A Canine Model of Septic Shock:

Balancing Animal Welfare and Scientific Relevance

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Abstract

A shock canine pneumonia model that permitted relief of discomfort using objective criteria was developed and validated. After intrabronchial *S. aureus* challenge, mechanical ventilation, antibiotics, fluids, vasopressors, sedatives and analgesics were titrated based on algorithms for 96 h. Increasing *S. aureus* (1 to 8 x 10⁹ cfu/kg) produced decreasing survival rates (p = 0.04). From 4 to 96 h, changes in arterial-alveolar oxygen gradients, mean pulmonary artery pressures, interleukin-1, serum sodium levels, mechanical ventilation and vasopressor support were ordered based on survival time [acute nonsurvivors (≤ 24 h, n = 8) ≥ subacute nonsurvivors (24 to 96 h, n = 8) ≥ survivors (≥ 96 h, n = 22) (all p < 0.05)]. In the first 12 h, increases in lactate and renal abnormalities were greatest in acute nonsurvivors (all p < 0.05). Compared to survivors, subacute nonsurvivors had greater rises in cytokines, liver enzymes and greater falls in platelets, white cell counts, pH, and urine output from 24 to 96 h (all p < 0.05). Importantly, these changes were not attributable to dosages of sedation which decreased in nonsurvivors (survivors: 5.0 ± 1.0 vs. nonsurvivors: 3.8 ± 0.7 ml/h/(fentanyl/midazolam/medetomidine); p = 0.02). In this model, the pain control regimen did not mask changes in metabolic function and lung injury or the need for more hemodynamic and pulmonary support related to increasing severity of sepsis. The integration into this model of both specific and supportive titrated therapies routinely used in septic patients may provide a more realistic setting to evaluate therapies for sepsis.

Keywords: sepsis, dog, *S. Aureus*
Introduction

More than two decades ago we developed a conscious canine model of *E. coli* peritonitis, which reproduced the cardiovascular profile of human septic shock (33, 36). This model did not employ analgesics or sedatives because their depressive cardiopulmonary effects would confound the changes and outcomes related to sepsis itself (33, 36, 39). The model was designed such that increases in bacteria dose or pathogenicity worsened cardiovascular dysfunction consistent with changes seen in patients with increasingly severe infections (16, 33, 36). In an initial series of studies, the model provided fundamental insights into the relative roles of microbes, microbial toxins and host mediators in the cardiovascular injury associated with sepsis (5, 11, 14, 16, 32, 35, 52). The model was then further validated in a series of studies showing that it reproduced the clinical effects of both standard and experimental therapies in sepsis (26, 34). Studies using this model were valuable to both drug researchers and the Food and Drug Administration in the development and application of new clinical therapies for sepsis (6, 10, 13, 15, 19, 20, 25, 27, 37, 38, 41-45, 49, 55).

Despite the model's scientific relevance, debate arose among two supervisory Animal Care and Use Committees at the National Institutes of Health in the late 1990’s as to whether the levels of pain and distress associated with the model were acceptable. An outside panel of experts concluded that although valuable, efforts were necessary to reduce the pain and distress associated with the model. In response, several approaches were investigated to relieve the discomfort caused by peritonitis including subcutaneous anesthetic injections, partial and total celiac plexus blockade (53), and the use of an epidural anesthetic (23, 53). These therapies, while proposed for clinical use during peritonitis, had not been commonly applied in patients. In our canine model, they either did not fully alleviate pain and distress or they caused
cardiovascular dysfunction and mortality rates to worsen thereby confounding the effects of sepsis (53).

We concluded that it would not be possible to balance the needs for both scientific relevance and humane animal treatment employing the canine peritonitis model. Given the continuing importance of a large animal model to understanding the pathogenesis of sepsis and for evaluating new treatments, we investigated whether it would be possible to fulfill both needs by employing a sedated and mechanically ventilated model of pneumonia. Prospectively determined criteria of a relevant and successful model were to closely simulate the clinical syndrome as encountered in humans and to be applicable to, and consistent with, the best practices of both human and veterinary medicine.

Methods

Experimental Design (Overview)

