

A rat model of multicompartmental traumatic injury and hemorrhagic shock induces bone marrow dysfunction and profound anemia

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Funding information

National Institute of General Medical Sciences, Grant/Award Number: NIH NIGMS R01 GM105893 and NIH NIGMS T32 GM-008721

Abstract

Background: Severe trauma is associated with systemic inflammation and organ dysfunction. Preclinical rodent trauma models are the mainstay of postinjury research but have been criticized for not fully replicating severe human trauma. The aim of this study was to create a rat model of multicompartmental injury which recreates profound traumatic injury.

Methods: Male Sprague–Dawley rats were subjected to unilateral lung contusion and hemorrhagic shock (LCHS), multicompartmental polytrauma (PT) (unilateral lung contusion, hemorrhagic shock, cecectomy, bifemoral pseudofracture), or naïve controls. Weight, plasma toll-like receptor 4 (TLR4), hemoglobin, spleen to body weight ratio, bone marrow (BM) erythroid progenitor (CFU-GEMM, BFU-E, and CFU-E) growth, plasma granulocyte colony-stimulating factor (G-CSF) and right lung histologic injury were assessed on day 7, with significance defined as p values <0.05 (*).

Results: Polytrauma resulted in markedly more profound inhibition of weight gain compared to LCHS ($p=0.0002$) along with elevated plasma TLR4 ($p<0.0001$), lower hemoglobin ($p<0.0001$), and enlarged spleen to body weight ratios ($p=0.004$). Both LCHS and PT demonstrated suppression of CFU-E and BFU-E growth compared to naïve ($p<0.03$, $p<0.01$). Plasma G-CSF was elevated in PT compared to both naïve and LCHS ($p<0.0001$, $p=0.02$). LCHS and PT demonstrated significant histologic right lung injury with poor alveolar wall integrity and interstitial edema.

Conclusions: Multicompartmental injury as described here establishes a reproducible model of multicompartmental injury with worsened anemia, splenic tissue enlargement, weight loss, and increased inflammatory activity compared to a less severe model. This may serve as a more effective model to recreate profound traumatic injury to replicate the human inflammatory response postinjury.

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KEYWORDS

anemia, inflammation, polytrauma, pseudofracture, shock

1 | INTRODUCTION

Polytrauma is defined as multiple severe injuries to two or more body regions or organ systems.^{1,2} Approximately 25%–50% of all trauma involves the thorax.³ While less common in blunt trauma, occurring in only about 1% of cases, hollow viscus injury is associated with a high mortality rate reaching almost 20%.⁴ Extremity fractures account for over 50% of all trauma hospital admissions.⁵ Injury severity score (ISS) provides an overall score for patients suffering multiple injuries and is intended to accurately represent the patient's degree of critical illness by classifying patients into the following categories: minor (1–3), moderate (4–8), serious (9–15), severe (16–24), and critical (25–75).^{6,7} Morbidity and mortality increase with the severity of injury denoted by ISS.⁸ In order to fully understand the underlying mechanisms and pathophysiology underlying severe injury such as systemic inflammation, multiorgan failure, coagulopathy, bone marrow dysfunction, it is crucial to utilize a realistic animal model of severe injury to represent severe traumatic injury in humans.

A variety of rodent models of varying levels of injury have been created to better study the response to injury. These models may include combinations of blunt thoracic injury, crush injury or lacerations to intra-abdominal organs, hemorrhagic shock, bone fractures, traumatic brain injury (TBI), and/or soft tissue trauma. Unfortunately, these models often tend to be studied for short time durations, such as 24 h, with none being studied for longer than 3 days.^{9–20} Thus, there is a need for a model of polytrauma which allows for longer duration of study in order to understand the persistence of the systemic effects of severe injury and subsequent recovery.

Severe trauma is associated with persistent anemia despite blood transfusions and resuscitation, which can last up to 6 months postinjury.²¹ This persistent anemia has been attributed to systemic inflammation, hypercatecholaminemia, mobilization of hematopoietic progenitor cells (HPC) from bone marrow, which is stimulated by granulocyte-colony stimulating factor (G-CSF), and bone marrow dysfunction.^{22,23} Human studies have even shown alterations in bone marrow messenger RNA (mRNA) and microRNA (miRNA) after severe injury.^{24,25} Current rodent models of lung contusion, hemorrhagic shock, or a combination of the two demonstrate these phenomena, but the underlying mechanisms behind trauma-induced bone marrow dysfunction remain to be elucidated.^{26,27} Thus, an animal model of more severe injury which can be studied for longer durations of time would allow for the investigations of underlying mechanisms of bone marrow dysfunction and further investigation into therapeutics to attenuate these effects.

To develop a greater understanding of the mechanisms of multiple injuries, reliable and reproducible animal models are required, fulfilling the ethical criteria of replacement, reduction and refinement as established by Russell and Burch.²⁸ In order to better

capture the severity of multicompartamental injuries that occur in humans to better understand bone marrow dysfunction after injury, a rodent model of polytrauma was developed with an ISS of approximately 27, thus implementing reverse translation.²⁹ Rodents were subjected to four traumatic injuries under general anesthesia: unilateral lung contusion (AIS 3), bilateral femoral pseudofracture to represent femur fractures (AIS 3), laparotomy with cecectomy (AIS 3), and multiple surgical soft tissue incisions in conjunction with hemorrhagic shock. Our aim was to characterize systemic inflammation, end-organ dysfunction, anemia, bone marrow dysfunction, and lung injury after 7 days. We hypothesized that this model of severe injury would produce worsened mortality and more severe evidence of systemic inflammation, anemia, bone marrow dysfunction and lung injury compared to a previous model of lung contusion and hemorrhagic shock alone.

2 | METHODS

2.1 | Animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA, USA) at age 8–10 weeks weighing 196–431 g were utilized. Rodents were kept in conventional housing in pairs with unrestricted access to standard irradiated pelleted diet and water. After arrival, rodents were allowed to acclimate to a 12-h light–dark cycle for at least 72 h and remained in their original cage for at least 10 days. If assigned to the same experimental group, rats stayed with their cage mate for the duration of the study; they were separated if allocated to different groups. Female animals were excluded from prior models of LCHS due to variability of the estrous cycle and previously shown protective effects of estrogen in hemorrhagic shock and injury and therefore were not included in this initial comparative study.³⁰ The Institutional Animal Care and Use Committee approved this animal protocol (IACUC protocols 201908271, 202011247). Care of all animals was compliant with the United States National Research Council's Guide for the Care and Use of Laboratory Animals.³¹ The ARRIVE guidelines were followed to ensure proper reporting of methods, results, and discussion (Appendix S1).³²

2.2 | Experimental design

Rats were randomly assigned to one of the following cohorts: naïve controls ($n=43$); lung contusion and hemorrhagic shock (LCHS, $n=20$); or polytrauma (PT, $n=27$). A power analysis performed showed that, assuming a greater than 80% incidence in control rats at baseline, a 30% change would require at least 8 rats per group.

