Conner, Jeff, Lammers, Daniel, Holtestaul, Torbjorg, Jones, Ian, Kuckelman, John, Letson, Hayley, Dobson, Geoffrey, Eckert, Matthew, and Bingham, Jason (2021) ***Combatting ischemia reperfusion injury from resuscitative endovascular balloon occlusion of the aorta (REBOA) using adenosine, lidocaine and magnesium: a pilot study.*** **Journal of Trauma and Acute Care Surgery, 91 (6) pp. 995-1001.**

https://doi.org/10.1097/TA.0000000000003388

Journal of Trauma and Acute Care Surgery, Publish Ahead of Print
DOI: 10.1097/TA.0000000000003388

Combatting Ischemia Reperfusion Injury from Resuscitative Endovascular Balloon Occlusion of the Aorta (REBOA) using Adenosine, Lidocaine and Magnesium: A Pilot Study

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Conflicts of Interest: No authors have any financial disclosures to report. G.D. is the inventor of the ALM concept for cardiac surgery, trauma and sepsis, however, he has no financial interests in any entity or organization

Disclosures: No disclosures

Meeting: This work was selected for an oral presentation at the 34th EAST Annual Scientific Assembly (virtual), January 13-14, 2021

ABSTRACT

Background:

REBOA, a minimally invasive alternative to resuscitative thoracotomy, has been associated with significant ischemia reperfusion injury (IRI). Resuscitation strategies utilizing adenosine, lidocaine, and magnesium (ALM) have been shown to mitigate similar inflammatory responses in hemorrhagic and septic shock models. This study examined the effects of ALM on REBOA-associated IRI using a porcine model.

Methods:

Animals underwent a 20% controlled hemorrhage followed by 30 minutes of supraceliac balloon occlusion. They were assigned to one of four groups: control (n=5), 4 hour ALM infusion starting at occlusion, 2 hour (n=5) and 4 hour (n=5) interventional ALM infusions starting at reperfusion. ALM cohorts received a post hemorrhage ALM bolus followed by their respective ALM infusion. Primary outcomes for the study assessed physiologic and hemodynamic parameters.

Results:

ALM infusion after reperfusion cohorts demonstrated a significant improvement in lactate, base deficit, and pH in the first hour following systemic reperfusion. At study endpoint, continuous ALM infusion initiated after reperfusion over 4 hours resulted in an overall improved lactate clearance when compared to the 2-hour and control cohorts. No differences in hemodynamic parameters were noted between ALM cohorts and controls.

Conclusion:

ALM may prove beneficial in mitigating the inflammatory response seen from REBOA-associated IRI as evidenced by physiologic improvements early during resuscitation. Despite this, further refinement should be sought to optimize treatment strategies.

Study Type: Animal Study, pre-clinical

Level of Evidence: N/A Animal Study

Key words: Adenosine Lidocaine Magnesium, REBOA, Noncompressible truncal hemorrhage, Ischemia reperfusion

BACKGROUND

Non-compressible truncal hemorrhage (NCTH) remains a leading cause of mortality in both military and civilian trauma populations.[1-3] Acute, life-saving measures, such as resuscitative thoracotomy (EDT) or resuscitative endovascular balloon occlusion of the aorta (REBOA), are frequently required for survival. These techniques work by temporarily occluding aortic blood flow proximal to the suspected injury. Despite their life-saving nature, each technique is potentially accompanied by a cohort of severe morbidities. While EDT offers the advantage of directly assessing the thoracic cavity and pericardium, the resultant morbidity associated with thoracotomy in critically ill patients has led to efforts to develop less invasive methods for the stabilization of non-compressible hemorrhage. Thus, in the absence of thoracic injuries, REBOA has become an increasingly attractive option due to its minimally invasive nature. Importantly, both techniques result in tissue ischemia distal to occlusion and require emergent surgical intervention for definitive hemorrhage control and restoration of distal blood flow.

