Comparative Efficacy Study of Two FDA Approved Complement Inhibitors in Pre-Hospital and Early Hospital Model of Traumatic Hemorrhage in Swine

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NOTICE AND SIGNATURE PAGE

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14. ABSTRACT- Prompt control of hemorrhage and adequate resuscitation are critical elements in managing hemorrhage patients. Conventional fluid resuscitation is designed to reestablish tissue perfusion; however, it fails to reduce concomitant inflammatory and coagulopathic responses. In fact, fluid resuscitation can further heighten inflammatory responses, worsen coagulopathy, and cause further cellular damage, all of which exacerbate trauma/hemorrhagic injury. The dysfunctional immune response triggered by trauma/hemorrhage is the basis for subsequent development of Multiple Organ Dysfunction (MOD). Early MOD is attributed to an overwhelming leukocyte driven pro-inflammatory response clinically defined as systemic inflammatory response syndrome (SIRS). Treatment with blood products or by targeting individual immune components has not improved clinical outcomes of inflammatory complications after trauma. Targeting the complement cascade is an alternative approach that could be employed to indirectly dampen multiple downstream immune effector functions that are activated during traumatic hemorrhage. The aim of our study was to compare the efficacy of a Complement C1 inhibitor (C1INH) and a Complement C5 inhibitor. Unfortunately, the partner responsible for providing the Complement C5 inhibitor withdrew their support prior to the start of this work. Through subsequent analysis of our findings from the model development process, it was determined that examining the effect of splenectomy on surgical outcomes and immune response would be the most judicious use of the remainder of the funds associated with this project.

The decision to perform a splenectomy in large animal models of hemorrhagic shock is controversial, as hemorrhage-induced splenic contraction returns erythrocytes into circulation. However, few studies have examined splenectomy in lethal models. We hypothesized that the spleen may play a role in decompensation as a volume sink, increasing early mortality. We performed splenectomies on 9 swine (NoSpl) and performed a subsequent polytrauma and hemorrhage and compared the findings to those from a historical polytrauma/hemorrhage group of 14 swine (Spl). Survival was significantly improved (from 43% to 89%, p=0.02) and the need for fluids was significantly slowed in the NoSpl group. Rapid crystalloid resuscitation in Spl animals caused erythrocyte, platelet, and plasma protein (among others) dilutions that disappeared in under 2h, suggesting the added fluid extravasated. Oxygen consumption was higher in the Spl group but was greater or equal to baseline at all-time points in both groups. Glucose dropped significantly in the Spl but not the NoSpl group, suggesting a potential hyper-metabolic state. Plasma sodium and potassium were also more disrupted in the Spl group. Inflammatory cytokines and leukocytes increased earlier in the NoSpl animals but were more resolved by 14h. Thromboelastography was similar between groups. Our findings support no splenectomy when studying early mortality from decompensation and splenectomy when studying late mortality from organ injury.

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1.0 EXECUTIVE SUMMARY

Prompt control of hemorrhage and adequate resuscitation are critical elements in managing hemorrhage patients. Conventional fluid resuscitation is designed to reestablish tissue perfusion; however it fails to reduce concomitant inflammatory and coagulopathic responses. In fact, fluid resuscitation can further heighten inflammatory responses, worsen coagulopathy, and cause further cellular damage, all of which exacerbate trauma/hemorrhagic injury. The dysfunctional immune response triggered by trauma/hemorrhage is the basis for subsequent development of Multiple Organ Dysfunction (MOD). Early MOD is attributed to an overwhelming leukocyte driven pro-inflammatory response clinically defined as systemic inflammatory response syndrome (SIRS). Treatment with blood products or by targeting individual immune components has not improved clinical outcomes of inflammatory complications after trauma. Targeting the complement cascade is an alternative approach that could be employed to indirectly dampen multiple downstream immune effector functions that are activated during traumatic hemorrhage. The aim of our study was to compare the efficacy of C1INH and a Complement C5 inhibitor. Unfortunately, the partner responsible for providing the Complement C5 inhibitor withdrew their support prior to the start of this work. Through subsequent analysis of our findings from the model development process, it was determined that examining the effect of splenectomy on surgical outcomes and immune response would be the most judicious use of the remainder of the funds associated with this project.

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2.0 INTRODUCTION

In recent conflicts, 90% of deaths from combat-related potentially survivable injuries (PSI) were the result of hemorrhage or its sequelae (1). Fatalities from PSI can caused by: 1) failure to stop the hemorrhage, 2) failure to compensate for the hypovolemia, and 3) failure of one or more organ systems. Though inter-related and triggered by the same injury, each of these is fundamentally a separate problem that must be addressed in the priority listed, i.e. if the hemorrhage is not stopped, no amount of cardiovascular compensation is going to be sufficient to prevent fatality. Likewise, decompensation may become fatal long before organ dysfunction develops or progresses to organ failure (2, 3). These multiple pathways to death add challenge and potential for misinterpretation of findings to pre-clinical studies of hemorrhagic shock (HS). For example, a treatment designed to prevent organ injury tested in a model with deaths caused by decompensation may be falsely rejected (blamed for deaths occurring via a decompensatory mechanism) or falsely supported (credited with a reduction in organ damage due to inclusion of data from early decompensating subjects with insufficient time to develop organ injury).

We recently encountered this difficulty in a 15-hour swine hemorrhage + polytrauma protocol (not published at the time that this report was written). The untreated group had relatively little organ damage as measured by histology and clinical biomarkers, making it difficult to determine if the study drug could reduce the organ injury. Despite this, there was a greater than 50% mortality (even excluding animals that died before treatment was given). If we increased the severity of shock to increase the organ damage, it is likely that the mortality from decompensation would have similarly increased, making it impossible to properly test treatment effects on organ dysfunction. We needed a way to reduce or prevent decompensation that would not invalidate the model for the purposes of pre-hospital combat casualty care.

For a solution, we looked to the spleen. It is well known that the spleen filters out older, less flexible erythrocytes for controlled destruction and recycling of iron and also that swine and canine spleens are contractile. In response to sympathetic stimulation, they will reduce in size, forcing high hematocrit blood back into circulation to aid in oxygen transport during a stressed state (4-6). While it was originally believed that the human spleen did not have this capability (7, 8), recent studies have shown that human spleens do in fact share this trait with swine, although the human spleen is smaller (4, 9) and the contraction may be short lived, making it more difficult to monitor (10-12). The question of whether or not to include splenectomy in large animal models of shock has been debated for many years. Most studies provide no clear rationale for it and there is "little evidence in the literature to support or refute the need to remove the spleen in acute porcine hemorrhagic shock models" (4). Some include splenectomy to remove the variability caused by inconsistent degrees of splenic contraction in response to hemorrhage (13, 14), while others argue that leaving the spleen in brings hematocrit closer to human, thereby improving applicability of the model. One group has compromised by spraying the exterior of the spleen with adrenaline to induce maximal contraction prior to excision (oral communication).

Though the focus has been on the change in oxygen carrying capacity brought on by the influx of circulating erythrocytes, splenic contraction serves an additional compensatory role through its effect on vascular volume. Regardless of how fast or how strong the heart is able to beat, cardiac output is ultimately limited by venous return, which in turn is dependent on the pressure gradient between the vena cava and the right atrium. *Unstressed volume* is the volume needed to fill the vasculature without stretching it. The volume in excess of this (i.e. *stressed volume*) stretches the vasculature and pressurizes the blood through elastic recoil (15). It is this pressure that ultimately drives the filling of the heart. Without stressed volume, there is no cardiac output.

The threshold between unstressed and stressed volume is dependent on the size of the vasculature. Vessels constrict in response to hemorrhage, reducing the threshold to make more of the total remaining volume count as stressed volume, thereby maintaining venous return (15). Constriction of the spleen has the same effect, improving our ability to compensate for hypovolemia (not just hypoxia). However, when the body is pushing its limits for compensation and the spleen has maximally contracted, it must then maintain that contraction or the subsequent increase in threshold could transform the remaining stressed volume to unstressed volume; barring an immediate influx of new fluid, this would cause venous return/cardiac output to plummet. Likewise, resuscitation is only useful for hemodynamics if it increases stressed volume. Added fluid will expand the part of the vasculature with the least resistance, i.e. arteries constrict with more force than veins, so generally, venous volume will increase before arterial volume in response to fluid (this is one reason that resuscitation does not always improve functional capillary density).

The spleen may represent another "weak" spot, such that some of the early resuscitation goes to refilling it. If the spleen increases its volume *non-elastically* in response to resuscitation instead of resisting it, that added volume will be unstressed and will not increase blood pressure. Not only is this not beneficial; it can be directly harmful depending on the fluid used. Crystalloids dilute plasma proteins, lowering oncotic pressure, resulting in the fluid rapidly leaking out as edema. Whether or not the vasculature or spleen can re-constrict as easily after this fluid escapes is unknown. While crystalloids may buy time, barring additional resuscitation, they may leave the individual in a worse condition than before: hypovolemic *and* edematous.

Our hypothesis is that the spleen represents not only a volume source, but a volume sink, increasing subsequent decompensation and reducing the effectiveness of resuscitation. The objective of this study was to determine if splenectomy improves the ability of swine to maintain a compensatory state after polytrauma, hemorrhage, and resuscitation, thereby increasing short-term (<14 h) survival and allowing time for the development of more organ damage.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

Swine model

Research was conducted in compliance with the Animal Welfare Act, the Animal Welfare Regulations, and the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AAALAC. The model is a modification of an existing polytrauma model incorporating a blunt chest trauma, an uncontrolled hemorrhage (penetrating liver injury), and a controlled hemorrhage (16). Because this study was undertaken as an effort to improve the animal model for future studies, there are some additional procedural differences, detailed below and summarized in **Figure 1**, between the splenectomized group (NoSpl, N=9) and historical group with the spleen intact (Spl, N=14).

