Spring 5-1-2011

Pathophysiology and Treatment of Septic and Traumatic Shock

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Pathophysiology and Treatment of Septic and Traumatic Shock

A Capstone Project Submitted in Partial Fulfillment of the Requirements of the Renée Crown University Honors Program at Syracuse University

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May 2011

Honors Capstone Project in Biology

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Date: ____________________
ABSTRACT

**Background.** While various medical treatments have been proven effective in the treatment of sepsis in animal models, the only current clinically accepted treatment of human sepsis is limited to the use of activated protein C. The complex pathogenesis of human sepsis presents a challenge in precisely duplicating the process of disease development in the particular animal models which are currently employed for preclinical testing. Further research examining the pathogenesis of this deadly condition is essential for the implementation of novel therapies that target distinct disease mechanisms. The objective of this 48-hour study is to utilize a clinical model that accurately replicates severe human sepsis along with gut ischemia/reperfusion (I/R). I/R further leads to the injury of multiple organs within the time period most closely mirroring disease progression in humans.

**Methodology.** The experimental protocol was approved by the Committee for the Humane Use of Animals at SUNY Upstate Medical University and complied with the National Institutes of Health Guidelines for the Use of Experimental Animals in Research. Five pigs were subjected to a “two-hit” injury involving the clamping of the superior mesenteric artery (SMA) for 30 minutes as well as a laparotomy used for insertion of a fecal clot, following appropriate administration of anesthetics and ventilation. A drain was inserted into this laparotomy wound twelve hours post injury. Monitoring of animals took place under a standard Intensive Care Unit setting over the course of 48 hours, with oxygen desaturation resolved by increasing FiO2. Hemodynamics were stabilized through administration of antibiotics and intravenous fluids as needed, while measurements of arterial and mixed venous blood gases as well as lung, kidney, liver, renal, and hemodynamic function measurements were recorded. Progression of the abdominal compartment syndrome was determined by monitoring bladder pressure changes. Serial measurements of peritoneal and plasma ascites were also taken for evaluation of cytokine concentration. Morphometric analysis was carried out using the organ tissues harvested and fixed at necropsy.

**Results.** All animals presented with polymicrobial sepsis. Over the course of 48 hours the lung, liver, kidney, and intestine showed ongoing deterioration and histopathological as well as clinical damage. This was found to occur in conjunction with increased levels of cytokines within the peritoneal fluid and serum.

**Conclusion.** In combining both sepsis and ischemia reperfusion injury, the animal model used in this study is valid for uncovering the intricate pathophysiological progression of septic shock and its transition to multiple organ dysfunction syndrome. This system mirrors the systemic inflammation and major organ systems dysfunction seen in humans. Through demonstrating the success of the current animal model,
prospective treatments may be developed through conducting sophisticated preclinical trials.
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ACKNOWLEDGEMENTS

I would like to thank Gary Nieman for guiding me through the research and learning process within the laboratory and for serving as an outstanding mentor by exposing me to both the challenging and rewarding aspects of scientific research. Thank you to Kathleen Snyder for teaching me the logistics of data collection throughout experiments, and also to medical resident, Shreyas Roy, and laboratory technician, Christopher Vieau, who played a key role in physically carrying out each surgical procedure. Histologist, Louis Gatto, as well as the numerous medical students and undergraduate students with whom I worked together to monitor all animals throughout experiments, and to subsequently collect and comprehensively analyze all of the data parameters provided essential assistance to me in my research. Dr. Fondy’s help in revising and evaluating my research work is very much appreciated. Many thanks to Dr. Belote and Dr. Segraves in the Biology Department at Syracuse University for providing me with the opportunity to present my research in front of an audience and in turn to receive useful feedback in order to strengthen my research.
INTRODUCTION

As the leading cause of hospitalized adult patients in the intensive care unit, 1.8 million cases of sepsis have been reported annually with nearly 2,000 deaths daily resulting from sepsis [1]. Human sepsis is typically caused by a site of infection containing replicating bacteria that persist for an extensive period of time. It is a syndrome that usually evolves in a continuum from systemic inflammatory response syndrome (SIRS) to sepsis combined with organ dysfunction, and to septic shock. As the severity of the disease increases, the frequency rate of positive blood cultures increases (sepsis [17%], severe sepsis [25%], septic shock [69%], suggesting that bacterial invasion can predict the expected disease outcome and mortality risk [2]. The mortality rate of these three stages is 25%, 30%, and 40-70%, respectively [3].

Animal studies are not easy to extrapolate to true clinical settings due to cross-species differences and variations in experimental design. Septic spread can be attributed to a series of causes including the state of activation of the target cell, the presence of nearby mediators, and the ability of the target cell to release other mediators. Animal studies have shed light on how such processes occur by analyzing apoptosis, fibrinolysis, organ and hormone function, tissue perfusion, and coagulation in animal models. Pathogens interact with the host immune response throughout the progression of the disease, leading from inflammation to immunosuppression via numerous mediators, such as
tumor necrosis factor α (TNF-α), interleukins, platelet activating factor, leukotrienes, thromboxane A₂, and activators of the complement cascade. Also important is the negative feedback of various mediators along with interleukin-4 and interleukin-8, which act as natural inflammatory suppressors. This shows that disease progression depends on the proper coordination and function of not only up-regulatory systemic factors, but also of down-regulatory that can be essential in active protection against infection.

Currently, protocolized management in the form of early goal oriented therapy is used in addressing preventative and early onset treatment of sepsis. Exogenous administration of activated protein C is used to support severely septic patients. Only experiments with relevant subjects, can develop the monitoring, diagnostic, and ventilation modalities that can lead to improved treatment of sepsis in eluding more effective patient support. Animal studies generally allow an opportunity for intervention even in the early stages of sepsis, when inflammatory cytokine levels continue to increase even though organ damage and vascular leakage are still minimal. In humans however, most treatments are started at a time when organ damage is already present, when many patients have activated a deleterious anti-inflammatory response following the protective proinflammatory response. Because proactive fluid therapy is rarely used in animal models, the effect of ventilators and vasopressors on a drug response is difficult to evaluate. In human sepsis, ubiquitous
mixed infections containing both Gram-negative and Gram-positive bacteria predominately with antibiotic treatment remaining only partially effective, unlike in animal models where the appropriate antibiotic may be selected to control bacterial proliferation [4].

