AWARD NUMBER: W81XWH-17-2-0019

TITLE: Assessment of the Immediate, Short-, and Long-Term Deleterious Consequences of Polytraumatic Injury in a Critical Care Non-Human Primate Model

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REPORT DATE: OCTOBER 2021

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
OCTOBER 2021	Final	1JUL2017 - 30JUN2021

4. TITLE AND SUBTITLE

Assessment of the Immediate, Short-, and Long-Term Deleterious Consequences of Polytraumatic Injury in a Critical Care Non-Human Primate Model

5a. CONTRACT NUMBER
W81XWH-17-2-0019

5b. GRANT NUMBER
W81XWH-17-2-0019

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Multi-organ failure (MOF) remains a significant cause of morbidity and mortality in trauma. The pathophysiology behind MOF is related to a maladaptive systemic inflammatory response syndrome (SIRS) triggered by the release of excessive inflammatory cytokines. Currently, the underlying mechanism for immune dysregulation is poorly understood, and there are no existing pre-clinical models, which accurately reflect the clinical scenarios that play out in severely injured patients prone to development of SIRS. The study will fill existing and emerging gaps in the Combat Casualty Care Program to improve treatment for service members injured in combat. Understanding and defining the physiologic and immunologic responses after polytraumatic injury in a stringent and relevant animal model will provide insight for the clinical management and for the development of innovative therapeutic interventions/strategies designed to improve the clinical outcome after combat-related trauma. The goal is to fully characterize the immune response in a clinically-relevant NHP trauma model in anticipation of identifying therapeutic targets to mitigate the associated SIRS/MOF following poly-traumatic injury.

15. SUBJECT TERMS
Trauma, non-human primate, systemic inflammatory response, multiple organ failure, animal models, inflammation, wound healing

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	26	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Multi-organ failure (MOF) remains a significant cause of morbidity and mortality in trauma. The pathophysiology behind MOF is related to a maladaptive systemic inflammatory response syndrome (SIRS) triggered by the release of excessive inflammatory cytokines. Currently, the underlying mechanism for immune dysregulation is poorly understood, and there are no existing pre-clinical models, which accurately reflect the clinical scenarios that play out in severely injured patients prone to development of SIRS. Further, outside of supportive care, there are limited existing strategies for preventing or treating SIRS following complex combat trauma. Moreover, this threatening immunological phenomenon has not been systematically studied as a potential important contributing risk factor for post-injury health complications. In this proposal, we are refining our non-human primate (NHP) poly-trauma model using prolonged ventilation and intensive care unit environment. The goal is to fully characterize the immune response in a clinically-relevant NHP trauma model in anticipation of identifying therapeutic targets to mitigate the associated SIRS/MOF following poly-traumatic injury.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Trauma, non-human primate, systemic inflammatory response, multiple organ failure, animal models, inflammation, wound healing

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Regulatory Approvals (Milestones): Local BUMED/ACURO/IACUC approval. CRADA amended. Approval of purchase contract for NHPs.

We completed our Regulatory Approvals milestones after receiving local BUMED/ACURO/IACUC approval, CRADA amendment, and approval of purchase contract for NHPs on 24-NOV-2017.

Specific Aim 1 Major Task 1 (Milestones): Completion of animal procurements, ICU facility, pre-experimental health monitoring of NHPs, recruitment/training of additional essential surgical and post-operative care staff.

Specific Aim 1, Major Task 1 completed. We procured animals, and recruited/trained additional essential surgical and post-operative care staff on 05-FEB-2018, performed pre-experimental health monitoring of NHPs, and developed an ICU protocol for care of NHPs on 26-FEB-2018.

Specific Aim 1 Major Task 2

Subtask 1: Polytraumatic injury studies with short-term ventilator support in an ICU setting.

We successfully performed a pilot study of maintaining sedation and ventilation of three non-injured NHPs for 72 hours at the request of the IACUC on 19Mar-2018 and 2-APR-2018.

In vivo experiments resumed NOV 2020 after COVID19 pandemic manpower restrictions were lifted and local command policy allowed for veterinary support of our protocol. One polytrauma animal model experiment was attempted (Animal ID 13C112, 16NOV2020). This animal did not survive the protocol as it suffered unrecoverable hemorrhagic shock secondary to unexpectedly high blood loss from the laparoscopically induced liver injury leading to cardiac arrest.

Two more animal studies of polytraumatic injury with ventilator and intensive care unit management were accomplished in March 2021. One animal was euthanized on postoperative day 2 after developing right hind-leg ischemia secondary to catheter-associated femoral arterial thrombus (Animal ID 12U036, 08MAR2021). The last animal was euthanized within 24 hours of index operation after developing sepsis leading to disseminated intravascular coagulopathy and unrecoverable coagulopathy, acidosis, and hypothermia, (Animal ID 12U009, 15MAR2021).

Subtask 2: Sample collections and processing to include baseline measurements through a 6-month post-operative time point.

Samples were collected and processed according to protocol to include baseline measurements through the early death of each animal.

Milestones: Completion of 6-month follow-on clinical assessments and sample collections

This milestone was not met as each animal that underwent the polytrauma protocol met early death before completion of the protocol.

Specific Aim 2 Major Task 1

Subtask 1: Assessment of short-term and long-term co-morbidities and survival (mortality). Determine the impact of mechanical ventilations.