Groups were larger than the minimum number because these data represent pooled data from multiple experiments to compare to prior models, during which various data points were collected. In total, 97 rats were enrolled: 90 assigned to groups and 7 used for donors for pseudofracture. These injury models represent common clinical scenarios including: blunt chest trauma and hemorrhage (LCHS) or blunt chest trauma, hemorrhagic shock, intestinal injury, and lower extremity fractures (PT). Due to the nature of cohorts having different incisions, group allocation could not be blinded during care of the animals.

LCHS was performed similarly to our previous study.³³ After induction of anesthesia with 100 mg/kg intraperitoneal ketamine (Akorn, Lake Forest, IL, USA) and 5 mg/kg xylazine (Akorn, Lake Forest, IL, USA), buprenorphine hydrochloride 0.05 mg/kg (Par Pharmaceutical, Chestnut Ridge, NY, USA) was given subcutaneously. Briefly, a right lung contusion was performed with a manual nail gun (Arrow, Saddle River, NJ, USA) applied over a 12 mm plate on the rodent's right axilla, similarly to previous studies. Cutdown of the left groin and right neck were performed to cannulate the left femoral artery and right internal jugular vein. A blood sample was obtained for a baseline complete blood count (CBC) prior to initiation of hemorrhagic shock. Mean arterial pressure (MAP) was measured continuously by connecting the arterial line to a blood pressure monitor (Columbus Instruments, Columbus, OH, USA). Next, blood was withdrawn at 1 mL/min from the venous cannula to produce a MAP of 30–35 mmHg which was maintained for 45 min. After this, half of the blood shed was reinfused via the venous cannula.

To create a pseudofracture solution to simulate bilateral femur fractures for the PT cohort, age-, sex- and weight-matched donor rats were anesthetized with inhaled isoflurane and euthanized via thoracotomy and exsanguination. Bilateral lower extremity bones (femur, tibia, fibula) were harvested under aseptic conditions, stripped of adherent muscle tissue, and transferred to a biological safety cabinet where they were crushed using a sterile mortar and pestle. During this process, 3 mL of normal saline was added to produce a pseudofracture solution as previously described.³⁴ Pseudofracture solution was stored at 4°C.

For PT, induction of general anesthesia was performed with isoflurane (Patterson Veterinary, Loveland, CA, USA). Then, 1 mg/kg sustained-release buprenorphine (ZooPharm, Laramie, WY, USA) was given subcutaneously. In addition to the right lung contusion and hemorrhagic shock described above, a midline laparotomy with cecectomy was performed.³⁵ Then, 150 µL of the pseudofracture solution from matched rats was injected into the periosteal space of the femur of each lower extremity using a 19 g needle. Rats were then resuscitated with 3 mL of subcutaneous normal saline after blood resuscitation.³⁵

Rodents were all euthanized on postoperative day 7, unless they met a prior humane endpoint. All rodents were evaluated, weighed and scored twice daily with set criteria as previously described.³⁶ Euthanasia was performed by terminal anesthesia with cardiac puncture and exsanguination.³⁷ The spleen and the right soleus muscle were collected and weighed. Acute muscle wasting has been

associated with decreased physical function after insult in critically ill patients, thus we sought to characterize acute muscle wasting by measuring soleus weight.³⁸

2.3 | Blood collection and analysis

During cardiac puncture, blood was collected in a 10 mL syringe with 0.1 mL heparinized saline (1000 units/mL). This sample was used to measure hemoglobin on a hematology analyzer (Zoetis, Parsippany-Troy Hills, NJ, USA). A small aliquot (100 µL) of whole blood was set aside for flow cytometry and the remainder centrifuged for plasma at 800 g for 10 min and kept in a –80°C freezer until further processing. Plasma analytes were measured using standard sandwich enzyme-linked immunosorbent assays (ELISA): toll-like receptor 4 (TLR4) (LSBio, Seattle, WA, USA), neutrophil gelatinase-associated lipocalin (NGAL) (Abcam, Cambridge, UK) and granulocyte colony-stimulating factor (G-CSF) (MyBioSource, San Diego, CA, USA) per manufacturer protocol. Toll-like receptor 4 (TLR4) is an established 'danger signal' which is released after cellular injury and is a sign of systemic inflammation.³⁹ NGAL is a biomarker of which is upregulated in acute kidney injury.⁴⁰

2.4 | Flow cytometry

Flow cytometry was performed similarly to our previously described method.³³ Briefly, 100 µL of whole blood was incubated with mouse anti-rat CD71-FITC (BD Biosciences, San Jose, CA, USA) along with rat anti-mouse CD117-APC (Invitrogen, Waltham, MA, USA) and run on a BD LSR II flow cytometer equipped with FACSDiva software (BD Biosciences) to quantitate CD117⁺CD71⁺ cells. Transferrin receptor-1, CD71, is an established marker for immature erythroid cells along with erythroid precursors.⁴¹ CD117, also known as c-kit, is expressed on early hematopoietic progenitor cells such as CFU-GEMM, CFU-E, and BFU-E.⁴² Circulating erythroid progenitors were defined as CD117⁺CD71⁺ cells in the peripheral blood.³³

2.5 | Bone marrow processing and erythroid progenitor culture

Bone marrow was processed from the right femur similarly to our previously described method.³³ Briefly, bone marrow was flushed with Iscove's Modified Dulbecco's Medium (IMDM) with 2% FBS and an automated cell counter was used to assess cellularity and viability with Trypan Blue. At this time, 10⁶ bone marrow cells were set aside for erythroid progenitor culture.

Erythroid burst-forming units (BFU-E), granulocyte, erythrocyte, monocyte, megakaryocyte colony-forming units (CFU-GEMM), and erythroid colony-forming units (CFU-E) growth was assessed with growth assays per prior methods.³³ A suspension of 1 × 10⁶ bone marrow-derived cells/mL was made in IMDM with 2% FBS and

transferred into 3 mL Methocult SF M3436 (CFU-E, BFU-E) and 3 mL HSC012 with added 5 μ mL rhEPO methylcellulose medium (CFU-GEMM). Cells were then plated in duplicate and then incubated at 37°C in 5% CO₂ for 14 days, after which a Nikon Eclipse TS100 inverted microscope was used to count cells manually by a single blinded observer.

2.6 | Histology

Sections of the right lung were obtained on the day of euthanasia and flushed and stored in formalin. After 24 h, tissues were transferred to 70% ethanol and then sectioned, stained in Hematoxylin and Eosin (H&E), and embedded in paraffin.³⁷ A blinded veterinary pathologist analyzed the lungs for injury using grading previously described; each category was graded from 0 (no injury) to 4 (severe changes) (Appendix S2).⁴³

2.7 | Statistical analysis

All statistical analyses were performed using GraphPad Prism version 9.5.0 (GraphPad Software, La Jolla, CA, USA). Comparisons between naïve, LCHS, and PT rodents for data of normal distribution were assessed for variance with a Brown-Forsythe test. For variables with equal variance, a one-way analysis of variance (ANOVA) with Tukey's post hoc test was performed. For variables with significant differences in variance, a Welch's ANOVA with Dunnett's T3 test was conducted. Comparisons between all three groups for data of non-normal distribution were performed with Kruskal-Wallis tests with Dunn's multiple comparisons test for correction. A Student's *t* test with Welch's correction or Mann-Whitney test were performed for comparisons between LCHS and PT depending on distribution. Data points were considered if identified by robust nonlinear regression analysis in GraphPad Prism (ROUT) or if they fell outside of 1.5 times the interquartile range below the first quartile or above the third quartile. Statistical significance was defined as $p < 0.05$ (*); all data are presented as mean \pm standard deviation for data of normal distribution or median with interquartile range for data of non-normal distribution.