Preparation

Briefly, 40±5 kg male Yorkshire crossbred swine were pre-medicated and anesthetized using atropine (0.04-0.4 mg/kg) and Telazol (6-8 mg/kg) before being transported to the procedural room. Immediately upon arrival, an ear vein catheter was placed followed by endotracheal intubation. Swine were maintained on isoflurane (1.0-3.0%) via an anesthesia machine and automatic ventilator (Draeger Medical Apollo Gas system and Infinity Explorer Monitoring System, Telford, PA) during instrumentation. The ventilator was set to an initial tidal volume of 10 ml/kg body weight, 20 breaths per minute, and peak pressure at 20 cm H_2O. Settings were adjusted as needed to maintain end tidal pCO_2 at approximately 40 mmHg. The fraction of inspired oxygen (FiO_2) was limited to simulate an austere environment where supplemental oxygen may not be available. In the NoSpl group, FiO_2 was kept between 0.21 and 0.3. Unfortunately, FiO_2

was not monitored closely in the Spl group and may have been as high as 0.4. Cut downs with blunt dissections were performed to isolate the carotid artery and jugular vein. A pressure transducer-tipped catheter (3.5Fr. Mikro-Tip, Millar Instruments, Inc., Houston, TX) was placed non-occlusively into the carotid artery for blood pressure monitoring. A Swan Ganz catheter (8Fr., 110cm, Q-Tip, heparin Coated; ICU Medical, San Clemente, CA) was advanced into the pulmonary artery via the jugular vein for measurement of continuous cardiac output via thermodilution (Q2 Plus SO_2Continuous Cardiac Output Computer, ICU Medical, San Clemente, CA). An additional cut down with blunt dissection was performed for isolation and cannulation of the femoral artery and vein (8 Fr side port/percutaneous catheter inducers; Argon Medical Devices, Athens, TX) for arterial hemorrhage, venous and arterial blood sampling, and intravenous infusion of resuscitation fluid. A second pressure transducer-tipped catheter was inserted through the hind port of the arterial catheter for blood pressure monitoring. Hemodynamic and cardiac data were collected utilizing the data acquisition instrumentation rack/Biomedical Data Recorder (DAQ) and Physiological Data Recorder program (Dynamic Research Evaluation Workstation- DREW, US Army Institute of Surgical Research, San Antonio, TX). Once lines were placed, a midline laparotomy was conducted for cystotomy. In NoSpl animals, the spleen was then isolated and vessels ligated using two umbilical cord tapes per blood vessel along with two sutures before cutting and complete removal of the spleen.

Prior to obtaining baseline readings, swine were transitioned from isoflurane to intravenous anesthesia through the ear vein catheter. The Spl group was given an initial bolus of sufentanil (2.5 μg/kg) and midazolam (0.1 mg/kg) followed by a continuous rate infusion (CRI) of sufentanil (1.0-20.0 μg/kg/h) and midazolam (0.07-0.15 mg/kg/h). However, due to a shortage in sufentanil supply, the NoSpl group was given propofol (3-12 mg/kg/h) and buprenorphine (2-8 ug/kg/h) in place of sufentanil and midazolam. Isoflurane was then reduced to reach a minimum alveolar concentration (MAC) ratio between 0.2 - 0.8. After a stabilization period of at least 10 minutes to adjust to the change in anesthetic, the baseline (BL) blood sample was drawn.

Poly-trauma Injury

To simulate a penetrating abdominal trauma, the left medial hepatic lobe of the liver was isolated, two lap sponges were placed (one below and one above) to capture any un-suctioned blood from the hemorrhage, and, using a custom scalpel, two cross-shaped puncture wounds (each of the 4 arms was 1” long) were made on the midline, 12 cm and 8 cm from the edge of the lobe. These wounds were allowed to bleed freely for 60 seconds. The uncontrolled hemorrhage volume was continuously quantified by suctioning shed blood into a canister (Vac-rite, disposable suction system, Baxter, Deerfield, IL) on a balance (SR16000, Mettler-Toledo, Greifensee, Switzerland). The two lap sponges were removed for weighing (to determine total uncontrolled hemorrhage), followed by immediate packing of the wound with five new lap sponges.

To create the lung contusion injury, we fired a captive bolt against a steel plate (3 inch by 5 inch, 1 cm thick, with 1 cm thick foam padding) to distribute the force of the injury. The plate was held against the ribs below the right front leg using a strap around the torso at consistent tension (15 lbs) to improve reproducibility. The animals were removed from the ventilator for a few seconds at the time of the lung injury to avoid creating tension pneumothorax injuries.

Immediately following completion of the liver and lung injuries, we began a controlled hemorrhage (100 ml/min) via the femoral artery. Blood was drawn until the mean arterial pressure (MAP) reached 30 mmHg. This signaled the start of shock (T = 0 h) and the drawing of the B0 blood sample. We then maintained a MAP of 30 ± 5 mmHg turning the pump on and off, as needed, until the full controlled hemorrhage was drawn. For the Spl animals, the controlled hemorrhage was 24 ml/kg body weight. However, since the uncontrolled hemorrhage was variable (3.1 ± 1.6 ml/kg), this led to inter-animal variations in the total hemorrhage. To improve the model going forward, the volume removed in controlled hemorrhage for the NoSpl animals was calculated so that the total hemorrhage (controlled + uncontrolled) would equal 24 mL/kg. With regards to total loss of volume, the two groups remained essentially equivalent, with the known 3.1-ml/kg greater hemorrhage in the Spl group offset by ~4.5 ml/kg of blood volume (estimate of splenic blood volume (17)) removed with the excision of the spleen in the NoSpl group.

After completion of hemorrhage, no additional blood was removed or fluid given until resuscitation at T = 1.5 h, at which time we replaced up to 3x the shed blood volume with Lactated Ringer's (LR) Fluid. Resuscitation started with a 60 ml bolus of LR given over one minute, followed by a rate of 1.5 ml/kg/min as needed to maintain MAP at 65 ± 5 mmHg. While NoSpl animals were allowed resuscitation fluids until the end at T = 14 h, in the original protocol (Spl group), resuscitation was halted at T = 2.5 h. However, this restriction only caused a slight reduction in the volume given to two animals (see Results), so overall, the resuscitation volume remained consistent between groups. After resuscitation, only maintenance fluids (LR) were given (0.5 ml/kg/h) for the remainder of the protocol. After collection of the final blood sample (see below), animals were euthanized with sodium pentobarbital (Euthasol; 120 mg/kg).

Differences between Spl and NoSpl group

In addition to those mentioned above, there were two other differences between the NoSpl and Spl procedures with regards to the order of injuries and the time of blood draws (**Figure 1**). First, in the Spl group the lung injury occurred prior to laparotomy. To reduce the amount of time between the start and completion of injuries going forward, the lung injury was moved to after the liver injury in the NoSpl group. For the Spl group, 13 ml blood samples were taken at baseline, at T = 0, T = 1.5h, at the end of resuscitation (T = ~ 2.5 h), and then hourly from that through T = ~13.5 h. The final blood draw, however, was taken 15 h after the lung injury (placing it at T = 14.4 h on average). To reduce temporal variability between the groups, for this study (NoSpl), the blood samples were taken at baseline, T = 0, T = 1.5h, and T = 2 h, then hourly through the final sample at T = 14 h. Due to the slight offset for the blood samples taken after the start of resuscitation, the NoSpl samples are compared to the following Spl sample (e.g. the B10 sample was at 10 h in NoSpl vs ~10.5 h in Spl).

Blood sample analysis

Complete blood cell count (CBC) was determined immediately after blood draw by HemaTrue hematology analyzer (Heska Corp. Loveland, CO). Blood gases and electrolytes were determined immediately after blood draw using the ABL 800 Flex blood analyzer system (Radiometer, Brea, CA). Thromboelastography was performed within an hour of blood draw with a Haemoscope 5000 (Haemonetics Corp., Braintree, MA). For each time point a 5 mL blood aliquot was centrifuged (15 minutes, 15,000 rcf) and the plasma aliquoted and frozen (-80 ºC) for later analysis. For cytokine and chemokine expression analysis, we used the MAGPIX System (Luminex, Austin, TX) with a Milliplex MAP Kit Porcine Cytokine/Chemokine Magnetic Bead Panel (PCYTMAG-23K, Millipore Sigma, Burlington, MA) per manufacturer instructions.

Arterial and venous oxygen concentrations (c_AO_2 and c_VO_2 in mL O_2 per dL blood) were calculated from the measured total hemoglogin (tHb in g/dL), oxygen saturation (sO_2 in %), and partial pressure of oxygen (PO_2 in mmHg) by the equation $cO_2 = (tHb \times 1.34 \times sO_2) + (PO_2 \times 0.003)$. Then oxygen delivery ($DO_2$ in L of O_2 per min) and oxygen extraction (VO_2 in L of O_2 per min) were calculated using the oxygen concentrations and cardiac output (CO in L/min) using the equations $DO_2 = CO \times c_AO_2 \times 0.01$ and $VO_2 = CO \times (c_AO_2 - c_VO_2) \times 0.01$.

Data Analysis

Data are expressed as Mean ± Standard Deviation. Continuous measures were analyzed by Student's t-test. Kaplan-Meier curves were analyzed by log-rank test. Significance was set to $p < 0.05$. Microsoft Excel was used for statistical analysis.

