

**Introduction:** The leading cause of death in military casualties is hemorrhage and most of these deaths occur prior to arrival at a medical treatment facility. Previously, we used a swine polytrauma model consisting of lung contusion, laparotomy, liver laceration, and 24 ml/kg controlled hemorrhage to test treatments to improve coagulopathy, inflammation, and organ function over the first ~14 h after injury. Coagulopathy, inflammation, and organ damage were mild, despite 55% mortality, leaving little room for improvement by treatment. The spleen is involved in compensation (and maybe decompensation) in swine, as it is a large reservoir (and potential sink) of unstressed (i.e. non-hemodynamically active) blood volume and cells. We hypothesized that splenectomy prior to polytrauma might give our model greater organ damage and coagulopathy, with less mortality due to decompensation. **Methods:** We compared mortality, coagulopathy, hemodynamics, and inflammatory mediators (organ damage to follow) in a pilot study of swine polytrauma with splenectomy (SPL group; N = 9) to historical control data (CONT; N = 11). **Results:** Total blood removal, including blood drained from the excised spleen, was 30 ml/kg in SPL group, compared to 27 ml/kg in the CONT group. Despite this increase, mortality in the SPL group was reduced to 11% (p=0.04 vs CONT). Liver re-bleeding was unchanged. Though equivalent by the end of shock, SPL subjects elevated heart rates faster in response to hemorrhage than CONT. SPL subjects maintained a higher mean arterial pressure (MAP) than CONT subjects from end of shock through 5 h post-injury, though diastolic pressure (reflective of vascular compensation) stayed elevated compared to CONT through 11 h post-injury. In contrast, surviving CONT subjects maintained their MAP by increasing pulse pressure, suggesting greater cardiac effort. Resuscitation was completed in  $\leq 1$  h in the CONT group, whereas at 5.5 h resuscitation was still only 90% complete on average in the SPL group. Hematocrit increased in the CONT group during the shock period, plummeted during resuscitation (likely the cause of a transient spike in cardiac output), recovered quickly afterwards as fluid extravasated, then gradually dropped off with decompensation. In contrast, there was only a gradual decrease in hematocrit in the SPL group and no spike in cardiac output. Inflammatory mediators were elevated by the end of shock in SPL versus CONT (significantly for GM-CSF, IFN-gamma, IL-1a, IL-2, IL-4, IL-6, IL-10, and TNF-alpha). After the start of resuscitation, however, these levels dropped and were significantly lower than CONT by 14 h post-injury for IL-1a, IL-1ra, IL-2, and IL-4. **Conclusions:** Our preliminary findings suggest that splenectomized swine are better able to hemodynamically compensate from polytrauma and may therefore be better suited for studies targeting late events in shock.