REBOA proponents advocate for the future possibility of its use in the prehospital setting to minimize ongoing hemorrhage. Military efforts mirror these thoughts as recent conflicts in the Middle East have placed an increased emphasis on minimizing time from injury to hemorrhage control.[4,5] Combat casualty populations experience high rates of penetrating and blast injury resulting in severe injury patterns that require invasive hemorrhage control techniques near the point of injury.[6] Consequently, reports of REBOA being deployed as close to the point of injury as possible have surfaced with successful use recently reported in the military prehospital setting.[7] Although hospital transport times within the United States have become increasingly

shorter, making point of injury REBOA arguably plausible, the deployed or combat setting provides unique set of challenges when transporting a critically injured patient. Thus, the forward application of REBOA must be carefully considered in light of the increased morbidity and mortality seen with aortic occlusion times greater than 30 minutes.[8] The current Joint Trauma System Clinical Practice Guidelines recommends that thoracic aortic occlusion (i.e. Zone I placement) times do not exceed 30 minutes for REBOA use, with the caveat that shorter occlusive times are preferred.[9] Civilian literature affirms these findings with shorter occlusion times correlating with improved short-term survival.[10] Additionally, animal models have demonstrated increased release of interleukin 6, higher incidence of acute respiratory distress syndrome, increased vasopressor requirements, and higher mortality rates with occlusion times greater than 30 minutes.[11, 12] Regional tissue ischemia has been shown to significantly increase the inflammatory response with occlusion times approaching 60 minutes.[13]

Aortic occlusion and malperfusion causes distal ischemia and irreversible organ damage that worsen with increasing occlusion times. Restoration of blood flow, although necessary, results in a clinical phenomenon called reperfusion injury that frequently worsens the physiologic insult by systemically releasing a multitude of inflammatory mediators. This process is collectively termed ischemia reperfusion injury (IRI). Current theories behind IRI suggest that following cessation of blood flow to the gut, mesenteric ischemia-induced inflammatory markers enter the lymphatic system and eventually the systemic circulation via the thoracic duct.[15, 16] Once in the systemic circulation, the associated cytokines are believed to be responsible for the high rates of trauma-related coagulopathy, multiorgan dysfunction, infection, and mortality.[16 - 19] Although the cornerstone of treating or preventing IRI is timely reperfusion, this is not always

possible in a far forward environment with limited surgical capabilities. Preventing IRI has been a focus of ongoing research for many years through techniques focused on decreasing metabolic demand, administration of antioxidant therapies, utilization of immunosuppressive therapies, and ischemic preconditioning. Recently, the use of adenosine, lidocaine, and magnesium (ALM) has demonstrated early promise in animal studies for IRI mitigation in various hemorrhagic shock and sepsis models. This drug combination has been well studied in the field of cardiac surgery demonstrating cardioprotective effects from post-operative IRI .[20, 21] Several animal studies have subsequently demonstrated the potential uses of ALM as a small volume resuscitative agent in hemorrhagic shock, cardiac arrest, traumatic brain injury, and sepsis.[22] These studies have demonstrated improved survival, decreased inflammatory states, correction of coagulopathy, and improved hemodynamics following severe and catastrophic blood loss.[23 - 26] Although the exact mechanism of action has not been fully elucidated, ongoing theories support the notion that ALM switches to a survival phenotype and reduces tissue metabolic demand through differential expression of the master genes of metabolism.[27]

While ALM has proven successful for mitigating IRI in various trauma models, its application during REBOA-based resuscitation has not been studied. To date, the majority of IRI mitigation efforts following REBOA have been mainly focused on decreasing aortic occlusion times or utilizing partial occlusion strategies.[28 - 30] Thus, the potential for pharmacologic strategies to decrease the IRI-associated inflammatory insult offers the opportunity for prolonging occlusion times and decreasing the REBOA-associated morbidities. This study sought to examine the physiologic effects of utilizing ALM in a REBOA-based IRI porcine model to mitigate the deleterious effects of IRI following reperfusion.

METHODS

Animal Care and Invasive Monitoring

Institutional Animal Care and Use Committee approval was attained (Protocol 219003) and all animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the Institute of Laboratory Animal Research. Adult male Yorkshire swine between 40 and 50 kg underwent general endotracheal anesthesia and adequate depth of anesthesia was ensured and monitored with pedal reflexes. The study was considered "non-survival." This ensured that mortality resulting from the protocol occurred under anesthesia and animals that survived to the experimental end point were euthanized.

