Original Article

A Novel Porcine Model of Septic Shock Induced by Acute Respiratory Distress Syndrome due to Methicillin-resistant *Staphylococcus aureus*

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Abstract

Background: Sepsis is one of the main causes of mortality in critically ill patients following progression to septic shock. To investigate the pathophysiologic changes of sepsis, we developed a novel porcine model of septic shock induced by acute respiratory distress syndrome (ARDS) due to methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia.

Methods: Twenty-six male Landraces (Lvyuanweiye, Beijing, China) weighing 30 ± 2 kg were divided into four groups: sham group (SH; $n = 5$); cotton smoke inhalation group (SM; $n = 6$); MRSA pneumonia group (MR; $n = 6$); and septic shock group with cotton smoke inhalation + MRSA pneumonia (SS; $n = 9$). Extensive hemodynamics, oxygen dynamics, and lung function were monitored for 24 h following the injury or until death. Tissues were collected, and histopathology evaluations were carried out.

Results: Blood cultures from 6 of 9 animals in the SS group were positive for MRSA. Two hours following the injury, decreased mean arterial blood pressure (60–70 mmHg) and cardiac index (<2 $L \cdot min^{-1} \cdot m^{-2}$) were observed in the animals in the SS group, while systemic vascular resistance index was increased. The hemodynamic characteristics of septic shock were only observed in the SS group but not significant in the other groups. The PO_2/FiO_2 in the SM and SS groups decreased to 300 and 100, respectively. In the SS group, extravascular lung water index increased to 20 ml/kg, whereas thoracopulmonary compliance decreased to 10 ml/H_2O after injury. Deterioration of pulmonary function in the SS group was more serious than the SM and MR groups. Severe lung injury in the SS group was confirmed by the histopathology evaluations. The lung injury confirmed by high-resolution thin-section computed tomography and histopathology in the SS group was more serious than those of other groups.

Conclusions: In the present study, we developed a novel porcine model of septic shock induced by ARDS due to severe MRSA pneumonia with characteristic hyperdynamic and hypodynamic phases in 24 h, which mimicked the hemodynamic changing of septic shock in human.

Key words: Acute Respiratory Distress Syndrome; Hemodynamic; Methicillin-resistant *Staphylococcus aureus*; Oxygen Dynamic; Septic Shock

Introduction

As a complex syndrome of multiple organ dysfunction induced by severe infection, sepsis is one of the main causes of mortality in critically ill patients following progression to severe sepsis or septic shock.[1] Although modern treatment strategies have been continually developed and improved, there are still remaining issues about the pathophysiologic development of sepsis. Pneumonia, which is one of the common causes of sepsis,[2,3] can lead to acute respiratory distress syndrome (ARDS). Both sepsis and ARDS are characterized by inflammation and organ dysfunction. In addition, severe sepsis is the most common etiology of ARDS, and the patients with sepsis-induced ARDS have higher mortality than patients with other risk factors of ARDS.[4]

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In the past two decades, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major cause of pneumonia and toxic shock syndrome (TSS). An increasing number of patients with community-acquired MRSA have been reported.[5] MRSA infection has also been found to be a predominant cause (38%) of ventilator-associated pneumonia in Surgical Intensive Care Units.[6] Furthermore, a recent epidemiology study has shown that 29.8% of the patients with MRSA pneumonia developed severe sepsis and 12.9% developed septic shock.[7] Since the frequency of pneumonia caused by MRSA is increasing and is associated with the high morbidity and mortality rates, it is important to understand the underlying pathophysiology and histopathology, which may lead to optimization of treatment strategy.

Several ovine models for sepsis caused by MRSA have been developed, in which sepsis was induced by infusing MRSA or *Pseudomonas aeruginosa* through the trachea after smoke inhalation.[8-10] However, these models are limited in their ability to mimic precisely the whole septic shock process and did not show the TSS symptoms. Several clinical features of human sepsis are used to validate the relevance of animal models, which include two distinct periods: the hyperdynamic phase and hypodynamic phase. Even all of these models showed an obvious hyperdynamic phase, none of them showed a hypodynamic phase in 24–48 h after injury. On the other hand, these models did not include oxygen dynamic and lung functional changes. To investigate the underlying mechanisms and to develop new therapeutic approaches, there is an urgent need to develop a septic model that mimics the human disease progression.

In this study, we developed a novel porcine model of septic shock that was induced by MRSA pneumonia after cotton smoke inhalation. The duration of the protocol was 24 h, and the model showed both significant hyper- and hypo-dynamic phases. Furthermore, the typical characteristics of lung function and pathology changes in ARDS were observed. It can be used for further investigation of MRSA-induced septic shock.

Methods

Animal preparation

The animal study was approved by the Animal Care and Use Committee of Beijing Chao-Yang Hospital, Capital Medical University, and the use of animals was in compliance with the National Research Council's 1996 Guide for the Care and Use of Laboratory Animals. The protocol was done in the laboratory of animals with approving of the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University.

Twenty-six healthy, male, Landraces (Lvyuanweiye, Beijing, China) with normal diet, aged 8–10 weeks, and weighing 30 ± 2 kg were used in this study. They were purchased from Beijing Lvyuan Weiye Laboratory Animal Center (License No. SCXK 11-00-002). Animals were fasted overnight and had free access to water. After premedication with intramuscular midazolam at 0.2 mg/kg, anesthesia was maintained with continuous intravenous infusion of pentobarbital (8 $mg \cdot kg^{-1} \cdot h^{-1}$) and fentanyl (5 $\mu g \cdot kg^{-1} \cdot h^{-1}$). Ringer's solution (15 $ml \cdot kg^{-1} \cdot h^{-1}$) was administered intravenously; the infusion speed was adjusted to maintain a sufficient preload. A 5-Fr PiCCO catheter (Pulsiocath PV2015L20; Pulsion Medical Systems, Munich, Germany) and central venous catheter were inserted into the descending aorta and the right atrium through the femoral artery and femoral vein, respectively. The arterial and central venous catheters were connected to an integrated bedside monitor (Philips Medical Systems; Best, Holland) for continuous hemodynamic and blood temperature monitoring.

All animals were intubated using a cuffed 6.5-mm endotracheal tube and mechanically ventilated with a volume-controlled ventilator (Evita 4; Dräger Medizintechnik, Lübeck, Germany) at a tidal volume of 8 ml/kg, respiratory frequency of 12 breaths/min, and positive end-expiratory pressure (PEEP) of 5 cmH_2O. The respiratory frequency and fraction of inspired oxygen (FiO_2) were adjusted to maintain an end-tidal concentration of carbon dioxide ($EtPCO_2$) of 35–40 mmHg and arterial oxygen saturation (SpO_2) at more than 90%, respectively.