The experiments described below were approved by the Animal Care and Use Committee of the Clinical Center at the National Institutes of Health. Thirty-eight purpose-bred beagles (12-28 months, 10-12 kg) were studied of which sixteen of these animals were controls for a subsequent intra-aortic balloon counterpulsation study. These sixteen animals received increasing doses of S. aureus and were managed using the same protocols as the other animals in this study. They were included to increase our ability to make inferences about causes of acute (≤24 h) and subacute (24 to 96 h) deaths. The protocol followed in these experiments is shown in fig. 1. Briefly, general anesthesia (propofol, isoflurane) and mechanical ventilation via an endotracheal tube were employed to perform a tracheostomy and to place percutaneous femoral arterial and external jugular vein catheters and a urinary bladder catheter. Following
tracheostomy, general anesthesia was discontinued and sedation (fentanyl, midazolam, and medetomidine) was started. Mechanical ventilation was initiated and maintained while animals received fluids and vasopressors for 96 h. At baseline, blood was cultured and blood samples were taken for analysis. A bronchoscope was then used at time 0 to place *Staphylococcus aureus* or sterile saline in the lower lobe of the right lung. During the first 4 h after *S. aureus* challenge while sepsis was developing, maintenance fluids (normasol-M with 27 mEq KC1 added; 2 ml/kg/h) and vasopressors (phenylephrine titrated to mean arterial pressure (MAP) > 80 mmHg) were administered. KCl was added to Normosol-M (dextrose, sodium chloride, potassium acetate, and magnesium acetate) to prevent hypokalemia found in pilot studies to occur with long fluid infusions. Phenylephrine was used to counteract the hypotensive effects related to sedation while sepsis was developing. After 4 h, when symptoms of sepsis were fully developed (based on prior experience with the model), vasopressor support was discontinued and intravascular hemodynamics and cardiac function were measured and blood samples taken. Treatment for sepsis was then initiated and individualized to the hemodynamics, oxygenation, and ventilation needs of each animal similar to human care. The level of vasopressor and ventilatory support was dictated by algorithms and adjusted according to continuously measured oxygen saturation, MAP, intermittent pulmonary capillary wedge pressure (PCWP) determinations and arterial blood gas monitoring (see *Mechanical Ventilation* and *Fluid and Vasopressors* below).

Antibiotics (ceftriaxone, 50 mg/kg, IV, q24) were started 4 h after bacterial inoculation and administered daily through day 4. Hemodynamic and blood value measurements were repeated at 8 h, and then again on days 1, 2, 3, 4. Animals alive at 96 h were considered survivors and subsequently were euthanized (Beuthanol, IV, 75 mg/kg).
**Surgical Procedures**

Animals were fasted for 12 h prior to surgery. After intravenous induction with propofol (4-6 mg/kg), tracheal intubation (Rusch, 6 F, Duluth, GA) and the initiation of mechanical ventilation (Fabius Tiro, Drager Medical, Telford, PA), anesthesia was maintained with isoflurane (0.5 – 1.5 %). An incision was made into the soft tissue of the tracheal wall and a tracheostomy tube was placed (2, 8). The free edges of the skin were then sutured around the tube. Betadine ointment was applied to the incision site.

**Catheter Placement**

Femoral arterial and external jugular venous catheters (Maxxim Medical, Athens, TX) were placed percutaneously using sterile technique (53). A 7 F pulmonary artery thermodilution catheter (Abbott Critical Care, Chicago, IL) was introduced through an 8 F introducer via the external jugular vein into the pulmonary artery. A 20 ga. arterial catheter was placed into the femoral artery. Using sterile technique, a urinary catheter (Cook, Foley 8 F, 55 cm) was placed in the bladder and connected to a collection system.

**Bacterial Preparation**

Frozen aliquots of *S. aureus* were thawed and inoculated into 250 ml of brain heart infusion (Gibco, Grand Island, NY). This isolate was sensitive to ceftriaxone. After incubation for 19 h, suspensions were centrifuged at 4° C, washed twice in PBS, and resuspended. The concentration of bacteria was determined turbidometrically. The suspension was then adjusted...
with PBS to produce a concentration of 0 to $8 \times 10^9$ colony forming units (cfu)/ml of \textit{S. aureus}.

The concentrations were confirmed by plating serial dilutions on the appropriate culture medium and counting colonies as previously described (32, 36).

\textit{Bacterial Inoculation}

Under continuous intravenous sedation (see above), animals received preoxygenation with 100\% O$_2$ for 5 min., the tracheal tube was removed and a sterile bronchoscope (Olympus BF 1T20, Lake Success, NY) was advanced via the tracheal stoma, under direct vision into the right lower lobe segmental bronchus. A pulmonary arterial thermodilution catheter was advanced via the suction port of the bronchoscope and wedged with the balloon inflated into a subsegmental bronchus (18). Ten ml of a solution with a known amount of \textit{S. aureus} bacteria (0 to $8 \times 10^9$ cfu/kg) or suspension without bacteria as a control was administered via the catheter into the subsegmental bronchus. The balloon was deflated and the bronchoscope and thermodilution catheter were removed.

\textit{Mechanical Ventilation}

The ventilator (Servovent 300, Siemans Medical, Sweden) was initially set with a fractional inspired oxygen concentration (FiO$_2$) = 25\%, positive end-expiratory pressure (PEEP) = 5 cm H$_2$O, tidal volume (TV) = 15 ml/kg, and respiratory rate (RR) = 15 breaths/min. If the O$_2$ saturation fell below 92\%, attempts to restore O$_2$ saturation to $\geq$ 92\% were made in the following incremental sequence: FiO$_2$ increased to 50\%, PEEP increased to 10 cm H$_2$O and then 12 cm H$_2$O, FiO$_2$ increased to 75\% and then to 100\%. The maximum settings were FiO$_2$ = 100\% and PEEP = 12 cm H$_2$O. Support was similarly reduced if the O$_2$ saturation was $\geq$ 93\% for 6 h. The
blood gas measures (q2 h until T8 and then q8 h thereafter) were used to set RR on the mechanical ventilator. RR was increased by increments of 5 breaths per min. to maintain PaCO2 under 35 mmHg, or decreased by 5 if pH > 7.35 and PaCO2 ≤ 30 mmHg. The maximum setting was 35 and the minimum 15 breaths per min. Plateau airway pressures were measured and maintained less than 35 cm H₂O.

