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A Model of Hemorrhagic Shock and Acute Lung Injury in Landrace–Large White Swine

Theodoros T Xanthos,[1,*] Xanthippi A Balkamou,[1] Kostantinos I Stroumpoulis,[1] Ioannis N Pantazopoulos,[2] Georgios I Rokas,[1] Georgios D Agrogiannis,[3] Georgios T Troupis,[1] Theano D Demestiha,[1] and Panagiotis N Skandalakis[1]

Traumatic injury is a leading cause of death worldwide for people between 5 and 44 y of age, and it accounts for 10% of all deaths. The incidence of acute lung injury, a life-threatening complication in severely injured trauma patients remains between 30% and 50%. This study describes an experimental protocol of volume-controlled hemorrhage in Landrace–Large White swine. The experimental approach simulated the clinical situation associated with hemorrhagic shock in the trauma patient while providing controlled conditions to maximize reproducibility. The duration of the protocol was 8 h and was divided into 5 distinct phases—stabilization, hemorrhage, maintenance, resuscitation, and observation—after which the swine were euthanized. Lung tissue samples were analyzed histologically. All swine survived the protocol. The hemodynamic responses accurately reflected those seen in humans, and the development of acute lung injury was consistent among all swine. This experimental protocol of hemorrhagic shock and fluid resuscitation in Landrace–Large White swine may be useful for future study of hemorrhagic shock and acute lung injury.

Hemorrhage, a leading cause of morbidity and mortality, is encountered frequently in hospital emergency rooms, operating rooms, and intensive care units. Marked loss of intravascular volume subsequently may lead to hemodynamic instability, decreased tissue perfusion, impaired delivery of O_2 and nutrients, cellular hypoxia, organ damage, and eventually death.[11] Gastrointestinal bleeding and trauma are the most common causes of hemorrhagic shock.[6]

In the last decades, extensive efforts have been made to elucidate the pathophysiologic mechanisms and immunologic alterations associated with severe hemorrhage.[12] The first response to blood loss is clot formation at the site of hemorrhage. With the progression of hemorrhage, catecholamines, antidiuretic hormone, and atrial natriuretic peptides are released, promoting arterial vasoconstriction and increases in heart rate. These mechanisms aim at increasing cardiac output and maintaining perfusion pressure. The loss of coronary perfusion pressure affects myocardial contractility whereas decreased cerebral blood flow results in loss of consciousness, coma, and death.[6,12] Fluid resuscitation is often the only treatment modality accessible to personnel who provide initial care to trauma patients. Most physicians begin resuscitating trauma patients according to the Advanced Trauma Life Support guidelines of the American College of Surgeons.[21] The current guidelines call for an aggressive fluid resuscitation regimen starting with a 2-L bolus of crystalloid (preferably Lactated Ringer) solution. Resuscitation continues with repeated boluses of Lactated Ringer solution, blood transfusion, and repair of surgically correctable causes of hemorrhage.[21]

One life-threatening consequence of traumatic hemorrhage is acute lung injury, which is associated with pulmonary edema due to increased capillary permeability and infiltration of inflammatory cells into the interstitium and airspaces. The incidence of acute lung injury in severely injured trauma patients remains between 30% and 50%, and associated mortality has been estimated to be 10%, depending on the severity of pulmonary dysfunction.[13]

The continuous emergence of alternative resuscitative strategies has led to the establishment of a broad variety of experimental conditions that enables researchers to assess the potential benefits of these interventions. Various animal species have been used in hemorrhagic shock experimentation. However, swine typically are used in biomedical research.[26] Clearly, intensive experimental efforts are needed if we are to reverse the deleterious consequences of trauma and hemorrhagic shock. The aim of the present study was to describe our experimental protocol for inducing hemorrhagic shock and acute lung injury in swine. This experimental approach simulated the clinical situation associated with hemorrhagic shock in the trauma patient while providing the controlled conditions that maximize reproducibility.

