Comparison of different resuscitation fluids in volume-controlled and uncontrolled hemorrhage models over time in swine (*Sus scrofa*)

R. Madelaine Paredes, PhD
Maria Castaneda, MS
Allyson A. Mireles, PhD, MS
Dylan Rodriguez, MSc
Col Joseph K. Maddry, MD, USAF, MC

FINAL REPORT

Date: October 30, 2023

59th Medical Wing
Office of the Chief Scientist
1632 Nellis, BLDG. 5406
JBSA Lackland AFB, TX 78236-7517

DECLARATION OF INTEREST

The views expressed are those of the authors and do not reflect the official views or policy of the Department of Defense or its Components. The experiments reported herein were conducted according to the principles set forth in the National Research Council's Guide for the Care and Use of Laboratory Animals (8th ed.), and the Animal Welfare Act of 1966, as amended. The views of are not necessarily the official views of, or endorsed by, the U.S. Government, the Department of Defense, or the Department of the Air Force. No Federal endorsement of manufacturer is intended. *This work was funded by Project Code Number AC16EM01.* *Authors are military service members, employees, or contractors of the US Government. This work was prepared as part of their official duties. Title 17 USC §105 provides that 'copyright protection under this title is not available for any work of the US Government.' Title 17 USC §101 defines a US Government work as a work prepared by a military service member, employee, or contractor of the US Government as part of that person's official duties.*

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

Qualified requestors may obtain copies of this report from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

COMPARISON OF DIFFERENT RESUSCITATION FLUIDS IN VOLUME-CONTROLLED AND UNCONTROLLED HEMORRHAGE MODELS OVER TIME IN SWINE (SUS SCROFA) – PART II

Michele F. Tavish

Michele F. Tavish, DHA
Program Analyst
En route Care Research Program

Robert T. Gerhardt, MD, MPH, FACEP, FAEMS, DHA
Director, Trauma and Clinical Care Research
59MDW /STT Office of the Chief Scientist

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 30, 2023	Final Report	October 2016 – December 2020

4. TITLE AND SUBTITLE

Comparison of different resuscitation fluids in volume-controlled and uncontrolled hemorrhage models over time in swine (Sus Scrofa) – Part II

5a. CONTRACT NUMBER

5b. GRANT NUMBER
AC16EM01

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
R. Madelaine Paredes, PhD; Maria Castaneda, MS; Allyson A. Mireles, PhD; Dylan Rodriguez, MSc; Col Joseph K. Maddry, MD, USAF, MC

5d. PROJECT NUMBER
AC16EM01

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
United States Air Force, 59th Medical Wing (59MDW/ST).
1255 Wilford Hall Loop, Building 4430
Lackland Air Force Base, 78236-9980

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Medical Support Agency (AFMSA)
2261 Hughes Ave
Suite 158
Lackland AFB, Tx 78236-9853

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT-

Purpose: The purpose of this project was to investigate different resuscitation fluids in volume controlled (CH) and uncontrolled hemorrhagic (UH) shock in swine. Blood products are ideal; however, they can be expensive, have a short shelf life and carry an infection risk. Hydroxocobalamin (HOC) is a small volume, small weight, safe, FDA-approved drug that improves SBP, reduces inflammation and is neuroprotective. HOC could be a useful adjunct to hemorrhagic shock in prehospital, tactical situations and could serve as a bridge for patient stabilization during evacuation to level III facilities during the resuscitation phase. **Methods:** Controlled (40%) and uncontrolled hemorrhagic shock induced in swine. Following hemorrhage, animals were randomized to receive treatment with either whole blood (WB), lactated ringers solution (LR), HOC, PRBC-cold stored platelets, PRBC- room temperature platelets, or Hextend. Animals were monitored for six hours after treatment. Hemodynamic parameters, blood gases and chemistries were collected throughout the duration of the experiment. Data is reported as mean ± SEM, statistical analysis was performed by ANOVA with a $p<0.05$. **Results**: Swine experienced a blood loss of 41%±0.02 for CH vs. 33%±0.07 for UH. During both UH and CH, HOC administration maintained a higher systolic blood pressure (SBP), cardiac output (CO), SPO_2, and vascular resistance (SVR) that was comparable to WB and above LR levels. Blood gas analysis revealed that during CH, lactate, potassium (K^+), O_2 and CO_2 levels were comparable to WB treatment. For UH, CO_2 and K^+ levels were also comparable to WB; however, CO_2 and lactate followed a similar trend to LR. During CH and following HOC administration, Ca^{2+} levels were elevated at several time points compared to WB and LR treatment. **Conclusion**: HOC administration resulted in improved hemodynamic parameters and Ca^{2+} levels compared to LR and were equivalent to WB in both controlled and uncontrolled hemorrhage. HOC may be a viable alternative when WB is not available.

15. SUBJECT TERMS- Swine, Hemorrhage, Hydroxocobalamin, HOC, resuscitation, Lactated Ringers

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT:**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Lt Col Joseph K Maddry
U	U	U	UU	23	**19b. TELEPHONE NUMBER** *(include area code)* **210-916-3693**

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

Traumatic hemorrhage has been a leading cause of death in the military and civilian environments. We have previously shown that pre-clinical treatment with Hydroxocobalamin (HOC), a U.S. FDA-approved drug, is as effective as whole blood and Hextend in maintaining mean arterial pressure (MAP) in a swine model of class III hemorrhage.

With this project, our goal was to show the efficacy of more accessible treatments available to our warfighters in the field. Based on the knowledge and results from previous studies, we hypothesized that 1) Hydroxocobalamin will increase systolic blood pressure (SBP) in our model of uncontrolled hemorrhagic shock with an efficacy comparable to Hextend. 2) Hydroxocobalamin will improve SBP without generating rebleeding or affecting coagulation. 3) Hydroxocobalamin will improve SBP and systemic vascular resistance and improve serum lactate with efficacy comparted to cold-stored platelets combined with packed red blood cells (PRBCs).

Towards the end of completing the study, we encountered a delay due to a national shortage of Demerol; however, once received, the lab staff was quick to schedule and aid in the completion of this project.

2.0 INTRODUCTION

Hemorrhagic shock is more commonly seen in critically injured civilian and military populations. More specifically, traumatic hemorrhage remains a leading cause of death in civilian and military environments accounting for 40% of civilian deaths and 50% of military deaths.[1,2] Injuries to extremities may be effectively treated with hemostatic bandages and tourniquets.[3,4]However, abdominal, thoracic, and groin hemorrhage may be lethal and not amenable to tourniquet application. Resuscitation therapies for these injury types continue being studied.[5] Hemorrhage occurring in the prehospital, tactical environment is most difficult to treat and better therapies for early resuscitation that are easily portable and lightweight are needed. To date, available resuscitation fluids have been used with mixed results and have not been very feasible to carry. In the late 90's, medics in Somalia were forced to carry 30 pounds of crystalloid fluids to the field and soldiers carried one-liter bags with IV sets weighing 2 pounds.[6] In addition to the excess weight encountered, researchers have confirmed that large volume crystalloid resuscitation can dilute coagulation factors and worsen survival.[7,8]