For the aforesaid reasons, a novel model of clinical relevance was implemented, emulating early sepsis progression through gradual organ function degradation and chronic pathogenesis evidenced by classic histopathologic abnormalities of sepsis observed in the liver, intestine, and kidney. This was made possible by conducting a “two-hit” injury model combining intraperitoneal infection and intestinal ischemia and reperfusion. This anatomically relevant, clinically applicable, unbiased replication of the progression of sepsis demonstrates a model that is expected to produce promising treatment options and in turn, the possibility of successful future clinical trials.
MATERIALS AND METHODS

The experiments performed under this study were approved by the Committee for the Humane Use of Animals at SUNY Upstate Medical University and complied with the National Institutes of Health Guidelines for the Use of Experimental Animals in Research [5].

A total of five pigs were used in this study, with a separate experiment conducted on each pig. None of the animals presented with co-morbidities at the start of the experiment, ensuring that outlying factors did not interfere with induced disease progression.

Anesthesia

Healthy female Yorkshire pigs weighing from 22 to 30 kg were treated intramuscularly with 0.01 mg/kg of glycopyrrolate, 5 mg/kg of telazol, and 2 mg/kg of xylazine prior to the start of the experiment. A 3 mg/mL ketamine plus xylazine (0.3 mg/mL) infusion was continuously administered using a 3M model 3000 infusion pump. This held the anesthesia rate steady at 100 mL/h throughout the duration of the experiment. All necessary adjustments to anesthesia administration rate were noted [5].

Tracheostomy and Mechanical Ventilation

Upon performing a tracheostomy the animals were connected to a Galileo ventilator (Hamilton Medical, Reno, NV). Low tidal volume mechanical ventilation was omitted in order to measure the progression of potential lung injury. The initial parameters were set to the following:
tidal volume (vt) 12 cc/kg, 15/mm titrated respiratory rate (RR) to maintain normal PaCO₂, 21% FiO₂, and 3 cmH₂O positive end-expiratory pressure (PEEP) [5].

**Surgical Preparation**

Blood chemistry, gas content, and arterial pressure measurements were collected via a left carotid artery catheter placed under sterile conditions. A triple lumen catheter was placed following a 4 cm right lateral neck incision and veinotomy. Antibiotics, fluids, and anesthesia were administered through this route. Pulmonary arterial pressure (PAP) and pulmonary artery wedge pressure (PAW), mixed venous blood gas collection, and cardiac output (CO), were all monitored using the inserted right internal jugular Swan-Ganz catheter. A Foley catheter inserted into the bladder allowed for measurements and analysis of urine output samples as well as bladder pressure monitoring via its connection to a pressure transducer leveled at mid-axillary line [5].

**Injury**

After performing a midline laparotomy, the superior mesenteric artery (SMA) was clamped for 30 minutes. Bowel discoloration as well as loss of mesenteric pulse confirmed that intestinal ischemia was indeed induced. The SMA was unclamped after 30 minutes and a fecal-blood clot was created following a 2 centimeter cecum enterotomy, from which 0.5 cc/kg of feces were obtained and combined with 22 cc/kg of blood. With the enterotomy remaining, the cecum was returned to its anatomical
position and the clot was implanted into the lower right quadrant of the abdominal cavity. Peritoneal fluids were collected via a catheter placed in the Morrison’s pouch and sutured to the skin outside of the body wall. T0 (signifying zero hours following injury) was the recorded time after the suturing of the abdomen. Twelve hours after injury (T12), the midline incision was reopened and abdomen was decompressed and allowed to passively drain for collection of ascites, which was flash-frozen to allow for examination of inflammatory mediators. Each animal was monitored for a total of 48 hours or until time of death [5].

**Fluids and Antibiotics**

A water bath was used to warm all fluids to 37°C. Prior to injury, animals were given a fluid bolus of lactated ringer (1 L, i.v.) over 30 minutes. After the T0 measurement, ampicillin (2g, i.v.) and flagyl (500 mg, i.v.) were administered over the course of 15 minutes, with this administration repeated at T12, T24, and T36. Mean arterial pressure (MAP) and urine output (UOP) were used as markers to adjust the intravenous infusion of lactated ringers, ensuring adequate volume status (UOP of above 0.5 cc/kg/h and MAP above 60mmHg). Fluid infusion or extraction recordings allowed for analysis of fluid balance throughout the experiment [5].

**Ventilator Adjustments**

If arterial saturation (SaO₂) dropped below 92%, oxygenation was maintained by increasing FiO₂. If this was inadequate, PEEP was
increased in increments of 2 cmH$_2$O, with pancuronium bromide (0.1 mg/kg, i.v.) administered to control breathing in the case of triggered ventilations while the pig was sedated [5].

**Hemodynamic Measurements**

All physiologic measurements were taken hourly, from T0 to T48. Edwards transducers were used for ECG monitoring as well as pulse oximetry, mean arterial pressure (MAP), central venous pressure (CVP), pulmonary artery pressure (PAP), and pulmonary artery wedge pressure (PAW) measurements. Cardiac output (CO) was measured by injecting three boluses of cold dextrose 5% and sodium chloride 0.45% solution at end-expiration and taking the average of the three separate measurements [5].

**Pulmonary Function Measurements**

All lung function parameters were recorded hourly, from T0 to T48. The following pulmonary parameters were measured using the Galileo ventilator: respiratory rate (RR), peak airway pressure (Pip), mean airway pressure (PEEP), auto PEEP, expiratory minute volume (EMV), and static compliance (Cstat) [5].

**Kidney Function**

Measurements of blood creatinine levels (through SUNY Upstate Medical University Clinical Pathology Department) as well as BUN levels (using Roche Cobras b221) were taken every hour for the first 6 hours and every 6 hours thereafter to assess kidney function. Measurements of UOP
were taken hourly and flash frozen samples of urine from T0, T12, T36, and T48 provided protein concentrations [5].