**Fluids and Vasopressors**

During the first 4 h after bacterial inoculation, before the full development of signs and symptoms of sepsis in this model (based on pilot studies), a phenylephrine infusion (10 mg/250 ml, titrated to effect) was used to maintain the animal’s blood pressure near normal at a mean of 80 mmHg (low normal for canines). This was to insure that sedation did not cause hypotension in any animal while sepsis was developing. During this time, maintenance intravenous fluid infusion of normasol-M with 27 mEq KCl added (2 ml/kg/h) was administered. Four hours after bacterial inoculation (T4), the phenylephrine infusion was turned off for a washout period of 10 min.

At T4, to simulate hemodynamic support as practiced clinically during sepsis, if PCWP was < 10 mmHg, a fluid bolus (0.9% sodium chloride, 20 ml/kg, Hospira, Lake Forest, IL) was given. If after 3 fluid boluses the MAP was < 80 mmHg, an infusion of norepinephrine (NE) was initiated. NE was adjusted incrementally (0, 0.2, 0.6, 1.0, and 2.0 µg/kg/min) to maintain a MAP between 80 and 110 mmHg (low normal for these animals). At subsequent time points (q2 h until T12 and q4 h thereafter) until the end of the study, if the PCWP was < 10 mmHg, an additional intravenous fluid bolus (20 ml/kg) was administered. Maintenance fluid infusion (Normasol-M with 27 mEq KCl added; 2 ml/kg/h) was continued from 4 to 96 h.
Other ICU Therapies

Other care was instituted based on standard veterinary practices for critically ill large animals requiring sustained mechanical ventilation in the clinical setting (21, 29). Every 4 h, the animal’s mouth was flushed with chlorhexidine solution and the eyes were lubricated with a sterile petroleum gel (50). The forelimbs were placed square with the slightly elevated head and the hind limbs were serially rotated between left and right position. Passive limb movement (fore and hind limbs) were performed every 4 h. Every 12 h, sterile saline (3 ml) was instilled in the trachea followed by tracheal suctioning. The inner cannula of the tracheostomy was cleaned with chlorhexidine and then rinsed with sterile saline two times each day or more frequently if secretions accumulate. All dressings of catheter sites were changed once daily. Throughout the study, a heated water blanket and other heavy blankets were used to maintain core temperature between 36.5° C and 37.5° C. Humidity in the tubing was maintained using a humidifier (Conchatherm III, Hudson RCI-AB) attached to the airway system. To protect the animals from stress induced stomach ulcers, famotidine (1 mg/kg IV q12 h), an H₂ blocker, was administered and to protect from venous thrombosis during mechanical ventilation and sedation, heparin (3000 IU IM, q8 h) was administered until the end of the study.

Physiologic Measurements

Cardiac output (CO), mean pulmonary artery pressure (MPAP), PCWP, and central venous pressure (CVP) were determined via a pulmonary arterial thermodilution catheter placed in the external jugular vein. MAP was measured and heart rate (HR) calculated via the femoral
arterial pressure recording. Left ventricular ejection fraction (LVEF) was determined by cardiac ultrasound (Sonos 5500, Philips Medical). The CO was standardized to the animal’s weight in kg (cardiac index, CI). These measurements were performed at baseline (prior to bacterial inoculation, T0), and 4, 24, 48, 72 and 96 h after intrabronchial bacterial inoculation.

Laboratory Data

Arterial and mixed venous blood gases were measured q2 h until T8 and q8 h thereafter with a blood gas system (ABL 500; Radiometer, Copenhagen, Denmark). Complete blood counts (model STK-S; Coulter Electronics, Hialeah, FL) and chemistries were performed with an automatic analyzer at 4, 6, 8, 24 h and every 24 h thereafter. Blood was also obtained for quantitative blood cultures (isolator tubes) (Wampole, Cranbury NJ) at 4, 8, 24 h and every 24 h thereafter.

Cytokines

Plasma was collected at baseline, 4, 24, 48, 72, and 96 h after inoculation. From these samples using the Searchlight® multiplex array system canine interleukin IL-2, IL-6, IL-10, tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ) and transforming growth factor beta -1 (TGFβ1) levels were measured (Pierce Biotechnology, Rockville IL).

Serum Protein Expression Profiling

Acute phase protein expression was globally analyzed to determine its relationship to the severity of infection. Serum was collected at baseline, 4, 24, 48, 72, and 96 h after inoculation. The serum was denatured and applied to ProteinChip copper metal affinity arrays (Ciphergen
Biosystems, Freemont, CA.) for analysis by surface enhanced laser desorption and ionization with time of flight mass spectrometry (SELDI-TOF) (Ciphergen ProteinChip Reader PBS IIc)(see Supplement A).