Different resuscitation fluids used to treat hemorrhagic shock

Current Tactical Combat Casualty Care (TCCC) guidelines list the different resuscitation fluids for hemorrhagic shock from most to least preferred depending on availability. Whole blood or blood components are the most preferred, followed by colloids (e.g. Hextend) and crystalloids (e.g. Lactated Ringers or Plasma-Lyte).[9] Hextend, a synthetic colloid is most frequently used because of the smaller weight and volume required. However, studies have not confirmed efficacy in hemorrhagic shock and some studies have demonstrated increased risks associated with its use.[10,11] In 2013, the FDA issued a warning related to the use of Hextend in critically-ill adult patients after receiving reports of associated mortality and renal insufficiency.[12] The civilian Advanced Trauma Life Support (ATLS) guidelines recommend initial resuscitation of hemorrhagic shock with 1 liter of crystalloid fluid, with the warning that infusion of more than 1.5 liters of crystalloid has been associated with increased mortality.[13] A recent review of the

prehospital fluid resuscitation protocols of 27 states reported the use of both lactated ringers and normal saline. The report concluded that more research is needed to determine the optimal fluid resuscitation in hypotensive trauma patients.[14]

Hypertonic saline has also been studied because of the small volume and potential benefit for head trauma. However, a large NIH-sponsored trial did not demonstrate a clear benefit.[15] Hemoglobin based oxygen carriers are attractive candidates, but the results of studies of these carriers have been limited by clinical side effects, lack of efficacy, and increased deaths.[16] Intravenous plasma may be effective for shock; however, it is difficult to deliver and store in prehospital, tactical settings.[17,18] In addition, plasma and other blood products are expensive and carry infection risks. Refrigerated (4^{O} C) platelets were the standard of care for blood loss related to acute hemorrhage until the mid-1980's and have an established safety record, including a decreased risk for bacterial contamination. The most common use for platelets has been in patients with hypoproliferative thrombocytopenia. Room temperature platelets survive in circulation longer, therefore they are indicated for prophylactic infusion. The hemostatic function of cold stored platelets is better, but room temperature platelets became the norm because they have both hemostatic function and survive longer. Cold-stored platelets have recently been approved by the Food and Drug Administration (FDA).[19] Platelets stored in this way retain blood clotting qualities for a longer period of time and are less likely to be contaminated by bacteria than the current standard-of-care room temperature stored platelets.[20] Cold-stored platelets show promise as a new resuscitation treatment. However, to be completely effective for treatment of hemorrhagic trauma, they require infusion with PRBCs. Early resuscitation with blood products is still under study at this time.[13]

A simple, durable, small-volume, portable drug that improves blood pressure and survival may be better to treat hemorrhagic shock in the tactical environment when blood products are not available.

During hemorrhagic shock and sepsis, nitric oxide is produced, 21 with high levels responsible for hypotension-associated complications. Treatments aimed at scavenging nitric oxide to treat hemorrhagic shock, such as hemoglobin oxygen based carriers (HBOC), have been proven successful.[22,23] However, most drugs studied in hemorrhagic shock that scavenge or reduce nitric oxide are not currently FDA approved or are known to have serious side effects.[24] One drug that may offer an off-label solution for better treatment of hemorrhagic shock is Hydroxocobalamin (Cyanokit), a safe, FDA-approved, effective drug used for cyanide-induced shock and cyanide toxicity.[25]

Hydroxocobalamin and its use for hemorrhagic shock

Hydroxocobalamin (HOC) is a durable, lightweight powder that is mixed in 90-180 mL of saline. It reduces cyanide toxicity by binding circulating cyanide and producing the nontoxic cyanocobalamin, which is excreted renally.25,26 In addition to its effectiveness and portability, HOC is considered a general stock medication in combat theater hospitals and in civilian ambulances; thus, it makes it attractive for the tactical environment and emergency physicians, paramedics, and pharmacists are often familiar with its administration.[27] Given the small volume needed for HOC administration, its infusion would not dilute coagulation factors compared to a crystalloid infusion (1,000-3,000 mL). Even though HOC has few serious adverse effects, some of the most common side effects reported following HOC injection vary from mild reactions

(including a rash and itchy skin on the site of injection, red body fluids, headache, chest discomfort and dizziness), to hypersensitive reactions (including anaphylaxis).[28,29]

Shock in combat can be the result of several causes and it is often not due to hemorrhage alone, but it can be manifested in combination with toxin/chemical warfare-induced hypotension. HOC has been studied as antidote against cyanide toxicity and it provides multiple benefits during shock [30-34] and sepsis.[35-37] In fact, HOC can improve cyanide induced shock and cardiac arrest possibly by reducing nitric oxide. Given that hemorrhagic shock and sepsis are also high in nitric oxide, and those states improve with reduction of nitric oxide, it is postulated that HOC could provide benefit to sepsis and hemorrhagic shock by reducing nitric oxide. Moreover, HOC may be used as an antidote against several types of chemical weapon exposures and chemical induced shock. Thus, ongoing studies will likely show that hydroxocobalamin may be a single treatment for several types of combat related shock.

Our study team has performed several studies using HOC and have demonstrated its effectiveness in volume-controlled hemorrhagic shock with observations over 1 hour in a preclinical setting.[30] In our study, blood pressure improved above baseline in all experimental animals with severe hypotension. The rise in blood pressure was likely due to nitric oxide scavenging.[38] We have also demonstrated that hydroxocobalamin can improve cardiac output, increase heart rate, reduce inflammatory markers, improve brain oxygenation, and reduce troponin leakage and myocardial damage (WHMC protocols FWH20070150A, FWH20080048A, FWH20100171A). We have also shown that hydroxocobalamin is as effective as whole blood in reversing hypotension and inhibiting rises in lactic acid in a Class III volume-controlled hemorrhagic model (WHMC protocol FWH20110148A). Manuscripts and abstracts from these experiments have been published.

Relevance of the current study

One limitation of our previous work evaluating resuscitation fluids in a controlled-hemorrhagic model was that the observation period was limited to one hour. Although the average military medical evacuation time is 55 minutes, under combat conditions and certain weather conditions, evacuation can and is delayed. Therefore, the effect of resuscitation treatments used over extended times must be examined. Moreover, our previous work has evaluated the efficacy of hydroxocobalamin in models in which 30% of total blood volume was lost.

With the study performed under this report, we have evaluated the use if hydroxocobalamin in a controlled hemorrhage model where 40% of the blood volume is removed, and in an uncontrolled hemorrhage model where systolic blood pressure decreased to 40-45 mmHg, representing approximately 40% blood loss.

The advantage of a volume-controlled hemorrhage model is that the amount of blood loss is consistent across experimental groups and findings from volume-controlled hemorrhage studies are reproducible across species. Furthermore, Frankel's model of a rapid rate of hemorrhage followed by a slower rate of hemorrhage, the model we employed in our study, has been suggested to be the best model of volume-controlled hemorrhage models.[39] Moreover, controlled hemorrhage studies continue to be used as valid research tools with data from the findings reported in peer-reviewed journals. Nevertheless, controlled hemorrhage may not precisely

replicate traumatic hypotension; hence, we also compared resuscitation fluids in a model of uncontrolled hemorrhage.