**Liver Function**

Samples were submitted to the SUNY Upstate Medical University Clinical Pathology Department for measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total and direct bilirubin, total protein, and coagulation parameters, as indicators of synthetic liver function [5].

**Coagulation Parameters**

Samples submitted to the SUNY Upstate Medical University Clinical Pathology Department were analyzed for prothrombin time (PT), international normalization ratio (INR), and activated partial thromboplastin time (PTT) [5].

**Blood Chemistry**

A Roche blood gas analyzer was used to measure blood gases at baseline, hourly for the first 6 hours following injury, and every 6 hours thereafter. Arterial and mixed venous blood samples were taken in order to measure pH, pCO₂, pO₂, SO₂, hematocrit, hemoglobin, sodium, potassium, chloride, ionized calcium, glucose, lactate, and BUN [5].

**Circulating Leukocytes and Platelets**

Samples were submitted to the SUNY Upstate Medical University Clinical Pathology Department for a complete blood count (CBC) with differential including the platelet and white blood cell (WBC) count [5].
Inflammatory Mediators

In order to obtain plasma, the blood collected was transferred to sodium citrate tubes and spun at 15°C at 3500 RPM for 10 minutes. Analysis of inflammatory mediators was subsequently performed using the plasma that was removed and snap-frozen in liquid nitrogen [5].

Peritoneal fluid was collected by injecting 20 mL of saline into the peritoneal catheter and aspirating the syringe back 1 minute later. The saline plus ascites which was collected was then spun at 15°C at 3500 RPM for 10 minutes, the supernate separated, and snap-frozen in liquid nitrogen for further inflammatory mediator analysis [5].

The bronchoalveolar lavage fluid (BALF) was obtained at necropsy when 60 mL of saline was used to lavage the right middle lobe. The collected BALF was spun at 15°C at 3500 RPM for 10 minutes, the supernate separated, and snap-frozen in liquid nitrogen for further inflammatory mediator analysis. [5]

ELISA assays were used to measure other inflammatory mediators including tumor necrosis factor alpha (TNF-α), interleukin (IL)-8, IL-6, IL-1β, IL-12, transforming growth factor beta (TGF-β), and IL-10.

An end point chromogenic LAL assay was used to attain endotoxin levels. Both anaerobic and aerobic bacteria were identified through analysis of the blood samples collected [5].
Necropsy

Hematoxylin and eosin were used to stain all excised specimens. Slides of these specimens were studied microscopically and any histological findings were photographed under low (4x), medium (10x), and high (40x) magnification [5].

After removal of the heart and lungs, the lungs were photographed after being inflated to peak airway pressure of 25cmH₂O. Two samples were sectioned off from the most medial section 3 cm from the distal lung tip. Dependent lung area specimens were sectioned off longitudinally by measuring 3 cm medially from the aortic groove. The bronchus to the right middle lobe of the heart was exposed, the right mainstream bronchus was clamped, the trachea was clamped, and the left lung was filled with 10% neutral buffered formalin and immersed in formalin for 48 hours before performing histological examination [5].

The kidney was divided along the central axis in order to obtain portions of the medulla and cortex, which were then fixed in 10% buffered formalin for at least 48 hours [5].

Proximal, mid-jejunum, and distal portions of the small intestine were excised and then fixed in 10% buffered formalin for at least 48 hours [5].

The center of the left lobe of the liver was severed in order to harvest a 3 cm section of liver, which was then fixed in 10% buffered formalin [5].
Edema Measurement

A wet weight/dry weight ratio (W/D ratio) was used to quantify edema measurements for the lung, kidney, intestine, and liver. Tissues from each of these organs were excised, minced, weighed, and allowed to dry inside of an oven at 60°C. Once the weight remained constant for a period of 24 hours, this dry weight was recorded. Control animals from previous experiments were used as comparative models for wet and dry weights in this study [5].
RESULTS

Three of the five pigs died prior to reaching T48, signifying a mortality rate of 60%. All five of the pigs survived to T18, 60% of the pigs survived to T46, and 40% of the pigs remained up to T48. Blood cultures tested positive for Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis. All animals presented with polymicrobial sepsis and bacteremia. Decreased cardiac output and mean arterial pressure indicated hypodynamic shock and hypotension [5].

Table 1 shows changes in lung function and hemodynamics. A P/F ratio below 300 without left ventricular failure served as the indicator of acute lung injury (ALI) according to the consensus definition [6]. Lung failure was noted due to a bladder pressure rising above 20mmHg, which demonstrated abdominal compartment syndrome according to the consensus definition [7]. Increased Ppeak, Pplat, and decreased Cstat served as markers of lung injury. Histological analysis further supported this, showing interstitial infiltration of granulocytes, as well as proteinaceous infiltrate evidenced by fibrinous deposits found in alveolar spaces as a probable correlation with focal alveolar atelectasis with dilated alveolar ducts. An elevated wet-to-dry ratio as compared with control models indicated advanced edema as a result of lung injury [5].
TABLE 1
Hemodynamic and Pulmonary Function