There was 100% mortality during this protocol leading to early termination of the protocol. Short-term comorbidities included hemorrhagic shock, catheter-associated arterial thrombus, limb ischemia, sepsis, septic shock, and disseminated intravascular coagulopathy. Early mortality rate was 100% as none of the animals studied in the polytrauma protocol survived beyond postoperative day 2. Mechanical ventilation provided adequate respiratory support for the duration of each truncated study.

Subtask 2: Clinical and molecular assessment of SIRS, ARDs, and MODS/MOF induction and organ system functional recovery kinetics.

- Tissues were stained with hematoxylin and eosin (H&E) staining and were examined by a pathologist
- To detect ARDS, immunostaining against myeloperoxidase (a marker of neutrophil infiltration) was used

Subtask 3: Assessment of systematic inflammatory, hemodynamic, metabolic, and trauma-induced immune cell activation responses, coagulation pathway activation, post-injury wound repair/healing.

- Complete Blood Count (CBC), coagulation and arterial blood gases (ABG) were collected
- Inflammatory response at the cellular and molecular levels was assessed using Fluorescence-activated cell sorting (FACS) and Luminex
- To detect cell death immunostaining against activated caspase-3, an indicator of apoptosis was performed
- Immunostaining against myeloperoxidase was performed to detect the neutrophil invasion
- Quantitative Polymerase Chain Reaction (qPCR) was done to detect expression of inflammatory genes associated with trauma

(Milestones): Completion of *in vivo* experiments and clinical assessment studies and data analysis.

In vivo experiments were completed after discussion with the funders in MAR2021 with ongoing data analysis through September 2021.

Specific Aim 2 Major Task 2

Subtask 1: Determine the long-term pathophysiology of polytraumatic injury on the respiratory, cardiac, genitourinary, gastrointestinal, and innate plus adaptive immune system as well as skin and soft tissue wound reparative/healing/regenerative process.

No long-term pathophysiologic determinations of polytraumatic injury were assessed as the model was modified to end with euthanization after a 5 day postoperative intensive care unit course. During the trial, all animals met early death within 2 days of surgically induced polytrauma.

(Milestones): Completion of in vivo experiments and clinical assessment studies and data analysis.

In vivo experiments concluded 15March2021 followed by data analysis.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities

The major activities accomplished included development of a critical care model for application in polytrauma injured non-human primates, pilot and sham testing of animals through the critical care model, and 3 animal studies of polytrauma injured non-human primates. Physiologic and laboratory parameters were studied for the animals that underwent the protocol and laboratory evaluation for markers of inflammation were performed.

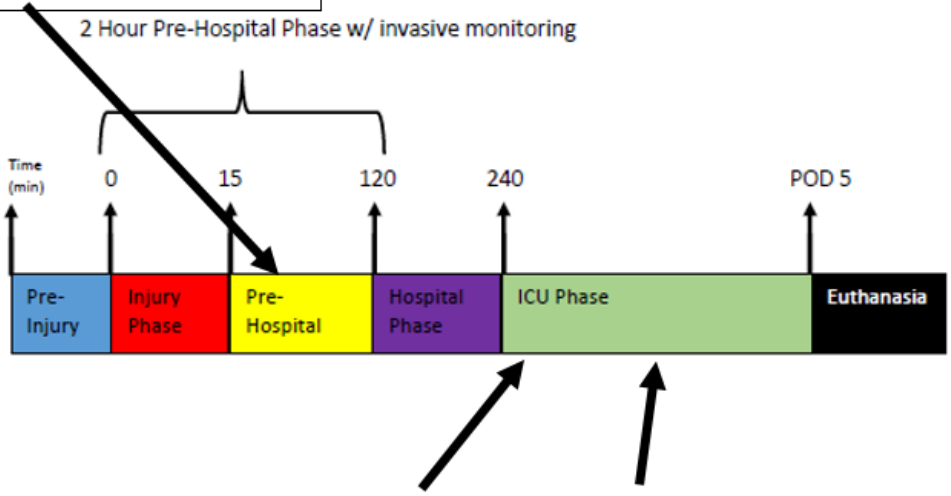
2) Specific objectives

- The main objective of this study was a proof of concept to maintain a non-human primate in an intensive care unit (ICU) setting for up to five days
- A second objective was to apply the ICU model to polytraumatic injured NHPs who sustained the injury pattern previously published by our group
- The third objective was characterization of the immune response after polytraumatic injury

3) Significant results / Key outcomes

For the polytrauma model, three animals were put through the revised protocol. The first animal (ID 13C112) suffered unrecoverable hemorrhagic shock from unexpectedly high blood loss immediately after liver injury and was euthanized intraoperatively. The second and third animals (ID 12U036 and 12U009, respectively) survived the injury and hospital phases, but had complications resulting in early euthanasia during the ICU phase of care. Figure 1 demonstrates the timeline of all three premature mortality outcomes in the injured animals.

NHP 1 becomes hemodynamically unstable immediately after liver injury is created on POD 0. Does not respond to initial crystalloid bolus per protocol. Abdomen is re-opened, there is clot present and no ongoing massive hemorrhage. Animal is euthanized for prolonged period of hypotension.



NHP 3 becomes hemodynamically unstable and hypothermic despite aggressive resuscitation in evening of POD0 and into POD1. Euthanized for prolonged hemodynamic instability.