## Background

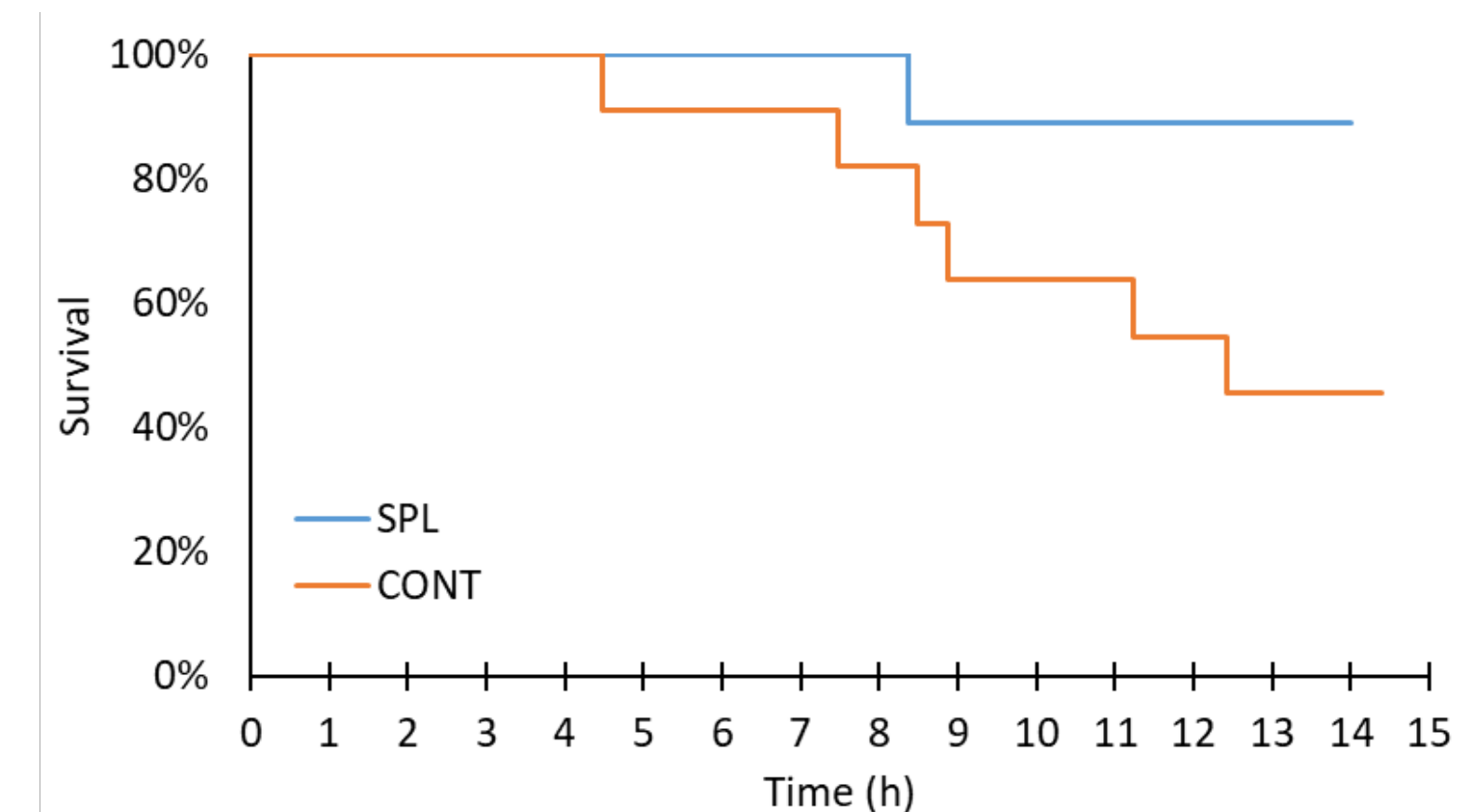
- Hemorrhage and its sequelae are the leading cause of death in military casualties with most of these deaths occurring prior to arrival at a medical treatment facility (1).
- Death due to hemorrhage typically occurs by 3 mechanisms: uncontrolled hemorrhage (immediate), decompensation (early), and organ failure (late).
- While it is simple in a laboratory setting to control the degree of hemorrhage, it can be difficult to separate decompensation from organ failure. Though we have observed in both trauma/hemorrhagic shock (T/HS) and splanchnic arterial occlusion shock models that increased organ damage is not required for death by decompensation (2,3), the mechanisms responsible for both may be occurring simultaneously. Therefore, a hypovolemia severe enough to trigger a given organ or system dysfunction may also result in unacceptable losses due to decompensation. This can be a major challenge during intervention studies. For example, we previously used a swine polytrauma model consisting of lung contusion, laparotomy, liver laceration, and 24 ml/kg controlled hemorrhage to test treatments to improve coagulopathy, inflammation, and organ function over the first ~14 h after injury. Coagulopathy, inflammation, and organ damage were mild, despite 55% mortality, leaving little room for improvement by treatment.
- The spleen is a major organ of blood volume reserve. It contracts during hypovolemia to maintain venous return to the heart. As such, it also has the potential to be a key organ during decompensation, as failure to remain constricted there could result in a loss of venous return and blood pressure.
- We hypothesized that splenectomy prior to polytrauma might give our model greater organ damage and coagulopathy, with less mortality due to decompensation.