**Sedation and Analgesia Management**

The level of sedation and analgesia was adjusted by a clinician or trained technician continuously at the bedside for 96 h after initiation of midazolam (0.2 mg/kg loading dose; 50 µg/kg/min infusion) and fentanyl (5 µg/kg loading dose; 0.7 µg/kg/min infusion). Both the fentanyl and midazolam infusion were increased in increments of one-fourth of the dose every 5 min until adequate sedation was obtained. Medetomidine infusion (2-5 µg/kg/min) was used to supplement sedation as needed as set by protocol (23). Criteria for adequacy of sedation were as follows: 1) the animal should be breathing comfortably in synchrony with the ventilator with jaw tone present but without voluntary limb movement; 2) the eyeballs should remain central in the orbit; 3) the animal should be unresponsive to light tactile stimuli. Criteria for reducing sedation in similar increments as noted above were monitored and included: 1) palpebral reflexes not present; 2) the animal is not responsive to painful stimuli (toe squeeze).

**Statistical Analysis**

Survival times were analyzed using a Cox Proportional Hazards model (7) with dose of bacteria treated as a continuous variable. Bacterial dose was then classified into three groups (low, medium, and high) and the survival rates across the groups were tested using a Mantel-Haenszel test (48). To analyze hemodynamic, laboratory, and measures of pulmonary and cardiac support data, animals were divided into three groups; survivors, subacute nonsurvivors
Results

Relationship between Survival and Bacterial Challenge

Increasing doses of intrabronchially inoculated \textit{S. aureus} (1 to $8 \times 10^9$ cfu/kg) resulted in dose dependent increases in mortality ($p = 0.04$; fig. 2A). In order to better characterize the ongoing pathophysiology and possible causes of death, the animals were separated based on timing of death (survivors, subacute deaths, and acute deaths; fig. 2B). Survivors ($n = 22$) received $4.0 \pm 0.4 \times 10^9$ cfu/kg (mean $\pm$ SE), while animals dying subacutely between 24 and 96 h received, $5.8 \pm 0.7 \times 10^9$ cfu/kg and those animals dying acutely within 24 h received $5.4 \pm 0.9 \times 10^9$ cfu/kg. Laboratory and hemodynamic results for control animals (no bacterial challenge)
that received all the same procedures over 96 h are reported in Supplement B to allow for comparison.

**Pulmonary Injury and Need for Supportive Therapies**

From 4 to 12 h after bacterial challenge, increases in arterial-alveolar oxygen gradient (A-aO₂) gradient, MPAP, and NE requirements and need for mechanical ventilatory support including FiO₂ levels and RR were ordered; acute nonsurvivors > subacute nonsurvivors > survivors (p < 0.02 to p < 0.0001). From 24 to 96 h after bacterial challenge, subacute nonsurvivors had increased A-aO₂ levels, MPAP, and NE requirements and the need for more mechanical ventilation support including FiO₂, PEEP, and RR compared to survivors (p < 0.003 to p < 0.008); (fig. 3A, C and 4A-D). PaO₂/FiO₂ ratio was not significantly different between groups, acutely (p=0.61) or subacutely (p=0.78) though the ratio fell during this time period from 4 to 96 h (fig. 3B).

**Nonpulmonary Abnormalities in Acute Nonsurvivors (< 24 h)**

Acute nonsurvivors had greater increases in lactate, hematocrit, and abnormalities of renal function (creatinine and blood urea nitrogen), then subacute nonsurvivors and survivors (p = 0.02 to p = 0.03); (fig. 3, 5 and 6). Changes in serum sodium and chloride were ordered; acute nonsurvivors > subacute nonsurvivors ≥ survivors (p = 0.04 to 0.02); (fig. 5). There were no significant differences between survivors and nonsurvivors (acute and subacute) in temperature, CI, EF, MAP, SVRI, PCWP, CVP, pH, base excess, potassium, bicarbonate, anion gap, amylase, albumin, number of fluid boluses, PEEP, sedation levels, and liver enzyme, platelet, polymorphonuclear, and total white blood cell counts (all p > 0.05); (fig. 3, 4, 5, 6, 7 and 8).
Nonpulmonary Abnormalities in Subacute Nonsurvivors (24 to 96 h)

Compared to survivors, subacute nonsurvivors had greater rises in liver enzymes (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline phosphatase), serum potassium, and hematocrit; lower pH, base excess, sodium, chloride, bicarbonate, and amylase levels. They also developed lower platelet, polymorphonuclear and total white blood cell counts. Urine volume and sedation requirements also decreased compared to survivors (all p = 0.04 to p = 0.003); (fig. 3, 4, 5, 6 and 7). There were no significant differences in temperature rise, frequency of positive blood culture or bacterial (cfu) levels, lactate levels, anion gap, renal function (creatinine and blood urea nitrogen levels), albumin, CI, EF, MAP, SVRI, PCWP, CVP, or number of fluid boluses received (all p > 0.05); (fig. 4, 6, 7 and 8).

Changes in Cytokine Levels

From 0 to 4 h, increases in IL-2 levels were ordered; acute nonsurvivors > subacute nonsurvivors > survivors (p = 0.01); (fig. 9). From 24 to 96 h, subacute nonsurvivors had greater rises in IL-2, IL-6, IL-10, IFNγ, and TGFβ1 levels then survivors (p = 0.02 to 0.008); (fig. 9). There were no significant differences in TNFα levels throughout the study.