<table>
<thead>
<tr>
<th>HR (s = 5')</th>
<th>T6 (n = 5')</th>
<th>T12 (n = 5')</th>
<th>T24 (n = 3')</th>
<th>T36 (n = 2')</th>
<th>T48 (n = 2')</th>
<th>P &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>122.4 ± 4.9</td>
<td>121.4 ± 4.5</td>
<td>113.0 ± 2.0</td>
<td>108.5 ± 3.5</td>
<td>103.0 ± 3.5</td>
<td>0.001</td>
</tr>
<tr>
<td>PAP</td>
<td>35.2 ± 3.5</td>
<td>36.8 ± 4.6</td>
<td>29.8 ± 3.5</td>
<td>24.7 ± 3.5</td>
<td>22.0 ± 2.5</td>
<td>0.001</td>
</tr>
<tr>
<td>CVP</td>
<td>13.0 ± 1.5</td>
<td>13.0 ± 1.5</td>
<td>13.0 ± 1.5</td>
<td>13.0 ± 1.5</td>
<td>13.0 ± 1.5</td>
<td>0.001</td>
</tr>
<tr>
<td>CO</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>1.5 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Temp</td>
<td>10.2 ± 1.5</td>
<td>10.2 ± 1.5</td>
<td>10.2 ± 1.5</td>
<td>10.2 ± 1.5</td>
<td>10.2 ± 1.5</td>
<td>0.001</td>
</tr>
<tr>
<td>PaO2</td>
<td>104 ± 1.5</td>
<td>104 ± 1.5</td>
<td>104 ± 1.5</td>
<td>104 ± 1.5</td>
<td>104 ± 1.5</td>
<td>0.001</td>
</tr>
<tr>
<td>PaCO2</td>
<td>104 ± 1.5</td>
<td>104 ± 1.5</td>
<td>104 ± 1.5</td>
<td>104 ± 1.5</td>
<td>104 ± 1.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

MAP = mean arterial pressure (mmHg); PAP = pulmonary artery pressure (mmHg); CVP = central venous pressure (mmHg); CO = cardiac output (L/min); Hct = hematocrit (mmHg); Temp = core temperature (°C); PaO2 = peak oxygen pressure (mmHg); PaCO2 = arterial carbon dioxide partial pressure (mmHg); PaO2 = arterial oxygen partial pressure (mmHg); EF = PaO2 fraction of inspired oxygen (FiO2).

Data are expressed as mean ± SEM.

Liver and kidney function abnormalities are a result of numerous fluctuations shown on the liver function panel, kidney function parameters, and via coagulation assessment as recorded in Table 3 and Table 4. Acute renal injury was first evidenced by a doubling of
creatinine levels throughout the experiments’ duration. Increasing intraabdominal pressure likely caused the average hourly UOP to drop despite consistent fluid resuscitation [5].

Histolopathological assessment of the kidneys showed early cortical tubular atrophy, interstitial and perivascular edema, damage in the renal medulla, and loss of tubular architecture combined with epithelial sloughing. The kidney glomeruli remained uninjured. Parameters which did not deviate from controls were the wet-to-dry ratio as well as bilirubin and alkaline phosphatase levels. The increase in aspartate aminotransferase (AST) was insignificant. The rise in international normalized ration (INR), partial thromboplastin time (PTT), and prothrombin time (PT) were clinically significant, but statistically were not seen as outliers [5].

TABLE 3

<table>
<thead>
<tr>
<th>Kidney Function</th>
<th>BL (n = 5)</th>
<th>TS (n = 5)</th>
<th>T12 (n = 5)</th>
<th>T24 (n = 5)</th>
<th>T36 (n = 2)</th>
<th>T48 (n = 2)</th>
<th>P &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>UOP</td>
<td>126.0 ± 23</td>
<td>30.6 ± 7.7</td>
<td>54.0 ± 26.3</td>
<td>70.3 ± 20.1</td>
<td>69.0 ± 30.0</td>
<td>31.5 ± 11.5</td>
<td>%</td>
</tr>
<tr>
<td>BUN</td>
<td>8.52 ± 0.65</td>
<td>10.81 ± 0.62</td>
<td>12.58 ± 1.62</td>
<td>16.77 ± 2.82</td>
<td>24.37 ± 5.43</td>
<td>23.0 ± 5.0</td>
<td>%</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.00 ± 0.0</td>
<td>1.00 ± 0.10</td>
<td>1.07 ± 0.12</td>
<td>1.35 ± 0.45</td>
<td>1.65 ± 0.65</td>
<td>2.10 ± 0.80</td>
<td>%</td>
</tr>
</tbody>
</table>

UOP = urine output (mL/h); BUN = blood urea nitrogen (mg dL⁻¹); Creatinine (mg dL⁻¹).
Data are expressed as mean ± SEM.

TABLE 4

<table>
<thead>
<tr>
<th>Liver Function Panel and Coagulation</th>
<th>BL (n = 5)</th>
<th>TS (n = 5)</th>
<th>T12 (n = 5)</th>
<th>T24 (n = 5)</th>
<th>T36 (n = 2)</th>
<th>T48 (n = 2)</th>
<th>P &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>34.0 ± 5.5</td>
<td>32.0 ± 6.3</td>
<td>41.6 ± 7.9</td>
<td>166.6 ± 54.0</td>
<td>41.7 ± 9.7</td>
<td>114.5 ± 77.5</td>
<td>%</td>
</tr>
<tr>
<td>ALT</td>
<td>35.2 ± 7.4</td>
<td>26.2 ± 2.3</td>
<td>23.8 ± 3.6</td>
<td>23.6 ± 3.1</td>
<td>37.9 ± 6.5</td>
<td>27.6 ± 6.0</td>
<td>%</td>
</tr>
<tr>
<td>ALK PHOS</td>
<td>208.8 ± 25.8</td>
<td>191.4 ± 21.8</td>
<td>160.0 ± 28.6</td>
<td>178.8 ± 29.8</td>
<td>173.7 ± 44.9</td>
<td>200.0 ± 25.0</td>
<td>%</td>
</tr>
<tr>
<td>PT</td>
<td>13.22 ± 5.3</td>
<td>14.66 ± 0.65</td>
<td>15.79 ± 0.49</td>
<td>18.12 ± 1.10</td>
<td>18.50 ± 11.42</td>
<td>19.35 ± 25.15</td>
<td>%</td>
</tr>
<tr>
<td>PTT</td>
<td>43.88 ± 5.22</td>
<td>39.30 ± 3.90</td>
<td>40.80 ± 4.77</td>
<td>32.32 ± 3.88</td>
<td>32.03 ± 6.09</td>
<td>22.90 ± 1.80</td>
<td>%</td>
</tr>
<tr>
<td>INR</td>
<td>0.99 ± 0.09</td>
<td>1.14 ± 0.5</td>
<td>1.25 ± 0.46</td>
<td>1.40 ± 0.11</td>
<td>2.66 ± 1.28</td>
<td>1.63 ± 0.23</td>
<td>%</td>
</tr>
</tbody>
</table>

AST = aspartate aminotransferase (U L⁻¹); ALT = alanine aminotransferase (U L⁻¹); ALK PHOS = alkaline phosphatase (U L⁻¹); PT = prothrombin time (s); PTT = partial thromboplastin time (s); INR = international normalized unit.
Data are expressed as mean ± SEM.
Histolopathological assessment of the liver revealed extensive interstitial edema throughout the connective tissue regions of the lobular septa and portal areas, accompanied by pronounced leukocyte infiltration into these regions. Sinusoid congestion as well as paracentral necrosis was observed along with loss of cellular integrity immediately surrounding the central vein. The wet-to-dry ratio did not deviate from the controls in either the kidney or liver [5].

