A canine model of septic shock: balancing animal welfare and scientific relevance

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More than two decades ago, our group (33, 36) developed a conscious canine model of Escherichia coli peritonitis, which reproduced the cardiovascular profile of human septic shock. 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 1990s 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, although 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 with the canine peritonitis model. Given the continuing importance of a large animal model in understanding the pathogenesis of sepsis and for the evaluation of 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.

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after sterile saline in the lower lobe of the right lung. During the first 4 h scope was then used at cultured, and blood samples were taken for analysis. A broncho-
ventilation via an endotracheal tube were employed before trache-
the tracheostomy, general anesthesia was discontinued and seda-
tion (fentanyl, midazolam, and medetomidine) was started. Me-
mentations, and arterial blood gas monitoring (see mechanical ventilation and blood gas monitoring (see Mechanical ventilation and fluid and vasopressors, below). Antibiotics (ceftriaxone, 50 mg/kg iv, every 24 h) 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, and 4. Animals alive at 96 h were considered survivors and subsequently were euthanized (Beuthanol; 75 mg/kg iv).

Surgical procedures. Animals were fasted for 12 h before surgery. After intravenous induction with propofol (4–6 mg/kg), tracheal intubation (6 Fr; Rusch, Duluth, GA), and the initiation of mechanical ventilation (Fabius Tiro, Drager Medical, Telford, PA), an incision was made into the soft tissue of the tracheal wall, and a tracheos-
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 with the use of sterile technique (53). A 7-Fr pulmonary artery thermodilution catheter (Abbott Critical Care, Chicago, IL) was in-
317x369duced through an 8-Fr introducer via the external jugular vein into the pulmonary artery. A 20-gauge arterial catheter was placed into the femoral artery. With the use of sterile technique, a 55-cm urinary catheter (Cook, Foley 8 Fr) 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 bidometrically. The suspension was then adjusted with PBS to pro-
duce a concentration of 0–8 \times 10^9 colony-forming units (cfu)/ml of S. aureus. The concentrations were confirmed by plating serial dilu-
tions on the appropriate culture medium and counting colonies as previously described (32, 36).

Bacterial inoculation. Under continuous intravenous sedation (see above), animals received preoxygenation with 100% O2 for 5 min, the tracheal tube was removed, and a sterile bronchoscope (Olympus BF IT20, Lake Success, NY) was advanced via the tracheal stoma, under direct vision into the right lower lobe segmental bronchus. A pul-
309nary arterial thermodilution catheter was advanced via the suction port of the bronchoscope and wedged with the balloon inflated into a subsegmental bronchus (18). Ten milliliters of a solution with a known amount of 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.

### 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 mo, 10–12 kg) were studied; 16 of these animals were used as controls for a subsequent intra-aortic balloon counterpulsation study. These 16 animals received increasing doses of Staphylococcus aureus and were managed with 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 (24–96 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 before trache-
335ostomy and before placement of percutaneous femoral arterial and a urinary bladder catheter. After the tracheostomy, general anesthesia was discontinued and seda-
tion (fentanyl, midazolam, and medetomidine) was started. Me-
327chanical 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 broncho-
316scope 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, mainte-
nance fluids (Normosol-M with 27 meq KCl added; 2 ml·kg⁻¹·h⁻¹) and vasopressors [phenylephrine titrated to mean arterial pressure (MAP) >80 mmHg] were administered. KCl was added to Normosol-M (dextrose, sodium chloride, potassium acet-
te, 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; next, intravascular hemo-
dynamics and cardiac function were measured, and blood samples were taken. Treatment for sepsis was then initiated and individu-
327lized 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 ac-
317cording to continuously measured oxygen saturation, MAP, inter-
317mittent pulmonary capillary wedge pressure (PCWP) determina-
tions, and arterial blood gas monitoring (see Mechanical ventila-
tion and Fluid and vasopressors, below). Antibiotics (ceftriaxone, 50 mg/kg iv, every 24 h) were started 4 h after bacterial inoculation