NHP 2 Lactate begins to rise in evening of POD 2, doppler signals in right lower extremity undetectable, suspect ischemic leg, cannot clear lactate, euthanized at T=48hr

Fig 1. Schematic timeline of the experimental protocol demonstrates the critical phases of care for each animal progressing through the protocol. NHP 1 references the animal who underwent this protocol on 16NOV2020 and succumbed to unrecoverable hemorrhagic shock during the injury phase of the protocol (ID 13C112). The other animals included NHP 2 (surgery 08MAR2021, ID 12U036) and NHP 3 (surgery 15MAR2021, ID 12U009). As demonstrated in this schematic, both animals that underwent the protocol in March were able to be supported through the initial stages but had complications resulting in early euthanasia during the ICU phase of care.

Case #1 – animal ID 13C112

Animal had successful intubation and initiation of general endotracheal anesthesia (GETA) as well as placement of invasive monitoring without complication. During the injury phase, the cecum then liver were injured per protocol. Immediately after liver laceration, the animal's blood pressure began to decline indicative of hemorrhagic shock. A crystalloid fluid bolus was given 15 minutes after injury, per our study design. The animal was a fluid non-responder and demonstrated early signs of cardiovascular collapse prompting the team to proceed euthanasia. Necropsy of the animal suggested the cause of death in this animal was hypovolemic cardiovascular collapse from the liver injury (Figure 2).

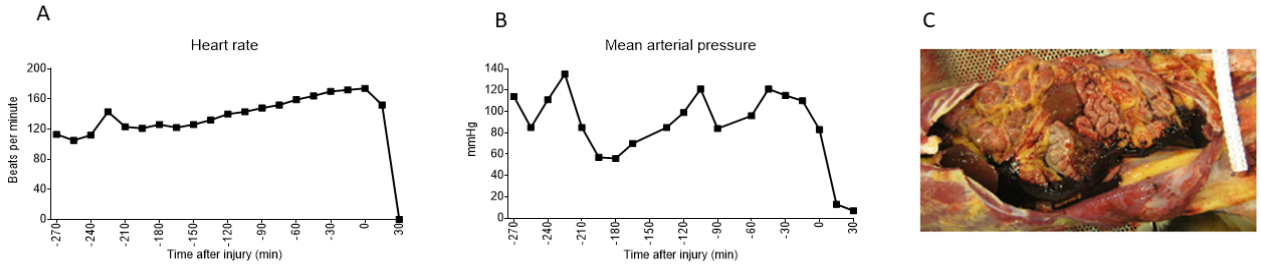


Fig 2. Selected hemodynamic parameters and necropsy specimen from animal ID 13C112. A&B. Heart rate and mean arterial pressure demonstrating abrupt cardiovascular collapse shortly after liver injury (T0). C. Abdominal cavity with dependent hematoma.

Case #2 – animal ID 12U036

After successful intubation and induction of GETA, the tier 3 animal had a reoperative groin site leading to challenging catheterization of the right femoral artery for invasive hemodynamic monitoring. The animal progressed through the injury, pre-hospital and entered the hospital phase. On postoperative day 1 (POD 1), the right femoral arterial line began to malfunction showing early signs of catheter related thrombosis. Per protocol, a series of heparin flushes were periodically used to maintain patency of the vessel and use of the line. On POD 2, evidence of ongoing catheter associated thrombosis was apparent and the function of this line could not be restored. Evidence of critical limb ischemia was apparent with absent distal pulses and Doppler signals. The lactate levels of this animal began to rise corresponding with the presentation of the ischemic limb. As the animal's lactate began to rise, evidence of shock was present and vasopressor support was started with norepinephrine. Vasopressor support gradually escalated with no signs of physiologic improvement with the ongoing insult of the ischemic limb causing irreversible shock. The decision was made to humanely euthanize the animal an hour later (48hr after injury).