Table 5 demonstrates large decreases in venous oxygen saturation (SvO$_2$), which points to decreased systemic oxygenation. Throughout experiments decreases were also seen in blood glucose levels, and to a lesser extent in chloride and sodium. At the acute injury phase, lactate rose, and then dropped as a result of animal resuscitation, only to rise again toward the end of the experiments as animals became increasingly ill [5].

<table>
<thead>
<tr>
<th>TABLE 5</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Blood Chemistry</th>
<th>BL (n = 5)*</th>
<th>T6 (n = 5)*</th>
<th>T12 (n = 5*)</th>
<th>T24 (n = 5*)</th>
<th>T36 (n = 5*)</th>
<th>T48 (n = 5*)</th>
<th>$P &lt; 0.05$</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.483 ± 0.029</td>
<td>7.412 ± 0.013</td>
<td>7.473 ± 0.020</td>
<td>7.509 ± 0.020</td>
<td>7.396 ± 0.008</td>
<td>7.404 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>2.75 ± 0.13</td>
<td>6.33 ± 0.40</td>
<td>1.98 ± 0.38</td>
<td>1.77 ± 0.23</td>
<td>4.67 ± 2.47</td>
<td>3.95 ± 1.75</td>
<td></td>
</tr>
<tr>
<td>SvO$_2$</td>
<td>81.5 ± 10.4</td>
<td>63.4 ± 8.5</td>
<td>46.8 ± 4.3</td>
<td>44.8 ± 3.5</td>
<td>53.0 ± 6.7</td>
<td>44.2 ± 6.4</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>100.5 ± 15.4</td>
<td>90.0 ± 10.6</td>
<td>69.3 ± 2.0</td>
<td>48.3 ± 3.8</td>
<td>44.9 ± 12.1</td>
<td>49.5 ± 13.0</td>
<td></td>
</tr>
<tr>
<td>Na$^+$</td>
<td>148.9 ± 5.0</td>
<td>141.7 ± 1.9</td>
<td>143.7 ± 2.0</td>
<td>139.8 ± 1.4</td>
<td>132.5 ± 1.6</td>
<td>137.1 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>K$^+$</td>
<td>3.22 ± 0.07</td>
<td>3.63 ± 0.15</td>
<td>4.23 ± 0.22</td>
<td>4.28 ± 0.22</td>
<td>4.95 ± 0.64</td>
<td>4.82 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>115.95 ± 3.67</td>
<td>112.40 ± 1.22</td>
<td>114.83 ± 2.03</td>
<td>108.87 ± 2.68</td>
<td>115.07 ± 1.07</td>
<td>113.30 ± 2.30</td>
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</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>1.05 ± 0.04</td>
<td>1.18 ± 0.04</td>
<td>1.06 ± 0.07</td>
<td>4.09 ± 3.60</td>
<td>1.02 ± 0.01</td>
<td>0.90 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

*pH = arterial pH; Lactate = plasma lactate concentration (mmol L$^{-1}$); SvO$_2$ = venous oxygen saturation (%); Glu = serum glucose concentration (mg dl$^{-1}$); Na$^+$ = mmol L$^{-1}$; K$^+$ = mmol L$^{-1}$; Cl$^-$ = mmol L$^{-1}$; Ca$^{2+}$ = mmol L$^{-1}$.
Data are expressed as mean ± SEM
$^*$P < 0.05 following RM ANOVA.

Histopathological analysis of the intestine showed surface epithelium degradation, sloughing of the lamina propria onto the intestinal lumen, and flattened denuded villi. Other changes in the upper right
mucosal compartment included signs of hypoxia with restricted blood flow through the end-capillary bed, caused by small blood capillary congestion. The substantially high wet-to-dry ratio as compared with controls correlates with the presence of the edema and dilated lymph vessels present in the serosa, a typical demarcation of acute peritonitis [5].

Both the ascites and plasma data collected pointed to a marked increase in the various cytokines present, as a result of the immune response upregulation following disease onset. Table 6 presents this data. BALF contained TNF-α, IL-1β, IL-6, IL-8, IL-10, and IL-12. Over time, TNF-α, IL-1β, IL-6, IL-8, and IL-14 levels were substantially increased when comparing baseline values to values at the conclusion of the experiments. While TNF-α and IL-1β levels rose systemically, IL-12 showed the opposite trend [5].