3 | RESULTS

3.1 | Polytrauma worsened mortality and systemic organ injury

Mortalities were included if a humane endpoint was reached prior to planned euthanasia on day 7 or as a result of an intraoperative or postoperative complication. Naïve controls did not have any mortality as a result of daily handling. The LCHS cohort had a mortality of 7.5%, whereas the PT group had a mortality of 13%.

Over the course of 7 days, rats in the naïve cohort gained on average 18% of their starting body weight. Both LCHS and PT did not gain as much weight over the course of 1 week compared to naïve rats (LCHS: 11% and PT: 4%; $p = 0.0007$ and $p < 0.0001$, respectively; Figure 1A). The PT group gained significantly less weight, with evidence of weight loss in some animals, at day 7 compared to LCHS ($p = 0.009$) (Figure 1A).

At day 7, there were no significant differences in plasma TLR4 levels between naïve and LCHS cohorts. Plasma TLR4 in the PT cohort was significantly elevated compared to naïve and LCHS rats (PT: 12.2 ± 5.0 ng/mL vs. naïve: 5.9 ± 1.4 ng/mL and LCHS: 4.7 ± 1.5 ng/mL; $p = 0.0003$ and $p < 0.0001$, respectively; Figure 1B). Regarding muscle wasting and renal injury, there was a significant decrease in soleus muscle weight in the PT group compared to LCHS alone (LCHS: 0.25 g, 0.19–0.30 g; PT: 0.14 g, 0.13–0.14 g; $p = 0.0003$). In terms of renal injury, plasma NGAL was significantly elevated in rats subjected to PT compared to LCHS alone (LCHS: $75\,805 \pm 15\,696$ pg/mL, PT: $254\,177 \pm 47\,771$ pg/mL; $p < 0.0001$). Together, these data demonstrate the systemic effects of polytrauma on mortality, weight loss, muscle wasting, renal injury (end-organ dysfunction) and systemic inflammation compared to LCHS alone.

3.2 | Polytrauma led to severe bone marrow dysfunction and anemia

Seven days after injury, the PT cohort had a hemoglobin count which was significantly lower than the LCHS cohort (Figure 2A). While LCHS showed minimal percentage change in hemoglobin between day 0 and day 7, the PT cohort had persistently lower hemoglobin at day 7 than day 0 (LCHS: -2.1% vs. PT: -11.6% ; $p = 0.002$; Figure 2B). The spleen to body weight ratios of the LCHS cohort and naïve rats were similar (Figure 2B). This ratio in the PT cohort was significantly elevated compared to both naïve and LCHS groups, suggestive of increased extramedullary hematopoiesis (Figure 2C).

There were no significant differences in the amount of live whole bone marrow cells at day 7 between LCHS or PT and naïve rats (Figure 3A). Similarly, there was no difference in the amount of live whole bone marrow cells between LCHS and PT rats (Figure 3A). There was no difference in growth of CFU-GEMM between cohorts (Figure 3B). However, both LCHS and PT demonstrated a drastic decline in CFU-E growth compared to the naïve cohort (Naïve: 60 ± 10 , LCHS: 52 ± 4 , PT: 48 ± 15 ; $p = 0.02$ and $p = 0.02$, respectively; Figure 3C). There was no significant difference in CFU-E growth between LCHS and PT groups ($p = 0.6$) (Figure 3C). Only PT had a decrease in BFU-E growth compared to naïve rats (Figure 3D). This demonstrates a more severe anemia after 1 week in the PT cohort compared to LCHS and significant erythroid progenitor growth suppression in both groups.

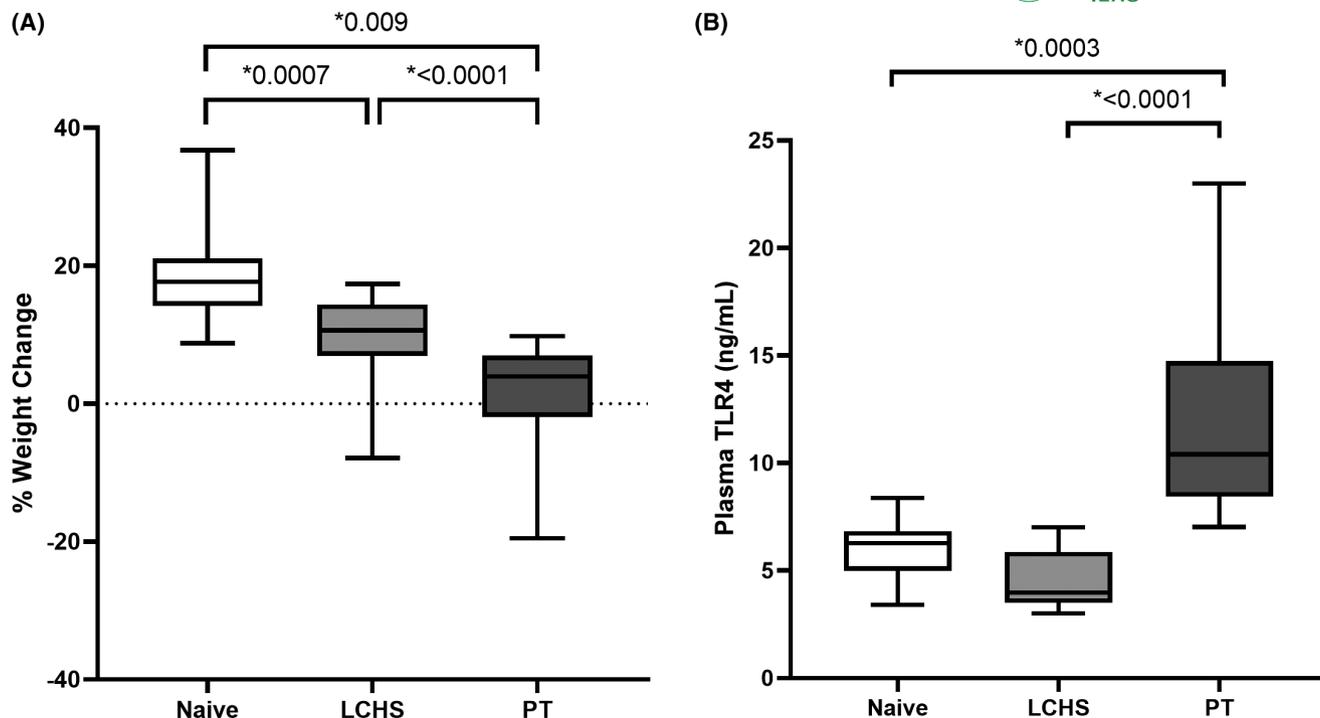
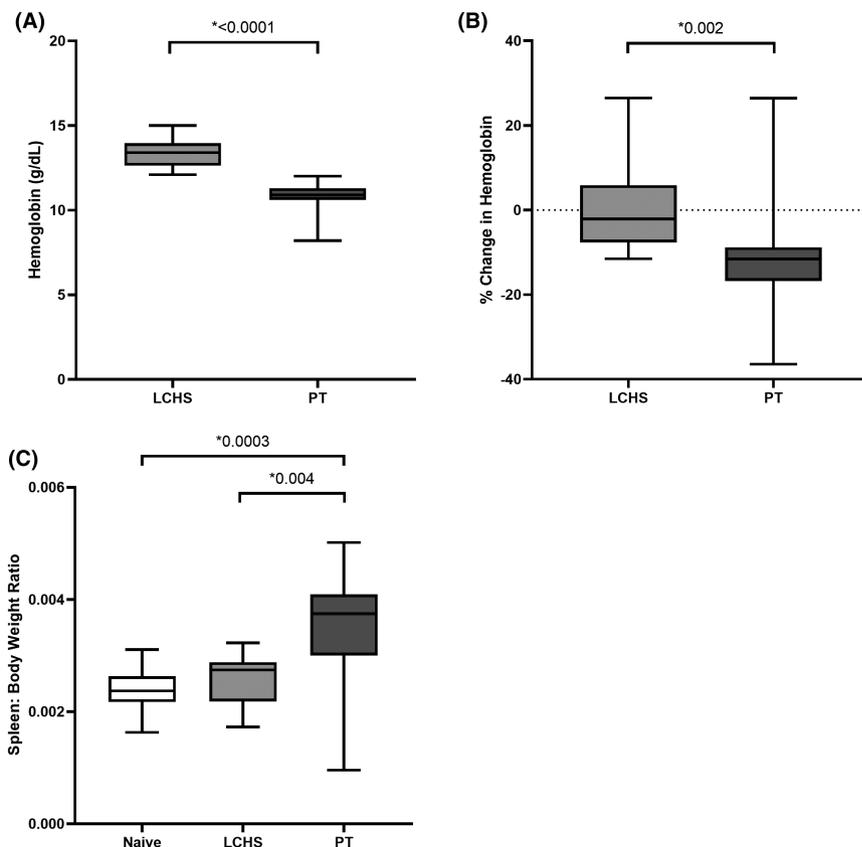