### Treatment

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Fig. 1. Study overview: treatments, laboratory measures, and procedures performed during the course of the 96-h study (time in hours on x-axis).
Mechanical ventilation. The ventilator (Servovent 300, Siemens Medical) was initially set with a fractional inspired oxygen concentration (Fi\textsubscript{O\textsubscript{2}}) = 25%, positive end-expiratory pressure (PEEP) = 5 cmH\textsubscript{2}O, tidal volume = 15 ml/kg, and respiratory rate (RR) = 15 breaths/min. If the \textsubscript{O2} saturation fell below 92%, attempts to restore \textsubscript{O2} saturation to ≥92% were made in the following incremental sequence: Fi\textsubscript{O2} increased to 50%, PEEP increased to 10 cmH\textsubscript{2}O and then to 12 cmH\textsubscript{2}O, and Fi\textsubscript{O2} increased to 75% and then to 100%. The maximum settings were Fi\textsubscript{O2} = 100% and PEEP = 12 cmH\textsubscript{2}O. Support was similarly reduced if the \textsubscript{O2} saturation was ≥93% for 6 h. The blood-gas measures (every 2 h until 8 h posttreatment and then every 8 h thereafter) were used to set RR on the mechanical ventilator. RR was increased by increments of 5 breaths/min to maintain arterial \textsubscript{PCO2} under 35 Torr or decreased by 5 if pH >7.35 and arterial \textsubscript{PCO2} ≤30 Torr. The maximum setting was 35 and the minimum was 15 breaths/min. Plateau airway pressures were measured and maintained at <35 cmH\textsubscript{2}O.

Fluids and suppressors. 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 ensure that sedation did not cause hypotension in any animal while sepsis was developing. During this time, a maintenance intravenous fluid infusion of Normosol-M with 27 meq KCl added (2 ml·kg\textsuperscript{-1}·h\textsuperscript{-1}) was administered. Four hours after bacterial inoculation, the phenylephrine infusion was turned off for a washout period of 10 min.

At 4 h after bacterial inoculation, 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 three fluid boluses 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\textsuperscript{-1}·min\textsuperscript{-1}) to maintain a MAP between 80 and 110 mmHg (low normal for these animals). At subsequent time points (every 2 h until 12 h posttreatment and every 4 h thereafter) until the end of the study, if the PCWP was <10 mmHg, an additional intravenous fluid bolus (20 ml/kg) was administered. A maintenance fluid infusion (Normosol-M with 27 meq KCl added; 2 ml·kg\textsuperscript{-1}·h\textsuperscript{-1}) was continued from 4 to 96 h.

Other intensive care unit 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 hindlimbs were serially rotated between left and right position. Passive limb movement (forelimbs and hindlimbs) 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 accumulated. 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 (Chatherrth III, Hudson RCI-AB) attached to the airway system. To protect the animals from stress-induced stomach ulcers, famotidine (1 mg/kg iv every 12 h), an H\textsubscript{2} blocker, was administered; to protect animals from venous thrombosis during mechanical ventilation and sedation, heparin (3,000 IU im, every 8 h) was administered until the end of the study.

Physiological measurements. Cardiac output, 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 was calculated via the femoral arterial pressure recording. Left ventricular ejection fraction (EF) was determined by cardiac ultrasound (Sonos 5500, Philips Medical). The cardiac output was standardized to the animal’s weight in kilograms [cardiac index (CI)]. These measurements were performed at baseline (before bacterial inoculation) and at 4, 24, 48, 72, and 96 h after intrabronchial bacterial inoculation.

Laboratory data. Arterial and mixed venous blood gases were measured every 2 h until 8 h after inoculation and every 8 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, and 24 h and every 24 h thereafter. Blood was also obtained for quantitative blood cultures (isolator tubes; Wampole, Cranbury, NJ) at 4, 8, and 24 h and every 24 h thereafter.

Cytokines. Plasma was collected at baseline and at 4, 24, 48, 72, and 96 h after inoculation. From these samples, using the Searchlight multiplex array system, canine IL-2, IL-6, IL-10, TNF-α, IFN-γ, and transforming growth factor-β1 (TGF-β1) levels were measured (Pierce Biotechnology, Rockville, IL).

Sedation and analgesia management. The level of sedation and analgesia was adjusted by a clinician or trained technician continously at the bedside for 96 h after initiation of midazolam (0.2 mg/kg loading dose; 50 μg·kg\textsuperscript{-1}·min\textsuperscript{-1} infusion) and fentanyl (5 μg/kg loading dose; 0.7 μg·kg\textsuperscript{-1}·min\textsuperscript{-1} infusion). Both the fentanyl and midazolam infusions were increased in increments of one-fourth of the dose every 5 min until adequate sedation was obtained. Medetomidine infusion (2–5 μg·kg\textsuperscript{-1}·min\textsuperscript{-1}) was used to supplement sedation as needed by set 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, and 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 and 2) animal not responsive to painful stimuli (toe squeeze).