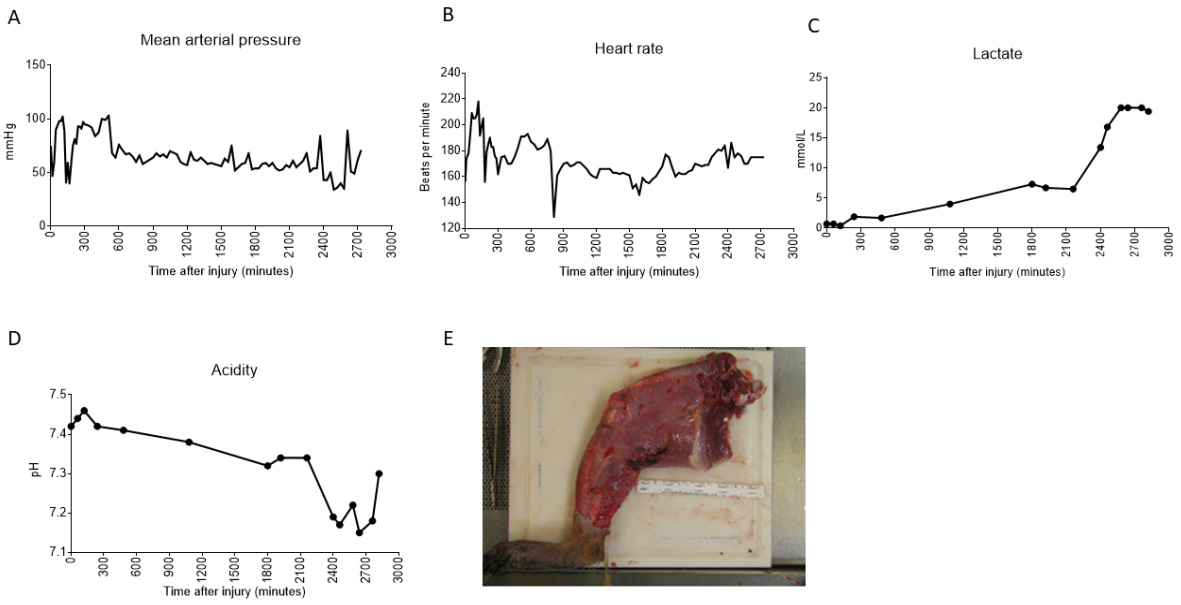


Fig 3. Selected hemodynamic parameters, laboratory trends, and necropsy specimen from animal ID 12U036. A. Mean arterial pressure demonstrating gradual downtrend and ultimately instability before euthanasia. B. Heart rate with sustained tachycardia during ICU course. C&D. Worsening lactic acidosis from limb ischemia after right femoral arterial thrombosis. E. Necropsy specimen with right hind leg musculature findings consistent with ischemic necrosis.

Case #3 – animal ID 12U009

A tier 3 animal was utilized for this run of the protocol with previous groin access operative sites. After successful intubation and induction of GETA, femoral cut downs were required for placement of invasive lines. The surgical protocol progressed uneventfully. At the conclusion of the operation the NHP demonstrated early evidence of catheter-associated thrombosis of the left femoral arterial line with poor waveform and poor backflow. Function of this line was unable to be salvaged with heparin and the catheter was removed. Distal pulses were able to be restored with heparin flushes through the extremity. The right femoral artery was accessed and catheterized. On routine laboratory evaluation, the NHP developed anemia that was initially responsive to blood transfusion, however at approximately 10 hours post-injury, the animal developed catheter-associated thrombosis of the right leg with poor wave forms and absent distal Doppler signals. The animal demonstrated progressive lactic acidosis and the arterial catheter was removed. Back bleeding of the distal artery could not be fully restored with heparin flushes which raised concern for ongoing limb ischemia.

The animal became anemic and an exploratory laparotomy was performed to rule out ongoing bleeding. No surgical source of bleeding was identified and oozing was noted from multiple intraabdominal sites. This finding was consistent in conjunction with the limb ischemia and with disseminated intravascular coagulation. During laparotomy, the pancreas appeared acutely edematous. Chemical pancreatitis was confirmed on laboratory evaluation. The decision was made to euthanize the animal with irreversible acidosis, hypothermia, and coagulopathy. Necropsy findings were consistent with a hypercoaguable state consistent with DIC and histopathologic evaluation was consistent with acute pancreatitis.

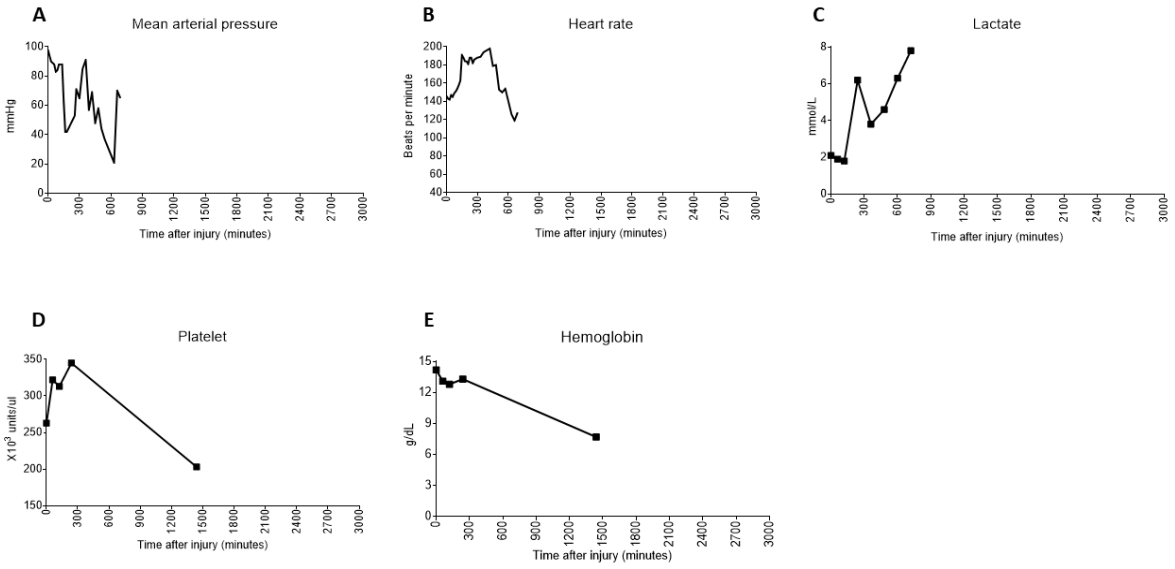


Fig. 4. Selected hemodynamic parameters, laboratory trends, rotational thromboelastography, and histological specimen from animal ID 12U009. A. Mean arterial pressure demonstrating hemodynamic instability and decline in MAP requiring support with vasopressors. B. Heart rate with initial sustained tachycardia progressing to cardiovascular collapse. C. Lactic acidosis initially responsive to blood transfusion, then progressive in the setting of right femoral arterial thrombosis. D. Platelet concentration demonstrating thrombopenia at the time other hypercoaguable abnormalities noted on thromboelastogrpahy. E. Hemoglobin concentration showing anemia at time hypercoaguable state consistent with widespread surface bleeding observed intraoperatively.