Protein Expression

Global assessment of serum protein expression by principal component analysis of the m/z spectra (supplement C) revealed significant time (two-way ANOVA versus time, p <
0.0001) and bacterial dose effects (two-way ANOVA versus dose, p = 0.035) with no interaction between time and bacterial dose (p = 0.62).

**Discussion**

In this canine model, increasing doses of intrabronchial *S. aureus* resulted in increased mortality. Lung injury and vasopressor and ventilatory support requirements were ordered with the greatest injury and support needed in animals dying acutely and the least in survivors. These treatments were based on algorithms very similar to the titrated care routinely provided to critically ill patients. Despite antibiotics and escalating cardiopulmonary support, nonsurvivors developed more severe nonpulmonary injuries. Acute nonsurvivors (<24 h) demonstrated renal dysfunction, lower serum sodium, increased IL-2, and elevation of arterial lactate. Subacute nonsurvivors (24 to 96 h) had lower urine output, decreased platelet and white blood cell counts, elevated liver enzymes, increased pro- and anti-inflammatory cytokines (IL-2, IL-6, IL-10, IFNγ, and TGFβ1), lower serum sodium and bicarbonate levels, and lower pH. The multisystem organ dysfunction seen in nonsurvivors in this model is similar to the systemic abnormalities that can develop in patients with pneumonia complicated by severe sepsis. Importantly, metabolic abnormalities, organ injury, and survival differences in the model were not caused by the administration of sedation and analgesia. As bacteria dose increased, animals developed more severe sepsis and lethality but had decreased needs for these treatments. Despite these reductions, with constant observation by veterinarians or other trained personnel, levels of pain and distress were always kept within set criteria (see methods), i.e. well within ranges of humane animal care. Thus, this model fulfilled our prospectively established goal to simulate the sepsis
syndrome encountered in humans while balancing the ethical concerns for the welfare of experimental subjects by incorporating the best practices of both human and veterinary medicine.

We chose a pneumonia model employing gram positive bacterium because this site and type of bacterial infection is one of the most frequently encountered causes of sepsis (4). The *S. aureus* bacteria used to infect these animals is a well characterized, virulent clinical isolate (32). Furthermore, we speculated based on clinical experience and later confirmed in these experiments that it would be possible with sedatives and narcotics to achieve the relief of pain and distress associated with pulmonary infection. We performed a tracheostomy to provide a secure airway for prolonged intubation (5 days) and believed that this would be less stressful and better tolerated than oral intubation as it is in human patients. We also provided ancillary therapies, such as low dose anticoagulation, H-2 receptor blockers, chlorhexidine mouthwashes, and side-to-side rotation of the animals, to simulate practices that are considered beneficial in the routine intensive care of both humans and animals.

Prior large animal models of ventilator dependent pneumonia have been developed in canines (28) and sheep (17, 31) but these have been of shorter duration lasting from 5 to 48 h. A feasibility study attempting to determine whether healthy pigs could be ventilated for up to 7 days demonstrated complications including iatrogenic hemorrhage, secondary pneumonia and acute respiratory distress as early as 44 h into the studies (22). These prior models sought to simulate some aspects of clinical intensive care including the monitoring of arterial and central venous pressures, cardiac output and electrocardiograms. However, while fluids were titrated to maintain systemic blood pressures, vasopressors, if present, were not adjusted to minimize the potentially harmful effects of an excessive catecholamine infusion rate. Furthermore, while respiratory rate was adjusted to normalize pH, neither FiO₂ nor PEEP levels were titrated to
minimize oxygen toxicity or barotrauma. Also, sedation and analgesia were not titrated in these prior models to limit their potential detrimental effects as cardiopulmonary injury worsened. In contrast to these prior models, the present model attempted to account for each of these elements of care which are routinely adjusted in critically ill patients. As a result, it was possible to successfully support large animals in the present study for periods at least as long as 96 h.

Our prior *E. coli* peritonitis model was extensively used to test the effects of new therapies or physiologic interventions. In order to do this, the doses of supporting therapies (e.g. fluids) remained constant throughout an experiment except for initial adjustments based on animal weight (49). This approach allowed better isolation of treatment effects from the potential confounding ones related to varying levels of supportive care. It also minimized the number of animals needed for statistical significance and made conclusions more directly attributable to the intervention studied. In contrast, in this new pneumonia model supportive treatments are adjusted to the animal’s physiologic needs. This poses both disadvantages and advantages for the study of new therapies or interventions. As it may be more difficult to differentiate the effects of new treatments themselves from those related to differences in the level of support animals receive, greater numbers of animals may need to be studied to account for this variability. Alternatively, titration of supportive therapies based on the individual needs of the animals is similar to clinical care, making the model potentially more relevant. Moreover, because increased requirements for mechanical ventilation and vasopressors were associated with worsened outcomes, these adjustments in treatment may themselves be clinically important secondary endpoints independent of survival. These parameters might therefore be used as outcome measures to substantiate trends in survival, minimize animal requirements, and provide clinically useful information on mechanisms of therapeutic benefit.
Ultimately, models of septic shock should provide insights into factors that contribute to death. In the current study, two clinically important parameters both acutely (≤24) and subacutely (24 to 96 h) significantly separated survivors from nonsurvivors: the degree of lung injury and the level of fall in the serum sodium. The more severe lung injury in nonsurvivors was measured by increases in A-aO₂ gradients, elevations in mean pulmonary artery pressures as well as the need for more mechanical ventilation support, including greater FiO₂ concentrations, higher PEEP levels and more rapid breathing rates. The fall in serum sodium in nonsurvivors was associated with corresponding decreases in serum bicarbonate levels. When corrected for the fall in serum albumin, the survivor and nonsurvivors alike had from 24 to 96 h, no change in serum anion gap. The fall in bicarbonate levels and pH in nonsurvivors were due to a nonanion gap metabolic acidosis because serum sodium and bicarbonate levels fell proportionately. Chloride rises or is maintained at least in the first 12 h. This is consistent with a hyperchloremic metabolic acidosis secondary to resuscitation fluids.