FIGURE 1 Percentage weight change from day 0 to day 7 (A) and day 7 plasma toll-like receptor 4 (TLR4) levels (B) between cohorts. LCHS, lung contusion and hemorrhagic shock; PT, polytrauma. Only statistically significant comparisons displayed (* $p < 0.05$).

FIGURE 2 Hemoglobin at day 7 (A), percentage change in hemoglobin from day 0 to day 7 (B) and day 7 spleen to body weight ratio (C) between cohorts. LCHS, lung contusion and hemorrhagic shock; PT, polytrauma. Only statistically significant comparisons displayed (* $p < 0.05$).



3.3 | Polytrauma induced erythroid progenitor cell mobilization

Flow data demonstrating identification of CD117⁺CD71⁺ cells from peripheral blood can be found in Figure 4A,B. Despite increases

in the percentage of circulating erythroid progenitor cells in both LCHS and PT compared to naïve, only LCHS reached statistical significance (Figure 4C). There was a small difference in peripheral blood erythroid progenitors between LCHS or PT ($p = 0.04$) (Figure 4C). Plasma G-CSF in the LCHS cohort was similar to

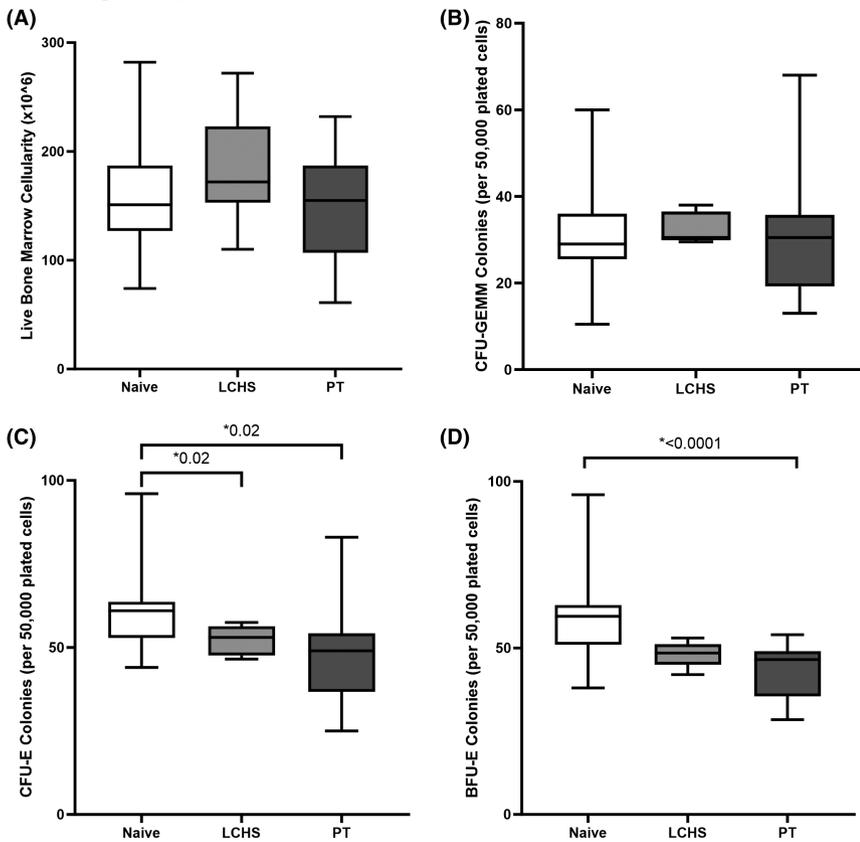


FIGURE 3 Whole bone marrow live cellularity (A), CFU-GEMM growth per 50000 plated cells (B), CFU-E growth per 50000 plated cells (C), and BFU-E growth per 50000 plated cells (D) between cohorts. LCHS, lung contusion and hemorrhagic shock; PT, polytrauma. Only statistically significant comparisons displayed ($*p < 0.05$).