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

In preparation for the execution of this project,

- Kick Off Meeting – 1 May 2015
- IRB/IACUC Approval – April 2015
- All experimental procedures completed – January 2019
- Data Analysis – May 2020
- Poster presentation – 42nd Annual Conference on Shock, Coronado, CA, June 2019
- Manuscript submitted to – Shock, submitted
- Dissemination of Results – October 2021

5.0 RISK ASSESSMENT

5.1 Risk Analysis: Following the development of an MTA, the company scheduled to provide the C5 inhibitor decided to end their association with this project. This raised the overall risk assessment of the initial project to high schedule and cost risk.

5.2 Technical Challenges: The lack of C5 inhibitor was insurmountable in completing the initial proposal. Further difficulties in sourcing sufentanil during the experiments described in this report provided a challenge by requiring the model to be altered following the initial design of the experiments.

6.0 TRANSITION PLAN

6.1 Military Relevance: Predictions for future conflicts indicate a potential for delayed evacuation, delayed damage control surgery, and austere environments. These conditions greatly increase the risk of death from decompensation even when tourniquets and hemostatic dressings have stopped hemorrhage. Hemodynamic support is required to prevent decompensation. The primary means of this support has been to reverse the hypovolemia as soon as possible with fluids, but operations far forward of controlled territory or in austere environments may mean that these fluids are limited to what the combat medic can carry. Additionally, care-under-fire conditions may delay the start of resuscitation. Fresh whole blood and blood components are considered ideal for volume replacement, but they are logistically difficult to bring to the front lines and are highly susceptible to spoilage with short shelf lives and poor temperature stability, in addition to the concerns that as a biologic, they may cause disease transmission or have compatibility issues in the recipient.

6.2 Transition Strategy

KRL 3 to KRL 4

Project will transition to replication in further military relevant models with well controlled and to confirm or reject hypotheses presented within report.

7.0 RESULTS

Survival, depth of shock, and hemodynamics

Though the total blood loss between groups was similar, the amount of controlled hemorrhage required to initially reduce MAP to 30 mmHg was 9.9±2.7 ml/kg in the Spl group as compared to 14.0±5.2 ml/kg in the NoSpl group ($p = 0.05$). Survival was significantly greater in the NoSpl group than in the Spl group (89% vs. 43%, $p = 0.02$) (**Figure 2**). Since lactate and base excess were similar between groups at all points in the experiment, it is likely that the greater mortality in the Spl group was not linked to the depth of shock (**Figure 3A and B**). Similarly, there were no differences in venous pH, except where it dropped more in the Spl group at B0, which may reflect the greater time since lung injury in the Spl group and which was equalized between groups by the end of shock at B1 (**Figure 3C**). Hemodynamically, we observed that mean arterial pressure (MAP) was significantly elevated in the NoSpl vs the Spl group for the first 6 h post-injury (**Figure 4A**). This was particularly evident at the end of shock (T = 1.5 h). Breaking MAP into its components of diastolic arterial pressure (DAP) and pulse pressure (PP), we observed that DAP in the NoSpl group remained significantly elevated through T = 10 h (**Figure 4B**). While PP in the Spl group was similar to NoSpl early on (except at the end of shock), it increased to be significantly greater than NoSpl PP by T = 14 h, Spl PP, at which point it was approximately double its baseline value (**Figure 4C**). There was an early trend for elevated heart rate (HR) in the NoSpl group (which was significant at the end of hemorrhage), but HR was equivalent between the two groups from the end of shock through the rest of the study (**Figure 4D**).

Fluids and volume effects

The effect of fluid resuscitation was profoundly different in Spl vs NoSpl animals. Though Spl animals were only resuscitated for 1 h, all but two animals reached the maximum 72 ml/kg of LR (and those two reached 67 and 68 ml/kg), indicating pressure stopped increasing with fluids which suggests a lack of resistance to the fluid influx (**Figure 5A**). In contrast, only the sole non-survivor in the NoSpl group reached the maximum resuscitation in under an hour of resuscitation. Most of the NoSpl animals needed little fluid to maintain their MAP above 60 mmHg. As a result, even by T = 5 h, only a third of NoSpl animals had reached maximum LR.

Spl animals respond to hemorrhage with splenic contraction. Because the hematocrit of blood in the spleen is much higher than that in circulation, this manifested as a significant increase in the circulating red blood cell (RBC) concentration, as measured by CBC, at B1 (**Figure 5B**). LR resuscitation caused a massive dilution of blood such that between B1 and B2, RBC concentration went from being significantly elevated in the Spl group to being significantly lowered relative to the NoSpl group. This change was short-lived, however, and the RBC concentration began to rise again until RBC concentration was again significantly greater than that of the NoSpl group, which did not receive an influx of RBCs from the spleen. After B4, there was a slow downward trend in RBC concentration in both groups, but RBC remained consistently and significantly higher in the Spl group. Since the resuscitation fluid was a crystalloid, a similar dilution response can be seen with total protein (though there was no influx of protein in the Spl group to match the influx of

RBCs) (**Figure 5C**). The differential response to fluid resuscitation was also apparent when we examined cardiac output (CO). During shock, CO was significantly higher in the NoSpl group (**Figure 5D**). Within 30 min of the start of resuscitation, NoSpl CO returned to baseline, whereas the Spl CO overshot baseline to become briefly significantly elevated compared to NoSpl. As discussed below, this overshoot likely reflects that the decreased plasma viscosity lowered resistance to flow, rather than improved cardiac function.

Oxygen dynamics

Our direct measurement of CO, hemoglobin, and arterial and venous blood gases enabled us to calculate oxygen transport. There was a small but significant difference between groups in measured arterial oxygen saturation (**Figure 6A**). However, the difference was present at baseline, possibly due to differences in the fraction of inspired oxygen settings between groups, and values stayed constant throughout the duration of the experiment (with the exception of the large variation at B8 from an animal that was 5 minutes from death). Since arterial oxygen saturation was lower in the group with higher survival, it is unlikely that it played an important role in mortality in our model. The steadiness of the values also suggests that the lung injury, at least when combined with mechanical ventilation, was not sufficient to significantly impair primary lung function. In contrast to arterial saturation, venous oxygen saturation varied considerably over the course of the experiment. SvO_2 started the same at baseline in both groups and plummeted during shock, though to a lesser extent in the NoSpl group, perhaps reflecting the improved CO in that group (longer RBC transit times allow more off-loading of oxygen). However, despite equivalent CO from B3 onward, SvO_2 remained elevated in the NoSpl group through B6, suggesting that there may have been additional factors at play. Oxygen delivery to tissues (DO_2) was similar between groups through the end of shock at B1, at which point delivery had fallen to approximately half of baseline (**Figure 6B**). For the Spl group, DO_2 returned to baseline levels at B2 with resuscitation, but levels rapidly rejoined those of the NoSpl group, which never recovered to baseline (despite better survival). The amount of oxygen being extracted from the circulation (VO_2) did not drop below baseline as a consequence of shock in either group (**Figure 6C**). Aside from a brief increase at B0, the NoSpl animals saw no real change in their VO_2. In contrast, the Spl VO2 was significantly greater than the NoSpl VO_2 at multiple time points after resuscitation. At approximately the same time, circulating glucose levels fell in the Spl group, but not in the NoSpl group, resulting in a significant difference between the groups (**Figure 6D**). Combined, these data suggest that the Spl animals, but not the NoSpl animals, may have been in a hyper-metabolic state.

Electrolytes

Splenectomy before polytrauma had a surprisingly large effect on plasma electrolytes. In both groups, there was a downward trend in sodium ions and upward trend in potassium ions (**Figure 7A and B**). However, this trend was greater in the Spl animals than in the NoSpl animals (note: if we first subtract baseline K^+ values, Spl is still significantly greater than NoSpl from B7 onward; data not shown). Calcium ions also dropped more in the Spl group (**Figure 7C**). Chloride ions in both groups dropped over time, and though there were significant differences between groups at nearly every time point, much of that was due to a large difference in baseline values, suggesting that splenectomy could have triggered an increase in chloride ions and shock a decrease (**Figure 7D**). If we subtract baseline values, the increase in chloride ions was significantly greater in the Spl group than the NoSpl group from B1 to B6 as well as at B9, suggesting there was an additional interaction between shock and splenectomy. These data suggest that a large fraction of the changes to electrolytes observed in shock may be tied to the splenic response.

Inflammation and coagulopathy

Platelet concentration closely reflected the earlier observed effects of shock on RBCs, except that the Spl group did not see the same increase in platelet concentration as it did in RBC concentration as a result of splenic contraction (**Figure 8A**). However, the Spl group did show the effect of the temporary dilution of all blood cells by LR resuscitation. In contrast, the level of white blood cells showed trends indicating changes beyond those caused by movement of fluid into and out of the vasculature. Lymphocytes concentration temporarily increased during shock in the Spl group, but not in the NoSpl group (**Figure 8B**). Both groups showed a trend for increasing relative levels of lymphocytes compared to platelets and RBCs at later time points. Circulating granulocytes increased sharply in both groups in response to shock (though the effect was greater in the NoSpl group) and continued to increase even during the dilution caused by LR (**Figure 8C**). Interestingly, after concentration peaked around T = 4 h for NoSpl and T = 5.5 h for Spl, granulocyte concentration dropped off in both groups faster than the drop in platelets or RBCs. Monocytes, similar to the granulocytes, appeared to increase in response to shock, especially in the NoSpl group (**Figure 8D**). The later gradual decrease in concentration, however, was more similar to that seen in platelets and RBCs.

We measured the plasma concentration of a number of inflammatory and anti-inflammatory cytokines. We found no differences in IL-1β, IL-8, IL-12, and IL-18 (data not shown). At the end of shock (B1), NoSpl animals had significantly elevated GM-CSF, IFN-γ, TNF-α, IL-1a, IL-1ra, IL-2, IL-4, IL-6, and IL-10 (**Table 1**). However, the situation reversed by the end of the experiment such that the concentration of all of these cytokines were lower (closer to baseline) in the NoSpl group (significantly lower for IFN-γ, IL-1a, IL-1ra, IL-2, IL-4, and IL-10).