Materials and Methods

After approval of the General Directorate of the Veterinary Services of Athens, Greece (permit no. K/8052/25-10-2007), and the Bioethics Committee of the University of Athens, 15 healthy swine (Landrace–Large White; age, 10 to 15 wk; weight, 19 ± 2 kg) of conventional microbiologic status and all coming from the same breeder (Validakis, Koropi, Greece) were included in this study. Swine were transported to the research facility 1 wk prior to experimentation and single housed in cages of 2 m^2 each in environmentally controlled room with 15 air changes hourly, 22 ± 2 °C, relative humidity of 55%, and a 12:12-h light:dark cycle

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[1]Department of Anatomy, [2]12th Department of Respiratory Medicine, Sotiria General Hospital and [3]Department of Pathology, University of Athens Medical School, Athens, Greece.
[*]Corresponding author. Email: theodorosxanthos@yahoo.com

(lights on, 0600 to 1800 h). During their stay, swine were fed with a commercially available food.[27]

Two days before the experimentation, all swine were sedated with ketamine hydrochloride (10 mg/kg IM; Merial, Lyon, France), midazolam (0.5 mg/kg IM; Roche, Athens, Greece), and atropine sulfate (0.05 mg/kg IM; Efar, Athens, Greece). Blood was withdrawn from the lateral auricular vein for CBC and standard biochemistry (glucose, sodium, potassium, liver, and urinary biochemistry) and chest radiography was performed, to ensure that the animals were healthy. The day before experimentation, the swine were food-fasted but had ad libitum access to water.

On the day of experimentation, swine were sedated as previously described[25] and transported to the operating room, where intravenous access was achieved through catheterization (20-gauge, BD Venflon, Luer-Lok, Helsinborg, Sweden) of the auricular vein. Anesthesia was induced with an intravenous bolus dose of propofol (2.0 mg/kg) and fentanyl (2 µg/kg; Janssen Pharmaceutica, Beerse, Belgium). While they were spontaneously breathing but anesthetized, the pigs were intubated with a 4.5 or 5.0 French endotracheal tube (MLT 4.5 or 5.0 Oral 27 mm, Mallinckrodt Medical, Greece) by using a size 3 bent metal laryngoscope blade. Auscultation and inflation of both lungs confirmed correct placement of the endotracheal tube. After the tracheal tube was secured to the upper jaw, hair was clipped from the ventral thorax to facilitate the use of self-adhesive electrodes. The swine then were placed supine on the operating table.

To achieve synchrony with the ventilator, additional propofol (1 mg/kg IV), cis-atracurium (0.15 mg/kg IV), and fentanyl (0.01 mg/kg IV) were administered. Swine were mechanically ventilated (ventiPac, Sims pneuPac, Lutton, UK) with 21% oxygen; anesthesia was maintained with infusion of propofol (150 µg/kg/min). Normocapnia was achieved by using continuous monitoring of end-tidal CO_2 (Nihon Kohden, Bergamo, Italy), and the respiratory frequency was adjusted to maintain end-tidal CO_2 at 35 to 40 mm Hg.

Five adhesive electrodes were attached to the ventral thorax of each swine, to accommodate electrocardiographic monitoring (Mennen Medical, Envoy, Papapostolou, Athens, Greece) by using leads I, II, III, aVR, aVL, aVF; heart rate was determined from the tracing. Pulse oximetry (Vet/Ox Plus 4700, Heska, Fribourg, Switzerland) was used to assess peripheral tissue oxygenation and was monitored throughout the experiment. The pulse oximeter sensor was placed on the tongue of each anesthetized, intubated pig.

For measurement of the aortic pressure, a normal saline-filled (model 6523, USCI CR, Bart, Papapostolou, Athens, Greece) arterial catheter was inserted and advanced into the descending aorta after surgical preparation of the right internal carotid artery. The systolic and diastolic aortic pressures were recorded; mean aortic pressure was determined electronically. The left internal jugular vein was surgically prepared and a catheter was inserted (5.5-French, 75 cm; Opticath, Abbott, Athens, Greece) for exsanguination and fluid loading. The right internal jugular vein was cannulated, and a Swan–Ganz triple-lumen 5-French balloon catheter was advanced through the right internal jugular vein into the right atrium. Correct placement was verified by checking arterial and venal waveforms.

Intravascular catheters were attached to pressure transducers that were aligned to the level of the right atrium. This allowed recording of central venous pressures. All catheters were flushed with isotonic saline containing 5 IU/mL heparin to prevent blood clot formation. Body temperature was monitored by a rectal temperature probe and was maintained between 37 and 38 °C with a heating blanket. Arterial blood was obtained by using heparinized syringes for analyzing pH, pO_2, pCO_2, base deficit, lactate, hemoglobin, and electrolytes with a blood-gas analyzer (Stat Profile, Critical Care Xpress, Nova Biomedical, Waltham, MA).