## Objective

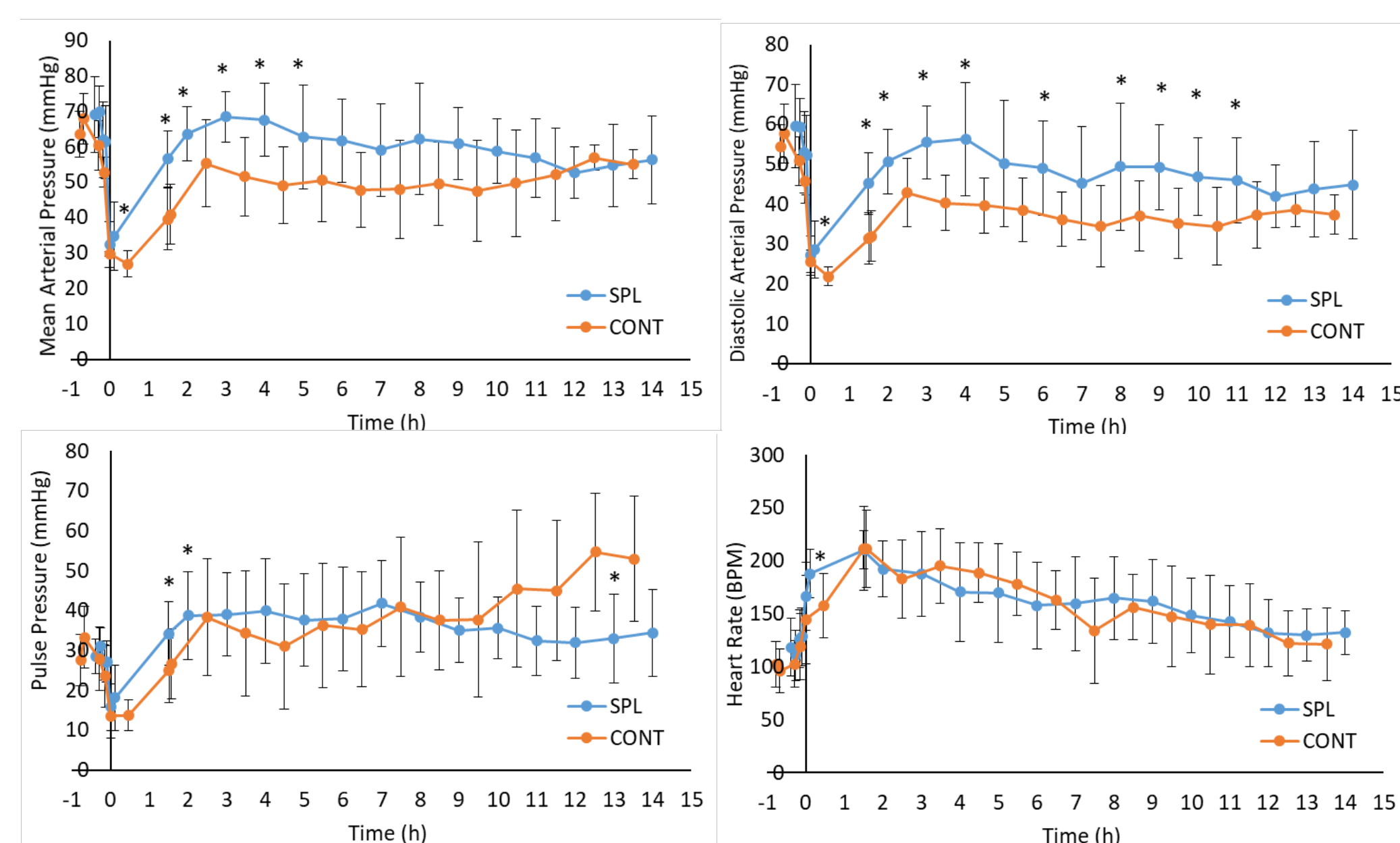
Our objective was to determine the effects of splenectomy compared to historical control data on mortality, coagulopathy, hemodynamics, and inflammatory mediators in a swine polytrauma model.