Thromboelastography was used to determine effects of splenectomy prior to polytrauma on coagulation. Relative to baseline, NoSpl animals tended to have faster split points (SP), and Spl animals to have slower ones, resulting in significant differences between the groups at numerous time points (**Figure 9A**). There were no significant differences in R time (not shown), however. This meant that Delta, the time between SP and R representing the thrombin burst, was often significantly shorter in the Spl group (**Figure 9B**). The rate of initial clot formation (Angle) was similar between groups and changed little over the course of the experiment (**Figure 9C**). There was a small but significant improvement in maximum clot strength (i.e. maximum amplitude, MA) in the NoSpl group in the earlier half of the experiment (**Figure 9D**). The Spl group was also a little slower to reach MA after shock, relative to baseline and to the NoSpl group (**Figure 9E**). There were no apparent differences in clot lysing, however, from either the shock or group effects based on the percent of clot lysis at 60 min (CL60; **Figure 9F**).

8.0 CONCLUSION/DISCUSSION

Shock period

Despite the overall significant difference in mortality, lactate and base excess were similar between groups at the end of shock, suggesting a similar degree of tissue that was under-perfused. However, even during the 1.5 h shock period, NoSpl animals were better able to maintain cardiac output and DAP, which is usually associated with vascular function. The amount of total protein dropped similarly between groups, suggesting a similar degree of auto-transfusion. Therefore, improved DAP and cardiac output suggest that a greater fraction of total vascular volume in the NoSpl group was stressed volume, especially if we assume that both groups were operating near the maximum possible cardiac output allowed by venous return during hypovolemia. Cardiac effort, as represented by HR and PP, which reflects trends in stroke volume (18), also appeared modestly increased in the NoSpl animals, as expected for the higher cardiac output. However, increased HR has been reported previously in other studies and is known to start with the splenectomy itself (4), suggesting that the NoSpl group may have had a head start on compensation (though this would not affect maximum cardiac output set by venous return). Combined, DAP and PP produced a significantly improved MAP in the NoSpl animals. The boost in RBC concentration during shock in the Spl group provided surprisingly little increase in the rate of arterial oxygen delivery during shock compared to NoSpl subjects, though it may have made a difference after resuscitation. Oxygen usage, in contrast, increased during shock in the Spl group, suggesting these animals were "working harder" but to less effect than NoSpl animals. If *total* oxygen delivery was a limiting factor, we would have expected oxygen usage to drop below baseline. That it did not drop in either group suggests there is sufficient oxygen for the tissues the red blood cells can reach, while the increased lactate and decreased pH and base excess indicate there are tissues not being reached.

Resuscitation and Intensive Care periods

One of the animals in the Spl group had decompensated to the point that administration of fluid, beginning at T = 1.5 h could not rescue it. However, even in the remainder of Spl animals the benefit of resuscitation was short-lived. In the first 30 min of resuscitation, 39 ± 5 ml/kg of fluid raised the Spl MAP by approximately 20 mmHg to 58 ± 5 mmHg. In contrast, the following 30 min of rescuscitation (32 ± 4 ml/kg) yielded no further improvement in MAP (61 ± 10 mmHg). This indicates that all of the later fluid (and likely some of the earlier fluid) contributed only to unstressed volume, i.e. the vasculature expanded to receive the fluid rather than resisting it. This crystalloid volume led to dilution of RBCs in the Spl group, but also to dilution of plasma proteins. The effects of this are two-fold. First, by reducing the viscosity of the blood, the resistance to flow through blood vessels is also reduced. If driving pressure is held constant, lowering resistance results in increased flow (Flow = Pressure/Resistance). This, better than any putative improvement in cardiac health, explains the large increase in cardiac output with resuscitation in the Spl group. Second, dilution lowers the oncotic pressure of the plasma. Starling's principle of flow in the capillary tells us that if oncotic pressure drops below hydrostatic pressure, fluid flow through the capillary wall will increase (even if capillary permeability to fluid remains the same) (19). Unless the dilution is being maintained exogenously, over time this outward flux will increase plasma protein concentration and therefore oncotic pressure. For shock resuscitated with crystalloid, this means that most, if not all, of the added volume will leak into the tissues, causing the vasculature to become hypovolemic once more and pressure to drop. This is exactly what was observed here in the Spl group, as RBC concentration rose over the next two hours following the end of resuscitation, indicating a loss of plasma volume, until RBC concentration was again significantly greater than that of the NoSpl group, which received no influx of RBCs from the spleen. The simultaneous drop in cardiac output supports the idea that blood dilution is why it rose so high.

Unlike the Spl group, NoSpl animals needed little fluid to maintain their target pressure, suggesting a greater portion of went directly to stressed volume. There was no great dilution of the blood, and no large changes in RBC concentration or cardiac output. Instead we observed a slow, steady drop in RBC concentration. This implies that, on average, the vascular volume was slowly growing from the time of injury onwards adding fluid to the plasma, first from endogenous sources then exogenous (e.g. venous relaxation causing a drop in capillary hydrostatic pressure and a net drive towards greater fluid retention in the vessels). This suggests that, at least in this model, decompensation may have started earlier than we expected and was ongoing even in survivors.

Interestingly, once the effects of the massive crystalloid resuscitation faded away, the survivors in the Spl group show the exact same trend in RBC concentration, offset only by what is presumably the influx of RBCs from the spleen during hemorrhage. Total protein, which was nearly identical between groups from B4 on, did not show the same downward trend as RBC concentration. This could indicate that the plasma protein concentration had dropped to the point that it was no longer higher than that of the incoming lymph fluid. Alternatively or additionally, new plasma protein synthesis may have been sufficient to match the deficit between the plasma and the incoming fluid.

Overall, we saw that resuscitation with a crystalloid provided only a short-term benefit to blood pressure. This may be sufficient when transit time to surgical care is expected to be short, but the benefit appears to be severely truncated in a prolonged field care scenario, as modeled here. These findings are in agreement with a recent report, describing equal benefit of pre-hospital crystalloid with plasma resuscitation only when pre-hospital transit times were under 20 minutes (20).

Mortality

From a rat splanchnic arterial occlusion model of shock, we previously put forward the observation that decompensation comes in two forms: 1) a slow form, in which, after being sympathetically stimulated by intestinal ischemia for hours, blood pressure would begin an hours-long decline leading to death, and 2) a rapid form, in which blood pressure would spontaneously plummet over the course of minutes, resulting in a much earlier death (3). More recently, we observed the same phenomenon in a rat hemorrhagic shock model and saw that the acute decompensation could occur more than an hour after the start of resuscitation and result in a fatal loss of pressure even while receiving fluid resuscitation (21 and data not yet published). Our hypothesis is that the acute death is a result of a failure in the efferent sympathetic signaling, whereas the slow deaths are the result of the vasculature losing the ability to respond to that signaling. In our rat models, we found that factors that increased the actual or perceived need for a sympathetic response to ischemia tended to increase the odds of acute decompensation, while factors that made the response easier improved survival.

In this swine model, based qualitatively on the rate of blood pressure decline, we saw that of the nine deaths in the Spl group, five appeared to be acute decompensations, three were slow decompensations, and one could not be assigned to either type, as blood pressure declined over an intermediate interval of ~35 minutes. The one death in the NoSpl group was a slow decompensation, though it appeared that another subject was also slowly decompensating and would likely have died within another hour or two. These results fit with theory, as any volume pooling in the spleen would logically tend to lower blood pressure in the Spl animals, which would be sensed by sympathetic afferents and interpreted as a need for greater sympathetic output, increasing the odds of acute decompensation. Likewise, the need to more powerfully constrict the venous vasculature to make up for the loss of stressed volume suggests that the Spl animals would experience a greater expenditure of energy and resources, increasing the odds of slow decompensation. The benefit to mortality seen here with splenectomy has also been seen in rats after enterectomy (22), another location believed to “pool” blood in shock. The increase in oxygen usage and decrease in glucose levels in the Spl group supports the idea that there was, indeed, more work being done. The creep in plasma sodium and potassium ions could represent either a shift in resources away from the sodium-potassium pump or a depletion of energy sources.

The fact that neither group experienced a decrease in oxygen extraction relative to baseline, even when oxygen delivery was lower, raises an additional consideration. Oxygen carrying capacity does not appear to be the limiting factor for these subjects. Certainly, improving oxygen delivery did not improve 14-h survival. Moreover, we saw no immediate change in lactate or base excess with resuscitation in either group, other than a little improvement in the Spl group that is just as likely to have resulted from dilution, suggesting resuscitation did not result in any arterial opening or improvement in functional capillary density. These data support the idea that treatments that return flow to under-perfused tissues, once fluids that will stay in the vasculature are readily available, may prove to be more beneficial than treatments that only increase oxygen in the places blood is already going.