The duration of the protocol was 8 h and was divided into 5 distinct phases: stabilization, hemorrhage, maintenance, resuscitation, and observation phase.

Stabilization phase. After induction of anesthesia and instrumentation for recording of hemodynamic variables, swine were allowed to stabilize for 45 min. Once a steady state was achieved, baseline measurements including all variables previously mentioned were obtained.

Hemorrhagic phase. After data collection, acute hemorrhage was induced by repeated removal of 5 mL/kg from the internal jugular vein in less than 5 min until 50% of the estimated total blood volume had been withdrawn. Total blood volume was estimated as 7% of total body weight. The procedure lasted approximately 20 min or less and was terminated if mean aortic pressure fell below 40 to 45 mm Hg. All parameters were obtained after completion of blood withdrawal.

Maintenance phase. Swine remained in hemorrhagic shock for an additional 90 min, during which no fluid was administered. This 90-min delay before the initiation of resuscitation allowed the animals to reach their nadir blood pressure and replicates the scenario of human trauma patients.

Infusion phase. After the 90-min shock period and after obtaining hemodynamic parameters and arterial blood gases, swine were resuscitated with 20 mL/kg Lactated Ringer solution in less than 20 min until the mean aortic pressure reached 90% of its baseline value. If mean aortic pressure remained excessively low, the same volume of fluid was infused according to the European guidelines for pediatric resuscitation.[2]

Observation phase. During the ensuing 4 h, swine were maintained for observation, and no further resuscitation was performed. Mean aortic pressure, central venous pressure, right atrial pressure, heart rate, and body temperature were monitored continuously throughout the 5 phases of the experiment. Body temperature was maintained within a strict range (38 to 39 °C). At the end of the observation phase, all swine were euthanized by using intravenous overdose of thiopental.

From each animal, a total of 6 lung tissue samples were collected from dependent and nondependent parts of both lower and upper lobes, immersed in 10% paraformaldehyde for at least 72 h, dehydrated through a graded alcohol series, embedded in paraffin, sliced into 0.4-µm thick sections, and stained with hematoxylin and eosin. Tissue samples were analyzed in a blinded fashion according to a previously described scoring system.[22] Features evaluated were focal thickening of the alveolar membranes, vascular congestion, alveolar edema, interstitial neutrophil infiltration, intraalveolar neutrophil infiltration, and alveolar hemorrhage. Each feature was assigned a score from 0 to 3 based on its absence (0) or presence to a mild (1), moderate (2), or severe (3) degree, and a total cumulative histology score was determined.

Statistics Continuous data are expressed as mean ± 1 SD, because all data were normally distributed. Categorical data are expressed as the 95% confidence interval of the mean. Measurements were compared between phases in the same animal by

using repeated-measures ANOVA. For all tests, P values less than 0.05 were considered to be statistically significant. All analyses were conducted by using SPSS 13.0 for Windows (SPSS, Chicago, IL).

Results

All swine included in this study were healthy and survived the entire acute hemorrhage–shock resuscitation protocol. Baseline variables after the stabilization phase are shown in Table 1.

The average blood volume loss was 35 mL/kg, corresponding to 50% of the circulating blood volume. Swine remained normocapnic (35 to 45 mm Hg) throughout the experiment. There were no statistically significant differences in SpO_2, pO_2, or pCO_2 among the 5 phases of the experiment. After hemorrhage, there was a rapid drop in mean aortic pressure and a return to near-baseline values with active fluid resuscitation (stabilization compared with hemorrhagic phase, $P < 0.001$; stabilization compared with maintenance phase, $P < 0.001$; stabilization compared with observation phase, $P < 0.05$; stabilization compared with infusion phase, P = nonsignificant; Table 1).