![](image.png)

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Baseline (SD)</th>
<th>Time 1 (SD)</th>
<th>Time 2 (SD)</th>
<th>Time 3 (SD)</th>
<th>Time 4 (SD)</th>
<th>Time 5 (SD)</th>
<th>P</th>
<th>P</th>
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</thead>
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<tr>
<td>TNF-α</td>
<td>321 ± 14.1</td>
<td>250 ± 48.1</td>
<td>141 ± 34.9</td>
<td>159 ± 27.0</td>
<td>631 ± 337.3</td>
<td>802 ± 874.1</td>
<td>0.00092</td>
<td>0.00032</td>
</tr>
<tr>
<td>Plasma (pg/mL)</td>
<td>NA</td>
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<td>NA</td>
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<td>NA</td>
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<tr>
<td>BALF (pg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.00008</td>
<td>0.000008</td>
</tr>
<tr>
<td>IL-6</td>
<td>391 ± 6.6</td>
<td>484 ± 73</td>
<td>180 ± 64</td>
<td>328 ± 124</td>
<td>572 ± 105</td>
<td>672 ± 135</td>
<td>0.00092</td>
<td>0.00032</td>
</tr>
<tr>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
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</tr>
<tr>
<td>IL-1β</td>
<td>230 ± 6.0</td>
<td>291 ± 49.6</td>
<td>146 ± 37.2</td>
<td>357 ± 49.2</td>
<td>389 ± 131.5</td>
<td>404 ± 81.3</td>
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<tr>
<td>IL-6</td>
<td>540 ± 60.4</td>
<td>310 ± 42.4</td>
<td>193 ± 43.0</td>
<td>178 ± 21.5</td>
<td>290 ± 74.2</td>
<td>401 ± 10.2</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>IL-10</td>
<td>31.7 ± 0.8</td>
<td>161 ± 102.1</td>
<td>32.0 ± 4.0</td>
<td>125.7 ± 56.9</td>
<td>40.4 ± 2.9</td>
<td>77.2 ± 0.5</td>
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</tr>
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<tr>
<td>TNF-β</td>
<td>3025 ± 4.9</td>
<td>3215 ± 5.7</td>
<td>3015 ± 5.2</td>
<td>2921 ± 6.3</td>
<td>2906 ± 5.4</td>
<td>2908 ± 5.4</td>
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<td>0.00032</td>
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<tr>
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<tr>
<td>BALF (pg/mL)</td>
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</tbody>
</table>

TNF-α = tumor necrosis factor; IL-β = interleukin-1β; IL-6, IL-8, IL-10, and IL-12 = transforming growth factor beta. Data are expressed as mean ± SEM. 

P < 0.05 following BMD ANOVA.
DISCUSSION

The ultimate goal of preclinical studies of potential sepsis treatments is for these studies to bear clinical relevance to humans so that they may eventually be translated into human trials and future effective treatments. Presently, animal models which have been employed may have proven effective in particular organisms, but due to the deviation from human pathophysiology and disease pathogenesis, many such studies have proven deleterious in humans or have resulted in a stalemate in terms of new treatment options for sepsis and its progression. Various scientists continue to discuss the relevance of several crucial factors in deciding which animals to use, how to induce injury, and management of disease progression. This includes consideration of genetic and sexual variability between humans and species being studied, as some respond differently to differing strains of viruses or bacteria, which may prove to be significant in how sepsis unravels. Similarly, the pre-disease health conditions of model organisms must be taken into account, as young animals are more likely to not have been exposed to previous insults. Pre-treated animals should also be viewed with skepticism because ill patients are often not pre-treated by the time disease onset is identified. Knock-out animals, such as mice which have been used throughout several studies, may have alternative genetic pathways to compensate for the insult inflicted during studies, the effects of which remain unknown. When small organisms are used, sometimes it becomes impossible to provide supportive therapy,
which may in itself become the cause of morality (i.e., lack of fluid resuscitation). Additionally, antibiotic agents must be used throughout effective studies because severely infected septic patients will generally always be treated with antibiotics. Furthermore, the basic aim of the study is to test the efficacy of various antibiotic and drug therapies, hence the need for a model organism whose response to particular drug treatments will mimic that of a human. In order to pinpoint such a model, drug dosage and virulence must be carefully measured [8]. Finally, because human sepsis often progresses relatively slowly in a chronic state, utilizing rapidly fatal models resulting from acute septic shock in which animal mortality is seen within only twelve hours may mask the manifestations of a true clinical septic response [9].

The animal model in this study complies with the majority of the aforementioned standards. However, using only female pigs may have limited the spectrum of results obtained due to the fact that gender was not randomized. Introducing comorbidities into the study, such as diabetes and COPD, which often complicate the evolution of human sepsis, may be useful for future research. Also, the use of only healthy adolescent pigs may not be fully representational of the true mean age (65 years of age) at which human sepsis is most likely to occur. On the other hand, the anatomy and physiology of pigs is similar to that of humans, which also allowed for replication of relevant etiology in this study. Mechanical ventilation, fluid resuscitation, and antibiotic therapy mimic standard
intensive care unit clinical procedures which human patients receive. Finally, each experiment was conducted over a length of time sufficient for generating data on markers that manifest themselves in a true case of human sepsis. Abiding by these guidelines proved to be effective in generating the multiple organ injury/dysfunction as well as systemic inflammatory responses, both of which are classic hallmarks in the pathogenesis of sepsis.

**Inflammatory Mediators and Coagulation**

Because sepsis is a dysfunction in the mechanism which normally maintains homeostasis, pro-inflammatory cytokines (IL-1, IL-6, and TNF) can slow down apoptosis as much as in activated neutrophils and macrophages [11]. Intestinal epithelium, as well as other tissues may suffer accelerated apoptosis, furthering tissue injury.

In this study, increase in TNF-α and decrease in IL-6 mirrored the profile of patients with severe septic shock. Because IL-12 is required for resistance to bacterial peritonitis [10], the decrease of this cytokine in our animals, as well as IL-10 found within the peritoneal fluid and BALF [5], shows the progressive suppression of both innate and T cell-dependent defense mechanisms, which are a critical phase in organ injury and consequently septic shock.

An increase in inflammation and coagulation occurs through induced pro-inflammatory mediators, endothelial injury, tissue factor (TF) release, and thrombin production. On the other hand fibrinogenolysis,
which normally counteracts pro-clotting forces, becomes suppressed [14].
The introduced endotoxin becomes bound to receptors found in mononuclear leucocytes (monocytes and macrophages), resulting in the secretion of multiple cytokines. When there is over-production of such cytokines, interleukin-1 (IL-1) and tumor necrosis factor (TNF) are the first cytokines to exhibit adverse effects. Cytokines produce specific effects through pathways using nitric oxide (NO), the metabolites of the arachidonic acid (prostaglandins, thromboxane and leukotrienes), and platelet activating factor (PAF). IL-1 and TNF stimulate the production of other cytokines, producing a vast cascade effect [13], showing that cytokines play a role in both coagulation abnormalities and vascular endothelial activation, both of which contribute to organ dysfunction as shown in Figure 1 below. Additionally, Protein C produces an essential anti-thrombotic pathway. Activation of this protein not only helps prevent excessive blood coagulation but it also diminishes the inflammatory response. In sepsis, activation of protein C is defective, resulting in inappropriate blood coagulation leading to inadequate organ perfusion and organ failure [12]. As previously discussed, this supports the effectiveness of current protein C therapies implicated in the treatment of sepsis.