Following anesthesia, a midline neck dissection exposed central vessels for invasive monitoring. A 5-French (Fr) carotid arterial line was placed for blood pressure monitoring and a 10-Fr sheath was used as a conduit for pulmonary artery catheter placement. A 7-Fr contralateral external jugular vein catheter was placed for central venous access, as well as to provide the conduit for controlled hemorrhage. Midline laparotomy was performed for placement of an intra-abdominal bladder catheter. The distal aorta was accessed at the level of the iliac artery for placement of a 7-Fr sheath and the REBOA. The REBOA was pre-placed in the supradiaphragmatic or Zone 1 position based on anatomic measurement. Normothermia was maintained during and after surgical instrumentation via conductive warming mechanisms. Animals received a weight-based maintenance intravenous crystalloid infusion during surgical instrumentation.

Experimental Design

Treatment groups consisted of: 1) Saline vehicle control (n = 5), 2) 4 hour ALM infusion beginning at aortic occlusion (n = 5), 3) 2 hour ALM infusion during reperfusion (n = 5), or 4) 4 hour ALM infusion during reperfusion (n = 5). All animals underwent 20% total blood volume hemorrhage using the 7-FR central venous catheter. During this time, animals did not receive any additional resuscitation and hemorrhage was completed as fast as animals could tolerate via a pressure-based hemorrhage to maintain a mean arterial pressure (MAP) greater than 40 mmHg. Blood was collected and stored in citrate bags for resuscitation used later in the protocol. ALM cohorts received a post-hemorrhage ALM bolus while control animals received saline. Each bolus was given over a 5 minute period followed by a 30 minute Zone 1 aortic occlusion via the REBOA catheter. All animals subsequently received their respective continuous ALM or saline infusions based on their experimental cohort (Figure 1). All animals were followed for four hours after systemic reperfusion.

ALM Dosing and Resuscitation Strategy

Animals under each ALM intervention arm received the same post hemorrhage bolus dose of the drug combination: 4 ml/kg 3% sodium chloride (NaCl) with 0.5 mM adenosine, 1.5 mM lidocaine, 1.25 mM magnesium sulfate ($MgSO_4$). The continuous infusion dose of ALM intervention consisted of: 0.9% NaCl with 8.98 mM adenosine, 16.62 mM lidocaine, 22.26 mM $MgSO_4$ at a rate of 2 ml/kg/hr. The continuous infusion was initiated either at time of aortic occlusion or at time of REBOA balloon deflation (reperfusion) depending on the experimental cohort. Saline vehicle controls received post hemorrhage bolus of 4 ml/kg 3% NaCl followed by a continuous infusion of 0.9% NaCl at a rate of 2 ml/kg/hr. Blood product resuscitation followed

the practice of hypotensive resuscitation with administration of prior hemorrhaged whole blood for MAP less than 40 mmHg. Following administration of blood products, 250 cc lactated ringer's bolus was available in order to maintain MAP above 40 mmHg.

Data Collection and Statistical Analysis

Physiologic parameters including basic vital signs, pulmonary artery catheter values, volume requirement and urine output were continuously measured. Laboratory evaluation included arterial blood gas, hemoglobin and hematocrit, lactate, prothrombin time and international normalized ratio and partial thromboplastin time. Measurements were obtained following induction and invasive line placement (baseline), post hemorrhage, systemic reperfusion (T0), 1 hour after reperfusion (T1), 2 hours after reperfusion (T2), and 4 hours after reperfusion (T4).

Standard descriptive statistics and analysis of variance (ANOVA) with Tukey post hoc test were performed using IBM SPSS statistics 24 (IBM Corp., Armonk, NY). Assessment of data normality was completed with Shapiro-Wilks. Comparisons were made between the experimental arms for all data points. Significance was set at a *p* value greater than 0.05.

RESULTS

A total of 20 animals weighing 47.6 kg (+/- 2.9 kg) were included in the study. Hemodynamics and laboratory values were comparable in all animals at baseline and after controlled hemorrhage (Table 1). Survival to study endpoint (4 hours after reperfusion) was 100% for the control and ALM cohorts that received their infusions following REBOA-balloon

deflation. All groups required resuscitation with shed blood in order to maintain MAP at goal (>40 mmHg). The cohort receiving ALM infusion during aortic occlusion demonstrated significantly worse survival (40%) compared to all other arms ($p < 0.05$) (Figure 2). The 3 animals that expired prior to study endpoint did so during the reperfusion time period and all experienced similar bradyarrhythmias, followed by cardiac arrest. Furthermore, this specific group required significantly more crystalloid boluses in order to maintain MAP > 40 mmHg ($p < 0.001$). No other groups required additional crystalloid boluses following shed blood resuscitation.