Experimental protocols

After 1 h recovery period, the baseline data were recorded. All animals were divided into four groups: sham group (SH; $n = 5$); cotton smoke inhalation group (SM; $n = 6$); MRSA pneumonia group (MR; $n = 6$); and septic shock group (SS; $n = 9$). The sham animals only received catheter implantations and mechanical ventilation. The pigs in the SM and SS groups were subjected to cotton smoke inhalation by means of a bee smoker filled with about 50 g of burning cotton toweling to induce smoke injury according to previously described methods.[8-10] Four sets of 12 inhalations (a total of 48) of cotton smoke were insufflated into the lungs, and the arterial carboxyhemoglobin level was measured using a portable monitor (Rad-57; Masimo Corp., Irvine, CA, USA) after each set of smoke inhalations to ensure that each animal received an equivalent dose of smoke.

Live MRSA, which was separated from the blood of a middle-aged male patient with TSS, was cultured to reach the mid-log phase on growth curve. Following the previous studies,[9,10] $2.5–3.0 \times 10^{11}$ colony-forming units (CFU) of live MRSA were suspended in 30-ml sterile saline and instilled into the lung lobes of the animals through a bronchoscope with or without smoke inhalation in the SS group and MR group, respectively: 10 ml was placed in the right lower and middle lobes, and the remaining 10 ml was placed in the left lung.

All animals were continuously monitored for 24 h or until death. At the end of the protocol, 10 ml of blood was drained into an aerobic blood culture bottle (BacT/ALERT FA; bioMérieux Inc., Durham, NC, USA) and cultured at 35.0 ± 1.5°C for 5 days. After a high-resolution computed tomography (HRCT) (SOMATOM Emotion

16-slice configuration; Siemens, Munich, Germany) scan of the chest, the animals were euthanized with a lethal infusion of potassium chloride after giving overdose of pentobarbital.

A part of the inferior lobe of the right lung was inflated with 10% formalin for histopathology. Spots of infection caused by the bacterial solution were avoided. Conventional hematoxylin and eosin staining and blinded histopathology evaluation were performed. The lung injury score was evaluated blindly by two independent pathologists as previously described,[11] and the average value was recorded. Tissue samples from the left bronchial airway and parenchyma were collected in 2% glutaraldehyde. Semi-thin (0.5 μm) sections were cut from the bronchial and parenchymal samples and were assessed by transmission electron microscopy (EM) (HT7700; Hitachi, Ibaraki Prefecture, Japan).

Hemodynamic measurements

Mean arterial pressures (MAPs), central venous pressure (CVP), heart rate (HR), and blood temperature were continuously recorded. The cardiac index (CI) was obtained by three central venous injections of 10-ml 0.9% saline (at 4°C) and recorded as the mean of three measurements at the baseline, 1, 2, 4, 8, 12, 16, 20, and 24 h following the injury. The CI was calculated by the thermodilution curve using the Stewart–Hamilton method. The intrathoracic blood volume index (ITBI), extravascular lung water index (ELWI), and systemic vascular resistance index (SVRI) were derived from the CI and blood pressure as previously described.[12-14] Maximum left ventricular contractility (dP_{max}) was calculated from the upslope of the arterial contour waveform.

Arterial and central venous blood samples for blood gas analysis (GEM Premier 3000 Blood Gas Analyzer; Instrumentation Laboratory, Lexington, MA, USA) were collected at the baseline and 1, 2, 4, 8, 16, and 24 h after injury. The white blood cell (WBC) and hemoglobin (HGB) counts were tested with a blood analyzer (Sysmex XE-2100; Sysmex Corp., Kobe, Japan) using the central venous blood sample at the same time points. The oxygen dynamics, including oxygen supply (DO_2), oxygen consumption (VO_2), and oxygen extraction rate (ERO_2), were calculated using the following formula:

$$DO_2 = CI \times (HGB \times 1.34 \times SaO_2 + 0.03 \times PaO_2) \times 10$$

$$VO_2 = CI \times (HGB \times 1.34 \times [SaO_2 - SvO_2] + 0.03 \times [PaO_2 - PvO_2]) \times 10$$

$$ERO_2 = VO_2/DO_2$$

Respiratory variables, including airway resistance (R), airway pressures, and thoracopulmonary pressure-volume (P-V) curves, were measured by the ventilator at the same time points as for the hemodynamics. P-V curves were obtained using the constant low-flow method, with flows of 9 L/min, a respiratory rate of 5 breaths/min, and a tidal volume of 500 ml.[15] At each time point, before the P-V curve was acquired, PEEP was set to 0 cmH_2O and pancuronium (0.1 mg/kg) was injected to eliminate spontaneous respiratory efforts.

Thoracopulmonary compliance (Ctp) was calculated as the slope rate of the linear portion of the P-V curve. The lower inflection point (Pl) was computed as the pressure corresponding to the intersection between the first inflation of 100 ml and the linear slope of the P-V curve. The percentage of dead space (Vd) was measured using the alveolar gas equation with the arterial blood gas and $EtPCO_2$:

$$Vd\ (\%) = (PaCO_2 - EtPCO_2)/PaCO_2.$$

High-resolution computed tomography scans

HRCT (1 mm thickness at 20 mm intervals) scan of the chest was made before the animals were euthanized. Two independent radiologists evaluated each CT scan according to a semiquantitative analysis system.[16] In brief, each section was graded from 0 to 4 according to the percentage of the abnormal volume: 0 = normal; 1 = <25%; 2 = 25–50%; 3 = 51–75%; and 4 = >75%. The overall score for each animal was obtained by averaging the scores of each section.

Statistical analysis

All data were analyzed using SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov method was used for normal distribution test, the data of which were reported as mean ± standard deviation. Continuous variables, including hemodynamics, oxygen dynamics, and respiratory parameters, were compared by repeated measurements and multivariate analysis of variance. $P < 0.05$ was considered statistically significant.

Results

Outcomes

No significant difference in baseline blood temperature was found among the four groups ($P = 0.942$). There was a rapid increase in blood temperature in the SS group, to 40.8 ± 0.2°C at 2 h postinjury, but it stayed normal in the SH group. The blood temperatures of the SM and MR groups also increased, but they were lower than that in the SS group. Significant differences were found among the four groups at each time point after 2 h [$P < 0.001$; Figure 1a]. Rashes were observed in 7/9 animals in the SS group. The WBC gradually increased in the SM, MR, and SS groups, particularly in the SS group, in which the highest count was more than 40 × 10^9/L. A significant difference in WBC count was found between the SS group and anyone of the other three groups at each time point 2 h after injury ($P < 0.001$). Blood temperature and WBC count in the MR group increased slowly than in the SM and SS groups [Figure 1b].

Two animals in the SS group died at 16 h and 20 h postinjury. All animals in the other three groups remained alive until the end of the protocol. No unexpected death was found in all groups. All blood cultures for MRSA from animals in the SH, SM, and MR group were negative, whereas 6/9 animals in the SS group were positive.

Hemodynamics

All parameters in the SH group stayed at a relatively normal level throughout the entire protocol. The HR increased very significantly in the SS group, which had a rate higher than 150 beats/min at 16 h postinjury. The HR increased in the SM and MR groups but was lower than that in the SS group [$P < 0.001$; Figure 2b]. No significant change in the ITBI was observed at all time points for all groups [all $P > 0.05$; Table 1].