In contrast to lung and metabolic abnormalities, there were no cardiovascular parameters that significantly separated survivors from nonsurvivors acutely or subacutely, although nonsurvivors did receive higher doses of norepinephrine throughout, and had lower trends for some cardiovascular performance measures (CI, LVEF, MAP). However, cardiac filling pressures, including central venous pressure and pulmonary capillary wedge pressure, were consistently elevated alike in survivors and nonsurvivors throughout. Furthermore, in this model, net fluid balance was overall positive, approximately 24 ml/kg/day over the 96 h experiment. When cardiovascular collapse did occur, it was in the setting of high cardiac filling pressures accompanied by infusions of large doses of vasopressors (40). Nonsurvivors acutely and subacutely had a rise in hematocrit. This does not appear to represent intravascular volume
depletion since cardiac filling pressures in nonsurvivors were, as noted above, similar to survivors and elevated throughout. Moreover, the nonsurvivors’ blood urea nitrogen was never elevated out of proportion to creatinine, as one would expect with intravascular volume depletion. The rise in hematocrit in nonsurvivors is most consistent with greater intravascular autotransfusion of blood from the spleen as described in this species in the setting of stress (51). Lastly, pancreatitis is a common and serious problem in this species (24). This was not likely contributing to death since serum amylases were normal in nonsurvivors and consistently lower than survivors.

The septic shock model developed here attempted to closely simulate the progression of severe sepsis as encountered when treating patients with pneumonia in the ICU. We sought to develop a tightly controlled, reproducible clinical environment in which the efficacy of new treatment protocols could later be studied. Over the 36 h after intrapulmonary bacterial challenge, like severe clinical cases of pneumonia (12, 54), animals developed increased temperatures, decreased white blood cell counts, and required progressively larger doses of vasopressor therapy and increased mechanical ventilatory support. In most survivors, compared to nonsurvivors, the need for therapeutic interventions were markedly less and did not progressively increase from 24 to 96 h. As seen clinically (12, 54), despite antibiotics, blood cultures for \textit{S. aureus} were intermittently positive, pro- and anti-inflammatory cytokines were elevated and multi-organ injury developed. Deaths were associated with severe lung injury, greater need for vasopressor and mechanical ventilation support, lower serum sodium and chloride levels. In addition, animals dying subacutely developed metabolic acidosis, acute renal dysfunction, elevated arterial lactate, liver function abnormalities, had greater increases in cytokines, lower white blood cell counts, lower platelet counts, and decreased urine output (3, 9,
30, 40, 46, 54). Despite these similarities in abnormalities to the human sepsis syndrome, we must still evaluate the effects of new therapies in the present pneumonia model and compare these results to those noted clinically in order to determine if this model is more or less predictive of efficacy in humans as our previous conscious peritonitis model. We believe this model successfully integrates humane animal care and routine clinical practices that are applicable to both the scientific investigation of septic shock as well as veterinary medicine at large.
FOOTNOTES

The authors do not have a commercial or other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding). The study was intramurally funded by the National Institutes of Health. Correspondences should be sent to: Steven Solomon, Ph.D., Critical Care Medicine Department, National Institutes of Health, Building 10, Room 2C145, Bethesda, MD  20892 or ssolomon@cc.nih.gov.
References


Figure Legends

1. **Study overview.** Treatments, laboratory measures, and procedures performed during the course of the 96 hour study.