Hemodynamics following reperfusion were similar between the control and the ALM arms that received continuous infusions upon REBOA-balloon deflation at all time points. Lactate levels at 4 hours after reperfusion (T4) were significantly lower ($p = 0.031$) in the 4 hour ALM post-deflation infusion group compared to both the control and the 2 hour ALM post-deflation infusion group (0.8 vs 1.9 and 2.3; Table 1). The overall trend in lactate from time of reperfusion to T4 is demonstrated in Figure 3 with the significant value at T4 highlighted for the ALM post reperfusion group. The 4 hour ALM cohort that received treatment infusion during aortic occlusion was excluded from the analysis after the 1 hour time point (T1) due to decreased sample size and inferior survival.

Following analysis of each individual value at their respective time points, changes in lactate, pH and base excess from point of reperfusion (T0) to T1 were reviewed. For this portion of the analysis, animals who received post-deflation ALM infusions were grouped together given the intervention received was the same until 2 hours after reperfusion. The combined values of

pH, base excess and lactate along with the change in values from T0 to T1 are shown in Table 2. When combined and compared to the control group, the ALM animals demonstrated a significant difference in the changes of lactate, pH and base excess in the first 1 hour following reperfusion ($p < 0.001$, $p = 0.048$, $p = 0.023$; Fig 5). Subset analysis of hemodynamics following hour 2 after reperfusion of the 4 hour and 2 hour post-deflation ALM infusion groups revealed a significantly ($p = 0.02$) lower heart rate in the 4 hour infusion group (mean = 85, 95% CI 72 - 98) compared to 2 hour infusion (mean 128, 95% CI 100 - 170), and no significant difference in mean arterial pressure at 4 hours after systemic reperfusion.

DISCUSSION

The ability to mitigate detrimental effects of IRI represents a highly desirable intervention in treatment of traumatically injured patients, especially in austere, far-forward military environments where time to definitive surgical intervention may be prolonged. Using small volume ALM therapy, we were able to demonstrate findings suggestive of an improved physiologic status in a porcine REBOA IRI model during the early resuscitative phases following reperfusion. To our knowledge, this is the first study assessing the effect of ALM on animals undergoing hemorrhage and aortic occlusion with REBOA. Despite this novel application, ALM has been well studied in a variety of other animal models and demonstrated positive results, including blunting the inflammatory response, improving coagulation parameters, and stabilizing hemodynamics for a multitude of critical illnesses. [27, 31, 32] We believe the work displayed by this study represents an advancement in understanding ALM-based resuscitation practices and provides a conceptual avenue for optimizing REBOA use in trauma care.

Initially known for its myocardial protectant properties when compared to other high potassium containing solutions used for cardioplegia, the use of ALM has demonstrated multiple systemic benefits that remain highly advantageous for traumatically injured patients. [20, 21] Due to its cardioplegic roots, concerns have been raised over its early use in trauma models due to fears for inducing cardiac arrest. However, through utilizing a lower dosing strategy, proponents of ALM have not demonstrated these effects in the multitude of hemorrhagic shock, sepsis, and traumatic brain injury models studied.[23-27, 31, 32] Interestingly, our study demonstrated fairly consistent bradyarrhythmia leading to cardiac collapse when the ALM was infused during aortic occlusion, proximal to the REBOA-balloon. This raised initial concerns regarding the solution's safety profile, however, these effects were not demonstrated when the infusion was initiated following REBOA-balloon deflation. In the REBOA model, it appears the ALM concentration was too high in the cardiopulmonary circuit during aortic occlusion. This finding demonstrates the importance of future studies examining the effect of varying the ALM dosing in combination with aortic occlusion. Other studies have used similar concentrations and dosing strategies with favorable outcomes, however, these were not in combination with REBOA. Importantly, all animals who received their ALM infusion following REBOA-balloon deflation did not display the previously seen arrhythmias. While this study did not directly assess the effects of initiating an ALM infusion distal to the REBOA-balloon during aortic occlusion, further studies utilizing this model with lower initial doses would help determine the etiology of these cardiac arrhythmias.