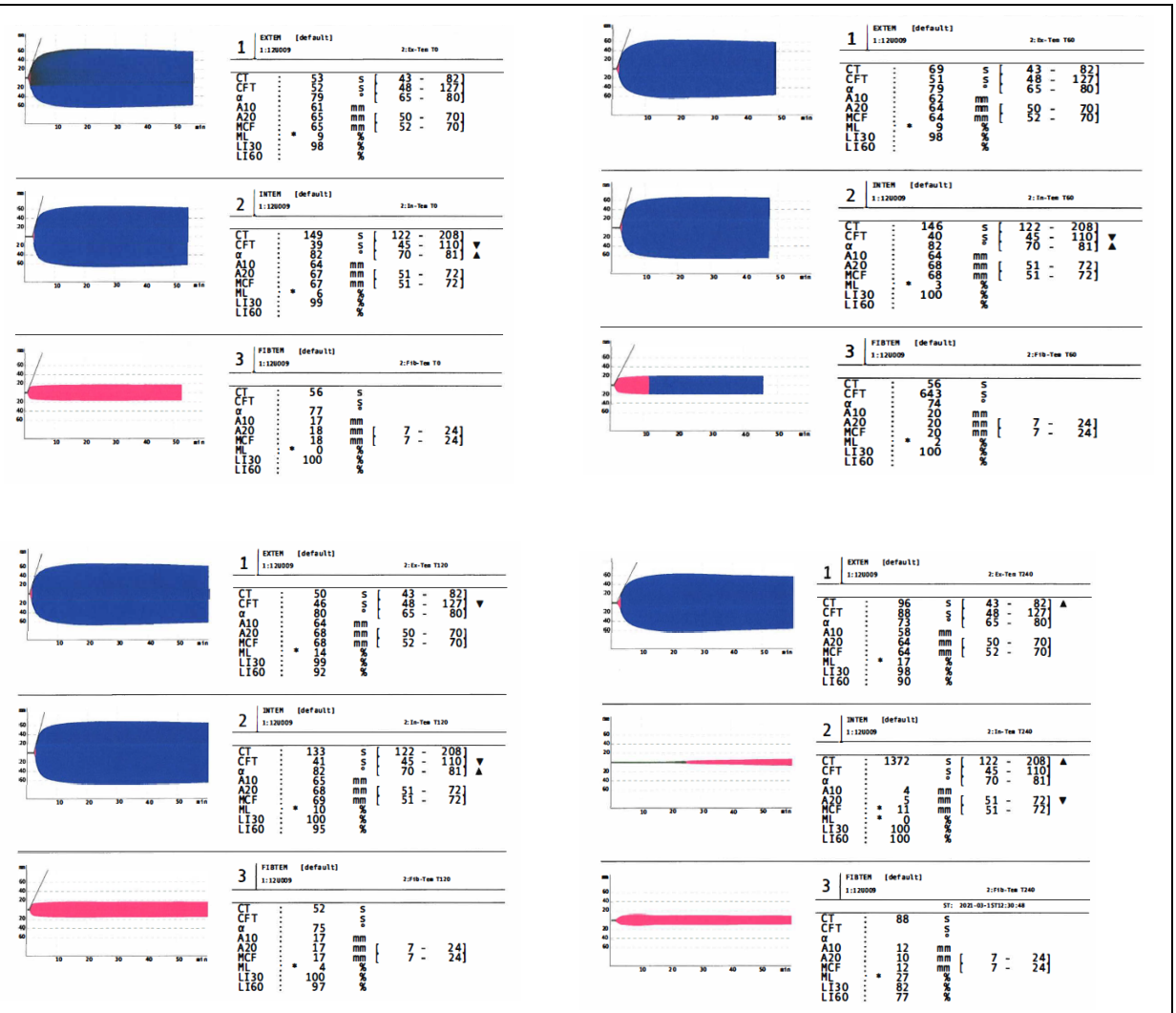


Fig. 5. Rotational thromboelastogram analysis in animal 12U009 at time on injury (top left), 60 minutes (top right), 120 minutes (bottom left) and 240 minutes (bottom right) after injury. Times 120 and 240 minutes after injury demonstrate interval development of a significant coagulopathy – most notably of the intrinsic pathway, with a prolonged clotting time (CT) and small clot amplitude (A20).

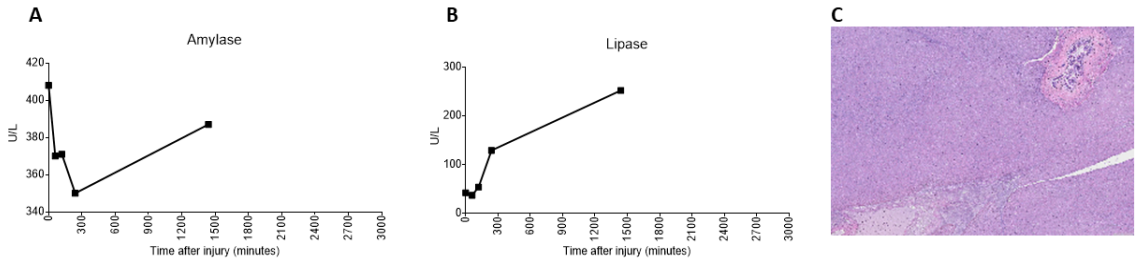


Fig. 6. Histologic section of the pancreas of animal 12U009 demonstrating diffuse necrosis consistent with operative and chemical observation of pancreatitis related to shock.