2. **Survival as a function of A) increasing *S. aureus* dose and B) survival times comparing survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h).**

3. **Pulmonary (Panel A - C), pancreatic function (Panel D), renal (Panel E & F), and liver (Panel G – J) during the 96 h after *S. aureus* challenge.** The two horizontal gray bars represent the normal range adjusted for size of the comparison groups. Data are presented as a change (mean ± SE) from a common origin, the mean value for all animals at baseline. In each panel, the p-value at 12 h was examined for ordering among survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h). The p-value at 96 h compares survivors and subacute nonsurvivors (> 24 – 96 h). To increase our ability to find significant differences, all renal and liver enzymes were analyzed combined and an overall p-value is provided in panels E & G. For comparison, the individual group values at baseline (mean ± SE) for survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h), respectively were for Panel A, A-aO$_2$ (mmHg), –33 ± 22, -13 ± 16, -15 ±55 (p = 0.63); Panel B, P$_{aO2}$/FiO$_2$ (mmHg) 7.1 ± 1.3, 6.0 ± 0.9, 6.2 ± 0.2 (p = 0.75); Panel C, MPAP (mmHg), 15 ± 1, 15 ± 1, 15 ± 1 (p = 0.66); Panel D, Amylase (U/L), 809 ± 59, 821 ± 32, 1032 ±144 (p = 0.09); Panel E, creatinine (mg/dL), 0.58 ± 0.02, 0.61 ± 0.03, 0.54 ± 0.03 (p = 0.37); Panel F, BUN (mg/dL), 9.3 ± 0.6, 11.8 ± 0.9, 8.6 ± 1.1 (p = 0.99); Panel G, AST (U/L), 37 ± 2, 38 ± 3, 39 ± 4 (p=0.70); Panel H, ALT, 34 ± 2, 35 ± 2, 39 ± 6 (p = 0.32); Panel I, LDH (U/L), 64
± 9, 103 ± 46, 69 ± 14 (p = 0.02); Panel J, Alkphos (mg/dL), 41 ± 12, 29 ± 3, 22 ± 2 (p = 0.27).

4. **Pulmonary (Panel A, B, C) and cardiovascular (Panel D, E) support measures and sedation infusion levels (Panel F) during the 96 h after *S. aureus* challenge.** Serial changes from baseline (mean ± SE) are shown for survivors (> 96 h), subacute (>24–96 h), and acute nonsurvivors (< 24 h). In each panel, the p-value at 12 h was examined for ordering among survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h). The p-value at 96 h compares survivors and subacute nonsurvivors (> 24 – 96 h).

5. **Acid base status (Panel A & B), electrolytes (Panel C-E), arterial lactates (Panel G) and anion gap (Panel H) during the 96 h after *S. aureus* challenge.** The format is similar to Fig. 3. Data are presented as a change (mean ± SE) from a common origin, the mean value for all animals at baseline. In each panel, the p-value at 12 h was examined for ordering among survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h). The p-value at 96 h compares survivors and subacute nonsurvivors (> 24 – 96 h). For comparison, the individual group values at baseline (mean ± SE) for survivors (>96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (< 24 h), respectively, were for Panel A, pH, 7.38 ± 0.01, 7.35 ± 0.02, 7.33 ± 0.03 (p = 0.03); Panel B, base excess (mmol/L), -6.2 ± 0.01, -6.3 ± 0.5, -7.5 ± 1.3 (P = 0.14); Panel C, sodium (mmol/L), 145 ± 0.4, 145 ± 0.7, 146 ± 0.8 (p = 0.25); Panel D, potassium (mmol/L), 3.7 ± 0.1, 3.7 ± 0.1, 3.9 ± 0.1 (p = 0.09); Panel E, chloride (mmol/L), 117 ± 1, 116 ± 1, 117 ±1 (p = 0.45); Panel F, bicarbonate (mmol/L), 17.8 ± 0.3, 18.3 ± 0.4, 17.6 ± 1.4 (p = 0.91);
Panel G, arterial lactate (mmol/L), 0.9 ± 0.1, 1.5 ± 0.3, 0.7 ± 0.02 (p = 0.88); and Panel H, anion gap (mmol/L), 13.4 ± 0.8, 13.9 ± 0.4, 15.4 ± 0.1 (p = 0.04).

6. **Circulating blood cell components during the 96 h after *S. aureus* challenge.** The format is similar to Fig. 3. Data are presented as a change (mean ± SE) from a common origin, the mean value for all animals at baseline. In each panel, the p-value at 12 h was examined for ordering among survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h). The p-value at 96 h compares survivors and subacute nonsurvivors (> 24 – 96 h). For comparison individual group values at baseline (mean ± SE) for survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (< 24 h were for Panel A, hematocrit (%), 36 ± 1, 32 ± 1, 36 ± 2 (p = 0.53); platelets (K/uL), 182 ± 11, 215 ± 13, 199 ± 20 (p = 0.29); white blood cells (K/uL), 6.1 ± 0.6, 6.2 ± 0.4, 5.2 ± 0.6 (p = 0.43); polymorphs (K/uL), 4.9 ± 0.6, 5.0 ± 0.5, 4.0 ± 0.6 (p = 0.73) and albumin 2.37 ± 0.05, 2.38 ± 0.06, 2.5 ± 0.06 (p = 0.2).

7. **Serial changes (mean ± SE) after *S. aureus* challenge during the 96 h after *S. aureus* challenge in A) temperature, B) blood cultures and C) urine volume.** In Panel B, the number in parentheses to the right of each mean value represents the percent of animals alive with positive cultures for *S. aureus*. Temperature data are presented as changes (mean ± SE) from a common origin, the mean value for all animals at baseline. For panel A, the p-value at 12 h was examined for ordering among survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h). For panels A, B, ad C, the p-value at 96 h compares survivors and subacute nonsurvivors (> 24 – 96 h). For comparison, the individual group values at baseline (mean ± SE) for survivors (> 96 h),
subacute nonsurvivors (> 24 - 96 h), and acute nonsurvivors (< 24 h) were 36.2 ± 0.1, 36.5 ± 0.2, 36.6 ± 0.2 (p = 0.09).