Our study utilized a 30-minute REBOA occlusion time based on the current military

clinical practice guidelines and a dosing strategy per expert guidance from leaders in ALM resuscitation. Despite this, it remains highly possible that our model contains much room for optimization. Although large animal models may be the best surrogate to human trials, animals display inherently different pathophysiology, and therefore, it is possible that a 30-minute REBOA inflation period does not create the same degree and temporal progression of physiologic insult experienced by humans. Furthermore, while the doses utilized were carefully chosen using expert opinion, REBOA-based ALM models may benefit from alternative dosing strategies compared to those that demonstrated benefit in previous hemorrhagic shock and sepsis models. One such approach may consist of ALM infusion distal to the balloon catheter during occlusion for reasons previously mentioned.

Early ALM studies have demonstrated promising results in catastrophically injured animal models. Notably, ALM bolus/infusion therapy was demonstrated to be associated with 100% survivability when compared to saline controls following a 60% hemorrhage within a rat model.[24] Increased survival was thought to be secondary to the improved hemodynamic stability via enhanced ventricular-arterial coupling. However further studies have also shown a decrease in systemic inflammation, improvements in trauma induced coagulopathies, and reductions in cerebral edema following traumatic brain injury.[25, 26] These findings were also translated in severe hemorrhagic shock porcine models confirming the physiologic benefits of ALM in large animals. [20] Interestingly, a recent study by How, *et al.* failed to demonstrate the previously reported benefits of ALM with regards to mortality, hemodynamics, and metabolic parameters when comparing an ALM-based resuscitation to the Tactical Combat Casualty Care standard of care for prehospital resuscitation during combat settings.[33] These findings, however, are not

without controversy as there remain concerns regarding the dosing regimens and strategies used in the previously mentioned study, including the use of the opioid buprenorphine, which has recently been shown to reduce ALM survival.[34,35] Similarly, our study failed to demonstrate any significant hemodynamic changes between the control and experimental groups. However, four-hour ALM infusion led to significantly decreased lactate at the completion of the study suggesting the potential for improvements in either tissue perfusion or lactate clearance.

Our focus mainly rested on the two experimental arms that were non arrhythmogenic. Each arm received equivalent ALM dosing for the pre-REBOA bolus and the continuous drip during the first two hours following REBOA-balloon deflation. When combining these arms, ALM displayed significantly improved changes in lactate, pH, and base excess compared to saline controls one hour following reperfusion. These physiologic improvements support the resuscitation benefits previously shown in early ALM studies. Conversely, the proposed hemodynamic effects of ALM were not demonstrated within our combined cohorts. Due to the study design, only the laboratory values and data points up to one hour following balloon deflation could be combined for evaluation.

There are several limitations to this study that should be considered. First, small sample sizes create a high possibility for type II error. The improvements seen within the combined ALM cohorts during the early reperfusion phase highlight this notion as the sample size was effectively doubled resulting in statistically significant findings. Moreover, as this study was the first to assess ALM with REBOA use, it remains possible that the induced physiologic insult within this model did not drive the same degree of hemodynamic and cardiovascular

derangements seen within previous studies. This is supported by the 100% survival rate of the control group and may account for the lack of hemodynamic differences between our study groups. Further model testing to assess varying degrees of hemorrhage, increased aortic occlusion times, and partial REBOA techniques should be evaluated in order to optimally define the potential role of ALM use with REBOA during acute traumatic resuscitation. Alternative laboratory testing such as coagulation and inflammatory parameters, tissue analysis, metabolic testing, and gene expression analysis could be implemented in future studies to gain insight into further ALM-associated changes at the organisms, tissue, and cellular levels. Finally, although large animal models embody the best representation of human physiology for nonclinical research, the complexities of the human inflammatory, cardiovascular, endocrine, and metabolic responses to traumatic insult are notoriously difficult to replicate within animal models resulting in heavily scrutinized findings prior to any human implementation.

ALM-based therapy may have a role in mitigating the inflammatory and physiologic response seen with REBOA-associated IRI as evidenced by physiologic improvements early during resuscitation. Despite this, the findings from this study suggest that further investigations assessing the use of ALM with REBOA should be performed prior to wide-scale adoption. The successful mitigation of IRI during REBOA may allow for prolonged aortic occlusion times while decreasing the morbidities associated with reperfusion. To date, efforts to do so have largely focused on partial REBOA techniques, however we believe that using pharmacologic therapies, such as ALM, to reduce IRI and decrease metabolic requirements represent a relatively unexplored avenue that may result in expanded indications for REBOA use, particularly in far-forward military and prehospital civilian setting.