## Methods

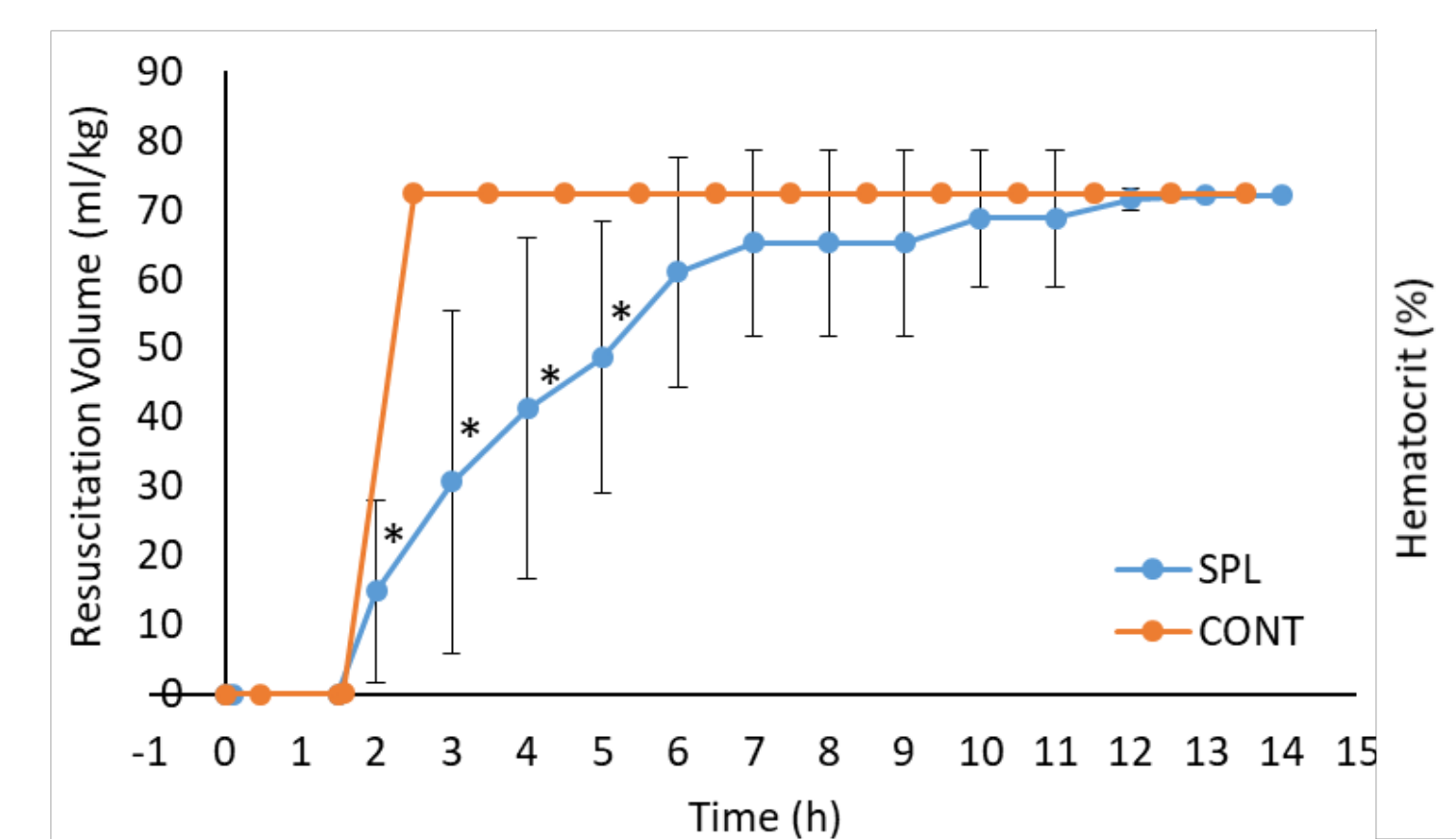
Anesthetized and mechanically ventilated male, Yorkshire swine ( $40 \pm 5$  kg) were instrumented for continuous measurement of blood pressure and cardiac output. They were subjected to laparotomy with (SPL, N = 9) or without (CONT, N = 11) splenectomy, blunt injury (lung contusion), liver laceration (~3 ml/kg uncontrolled hemorrhage), and controlled hemorrhage (24 ml/kg). For the controlled hemorrhage, blood was drawn until mean arterial pressure (MAP) reached 30 mmHg (T=0 h). MAP was kept there by intermittent withdrawal until the full volume was drawn. At T=1.5 h, swine were resuscitated with up to 3x shed volume of LR over 1 h and observed for an additional 12.5-13 h. Due to a change in the protocol, time points and blood samples are offset after the start of resuscitation by ~0.5 h between the two groups (we compare CONT data to the following SPL time point). Blood was sampled regularly for blood gases, hematocrit, cytokines, markers of tissue damage, complete blood cell count, and thromboelastography. Tissues were fixed post-mortem and will be examined for histological damage by a trained, blinded pathologist. Data expressed as mean  $\pm$  SD. \* p<0.05. NS = not significant.



**Figure 1 Effects of splenectomy on survival vs. time.** Start of shock at T = 0 h. Start of resuscitation at T = 1.5 h. Total blood removal, including blood drained from the excised spleen, was 30 ml/kg in SPL group, compared to 27 ml/kg in the CONT group. Despite this increase, mortality in the SPL group was reduced to 11% (p=0.04 vs CONT) Liver re-bleeding was similar between groups ( $7.9 \pm 3.3$  ml/kg in SPL,  $8.1 \pm 3.0$  ml/kg in CONT; NS).