Our findings suggest that splenectomy provides a method by which the mortality of a swine model due to decompensation can be tuned, allowing for study of organ damage mechanisms after severe hemorrhage. That said, it is unlikely that splenectomy will reduce death in every hemorrhagic shock model. A model with greater hemorrhage could potentially overwhelm the compensatory mechanisms, regardless of splenectomy. Likewise, modifications that make compensation more difficult in other ways may also overwhelm the benefit of splenectomy. For example, in a recent pilot study using this model, we saw that addition of a large pre-surgical dose of opioid analgesic returned the model to a high mortality outcome despite splenectomy (opioids interfere with vessel constriction (23)). Unfortunately, most of the previous studies that examined the effect of splenectomy on hemorrhagic shock were performed at the opposite extreme, in models that were not lethal to begin with, either because of a lower hemorrhage volume (24) insufficient to elicit a strong splenic response (25) or because the hemorrhage was performed slowly over a long period of time (6), maximizing movement of extravascular fluid into the vascular space, reducing the effective severity. A more recent study also compared swine with and without splenectomy in a pressure-controlled hemorrhage model with similarities to ours (4). However, in their splenectomy group, hematocrit was significantly higher than the sham splenectomy hematocrit prior to hemorrhage, leading to equivalent hematocrits at the end of shock and suggesting greater ejection from spleen handling during their splenectomy. Despite this, target pressure was reached with less blood removal in the splenectomy than the sham splenectomy group in their study. That is the intuitive result since the spleen is not there to provide reserve volume, but is opposite from what we observed. This could indicate that our NoSpl group was already in a greater compensatory state due to the earlier uncontrolled hemorrhage from the liver injury. Regardless, their model was only 2 h in duration post hemorrhage and, like the other studies mentioned, non-fatal. Ultimately, the lack of lethality in these models may have limited their ability to detect differences caused by splenectomy.

Inflammation and Coagulopathy

During the shock period, the NoSpl seemed to have entered a state of higher inflammation than the Spl group. This was reflected by early increases in both cytokine concentrations and concentrations of granulocytes and monocytes. It has been suggested previously that increased inflammation via priming (the two-hit model) could be one of the potential effects of surgical trauma from splenectomy (26). Alternatively, the increased inflammation we observed may be connected to the NoSpl group having to initially work harder to compensate for hypovolemia without the volume boost that would normally come from the spleen. Regardless, with increasing time we found that the NoSpl cytokines concentrations decreased towards baseline, becoming significantly lower than Spl values by the end of the experiment, suggesting that the inflammation, or at least the inflammatory trigger, may have been resolving. In contrast, there were almost no group differences in leukocyte concentrations between groups following the shock period, though we did see a sharp drop in granulocytes in both groups suggesting the cells were either extravasating or becoming trapped in the vasculature (e.g. the lungs). There was a temporary boost to circulating lymphocytes in the Spl group that did not fit the above patterns. Additional studies may be required to determine the cause of this. Splenectomy had only minor effects on coagulability, aside from a little acceleration in the earliest part of the coagulation cascade and the brief dilution of Spl platelets at B2.

Limitations

This study came about out of an opportunity to improve our animal model and employs comparisons to historical controls, rather than a prospective, randomized approach. This resulted in a number of limitations and factors that need to be weighed when considering the findings. First, though the swine were obtained from the same vendor, there is the possibility of differences between swine (Spl) received at the time of the initial study and those of this study (NoSpl). Another major concern is the difference in anesthetic agents (sufentanil/midazolam for Spl; propofol/buprenorphine for NoSpl). Though we maintained a similar depth of anesthesia, it is not known what, if any, effects on compensation that this change may have caused. Lastly, though the total volume of blood removed was similar in both groups, the proportion removed by controlled hemorrhage was different. It is unclear, what, if any, effects this might have.

Conclusions

There are differing opinions in the literature whether or not to include splenectomy in large animal models of shock, but this method has only been tested in brief, non-lethal models. To our knowledge, ours is the first study to look at the effects of splenectomy on hemorrhagic shock that was potentially lethal and that was observed for more than a few hours. Splenectomy had a profound protective effect against mortality from decompensation within the first 14 h after injury in our model, without affecting lactate levels or base excess, suggesting a similar level of "shock." Improved survival with splenectomy occurred despite a decreased, or unaffected, oxygen delivery, suggesting oxygen debt is not playing a large role in early mortality. Our results suggest that the decision whether or not to include splenectomy as part of an experimental model of severe hemorrhagic shock should depend most on whether the investigator is studying "early" death from decompensation or "late" death from organ failure. To study early death, leaving the spleen intact may allow the spleen to continue to play its role in cardiovascular collapse. In contrast, to study the effects of severe hemorrhagic shock on organ damage, or to study a treatment to reduce such, splenectomy may be helpful to avoid early losses of animals that could skew data due to decompensation independent of organ damage. Experimental designs should also consider that in our study, splenectomy caused greater inflammation during the shock period but resolved it faster in the observation period. Trauma and hemorrhage can lead to mortality and morbidity by multiple pathways. Treatments that block one pathway may amplify another, therefore it is critical to know which mechanisms are being affected by treatments under evaluation in animal studies. We hope that our findings will provide the rationale for more judicious use of splenectomy in the future.

9.0 DELIVERABLES

9.1 Publications: Manuscript currently under review at *Military Medicine*.

9.2 Presentations:

Effects of splenectomy in a pre/early hospital model of traumatic hemorrhage in swine was presented at:

- 42nd Annual Conference on Shock, Coronado, CA, June 2019
- San Antonio Military Health System and Universities Research Forum, June, 2019
- Military Health System Research Symposium, Kissimmee, FL, August 2019

10.0 COST

The overall cost associated with this effort was $1,700,000.

2015: 800k
2016: 900k

11.0 REFERENCES

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TABLES AND FIGURES:

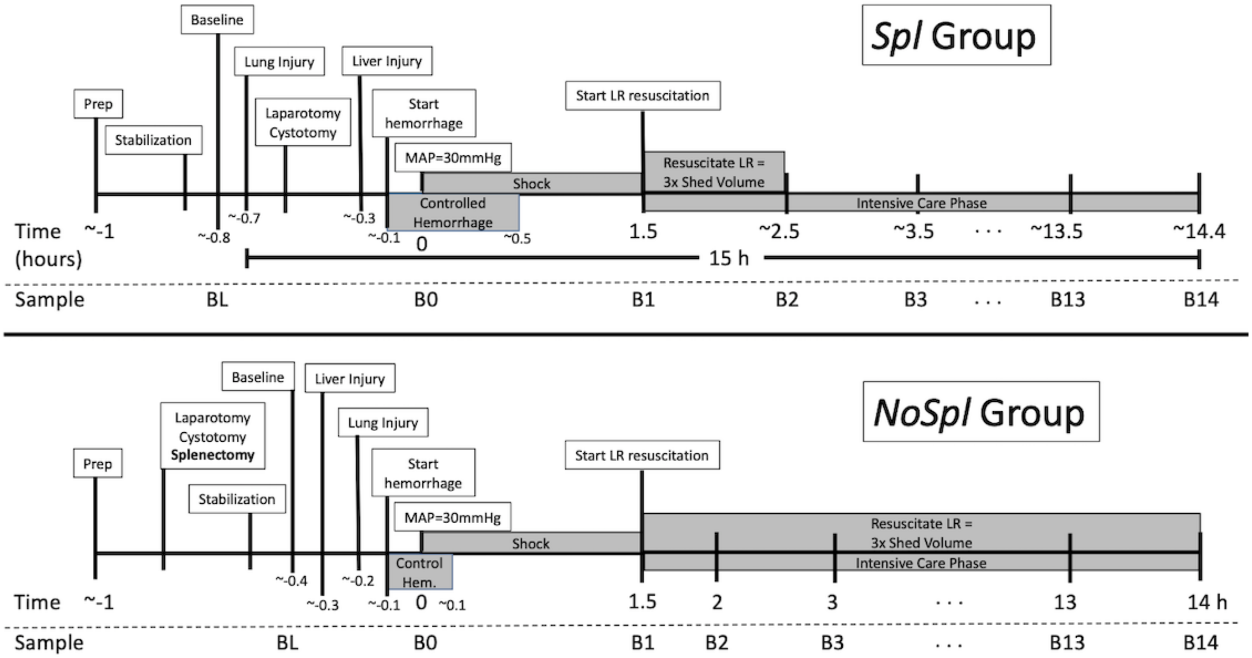

Figure 1: Experimental timelines of the historical group with spleens (Spl group; top) and the new study using splenectomized swine (NoSpl; bottom). The main differences are in the timing of the lung injury (prior to laparotomy in Spl versus after liver injury in NoSpl) and in the timing of the later blood draws (hourly after the end of resuscitation until the final draw 15 h after lung injury in the Spl group versus on the hour after the start of resuscitation in the NoSpl group). Splenectomy and/or these changes resulted in quicker completion of hemorrhage in the NoSpl group and quicker completion of resuscitation in the Spl group.

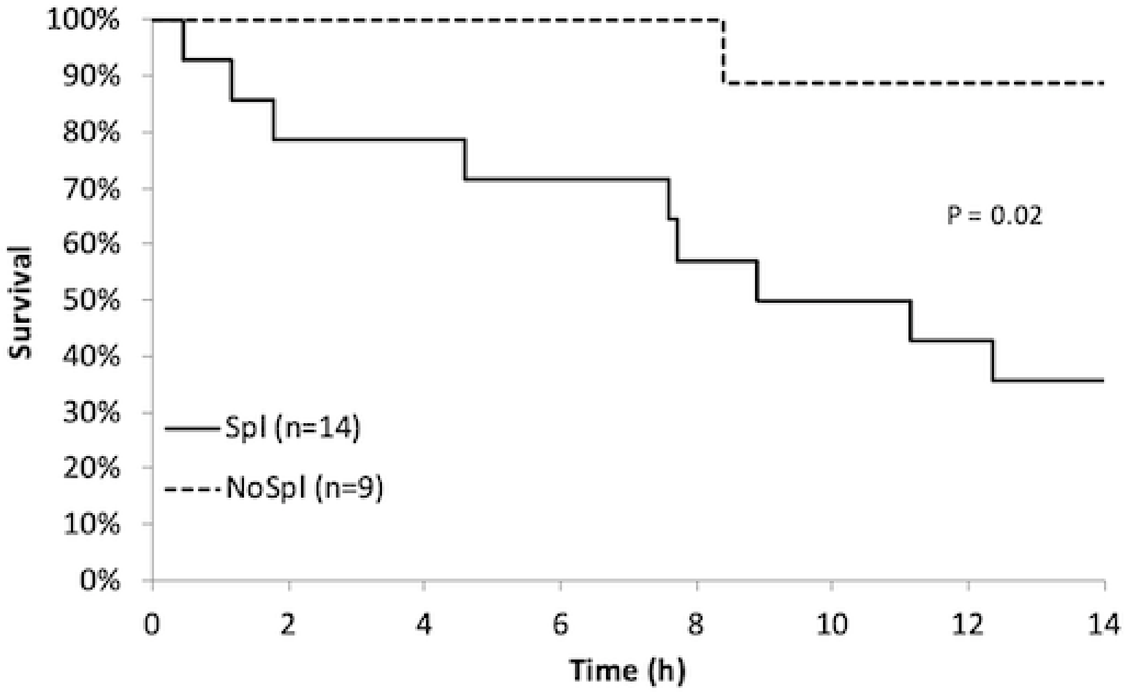

Figure 2: Effects of splenectomy on survival vs. time. Start of shock at T = 0 h. Start of resuscitation at T = 1.5 h. Nine animals of fourteen (57%) died in the Spl group compared to one of nine (11%) in the NoSpl group (p = 0.02 by log-rank analysis).