Prolonged PT, PTT, and elevated INR levels along with histopathologic confirmation of microthrombosis within all organs, verified coagulation abnormalities in all animals [5]. The decrease in platelet count of all animals in this study are consistent with the role that activated platelets play in generating microvascular clots, propagated in the inflammatory
response, leading to the thrombocytopenia observed in critically ill patients, due to platelet consumption [13].

Figure 1: Progression of the inflammation-coagulation autoamplification loop [31].

Pulmonary Function

Acute respiratory distress syndrome (ARDS) is associated with increased mortality rates, with sepsis being the most frequent risk factor for ARDS. The two conditions share the same complex mediator-facilitated process involving different cellular components and an important inter-relation between inflammation and coagulation. As a result of the action of various mediators, capillary permeability changes appear, resulting in pulmonary edema, as histopathology analysis in this study shows, and an increase in extravascular lung water, as observed in comparison with controls within this study. Mortality rates from ARDS remain at 30–50%, with outcome usually better in trauma than in sepsis.
[15]. In 80% of cases death is due to multiple organ failure, reflecting the systemic nature of the disease’s progression [15].

Intraalveolar and intravascular fibrin deposition are a common feature found in acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) [17]. These fibrin deposits increase vascular permeability, which in turn exaggerates the inflammatory response, further activating endothelial cells to produce proinflammatory cytokines and other mediators [16]. There was evidence of this in a study which confirmed the presence of TNF-α, IL-1β, IL-6, IL-8, and IL-10 in BALF [5]. From here an accumulation of activated neutrophils and modulating immunoregulatory responses become stimulated in the lung. The endotoxin implantation in this study is responsible for producing fibrin deposits in the lung and other organs, and these widespread intravascular alterations are believed to have contributed to the multiple organ dysfunction seen in this model of sepsis.

Increases in peak and plateau pressures along with a marked decrease in static compliance and P/F ratio provided proof of acute lung injury. Anatomical findings of alveolar collapse (shown in Figure 2 below), hyaline membrane formation, and accumulation of lymphocytes were consistent with profiles of traditional autopsy findings in severely septic patients [5]. Subsequent fibrosis leads to further thickened alveolar septa.
Figure 2: Shows alveoli that are collapsed or distended. Many contain dense proteinaceous debris, desquamated cells, and hyaline membranes [33]. The topmost arrow points to collapsed alveolar spaces and the bottommost arrow shows a hyaline membrane.

Kidney Function

As described in Figure 3 below, acute renal failure (ARF), the most common renal manifestation of sepsis, is not an isolated event but often a component of multiple organ dysfunction syndrome (MODS) which may complicate sepsis or septic shock, important risk factors for ARF. This demonstrates that similar mechanisms operate in inducing the dysfunction of a variety of organ system. Rising BUN and creatinine levels, both of which occurred in the animals in this study, were indications of late-stage acute renal injury. Renal dysfunction typically proceeds after lung dysfunction, which was consistent with findings in this study [5].

During severe sepsis, in addition to activation of cellular response system and overwhelmingly increased production of inflammatory humoral mediators, an activation of the sympathico-adrenal axis with
increased plasma levels of epinephrine, of the renin-angiotensin aldosterone system (RAAS), with elevated levels of angiotensin II and a rise in vasopressin levels are present as part of the host response [18]. These shifts contribute to the clinical manifestations of sepsis, including the hemodynamic changes characterized by vasodilation, a hyperdynamic circulation, and microcirculatory changes contributing to inefficient oxygen extraction [18]. Although often there is no consistent or typical renal histopathological pattern indicating the severity of kidney injury [19], the renal histopathology observed pertained to the convoluted tubules’ cortex as well as interstitial and perivascular edema [5].

Ultimately, sepsis causes a reduction in mean arterial pressure (MAP), which affects systemic vasculature as mean arterial pressure decreases below the auto-regulatory range in which renal blood flow is normally maintained [19]. This leads to renal vasoconstriction. This study demonstrated a gradual drop in urine output in all animals, remaining substantially lower than baseline measurements despite fluid resuscitation [5].
**Liver Function**

The liver plays a major role in host defense mechanisms in septic patients. Here, Kupffer cells perform bacterial scavenging, inactivate bacterial products, and clear or produce inflammatory mediators. Hepatocytes shift their metabolic pathway toward gluconeogenesis, amino-acid uptake, and increased synthesis of complement and coagulation factors and protease inhibitors with the help of receptors for a variety of proinflammatory cytokines [22]. In sepsis, the acute-phase protein (APP) response contributes to an upregulated coagulation state by

---

**Figure 3:** Detailed progression of acute renal failure (ARF) as seen in septic shock [32].
inhibiting protein C and decreasing liver synthesis of antithrombin and protein C [21]. The liver’s increased production of thrombin-activated fibrinolytic inhibitor promotes fibrinolysis inhibition. Such hepatic inflammatory and altered coagulation in sepsis may have harmful effects on the liver as Figure 4 reveals. This study showed hepatic dysfunction which causes diminished lactate clearance, via the elevated serum lactate level observed at the conclusion of the experiment [5]. Activated Kupffer cells that release chemokines, attract blood neutrophils into the liver, and activate them, may in turn cause microcirculatory instability, hepatocyte injury, fibrin deposition, endotoxin spillover, and multiple organ failure [22].

Figure 4: Hepatic cytokine production in sepsis and extrahepatic bacterial infections.