Statistical analysis. Survival times were analyzed by 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 with a Mantel-Haenszel test (48). To analyze hemodynamic, laboratory, and measures of pulmonary and cardiac support data, animals were divided into three groups: survivors (survival time ≥96 h), subacute nonsurvivors (survival times between 24 and 96 h), and acute nonsurvivors (survival times ≤24 h). Baseline differences among the three groups were tested by one-way ANOVA. Hemodynamic and laboratory analyses were performed by computing the change in each variable from baseline to the follow-up time points for each animal. During the first 24 h, data were analyzed by a three-way ANOVA procedure (47). The three factors in the ANOVA model included group (survivors, subacute nonsurvivors, and acute nonsurvivors), dog nested within group, and time. An interaction between group and time was included in the model, and a test for ordered effects was performed (1). Interactions between dog and time formed
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 \( 4.0 \pm 0.4 \times 10^6 \) cfu/kg (mean ± SE), whereas animals dying subacutely between 24 and 96 h received \( 5.8 \pm 0.7 \times 10^6 \) cfu/kg and those animals dying acutely within 24 h received \( 5.4 \pm 0.9 \times 10^6 \) cfu/kg. Laboratory and hemodynamic results for control animals (no bacterial challenge) that received all of the same procedures over 96 h are reported in supplement B in supplementary materials 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, 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 arterial-alveolar oxygen gradient levels, MPAP, and NE requirements and the need for more mechanical ventilation support including FiO₂, PEEP, and RR compared with survivors \( (P < 0.003 \) to \( P < 0.008 \)) (Fig. 3, A and C, and 4, AD). Arterial P__₀₂_to_P__₀₂ ratio was not significantly different between groups, acutely \( (P = 0.61) \) or subacutely \( (P = 0.78) \), although the ratio fell during this time period from 4 to 96 h (Fig. 3B).

**Nonpulmonary abnormalities in acute nonsurvivors** \((\leq 24 \) 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, systemic vascular resistance index, 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 \( P > 0.05 \)) (Figs. 3–8).

**Nonpulmonary abnormalities in subacute nonsurvivors** \((24–96 \) h). Compared with survivors, subacute nonsurvivors had greater rises in liver enzymes (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline phosphatase), serum potassium, and hematocrit and lower pH, base excess, sodium, chloride, bicarbonate, and amylase levels. They also developed lower platelet and polymorphonuclear and total white blood cell counts. Urine volume and sedation requirements also were decreased compared with survivors (all

**RESULTS**

**Relationship between survival and bacterial challenge.** Increasing doses of intrabronchially inoculated *S. aureus* \((1 \) to \( 8 \times 10^9 \) cfu/kg) resulted in dose-dependent increases in mortality \( (P = 0.04; \) Fig. 2A). To better characterize the

**Fig. 2.** Survival as a function of increasing *S. aureus* dose \( (A) \) and survival times \( (B) \) comparing survivors (>96 h until death), subacute nonsurvivors (>24 to 96 h until death), and acute nonsurvivors (<24 h until death). cfu, Colony-forming units.

the error term. After the first 24 h, data were analyzed with a similar three-way ANOVA, although only two groups of animals (survivors and subacute nonsurvivors) remained for analyses. Descriptive \( P \) values are presented as a method to compare the magnitude of effects across different hemodynamic and laboratory parameters, with \( P = 0.05 \) used to declare statistical significance.