Finally, given the potential benefits of hydroxocobalamin in different types of injuries that induce shock, including hemorrhage, sepsis or chemical terrorism, the use of hydroxocobalamin would be beneficial, thus offering one safe drug for several types of combat induced shock in a tactical environment.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

Aims/Objectives

1) To determine if hydroxocobalamin is effective in improving volume-controlled and uncontrolled hemorrhagic shock when administered to maintain a target systolic BP (SBP) of 80 to 90mmHg over 6 hours

Hypothesis - We hypothesized that hydroxocobalamin would increase systolic blood pressure (primary outcome) and systemic vascular resistance and would improve serum lactate in our model of uncontrolled hemorrhagic shock with equal efficacy compared to Hextend®.

Rationale - A safe, small volume, durable drug is needed for early resuscitation in the field and in the emergency department for uncontrolled hemorrhagic shock. Hydroxocobalamin is an FDA approved drug for cyanide toxicity that has been successful in the off-label treatment of sepsis and controlled hemorrhagic shock in a large animal model of swine (*Sus scrofa*). It is postulated that hydroxocobalamin scavenges nitric oxide, which is released in shock. Hydroxocobalamin also improves cardiac output, decreases inflammation, and improves brain oxygenation and is less likely to worsen coagulopathy as compared to crystalloid fluids. Thus, our plan was to evaluate this drug as an equivalent to Hextend® which is the current resuscitation fluid in the deployed setting, in a clinically relevant validated model of uncontrolled hemorrhagic shock.

2) To determine if hydroxocobalamin has fewer negative effects compared to Hextend® in improving volume-controlled and uncontrolled hemorrhagic shock when each are administered to maintain a target systolic BP (SBP) of 80 to 90mmHg over 6 hours

Hypothesis - We hypothesized that hydroxocobalamin would improve systolic blood pressure in our clinically relevant, validated swine model of uncontrolled hemorrhagic shock with equal efficacy to Hextend® without generating rebleeding. We also hypothesized that hydroxocobalamin would not affect coagulation and may be superior to Hextend in that regard.

Rationale – Hydroxocobalamin is a promising drug for hemorrhagic shock. It is a small volume, safe, FDA approved drug, which in non-hemorrhagic shock models has shown to mitigate inflammation, brain hypoxia, nitric oxide dysregulation, and acidosis. In our prior experiments we have shown that hydroxocobalamin can improve systolic blood pressure in a Class III controlled hemorrhagic model comparable to Hextend® with a mean systolic blood pressure over time of 75 mmHg which is well within the TCCC guidelines for maintenance of systolic blood pressure without creating a rebleed situation. Moreover, our experiments have shown that hydroxocobalamin does not have an effect on platelets, PT and PTT or TEG K values when used in a volume-controlled hemorrhagic shock model evaluated over 1 hour. However, there is some evidence that Hextend® may decrease platelets and prolong clotting time.

3) To determine if hydroxocobalamin is comparable to cold-stored platelets or room temperature platelets with PRBC in improving volume-controlled and uncontrolled hemorrhagic shock when administered to maintain a target systolic BP (SBP) of 80 to 90 mmHg over 6 hours

Hypothesis - We hypothesized that hydroxocobalamin would increase systolic blood pressure (primary outcome) and systemic vascular resistance and would improve serum lactate in our model of uncontrolled hemorrhagic shock with equal efficacy compared to cold-stored platelets or room temperature platelets with PRBC.

Rationale - A safe, small volume, durable drug is needed for early resuscitation in the field where blood products, including cold-stored platelets are not readily available for hemorrhagic shock.

Hydroxocobalamin is an FDA approved drug and has been successful in our volume-controlled hemorrhagic shock models when administered as a one-time bolus. There are no studies evaluating the effects of hydroxocobalamin when repeatedly administered to maintain a target systolic BP (SBP) of 80 to 90mmHg over 6 hours.

Primary Outcome Variable
The major outcome parameters were survival and the slope of the blood pressure-time curve from beginning of drug treatment to the end of observation, 6 hours later.

Secondary Outcome Variable
Secondary outcome parameters were heart rate, near Infrared spectrometry of brain and kidney, cardiac output, and death of animals prior to euthanasia. We also measured serum and brain (cerebral spinal fluid) laboratory values indicative of inflammation, hemorrhagic insult (hypoxia) and cellular stress. Clinical researchers have used microdialysis technology in brain injury to measure ischemia and cellular damage in hypotension and in brain trauma.

Methods
Seventy-two (n=72) Yorkshire swine (*Sus scrofa*), males and females, weighing between 70-90 kg were procured from a local United States Department of Agriculture registered vendor Swine weighing between 65-85kg were fasted overnight except for water ad lib. Anesthesia was induced with Telazol-Ketamine. Animals were intubated and anesthesia maintained with a mixture of oxygen and isoflurane. A foley was placed in all females and a suprapubic catheter in males, arterial PCO_2 maintained between 35 - 40 mmHg and FiO_2 kept at 0.40 during line placement. Animal temperature was maintained between 37.5 and 40 ^oC throughout the experiment (i.e. warming bed, warming fluids, warming blankets). Isoflurane was maintained between 2.5% and 3% during line placement. Prior to hemorrhage, the isoflurane was titrated down to 1%, FiO_2 titrated down to 0.21-0.26 and animals were given the first dose of Demerol (50mg/hr) and Midazolam (20mg/hr). After a 10-minute stabilization period, animals underwent either a 40% controlled hemorrhage (CH, n=36) utilizing a modified Frankel and White method (2mL/kg/min for 7 minutes and 1.2mL/kg/min for 17 minutes), or an uncontrolled hemorrhage (UH, n=36) by transecting both the femoral artery and vein and allowed to freely bleed until an SBP of 30 mmHg was reached. Following hemorrhage, animals were randomized to receive treatment with 500 mL of either whole blood (WB), LR, Hextend, PRBC with cold stored

platelets, PRBC with room temperature platelets or HOC (150mg/kg). Supplemental Ca^{2+} was given to the WB group only (1g/10 mL of $CaCl_2$). Animals were monitored for six hours after treatment. Hemodynamic parameters, blood gases and chemistries were collected throughout the duration of the experiment. Measurement of invasive parameters including blood pressure, heart rate, cardiac output (CO), SpO_2, core temperature, pulmonary artery pressure (PA), central venous pressure (CVP) and systemic vascular resistance (SVR) were recorded every minute from the start of the hemorrhage until 6 hours post- treatment.

Serum and cerebral spinal fluid were collected at the indicated time points and frozen at –80°C. At the completion of the experiment, cytokines were analyzed in duplicate via a Multiplex bead-based assay (Milliplex) according to manufacturer's instructions. Interleukin (IL)-1b, IL-6, IL-10 and Tumor necrosis factor (TNF)-a were analyzed. Heat shock protein 70 (HSP70) was analyzed by ELISA (R&D systems) following manufacturer's instructions.