**Figure 2 Effects of splenectomy on blood pressure and heart rate vs. time (A: mean, B: diastolic, C: pulse pressure, D: heart rate).** SPL subjects maintained a higher MAP for ~5 hours (the later improvement in CONT MAP was due in part to the death and subsequent exclusion of subjects with lower pressures). However, diastolic pressure (reflective of vascular compensation) stayed elevated compared to CONT through 11 h post-injury. In contrast, surviving CONT subjects maintained their MAP by increasing pulse pressure, suggesting greater cardiac effort. Though equivalent by the end of the shock period, SPL subjects elevated heart rates faster in response to hemorrhage than CONT. This may be why they completed hemorrhage faster ( $6 \pm 6$  vs.  $28 \pm 19$  min, p=0.004, not shown).



**Figure 3 Resuscitation volume (A), hematocrit (B), and cardiac output (C) vs. time.** (A) By T = 5 h, only 1/3rd of SPL subjects had reached their maximum resuscitation, compared to all of the CONT subjects within 1 h of starting resuscitation. (B) Hematocrit was higher in SPL than CONT at the start of hemorrhage (likely due to handling of the spleen during removal), but their positions reversed by the end of hemorrhage as the CONT spleen was able to discharge its stored red blood cells. Positions reversed again during at the time points following the start of resuscitation as the CONT group accepted all of its allowed LR in that short span of time. The lack of improvement in pressure suggests the volume was all be likely filled back up rather than resisting the influx). However the cr causing fluid to leak out through capillaries, hence the subsequent ris two groups, except at the time point after the start of resuscitation w decreased plasma viscosity lowering resistance to flow.

**Table 1 Cytokines**

	GM-CSF CONT	SPL	I C
End Shock	-0.006 $\pm$ 0.034	0.018 $\pm$ 0.005*	2.1
After Start of Resuscitation	-0.001 $\pm$ 0.054	0.021 $\pm$ 0.009	5.6
Final	0.030 $\pm$ 0.012	0.010 $\pm$ 0.021	7.2
IL-1ra			
End Shock	8.58 $\pm$ 9.23	18.15 $\pm$ 8.78	0.0
After Start of Resuscitation	31.06 $\pm$ 25.72	23.05 $\pm$ 12.85	-0.0
Final	52.53 $\pm$ 15.50	11.95 $\pm$ 13.66*	0.1
IL-6			
End Shock	0.15 $\pm$ 0.15	0.27 $\pm$ 0.07*	0.0
After Start of Resuscitation	0.33 $\pm$ 0.24	0.33 $\pm$ 0.08	-0.0
Final	0.37 $\pm$ 0.20	0.23 $\pm$ 0.37	0.3

Values shown are change from baseline. All units are ng/ml. End sho for SPL and 2.5 h for CONT. Final is T = 14 h for SPL and 14.5 h fo differences) were IL-1b, IL-8, IL-12, and IL-18.

## Discussion

Splenectomy had a powerful effect on the compensatory response to hemorrhage, as shown by increased heart rate and blo even more important effect during resuscitation. Subjects with spleens needed fluids earlier and rapidly accepted the fluid v hematocrit suggests much of that fluid then exited as potentially harmful edema. These subjects then continued to decomp comparison, splenectomized subjects had significantly lower mortality and, though they needed fluid, fluid appeared more and mean arterial pressure, reducing the rate at which fluids were required. This supports the role of the spleen as an important organ for decompensation. Splenectomy also had effects on the inflammatory profile and on coagulopathy (splenectomy improved clotting time and strength; not shown), though not on re-bleed from the liver. These effects may also need to be considered when planning studies.

## Conclusions

This data supports our claim that the spleen potentiates decompensation and therefore that studies with early mortality or decompensation as outcomes should leave the spleen in, while studies aimed at subsequent dysfunction may wish to remove it.

- (1) Eastridge BJ, et al. Death on the combat casualty care. *The jou* 5):S431-437.
- (2) Penn AH, Schmid-Schönbein G collapse and sudden death vi
- (3) Penn AH, et al. Fatty acid-satur in a rat model of hemorrhagic 2019 epub ahead of print.

## Disclaim

The views expressed do not reflect Experiments were conducted accor and Use of Laboratory Animals and Health Program/Joint Program Con