Heart rate increased significantly from baseline (stabilization phase) values during hemorrhage ($P < 0.001$) and shock maintenance ($P < 0.001$), remained higher than baseline after fluid replacement ($P < 0.001$), and returned to baseline during the observation phase of the experiment. Right atrial mean pressure decreased significantly from baseline values during the hemorrhagic ($P < 0.05$) and maintenance phases ($P < 0.05$), whereas no significant difference was observed between stabilization and infusion or observation phases.

pH did not vary after hemorrhage but significantly ($P = 0.05$) decreased during the maintenance phase, decreased further during infusion ($P < 0.001$), and returned to near-baseline values at the end of the experiment (Table 1). Blood lactate levels were significantly ($P < 0.001$) elevated during all phases of the experiment when compared with baseline values. Administration of Ringer Lactated solution had no significant effect on pH when compared with that during the hemorrhagic phase. Hemoglobin was decreased throughout the experiment ($P < 0.05$ for all comparisons). Whereas potassium increased significantly ($P < 0.05$) from baseline during the maintenance phase and then returned to baseline levels, sodium did not vary throughout the experiment (Table 1).

Lung tissue from all swine was severely impaired due to pulmonary neutrophil infiltration and alveolar edema (Figure 1). The 95% confidence interval of the mean for the various histologic features assessed is shown in Table 2.

Discussion

Our aim was to refine current experimental technique and reproduce conditions of hemorrhagic shock by using a model of controlled hemorrhage that would be easily reproducible and clinically relevant in its replication. In addition, we simulated 3 distinct components of patient care: an injury phase, a prehospital or transport phase, and a treatment phase. During the potential treatment phase, various interventions can be applied, such as different resuscitation fluids and protocols, hemoglobin carriers, and operative techniques. The most important refinement in the current study is the consistency in inducing acute lung injury, namely the histologic changes associated with the condition. Therefore, our swine model may be of great use in evaluating strategies to modify shock and acute lung injury.

Physiologic parameters correlated as expected with the simulated clinical phases during the course of the study. Controlled hemorrhage caused a more than 50% decrease in the mean aortic pressure. Lactated Ringer solution was used to resuscitate the swine. The volume and type of solution that we used as well as the way it was infused simulated the initial approach for fluid resuscitation in trauma and hemorrhagic shock in the prehospital setting and emergency department.[2] The effect of a resuscitation treatment should not be judged only during active volume restoration but also after treatment.[3] In our experimental protocol, we targeted a fluid resuscitation to 90% of the baseline blood pressure. The large volume of Lactated Ringer solution infused resulted in effective hemodynamic performance due to expansion of the intravascular volume. After infusion, hemodynamic variables decreased, probably because crystalloids entered the interstitial space due to lack of intrinsic colloid osmotic pressure.[7]

Patients with massive hemorrhage can experience conditions ranging from severe hypovolemia, in which blood volume decreases with no changes in hemoglobin concentration, to isovolemic anemia, in which extreme decreases in hemoglobin concentration occur with normal or even increased blood volume.[5] In our experiment, hemoglobin concentration dropped quickly after injury and controlled hemorrhage and remained low throughout the 7-h study. An explanation for this result is that aggressive fluid replacement can produce isovolemic anemia, which is characterized by adequate blood volume but decreased hemoglobin concentration and low oxygen-carrying capacity. Isovolemic anemia occurs when blood for transfusion is not readily available or in patients who are bleeding but refuse to accept blood products.[5] The ability to induce experimental isovolemic anemia in the swine without having the hemoglobin concentration drop below 4 g/dL, which is equivalent to a dissolved oxygen concentration less than 10 mL/min/kg (critical dissolved oxygen concentration),[25] makes our swine model easily reproducible.

Increases in tissue lactate concentration and acidosis correlate with depletion of high-energy phosphate compounds and cellular dysfunction. Because lactic acid is a product of anaerobic metabolism, elevated lactate levels represent inadequate tissue oxygen delivery. This decrease in oxygen delivery represents a combination of tissue hypoperfusion and hypoxemia and prompts a systematic diagnostic evaluation in human patients.[2] Several studies have shown a strong correlation between blood lactate levels and the risk of morbidity and mortality in various clinical situations including circulatory shock, septic shock, severe hypoxemia, liver failure, and diabetes. In one study of serial lactate levels in trauma patients over 48 h, subsequent levels were significantly lower in the survivors compared with nonsurvivors.[1] The authors suggested that the ability to clear lactate predicts survival in severely injured patients.[1]

After severe trauma, lactate is a universally accepted, clinically useful indicator of tissue hypoperfusion or hypoxia.[17] A high lactate concentration follows severe insults because excessive mobilized substrates cannot be processed through the Krebs cycle.[16] As a result, intracellular pyruvate builds up and is converted to lactate, which then is taken to the liver to be converted into glucose (the Cori cycle). In our study, the mean baseline lactate level (1.62 ± 0.77 M) increased to 3.52 ± 1.81 M at 20 min after the initiation of hemorrhage and to 6.97 ± 3.78 M at 90 min thereafter.