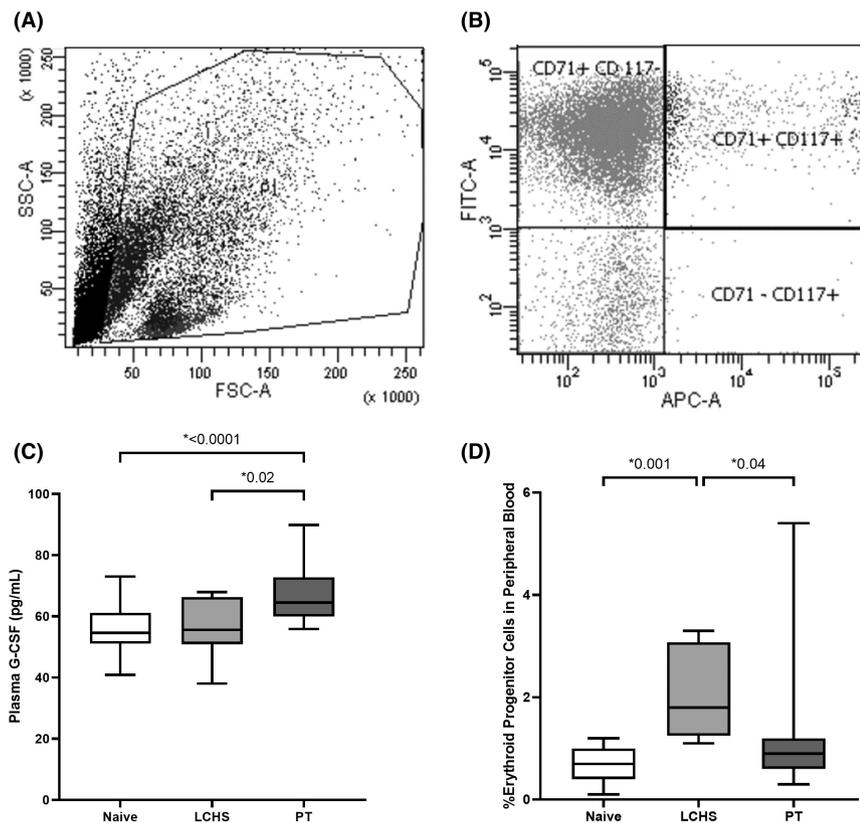


FIGURE 4 (A, B) Sample flow demonstrating impact of injury on CD117⁺CD71⁺ hematopoietic stem cell mobilization. Peripheral red cell-lysed blood cells labeled using APC rat anti-mouse CD117 and FITC mouse anti-rat CD71. (C) Circulating plasma hematopoietic stem cells. (D) plasma G-CSF levels. LCHS, lung contusion and hemorrhagic shock; PT, polytrauma. Only statistically significant comparisons displayed ($*p < 0.05$).

naïve (Figure 4D). However, the PT group had significantly elevated plasma G-CSF levels compared to both naïve and LCHS (PT: 67 ± 8 pg/mL vs. naïve: 56 ± 8 pg/mL and LCHS: 56 ± 10 pg/mL;

$p = 0.0001$ and $p = 0.02$, respectively; Figure 4D). Together, these data demonstrate mobilization of erythroid progenitors after injury in both LCHS and PT.

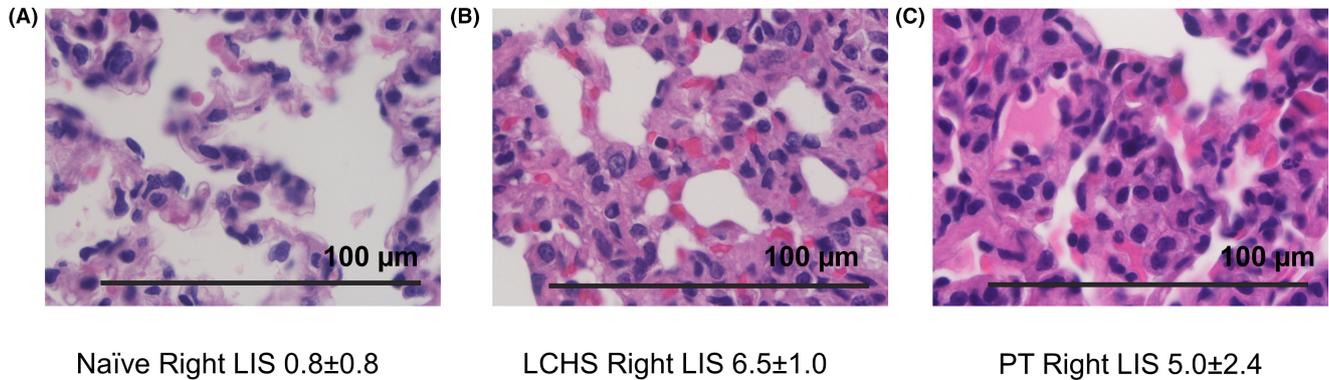


FIGURE 5 Sample right lung histology from naïve (A), LCHS (B), and PT (C) cohorts. LCHS, lung contusion and hemorrhagic shock; PT, polytrauma. Scale bar represents $100 \mu\text{m}$. LIS, lung injury score.

3.4 | Lung injury is evident histologically after lung contusion in both LCHS and PT

Sample right lung histology from the naïve, LCHS and PT cohorts can be found in Figure 5A–C. Both the LCHS and PT cohorts demonstrated evidence of poor alveolar wall integrity, interstitial edema, and the presence of neutrophils in lung tissue compared to naïve. The right lung injury scores for both LCHS and PT were significantly higher than the naïve cohort (Naïve: 0.8 ± 0.8 , LCHS: 6.5 ± 1.0 , PT: 5.0 ± 2.4 ; $p=0.001$ and $p=0.001$, respectively; Figure 6). There was no difference in lung injury scores between LCHS and PT (Figure 6). This demonstrates that the levels of injury in LCHS and PT are similar after lung contusion as part of each model.

4 | DISCUSSION

Multicompartmental injury consisting of unilateral lung contusion, cecectomy, bilateral femoral pseudofracture, and hemorrhagic shock induced weight loss, systemic inflammation, anemia, spleen enlargement suggestive of extramedullary hematopoiesis, and plasma G-CSF levels which were worse than lung contusion and hemorrhagic shock alone after 7 days. In addition, the cohort subjected to polytrauma showed suppression of erythroid progenitor growth, mobilization of bone marrow erythroid progenitor cells to the peripheral blood and histologic lung injury. This model represents a reproducible animal model of severe injury that simulates the condition of most critically ill trauma patients, allowing further study of mechanisms underlying bone marrow dysfunction and other postinjury phenomena.

Our PT cohort demonstrated increased mortality, significant weight loss, acute kidney injury, muscle wasting, and elevated plasma TLR4 (systemic inflammation) compared to the LCHS alone. The increased mortality in polytrauma compared to less severe injury is similar to other rodent studies, although these involved different injuries than this model.¹⁹ This trend in weight loss is similar to another study of TBI with hemorrhagic shock in rats; Qi et al saw an initial decline in weight after injury that did not recover to the level of

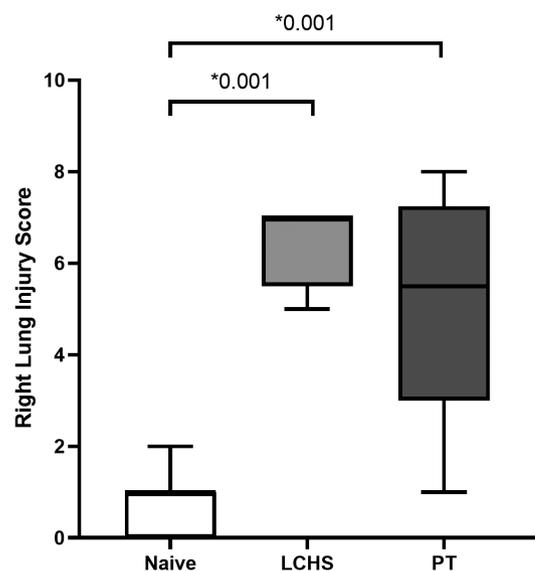


FIGURE 6 Right lung injury scores at day 7. LCHS, lung contusion and hemorrhagic shock; PT, polytrauma. Only statistically significant comparisons displayed ($*p < 0.05$).

sham animals over the course of a week out to 28 days.³⁹ PT induced worse end-organ dysfunction as represented by significantly worse acute kidney injury, similar to another model of less severe polytrauma.¹² PT also induced more acute muscle wasting; while other models of polytrauma did not investigate muscle wasting, human studies have correlated acute muscle wasting in critically ill sepsis patients with worse physical function after discharge.³⁸ Our observation of increased systemic inflammation in PT compared to the less severe LCHS cohort is similar to multiple other studies comparing a polytrauma model to isolated injuries, although the duration of the model was shorter than ours.^{9,10,12,19} Our lung histology findings after lung contusion in the setting of polytrauma are similar to other studies that found similar evidence of alveolar wall disruption and edema.^{10–12} We were surprised to find that the lung injury within the polytrauma cohort was not worse than LCHS alone, given findings of others which demonstrate lung injury related to extrapulmonary

injuries.^{17,44} It is possible that the combination of injuries in this PT model were not severe enough to induce additional lung injury as a result of extrapulmonary insults or that such differences would have been observed in the more acute period.