Inflammatory parameters were obtained at 1, 2 and 4hr after injury in accordance with previous findings that the inflammatory response to polytrauma is most dynamic and peaks within several hours post-injury. Injured animals demonstrated the expected inflammatory response to trauma while sham animals did not.

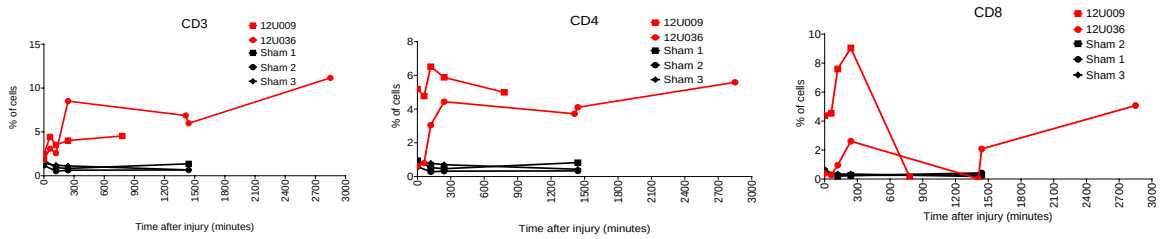


Fig. 7. FACS analysis demonstrated inflammatory response in injured animals (red lines) compared to sham animals (black lines). In the injured animals there is sustained elevation in CD3 and CD4 consistent with previous studies of the trauma inflammatory response. There is a spike in CD8 in injured animals at time 300 min following injury.

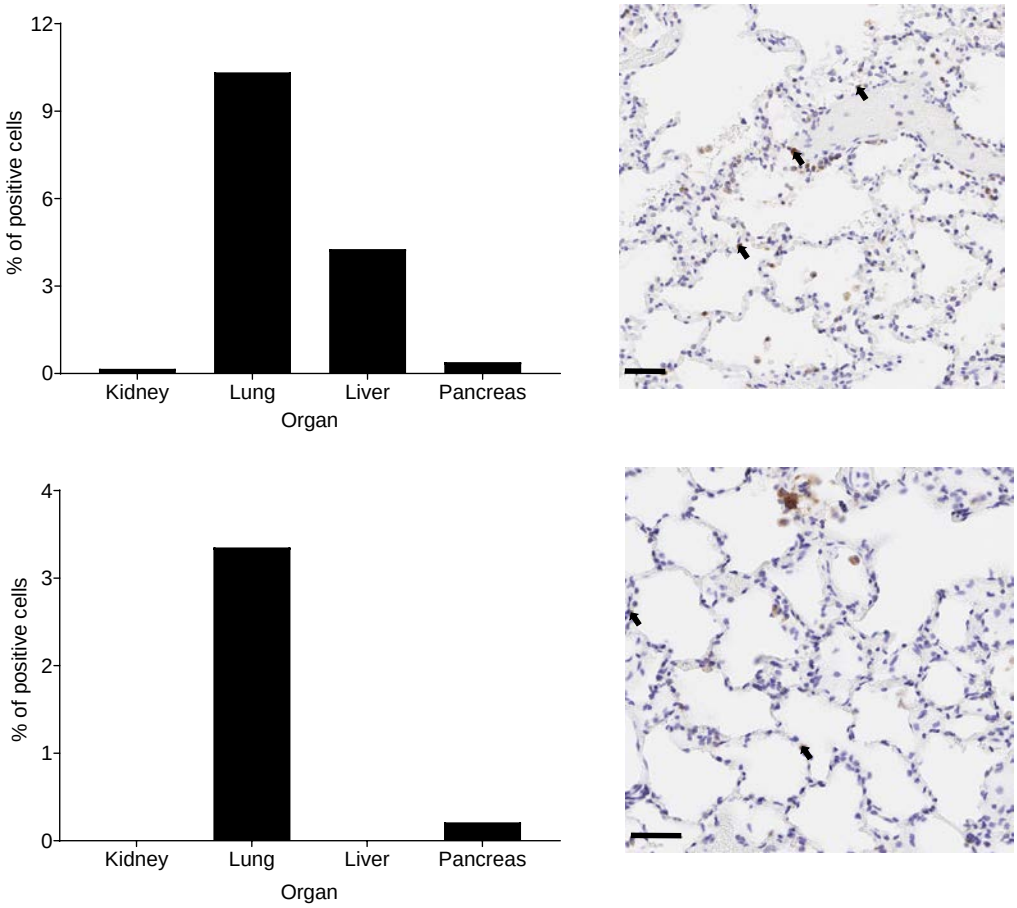


Fig. 8. Sections from animal ID 12U009 (top) or 12U036 (bottom) were stained with active caspase 3 to detect apoptotic cells. Right: representative image of the lung. An increase in positive cells was seen in the lungs of injured animals, consistent with early ARDS. Arrows indicate positive cells. Scale bar, 50 microns.