**Fig. 3.** Pulmonary \((A–C), \) pancreatic \((D), \) renal \((E \) and \( F), \) and liver \((G–J) \) function during the 96 h after *S. aureus* challenge. The 2 horizontal gray bars represent the normal range adjusted for size of the comparison groups. Data are presented as change (means ± SE) from a common origin (mean value for all animals at baseline). In each panel, \( P \) value at 12 h was examined for ordering among survivors, subacute nonsurvivors, and acute nonsurvivors. \( P \) value at 96 h compares survivors and subacute nonsurvivors. To increase our ability to find significant differences, all renal and liver enzymes were analyzed combined, and an overall \( P \) value is provided in \( E \) and \( G . \) For comparison, individual group values at baseline (means ± SE) for survivors, subacute nonsurvivors, and acute nonsurvivors, \( P \) value at 96 h compares survivors and subacute nonsurvivors. To increase our ability to find significant differences, all renal and liver enzymes were analyzed combined, and an overall \( P \) value is provided in \( E \) and \( G . \) For comparison, individual group values at baseline (means ± SE) for survivors, subacute nonsurvivors, and acute nonsurvivors, respectively, are as follows: in \( A , \) arterial-alveolar oxygen gradient (mmHg) = \(- 33 \pm 22, - 13 \pm 16, \) and \(- 15 \pm 55 (P = 0.63) \); in \( B , \) ratio of arterial \( P_{\text{A/O}} \) (PaO₂) to fractional inspired oxygen concentration (FiO₂) (mmHg) = \( 7.1 \pm 1.3, 6.0 \pm 0.9, 6.2 \pm 0.2 (P = 0.75) \); in \( C , \) mean pulmonary arterial pressure (mmHg) = \( 15 \pm 1.15 \pm 1, 15 \pm 1 (P = 0.66) \); in \( D , \) amylase (U/l) = \( 809 \pm 59, 821 \pm 32, 1032 \pm 144 (P = 0.09) \); in \( E , \) blood urea nitrogen (mg/dl) = \( 9.3 \pm 0.6, 11.8 \pm 0.9, 8.6 \pm 1.1 (P = 0.99) \); in \( F , \) creatinine (mg/dl) = \( 0.58 \pm 0.02, 0.61 \pm 0.03, 0.54 \pm 0.03 (P = 0.37) \); in \( G , \) alanine aminotransferase = \( 34 \pm 2.35 \pm 2, 39 \pm 6 (P = 0.32) \); in \( H , \) aspartate aminotransferase (U/l) = \( 37 \pm 2, 38 \pm 3, 39 \pm 4 (P = 0.70) \); in \( I , \) alkaline phosphatase (mg/dl) = \( 41 \pm 12, 29 \pm 3, 22 \pm 2 (P = 0.27) \); in \( J , \) lactate dehydrogenase (U/l) = \( 64 \pm 9, 103 \pm 46, 69 \pm 14 (P = 0.02) \).
CANINE MODEL OF SEVERE BACTERIAL PNEUMONIA

A. Arterial-alveolar Oxygen Gradient

B. PaO₂/FiO₂

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

Time (h)
Fig. 4. Pulmonary (A–C) and cardiovascular (D and E) support measures and sedation infusion levels (F) during the 96 h after S. aureus challenge. Serial changes from baseline (mean ± SE) are shown for survivors, subacute nonsurvivors, and acute nonsurvivors. In each panel, *P* value at 12 h was examined for ordering among survivors, subacute nonsurvivors, and acute nonsurvivors. *P* value at 96 h compares survivors and subacute nonsurvivors.
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, IFN-\( \gamma \), and TGF-\( \beta_1 \) levels than survivors (\( P = 0.02 \) to 0.008) (Fig. 9). There were no significant differences in TNF-\( \alpha \) levels throughout the study.