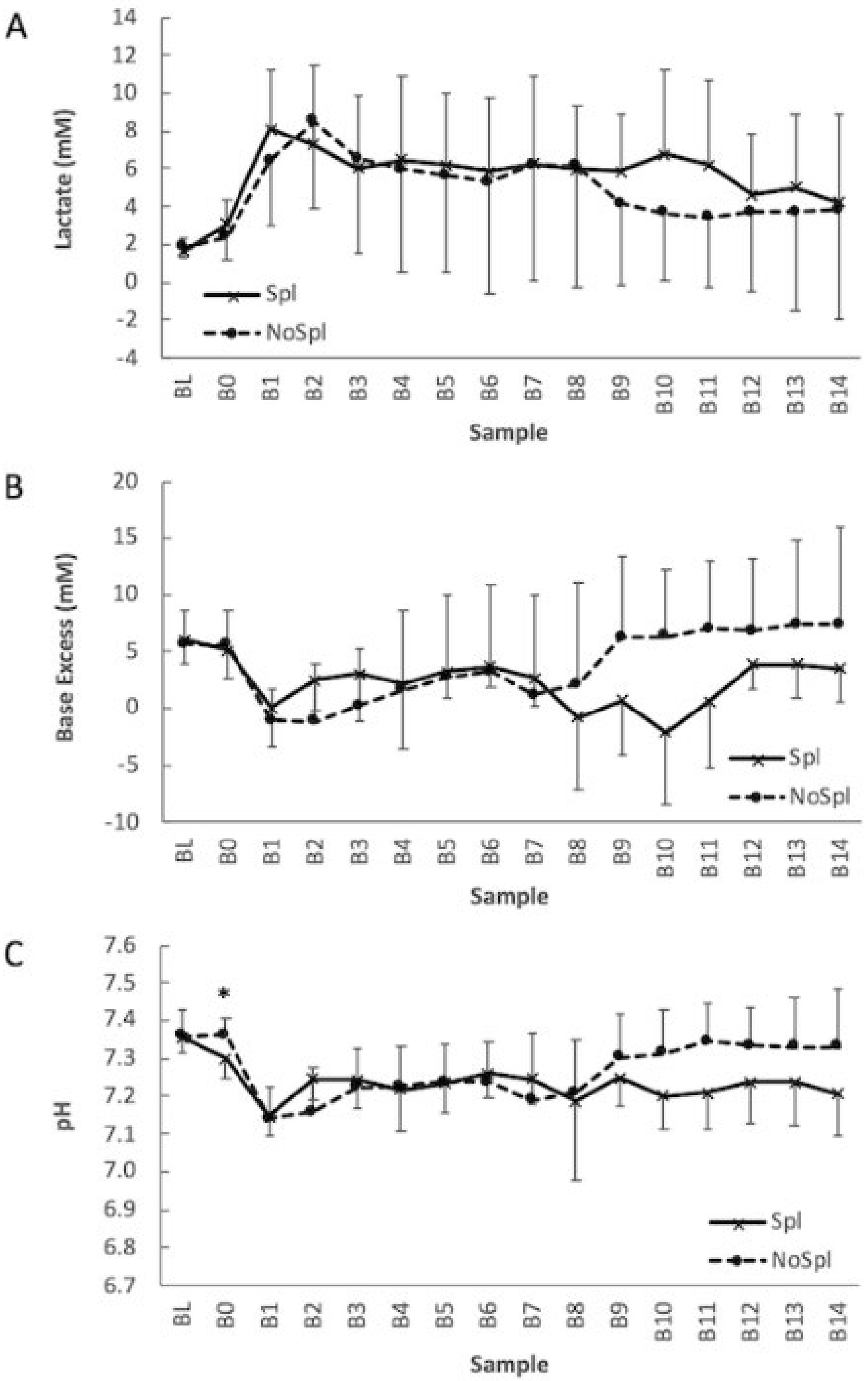

Figure 3: **Indices of the depth of shock**. Lactate (**A**), base excess (**B**), and venous pH (**C**). Data expressed as mean ± SD. N_{Spl} = 14 and N_{NoSpl} = 9 at baseline, decreasing to 6 and 8, respectively by B14. * $p < 0.05$ between groups.

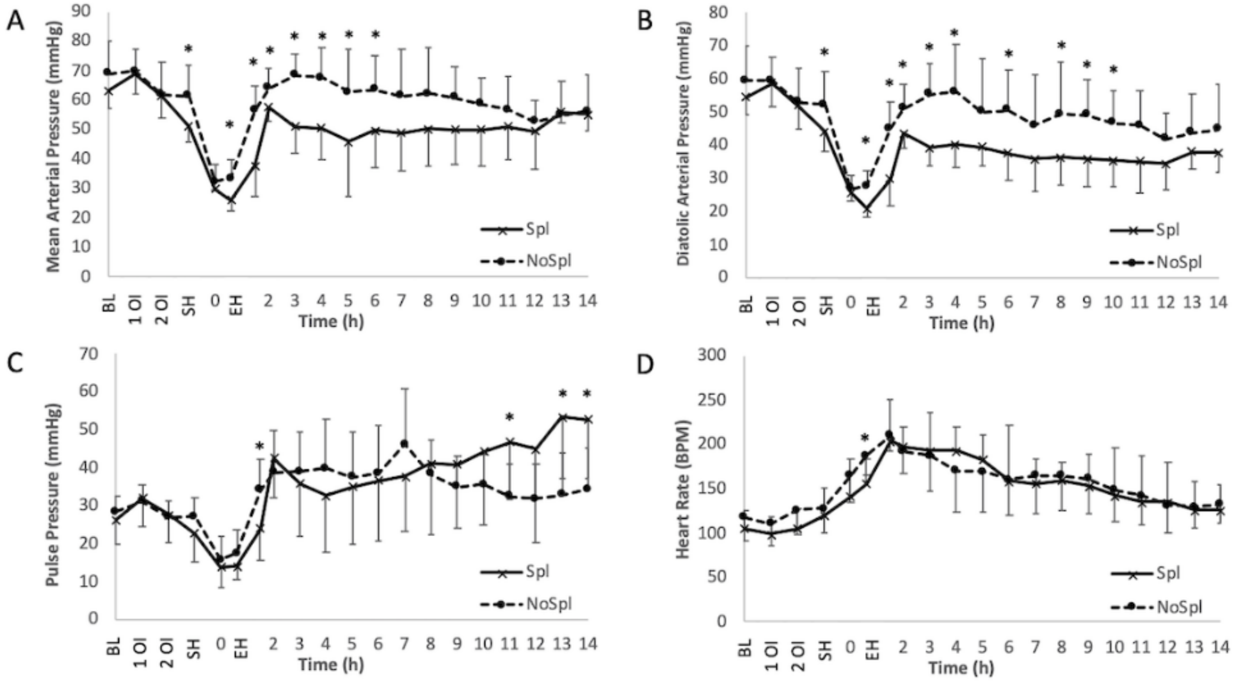

Figure 4: Hemodynamic parameters. Mean arterial pressure (**A**), diastolic arterial pressure (**B**), pulse pressure (**C**), and heart rate (**D**). Data expressed as mean ± SD. N_{Spl} = 14 and N_{NoSpl} = 9 at baseline, decreasing to 6 and 8, respectively by B14. * p <0.05 between groups.