In the SS group, MAP decreased to 60–70 mmHg at 2 h postinjury [Figure 2a], and CVP increased gradually [Figure 2f], significant differences were found among the SS group and the other three groups at each time point starting at 2 h postinjury ($P < 0.05$). The changes of CI and SVRI in the SS group were in the opposite direction: the CI increased at 2 h postinjury and then gradually decreased [Figure 2c], whereas the SVRI decreased at the same time point and then gradually increased. SVRI in the SM and MR groups increased slightly before 8 h postinjury, and stayed relatively high in the MR group, but it recovered after that in the SM group [Figure 2d]. The change in dP_{max} was identical to that in CI [Figure 2e].

Respiratory parameters

No significant differences in respiratory parameters were found among the four groups at baseline. Vd, R, Pl, and ELWI increased and Ctp decreased after injury in the SM, MR, and SS groups. In the SM group, Ctp was improved by positive pressure ventilation for up to 8 h postinjury, but deteriorated afterward [Figure 3a], whereas Vd, R, and ELWI stayed at a high level [Figure 3b-3d]. The deterioration of Ctp in the MR group was the mildest in the three lung injury groups. All parameters of the SS group deteriorated gradually and were the worst among the three lung injury groups. Significant differences were found between the SS group and the other three groups at each time point after 4 h injury ($P < 0.05$).

Pl increased rapidly at 2 h postinjury in the SM and SS groups, being higher in the SS group. It increased gradually in the MR group. A significant difference was found at each time point [$P < 0.001$; Table 2].

Oxygen dynamics

All parameters in the SH group were stable and normal. The ratio of the partial pressure of arterial oxygen (PO_2) to the FiO_2 in the SM and SS groups gradually decreased after cotton smoke inhalation and was lower than 300 after 8 h and 2 h, respectively. It was lower in the SS group than in the SM group at each time point; a significant difference was found between two groups at each time point after injury ($P < 0.001$). PaO_2/FiO_2 in the MR group stayed normal during the whole protocol [Figure 4c].

PH values in the SS group decreased progressively [Figure 4a], whereas lactic acid (Lac) level increased [Figure 4b]. Significant differences were found between the SS group and the other three groups ($P < 0.001$). In the SS group, DO_2 and VO_2 increased and reached to their peak values at 2 h postinjury and then decreased to the nadir, but ERO_2 stayed at a high level by the end of the protocol. In the SH and SM groups, DO_2, VO_2, and ERO_2 were stable and stayed normal throughout the entire protocol. In the MR group, there was a slight increase in VO_2 with a significant increase of ERO_2 ($P < 0.05$ vs. the SH group) 2 h postinjury [Figure 4d-4f].

High-resolution computed tomography scans

X-ray transmission values of lung decreased in the SM, MR, and SS groups. The most common findings were ground glass-like appearance, serious effusion, and consolidation. The distribution of lesions was severe in both lower lungs; nodules were scattered in all lung fields. Peribronchial thickening and mucus plugging were also found [Figure 5].

The averaged CT grades of the SH, SM, MR, and SS groups were 0.15 ± 0.13, 1.64 ± 0.28, 1.68 ± 0.24, and

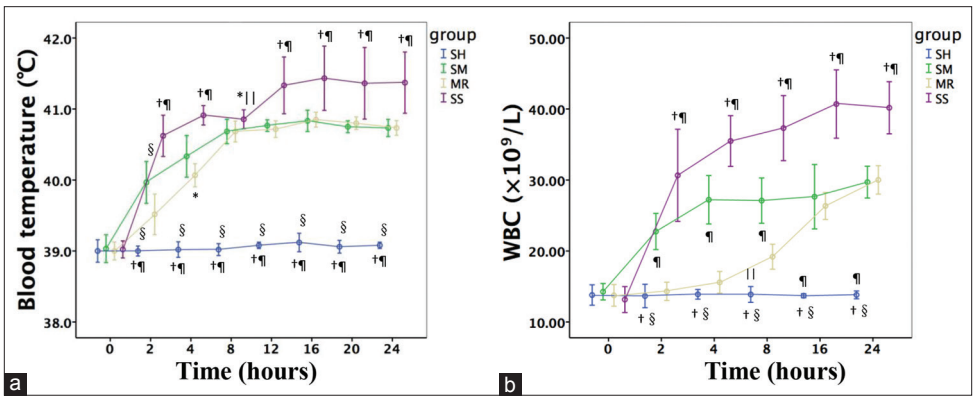

Figure 1: Changes in blood temperature and WBC. (a) Blood temperature in the SM and SS groups increased progressively from 2 h postinjury. It was higher in the SS group than in the SM and MR group; significant differences were found between the SS group and other two lung injury groups (SM and MR) at each time point after 2 h. Blood temperature in the SH group stayed normal at all time points. (b) Similar changes with blood temperature were found in the WBC count among the four groups. The increasing of blood temperature and WBC in the MR group was slower than those in the SM and SS group. WBC: White blood cell; SH: Sham group; SM: Cotton smoke inhalation group; MR: Methicillin-resistant *Staphylococcus aureus* pneumonia group; SS: Septic shock group. *$P < 0.05$ versus the SM group; †$P < 0.01$ versus the SM group; §$P < 0.01$ versus the SS group; ‖$P < 0.05$ versus the MR group; ¶$P < 0.01$ versus the MR group.