Immediately after the animal was euthanized, the brain was retrieved by the veterinary pathologist from 4 study animals in each treatment group. Animals designated for brain histology were assigned at the beginning of the study via randomization using www.random.org. Sections from cerebral cortex, hippocampus, putamen, cerebellum and brainstem from both hemispheres were evaluated microscopically by a board-certified veterinary pathologist to determine if there was microscopic evidence of acidophilic neuronal necrosis (shrunken neuron with deeply acidophilic cytoplasm and pyknotic nucleus). For histology analysis, tissue sections were fixed in 10% neutral buffered formalin, processed into 5µm hematoxylin and eosin (H&E) stained tissue sections, and evaluated microscopically for evidence of acidophilic neuronal necrosis (shrunken neuron with deeply acidophilic cytoplasm and pyknotic nucleus). A histologic scoring scale was utilized to score the number of necrotic neurons present in the examined sections with 0 = none, 1 = 1 necrotic neuron/hpf, 2 = 2-3 necrotic neurons/hpf and 3 = >3 necrotic neurons/hpf. Any other microscopic lesions were scored in a severity scale of 0-5 (none, minimal, mild, moderate, marked, severe).

Statistical Analysis

Data is reported as mean ± SEM, statistical analysis was performed by repeated measures mixed models ANOVA to evaluate the differences between groups with a $p<0.05$.

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

- Kick Off Meeting – April 2017
- IACUC Approval – February 2016
- All experimental procedures completed – August 31, 2020
- Data Analysis – completed – October 2022
- Manuscript Published to JTACS – May 2023

MHSRS 2020: PAO 20255, approved 11 March 2020, "Comparison of Different Resuscitation Fluids Over Time in Volume-Controlled and uncontrolled Hemorrhage Models in Swine (*Sus scrofa*)"

MHSRS 2020: PAO 20290, approved 06 April 2020, "Comparison of Cold-Stored Platelets Combined with Packed Red Blood Cells vs. Room Temperature Platelets Combined with Packed

Red Blood Cells Over Time in Volume Controlled and Uncontrolled Hemorrhage Models in Swine (*Sus scrofa*)"

SHOCK Society Annual 2020 Conference: PAO 20130, approved 24 January 2020, "Comparison of Different Resuscitation Fluids Over Time in Volume-Controlled and uncontrolled Hemorrhage Models in Swine (*Sus scrofa*)"

These abstracts were cleared by 59 CIRS and Public Affairs but never presented in person due to COVID- 19 pandemic.

- Manuscript submitted to – the 2023 Military Supplement for The Journal of Trauma and Acute Care Surgery, January 2023.
- Dissemination of Results – (in progress: manuscript listed above and current final report).

5.0 RISK ASSESSMENT

5.1 Risk Analysis:

Medium Risk was experienced during the execution of the project that delayed its completion, which included:
1) Towards the end of completing the study we encountered a delay due to a national shortage of Demerol.
2) Due to the amount of training and research protocols that are conducted at the CIRS laboratory each year, we sometimes encountered delays in scheduling, however CIRS staff was always willing to schedule our team months in advance.
3) Even though the use of homologous blood and blood products is known to produce adverse immune reactions, we only encountered very few animals with possible allogenic responses during procedures.

5.2 Technical Challenges

In addition to the risks mentioned above, no other technical challenges were found during procedures.

6.0 TRANSITION PLAN

6.1 Military Relevance

During military conflicts, troops often suffer from hemorrhagic shock. Despite our advances in military medicine, acute hemorrhage is still the leading cause of battlefield deaths. Crystalloid and colloid fluids are heavy to carry around and are not clearly effective. This creates a need for easily stored and transportable treatments for blood volume replacement in both military and civilian environments. Blood products are ideal, however, research on their usage is ongoing and despite their biological properties, many of them can be expensive, have a short shelf life, and carry an infection risk. Hydroxocobalamin (HOC) is a small volume, small weight, safe, FDA approved drug that improves systolic blood pressure, reduces inflammation, and is neuroprotective. HOC is given as a one-time bolus and is familiar to pharmacists, paramedics, and emergency physicians. It has worked very well in other forms of shock (controlled hemorrhage, sepsis and cyanide). Therefore, HOC could be a useful adjunct to hemorrhagic shock in prehospital, tactical situations. If this

project is successful, HOC could serve as a bridge for patient stabilization during evacuation to level III facilities during the resuscitation phase. This would address the gap of lack of blood products available for resuscitation at the point of injury.

6.2 Transition Strategy

The results from this research suggest that HOC can be used to stabilize casualties undergoing hemorrhagic shock. HOC administration resulted in improved hemodynamic parameters and Ca^{2+} levels compared to LR and were equivalent to WB in both controlled and uncontrolled hemorrhage. HOC may be a viable alternative when WB is not available. This would allow our warfighters more options for resuscitative measures both in the field and En route; therefore, improving medical operations and potentially saving lives. The results here could guide future studies to ultimately change the Joint trauma system (JTS) clinical practice guidelines (CPGs) for the use of HOC in cases of hemorrhagic shock when whole blood is not available. This study started with a KRL 3 and ended with a KLR 6.

7.0 RESULTS

7.1 Survival

In terms of survival, a comparison between HOC and each of the treatment groups was performed for the CH cohort and the UH cohort. The Kaplan-Meier survival curves indicate that for the CH no significant differences were found between HOC and any of the groups within that cohort (log-rank $p = 0.1694$) (Figure 2). However, survival curves suggests that the HOC group was comparable to the other treatment groups in the controlled hemorrhage. For the UH cohort, log-rank analysis found overall significant differences between cohorts (log-rank $p = 0.0299$). However, no significant differences were found between HOC and the other groups. The survival graph suggests that the HOC group was comparable to whole blood (WB), with a trend for a slight improvement compared to the other treatment groups; however, no significant differences were found under our test conditions (Figure 2).

Several swine died in the treatment groups for both CH and UH. To note, there was a slight increase in the number of animals that died throughout the procedures in the CH cohort compared to the UH cohort. However, in terms of mortality, no statistical differences were found between HOC and any of the other treatment groups (Table 2a and 2b).

7.2 Blood Pressure Slope Over Time

From the time of treatment to the end of the study (6 hours observation), we found that for the CH cohort, there was a significant difference between the sBP slope for HOC compared to that of LR-treated animals ($p=0.0002$), with an sBP mean of 71.4 ± 7.7 mmHg for HOC vs 58.2 ± 9.8 mmHg for LR. No differences were found between the slope of HOC compared to any of the other groups (Supplementary Table 2). The sBP slope for LR was different compared to PRBC + RT PT ($p<0.0001$), PRBC + cold PT ($p=0.0002$) and Hex ($p=0.0075$) with mean sBPs (in mmHg) of 60.3 ± 3.8, 64.3 ± 4 and 58.1 ± 3.5 respectively. For the UH cohort, a significant difference in the sBP slope was found between HOC, LR and WB compared to PRBC + cold PT ($p<0.0001$) with a sBP (in mmHg) mean of 69.7 ± 6.6 for HOC, 72.4 ± 5.4 for WB, 56.2 ± 7.3 for LR.

7.3 Hemodynamic Parameters and Arterial Blood Gas (ABG) analysis.

7.3.1 Overall hemodynamic parameters and ABG's for whole blood (WB), Lactated Ringers (LR) and Hydroxocobalamin (HOC).

Swine experienced a blood loss of 41%±0.02 for Controlled Hemorrhage (CH) vs. 33%±0.07 for Uncontrolled Hemorrhage (UH). During both UH and CH, HOC administration maintained a higher systolic blood pressure (SBP), cardiac output (CO), SPO_2, and vascular resistance (SVR) that was comparable to WB and above LR levels (Table 1). Blood gas analysis revealed that during CH, lactate, potassium (K^+), O_2 and CO_2 levels were comparable to WB treatment. For UH, CO^2 and K^+ levels were also comparable to WB; however, CO_2 and lactate followed a similar trend to LR. During CH and following HOC administration, Ca^{2+} levels were elevated at several time points compared to WB and LR treatment; comparable levels were observed during UH between the three cohorts (see table 1).