Protein expression. Global assessment of serum protein expression by principal component analysis of the \( m/z \) spectra (see supplement C in supplemental materials) revealed significant time (two-way ANOVA vs. time, \( P < 0.0001 \)) and bacterial dose effects (two-way ANOVA vs. 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 vasoressor 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 (\( \leq 24 \) h) demonstrated renal dysfunction, lower serum sodium, increased IL-2, and elevated 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, IFN-\( \gamma \), and TGF-\( \beta_1 \)), lower serum sodium and bicarbonate levels, and lower \( \mathrm{pH} \). 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 MATERIALS AND 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 that employed 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 that attempted 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 pressure and CVP, cardiac output, and electrocardiograms. However, although fluids were titrated to maintain systemic blood pressures, vasoressors, if present, were not adjusted to minimize the potentially harmful effects of an excessive catecholamine infusion rate. Furthermore, although RR was adjusted to normalize \( \mathrm{pH} \), neither \( F_{O_2} \) 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 that 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 physiological interventions. 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 physiological needs. This poses both disadvantages and advantages for the study of new therapies or interventions. Because 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 vasoressors are associated with worsened outcomes, these adjustments in treat-
Fig. 5. Acid base status (A and B), electrolytes (C–E), arterial lactate (G), and anion gap (H) during the 96 h after *S. aureus* challenge. The format is similar to Fig. 3. Data are presented as change (means ± SE) from a common origin (mean value for all animals at baseline). In each panel, *P* value at 12 h was examined for ordering among survivors, subacute nonsurvivors, and acute nonsurvivors. *P* value at 96 h compares survivors and subacute nonsurvivors. For comparison, individual group values at baseline (mean ± SE) for survivors, subacute nonsurvivors, and acute nonsurvivors were as follows: in A, pH = 7.38 ± 0.01, 7.35 ± 0.02, 7.33 ± 0.03 (*P* = 0.03); in B, base excess (mmol/l) = −6.2 ± 0.01, −6.3 ± 0.5, −7.5 ± 1.3 (*P* = 0.14); in C, sodium (mmol/l) = 145 ± 0.4, 145 ± 0.7, 146 ± 0.8 (*P* = 0.25); in D, potassium (mmol/l) = 3.7 ± 0.1, 3.7 ± 0.1, 3.9 ± 0.1 (*P* = 0.09); in E, chloride (mmol/l) = 117 ± 1, 116 ± 1, 117 ± 1 (*P* = 0.45); in F, bicarbonate (mmol/l) = 17.8 ± 0.3, 18.3 ± 0.4, 17.6 ± 1.4 (*P* = 0.91); in G, arterial lactate (mmol/l) = 0.9 ± 0.1, 1.5 ± 0.3, 0.7 ± 0.02 (*P* = 0.88); and in H, anion gap (mmol/l) = 13.4 ± 0.8, 13.9 ± 0.4, 15.4 ± 0.1 (*P* = 0.04).

Fig. 6. Circulating blood cell components during the 96 h after *S. aureus* challenge. The format is similar to Fig. 3. Data are presented as change (means ± SE) from a common origin (mean value for all animals at baseline). In each panel, *P* value at 12 h was examined for ordering among survivors, subacute nonsurvivors, and acute nonsurvivors. *P* value at 96 h compares survivors and subacute nonsurvivors. For comparison, individual group values at baseline (mean ± SE) for survivors, subacute nonsurvivors, and acute nonsurvivors were as follows: in A, hematocrit (%) = 36 ± 1, 32 ± 1, 36 ± 2 (*P* = 0.53); in B, platelets [1,000 (K)/μl] = 182 ± 11, 215 ± 13, 199 ± 20 (*P* = 0.29); in C, white blood cells (K/μl) = 6.1 ± 0.6, 6.2 ± 0.4, 5.2 ± 0.6 (*P* = 0.43); in D, polymorphonuclear cells (K/μl) = 4.9 ± 0.6, 5.0 ± 0.5, 4.0 ± 0.6 (*P* = 0.73); and in E, albumin (g/dl) = 2.37 ± 0.05, 2.38 ± 0.06, 2.5 ± 0.06 (*P* = 0.2).
ment 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 present 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 arterial-alveolar oxygen gradients, elevations in MPAP, and 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 was 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 NE throughout and had lower trends for some cardiovascular performance measures (CI, left ventricular EF, MAP). However, cardiac filling pressures, including CVP and PCWP, were consistently elevated similarly in survivors and nonsurvivors throughout. Furthermore, in this model, net fluid balance was overall positive, ~24 ml·kg⁻¹·day⁻¹ 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 because cardiac filling pressures in nonsurvivors were, as noted above, similar to survivors and elevated throughout. Moreover, the nonsurvivors’ blood urea nitrogen levels were 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 did not likely contribute to death because serum amylase levels were normal in nonsurvivors and consistently lower than levels in 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 intensive care unit. 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

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**Fig. 7.** Serial changes (means ± SE) during the 96 h after *S. aureus* challenge in temperature (A), blood cultures (B), and urine volume (C). In B, nos. in parentheses represent the percentages of animals alive with positive cultures for *S. aureus*. Temperature data are presented as changes (mean ± SE) from a common origin (mean value for all animals at baseline). For A, P value at 12 h was examined for ordering among survivors, subacute nonsurvivors, and acute nonsurvivors. For comparison, the individual group values at baseline (means ± SE) for survivors, subacute nonsurvivors, and acute nonsurvivors were 36.2 ± 0.1, 36.5 ± 0.2, and 36.6 ± 0.2 (P = 0.09).
animals developed increased temperatures and decreased white blood cell counts and required progressively larger doses of vasopressor therapy and increased mechanical ventilatory support. In most survivors, compared with nonsurvivors, the need for therapeutic interventions was markedly less and did not progressively increase from 24 to 96 h. As seen clinically (12, 54), despite antibiotics, blood cultures for *S. aureus* were intermittently positive, pro- and anti-inflammatory cytokines were elevated, and multiorgan injury developed. Deaths were associated with severe lung injury, greater need for vasopressor and mechanical ventilation support, and lower serum sodium and chloride levels. In addition, animals dying subacutely developed metabolic acidosis, acute renal dysfunction, and elevated arterial lactate and liver function abnormal-

