heart rate (per min)</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.2</td>
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<tr>
<td>End diastolic volume (ml)</td>
<td>8.1 ± 1.5</td>
<td>8.9 ± 2.0</td>
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<tr>
<td>Stroke volume (ml)</td>
<td>7.8 ± 1.6</td>
<td>6.4 ± 1.1</td>
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<tr>
<td>Cardiac output (ml/min)</td>
<td>7.5 ± 1.5</td>
<td>5.9 ± 1.1</td>
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Supplementary Table 2. Intra- and inter-observer variability of echocardiography-derived measurements in severely septic (poor prognosis) animals (6h post-insult).
Clinical Scoring

Blood Pressure

Core Temperature

Base Deficit

Arterial Lactate

Sham-operated control
Good prognosis sepsis
Poor prognosis sepsis

246x153mm (600 x 600 DPI)
For Peer Review

End Diastolic Volume

(ml)

Stroke Volume

(ml)

Heart Rate

(per/min)

Cardiac Output

(ml/min)

B  F  B  F

6h  24h

6h  24h

Sham-operated control  Good prognosis sepsis  Poor prognosis sepsis

203x179mm (600 x 600 DPI)
Study 1

- Venous line insertion
- Arterial line insertion, tracheostomy, tPO$_2$ probe insertion
- Fluid infusion
- Oxygen challenge test

Study 2

- Venous line insertion
- Arterial line insertion, tracheostomy, tPO$_2$ probe insertion
- Fluid infusion
- Oxygen challenge test

Time (hours)

FiO$_2$

- 0.21
- 0.3
- 0.6
- 1.0
- 0.21

Instrumentation

- Venous line insertion
- Arterial line insertion, tracheostomy, tPO$_2$ probe insertion

- 25 ml/kg
- 10 ml/kg
242x153mm (600 x 600 DPI)