Table 1. Hemodynamic variables and blood gases after the various phases of the experiment

Phase	Mean aortic pressure (mm Hg)	Heart rate (bpm)	Right atrial mean pressure (mm Hg)	pH	Lactate (M)	Na (mEq/L)	K (mEq/L)	Hemoglobin (g/dL)
Stabilization	88.6 ± 12.1	116.5 ± 13.8	8.2 ± 1.9	7.42 ± 0.07	1.62 ± 0.77	139.3 ± 2.6	3.6 ± 0.4	11.0 ± 0.7
Hemorrhagic	36.9 ± 10.6	148.2 ± 41.2	6.0 ± 1.8	7.42 ± 0.06	3.52 ± 1.81	137.5 ± 3.1	4.4 ± 0.9	8.4 ± 1.2
Maintenance	41.6 ± 16.5	144.3 ± 42.6	6.8 ± 2.0	7.31 ± 0.03	6.97 ± 3.78	137.4 ± 2.4	4.9 ± 0.6	7.9 ± 1.1
Infusion	74.9 ± 8.0	143.1 ± 34.3	7.2 ± 1.5	7.29 ± 0.06	7.76 ± 4.03	137.6 ± 2.9	4.2 ± 0.7	6.4 ± 1.0
Observation	60.8 ± 18.4	116.8 ± 18.0	7.4 ± 1.7	7.36 ± 0.02	7.61 ± 2.33	137.4 ± 3.6	3.9 ± 0.9	6.9 ± 1.1

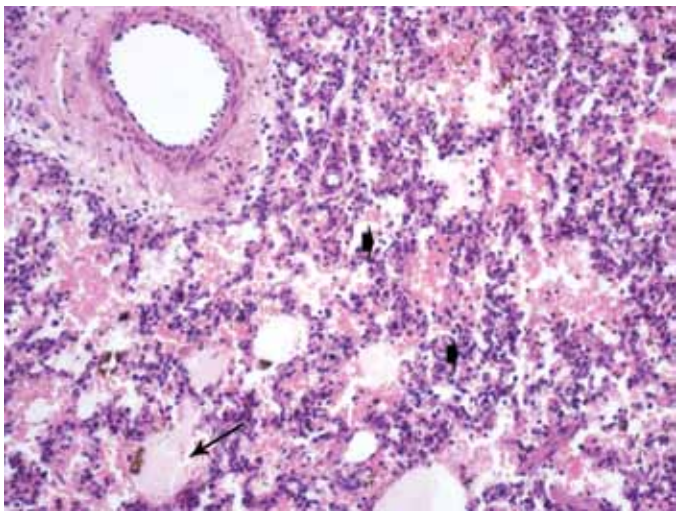

Figure 1. Representative field of lung, demonstrating severe distortion of parenchymal architecture with evidence of edema (arrow) and interstitial and alveolar inflammatory infiltrates (arrowheads). Hematoxylin and eosin stain; original magnification, ×100.

Table 2. Confidence interval (95%) for means of incidence of histologic features

	Lower bound	Upper bound
Focal thickening	1.21	1.59
Vascular congestion	1.54	2.06
Alveolar edema	1.06	1.94
Alveolar hemorrhage	1.61	2.34
Interstitial neutrophil infiltration	1.46	2.44
Intraalveolar neutrophil infiltration	0.26	1.34

Serum lactate levels in our swine were also increased markedly after resuscitation, suggesting tissue hypoperfusion; this finding does not correlate with the amount of fluid given. In patients in hemorrhagic shock, infusion of lactate-based solutions resulted in improvement of vital signs, improvement of metabolic acidosis, and rapid clearance of lactate.[15] The concerns about worsening lactic acidosis with infusion of lactate are based largely on an incomplete understanding of pyruvate and lactate metabolism. Lactated Ringer solution is produced by adding sodium lactate and other electrolytes to sterile water. The sterile water is actually acidic (pH 5 to 7) due to interactions with air and the plastic container, and the addition of sodium lactate increases pH slightly to between 6.0 and 7.5. Therefore, the lactate acts as a base and cannot cause acidosis.[15] We conclude that the elevated lactate levels in our swine resulted from the ongoing tissue hypoperfusion. Lactated Ringer solution is used widely in patients with hemorrhage because it closely approximates the fluid being lost from the circulatory system.[11]