This severe injury model demonstrated worsened anemia associated with enlargement of the spleen and suppression of bone marrow erythroid progenitor growth. There is a paucity of data investigating anemia, spleen size, and bone marrow dysfunction in the setting of polytrauma. In less severe injury, rodent models demonstrate similar persistent anemia at 7 days.^{27,45–47} Splenic enlargement was also only found in the PT cohort, similar to other studies which did not show changes in spleen size after LCHS alone.²⁷ We identified similar suppression of both CFU-E and BFU-E growth after LCHS and PT to that found in other studies of less severe injury.^{45,47–49} While we did not identify the differences in CFU-GEMM seen in some other rodent studies of injury, it is possible that polytrauma may induce alterations later in erythropoiesis.^{45,47–49} A recent study showed that terminal erythropoiesis is altered after injury and stress by changing the structure of erythroblastic islands.⁵⁰ Therefore, the underlying mechanisms of bone marrow dysfunction in severe injury are complex and could be attributed to terminal erythropoiesis and other mechanisms which need to be better understood.

While PT rats showed increased circulating erythroid progenitors in the peripheral blood, this did not reach statistical significance compared to controls as seen in other studies.^{46,51} However, other studies of less severe injury only identified mobilization of erythroid progenitors with the addition of restraint stress to simulate the intensive care unit.^{27,47} Therefore, perhaps this additional restraint stress may impact the mobilization of erythroid progenitors to the peripheral blood in addition to injury on day seven. We were surprised to observe that the LCHS cohort did not have elevated plasma G-CSF compared to naïve controls, unlike other studies.^{47,51} PT did result in elevated plasma G-CSF levels compared to naïve and LCHS rats though, which implicates hematopoietic progenitor mobilization after severe injury, and could be a result of multicompartmental injury. Further studies examining restraint stress following polytrauma are warranted.

This study has limitations. While previous models from our group utilized ketamine and xylazine for anesthesia, we have since transitioned to using inhaled anesthetics, which may be a confounding factor in comparing LCHS and PT. However, there is no evidence of either anesthetic impacting weight or the bone marrow in the literature to suggest this. One group demonstrated that isoflurane can attenuate lung injury, but this was in a murine model of lung injury induced by zymosan and not lung contusion.⁵² Another study investigated the impact of anesthesia on systemic inflammation in a rodent model of burns, and found that both isoflurane and ketamine xylazine can affect different biomarkers of inflammation, although TLR4 specifically has not been studied.⁵³ Despite the different anesthesia used for PT and LCHS, due to the lack of impact on the bone marrow, our group chose to not perform LCHS with isoflurane to avoid the use of additional animals. In addition, due to the severity of injuries in the PT model, our veterinarians recommended postinjury

resuscitation with normal saline which LCHS animals did not receive. We attributed the splenomegaly observed in the PT cohort to extramedullary hematopoiesis, although other etiologies could include congestion, infiltration, or increased splenic function.⁵⁴ Pseudofracture is not equivalent to femur fractures; however, this technique has been shown to induce similar physiologic changes to a femur fracture and prevents disruption of the bone marrow microenvironment for study.³⁴ This method also allows for an ethically acceptable longer duration of study to ensure mobility of the animal postinjury. This model of polytrauma did not include common injuries such as traumatic brain injury or intra-abdominal solid organ injury, which are not uncommon in blunt trauma. It also only included one age group of animals and did not include females; both sex and age have been shown to affect postinjury outcomes.^{55,56} Future iterations of polytrauma should consider including these injuries along with females and a variety of age groups. Longer durations of study should also be investigated to understand recovery from such severe injury. Finally, animals were either housed alone or separated depending on group allocation, which may have affected results given evidence that social isolation alone is a stress to animals.⁵⁷

5 | CONCLUSIONS

In summary, this novel model of polytrauma induced systemic inflammation, end-organ dysfunction, muscle wasting, anemia and bone marrow dysfunction, which allows further understanding of postinjury phenomena affecting critically ill patients. Future studies should consider the inclusion of female subjects, a variety of age groups, the addition of stress to simulate an intensive care unit stay, and postinjury infection to simulate sepsis in this population.

AUTHOR CONTRIBUTIONS

LSK, JAM, EEP, KBK, and AAM conceptualized the study design. LSK, JAM, EEP, KBK, and EMW contributed to data curation. LSK, JAM, KBK, LEB and AMM contributed to data analysis and interpretation. LSK and JAM wrote the original draft of the manuscript and LEB, PAE and AMM contributed critical revisions to produce the final manuscript.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Animal Care Services for their guidance and input regarding the design of this protocol along with their assistance with rodent care for this study.

FUNDING INFORMATION

This research was supported by the National Institutes of Health. AMM was supported by NIH NIGMS R01 GM105893. LSK and JAM were supported by postgraduate training grant NIH NIGMS T32 GM-008721 in burns, trauma, and perioperative injury.

CONFLICT OF INTEREST STATEMENT

The authors have no relevant conflicts of interest.

ETHICS STATEMENT

The Institutional Animal Care and Use Committee approved this animal protocol (IACUC protocols 201908271, 202011247).