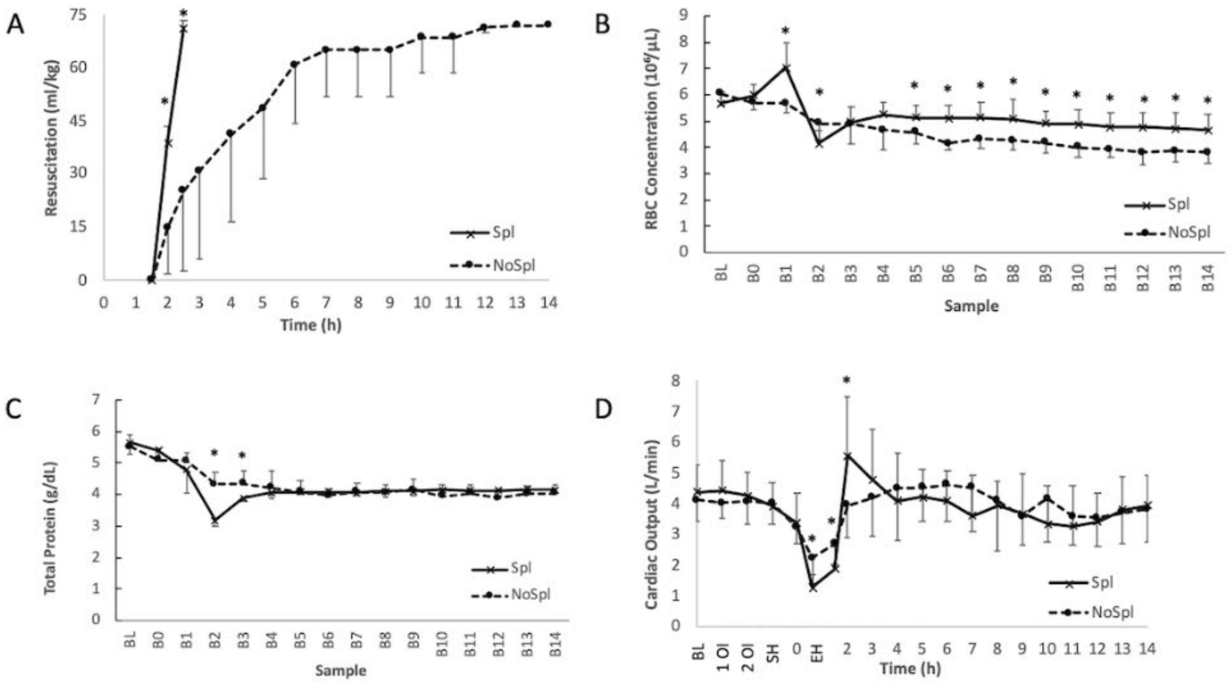

Figure 5: **Effects of resuscitation.** Resuscitation volume vs. time (A), red blood cell concentration in samples (B), plasma protein in samples (C), and cardiac output vs. time (D). Data expressed as mean ± SD. N_{Spl} = 14 and N_{NoSpl} = 9 at baseline. * $p < 0.05$ between groups. (A) Resuscitation was ended at 2.5 h in the Spl group (though two animals were still slightly shy of the 72 ml/kg maximum resuscitation volume). (D) Timing of baseline (BL), first organ (lung or liver) injury (1 OI), second organ (liver or lung) injury (2 OI), start of hemorrhage (SH), and end of hemorrhage (EH) for cardiac output are not to scale (see Figure 1 for approximate timing of these events).

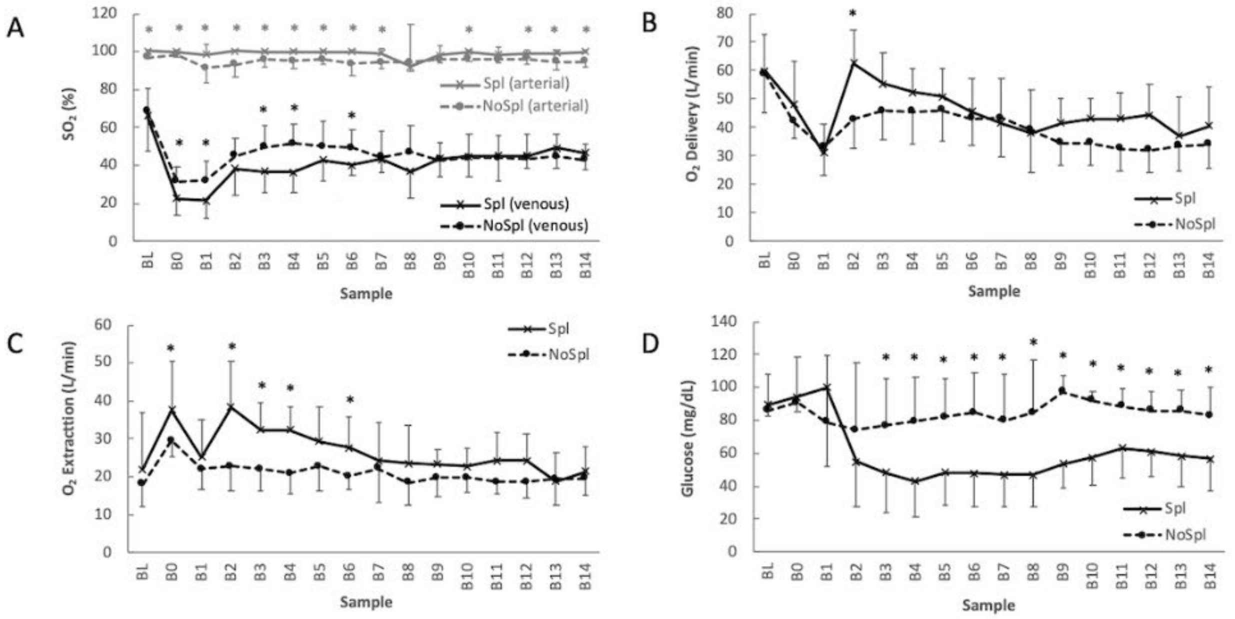

Figure 6: Oxygen and glucose dynamics. Arterial and venous blood oxygen saturation (SO_2) (A), oxygen delivery (B), oxygen extraction (C), and blood glucose levels (D). Data expressed as mean ± SD. N_{Spl} = 14 and N_{NoSpl} = 9 at baseline, decreasing to 6 and 8, respectively by B14. * $p < 0.05$ between groups.

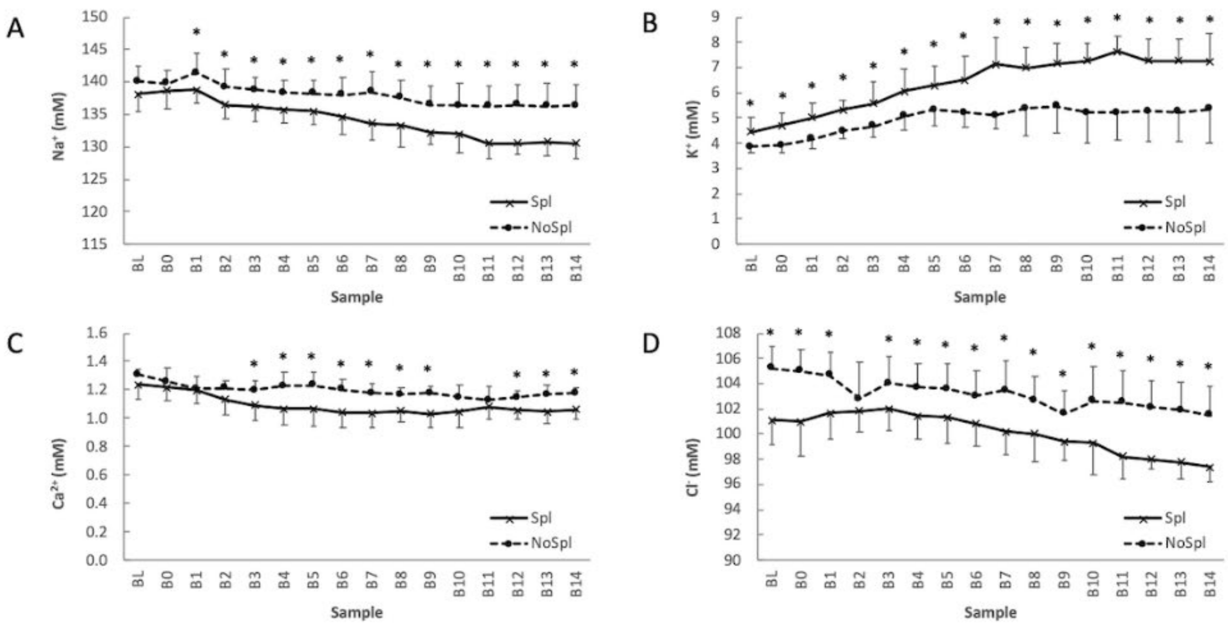

Figure 7: Electrolytes. Sodium (A), potassium (B), calcium (C), and chloride (D) ion concentrations. Data expressed as mean ± SD. N_{Spl} = 14 and N_{NoSpl} = 9 at baseline, decreasing to 6 and 8, respectively by B14. * p <0.05 between groups.

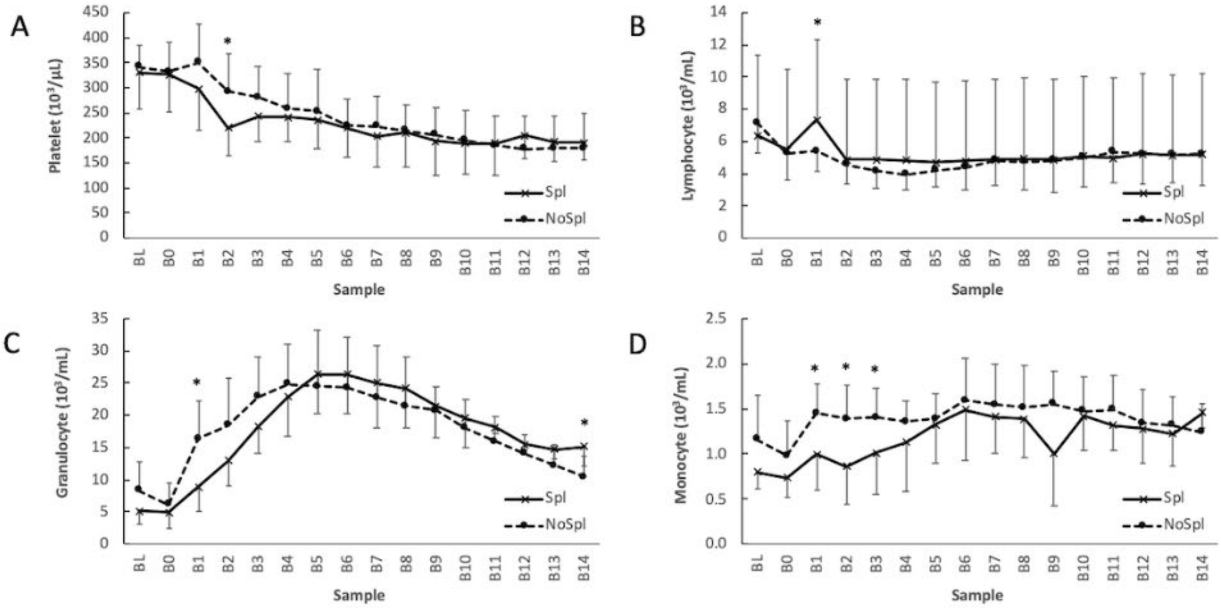

Figure 8: Blood platelet and leukocyte concentrations. Platelet (A), lymphocyte (B), granulocyte (C), and monocyte (D) concentrations. Data expressed as mean ± SD. N_{Spl} = 14 and N_{NoSpl} = 9 at baseline, decreasing to 6 and 8, respectively by B14. * $p < 0.05$ between groups.