8. **Cardiac function (Panel A & B), systemic (Panel C & D), and cardiac filling**

pressures (Panel E & F) during the 96 h after *S. aureus* challenge. The format is similar to Fig. 3. Data are presented as a change (mean ± SE) from a common origin, the mean value for all animals at baseline. In each panel, the p-value at 12 h was examined for ordering among survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h). The p-value at 96 h compares survivors and subacute nonsurvivors (> 24 – 96 h). For comparison individual group values at baseline (mean ± SE) for survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (< 24 h), respectively, were for Panel A, CI (L/kg/min), 1.8 ± 0.2, 2.2 ± 0.3, 2.3 ± 0.2 (p = 0.26); Panel B, EF (%), 60 ± 1, 60 ± 3, 58 ± 2 (p = 0.59); Panel C, MAP (mmHg), 85 ± 2, 85 ± 3, 84 ± 3 (p = 0.89), Panel D, SVRI (dynes/s•cm⁻⁵), 4.3 ± 0.3, 3.2 ± 0.4, 3.0 ± 1.4 (p = 0.27); Panel E, PCWP (mmHg), 8.7 ± 0.8, 8.9 ± 1.5, 9.4 ± 1.2 (p = 0.66); and Panel F, CVP (mmHg), 5.4 ± 0.7, 4.6 ± 0;8, 2.9 ± 1.4 (p = 0.26).

9. **Cytokine levels during the 96 h after *S. aureus* challenge (Panels A-F).** The format is similar to Fig. 3. Data are presented as a change (mean ± SE) from a common origin, the mean value for all animals at baseline. In each panel, the p-value at 4 h was examined for ordering among survivors (> 96 h), subacute nonsurvivors (> 24 – 96 h), and acute nonsurvivors (≤ 24 h). The p-value at 96 h compares survivors and subacute nonsurvivors (> 24 – 96 h). For comparison, the individual group values at baseline (mean ± SE) for survivors (> 96 h), subacute nonsurvivors (24 – 96 h), and acute nonsurvivors (<24 h), respectively, were for Panel A, IL2 (pg/ml), 4.55 ± 0.03, 5.0 ± 0.0, 5.2 ± 0.6 (p = 0.01);
Panel B, IL6 (pg/ml*10^5), 0.006 ± 0.002, 0.004 ± 0.002, 0.002 ± 0.002 (p = 0.09); Panel C, IL10 (pg/ml), 12 ± 2, 10 ± 0, 28 ± 18 (p = 0.26); Panel D, TNFα (pg/ml), 1.5 ± 0.6, 1.5 ± 0.9, 3.3 ± 2.0 (p = 0.68); Panel E, IFNγ (pg/ml), 15 ± 4, 14 ± 2, 12 ± 0 (p = 0.4); Panel F, TGFβ1 (pg/ml*10^4), 8.37 ± 1.0, 5.5 ± 2.8, 9.1 ± 1.2 (p = 0.9).
## Treatment

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<td>Sedation</td>
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## Laboratory Measures

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**General anesthesia**

- Tracheostomy
- Catheter placement
- Bacterial inoculation

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Figure 1
**Figure 2.**

**A.**

Bar graph showing bacterial dose (\(x 10^9\) cfu) versus percent surviving. The bars represent different groups with varying bacterial doses:

- n=2 for drought
- n=3 for group
- n=4 for group 1
- n=13 for group 2
- n=5 for group 3
- n=2 for group 4
- n=9 for group 5

The p-value for the comparison is 0.04.

**B.**

Survival curve showing percent surviving versus time (h) for different groups:

- Survivor N=22
- Non-Survivor >24-96 h N=8
- Non-Survivor <24 h N=8
Figure 3.

A. Arterial-alveolar Oxygen Gradient

B. PaO$_2$/FiO$_2$

C. Mean Pulmonary Arterial Pressure

D. Amylase

E. Blood Urea Nitrogen

F. Creatinine

G. Alanine Aminotransferase

H. Aspartate Aminotransferase

I. Alkaline Phosphatase

J. Lactate Dehydrogenase

* $p = 0.02$

** $p = 0.32$

+ $p = 0.31$

++ $p = 0.0004$

+++ $p = 0.00004$
Figure 7.

A. Temperature

B. Blood Culture

C. Urine Volume
Figure 8.

A. Cardiac Index

B. Ejection Fraction

C. Mean Arterial Pressure

D. Systemic Vascular Resistance Index

E. Pulmonary Capillary Wedge Pressure

F. Central Venous Pressure

Copyright Information
Figure 9.

A. IL2

B. IL6

C. IL10

D. TNFa

E. IFNg

F. TGFb1

Survivor
Non-Survivor 24-96 h
Non-Survivor <24 h

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