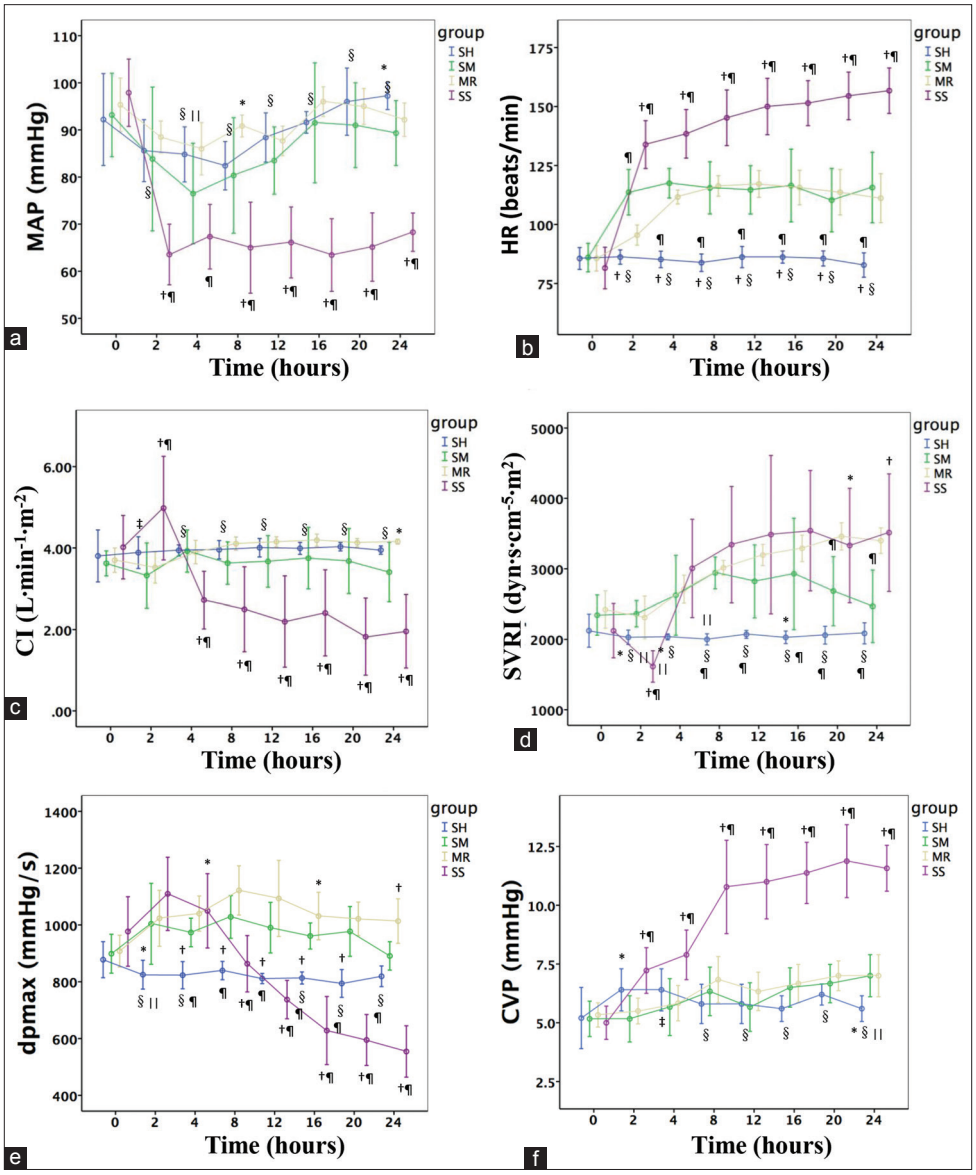

Figure 2: Hemodynamic changes. (a) MAP decreased to 60–70 mmHg exactly 2 h after injury in the SS group; significant changes were found between the SS and other three groups. (b) HR increased significantly in the lung injury groups (SM, MR, and SS), especially in the SS group. Very significant differences were found between the SS and anyone of other three groups at each time point after 2 h postinjury. (c and d) In the SS group, the changes in the CI and SVRI were opposing: The CI increased at 2 h and then gradually decreased, whereas the SVRI decreased at 2 h and then gradually increased. (e) The change in dP_{max} was identical to that in CI. (f) The CVP of individuals in the SS group increased progressively, but remained normal in the other three groups. CI: Cardiac index; CVP: Central venous pressure; dP_{max}: Index of left ventricular contractility; HR: Heart rate; MAP: Mean artery pressure; SH: Sham group; SM: Cotton smoke inhalation group; MR: Methicillin-resistant *Staphylococcus aureus* pneumonia group; SS: Septic shock group; SVRI: Systemic vascular resistance index. *$P < 0.05$ versus the SM group; †$P < 0.01$ versus the SM group; ‡$P < 0.05$ versus the SS group; §$P < 0.01$ versus the SS group; ‖$P < 0.05$ versus the MR group; ¶$P < 0.01$ versus the MR group.

3.51 ± 0.28, respectively. Significant differences were found among the four groups ($P < 0.001$) except the SM versus MR groups.

Histopathology

Significant histopathologic lung injuries were found in the SM, MR, and SS groups. The alveoli were infiltrated by neutrophils, lymphocytes, and macrophages; the microthrombus was formatted and neutrophils were aggregated in the capillaries; the alveoli were edematous and filled with protein liquid; the interalveolar septum was thickened by infiltration of inflamed cells [Figure 6a-6d]. The SH group showed normal lung morphology. The lung injury scores of the SH, SM, MR, and SS groups were 0.60 ± 0.55, 2.50 ± 0.55, 2.33 ± 0.25, and 3.33 ± 0.71, respectively. Significant differences were found among the four groups ($P < 0.01$) except the SM versus the MR groups.

In the SS group, MRSA was found by EM in the parenchymal area. Type I alveoli and capillary epithelium showed extensive injuries and their bandings being loose and disordered. The chromatin and nucleus vacuolar of Type II

Table 1: ITBI at each time point for all groups (ml/m²)

Time points	SH (n = 5)	SM (n = 6)	MR (n = 6)	SS (n = 9)	F	P
Baseline	754.0 ± 31.6	760.5 ± 34.5	752.5 ± 20.1	746.9 ± 20.7	0.278	0.840
2 h	777.6 ± 37.5	747.8 ± 38.1	748.0 ± 21.9	746.9 ± 33.5	1.086	0.378
4 h	754.8 ± 20.0	769.2 ± 35.3	757.8 ± 23.4	755.6 ± 43.9	0.240	0.868
8 h	757.4 ± 27.0	763.5 ± 30.8	751.3 ± 33.0	765.1 ± 21.1	0.313	0.816
12 h	766.4 ± 36.3	784.2 ± 24.1	750.7 ± 12.5	755.3 ± 17.6	2.500	0.089
16 h	748.7 ± 31.7	768.9 ± 30.5	771.1 ± 11.3	745.3 ± 35.9	1.291	0.305
20 h	765.6 ± 25.4	755.3 ± 31.1	758.5 ± 15.2	755.7 ± 26.3	0.192	0.901
24 h	763.2 ± 38.6	748.8 ± 26.5	747.3 ± 20.5	746.9 ± 35.0	0.343	0.795

The ITBI was stable in all groups at all-time points; no significant differences were found (all P>0.05). ITBI: Intrathoracic blood volume index; SH: Sham group; SM: Cotton smoke inhalation group; MR: Methicillin-resistant *Staphylococcus aureus* lung infection group; SS: Septic shock group.

Table 2: PI at each time point for all groups (mmHg)

Time points	SH (n = 5)	SM (n = 6)	MR (n = 6)	SS (n = 9)	F	P
Baseline	1.66 ± 0.23	1.68 ± 0.15	1.63 ± 0.10	1.71 ± 0.12	0.336	0.799
2 h	1.64 ± 0.23*,†	7.43 ± 1.34†,§	2.15 ± 0.29†	10.38 ± 1.15	129.839	<0.001
4 h	1.60 ± 0.24*,†,‡	6.53 ± 0.76†,§	2.98 ± 0.56†	10.84 ± 1.20	132.884	<0.001
8 h	1.30 ± 0.36*,†,§	6.45 ± 0.76†,§	3.32 ± 0.37†	10.84 ± 1.20	176.495	<0.001
12 h	1.62 ± 0.22*,†,§	6.15 ± 0.42†,§	3.93 ± 0.34†	10.61 ± 1.01	231.133	<0.001
16 h	1.42 ± 0.13*,†,§	6.20 ± 0.39†,§	4.97 ± 0.41†	10.46 ± 0.90	256.670	<0.001
20 h	1.44 ± 0.17*,†,§	6.17 ± 0.36†,§	4.97 ± 0.40†	10.40 ± 0.88	263.398	<0.001
24 h	1.44 ± 0.28*,†,§	6.05 ± 0.24†,§	5.10 ± 0.24†	10.41 ± 0.66	462.025	<0.001

*P<0.01 versus the SM group; †P<0.01 versus the SS group; ‡P<0.05 versus the MR group; §P<0.01 versus the MR group. Pl stayed at a high level after 2 h of the model being established in the SM and SS groups. It was higher in the SS than in the SM group. A significant difference was found for each time point except baseline (all P<0.001). Pl: Lower inflection point; SH: Sham group; SM: Cotton smoke inhalation group; MR: Methicillin-resistant *Staphylococcus aureus* lung infection group; SS: Septic shock group.

alveoli epithelium were degenerated; their mitochondria swelled, and their cristae disappeared [Figure 6e-6g].