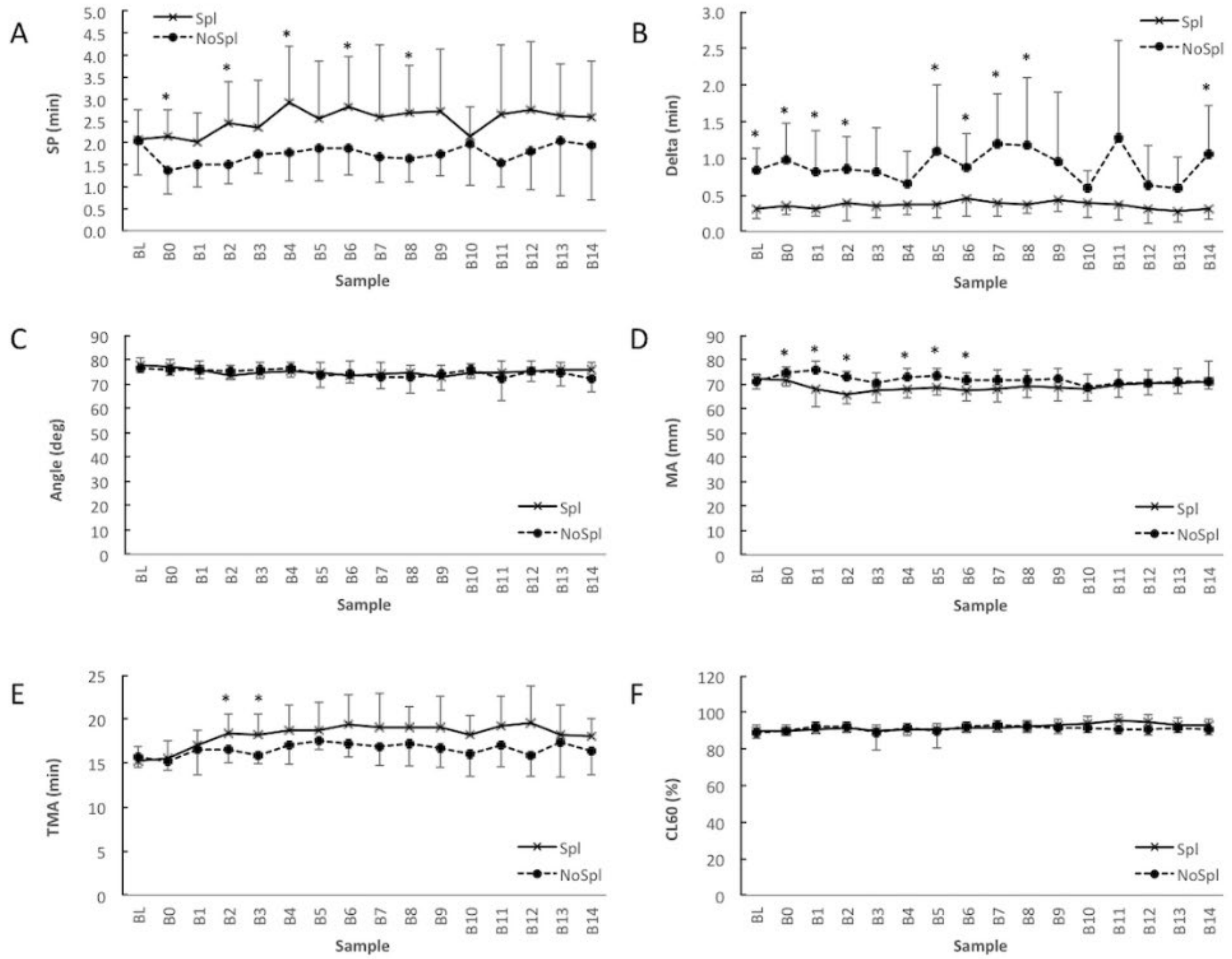

Figure 9: **Coagulation parameters measured by thromboelastography.** Split point (SP) (A), difference between the time amplitude = 2 mm ("R") and SP representing the thrombin burst (B), angle between the horizontal axis and the line formed by connecting the start of clot formation to the inflection point at which the first derivative starts decreasing (C), the maximum amplitude (MA) representing the maximum strength of the clot (D), the time to reach maximum amplitude (TMA) (E), and the amplitude (as a % of MA) 60 min after TMA (CL60) (F). Data expressed as mean ± SD. N_{Spl} = 14 and N_{NoSpl} = 9 at baseline, decreasing to 6 and 8, respectively by B14. * p <0.05 between groups.

Table 1 Cytokines

GM-CSF		IFNg		TNFa	
Spl	NoSpl	Spl	NoSpl	Spl	NoSpl

B0	0.000 ± 0.013	0.002 ± 0.006		0.2 ± 0.5	0.7 ± 0.5	*	0.01 ± 0.05	0.01 ± 0.01	
B1	-0.006 ± 0.034	0.024 ± 0.018	*	2.2 ± 1.8	4.8 ± 1.6	*	0.02 ± 0.04	0.07 ± 0.04	*
B2	-0.001 ± 0.054	0.023 ± 0.010		5.6 ± 6.9	4.8 ± 1.7		0.06 ± 0.10	0.07 ± 0.03	
B7	0.021 ± 0.043	0.027 ± 0.017		8.0 ± 2.8	5.2 ± 1.8	*	0.09 ± 0.09	0.08 ± 0.04	
B14	0.030 ± 0.012	0.016 ± 0.018		7.2 ± 2.7	3.1 ± 2.8	*	0.10 ± 0.12	0.05 ± 0.05	

	IL-1a			IL-1ra			IL-2		
	Spl	NoSpl		Spl	NoSpl		Spl	NoSpl	
B0	0.000 ± 0.008	0.001 ± 0.002		1 ± 2	3 ± 3		-0.01 ± 0.07	0.00 ± 0.03	
B1	0.001 ± 0.013	0.011 ± 0.004	*	9 ± 9	23 ± 13	*	0.00 ± 0.10	0.12 ± 0.05	*
B2	-0.005 ± 0.022	0.010 ± 0.008		31 ± 26	31 ± 15		-0.02 ± 0.20	0.12 ± 0.10	
B7	0.017 ± 0.007	0.009 ± 0.007	*	59 ± 24	31 ± 16	*	0.21 ± 0.09	0.12 ± 0.10	
B14	0.011 ± 0.007	0.001 ± 0.009	*	53 ± 15	21 ± 20	*	0.18 ± 0.03	0.03 ± 0.13	*

	IL-4			IL-6			IL-10		
	Spl	NoSpl		Spl	NoSpl		Spl	NoSpl	
B0	-0.07 ± 0.26	0.01 ± 0.03		0.00 ± 0.03	0.02 ± 0.02	*	0.01 ± 0.14	0.01 ± 0.03	
B1	-0.17 ± 0.39	0.11 ± 0.08	*	0.15 ± 0.15	0.29 ± 0.10	*	0.05 ± 0.24	0.21 ± 0.05	*
B2	-0.35 ± 0.65	0.07 ± 0.10		0.33 ± 0.24	0.36 ± 0.11		-0.03 ± 0.31	0.20 ± 0.11	*
B7	0.13 ± 0.14	0.06 ± 0.11		0.41 ± 0.49	0.17 ± 0.09		0.38 ± 0.22	0.22 ± 0.14	
B14	0.08 ± 0.03	-0.02 ± 0.10	*	0.37 ± 0.20	0.16 ± 0.26		0.38 ± 0.11	0.10 ± 0.20	*

Values shown are change from baseline. All units are ng/ml. Measured but not shown (no significant differences) were IL-1b, IL-8, IL-12, and IL-18.

12.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

BL – Baseline

$_{CA}O_2$ – Arterial oxygen concentration

$_{CV}O_2$ – Venous oxygen concentration

CBC – Complete blood cell count

CL60 – Clot lysis at 60 minutes

CO – Cardiac output

CRI – Continuous rate infusion

DAP – Diastolic arterial pressure

DO_2 – Oxygen delivery

FiO_2 – Fraction of inspired oxygen

Fr – French

GM-CSF – Granulocyte/macrophage colony stimulating factor

HR – Heart rate

HS – Hemorrhagic shock

IFN - Interferon

IL - Interleukin

LR - Lactated Ringer’s

MA – Maximum amplitude

MAC – Minimum alveolar concentration

MAP – Mean arterial pressure

NoSpl – No spleen (performed splenectomy)

pCO_2 – Partial pressure of carbon dioxide

pO_2 – Partial pressure of oxygen

PP – Pulse pressure

PSI – Potentially survivable injuries

RBC – Red blood cell

sO_2 – Oxygen saturation

SP – Split point

Spl – With spleen

SvO_2 – Mixed venous oxygen saturation

tHb – Total hemoglobin

TMA – Time to reach maximum amplitude

VO_2 – Oxygen extraction