Hemorrhage induced a rapid increase in serum potassium in our swine, at 90 min after blood removal. Arterial plasma potassium concentrations increase after severe hemorrhage,[23] consistent with decreased activity of ATP-dependent Na^+–K^+ ATPase,[4] and are significantly higher in nonsurvivors than survivors after hemorrhage.[14] Possible mechanisms include loss of available Na^+–K^+ ATPase activity or substrate, presence of an in vivo Na^+–K^+ ATPase inhibitor, or uncoupling of Na^+-K^+ ATPase activity from potassium transport.[4] Furthermore the ischemia-induced loss of hepatic potassium could partially account for the increase in extracellular potassium because the liver during shock experiences a large decrease in blood flow, which is not reversed after resuscitation with Lactated Ringer solution.[24]

The anesthesia used in experimentally induced hemorrhage models can be a confounding factor because of its effect on cardiovascular function.[14] Intraperitoneal pentobarbital was used in several studies of pressure-controlled hemorrhagic shock in a rat model.[10,20] Although ketamine has been used, it appeared to be equivalent or inferior to isoflurane anesthesia in swine hemorrhagic shock.[6] In the present study, we chose propofol to maintain anesthesia because it is an underused anesthetic in experimental protocols mainly because of its potent cardiovascular depressant effect. Other authors suggest that propofol suppresses the protective sympathoexcitatory response (increased heart rate, increased peripheral resistance) that maintains blood pressure in response to a loss of 35% of blood volume.[14] However, although propofol may have more pronounced cardiodepressant and vasodilatory effects than does isoflurane, the cardiovascular effects of propofol are dependent on administration rate (that is, a slow induction has minimal cardiovascular effects). Furthermore, anesthesia in the current study was maintained by using continuous infusion, which has been characterized by hemodynamic stability and has not been proven to have significantly more cardiovascular depressant effects than does isoflurane anesthesia.[18] Further work[9] suggests that hemorrhagic shock alters the pharmacokinetics and pharmacodynamics of propofol, mainly because of changes in intercompartmental clearances. In our view, propofol—when carefully titrated—can be suitable (although expensive) for use in experimental protocols for hemorrhage research.

There are many well-established protocols for shock preparations, and the cardiovascular and hemodynamic responses of swine often parallel those in humans rather closely. Hemorrhagic shock can be modeled in pigs by a wide variety of means. We developed a stable experimental model in which hemodynamics did not deteriorate during hemorrhage and the compensatory response was not blunted thereafter, making this swine model

highly reproducible and clinically relevant. A consistent severe injury profile was achieved, after which experimental interventions could be applied. Rodents have been used previously as hemorrhagic shock models.[28] The swine model has a resting heart rate that averages that of humans (60 to 100 bpm), whereas that of mice is approximately 500 bpm with an extremely short refractory period and that of rats averages from 260 to 450 bpm.[26] Given that heart rate is expected to increase after acute blood loss, monitoring heart rate may be difficult in these rodent species.

Animal models are used for 2 relatively distinct applications: investigating pathogenetic mechanisms and assessing therapeutic modalities. Control and reproducibility of the model are important. In this regard, pressure- and volume-controlled models are preferred to uncontrolled hemorrhage models. In our experimental protocol, we used a fixed-volume hemorrhage model. A key advantage of this model is its applicability to elucidating the hemodynamic response specific to a designated volume of blood loss.[12]

Our experimental protocol has several limitations. First our swine model of controlled hemorrhage does not take into account any potential associated traumatic injuries, which could contribute to systemic inflammation and subsequent organ damage. Moreover, the general anesthesia used in our model affects the hemodynamic response to hemorrhage, as mentioned earlier. The most noteworthy limitation of the current study was that lung injury was evaluated early after the initiation of hemorrhagic shock. In different studies, the observation phase ranges from 3 to 24 h.[8,19] However, the facts that acute lung injury occurred early in the experimental period and developed in all subjects make this a useful model of hemorrhagic shock.

Despite these limitations, we conclude that our protocol of hemorrhagic shock and fluid resuscitation in Landrace–Large White swine may be useful for further research addressing hemorrhagic shock and acute lung injury.

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