Microcirculatory instability often leads to capillary leakage, manifested as low serum albumin and total protein levels as seen in this
study [5]. Elevated AST and INR values in this study correspond with liver injury as seen in human septic shock [23]. As literature indicates, hepatosplanchnic hypoperfusion, which often leads to such hepatic dysfunction, may be reversed with fluid support [23]. Histopathological observation showed non-specific damage, but was consistent with the typical presence of centriacinar necrosis, prominent Kupffer and endothelial cells, and minimal inflammation [20] [5].

**Gastrointestinal Function**

The difficulty in assessing gut function at the bedside has left no clear indicator(s) of acute intestinal dysfunction. However, the gut has been introduced as an important initiator of organ injury following septic shock as explained further [24]. This may occur when the overgrowth of bacteria in the gastrointestinal tract compromises the normal barrier function of the gut and subsequently allows bacteria and endotoxins to be translocated into the systemic circulation [24], which is shown in Figure 4. This causes an extension of septic shock.

This study was able to show an increase in inflammatory peritoneal fluid and intestinal edema substantial enough to contribute to abdominal compartment syndrome [5]. Because this syndrome often affects the function of other organs, it is suggestive of a pathway by which intestinal injury leads to a worsening prognosis for severe sepsis and septic shock patients [5]. Evidence of significant intestinal edema and a large-scale volume of ascites suggest intestinal injury. This is confirmed by observed
widespread histopathologic injury as substantiated by denudated villi and epithelial sloughing [5].

CONCLUSION

This translational animal model of trauma-induced shock and bacterial sepsis successfully mimics the human pathophysiology of traumatic and septic shock. It can help direct future patient treatment through improved diagnosis, clinical care management, and effective therapy.
REFERENCES


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CAPSTONE SUMMARY

I began conducting research at the Cardiopulmonary Critical Care laboratory at Upstate Medical University beginning in June 2009. Through working with medical residents, doctors, students, and various researchers, I have had the opportunity to participate in translational research conducted on Yorkshire pigs in order to investigate the evolution of sepsis and septic shock to multiple organ dysfunction syndrome (MODS). Translational research is unique and advantageous in that scientists provide clinicians with new treatments and tools for patient care and for assessment of their impact, while clinical researchers make novel observations about the nature and progression of disease, often stimulating cutting edge research investigations. Elucidating the physiological progression of this disease process is important for generating relevant data regarding improved preventative, monitoring, and treatment options, which may hopeful be used in future clinical trials on humans.

Human sepsis is typically caused by a site of infection containing replicating bacteria that persists for an extensive period of time. It is a syndrome that usually evolves in a continuum from systemic inflammatory response syndrome (SIRS) to sepsis combined with organ dysfunction to septic shock. While various medical treatments have been proven effective in the treatment of sepsis in animal models, the only current effectual treatment of human sepsis is limited to the use of activated protein C. The complex pathogenesis of human sepsis presents a
challenge in precisely duplicating the process of disease development in
the particular animal models that are currently employed for preclinical
testing. The objective of this 48 hour study was to utilize a clinical model
that accurately replicates severe human sepsis along with gut
ischemia/reperfusion (the blocking off of blood flow to a particular
regions followed by subsequent restoration of blood flow to this area),
进一步 leading to the injury of multiple organs within the time period most
closely mirroring disease progression in humans.

Pigs were subjected to a “two-hit” injury involving the clamping of
the superior mesenteric artery for 30 minutes as well as a laparotomy used
for insertion of a fecal clot, following appropriate administration of
anesthetics and ventilation. Twelve hours after the injury was inflicted,
the abdomen was reopened, dressed and allowed to drain passively. The
animals were followed for the remainder of the 48-hour study and
received treatment with fluids and antibiotics according to standard
Intensive Care Unit practices. Physiologic parameters, laboratory data, and
inflammatory mediator levels were collected regularly and recorded
throughout the study. Hemodynamics, also known as blood flow
parameters, were stabilized through administration of antibiotics and
intravenous fluids as needed, while measurements of arterial and mixed
venous blood gases as well as lung, kidney, liver, renal, and hemodynamic
function measurements were recorded. Progression of the abdominal
compartment syndrome was determined by monitoring bladder pressure
changes. Serial measurements of peritoneal and plasma ascites were also taken for evaluation of cytokine concentration, which was important for determining the onset, spread, and stage of disease development, seeing as cytokines are small cell-signaling protein molecules generated during the immune response and are telling of the degree of injury and/or response to a physiological insult. Morphometric analysis, or examination of variations in size, shape, and pathology of the organism on a micro and macro scale, was carried out using the organ tissues harvested and fixed at necropsy. After 48 hours the animals were sacrificed (if they were not yet deceased) with bolus barbiturate and necropsy was performed followed by histologic analysis.

Through watching and emulating operative and post-operative techniques, such as inserting catheters, or assisting during a necropsy, I was able to fundamentally understand, at the most basic level, how, why, and which vital measurements are monitored during experiments and translate this knowledge into understanding how numbers correlate with the functions of an infected living organism. Routine questioning and hands-on testing of acquired skills by my mentor/advisor ensured that I not only understood the significance of my findings, but was able to independently explain and measure the necessary information in order to carry out my project.

In combining both sepsis and ischemia reperfusion injury, the animal model used in this study should be valid for uncovering the
intricate pathophysiological progression of septic shock and its transition to multiple organ dysfunction syndrome, as it mirrors the systemic inflammation and major organ systems dysfunction seen in humans. The usefulness and validity of this animal model is further evidenced by the presence of polymicrobial sepsis in all animals with a progressive deterioration of organ function, histopathological and clinical injury, and development of acute lung injury and abdominal compartment syndrome over the duration of the 48-hour experiments. Each of these conclusions was drawn through the precise observation of increases in multiple cytokines within peritoneal and serum fluid, which parallels the dysfunction of major organ systems. The novel findings of this study have significant implications for the future reduction of mortality from the leading cause of hospitalization for adult patients in the intensive care unit: sepsis.