7.3.2 Overall hemodynamic parameters and ABG's for packed red blood cells (PRBCs) with Cold Stored Platelets (PRBC + CSP) and PRBCs with Room Temperature Platelets

Hemodynamic variables including heart rate (HR) and cardiac output (CO) obtained throughout the experiment that included either controlled hemorrhage (CH) or uncontrolled hemorrhage (UH). Treatment groups comparing between PRBCs with Cold Stored Platelets (PRBC + CSP) and PRBCs with Room Temperature Platelets were performed.

Values shown correspond to the mean ± standard error for heart rate (HR) and Cardiac output (CO). Statistical analysis was performed by a Two-way ANOVA analysis with multiple comparisons. This analysis revealed no statistical significant differences between groups under the conditions of our experiments.

7.4 Serum and CSF cytokine analysis:

Analyte quantification analysis was performed for IL-1b, IL-6, IL-10, TNFα and HSP-70, but results were inconclusive as levels of analytes were found to be below the assay's limit of detection for multiple samples.

7.5 Pathology:

After performing brain histology analysis, no significant lesions were found in brain sections of experimental animals. In very rare cases were there were minor findings, those seemed to be artefactual and likely clinically insignificant to the objectives and outcomes of the study.

8.0 CONCLUSION/DISCUSSION

HOC administration resulted in improved hemodynamic parameters and Ca^{2+} levels compared to LR and were equivalent to WB in both controlled and uncontrolled hemorrhage. HOC may be a

viable alternative when WB is not available. This allows our warfighters more options in the field and En route.

9.0 DELIVERABLES

9.1 Publications:

Journal of Trauma and Acute Care Surgery 2023 95(2S):p S120-S128, August 2023. | *DOI:* 10.1097/TA.0000000000004049: "Comparison of Hydroxocobalamin with other resuscitative fluids in volume-controlled and uncontrolled hemorrhage models in swine (Sus scrofa)" Paredes, R. Madelaine PhD; Castaneda, Maria MS; Mireles, Allyson A. PhD; Rodriguez, Dylan MS; Maddry, Joseph MD. PAO 23054, approved 2/10/2023

9.2 Presentations:

MHSRS 2020: PAO 20255, approved 11 March 2020, "Comparison of Different Resuscitation Fluids Over Time in Volume-Controlled and uncontrolled Hemorrhage Models in Swine (Sus scrofa)"

MHSRS 2020: PAO 20290, approved 06 April 2020, "Comparison of Cold-Stored Platelets Combined with Packed Red Blood Cells vs. Room Temperature Platelets Combined with Packed Red Blood Cells Over Time in Volume Controlled and Uncontrolled Hemorrhage Models in Swine (Sus scrofa)"

SHOCK Society Annual 2020 Conference: PAO 20130, approved 24 January 2020, "Comparison of Different Resuscitation Fluids Over Time in Volume-Controlled and uncontrolled Hemorrhage Models in Swine (Sus scrofa)"

These abstracts have been cleared by 59 CIRS and Public Affairs but were never presented in person due to COVID- 19 pandemic.

10.0 COST

This proposal (AC16EM01) was funded by the Air Force Medical Support Agency (AFMSA) under the Emergency Preservation and Resuscitation Program (EPR) in the amount of $1,096,000. $546K was received in June 2016 and $526K was received in February 2017. All funds were expended.

11.0 REFERENCES

1. Holcomb JB, McMullin NR, Pearse L, et al. Causes of death in U.S. Special Operations Forces in the global war on terrorism: 2001-2004. *Ann Surg*. Jun 2007;245(6):986-91. doi:10.1097/01.sla.0000259433.03754.98
2. Kisat M, Morrison JJ, Hashmi ZG, et al. Epidemiology and outcomes of non-compressible torso hemorrhage. *J Surg Res*. Sep 2013;184(1):414-21. doi:10.1016/j.jss.2013.05.099
3. Beekley AC, Sebesta JA, Blackbourne LH, et al. Prehospital tourniquet use in Operation Iraqi Freedom: effect on hemorrhage control and outcomes. *J Trauma*. Feb 2008;64(2 Suppl):S28-37; discussion S37. doi:10.1097/TA.0b013e318160937e
4. Wedmore I, McManus JG, Pusateri AE, et al. A special report on the chitosan-based hemostatic dressing: experience in current combat operations. *J Trauma*. Mar 2006;60(3):655-8. doi:10.1097/01.ta.0000199392.91772.44

5. Pepe PE, Dutton RP, Fowler RL. Preoperative resuscitation of the trauma patient. *Curr Opin Anaesthesiol*. Apr 2008;21(2):216-21. doi:10.1097/ACO.0b013e3282f60a46
6. Mabry R, McManus JG. Prehospital advances in the management of severe penetrating trauma. *Crit Care Med*. Jul 2008;36(7 Suppl):S258-66. doi:10.1097/CCM.0b013e31817da674
7. Bickell WH, Wall MJ, Jr., Pepe PE, et al. Immediate versus delayed fluid resuscitation for hypotensive patients with penetrating torso injuries. *N Engl J Med*. Oct 27 1994;331(17):1105-9. doi:10.1056/NEJM199410273311701
8. Borgman MA, Spinella PC, Perkins JG, et al. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. *J Trauma*. Oct 2007;63(4):805-13. doi:10.1097/TA.0b013e3181271ba3
9. Joint Trauma System.TCCC Guideliness 1 August 2018. Accessed April 15.
10. Mutter TC, Ruth CA, Dart AB. Hydroxyethyl starch (HES) versus other fluid therapies: effects on kidney function. *Cochrane Database Syst Rev*. Jul 23 2013;(7):CD007594. doi:10.1002/14651858.CD007594.pub3
11. Sondeen JL, Prince MD, Kheirabadi BS, et al. Initial resuscitation with plasma and other blood components reduced bleeding compared to hetastarch in anesthetized swine with uncontrolled splenic hemorrhage. *Transfusion*. Apr 2011;51(4):779-92. doi:10.1111/j.1537-2995.2010.02928.x
12. Federal Drug Administration. Hextend Warning. Accessed April 15 a.
13. American College of Surgeons. ATLS 10th edition offers new insights into managing trauma patients. Bulletin of the American College of Surgeons. Accessed April 15 a. 2018;(June 1)
14. Dadoo S, Grover JM, Keil LG, et al. Prehospital Fluid Administration in Trauma Patients: A Survey of State Protocols. *Prehospital emergency care*. Sep-Oct 2017;21(5):605-609. doi:10.1080/10903127.2017.1315202
15. Bulger EM, May S, Kerby JD, et al. Out-of-hospital hypertonic resuscitation after traumatic hypovolemic shock: a randomized, placebo controlled trial. *Ann Surg*. Mar 2011;253(3):431-41. doi:10.1097/SLA.0b013e3181fcdb22
16. Natanson C, Kern SJ, Lurie P, et al. Cell-free hemoglobin-based blood substitutes and risk of myocardial infarction and death: a meta-analysis. *JAMA*. May 21 2008;299(19):2304-12. doi:10.1001/jama.299.19.jrv80007
17. Shuja F, Finkelstein RA, Fukudome E, et al. Development and testing of low-volume hyperoncotic, hyperosmotic spray-dried plasma for the treatment of trauma-associated coagulopathy. *J Trauma*. Mar 2011;70(3):664-71. doi:10.1097/TA.0b013e31820e83be
18. Sunde GA, Vikenes B, Strandenes G, et al. Freeze dried plasma and fresh red blood cells for civilian prehospital hemorrhagic shock resuscitation. *The journal of trauma and acute care surgery*. Jun 2015;78(6 Suppl 1):S26-30. doi:10.1097/ta.0000000000000633
19. Department of Health Affairs. 7700 Arlington BLVD FC, VA, Department of Health Affairs. . FDA approves cold-stored platelets for resuscitation. . 2015;
20. Reddoch KM, Pidcoke HF, Montgomery RK, et al. Hemostatic function of apheresis platelets stored at 4 degrees C and 22 degrees C. *Shock*. May 2014;41 Suppl 1(0 1):54-61. doi:10.1097/SHK.0000000000000082
21. Musser JB, Bentley TB, Griffith S, et al. Hemorrhagic shock in swine: nitric oxide and potassium sensitive adenosine triphosphate channel activation. *Anesthesiology*. Aug 2004;101(2):399-408. doi:10.1097/00000542-200408000-00021