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REFERENCES

- Paffrath T, Lefering R, Flohe S, TraumaRegister DGU. How to define severely injured patients? – an injury severity score (ISS) based approach alone is not sufficient. *Injury*. 2014;45(Suppl 3):S64-S69. doi:10.1016/j.injury.2014.08.020
- Pape HC, Lefering R, Butcher N, et al. The definition of polytrauma revisited: an international consensus process and proposal of the new 'Berlin definition'. *J Trauma Acute Care Surg*. 2014;77(5):780-786. doi:10.1097/TA.0000000000000453
- Hunt PA, Greaves I, Owens WA. Emergency thoracotomy in thoracic trauma—a review. *Injury*. 2006;37(1):1-19. doi:10.1016/j.injury.2005.02.014
- Watts DD, Fakhry SM, Group EM-IHVIR. Incidence of hollow viscus injury in blunt trauma: an analysis from 275,557 trauma admissions from the east multi-institutional trial. *J Trauma*. 2003;54(2):289-294. doi:10.1097/01.TA.0000046261.06976.6A
- DiMaggio C, Ayoung-Chee P, Shinseki M, et al. Traumatic injury in the United States: in-patient epidemiology 2000–2011. *Injury*. 2016;47(7):1393-1403. doi:10.1016/j.injury.2016.04.002
- Palmer CS, Gabbe BJ, Cameron PA. Defining major trauma using the 2008 abbreviated injury scale. *Injury*. 2016;47(1):109-115. doi:10.1016/j.injury.2015.07.003
- Baker SP, O'Neill B, Haddon W Jr, Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma*. 1974;14(3):187-196.
- Patel N, Harfouche M, Stonko DP, Elansary N, Scalea TM, Morrison JJ. Factors associated with increased mortality in severe abdominopelvic injury. *Shock*. 2022;57(2):175-180. doi:10.1097/SHK.0000000000001851
- Gentile LF, Nacionales DC, Cuenca AG, et al. Identification and description of a novel murine model for polytrauma and shock. *Crit Care Med*. 2013;41(4):1075-1085. doi:10.1097/CCM.0b013e318275d1f9
- Weckbach S, Hohmann C, Braumueller S, et al. Inflammatory and apoptotic alterations in serum and injured tissue after experimental polytrauma in mice: distinct early response compared with single trauma or "double-hit" injury. *J Trauma Acute Care Surg*. 2013;74(2):489-498. doi:10.1097/TA.0b013e31827d5f1b
- Weckbach S, Perl M, Heiland T, et al. A new experimental polytrauma model in rats: molecular characterization of the early inflammatory response. *Mediat Inflamm*. 2012;2012:890816. doi:10.1155/2012/890816
- Denk S, Weckbach S, Eisele P, et al. Role of hemorrhagic shock in experimental polytrauma. *Shock*. 2018;49(2):154-163. doi:10.1097/SHK.0000000000000925
- Denk S, Wiegner R, Hones FM, et al. Early detection of junctional adhesion molecule-1 (JAM-1) in the circulation after experimental and clinical polytrauma. *Mediat Inflamm*. 2015;2015:463950. doi:10.1155/2015/463950
- Darlington DN, Craig T, Gonzales MD, Schwacha MG, Cap AP, Dubick MA. Acute coagulopathy of trauma in the rat. *Shock*. 2013;39(5):440-446. doi:10.1097/SHK.0b013e31829040e3
- Mira JC, Nacionales DC, Loftus TJ, et al. Mouse injury model of Polytrauma and shock. *Methods Mol Biol*. 2018;1717:1-15. doi:10.1007/978-1-4939-7526-6_1
- Nicholson SE, Merrill D, Zhu C, et al. Polytrauma independent of therapeutic intervention alters the gastrointestinal microbiome. *Am J Surg*. 2018;216(4):699-705. doi:10.1016/j.amjsurg.2018.07.026
- Wu X, Dubick MA, Schwacha MG, Cap AP, Darlington DN. Tranexamic acid attenuates the loss of lung barrier function in a rat model of polytrauma and hemorrhage with resuscitation. *Shock*. 2017;47(4):500-505. doi:10.1097/SHK.0000000000000758
- Chen J, Wu X, Keese J, Liu B, Darlington DN, Cap AP. Limited resuscitation with fresh or stored whole blood corrects cardiovascular and metabolic function in a rat model of polytrauma and hemorrhage. *Shock*. 2017;47(2):208-216. doi:10.1097/SHK.0000000000000748
- Probst C, Mirzayan MJ, Mommsen P, et al. Systemic inflammatory effects of traumatic brain injury, femur fracture, and shock: an experimental murine polytrauma model. *Mediat Inflamm*. 2012;2012:136020. doi:10.1155/2012/136020
- Wichmann MW, Ayala A, Chaudry IH. Severe depression of host immune functions following closed-bone fracture, soft-tissue trauma, and hemorrhagic shock. *Crit Care Med*. 1998;26(8):1372-1378. doi:10.1097/00003246-199808000-00024
- Kelly LS, Munley JA, Kannan KB, et al. Anemia recovery after trauma: a longitudinal study. *Surg Infect*. 2023;24(1):39-45. doi:10.1089/sur.2022.299
- Munley JA, Kelly LS, Mohr AM. Adrenergic modulation of erythropoiesis after trauma. *Front Physiol*. 2022;13:859103. doi:10.3389/fphys.2022.859103
- Bonig H, Papayannopoulou T. Mobilization of hematopoietic stem/progenitor cells: general principles and molecular mechanisms. *Methods Mol Biol*. 2012;904:1-14. doi:10.1007/978-1-61779-943-3_1
- Kelly LS, Apple CG, Darden DB, et al. Transcriptomic changes within human bone marrow after severe trauma. *Shock*. 2022;57(1):24-30. doi:10.1097/SHK.0000000000001826
- Apple CG, Miller ES, Kannan KB, et al. The role of bone marrow microRNA (miR) in erythropoietic dysfunction after severe trauma. *Surgery*. 2021;169(5):1206-1212. doi:10.1016/j.surg.2020.11.029
- Doobay GD, Miller ES, Apple CG, et al. Mediators of prolonged hematopoietic progenitor cell mobilization after severe trauma. *J Surg Res*. 2021;260:315-324. doi:10.1016/j.jss.2020.11.084
- Alamo IG, Kannan KB, Loftus TJ, Ramos H, Efron PA, Mohr AM. Severe trauma and chronic stress activates extramedullary erythropoiesis. *J Trauma Acute Care Surg*. 2017;83(1):144-150. doi:10.1097/TA.0000000000001537
- Russell WMS, Burch RL. *The Principles of Humane Experimental Technique*. Methuen & Co. Ltd.; 1959.
- Efron PA, Mohr AM, Moore FA, Moldawer LL. The future of murine sepsis and trauma research models. *J Leukoc Biol*. 2015;98(6):945-952. doi:10.1189/jlb.5MR0315-127R
- Bosch F, Angele MK, Chaudry IH. Gender differences in trauma, shock and sepsis. *Mil Med Res*. 2018;5(1):35. doi:10.1186/s40779-018-0182-5
- Guide for the Care and Use of Laboratory Animals. *The National Academies Collection: Reports funded by National Institutes of Health*. 8th ed. National Academies Press; 2011.
- Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *BMJ Open Sci*. 2020;4(1):e100115. doi:10.1136/bmjos-2020-100115
- Kelly LS, Munley JA, Pons EE, et al. Mechanisms of improved erythroid progenitor growth with removal of chronic stress after trauma. *Surgery*. 2022;172(2):759-765. doi:10.1016/j.surg.2022.04.056
- Darwiche SS, Kobbe P, Pfeifer R, Kohut L, Pape HC, Billiar T. Pseudofracture: an acute peripheral tissue trauma model. *J Vis Exp*. 2011;(50):2074. doi:10.3791/2074
- Munley JA, Kelly LS, Pons EE, et al. Multicompartmental traumatic injury and the microbiome: shift to a pathobiome. *J Trauma Acute Care Surg*. 2023;94(1):15-22. doi:10.1097/TA.0000000000003803