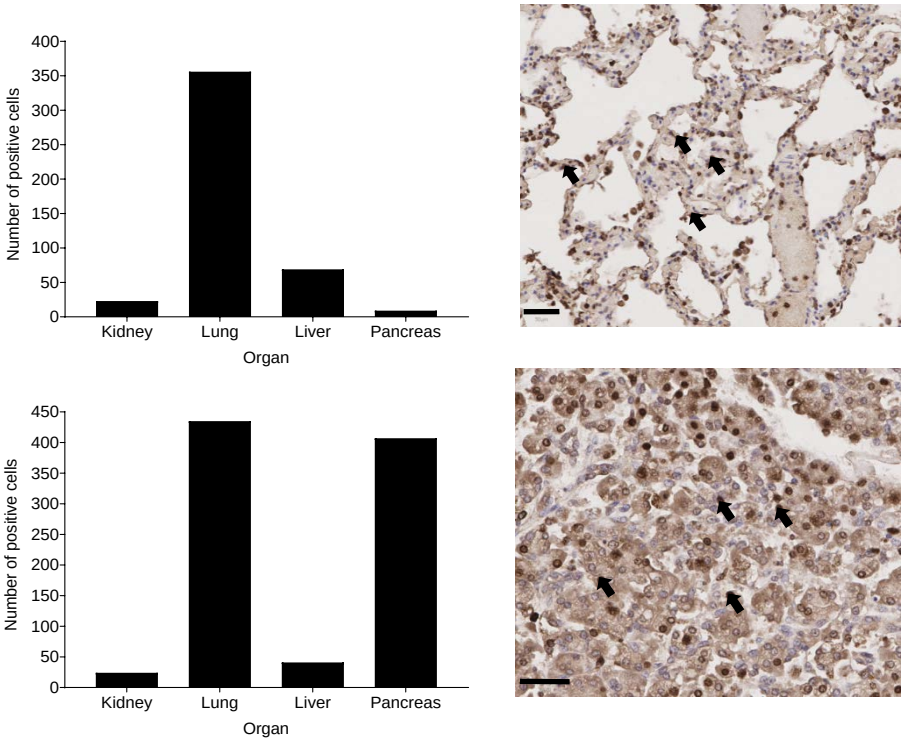


Fig. 9. Sections from animal ID 12U009 (top) or 12U036 (bottom) were stained with Myeloperoxidase to detect neutrophil cells. Top right: representative image of the lung from animal ID 12U009. Increased level of neutrophil cells in the lungs support the increase in inflammatory response and ARDS. Bottom right: representative image of the pancreases from animal ID 12U036. Abundant neutrophil infiltration is consistent with pancreatitis. Arrows indicate positive cells. Scale bar, 50 microns.

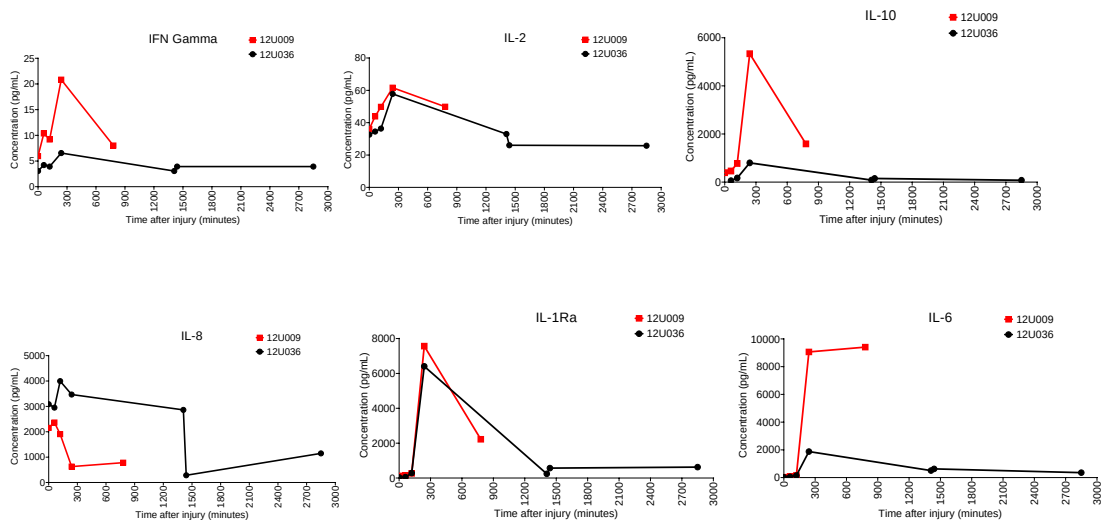


Fig. 10. Cytokine expression in injured animals was consistent with prior findings – demonstrating within several hours after injury an increase to a peak in the pro-inflammatory mediators IL-2, IL-6, and IFN Gamma; the anti-inflammatory mediator IL-10, and the immunomodulatory IL-1Ra. These findings are consistent with what is clinically observed in human trauma data. The significant decrease in IL-8 observed in this model has been observed in previous NHP trauma models. IL-8 is a potent chemotactic factor for neutrophil recruitment and has been associated with the development of multiple organ dysfunction in trauma patients. It has been theorized that the decreased systemic levels of IL-8 in NHP trauma models may be caused by robust activation of hepatic granulocytes in response to liver injury and/or hemorrhage, or potentially a species-specific phenotypic response to hemorrhagic shock.