Discussion

Since the etiology and pathophysiology of septic shock are complicated, it is difficult to make a perfect animal model. Nevertheless, the hemodynamic features of septic shock have been well defined, that is, the hyperdynamic and hypodynamic phases. The hyperdynamic phase is characterized by low SVRI and high CI,[17-19] and the hypodynamic phase is characterized by high SVRI and low CI. Many animal models have been established to mimic the hemodynamic changes of septic shock in humans,[8-10] but none of them have shown the two phases.

Murakami *et al*. developed an ovine model by instilling live *P. aeruginosa* in a smoked lung.[8] The hyperdynamic phase of this model lasted for 42 h after lung injury and no hypodynamic phase was observed. Another ovine model using MRSA did not show the hypodynamic phase until 24 h after the lung injury.[9] The incomplete hemodynamic changes of these models make them difficult for studying the disease progression as well as intervention of septic shock.

Our experiments were designed to study the effects of lung injury (cotton smoke inhalation), MRSA infection, and lung injury plus MRSA infection on the development of septic shock. A high bacterial clearance capacity by species-specific pulmonary intravascular macrophages has been confirmed by a previous study.[20] Therefore, damage of the protective barrier of lung by smoke inhalation could be necessary for MRSA to spread to the blood stream to induce bacteremia. This was confirmed by results from our study that infusion of MRSA alone did not induce MRSA bacterium in the MR group. Instead, for the SS group, in which the animals received both smoke inhalation and bacterium infusion, MRSA was positive in most of the animals' bloodstream, indicating the MRSA bacteremia. Under EM examination, MRSA was found in the macrophage of the SS group. A spreading of the infection through the bloodstream is the main reason for TSS; many manifestations of which – such as increasing body temperature, hypotension, and rash – have been observed in our model.

In the present study, we have developed a 24-h animal model for septic shock. Both hyperdynamic and hypodynamic phases have been seen in the SS group by the MRSA infection. The CI increased to the peak, whereas MAP and SVRI decreased to the nadir at 2 h after the MRSA infusion. From that time point, CI decreased and SVRI increased gradually, whereas MAP stayed at the relatively low level of 60–70 mmHg. However, such characteristic hemodynamics were not observed in the SM and MR groups, indicating that both lung injury and MRSA infusion are important for the development of septic shock animal model. Throughout the protocol, Ringer's solution was infused to maintain a sufficient preload on the heart to maintain a stable ITBI, which ensured that any hemodynamic change was due to the

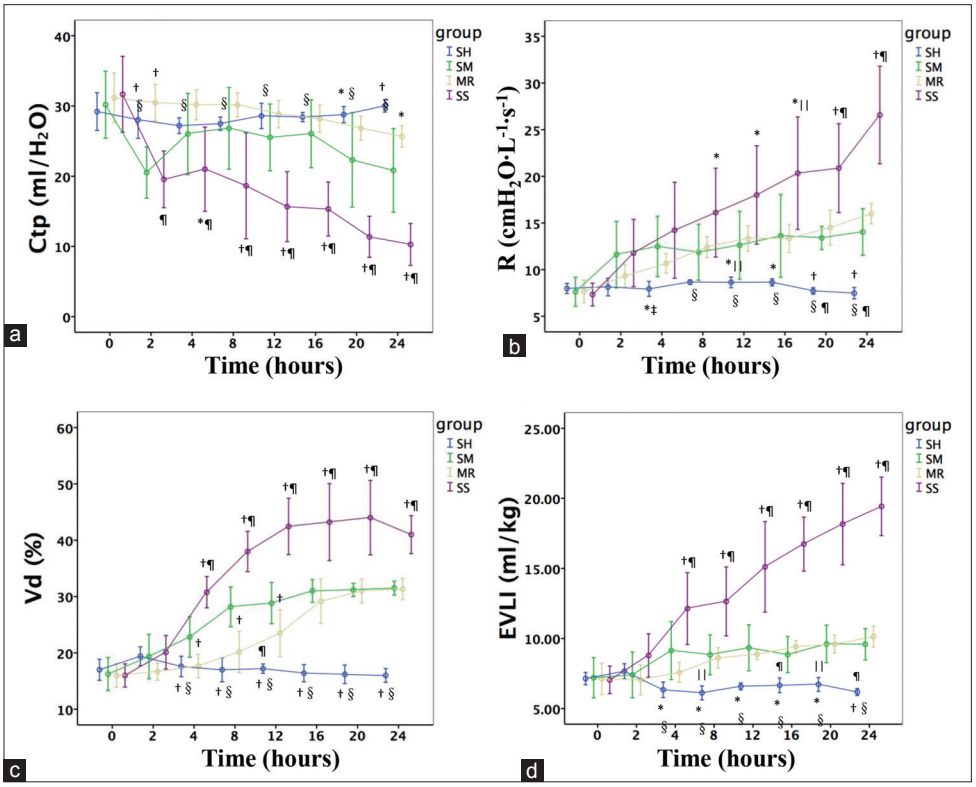

Figure 3: Changes in respiratory parameters. All parameters in the SS group deteriorated gradually and the changes were more obvious than those in the SM and MR groups. Significant differences were found between the SS and anyone of other two lung injury groups (SM and MR) at each time point after 2 h injury. (a) Ctp of the SM group was improved before 8 h by positive pressure ventilation but deteriorated after that. (b-d) Vd, R, and ELWI increased after injury in the SM, MR, and SS groups, whereas they were stable in the SH group. Ctp: Thoracopulmonary compliance; ELWI: Extravascular lung water index; R: Airway resistance; P-V: Pressure-volume; SH: Sham group; SM: Cotton smoke inhalation group; MR: Methicillin-resistant *Staphylococcus aureus* pneumonia group; SS: Septic shock group; Vd: Dead space. *$P < 0.05$ versus the SM group; †$P < 0.01$ versus the SM group; ‡$P < 0.05$ versus the SS group; §$P < 0.01$ versus the SS group; ‖$P < 0.05$ versus the MR group; ¶$P < 0.01$ versus the MR group.

injury management. Throughout the protocol, dP_{max}, which is related to the contractility of the myocardium, also showed a similar change as CI, which may be partly due to the change of reserved cardiac function. Many of the cytokines and toxicants induced by sepsis could depress the myocardium and thus decrease the dP_{max}.