![Cardiac function (A and B) and systemic (C and D) and cardiac filling pressures (E and F) during the 96 h after *S. aureus* challenge. The format is similar to Fig. 3. Data are presented as change (means ± SE) from a common origin (mean value for all animals at baseline). In each panel, *P* value at 12 h was examined for ordering among survivors, subacute nonsurvivors, and acute nonsurvivors. *P* values at 96 h compare survivors and subacute nonsurvivors. For comparison, individual group values at baseline (means ± SE) for survivors, subacute nonsurvivors, and acute nonsurvivors, respectively, were as follows: in A, cardiac index (l·kg⁻¹·min⁻¹) = 1.8 ± 0.2, 2.2 ± 0.3, 2.3 ± 0.2 (*P* = 0.26); in B, ejection fraction (%) = 60 ± 1, 60 ± 3, 58 ± 2 (*P* = 0.59); in C, mean arterial pressure (mmHg) = 85 ± 2, 85 ± 3, 84 ± 3 (*P* = 0.89); in D, systemic vascular resistance index (dyn⁻¹·cm⁻⁵) = 4.3 ± 0.3, 3.2 ± 0.4, 3.0 ± 1.4 (*P* = 0.27); in E, pulmonary capillary wedge pressure (mmHg) = 8.7 ± 0.8, 8.9 ± 1.5, 9.4 ± 1.2 (*P* = 0.66); and in F, central venous pressure (mmHg) = 5.4 ± 0.7, 4.6 ± 0.8, 2.9 ± 1.4 (*P* = 0.26).

(12, 54), animals developed increased temperatures and decreased white blood cell counts and required progressively larger doses of vasopressor therapy and increased mechanical ventilatory support. In most survivors, compared with nonsurvivors, the need for therapeutic interventions was markedly less and did not progressively increase from 24 to 96 h. As seen clinically (12, 54), despite antibiotics, blood cultures for *S. aureus* were intermittently positive, pro- and anti-inflammatory cytokines were elevated, and multiorgan injury developed. Deaths were associated with severe lung injury, greater need for vasopressor and mechanical ventilation support, and lower serum sodium and chloride levels. In addition, animals dying subacutely developed metabolic acidosis, acute renal dysfunction, and elevated arterial lactate and liver function abnormal-
ities and 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 with those noted clinically to determine whether this model is more or less predictive of efficacy in humans as our previous conscious peritonitis model. We believe that 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.

Fig. 9. Cytokine levels during the 96 h after S. aureus challenge (A–F). Format is similar to Fig. 3. Data are presented as change (means ± SE) from a common origin (mean value for all animals at baseline). In each panel, P value at 4 h was examined for ordering among survivors, subacute nonsurvivors, and acute nonsurvivors. P values at 96 h compare survivors and subacute nonsurvivors. For comparison, the individual group values at baseline (means ± SE) for survivors, subacute nonsurvivors, and acute nonsurvivors, respectively, were as follows: in A, IL-2 (pg/ml) = 4.55 ± 0.03, 5.0 ± 0.0, 5.2 ± 0.6 (P = 0.01); in B, IL-6 [pg/ml (×10^3)] = 0.006 ± 0.002, 0.004 ± 0.002, 0.002 ± 0.002 (P = 0.09); in C, IL-10 (pg/ml) = 12 ± 2, 10 ± 0, 28 ± 18 (P = 0.26); in D, TNF-α (pg/ml) = 1.5 ± 0.6, 1.5 ± 0.9, 3.3 ± 2.0 (P = 0.68); in E, IFN-γ (pg/ml) = 15 ± 4, 14 ± 2, 12 ± 0 (P = 0.4); in F, transforming growth factor (TGF)-β1 [pg/ml (×10^3)] = 8.37 ± 1.0, 5.5 ± 2.8, 9.1 ± 1.2 (P = 0.9).
GRANTS

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REFERENCES