36. Munley JA, Kelly LS, Park G, et al. Multicompartmental traumatic injury induces sex-specific alterations in the gut microbiome. *J Trauma Acute Care Surg.* 2023;95(1):30-38. doi:[10.1097/TA.0000000000003939](https://doi.org/10.1097/TA.0000000000003939)
37. Munley JA, Kelly LS, Gillies GS, et al. Multicompartmental trauma induces persistent inflammation and organ injury. *J Surg Res.* 2024;293:266-273. doi:[10.1016/j.jss.2023.08.033](https://doi.org/10.1016/j.jss.2023.08.033)
38. Cox MC, Booth M, Ghita G, et al. The impact of sarcopenia and acute muscle mass loss on long-term outcomes in critically ill patients with intra-abdominal sepsis. *J Cachexia Sarcopenia Muscle.* 2021;12(5):1203-1213. doi:[10.1002/jcsm.12752](https://doi.org/10.1002/jcsm.12752)
39. McGhan LJ, Jaroszewski DE. The role of toll-like receptor-4 in the development of multi-organ failure following traumatic haemorrhagic shock and resuscitation. *Injury.* 2012;43(2):129-136. doi:[10.1016/j.injury.2011.05.032](https://doi.org/10.1016/j.injury.2011.05.032)
40. Devarajan P. Neutrophil gelatinase-associated lipocalin (NGAL): a new marker of kidney disease. *Scand J Clin Lab Invest Suppl.* 2008;241:89-94. doi:[10.1080/00365510802150158](https://doi.org/10.1080/00365510802150158)
41. Dong HY, Wilkes S, Yang H. CD71 is selectively and ubiquitously expressed at high levels in erythroid precursors of all maturation stages: a comparative immunohistochemical study with glycophorin A and hemoglobin A. *Am J Surg Pathol.* 2011;35(5):723-732. doi:[10.1097/PAS.0b013e31821247a8](https://doi.org/10.1097/PAS.0b013e31821247a8)
42. Broudy VC. Stem cell factor and hematopoiesis. *Blood.* 1997;90(4):1345-1364.
43. Loftus TJ, Thomson AJ, Kannan KB, et al. Effects of trauma, hemorrhagic shock, and chronic stress on lung vascular endothelial growth factor. *J Surg Res.* 2017;210:15-21. doi:[10.1016/j.jss.2016.10.023](https://doi.org/10.1016/j.jss.2016.10.023)
44. Perl M, Lomas-Neira J, Venet F, Chung CS, Ayala A. Pathogenesis of indirect (secondary) acute lung injury. *Expert Rev Respir Med.* 2011;5(1):115-126. doi:[10.1586/ers.10.92](https://doi.org/10.1586/ers.10.92)
45. Bible LE, Pasupuleti LV, Gore AV, Sifri ZC, Kannan KB, Mohr AM. Chronic restraint stress after injury and shock is associated with persistent anemia despite prolonged elevation in erythropoietin levels. *J Trauma Acute Care Surg.* 2015;79(1):91-96; discussion 96-7. doi:[10.1097/TA.0000000000000686](https://doi.org/10.1097/TA.0000000000000686)
46. Loftus TJ, Kannan KB, Mira JC, Brakenridge SC, Efron PA, Mohr AM. Modulation of the HGF/c-met Axis impacts prolonged hematopoietic progenitor mobilization following trauma and chronic stress. *Shock.* 2020;54(4):482-487. doi:[10.1097/SHK.0000000000001506](https://doi.org/10.1097/SHK.0000000000001506)
47. Alamo IG, Kannan KB, Ramos H, Loftus TJ, Efron PA, Mohr AM. Clonidine reduces norepinephrine and improves bone marrow function in a rodent model of lung contusion, hemorrhagic shock, and chronic stress. *Surgery.* 2017;161(3):795-802. doi:[10.1016/j.surg.2016.08.043](https://doi.org/10.1016/j.surg.2016.08.043)
48. Alamo IG, Kannan KB, Bible LE, et al. Daily propranolol administration reduces persistent injury-associated anemia after severe trauma and chronic stress. *J Trauma Acute Care Surg.* 2017;82(4):714-721. doi:[10.1097/TA.0000000000001374](https://doi.org/10.1097/TA.0000000000001374)
49. Mohr AM, ElHassan IO, Hannoush EJ, et al. Does beta blockade postinjury prevent bone marrow suppression? *J Trauma.* 2011;70(5):1043-1049. doi:[10.1097/TA.0b013e3182169326](https://doi.org/10.1097/TA.0b013e3182169326)
50. Kelly LS, Munley JA, Pons EE, et al. Multicompartmental trauma alters bone marrow erythroblastic islands. *J Trauma Acute Care Surg.* 2023;94(2):197-204. doi:[10.1097/TA.0000000000003821](https://doi.org/10.1097/TA.0000000000003821)
51. Baranski GM, Offin MD, Sifri ZC, et al. Beta-blockade protection of bone marrow following trauma: the role of G-CSF. *J Surg Res.* 2011;170(2):325-331. doi:[10.1016/j.jss.2011.03.059](https://doi.org/10.1016/j.jss.2011.03.059)
52. Li JT, Wang H, Li W, et al. Anesthetic isoflurane posttreatment attenuates experimental lung injury by inhibiting inflammation and apoptosis. *Mediat Inflamm.* 2013;2013:108928. doi:[10.1155/2013/108928](https://doi.org/10.1155/2013/108928)
53. Al-Mousawi AM, Kulp GA, Branski LK, et al. Impact of anesthesia, analgesia, and euthanasia technique on the inflammatory cytokine profile in a rodent model of severe burn injury. *Shock.* 2010;34(3):261-268. doi:[10.1097/shk.0b013e3181d8e2a6](https://doi.org/10.1097/shk.0b013e3181d8e2a6)
54. McKenzie CV, Colonne CK, Yeo JH, Fraser ST. Splenomegaly: pathophysiological bases and therapeutic options. *Int J Biochem Cell Biol.* 2018;94:40-43. doi:[10.1016/j.biocel.2017.11.011](https://doi.org/10.1016/j.biocel.2017.11.011)
55. Fu CY, Bajani F, Bokhari M, et al. Age itself or age-associated comorbidities? A nationwide analysis of outcomes of geriatric trauma. *Eur J Trauma Emerg Surg.* 2022;48(4):2873-2880. doi:[10.1007/s00068-020-01595-8](https://doi.org/10.1007/s00068-020-01595-8)
56. Choudhry MA, Bland KI, Chaudry IH. Trauma and immune response—effect of gender differences. *Injury.* 2007;38(12):1382-1391. doi:[10.1016/j.injury.2007.09.027](https://doi.org/10.1016/j.injury.2007.09.027)
57. Krugel U, Fischer J, Bauer K, Sack U, Himmerich H. The impact of social isolation on immunological parameters in rats. *Arch Toxicol.* 2014;88(3):853-855. doi:[10.1007/s00204-014-1203-0](https://doi.org/10.1007/s00204-014-1203-0)

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kelly LS, Munley JA, Pons EE, et al. A rat model of multicompartmental traumatic injury and hemorrhagic shock induces bone marrow dysfunction and profound anemia. *Anim Models Exp Med.* 2024;7:367-376. doi:[10.1002/ame2.12447](https://doi.org/10.1002/ame2.12447)