22. Moon-Massat P, Scultetus A, Arnaud F, et al. The effect HBOC-201 and sodium nitrite resuscitation after uncontrolled haemorrhagic shock in swine. *Injury*. May 2012;43(5):638-47. doi:10.1016/j.injury.2010.10.013
23. White NJ, Wang X, Bradbury N, et al. Fluid resuscitation of uncontrolled hemorrhage using a hemoglobin-based oxygen carrier: effect of traumatic brain injury. *Shock*. Feb 2013;39(2):210-9. doi:10.1097/SHK.0b013e31827fd62b
24. Kan WH, Hsu JT, Schwacha MG, et al. Selective inhibition of iNOS attenuates trauma-hemorrhage/resuscitation-induced hepatic injury. *Journal of applied physiology (Bethesda, Md : 1985)*. Oct 2008;105(4):1076-82. doi:10.1152/japplphysiol.90495.2008
25. FDA Approves Drug to Treat Cyanide Poisoning. U S Food and Drug Administration 2006.
26. Hall AH, Dart R, Bogdan G. Sodium thiosulfate or hydroxocobalamin for the empiric treatment of cyanide poisoning? *Annals of emergency medicine*. Jun 2007;49(6):806-13. doi:10.1016/j.annemergmed.2006.09.021
27. Shepherd G, Velez LI. Role of hydroxocobalamin in acute cyanide poisoning. *Ann Pharmacother*. May 2008;42(5):661-9. doi:10.1345/aph.1K559
28. Shapeton AD, Mahmood F, Ortoleva JP. Hydroxocobalamin for the Treatment of Vasoplegia: A Review of Current Literature and Considerations for Use. *J Cardiothorac Vasc Anesth*. Apr 2019;33(4):894-901. doi:10.1053/j.jvca.2018.08.017
29. Technologies. CPIMM.
30. Bebarta VS, Garrett N, Boudreau S, et al. A prospective, randomized trial of intravenous hydroxocobalamin versus whole blood transfusion compared to no treatment for Class III hemorrhagic shock resuscitation in a prehospital swine model. *Acad Emerg Med*. Mar 2015;22(3):321-30. doi:10.1111/acem.12605
31. Bebarta VS, Garrett N, Boudreau S, et al. Intravenous Hydroxocobalamin Versus Hextend Versus Control for Class III Hemorrhage Resuscitation in a Prehospital Swine Model. *Mil Med*. Nov 5 2018;183(11-12):e721-e729. doi:10.1093/milmed/usy173
32. Bebarta VS, Pitotti RL, Dixon P, et al. Hydroxocobalamin versus sodium thiosulfate for the treatment of acute cyanide toxicity in a swine (Sus scrofa) model. *Annals of emergency medicine*. Jun 2012;59(6):532-9. doi:10.1016/j.annemergmed.2012.01.022
33. Bebarta VS, Pitotti RL, Dixon PS, et al. Hydroxocobalamin and epinephrine both improve survival in a swine model of cyanide-induced cardiac arrest. *Annals of emergency medicine*. Oct 2012;60(4):415-22. doi:10.1016/j.annemergmed.2012.02.002
34. Bebarta VS, Tanen DA, Boudreau S, et al. Intravenous cobinamide versus hydroxocobalamin for acute treatment of severe cyanide poisoning in a swine (Sus scrofa) model. *Annals of emergency medicine*. Dec 2014;64(6):612-9. doi:10.1016/j.annemergmed.2014.02.009
35. Broderick KE, Feala J, McCulloch A, et al. The nitric oxide scavenger cobinamide profoundly improves survival in a Drosophila melanogaster model of bacterial sepsis. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. Sep 2006;20(11):1865-73. doi:10.1096/fj.06-5780com
36. Sampaio AL, Dalli J, Brancaleone V, et al. Biphasic modulation of NOS expression, protein and nitrite products by hydroxocobalamin underlies its protective effect in endotoxemic shock: downstream regulation of COX-2, IL-1β, TNF-α, IL-6, and HMGB1 expression. *Mediators of inflammation*. 2013;2013:741804. doi:10.1155/2013/741804
37. Bebarta VS, Garrett N, Maddry JK, et al. A prospective, randomized trial of intravenous hydroxocobalamin versus noradrenaline or saline for treatment of lipopolysaccharide-induced

hypotension in a swine model. *Clin Exp Pharmacol Physiol*. Mar 2019;46(3):216-225. doi:10.1111/1440-1681.13060
38. Gerth K, Ehring T, Braendle M, et al. Nitric oxide scavenging by hydroxocobalamin may account for its hemodynamic profile. *Clinical toxicology (Philadelphia, Pa)*. 2006;44 Suppl 1:29-36. doi:10.1080/15563650600811805
39. Frankel DA, Acosta JA, Anjaria DJ, et al. Physiologic response to hemorrhagic shock depends on rate and means of hemorrhage. *J Surg Res*. Dec 2007;143(2):276-80. doi:10.1016/j.jss.2007.01.031

FIGURES AND TABLES:

Table 1a. Controlled hemorrhage cohort, swine characteristics by treatment group

	Variable	HOC	Lactated ringers	Whole blood	Hextend	PRBC + cold plts	PRBC + RT- plts	p
Controlled Hemorrhage	Male gender	3 (50%)	5 (83%)	2 (33%)	2 (33%)	4 (67%)	3 (50%)	0.4706
	Weight, kg	73.00 (6.84)	75.00 (7.04)	76.17 (6.59)	71.83 (5.64)	75.00 (6.29)	73.17 (6.43)	0.8632
	Height, in	63.00 (1.10)	65.17 (3.06)	63.33 (1.97)	63.50 (3.51)	65.00 (3.22)	62.67 (0.82)	0.4046
	BSA	1.76 (0.09)	1.82 (0.12)	1.80 (0.09)	1.76 (0.12)	1.83 (0.13)	1.75 (0.08)	0.7110
	Total vol of blood out by mL	2098 (482.6)	2299 (513.9)	1963 (314.7)	2073 (96.06)	1908 (568.1)	1883 (423.8)	0.5749
	Total vol of blood out by weight	2052 (481.3)	2251 (520.6)	1924 (293.8)	1983 (107.3)	1836 (541.0)	1862 (382.6)	0.5554
	Percent blood by mL	0.43 (0.08)	0.45 (0.06)	0.40 (0.09)	0.44 (0.03)	0.39 (0.13)	0.39 (0.10)	0.7289
	Percent blood by weight	0.42 (0.08)	0.44 (0.06)	0.39 (0.08)	0.42 (0.04)	0.38 (0.12)	0.39 (0.10)	0.7365
	Total pig blood volume	4818 (451.5)	5062 (637.2)	5027 (434.6)	4741 (372.0)	4950 (415.3)	4829 (424.5)	0.8052
	Mortality	3 (50%)	5 (83%)	3 (50%)	1 (17%)	2 (33%)	2 (33%)	0.2781

Values are count (percentage) or mean (standard deviation).

Table 1b. Uncontrolled hemorrhage cohort, swine characteristics by treatment group

	Variable	HOC	Lactated ringers	Whole blood	Hextend	PRBC + cold plts	PRBC + RT- plts	P
Uncontrolled Hemorrhage	Male gender	2 (33%)	5 (83%)	4 (67%)	1 (17%)	4 (67%)	5 (83%)	0.1334
	Weight, kg	71.00 (8.97)	70.67 (4.76)	70.50 (3.51)	67.83 (3.87)	72.83 (5.81)	73.67 (4.41)	0.5474
	Height, in	61.67 (2.34)	60.33 (7.42)	60.50 (7.18)	61.00 (1.41)	63.50 (2.26)	63.67 (1.97)	0.6736
	BSA	1.72 (0.13)	1.66 (0.14)	1.75 (0.04)	1.67 (0.06)	1.77 (0.07)	1.78 (0.08)	0.1412
	Total vol of blood out by mL	1602 (649.1)	1381 (349.0)	1484 (299.7)	1426 (128.0)	1792 (391.3)	1743 (369.8)	0.3795
	Percent blood by mL	0.34 (0.10)	0.30 (0.07)	0.32 (0.05)	0.32 (0.04)	0.38 (0.06)	0.36 (0.08)	0.4027
	Total pig blood volume	4615 (582.8)	4640 (314.0)	4642 (239.0)	4409 (251.5)	4748 (401.2)	4801 (296.3)	0.5485
	Mortality	1 (17%)	3 (50%)	0 (0%)	0 (0%)	4 (67%)	2 (33%)	0.0630

Values are counts (percentage) or mean (standard deviation).

Figure 1: Survival

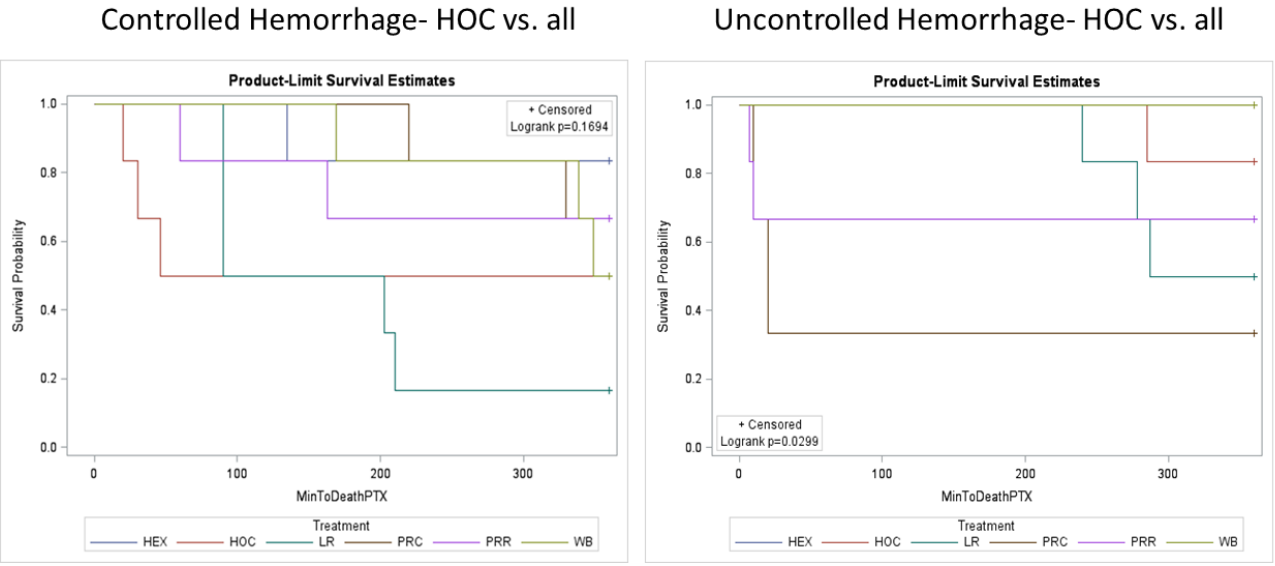
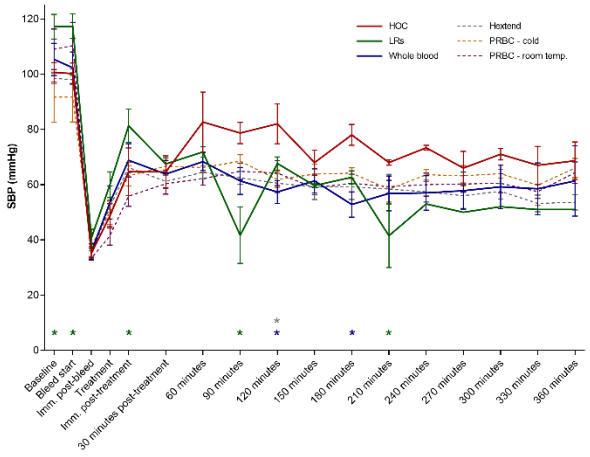
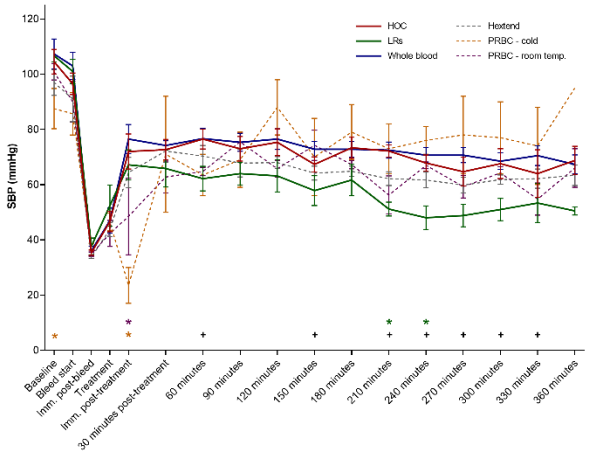
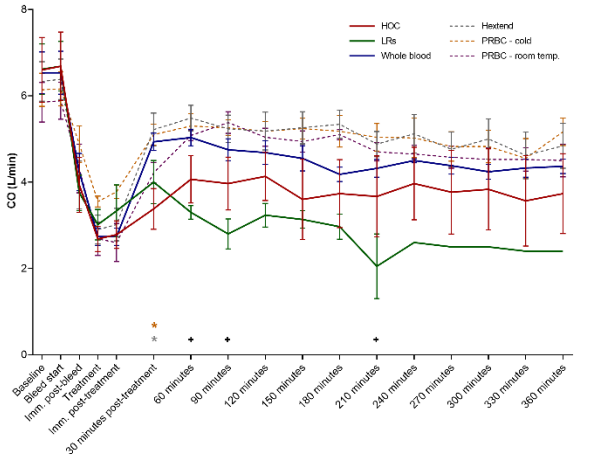