With regard to oxygenation, hypoxemia, which was indicated by the PaO_2/FiO_2, was observed in both the SM and SS groups, but more serious hypoxemia was seen in the SS group, which was due to ARDS induced by MRSA infection. PaO_2/FiO_2 stayed normal in the SH and MR groups. The changes in DO_2 and VO_2 in the SS group were similar in terms of CI, because they are two parameters that are mainly derived from CI, SaO_2, and SvO_2. We adjusted FiO_2 to maintain a stable SaO_2 level at least 90% at all times throughout the experimental protocol, so the changes were due to CI and SvO_2. Since CI decreased significantly after 4 h postinjury in the SS group, it was not surprising that DO_2 also decreased.

Sepsis shows unbalanced oxygen delivery and consumption, that is, increased VO_2 and decreased DO_2. The body takes in almost all its oxygen from low DO_2, which was the reason that ERO_2 in the SS group maintained high throughout the entire process. Even though it cannot maintain the body metabolism of oxygen, VO_2 increases when DO_2 increases. The increase in Lac could confirm an increase in anaerobic glycolysis, which is due to the mismatch between DO_2 and VO_2. Whereas in the MR group, even ERO_2 was increased; normal CI was able to maintain sufficient DO_2 with a relative high VO_2.

As a model of ARDS induced by pneumonia, this model showed characteristic changes in respiratory parameters of lung function. The gradual increase in R, Vd, Pl, and ELWI and the deterioration in Ctp demonstrated the poor lung function induced by the smoke injury and MRSA infection. We kept PEEP at 5 cmH_2O, which can decrease alveolar collapse and thus improve lung function. Nevertheless, lung function deteriorated in the SS groups by the severe MRSA infection, which was not significant in the SM and MR groups.

ARDS was also confirmed by typical changes in HRCT and histopathology. Significant differences in injury grade and score evaluated by HRCT and histopathology were found in the SS group.

In this porcine model, the hyperdynamic phase occurred within 2 h after injury and then progressed to the hypodynamic phase, both of them were the obvious manifestations of septic shock. None of the previous models

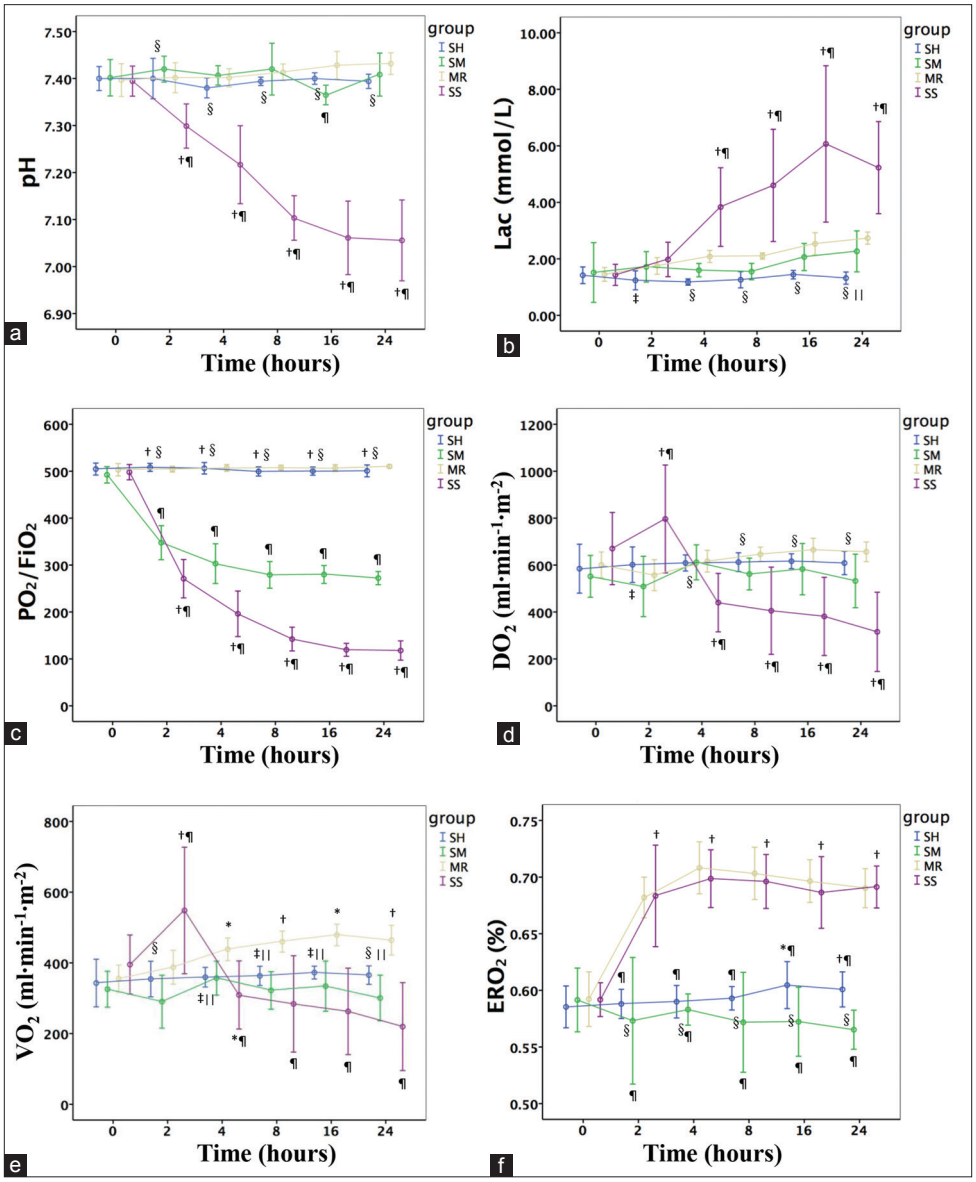

Figure 4: Changes in oxygen dynamics. (a and b) In the SS group, pH decreased progressively, whereas Lac increased. Significant differences were found between the SS group and anyone of other three groups after 2 h. (c) The PO_2/FiO_2 of the SM and SS group decreased gradually after smoke inhalation. It was lower in the SS group than in the SM group. Very significant differences were found between anyone of them and the SH group. (d-f) DO_2 and VO_2 in the SS group increased to peak at 2 h and then decreased to the nadir, but ERO_2 stayed at a high level until the end of the protocol. DO_2, VO_2, and ERO_2 in the SH and SM group stayed normal throughout the entire protocol. VO_2 and ERO_2 in the MR group increased significantly after 2 h of injury. DO_2: Oxygen supply; ERO_2: Oxygen extraction rate; Lac: Lactic acid; SH: Sham group; SM: Cotton smoke inhalation group; MR: Methicillin-resistant *Staphylococcus aureus* pneumonia group; SS: Septic shock group; VO_2: Oxygen consumption. *$P < 0.05$ versus the SM group; †$P < 0.01$ versus the SM group; ‡$P < 0.05$ versus the SS group; §$P < 0.01$ versus the SS group; ‖$P < 0.05$ versus the MR group; ¶$P < 0.01$ versus the MR group.

have shown these characteristics in such a short period. Our model successfully mimics the pathophysiologic changes during septic shock with ARDS in terms of hemodynamics, oxygen dynamics, and lung functional changes. All of these changes were due to the MRSA infusion after lung injury by smoke inhalation, but not by the smoke inhalation or MRSA infusion alone. These results would provide important insights into the mechanisms of the host response to pathogens. All of these features of our model make it possible for further investigation of the mechanisms and the development of interventions to septic shock.