AUTHOR CONTRIBUTIONS:

J.C. and D.L conducted the literature search. All authors contributed to the study design. J.C., T.H, and I.J. collected the data. J.C, D.L. and J.K. interpreted the data. J.C. and D.L. wrote the article. All authors critically revised the final article.

ACKNOWLEDGEMENTS:

We would like to thank the staff of the Department of Clinical Investigation and the Live Animal Research Staff for their contributions in carrying out the study.

CONFLICTS OF INTEREST

There are no conflicts of interest

SOURCE OF FUNDING

This study was supported by a ZOLL Foundation research grant and a Geneva Foundation research grant.

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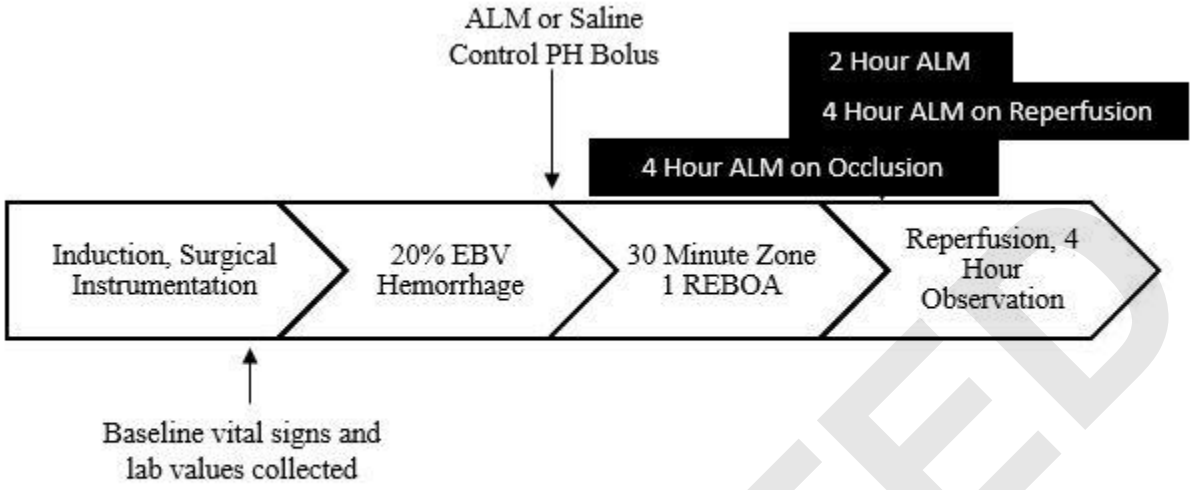
FIGURE LEGENDS:

Figure 1. Experimental Outline

Figure 2. Kaplan-Meier curve demonstrating decreased survival in 4 hour ALM occlusion group compared to all other groups.