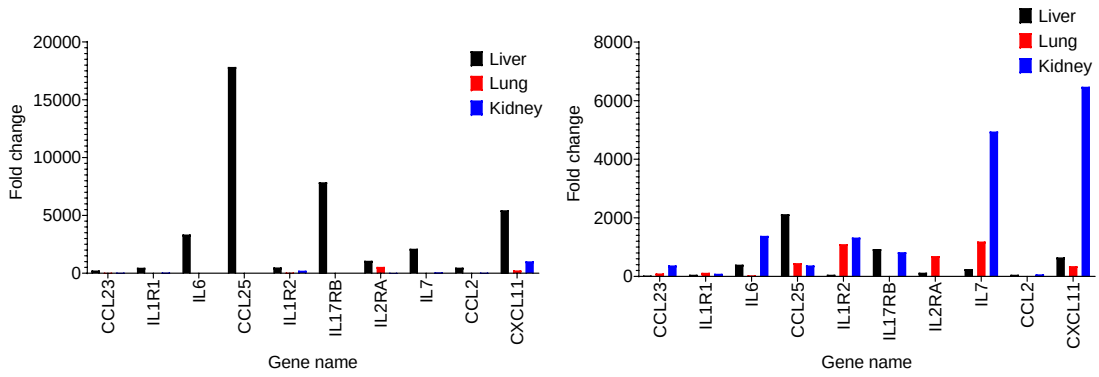


Fig. 11. Fold expression of representative inflammation genes in the liver, lung and kidney of animal 12U009 (left) and 12U036 (right) is shown compared to a house keeping gene (GAPDH). The complications related to limb ischemia, pancreatitis, and DIC makes the gene expression profile difficult to interpret in the animals studied.

What was accomplished?
Include participation in conferences, workshops, and seminars not listed under major activities.

Collaboration with Dan Chertow, MD, MPH, a PI at the NIH who has published data on the use of a non-human primate model has allowed for the development of the critical care portion of this project and ongoing interaction with him and his group has allowed for feedback on our model and improvements in our study.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

This project will be closed out with no additional work anticipated.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

The project applied a prolonged ICU care protocol previously utilized to study Ebola with ventilator support for uninjured NHPs and demonstrated successful sedation under this protocol with alfaxalone. Neither of these goals has previously been achieved in a non-human primate trauma model.

The project demonstrated the high mortality associated with combined septic and hemorrhagic shock in a NHP model. With the complexity of this model, utilizing tier 3 animals during supply shortages of animals due to the COVID19 pandemic resulted in multiple unanticipated complications and the premature mortality of all animals. The combined hemorrhagic and septic shock in this population led to complications related to DIC which was not seen in either the SHAM animals or in other non-traumatic applications of this protocol.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*

- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

To mitigate the problems with availability of NHPs during the COVID19 pandemic limiting shipping of animals from our typical suppliers overseas, tier 3 animals were utilized for this protocol. An unexpected complication encountered during these trials included difficulty with obtaining arterial access for hemodynamic monitoring in the groin. All tier 3 animals previously had surgical access at these sites as well as multiple blood draws from these sites which made operative access challenging, though not prohibitive for placing a femoral arterial line. Unfortunately, as the line remained in place for ongoing monitoring after injury, both animals that progressed to the hospital phase of care demonstrated an unanticipated hypercoaguability resulting in acute limb ischemia in NHP2 and DIC in NHP 3. This physiology may be related to the reoperative groin site access with endothelial damage from previous lines and blood draws, or it may be related to the traumatic injuries sustained causing a hypercoaguable state. In the future, consideration should be made for using tier 1 animals to limit the confounding circumstances leading to line failure and limb ischemia.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

To mitigate the problems with availability of NHPs during the COVID19 tier 3 animals were utilized for this protocol. This may have direct impact on outcome data for this protocol as underlying medical conditions and previous interventions may play a role in the inflammatory response of the animal. This problem was considered by the group before deciding to proceed with this population of available animals understanding that this may impact outcomes. Ultimately, this decision may have contributed to the early mortality of all injured animals in the protocol.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

During the COVID19 pandemic, animal work was shut down due to personnel restrictions both at the department level and in the support staff with the veterinary services branch. Funds were utilized to maintain salary commitments to our employees during this time.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Nothing to Report.

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

- Rex E. Atwood MD, Dana M. Golden, Stephen A. Kaba, and Matthew J. Bradley **(2020).** Characterization of the Cortisol Response to Traumatic Hemorrhage and Intra-Abdominal Sepsis Models in Cynomologus Macaques. Molecular and Cellular Endocrinology (**submitted**)

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year*

(international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Nothing to report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *biospecimen collections;*

- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name:	Dr. Stephen Ahlers
Project Role:	PI
Nearest person month worked:	2
Contribution to Project:	Overall project coordination, experimental procedures/intensive care unit animal care
Name:	LCDR Carolyn Gosztyla
Project Role:	Associate PI
Nearest person month worked:	9
Contribution to Project:	Experimental procedures/intensive care unit NHP management
Name:	Dr. Yaron Dayani
Project Role:	Associate PI
Nearest person month worked:	9
Contribution to Project:	Experimental procedures/intensive care unit NHP management
Name:	Dr. Patrick Benoit
Project Role:	Surgical Research Resident
Nearest person month worked:	8
Contribution to Project:	Experimental procedures/intensive care unit NHP management
Name:	Dr. Rathnayaka Gunasingha
Project Role:	Surgical Research Resident
Nearest person month worked:	8
Contribution to Project:	Experimental procedures/intensive care unit NHP management
Name:	Crystal Leonhardt
Project Role:	Protocol Coordinator
Nearest person month worked:	No change
Contribution to Project:	Experimental procedures, blood draws, laboratory analysis, animal care
Name:	Stephen Kaba
Project Role:	Senior Scientist
Nearest person month worked:	No Change
Contribution to Project:	Laboratory analysis
Name:	Andrew Yurko
Project Role:	Research Assistant
Nearest person month worked:	No change
Contribution to Project:	Assistance with experimental procedures, blood draws, animal

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations wre involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.