Unlike other animals such as rodents, cats, and dogs, which are relatively resistant to endotoxin,[21] pigs were selected for models of sepsis because they have a similar sensitivity to endotoxin as in humans. Direct infusion endotoxin is not a good model for sepsis because it cannot mimic the whole process of infection by a bacterium. The advantage of using pigs rather than other large nonprimate animals is that the porcine renal and cardiovascular systems, gastrointestinal physiology, and anatomy are all very similar to those in humans, making it an excellent experimental species.[22] Previous studies have shown that pigs develop

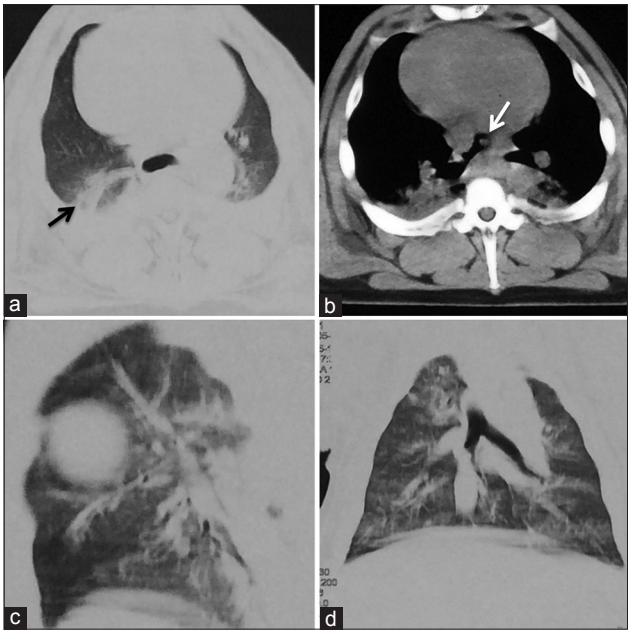

Figure 5: HRCT scans of the SS group. (a and b) The transverse section of the lung window and the longitudinal diaphragm window of HRCT, respectively, showing the consolidation of double lower lung. The black arrow shows the pleural thickening, and the white arrow shows the mucous plugging. (c and d) The vertical plane and the coronal section of lung, respectively, showing the serious effusion with the ground glass-like appearance. All sections were from the SS group. HRCT: High-resolution computed tomography; SS: Septic shock group.

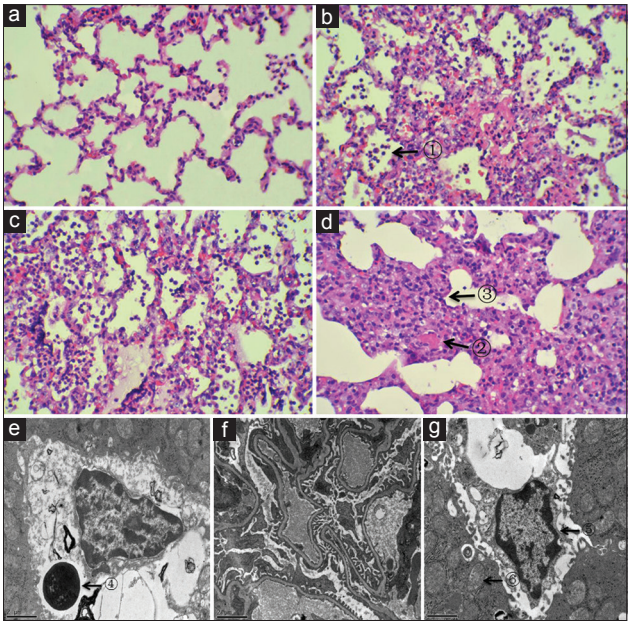

Figure 6: Histopathologic figures. (a-d) Histopathologic slides under LM (H and E, ×200) from the SH group, SM group, MR group, and SS group, respectively. The slide was normal in the SH group, but very significant lung injuries were found in the SM, MR, and SS groups. ①Neutrophils, lymphocytes, and macrophages infiltrated in the alveoli; ②microthrombus formatted in the capillaries; ③interalveolar septum thickened by the infiltration of inflammatory cells. (e-g) Histopathologic figures from the SS group under EM (bars: 2 μm in e and f, 1 μm in g). (e) ④MRSA was found in the parenchymal area. (f) The bandings of Type I alveoli and capillary epithelium were loose and disordered. (g) ⑤The chromatin and nucleus vacuolar of Type II alveoli epithelium degenerated; ⑥The mitochondria swelled and the cristae disappeared. LM: Light microscope; EM: Transmission electron microscope; SH: Sham group; SM: Cotton smoke inhalation group; MR: Methicillin-resistant *Staphylococcus aureus* pneumonia group; SS: Septic shock group; MRSA: Methicillin-resistant *Staphylococcus aureus*.

sepsis, and even consequent multiple organ dysfunction, after intravenous inoculation with *S. aureus*.[23,24]

One commonly used approach to study the mechanism of sepsis is to inoculate animals with pure or mixed bacterial flora in an attempt to mimic the condition of bloodstream infection such as TSS.[25-27] However, high doses of bacteria infused into a vein do not typically colonize and replicate in the host, often as a result of rapid complement-mediated lysis. The reactions of the host are hypersensitivity and toxemia, rather than a true model of sepsis.[28,29]

One early study compared porcine models with venous infusion of *P. aeruginosa*, *S. aureus*, and *Escherichia coli*.[30] It demonstrated that, although causing persistent pulmonary hypertension, gram-positive *S. aureus* did not result in hypoxemia, pulmonary edema, or an increased shunt fraction. This may be due to the fact that the bacteria infused into the vein were cleared by the liver and spleen first, and therefore most of them bypassed the lung.[31] Thus, rather than infection, the reaction is more akin to intoxication, even when bacteria have been cultured *in vitro* and a known, tightly controlled number of CFU of bacteria have been administered.[29]

The primary systemic challenges of live bacterial injection in the absence of a focus of infection makes such models lack the necessary immune reaction characteristics of human sepsis and only produces a hypodynamic circulatory response with limited survival time, which does not correlate with clinical disease. Our model of lung injury combined with MRSA infection, however, is characterized by both phases of septic shock, which mimic the human disease progression.

In conclusion, in this study, we developed a porcine model of septic shock after ARDS induced by MRSA with a short period. The hemodynamics, oxygen dynamics, and lung function responses were similar to those observed in humans. The data from this model are useful for further investigation of the treatment of sepsis and can be adapted to clinical trial designs.

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Conflicts of interest

There are no conflicts of interest.