Figure 3. ALM Cohorts vs Control Lactate Trend

Figure 1



Key: EBV – estimated blood volume; PH – post hemorrhage

Figure 2

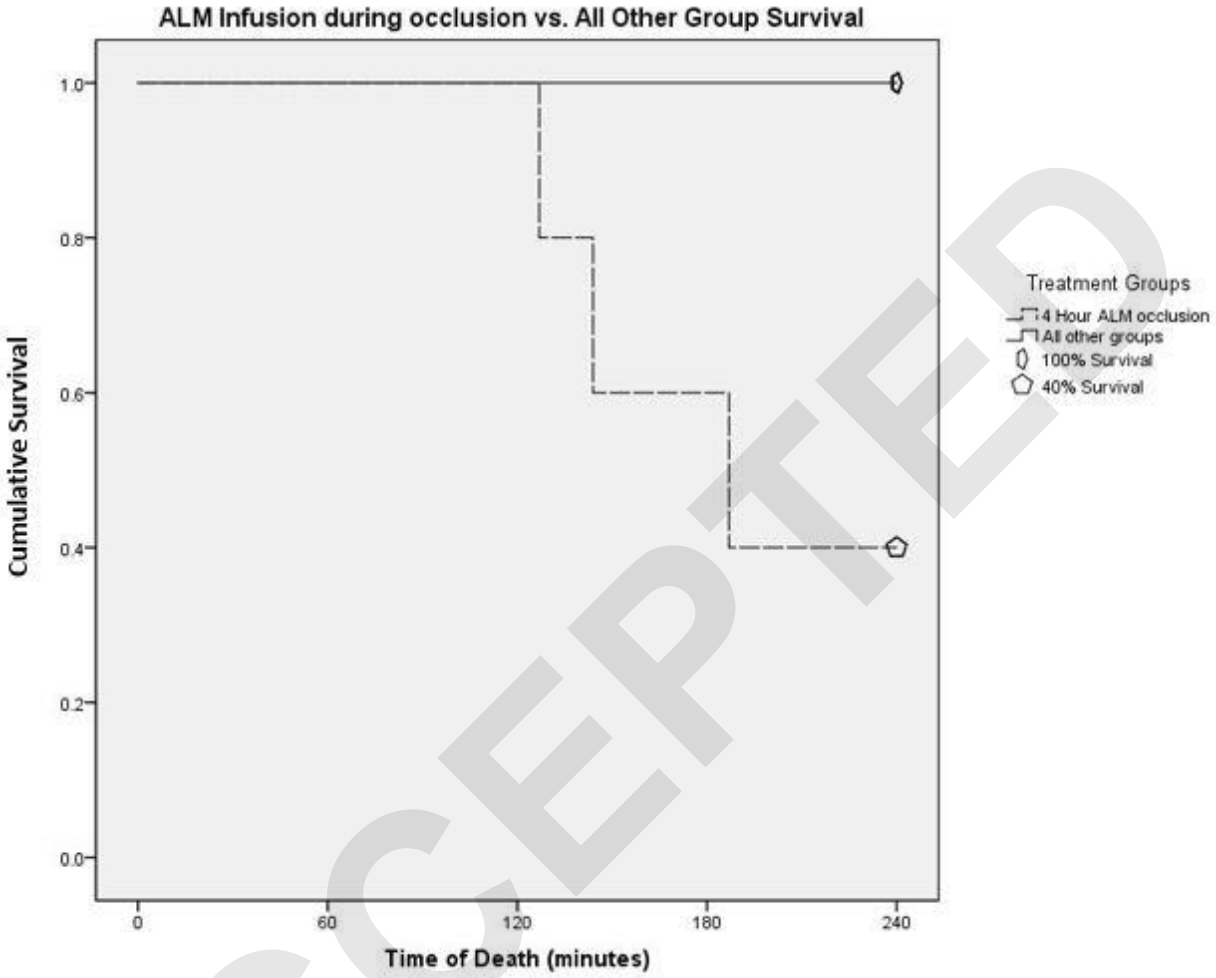
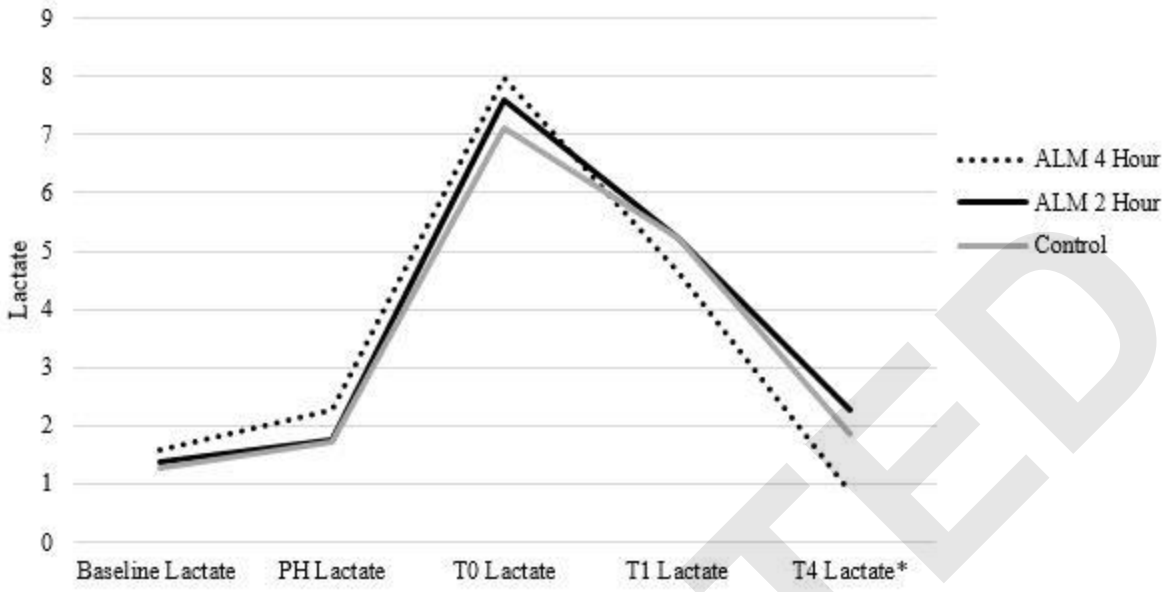