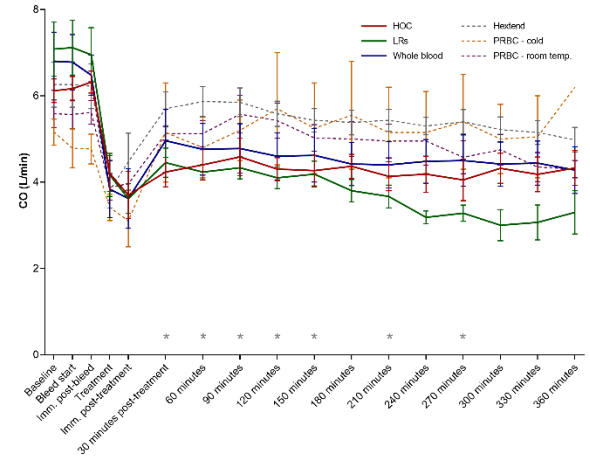
Figure 1: Survival time for Controlled Hemorrhage treatment groups (left) and uncontrolled hemorrhage treatment groups (right) - HOC vs all treatment groups: Survival time between groups was analyzed using Kaplan-Meier survival curves and log-rank p values. For **CH treatment groups (left)**, no significant differences were found between treatment groups (log-rank p = 0.1694). For **UH treatment groups (right)**, log-rank analysis found significant differences between groups (log-rank p = 0.0299). However, no significant differences were found between HOC and the other groups.

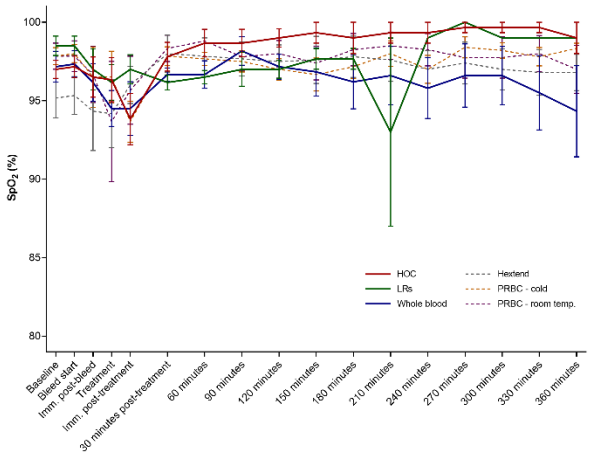
Controlled hemorrhage **Uncontrolled hemorrhage**











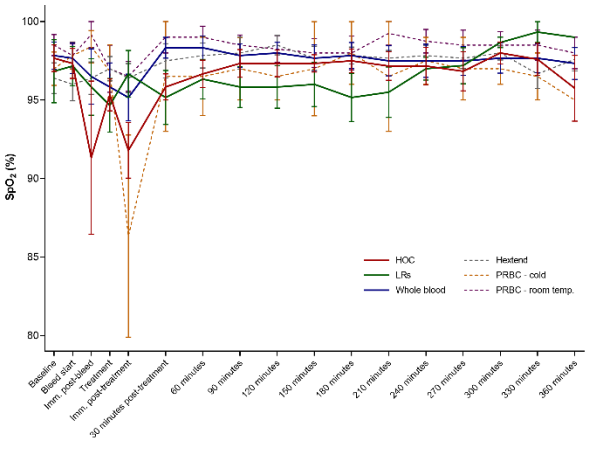


Figure 2. Hemodynamic parameter comparison for all controlled hemorrhage treatment groups (left) and uncontrolled hemorrhage treatment groups (right). Data are shown as the mean ± standard error. Significant differences ($p<0.05$ after adjusting for multiple comparisons) are represented using green stars (*) for HOC vs. LRs, blue stars (*) for HOC vs. whole blood, gray stars (*) for HOC vs. Hextend, orange stars (*) for HOC vs. cold PRBC, purple stars (*) for HOC vs. room temperature PRBC, and black crosses ($^{+}$) for LRs vs. whole blood.

Table 2a. Percent Mortality for each group within CH and UH cohorts.

	Variable	HEX	HOC	LR	PRBC + cold plt	PRBC + RT-plt	WB	p for overall chi square
Controlled Hemorrhage (CH)	Mortality	1 (17%)	3 (50%)	5 (83%)	2 (33%)	2 (33%)	3 (50%)	0.2781
Uncontrolled Hemorrhage (UH)	Mortality	0 (0%)	1 (17%)	3 (50%)	4 (67%)	2 (33%)	0 (0%)	0.0630

Table 2b. Mortality comparison between groups for each cohort.

	Controlled Hemorrhage (CH)	Uncontrolled Hemorrhage (UH)
Groups compared	**p for chi square**	**p for chi square**
HOC vs. HEX	0.5455	0.9999
HOC vs. LR	0.5455	0.5455
HOC vs. PRBC + cold plt	0.9999	0.2424
HOC vs. PRBC + room temp plt	0.9999	0.9999
HOC vs. WB	0.9999	0.9999
WB vs. LR	0.5455	0.1818
PRBC + cold plt vs. PRBC + room temp plt	0.9999	0.5671
PRBC + cold plt vs. Hex	0.9999	0.0606
PRBC + cold plt vs. LR	0.2424	0.9999
PRBC + cold plt vs. WB	0.9999	0.0606
PRBC + room temp plt vs. Hex	0.9999	0.4545
PRBC + room temp plt vs. LR	0.2424	0.9999
PRBC + room temp plt vs. WB	0.9999	0.4545

12.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

Advanced Trauma Life Support (ATLS)
Arterial blood gas (ABGs)
Cardiac output (CO)
Clinical Investigations & Research Support (CIRS)
Controlled Hemorrhage (CH)
Cold Stored Platelets (CSP)
Food and Drug Administration (FDA)
Hemoglobin oxygen-based carriers (HBOC)
Hydroxocobalamin (HOC)
Improvised explosive device (IED)
Joint Trauma System (JTS)
Knowledge Readiness Level (KRL)
Lactated Ringers (LR)
Mean arterial pressure (MAP)
National Institutes of Health (NIH)
Packed Red Blood Cells (PRBC)
Platelets (PT)
Potassium (K^+)
Room Temp (RT)
Pulse oximetry (SPO_2)
Systolic blood pressure (SBP)
Tactical Combat Casualty Care (TCCC)
Uncontrolled Hemorrhage (UH)
Vascular resistance (SVR)
Whole blood (WB)