References

1. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003;348:1546-54. doi: 10.1056/NEJMoa022139.
2. Hayakawa M, Saito S, Uchino S, Yamakawa K, Kudo D, Iizuka Y, *et al.* Characteristics, treatments, and outcomes of severe sepsis of 3195 ICU-treated adult patients throughout Japan during 2011-2013. J Intensive Care 2016;4:44. doi: 10.1186/s40560-016-0169-9.
3. Yang Y, Xu F, Shi LY, Diao R, Cheng YS, Chen XY, *et al.* Efficacy and significance of various scores for pneumonia severity in the management of patients with community-acquired pneumonia in China. Chin Med J 2012;125:639-45. doi: 10.3760/cma.j.issn.0366-6999.2012.04.015.
4. Stapleton RD, Wang BM, Hudson LD, Rubenfeld GD, Caldwell ES, Steinberg KP. Causes and timing of death in patients with ARDS. Chest 2005;128:525-32. doi: 10.1378/chest.128.2.525.
5. Li DZ, Chen YS, Yang JP, Zhang W, Hu CP, Li JS, *et al.* Preliminary molecular epidemiology of the *Staphylococcus aureus* in lower respiratory tract infections: A multicenter study in China. Chin Med J 2011;124:687-92. doi: 10.3760/cma.j.issn.0366-6999.2011.05.010.
6. Woske HJ, Röding T, Schulz I, Lode H. Ventilator-associated pneumonia in a surgical intensive care unit: Epidemiology, etiology and comparison of three bronchoscopic methods for microbiological specimen sampling. Crit Care 2001;5:167-73.
7. Paulsen J, Mehl A, Askim Å, Solligård E, Åsvold BO, Damås JK. Epidemiology and outcome of *Staphylococcus aureus* bloodstream infection and sepsis in a Norwegian county 1996-2011: An observational study. BMC Infect Dis 2015;15:116. doi: 10.1186/s12879-015-0849-4.
8. Murakami K, Bjertnaes LJ, Schmalstieg FC, McGuire R, Cox RA, Hawkins HK, *et al.* A novel animal model of sepsis after acute lung injury in sheep. Crit Care Med 2002;30:2083-90. doi: 10.1097/01.CCM.0000026330.66508.5F.
9. Enkhbaatar P, Joncam C, Traber L, Nakano Y, Wang J, Lange M, *et al.* Novel ovine model of methicillin-resistant *Staphylococcus aureus*-induced pneumonia and sepsis. Shock 2008;29:642-9. doi: 10.1097/shk.0b013e318158125b.
10. Jonkam CC, Bansal K, Traber DL, Hamahata A, Maybauer MO, Maybauer DM, *et al.* Pulmonary vascular permeability changes in an ovine model of methicillin-resistant *Staphylococcus aureus* sepsis. Crit Care 2009;13:R19. doi: 10.1186/cc7720.
11. Hofbauer B, Saluja AK, Bhatia M, Frossard JL, Lee HS, Bhagat L, *et al*. Effect of recombinant platelet-activating factor acetyihydrolase on two models of experiment acute pancreatitis. Gastroenterology 1998;115:1238-47.
12. Martin GS, Eaton S, Mealer M, Moss M. Extravascular lung water in patients with severe sepsis: A prospective cohort study. Crit Care 2005;9:R74-82. doi: 10.1186/cc3025.
13. Neumann P. Extravascular lung water and intrathoracic blood volume: Double versus single indicator dilution technique. Intensive Care Med 1999;25:216-9.
14. Sakka SG, Rühl CC, Pfeiffer UJ, Beale R, McLuckie A, Reinhart K, *et al*. Assessment of cardiac preload and extravascular lung water by single transpulmonary thermodilution. Intensive Care Med 2000;26:180-7.
15. Lu Q, Vieira SR, Richecoeur J, Puybasset L, Kalfon P, Coriat P, *et al.* A simple automated method for measuring pressure-volume curves during mechanical ventilation. Am J Respir Crit Care Med 1999;159:275-82. doi: 10.1164/ajrccm.159.1.9802082.
16. Bergin CJ, Bell DY, Coblentz CL, Chiles C, Gamsu G, MacIntyre NR, *et al.* Sarcoidosis: Correlation of pulmonary parenchymal pattern at CT with results of pulmonary function tests. Radiology 1989;171:619-24. doi: 10.1148/radiology.171.3.2717731.
17. Groeneveld AB, Bronsveld W, Thijs LG. Hemodynamic determinants of mortality in human septic shock. Surgery 1986;99:140-53.
18. Parker MM, Shelhamer JH, Bacharach SL, Green MV, Natanson C, Frederick TM, *et al*. Profound but reversible myocardial depression in patients with septic shock. Ann Intern Med 1984;100:483-90.
19. Parker MM, Shelhamer JH, Natanson C, Alling DW, Parrillo JE. Serial cardiovascular variables in survivors and nonsurvivors of human septic shock: Heart rate as an early predictor of prognosis. Crit Care Med 1987;15:923-9.
20. Crocker SH, Eddy DO, Obenauf RN, Wismar BL, Lowery BD. Bacteremia: Host-specific lung clearance and pulmonary failure. J Trauma 1981;21:215-20.
21. Piper RD, Cook DJ, Bone RC, Sibbald WJ. Introducing critical appraisal to studies of animal models investigating novel therapies in sepsis. Crit Care Med 1996;24:2059-70.
22. Swindle MM, Smith AC. Comparative anatomy and physiology of the pig. Scand J Lab Anim Sci 1998;25:1-10.
23. Leifsson PS, Iburg T, Jensen HE, Agerholm JS, Kjelgaard-Hansen M, Wiinberg B, *et al.* Intravenous inoculation of *Staphylococcus aureus* in pigs induces severe sepsis as indicated by increased hypercoagulability and hepatic dysfunction. FEMS Microbiol Lett 2010;309:208-16. doi: 10.1111/j.1574-6968.2010.02042.x.
24. Nielsen OL, Iburg T, Aalbaek B, Leifsson PS, Agerholm JS, Heegaard P, *et al.* A pig model of acute *Staphylococcus aureus* induced pyemia. Acta Vet Scand 2009;51:14. doi: 10.1186/1751-0147-51-14.
25. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock – A review of laboratory models and a proposal. J Surg Res 1980;29:189-201.
26. Fink MP, Heard SO. Laboratory models of sepsis and septic shock. J Surg Res 1990;49:186-96.
27. Deitch EA. Animal models of sepsis and shock: A review and lessons learned. Shock 1998;9:1-11.
28. Buras JA, Holzmann B, Sitkovsky M. Animal models of sepsis: Setting the stage. Nat Rev Drug Discov 2005;4:854-65. doi: 10.1038/nrd1854.
29. Cross AS, Opal SM, Sadoff JC, Gemski P. Choice of bacteria in animal models of sepsis. Infect Immun 1993;61:2741-7.
30. Dehring DJ, Crocker SH, Wismar BL, Steinberg SM, Lowery BD, Cloutier CT. Comparison of live bacteria infusions in a porcine model of acute respiratory failure. J Surg Res 1983;34:151-8.
31. Niehaus GD, Schumacker PR, Saba TM. Reticuloendothelial clearance of blood-borne particulates: Relevance to experimental lung microembolization and vascular injury. Ann Surg 1980;191:479-87.