FIGURE 3. ALM Cohorts vs Control Lactate Trend



Key: PH, post hemorrhage; T0, time of reperfusion/REBOA deflation; T1, one hour after reperfusion; T4, 4 hours after reperfusion; *, $p < 0.05$

Table 1. Individual group values are shown, significant comparisons are highlighted

	Control					4 Hour ALM post reperfusion					2 Hour ALM post reperfusion					4 Hour ALM on occlusion				
	B	PH	T0	T1	T4	B	PH	T0	T1	T4	B	PH	T0	T1	T4	B	PH	T0	T1	T4
Hemodynamics																				
Heart rate, bpm	79.4	87.6	142.4	105.4	100.2	83.8	112.3	133.75	113.5	85⸸	83.3	125.8	152	129	128.5	98.2	136.2	136.4	105.8	130.5
MAP, mmHg	52.8	42	54	51.8	45.2	65.5	55.25	63.5	54.3	52.8	60.3	46.5	58.5	55.8	49.3	64.4	45	49.6	47.6	63.5
CO, L/min	3.1	2.6	3.8	4.6	4.1	3.5	3.4	5.4	4.9	3.7	3.2	3.2	4.4	4.6	4.4	3.6	2.7	3.3	4.3	5.2
Lab Values																				
pH	7.52	7.49	7.28	7.39	7.46	7.48	7.47	7.22	7.4	7.48	7.58	7.57	7.32	7.48	7.52	7.52	7.46	7.29	7.31	7.31
Lactate, mg/dL	1.3	1.7	7.1	5.2	1.9	1.5	2.1	7.8	4.2	0.8*	1.4	1.7	7.6	5.1	2.3	0.9	2.1	8.4	7.5⸸*	1.5
Base Excess	8.6	7.8	-2.4	2.2	7.2	8.3	7.5	-4.75	3	9.75	13.3	11.5	-1	4.5	9	11	7.8	-6	-2.4	5
Hematocrit, %	24.2	24.4	30.2	26.6	25.6	21	21.5	23	22.8	22.8	23.5	25.8	29.5	26.8	28	26.4	28.8	26.8	29.5	28
Hemoglobin, g/dL	8.2	8.3	10.3	9.1	8.8	7.1	7.3	7.9	7.5	7.1	8	8.8	10	9.1	9.5	8.9	9.8	9.1	10.1	9.3
Potassium, mg/dL	4.2	4.4	3.9	4.9	6.7	3.9	4	3.7	4.1	5.6	3.9	3.9	3.8	4.5	6	4.4	3.6	4.9	5.7	6.7

B, baseline; PH, post hemorrhage; T0, time of systemic reperfusion; T1, 1 hour after reperfusion; T4, 4 hours after reperfusion.

, $p < 0.05$ when compared to 2 hour ALM group and control; ⸸, $p < 0.05$ when compared to 2 hour ALM; ⸸, $p < 0.05$ when compared to all groups; ⸸⸸, n = 2 (40% survival)

TABLE 2. Lactate, pH and base excess for control and ALM cohorts: baseline to one hour after reperfusion

	Control					ALM				
	B	**PH**	**T0**	**T1**	**Δ**	**B**	**PH**	**T0**	**T1**	**Δ**
Lactate	1.27	1.73	7.11	5.23	1.88	1.49	2.03	7.82	4.90	2.91*
pH	7.52	7.49	7.28	7.39	0.10	7.53	7.50	7.25	7.41	0.15*
Base Excess	8.60	7.80	-2.40	2.20	4.6	9.78	8.44	-3.89	2.89	6.78*

B, baseline; PH, post hemorrhage; T0, time of systemic reperfusion; T1, 1 hour after reperfusion; Δ**,** change in value from T0 to T1.

*, $p < 0.05$ compared to control group